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Acclimation of *Teucrium polium* Plants to Seasonal Variations by Alterations in the Activities of Antioxidant Enzymes and Protein Accumulation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNK and MM designed the study, collected plant samples, analyzed growth, physiological and biochemical parameters and wrote the first draft of the manuscript. Authors ZKA and KAA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Any change in climatic conditions affects growth and productivity directly or indirectly. The present study was carried out to investigate the impact of varying climatic conditions of Tabuk region of Saudi Arabia on the performance of *Teucrium polium* plants*.* Plants were collected under varying environmental conditions of February, May, August and November 2014. Performance of the plants was assessed in terms of fresh weight (FW) and dry weight (DW), leaf chlorophyll content (Chl), carbonic anhydrase (CA) activity, hydrogen peroxide (H_2O_2) content, leaf relative water content (LRWC), activites of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) and leaf protein content. The results show that plants collected during August gave lower values for all the studied parameters except H_2O_2 content which was highest. Therefore, climatic conditions of November were found most suitable for proper growth and development of plants, as exhibited by higher values of most of the parameters except H_2O_2 content which gave lowest values during November. Plants sampled during November gave 24.8%,

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61.8%, 48.1% and 12.0% higher values for FW, DW, total Chl content and activity of CA enzyme, respectively, than the plants collected during August which gave lowest values for these parameters. Moreover, plants collected during November again gave 0.5%, 66.4%, 34.6% and 53.3% higher values for SOD, CAT and POX and leaf protein content, respectively, than the plants collected during August. Thus, the present study suggested that the climatic conditions of Tabuk region during November suited best for the growth and development of *Teucrium polium* plants.

Keywords: Antioxidant enzymes; chlorophyll; protein; temperature; Teucrium polium.

1. INTRODUCTION

Uncontrolled anthropogenic activities coupled with extreme exploitation of natural resources severely galvanized atmospheric CO_2 concentrations to 387 ppm and predicted to fall in the range 550-900 ppm by the end of the 21st century [1]. Excessive accumulation of CO_2 absorbs heat leaving the earth's atmosphere that result in increasing surface temperatures and wider swings in weather, which are likely to reduce the performance of land plants [2,3] and expected to cause severe loss to genetic diversity by 2080 [4]. High temperature stress induces several physiological, biochemical and molecular responses in several plants [5], which adversely affect the plants and also at the same time help the plants to adapt to such conditions. Elevated temperature and enhanced solar radiations contribute to the accumulation of chemically active molecules and reactive oxygen species (ROS) in plant cells. Over production of ROS causes pigment bleaching, lipid peroxidation, protein damage, inactivation of enzyme activities [6], which ultimately culminate in the alterations in metabolism and structure of plants, especially cell membranes and many basic physiological processes such as photosynthesis, respiration, and water relations [7]. Chlorophyll is considered as one of the crucial factors in determining the performance of a plant, while chloroplasts are extremely prone to ROS production that may cause inactivation of chloroplast enzymes leading to the reduction in the rate of photosynthesis [8]. Carbonic anhydrase (CA) is the enzyme that regulates the availability of CO_2 to ribulosebisphosphate carboxylase (rubisco) by catalyzing the reversible hydration of CO_2 [9].

However, to cope with such stressful environmental conditions plants are equipped with a system of defense, orchestrated by a network of antioxidant enzymes and various proteins. Of these, the antioxidants superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) have been shown to protect plants against ROS. Enzyme SOD dismutates superoxide radicals to H_2O_2, and CAT and POX further detoxify H_2O_2 into water and oxygen [10].

Tabuk, the northwestern province of Saudi Arabia, is characterized by highly varied environmental conditions from extreme cold to extreme hot which significantly affect the performance of plants throughout the year. *Teucrium polium* L. (family Lamiaceae) is a dwarf, pubescent and aromatic shrub possessing oval leaves with aromatic margins and dense heads of white flowers. The plant has been used for over 2000 years in traditional medicine of Arabian Peninsula due to its diuretic, hypoglycemic, diaphoretic, antipyretic, antispasmodic and cholagogic properties [11-13]. Although several studies under controlled conditions have been performed to study the impact of temperature on various attributes of plants, the study of the impact of natural temperature and environmental fluctuations on growth, physiological and biochemical attributes of plants will be of crucial importance.

Although several studies under controlled conditions have been performed to study the impact of temperature on various attributes of plants but to study the impact of natural temperature and environmental fluctuations on growth, physiological and biochemical attributes of plants will be of crucial importance which will explore new insight in to response of plants to their environmental oscillations. The present investigation was carried out to evaluate the response of *T. polium* plants to varying climatic conditions in terms of growth, physiological and biochemical parameters.

2. MATERIALS AND METHODS

2.1 Study Area and Plant Collection

The study was conducted in Tabuk region (760 meter above sea level; 28°22'N, 36°36'E), the northwestern province of Saudi Arabia. At the collection site five wildly grown healthy plants were collected randomly on 10th day of February, May, August and November 2014. Environmental data of the collection site is given in Table 1.

2.2 Study of Growth Parameters

Growth parameters of the collected plant specimens were assessed in terms of fresh and dry weight. To determine fresh weight, plants were uprooted and washed to remove surface-adhered soil particles. Dry weight of plants was recorded after drying the plants at 80℃ for 24 h in a hot air oven.

2.3 Estimation of Physiological and Biochemical Parameters

The activity of CA (E.C. 4.2.1.1) in fresh leaves was measured using the method described by Dwivedi and Randhawa [14]. The enzyme activity was estimated by titrating reaction mixture against 0.05 N HCl using methyl red as an indicator. The enzyme was expressed as µM CO_2 kg^{-1} leaf FW s^{-1}.

Chlorophyll (Chl) content in fresh leaves was determined with 100% acetone using the method of Lichtenthaler and Buschmann [15]. The optical density of the pigment solution was recorded at 662, 645 nm to determine Chl-*a* and Chl-*b* content, respectively, on a spectrophotometer (CE 2021, Cecil, Cambridge, England).

Response of plants to oxidative stress was determined in terms of hydrogen peroxide (H_2O_2) content which was determined according to Velikova et al. [16]. Homogenate of fresh leaves was centrifuged at 12,000 rpm for 15 min, and 0.5 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The absorbance of supernatant was recorded at 390 nm using a spectrophotometer (CE 2021, Cecil, Cambridge, England).

To evaluate water status of plants, leaf relative water content (LRWC) was measured by the method of Yamasaki and Dillenburg [17]. Values for leaf FW, turgid weight (TW) and DW were used to calculate LRWC using the equation below:

$$\text{LRWC (\%)} = [(\text{FW-DW})/(\text{TW-DW})] \times 100$$

To determine the activities of antioxidant enzymes, leaf tissues were homogenized with three volumes (w/v) of an ice-cold extraction buffer (50 mM Tris–HCl, pH 7.8, 1 mM EDTA, 1 mM $MgCl_2$ and 1.5% (w/w) polyvinylpyrrolidone). The homogenate was centrifuged at 15,000 g for 20 min at 4℃. The supernatant was used as the crude extract for the assay of enzyme activities. Activities of SOD (E.C. 1.15.1.1), CAT (E.C. 1.11.1.6), and POX (E.C. 1.11.1.7) were determined by the method of Beauchamp and Fridovich [18], Cakmak and Marschner [19], and Upadhyaya et al. [20], respectively. Total leaf protein content was determined according to Bradford [21] using bovine serum albumin as a standard.

The chemicals used for the analyses of various parameters were supplied by Loba Chemie Pvt. Limited, Mumbai, India.

2.4 Statistical Analysis

Five replicates were taken in to account. Data were analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). When F value was found to be significant at 5% level of probability, least significant difference (LSD) was calculated. Standard error of the replicates was also calculated.

Table 1. Climatic characteristics of the study area during four months of the study

Collection time	Maximum temperature (℃)	Minimum temperature (℃)	Mean temperature (℃)	Average rain fall (mm)	Relative humidity (Max)	Radiation (mm day^{-1})
February	21.62	6.41	13.83	0.12	41.82	5.87
May	33.58	16.45	25.59	0	25.39	8.71
August	37.68	19.81	29.13	0	22.58	8.28
November	25.54	10.15	17.41	0.21	43.53	4.72

3. RESULTS AND DISCUSSION

The results exhibit that temperature has a significant effect on growth, physiological and biochemical attributes of *T. polium.*

Regarding growth parameters, highest fresh weight and dry weight was recorded in the plants collected during November, whereas, plants collected during August gave lowest value and were statistically similar to the plants sampled in May. *T. polium* plants collected in November gave 24.8% and 61.8% higher values for fresh and dry weight, respectively, than the plants collected in August which gave lowest values (Table 2). Higher values of fresh and dry weights during November can be explained on the basis of climatic characteristics (Table 1) such as optimum temperature, improved rain fall and humidity, and suppressed solar radiations, all these collectively provided suitable growth conditions to the plants which ultimately contributed to enhanced fresh weight and dry matter accumulation. On the other hand, during the remaining month lower rain falls and humidity but higher level of temperature and solar radiations adversely affected growth of plants as witnessed by lower values of fresh and dry weights (Table 2). Low temperature has been shown as one of the important factors that limits growth and productivity of plants by inhibiting biosynthetic activities and normal function of physiological processes [22]. It is well documented that temperature lower than the normal level may lead to chilling injuries that are reflected in the form of chlorosis and browning of leaves [23] as shown by the lower values of Chl content (Table 2) which ultimately contributed to reduced fresh and dry weights under lower temperature. In contrast to low temperature, high temperature stress also adversely affects growth of plants by reducing water content (Fig. 1B) Chl content (Table 2) and inducing oxidative stress leading to losses in growth and productivity of plants [24,25].

Chlorophyll is one of the important parameters in determining growth and yield performance of a plant. Table 2 shows a significant difference in Chl-*a*, Chl-*b*, total Chl content and Chl-*a*:*b* ratio in response to seasonal variations. Highest values of these attributes were recorded in the month of November, except Chl-*a*:*b* ratio which was highest in August, whereas, lowest values for all these Chl characteristics were recorded during August, except Chl-*a*:*b* ratio that was lowest during February. An increase of 39.4%, 60.8%, 48.1% in Chl-*a*, Chl-*b* and total Chl content was recorded, respectively, during November as compared with the plants collected in August (Table 2). The results indicate that Chl-*b* is more sensitive than Chl-*a* to the climatic fluctuations. Exposure of plants to UV-B radiation, low temperature, large diurnal temperature variations adversely affect chlorophyll biosynthesis [26], furthermore, an enhanced activity of chlorophyllase, the enzyme responsible for the degradation of chlorophyll molecule, has been recorded in plants exposed to several environmental stresses [27]. Highest enzyme activity of CA was registered in the month of November, being at par with the plants sampled in February. However, lowest enzyme activity was recorded in August followed by May. Plants collected in November gave 12.0% higher CA activity than the plants sampled during August (Table 2). The enzyme CA which catalyzes reversible hydration of CO_2 and maintained its constant supply to rubisco, resulted in optimum rate of photosynthesis and thus more dry matter accumulation in the plants (Table 2). Our results are in agreement with the findings of Hayat et al. [28] who observed a significant decrease in CA activity in the plants grown under high temperature stress.

It is clear from Fig. 1A that elevation in temperature has a concomitant increase in H_2O_2 content which reached at its highest in the month of August which reached its highest value in the month of August, however, these values were at par with that of the values recorded in May. On the other hand, in November a decline in H_2O_2 content was recorded which exhibited parity to the level of H_2O_2 content recorded in February. During highest temperature of August, an increase of 38.01% in H_2O_2 content was recorded as compared with the lowest values recorded in November (Fig. 1A). Temperature stresses such as heat, cold or freezing are the major causes of ROS production in plants that cause cellular damage through peroxidation of lipids, proteins, nucleic acids and Chl bleaching [29,30] leading to losses to growth and yield of plants. Moreover, enhanced UV radiations significantly contribute to over production of H_2O_2 in plants. Table 1 indicates higher values of radiations during the colder and higher temperature conditions of February, May and August, which might have contributed to higher accumulation of H_2O_2 content during these months (Fig. 1A).

Table 2. Impact of variable climatic conditions on fresh weight, dry weight, Chl content and CA activity of *Teucrium polium* collected in four different months

Collection time	Fresh weight (g)	Dry weight (g)	Chl-*a* (mg/g FW)	Chl-*b* (mg/g FW)	Total Chl (mg/g FW)	Chl-*a:b*	CA activity (µmol CO_2 kg $^{-1}$ leaf FW s^{-1})
February	42.71±1.21	12.83±0.71	1.44±0.07	1.20±0.13	2.64±0.17	1.20±0.02	389.07±3.56
May	39.10±1.13	10.66±0.83	1.11±0.08	0.86±0.09	1.97± 0.08	1.29±0.07	366.25±4.12
August	37.64±0.86	9.73±0.66	1.09±0.02	0.74±0.04	1.8 3±0.01	1.47±0.04	351.41±3.29
November	46.95±0.92	15.75±1.24	1.52±0.05	1.19±0.06	2.71±0.03	1.28±0.01	393.54±2.32
LSD at 5%	3.59	2.07	0.06	0.09	0.04	0.05	5.82

Data are mean of five independent replicates (±S.E.)

Results show that variation in temperature has significant impact on water status of plants. LRWC in the month of November was 57.49% higher than the values recorded in any month during the study. On contrary, lowest water content of 39.20% was registered in the month of August (Fig. 1B). Values of LRWC recorded during November were almost similar to that of the value registered in February. Increasing temperature has direct impact on stomatal conductance, a transpiration rate and leaf water potential which directly affects water status of plants [31]. Moreover, higher rainfall and humidity, and lower temperature during November (Table 1) may have facilitated the plants with reduced transpiration rate which resulted in improved water status of the plants (Fig. 1B).

Efficiency of plants to counteract abiotic stress induced oxidative stress was evaluated by determining the activities of antioxidant enzymes (SOD, CAT and POX). Results show a parallel increase in the activities of antioxidant enzymes with an increase in temperature from February to May, while a decline in the activities was noted in August reaching the lowest levels compared to all the values recorded in any month (Fig. 2A). However, in November an increase of 10.5%, 66.4% and 34.6% was recorded in the activities of SOD, CAT and POX, respectively, than the lowest values recorded in August. To cope with stress induced oxidative stress, plants are equipped with a system of antioxidative enzymes such as SOD, CAT and POX. These are chloroplastic or cytosolic enzymes which detoxify H_2O_2. SOD converts superoxide radicals to H_2O_2, while CAT and POX dismutate H_2O_2 into water and oxygen. Activities of antioxidant enzymes have been shown to increase in response to high temperature stress [32]; however, Yong et al. [33] observed that exposure of strawberry plants to low temperature stress initially enhanced the level of ROS and subsequently the activities of antioxidant enzymes; however, at later stages of low temperature stress, a decrease or no change in enzyme activities was recorded. Thus, lower level of H_2O_2 content under the lower temperature of November was due to enhanced activities of SOD, CAT and POX, while reverse is true for the plants collected during August which gave higher level of H_2O_2 content coupled with suppressed activities of antioxidative enzymes (Fig. 2A).

Perusal of data shows that leaf protein content also exhibits a significant variation under varying climatic conditions of the four months. Plants collected during the highest recorded temperature of August, being statistically equal with the values recorded in May, exhibit lowest leaf protein content, whereas, highest protein content was recorded during the month of November. Plants of *T. polium* during November gave 53.3% more protein content than the plants sampled during August which being at par with values recorded during February, gave the lowest values (Fig. 2B). Our results corroborate the findings of Das et al. [34] who observed a decrease in protein concentration in the plants exposed to high temperature stress. High temperature causes membrane injuries, depletion of cell reserves and loss of catalytic activities of enzymes [7]; all these contributed to reduced activities of CA, SOD, CAT and POX enzymes and enhanced accumulation of H_2O_2 which ultimately resulted in reduced fresh and dry weights of the plants grown under high temperature conditions.

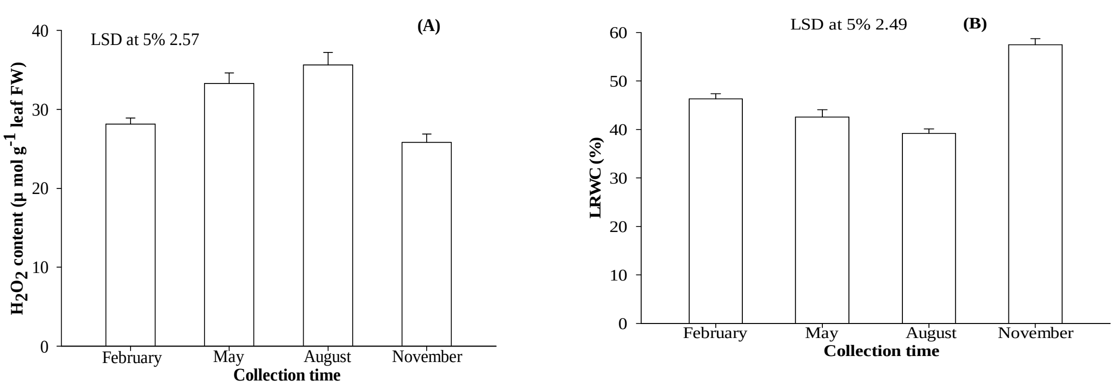

Fig. 1. Impact of variable climatic conditions on H_2O_2 content (A), leaf relative water content (LRWC) (B) in *Teucrium polium* during four different months (Vertical bars ± S.E.)

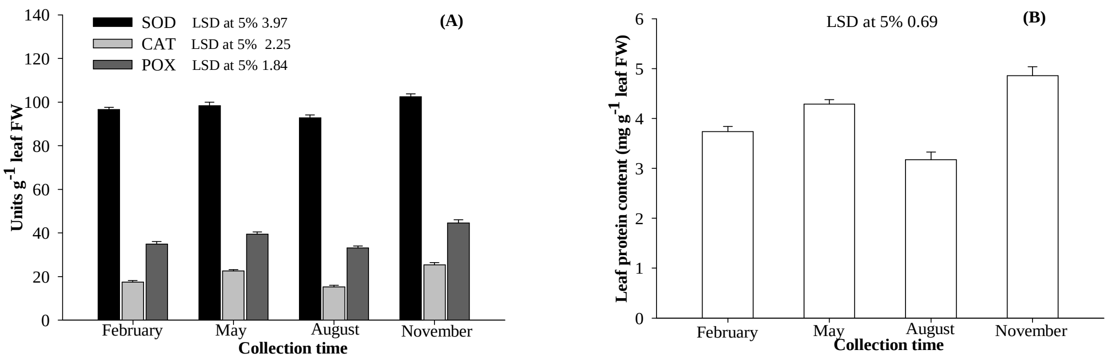

Fig. 2. Impact of variable climatic conditions on activities of SOD, CAT and POX (A) and leaf protein content (B) in *Teucrium polium* during four different months (Vertical bars ± S.E.)

4. CONCLUSION

On the basis of assessment of growth, physiological and biochemical parameters, it can be concluded that temperature and humidity of November was found better than in August to ensure proper growth and development of *T. polium* plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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