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Impact of varying elevations on growth and activities of antioxidant enzymes of some medicinal plants of Saudi Arabia

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ABSTRACT

Tabuk is the northwestern province of Saudi Arabia which is characterized by highly variable climatic conditions that ranges from extreme cold to extreme hot which not only supports the growth of a huge variety of medicinally important plants but also modulates the inherent medicinal potential of these plants. Keeping these points in view, the present work was designed to evaluate the effect of climatic conditions on growth physiological and biochemical attributes of five medicinal plants namely, *Artemisia judaica*, *Achillea fragrantissima*, *Teucrium polium*, *Lavandula pubescens* and *Retama raetam*. We collected each plant species from four different elevations of Tabuk region namely Jabal Al-Lawz (2580 m asl: higher elevation), Jabal Al-Harrah (1370 m asl; medium elevation), Jordan Road (760 m asl; lower elevation) and Tabuk City (760 m asl; lower elevation). The plants growing at medium elevation of 1370 m exhibited better growth and physiological performance than the others that may be ascribed to enhanced leaf water, Chl and protein content as we recorded for these plants. Maximum and minimum accumulation of secondary metabolites corresponded to the plants grown at the higher and lower altitude, respectively. The antioxidant enzyme activities were highest at 1370 m asl that might have contributed to the improved growth and physiological attributes. Consequently, the results obtained in this study strongly suggest that plants grown at medium elevation of 1370 m asl possess higher activity of antioxidant enzymes, whereas, plants at higher elevation of 2580 m are best for medicinal use because they accumulated higher level of secondary metabolites.

1. Introduction

Tabuk region, the northwestern province of Saudi Arabia, is characterized by its diversified topography ranging from plains to the mountains of low and high elevations which create highly variable environmental conditions encompassing through extreme cold to extreme hot climate. Diversified topography coupled with varied environmental conditions supports the growth of an array of medicinal plants.

Elevations drastically influence environmental factors, such as quick seasonal and daily variations in temperature, low atmospheric pressure, and depressed CO_2 concentration, short period of vegetation and enhanced intensity of solar UV radiation, which set the plants under serious stress. Plants show a significant morphological, physiological and biochemical responses to increasing altitude, which include decrease in stem height, stem diameter, biomass production [1], specific leaf area, and increases in the leaf thickness [2,3]. High light intensities at higher altitude induce excessive accumulation of reactive oxygen species (ROS) leading to pigment bleaching, lipid peroxidation, protein damage, inactivation of enzyme activities and ultimately cell death [4–6]. The enzyme carbonic anhydrase (CA) is found in abundance in the photosynthesizing tissues of both C_3 and C_4 plants and regulates the availability of CO_2 to ribulose bisphosphate carboxylase (rubisco) by catalyzing the reversible hydration of CO_2 [7]. Higher altitude restricts uptake of CO_2 that consequently decreases carbon assimilation due to non-availability of oxidized $NADP^+$ for acceptance of electrons during photosynthesis, a significant factor accorded to the formation of ROS [8].

Therapeutic use of plants is as old as human civilization and medicinal plants are integral part of healthcare system which is witnessed by the fact that 80% of the population still relies on a traditional system of medicine, based on herbal drugs [9]. Because of no side effects, the interest in medicinal products of plant has increased considerably all over the world. Of these, *Artemisia judaica* L. (family Asteraceae) called as 'wormwood' is a perennial shrub with pubescent and fragrant leaves is a highly resistant plant against adverse environmental conditions and very effective in stabilizing the habitat. *Achillea fragrantissima* (family Asteraceae), has been extensively used in Arabian folk medicine due its hypoglycemic properties. *Teucrium polium* L. (family Lamiaceae) is one of the most common medicinal plants of Arabian Peninsula that has been used for over 2000 years in traditional medicine due to its diuretic, diaphoretic, antipyretic, antispasmodic and cholagogic properties [10]. *Lavandula pubescens* Decne. (family Lamiaceae), is well known for its essential and aromatic oil, used in cosmetics while

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decoction of leaves is given in headache and cold [11]. *Retama raetam* (Forssk.) Webb & Berthel. (family Fabaceae), commonly known as 'raetam' or 'broom bush' is a shrub that grows widely in Saudi Arabian deserts. The plant possesses antibacterial, antifungal, antihypertensive, anti-oxidant, antiviral, diuretic and hypoglycemic properties [12,13]. *R. raetam* has long been used as abortifacient, purgative and a vermifuge in the traditional system of medicine. All these five plants viz. *A. judaica*, *A. fragrantissima*, *T. polium*, *L. pubescens* and *R. raetam* are commonly grown in all parts of Tabuk region and are extensively used in the traditional system of medicine of the region.

Although extensive research has been carried out on the altitudinal variation in morphological, physiological and biochemical characteristics of tree plants, but meager information is available on the changes in growth, lipid peroxidation, activities of antioxidant enzymes and on the accumulation of secondary metabolites of medicinal plant species growing under stressful conditions at various altitudes. Considering the importance of above mentioned plants in view, the present study was performed to test the hypothesis that variation in altitude significantly affects the activities of antioxidant enzymes and secondary metabolite concentration which plays a role in the acclimation of plants to abiotic stress induced impairment in plants.

2. Materials and methods

2.1. Study area

The study was conducted in Tabuk region, located in the northwest of Saudi Arabia. The region is characterized by its hyper-arid climate and occasional low precipitations during December to February coupled with high evaporation rate.

Plants were collected from four different locations of the region i.e. Jabal Al-Lawz (28°39′N, 35°18′E), Jabal Al-Harrah (23°5′N, 39°47′E), Jordan Road (28°56′N, 36°19′E) and Tabuk City (28°22′N, 36°36'E), which are located at an elevation of 2580 m above sea level (m asl: high), 1370 m asl (medium) and 760 m asl (low) respectively. Plant species from Tabuk City were collected within the periphery of 15 km. The purpose of plant collection from Jordan Road and Tabuk City was to compare the performance of plants growing at same elevations but at different locations. Environmental data of each collection site, shown in Table 1, were provided by the department of civil engineering, university of Tabuk, Saudi Arabia.

Five plant species namely *A. judaica*, *A. fragrantissima*, *T. polium*, *L. pubescens* and *R. raetam* were collected at flowering stage from above mentioned four sites. At each collection site four 30 m × 30 m plots, at a distance of 20 m each, were established randomly. Total 16 plots were organized during the study. Four replicates of each plant from each plot were collected randomly and four healthy plants were finally selected for assessing following parameters.

2.2. Growth parameters

Growth parameters for the above mentioned plants species were assessed in terms of plant height, plant fresh weight and dry weight.

For fresh weight, plants were uprooted and washed to remove surface adhered soil particles then the water was blotted by wrapping the plants in blotting papers. After removing the plants from blotting papers fresh weight for each plant was recorded. Dry weight of plants was recorded after drying the plants at 80 °C for 24 h in a hot air oven.

2.3. Physiological and biochemical parameters

2.3.1. Determination of hydrogen peroxide (H_2O_2) content

The hydrogen peroxide (H_2O_2) content in fresh leaf samples was determined according to Velikova et al. [14]. The content of H_2O_2 was calculated by comparison with a standard calibration curve, plotted by using different concentrations of H_2O_2. The absorbance of supernatant was recorded at 390 nm. H_2O_2 content was expressed as μ mol g^{-1} leaf FW.

2.3.2. Estimation of leaf protein content

Protein content was measured according to Bradford [15] using bovine serum albumin as standard. The absorbance was read spectrophotometrically (CE 2021, Cecil, Cambridge, England) at 595 nm.

2.3.3. Determination of carbonic anhydrase (CA) activity

The activity of carbonic anhydrase (CA: E.C. 4.2.1.1) was measured using titration method of Dwivedi and Randhawa [16]. The reaction mixture was titrated against HCl using methyl red as indicator. The enzyme was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

2.3.4. Leaf chlorophyll (Chl) content

Total chlorophyll content in the leaves was estimated according to Lichtenthaler and Buschmann [17]. The optical density (OD) of the pigment solution was recorded at 662 and 645 nm to determine chlorophyll a and chlorophyll b content, respectively, using a spectrophotometer (CE 2021, Cecil, Cambridge, England). Total chlorophyll content was assessed by totaling chlorophyll a and b contents. The photosynthetic pigment, thus measured, was expressed as mg g^{-1} leaf FW.

2.3.5. Leaf relative water content (LRWC)

To evaluate water status of plants, leaf relative water content (LRWC) was measured by the method of Yamasaki and Dillenburg [18]. For each treatment 10 pieces of leaves were taken. These leaf pieces were used to measure fresh mass (FM), turgid mass(TM), and dry mass (DM). The values for FM, TM and DM were used to calculate LRWC using the equation below.

$$\text{LRWC } (\%) = [(\text{FM}-\text{DM})/(\text{TM}-\text{DM})] \times 100$$

2.3.6. Assay 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 °C. The supernatant was used as the crude extract for the assay of enzyme activities in unit g^{-1} leaf FW.

Activity of superoxide dismutase (SOD: E.C. 1.15.1.1) was determined according to Beauchamp and Fridovich [19] by following the photo-reduction of nitro blue tetrazolium (NBT). Non-illuminated and

Table 1
Climatic characteristics of four collection sites.

Collection sites	Characteristics					
	Elevation (m asl)	Maximum temperature (°C)	Minimum temperature (°C)	Mean temperature (°C)	Annual rain fall (mm)	Humidity (%)
Jabal Al-Lawz	2580 (high)	34.7	−4.3	16.6	38	31.49
Jabal Al-Harrat	1370 (medium)	42.6	−2	19.4	40	35.26
Jordan Road	760 (low)	43.8	2.1	20.4	46	33.63
Tabuk City	760 (low)	43.8	2.1	20.4	46	33.63