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ORIGINAL ARTICLE

Ecological variations and role of heat shock protein in *Artemisia judaica* L. in response to temperature regimes of Tabuk, Saudi Arabia

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Abstract *Artemisia judaica* L. (Compositae) are shrubby herbs growing wildly in Tabuk region and distributed in the desert regions. This region is characterized by extremely variable environmental conditions where the temperature varies from extreme low to extreme high. These temperature regimes have a profound effect on morphology, growth physiology and biochemistry of the plants. The plant samples were collected from Tabuk–Jordan road (760 m above sea level) in the month of January, April, July and October 2013 to evaluate the effect of temperature dynamics on *A. judaica* L. in four different seasons. Physiological, biochemical alterations and heat shock proteins (HSPs) were studied during these seasons in order to evaluate the environmental adaptation and stress tolerance in response to temperature variations. Plant growth parameters showed a significant increase in height, fresh and dry matter accumulation, total chlorophyll, nitrogen, phosphorus, potassium, artemisinin and leaf relative water contents investigated in the month of April and October. Growth of plant was suppressed and an active role of carbonic anhydrase (CA), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) was observed to cope with the extreme low temperature in January and extreme high temperature in July 2013. However, the plants collected in October and April did not show a statistical difference. Inductions in the expression of HSP90 were recorded in all the plants collected during April and October 2013 with no statistically significant difference. Therefore, based on the results it is recommended that during April and October the environmental conditions are best suitable for growth, development and medicinal use of *Artemisia*.

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1. Introduction

Temperature stress is becoming the major concern for plant scientists worldwide due to the changing climate. Temperature stress has devastating effects on plant growth and metabolism, as these processes have optimum temperature limits in every

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Table 1 Temperature regimes in four different seasons of Tabuk, Saudi Arabia, in 2013 (recorded on 15th day of each month).

Temperature regimes (°C)	January 2013	April 2013	July 2013	October 2013
Average	10.4	23.9	34.6	22.2
Maximum	19.0	32.0	42.0	29.0
Minimum	2.0	17.5	26.0	15.0

plant species. Plants are unremittingly exposed to environmental stimuli that influence the development, growth and the productivity. High and low temperatures, mineral imbalance, excess or insufficient light and lack of water are stressors that compromise productivity (Lawlor, 2002). High temperature (HT) stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Hasanuzzaman et al., 2013; Wahid, 2007). Plant responses to HT vary with the extent of the temperature increase, its duration, and the plant type. Worldwide, extensive agricultural losses are attributed to heat, often in combination with drought or other stresses (Mittler, 2006).

Low temperature (LT) or cold stress is another major environmental factor that often affects plant growth and crop productivity and leads to substantial crop losses (Xin and Browse, 2000; Sanghera et al., 2011). Chilling stress results from temperatures cool enough to produce injury without forming ice crystals in plant tissues, whereas freezing stress results in ice formation within plant tissues. Plants differ in their tolerance to chilling (0–15 °C) and freezing (< 0 °C) temperatures.

The cellular changes induced by either HT or LT include responses which lead to the excess accumulation of toxic compounds, especially reactive oxygen species (ROS). The end result of ROS accumulation is oxidative stress (Awasthi et al., 2015; Mittler, 2002; Yin et al., 2008; Suzuki and Mittler, 2006). Plants have evolved a variety of responses to extreme temperatures which minimize damages and ensure the maintenance of cellular homeostasis (Kotak et al., 2007). A considerable amount of works have explored that there is a direct link between ROS scavenging and plant stress tolerance under temperature extremes (Suzuki and Mittler, 2006). Thus, the improvement of temperature stress tolerance is often related to enhanced activities of enzymes involved in antioxidant systems of plants. Any change in climatic conditions results in accumulation of chemically active molecules and free radicals in plant cells cause alterations in metabolic processes (Asada, 1999; Merzayak, 1999). To endure such unfavorable conditions plants are equipped with a system of antioxidant enzymes and heat shock proteins (HSPs) which inhibit free radical processes (Keniya et al., 1993; Zen'kov and Men'shikova, 1993).

Artemisia judaica L. grow wildly in Tabuk region, northwestern part of Saudi Arabia. For many years, *A. judaica* have enjoyed a reputation among herb experts in Egypt and Saudi Arabia as traditional medicinal herbs. Tabuk region is characterized by highly variable environmental conditions from extremely low to high temperatures (Table 1). These episodic variations in temperature significantly affect the medicinal properties of the plants. Therefore, in this research we investigated the impact of sporadic dynamics of temperature on an important medicinal plants *A. judaica* L. in terms of its growth, physiological and its biochemical attributes.

2. Materials and methods

2.1. Plant collection

In this research a medicinally important plant of Tabuk namely *A. judaica* was selected. It's from the family *Asteraceae* an annual, perennial, or shrub; leaves alternate strongly aromatic, densely grayish, tomentose low shrub, 30–80 cm; stems many branched from the base.

Plant samples were collected from Tabuk-Jordan road (760 m above sea level) on 15th day of January, April, July and October 2013 to evaluate the effect of temperature dynamics on *Artemisia* plants.

2.2. Fresh and dry weight analysis

For fresh weight the plants were uprooted and washed to remove surface adhered soil particles and wrapped in blotting papers. Dry weight of plants was recorded after drying the plants at 80 °C for 24 h in hot air oven.

2.3. Analysis of minerals

The nitrogen and phosphorus contents were determined using the method of Lindner (1944) and Fiske and Subba-Row (1925) respectively. Potassium was determined with a flame photometer (AIMIL).

2.4. Estimation of carbonic anhydrase activity

Activity of carbonic anhydrase (E.C. 4.2.1.1) was measured using the method as described by Dwivedi and Randhawa (1974). The enzyme was expressed as $\mu M\ CO_2\ kg^{-1}$ leaf $FW\ s^{-1}$. Total chlorophyll contents in leaves were estimated using the method of Lichtenthaler and Buschmann (2001). Leaf relative water content (LRWC) was measured by adopting the method of Yamasaki and Dillenburg (1999) using following formula:

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

2.5. Estimation of leaf protein content

Leaf protein content was determined according to Bradford (1976) using BSA as a standard.

2.6. Western blot analysis

For the estimation of heat shock proteins (HSPs), cytosolic extracts were prepared and frozen plant material was ground in liquid nitrogen. Tissue was thawed in electrophoresis buffer

(Laemmli, 1970) without b-mercaptoethanol. Protein concentration was determined according to Bradford (1976). Twenty micrograms of total protein was boiled in SDS-sample buffer in the presence of b-mercaptoethanol and separated by SDS–PAGE (Laemmli, 1970). After electrophoresis, proteins were blotted onto nitro cellulose membrane. The membranes were blocked with 5% (w/v) non-fat dry milk in phosphate buffered saline and incubated with an antibody against Hsp90 which was detected using R2 antiserum (Krishna et al., 1997). Antibody was applied in a 1:2000 dilution. Detection was performed using an anti-rabbit antibody conjugated to horseradish peroxidase.

2.7. Antioxidant enzymes activities

For antioxidant enzymes assay 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. Superoxide dismutase (SOD; E.C. 1.15.1.1) activity was determined according to Beauchamp and Fridovich (1971) by following the photo-reduction of nitro blue tetrazolium (NBT). Activity of CAT (E.C. 1.11.1.6) was measured according to Cakmak and Marschner (1992). Activity of POX (E.C. 1.11.1.7) was assayed by the method of Upadhyaya et al. (1985). Secondary metabolites were estimated using dry leaf material (1 g) for the estimation of artemisinin modified to a compound Q260 and quantified using HPLC method (Zhao and Zeng, 1986).

3. Statistical analysis

Statistical analysis of data obtained from research was done using five replicates of collected plants for each season. The data were analyzed statistically using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). In applying the *F* test, the error due to replicates was also determined. When *F* value was found to be significant at 5% level of probability, critical difference (CD) was calculated.

4. Results

4.1. Effect on plant growth and physiological attributes

It is evident from the results that *Artemisia* plants, collected in four different seasons of Tabuk region, exhibited diverse pattern of growth, physiological and biochemical attributes (Table 2). Plants collected in April 2013 exhibited highest values for plant height (60.66 cm), whereas the significant reduction in plant height was recorded in July 2013 (45.35 cm) which was the least value among the four seasons. However, the plants collected in October and April did not show a statistical difference. A highest (50.54 g) value for fresh weight per plant was recorded in April 2013, whereas it was lowest (35.67 g) in July 2013. However, there was no statistical difference in January and July 2013.

Plants exhibited maximum (25.73 g) dry matter accumulation and minimum (15.61 g) during April and July, respectively. However, the plants showed almost similar dry matter accumulation pattern in April–October and January–July 2013. For nitrogen, phosphorus and potassium (NPK) uptake by plants a similar pattern was observed. The results on these growth parameters showed that the plants investigated in January and July gave lower values for the growth parameters as compared to plants collected in April and October.

Table 2 shows that the plants investigated in October gave highest values for leaf chlorophyll contents whereas; plants in July gave lowest values for the same parameter. There was a significant increase in leaf chlorophyll content observed in October 2013. The data show that leaf protein content increased in April as compared to the plants observed in January–July 2013. A significant difference in protein contents in plants was observed in different seasons of Tabuk. An increase in secondary metabolites was recorded in plants grown in April and October. Highest levels of artemisinin content in *A. judaica* was observed in October and lowest in July 2013.

CA enzyme in plants was found highly active in October and least in January 2013. The plants in April and July showed no significant differences in between ($F_{3,\ 60.1}$) (Fig. 1).

4.2. Alteration in antioxidant enzyme activities

Leaf antioxidant systems can prevent or alleviate the damage caused by the reactive oxygen species under stressful conditions. Our study showed that the plant *Artemisia* grown in July showed highest values for SOD enzyme activity as compared to January ($P < 0.001$; $F_{3,\ 35.8}$) while the plants grown in April and October showed no significant differences (Fig. 2A). A similar type of pattern was observed in the activity of CAT with the significant differences in the month of January and July ($P < 0.001$; $F_{3,\ 112.4}$) (Fig. 2B).

Phenols are oxidized by POD and primarily by PPO, and this latter enzyme catalyzing the oxidation of the o-diphenols to o-diquinons, as well as hydroxylation of monophenols (Thypyapong et al., 1995). In the present study we found that the POD activity was highest in July as compared to the other seasons ($P < 0.001$; $F_{3,\ 183.4}$) (Fig. 2C).

4.3. Effect of temperature variation on the expression of heat shock protein90 (HSP90)

It is apparent from Fig. 3 that in July as temperatures increased the expression of HSP90 was also increased. An induction in the expression of HSP90 was recorded in all the plants collected during April and October 2013 with no significant difference.

5. Discussion

In this research we studied growth, morphological, physiological and biochemical attributes of an important medicinal plant *A. judaica* in response to varying temperature conditions of Tabuk, Saudi Arabia. The plant species investigated in April and October 2013 showed optimum growth (plant height, fresh weight, dry weight accumulation and uptake of NPK) whereas the same parameters showed a significant decrease in the plant samples investigated in July and January 2013. Prakash et al. (2011) observed an increase in morphological characteristics of plants in suitable environmental

Table 2 Effect on plant growth and physiological attributes of *Artemisia judaica* to temperature regimes of Tabuk, Saudi Arabia.

Plant parameters	January 2013	April 2013	July 2013	October 2013	CD at 5%
Height (cm)	50.78 ± 3.10	60.66 ± 2.63	45.35 ± 2.52	55.54 ± 3.01	4.25
Fresh weight (g)	38.77 ± 3.41	50.54 ± 4.31	35.67 ± 1.05	45.87 ± 2.02	6.18
Dry weight (g)	18.28 ± 1.91	25.73 ± 1.53	15.61 ± 1.81	20.22 ± 2.21	2.58
Leaf relative water contents (%)	57.45 ± 2.61	60.77 ± 0.95	39.56 ± 1.22	65.75 ± 2.52	2.67
Leaf protein (mg/g)	4.60 ± 0.21	5.91 ± 0.13	4.53 ± 0.16	4.92 ± 0.26	0.31
Leaf chlorophyll (mg^{-1} leaf FW)	1.75 ± 0.01	1.85 ± 0.03	1.62 ± 0.02	2.75 ± 0.02	0.04
Nitrogen (mg 100 mg^{-1}DW)	2.743 ± 0.135	3.169 ± 0.152	2.240 ± 0.173	2.810 ± 0.184	0.236
Phosphorus (mg 100 mg^{-1}DW)	0.480 ± 0.055	0.635 ± 0.048	0.448 ± 0.057	0.596 ± 0.038	0.048
Potassium (mg 100 mg^{-1}DW)	0.923 ± 0.067	1.448 ± 0.056	1.232 ± 0.068	1.249 ± 0.044	0.095
Artemisinin (μg/g DW)	540.79 ± 3.52	565.94 ± 6.22	535.59 ± 5.54	590.88 ± 4.63	7.91

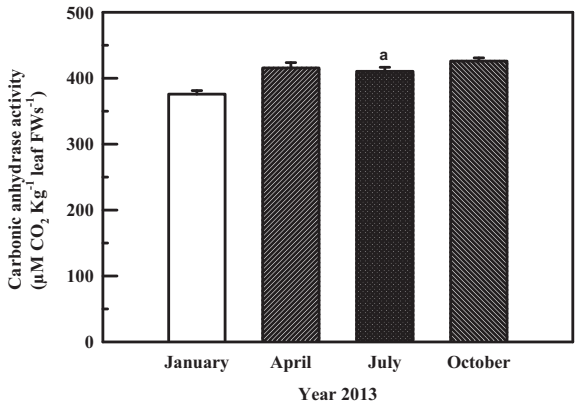

Figure 1 CA activity of *Artemisia judaica* in response to temperature regimes of Tabuk, Saudi Arabia.

conditions and a decrease in this parameter observed beyond a certain limit. Sudden and extreme increase in temperature is accompanied with more stressful conditions which affect growth and development of plant species. Growth patterns of *Artemisia* plants, fresh and dry matter accumulation and nutrient contents (NPK) were studied as they indicates primary productivity in response to temperature regimes (Smith, 2007). Temperature not only acts as an important limiting factor for enzymatic activities and metabolism of plants but also regulates cell division, translocation of food and photosynthesis in plants. A temperature of 30° is optimal for most biochemical processes (Devlin and Witham, 1986) in plants. Effect of temperature also pronounced when duration of treatment exceeded (Li et al., 1995).

The reduced growth characteristic in the studied plants at elevated temperature can also be explained on the basis of the fact that plants grown in July are exposed to greater irradiance, large diurnal fluctuations of temperature, increased rate of transpiration due to high wind velocity, reduced partial pressure of gases, limited water and nutrient supply and a narrow time window for growth and development of plants (Körner et al., 1989; Streb et al., 1998).

Regarding physiological and biochemical parameter, CA activity was recorded higher in plant species grown in April and October 2013 and it was lower in plants investigated in July and January 2013. CA is the enzyme which catalyzes reversible hydration of CO_2 and maintained its constant supply to rubisco resulting in optimum rate of photosynthesis and thus more dry matter accumulation. Therefore, increased CA activity might have been one of the reasons for improved growth performance of plants at optimum temperature in April and October 2013. It was also reported that while transpiration rates increased with lower atmospheric pressure at higher temperature, corresponding increase in CO_2 uptake was higher than expected (Gale, 1973), thus there is a possibility of greater non-stomatal capability for CO_2 uptake in plants at higher temperatures.

Leaf chlorophyll content is another important parameter in determining growth and yield performance of a plant. A progressive increase in chlorophyll content was recorded in October and July respectively. However, a significant decrease was noted at highest temperature during the month of July 2013. The reason behind decrease in chlorophyll content at higher temperatures may be due to the photo-oxidation of pigments in the presence of high radiations (Prakash et al., 2011). A significant decrease in leaf relative water contents was recorded with a concomitant increase in temperature. The decrease in leaf relative water contents at higher temperatures in July is due to high wind velocity, large diurnal fluctuations in temperature and limited supply of water and nutrients (Gale, 1973). This results from the higher total radiation absorbed by leaves, increase in the diffusion coefficient of water vapor in air and intern increase in transpiration at reduced barometric pressure and the increased density gradient of H_2O vapor from the leaf to the ambient air (Gale, 1973; Cohen et al., 1981).

An extreme increase and decrease in temperature causes generation of several detrimental effects and set the plants under stress leading to the generation of reactive oxygen species (ROS). However, to cope with such stressful conditions plants are equipped with antioxidant enzymes such as SOD, POD, and CAT. These enzymes prevent or alleviate the damage caused by ROS and set the plants to perform normally even under stressful conditions. The results exhibit that SOD, POD and CAT show a parallel increase in the activities with increasing temperature giving highest values. Thus, increased antioxidant enzyme activities protected the plants from stressful environment which is reflected in terms of improved growth and dry matter and protein accumulation, CA activity and leaf Chl content in the plants grown in July and January 2013. On the other hand, plants grown in April and October showed a decline in antioxidant enzyme activities and enhanced growth, physiological and biochemical

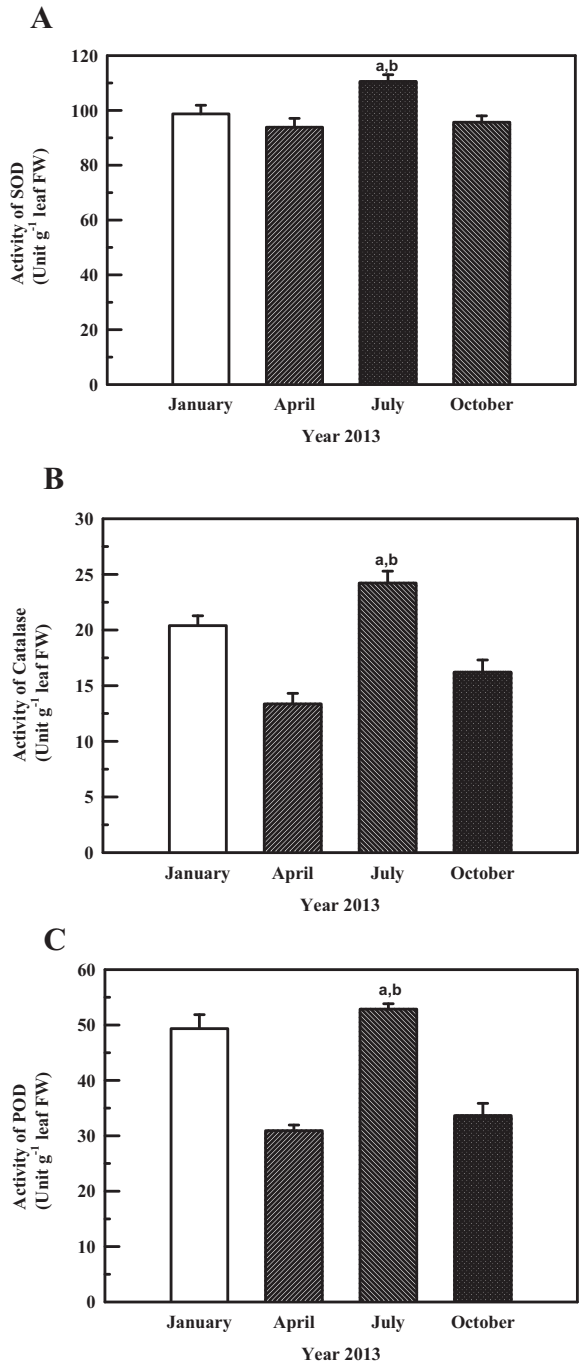

Figure 2 Variation in antioxidant enzyme activities of *Artemisia judaica* to temperature regimes of Tabuk, Saudi Arabia. (A) enzymatic activity of SOD, (B) activity of CAT and C. POD activity.

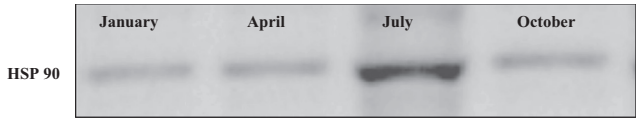

Figure 3 Expression of HSP 90 of *Artemisia judaica* in response to temperature regimes of Tabuk, Saudi Arabia.

parameters including protein accumulation. Similarly, the inhibition in the activities of antioxidant enzymes during optimum temperature was recorded by Wang et al. (2009) and Streb et al. (1998).

It is shown that under extreme conditions the protective mechanism of antioxidant system is activated. The higher is the antioxidant activity, the more resistant is the species toward the stressor. Besides these, to counter elevated temperatures plant accumulates heat shock proteins (HSPs). HSPs protect proteins, membranes and cellular components during temperature stress; facilitate repair and degradation of denatured proteins under stressful events (Parsell and Lindquist, 1993;, Wang et al., 2004). As shown in Fig. 3, HSP90 was strongly induced under elevated temperature; however, no significant difference in the expression of HSP90 was recorded among the plants grown under optimum temperature in April and October 2013. These results corroborate the findings of (Ludwig-Muller et al., 2000) who reported enhanced expression of HSP90 under temperature stress.

Xu et al. (2013) also reported that HSP90 genes function differently in response to temperature stress, they observed that HSP90 could be strongly induced under high temperature; osmotic and salt stress but not show any physiological stress under low temperature conditions.

6. Conclusion

Based on the results of this research it is recommended that during April and October the environmental conditions are best suitable for growth, development and medicinal use of *Artemisia*.

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