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VARIATIONS IN THE PROLINE AND TOTAL PROTEIN CONTENTS IN *ORIGANUM SIPYLEUM* L. FROM DIFFERENT ALTITUDES OF SPIL MOUNTAIN, TURKEY

BENGU TURKYILMAZ UNAL[1*], AYKUT GUVENSEN[2], AYLIN ESIZ DEREBOYLU[2] AND MUNIR OZTURK[2]

[1]*Nigde University, Ulukışla Vocational School, Horticulture Dept., Ulukışla-Nigde, Turkey*
[2]*Ege University, Science Faculty, Botany Department, Bornova- Izmir, Turkey*
[*]*Corresponding author's e-mail: "Bengu Turkyilmaz" <bengu35540@hotmail.com>,*

Abstract

Soil samples and leaves of *Origanum sipyleum* L., were collected from 5 different altitudes of Spil mountain situated in the State of Manisa in the West Anatolian part of Turkey. The soils were analysed to determine pH, organic matter content, calcium carbonate and other chemical constituents. The photosynthetic pigment, proline and total protein contents of leaves were also determined. Results obtained showed that *O. sipyleum* grows on sandy-loam soils, rich in organic matter content, with pH varying between 6.11-6.97. It prefers slightly acidic and neutral soils, rich in N and P, but poor in K. The physiological analyses revealed that total protein and proline contents increased whereas photosynthetic pigment decreased at 520 and 790m altitudes. All parameters decreased at 1020 and 1150m altitudes. A statistically significant correlation was observed at higher altitudes.

Introduction

Several environmental factors adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide (Ozturk & Szaniawiski, 1981; Ozturk *et al.,* 1986, 2011; Ashraf & Foolad, 2007; Cha-Um *et al.,* 2010; Suriyan *et al.,* 2010; Ali *et al.,* 2011; Shafi *et al.,* 2011; Akça & Samsunlu, 2012; Berber & Önlü. 2012; Bakht *et al.,* 2012; Ejaz *et al.,* 2012, Hamayun *et al*., 2010). The altitude also plays an important role in this connection, because it is associated with light intensity, UV-B radiation, air temperature, ozone density, wind exposure, soil fertility etc. At increased altitudes, plants are exposed to higher light intensities and lower mean temperatures. So, plants have developed mechanisms to prevent damage caused by photodestruction, freezing and chilling. Photosynthesis is highly efficient at low temperatures and adapted to high irradiance in several high mountain plants (Korner & Diemer, 1987; Kofidis *et al*., 2003, Shinwari *et al*., 1998). Proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Ozturk & Demir, 2002; Hsu *et al*., 2003; Kavi Kishore *et al*., 2005). It ameliorates the stress tolerance by preventing membrane and protein damage (Santoro *et al*., 1992; Santarius, 1992). Some studies have shown that altitudinal stress increases accumulation of proline and total protein, while the chlorophyll content decreases (Yadeghari *et al*., 2008; Koc *et al*., 2010).

Lamiaceae includes 45 genera, 546 species, and 730 taxa, widely distributed in the Mediterranean area of Turkey, which is regarded as an important gene centre for the family. The ratio of endemism lies around 42.2 % (Karik *et al*., 2007), the rate among Turkish *Origanum* species being 63% (Esen *et al*., 2007). *Origanum* is one of the major export products of Turkey. The production has started under the field conditions in the Aegean and Mediterranean regions, as an alternative product instead of tobacco (Koksal *et al.*, 2010).

Origanum sipyleum is 80cm tall perennial semi-shrub, with more than one stiff stem (Davis, 1982). The pink flowers are located in bunches on the stem ends. It usually likes warm climates and grows well in arid soils, rich in nutrient, mostly calcareous like all other *Origanum* taxa (Davis, 1982). It is an endemic to Western Anatolia and grows on calcareous rocks, hillsides, in Pine groves and inside the marquis, flowering from April to October. The altitudinal distribution lies between from 100 to 1500m in the West and South Anatolian regions. The life span of the plant is about 3-4 years under favorable climatic conditions. The species is included under lower risk and the least concern in the Red Data Book of Turkey; but will be under risk in the near future because of its over exploitation, loss of habitat, excess consumption and climate change (Baytop, 1999; Isık *et al*., 1997). Therefore there is a great need for conservation of this medicinally important endemic species.

It is commonly known as "Bayircayi" or "Guve otu" and is used for stomach ache and cough therapy. The main components of essential oil are carvacrol, thymol, γ-terpinene and p-cymene (Baser *et al*., 1992; 2000). Traditionally vegetative parts and biochemical extracts of the plant have been used as medicinal tea or food additives and dried plants have also been used for the production of essential oil and an aromatic water or hydrosol (Ozkan *et al*., 2007; Koksal *et al*., 2010). In this study the soils and some physiological parameters of *O. spiyleum* from different altitudes were analysed, keeping in view the expectations of climate change in the future.

Material and Methods

Soil analysis: Soil samples were collected at random from 8 sites from 20cm depth using a stainless steel auger at five altitudes (300m, 520m, 790m, 1020m and 1150m). These were pooled together, stored in plastic bags, sealed and labeled. Soil samples were dried in an air-forced oven at 40°C. The dried samples were sieved to remove stones and plant residues and were ground in a stainless steel mill and passed through a 2-mm sieve.

Soils textures were determined with "Bouyoucos Hydrometer Method" (Bouyoucos, 1962), pH with "Beckman pH meter", $CaCO_3$ with Scheibler Calcimeter, total salt content by Conductivity Bridge apparatus and organic matter content according to the methods outlined in detail by Ozturk *et al*., (1997). N, P, K in the samples were determined after wet digestion, using Kjeldahl method and methods given by Bingham (1949) and Pizer (1967) respectively. Results were evaluated according to Ozturk *et al*., (1989).

Plant material and sampling: Native populations of *O. sipyleum* plants were studied at five altitudes (300m, 520m, 790m, 1020m and 1150m) on Spil mountain, Manisa-Turkey during the flowering spring season, The fully expanded actively synthesizing green leaves were collected randomly from 3-5 plants and stored at -80^{0}C till further analysis.

Photosynthetic pigment contents: The leaf samples were extracted with 80% acetone and absorbance of supernatants was measured spectrophotometerically. Chlorophyll a was determined at wavelength 663 nm and b at 645 nm, and total chlorophyll at 652 nm and carotenoids at 450nm following the method given by Linchtenthaler (1987).

Total protein content: Total soluble protein content was determined according to Bradford (1976) using BSA as a standard. In the Bradford assay, protein concentration is determined by quantifying the binding of the dye, Coomassie Brilliant Blue G-250, to the unknown protein solution, as compared to known standards. Tubes containing 100 µl aliquots of known concentrations of Bovine Serum Albumin (BSA; 0.156mg l^{-1} to 10mg l^{-1} in 0.15M NaCl), were prepared. Blank tubes containing 100µl of 0.15M NaCl were also prepared. One ml Coomassie Brilliant Blue solution was added to each tube and the mixtures vortexed. The reactions were left at room temperature for 2 min. The absorbance at wavelength of 595nm was determined against the blank and the standard curve of absorbance versus protein concentration plotted (Copeland, 1994).

Proline content: Proline was determined according to the modified method of Bates *et al*., (1973). Leaf samples were homogenized in 3% (w/v) sulfosalicylic acid solution and then centrifuged. The supernatant was taken into a test tube to which glacial acetic acid and acid ninhydrin solution were added. Tubes were incubated in boiling water bath for 1 h and then allowed to cool to room temperature. After adding toluene, the mixture was vortexed and allowed to separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm in a spectrophotometer. The concentration was calculated from a proline standard curve and expressed as µmol/g FW.

Statistical analysis: Experimental data were analyzed with the SPSS statistical computer package (SPSS for WINDOWS, Standard Version 16.0) with Turkey test at $p<0.05$ level, and standard errors (±) were also calculated.

Results and Discussion

The results on the physico-chemical chracteristics of soil samples from five successive altitudinal levels in native habitats of *O. sipyleum* are presented in Tables 1 and 2.

The plants flourish well on sandy-silty soils, with pH varying between 6.11-6.97. The plant prefers slightly acidic to neutral soils (Table 1). The soils are extremely calcareous and non saline in nature, rich in N and P, but poor in K. The samples are abundant in organic matter (Table 2).

Table 1. Physical analysis of the soils supporting *Origanum sipyleum* at different altitudes.

Altitude (m)	Sand (%)	Silt (%)	Clay (%)	Texture	pH	Total salt (%)	$CaCO_3$ (%)
300	69.41	15.29	15.30	sandy-silt	6.70 (neutral)	0.02 (non saline)	7.31 (extremely calcareous)
520	65.37	15.31	19.32	sandy-silt	6.11 (slightly acidic)	0.042 (non saline)	7.23 (extremely calcareous)
790	71.50	7.25	21.25	sandy-silt	6.28 (slightly acidic)	0.01 (non saline)	7.32 (extremely calcareous)
1020	59.37	19.30	21.33	sandy-silt	6.65 (neutral)	0.05 (non saline)	7.36 (extremely calcareous)
1150	49.44	29.28	21.28	sandy-silt	6.97 (neutral)	0.03 (non saline)	7.32 (extremely calcareous)

Table 2. Chemical analysis of the soils supporting *Origanum sipyleum* at different altitudes.

Altitude (m)	Organic matter (%)	N (kg/da)	P_2O_5 (kg/da)	K_2O (kg/da)
300	10.5 (high)	0.7 (hyper)	41 (hyper)	0.8 (hypo)
520	18.1 (high)	2.3 (hyper)	41 (hyper)	2.5 (hypo)
790	21.1 (high)	5.5 (hyper)	41 (hyper)	6.5 (hypo)
1020	20.1 (high)	1.6 (hyper)	25.6 (hyper)	2.2 (hypo)
1150	17.5 (high)	1.0 (hyper)	36.9 (hyper)	1.0 (hypo)

A comparison of our soil analysis data with that of other workers reveals that some *Origanum* species together with several Mediterranean plants prefer neutral or slightly alkaline soils like *O. sipyleum.* Calcium carbonate content of the soils for all species ranged from 7.23 to 7.36%. *O. sipyleum* also prefers high calcareous soils. The soils of *O. leptocladum* are very rich in Calcium carbonate too (Scheffer & Schactschabel, 1956). The soil salinity values of *O. spiyleum* vary from 0.010 to 0.042% (Table 1). This may indicate that this species is not a salt tolerant one. The values of potassium content were 0.8-6.5 % for all altitudes (Table 2). According to the potassium classification of Pizer (1967), it can be said that *Origanum* generally prefers soils, deficient in potassium. The study of Gonuz and Ozorgucu (1999) reports that potassium in the soil of *O. onites* is 0.7%. This is lower than our findings for *O. sipyleum* (Table 2). Species such as *A. aestivus*, *C. ovata* and *C. spinosa* likewise prefer soils poor in potassium while *P. lentiscus*, a plant of the same region, prefers soils rich in potassium (Ozturk & Atac, 1982; Pirdal, 1989; Ozdemir & Ozturk, 1996). Average organic matter content in the soils ranges between 10.5 and 21.1% (Table 2). They are rich according to Petri and Wagner's classification (1978). It is known that *P. lentiscus* (Ozturk & Atac, 1982), *Inula viscosa* (Pirdal, 1980) and *C. ovata* (Ozdemir & Ozturk 1996), collected from the area prefer very rich and rich soil.

The concentration of chl a, chl b, total chl and chl a:b in the leaves of *O. sipyleum* decrease with increase in altitude. There were significant differences at 1020m and 1150m (Table 3).

The carotenoid content decreases with an increase in the altitude. The proline and total protein contents in the leaves were found to be max. at 790m while min. at 300m altitude. There was an increase in their levels from 300m to 790m, but the levels decreased at 1020m and 1150m. The differences were statistically significant at 790m (Table 4).

Table 3. Chlorophyll content in the leaves of *Origanum sipyleum* collected from different altitudes.

Altitude (m)	Chl a ($mg^{-1}FW$)	Chl b ($mg^{-1}FW$)	Total Chl ($mg^{-1}FW$)	Chl a/b ($mg^{-1}FW$)
300	1.898 ± 0.128^{a}	0.901 ± 0.049^{a}	2.798 ± 0.178^{a}	2.104 ± 0.029^{a}
520	1.622 ± 0.107^{a}	0.798 ± 0.074^{a}	2.420 ± 0.180^{a}	2.032 ± 0.067^{a}
790	1.611 ± 0.025^{a}	0.791 ± 0.015^{a}	2.403 ± 0.032^{a}	2.035 ± 0.012^{a}
1020	0.740 ± 0.025^{b}	0.419 ± 0.015^{b}	1.157 ± 0.062^{b}	2.003 ± 0.088^{b}
1150	0.445 ± 0.151^{b}	0.256 ± 0.031^{b}	0.701 ± 0.259^{b}	1.774 ± 0.195^{a}

Means in columns followed by different letters are significantly different at $p<0.05$ (Tukey test)

Table 4. Carotenoid, proline and total protein contents in the leaves of *Origanum sipyleum* collected from different altitudes.

Altitude (m)	Carotenoid ($mg^{-1}FW$)	Total protein (mg/ml)	Proline ($\mu mol\ g^{-1}$)
300	8.678 ± 0.355^{a}	1.366 ± 0.057^{a}	5.458 ± 1.060^{a}
520	8.055 ± 0.117^{a}	1.456 ± 0.023^{a}	9.399 ± 1.949^{ab}
790	8.096 ± 0.132^{a}	1.590 ± 0.090^{b}	9.575 ± 1.836^{b}
1020	3.164 ± 0.526^{b}	1.357 ± 0.134^{a}	9.337 ± 1.561^{ab}
1150	2.423 ± 0.796^{b}	1.338 ± 0.086^{a}	8.556 ± 1.277^{ab}

Means in columns followed by different letters are significantly different at $p<0.05$ (Tukey test)

High mountain environmental conditions characterized by high intensity of solar ultraviolet (UV) radiation, sharp seasonal and daily differences of temperature, low atmospheric pressure, decreased concentration of CO_2, short period of vegetation lead to an accumulation of chemically active molecules and free radicals in plant cells, capable of changing the direction of metabolic processes (Larcher, 1988; Abbasi *et al*., 2011). Especially low temperature and UV-B radiation are abiotic stresses that cause plant damage at high altitudes. UV-B radiation has many direct and indirect effects on plants, including damage to DNA, proteins and membranes; alterations in transpiration and photosynthesis; and changes in proline and protein amounts, growth, development and morphology (Teramura & Sullivan, 1994; Jansen *et al.,* 1998; Salama *et al*., 2011). Cold acclimation is a complex process involving a number of biochemical and physiological changes, associated with the accumulation of sugars, several types of proteins, lipids, abscisic acid and other products of altered metabolism (Pinedo *et al.,*2000; Szalai *et al*., 2000; Atici *et al*., 2003).

In our study, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of *O. spiyleum* leaves decreased with increasing altitude. Statistical analysis for photosynthetic pigments is significant at 1020 and 1150m (Table 3 and Table 4). According to Jan *et al.*, (2011) a lower percentage of chlorophyll was found with increasing height in *Chichorium intybus* plants. The concentration of total chlorophyll (chl) and carotenoids in the fresh leaves of 10 wild and 6 cultivated plants too has been reported to have decreased with increase in altitude (Todaria *et al*., 1980).

The activation of protein synthesis in plants, in combination with the antioxidative system, plays an protective role under the stressful conditions of high mountains. Synthesis of specific proteins is an important mechanism involved in increasing cold tolerance. Low temperature can result in the synthesis of stress proteins (Hughes & Dunn, 1996). The proline accumulation in response to various abiotic stresses such as drought, salinity, chilling stress (Rhodes, 1987; Delauney & Verma, 1993; Samaras *et al*., 1995; Rhodes *et al.*, 1999; Ali *et al.*, 1999; Gilmour *et al*., 2000) plays a major role in plant defense system.

Our data indicates that the proline and total protein contents significantly increased at 790 m altitude, but decreased at 1050 and 1120m altitudes (Table 4). Prakash *et al*., (2011) have studied the behaviour of *Plantago major* plants, the chlorophyll and carotenoids have showed a decreasing trend as altitude increased, and the amount of soluble proteins was higher at 1800 m and 2500 m altitude respectively.

It is a well known fact that proline and soluble protein concentrations are significantly higher in alpine plants than in steppe plants. These therefore play a very important role in the stress resistance of the alpine plants (Oncel *et al*., 2004). Bano *et al*., (2009) have reported that there is a certain critical level of proline, protein, sugar and ABA to be attained at high altitudes. In Chkhubianishvili *et al*., (2009)'s experiments the structural and functional peculiarities of leaves of dandelion, plantain and clover, growing under high mountain conditions of the Minor Caucasus have revealed the tendency of protein accumulation in alpine zone. The supposition was offered that increase in the protein content may be linked to the accumulation of shock proteins as a result of increased UV-B radiation. In some poplar species, enhanced UV-B radiation had a significant effect on proline accumulation (Ren *et al*., 2007). Also, in cold acclimated plants such as hybrid patato (Rhodes *et al*., 1999) proline content was higher as a response to the stress.

The data on the amount of photosynthetic pigments, proline and total protein in the leaves of *O. sipyleum* from Spil mountain leads us to concluded that adaptation of plants to altitudinal conditions is expressed through specific peculiarities of metabolic changes leading to regulation of proline and protective proteins upto 790m. At higher altitudes abscisic acid and antioxidative enzyme systems are used for adaptation. We can conclude that investigations on the ABA and antioxiative enzyme contents in the plants of *O. sipyleum* growing at higher altitude are required for further conclusions.

The current ecological and physiological characteristics of *O. spiyleum*L. populations on Spil mountain will pave way for their future conservation.

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