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Effects of altitude on anatomy and concentration of crocin, picrocrocin and safranal in *Crocus sativus* L

Fatemeh Zarinkamar*, Somaye Tajik, Saeideh Soleimanpour

Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran

***Corresponding author: zarinkamar@modares.ac.ir**

Abstract

Saffron is the most expensive spice in the world. It is made from the dried stigmas of the saffron flower (*Crocus sativus* L). This species is cultivated in environments with very different climatic conditions. In this study, saffron samples from different altitudes, Ghaen with altitude of 1400m (samples *A*) and Tabas with altitude of 700m (samples *B*), were collected from 3- and 6-year-old saffron plants repeatedly in the years 2007-2008. Then, three major metabolites (crocin, picrocrocin and safranal) were quantified in 3- and 6-year-old plants in both altitudes by high performance liquid chromatography. The results indicated that saffron samples *A* had higher concentration of these constituents in comparison to that of samples *B*. Also, comparative study of these metabolites between 3- and 6-year-old plants in each region revealed increased amounts of saffron components in 3-year-old plants in comparison to 6-year-old ones. Anatomical studies of leaves showed significant differences between two reigons characteristics including thickness of cuticle, width of epidermis outer cell wall, diameter of cortex and palisade cells, trichomes, and depth of crypts. Scanning results of leaves and stigmas by SEM also showed different characteristics in the two regions.

Keywords: Anatomy, *Crocus sativus*, Crocin, Picrocrocin, Safranal, Altitude.
Abbreviations: pq, parenchyma; cr, crypt; tr, trichoms; gl, gland; s, stomata; s.ch, stomata chamber; x, xylem; ph, phloem; sc, sclerenchyma.

Introduction

Crocus sativus from Iridaceae family is an autumn-flowering plant with permanent underground stem bases called bulbs or corms (Fernández, 2004). *Crocus sativus* is a triploid sterile plant that propagates by corms. This vegetative cultivation offers advantages in maintaining the genetic characteristics of the plants (Castillo *et al* 2005). *Crocus* species have basal, grass-like, dark green leaves with a whitish median strip. On the lower surface of the leaves, there are two deep grooves on either side of the flattish keel. The leaves appear simultaneously with flowers or after flowering. Each corm produces 6-9 leaves (Fernández, 2004). Saffron is the dried stigmas of *Crocus sativus* and the most expensive spice used in industry, with many different uses as drug, textile dye, and culinary adjunct. It is mainly valued as a food additive for tasting, flavoring and coloring, as well as for its therapeutic properties e.g., antitumor activity (Lozano *et al*., 1999). Saffron is a perennial crop well adapted to arid and semi-arid lands which produces stigmas annually. It is also adaptable to temperate and sub-tropical climates, and can be grown on soils varying from sandy to well-drained clay loams (Sheykhdavodi *et al.,* 2010). Although the source of saffron is unknown, it apparently originated in the area of Iran, Turkey and Greece, but now it is also successfully cultivated in such European countries as Spain, Italy, France, and Switzerland, as well as in Morocco, Egypt, Israel, Azerbaijan, Pakistan, India, New Zealand, Australia and Japan. While the world's total annual saffron production is estimated to be 190 tons, Iran produces about 90% of the total. The stigmas contain different compounds like carbohydrates, minerals, vitamins, pigments such as carotenes, and flavonoids (Abdullaev, 2007). Picrocrocin ($C_{16}H_{26}O_7$) is considered to be the main bitter principle of saffron. It is a monoterpene glycoside precursor of safranal ($C_{10}H_{14}O$), the major volatile oil responsible for the aroma. Action of β-glucosidase on picrocrocin liberates the aglycone4-hydroxy-2, 6, 6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, $C_{10}H_{16}O_2$), which is transformed to safranal by dehydration during the drying process of the plant material. The color of saffron comes from the water-soluble glycosidic cis- and trans-carotenoids, crocins, and glucosyl esters of crocetin (8, 80-diapocarotene-8, 80-dioic acid; $C_{20}H_{24}O_4$) (Lage and Cantrell, 2009). These saffron apocarotenoids (carotenoid-derived metabolites) are derived by bio-oxidative cleavage of zeaxanthin by a 7, 8-7', 8 cleavage reaction, followed by specific glucosylation steps. Recently, two enzymes involved in saffron apocarotenoid formation have been isolated: the Crocus zeaxanthin 7,8(7',8')-cleavage dioxygenase gene (*CsZCD*), which codes for a chromoplast enzyme that initiates the biogenesis of crocetin glycosides and picrocrocin and UGTCs2, which transfers Glc molecules to crocetin (Castillo *et al*., 2005). Various factors affect the quantity of secondary metabolites, as well as morphological and anatomical features of the plants (Krishnan *et al*., 2000). Plants suffered from drought stress develop morphological and physiological mechanisms which allow them to adapt and survive. These mechanisms mainly comprise a reduction of the leaf size, leaf rolling, leaf pubescence, sunken stomata, increase of mesophyll and accumulation of mucilage and other secondary metabolites in the tissue (Kofidis and Basabalidis, 2002). Various factors,

Table 1. Soil characteristic in both altitudes

Clay %	Silt %	Sand %	Mn	Zn	Cu	Fe	%OC	K ppm	P ppm	%N	% SP	EC Ds/m	PH	depth	Characterestic
24	26	46	18	3.6	1.1	3	1.7	400	20	0.06	34	2	7.77	0-30	Ghaen 1400m
20	28	40	20	4	0.9	3.5	1.9	380	19	0.06	34.3	1.5	7.8	0-30	Tabas 700m

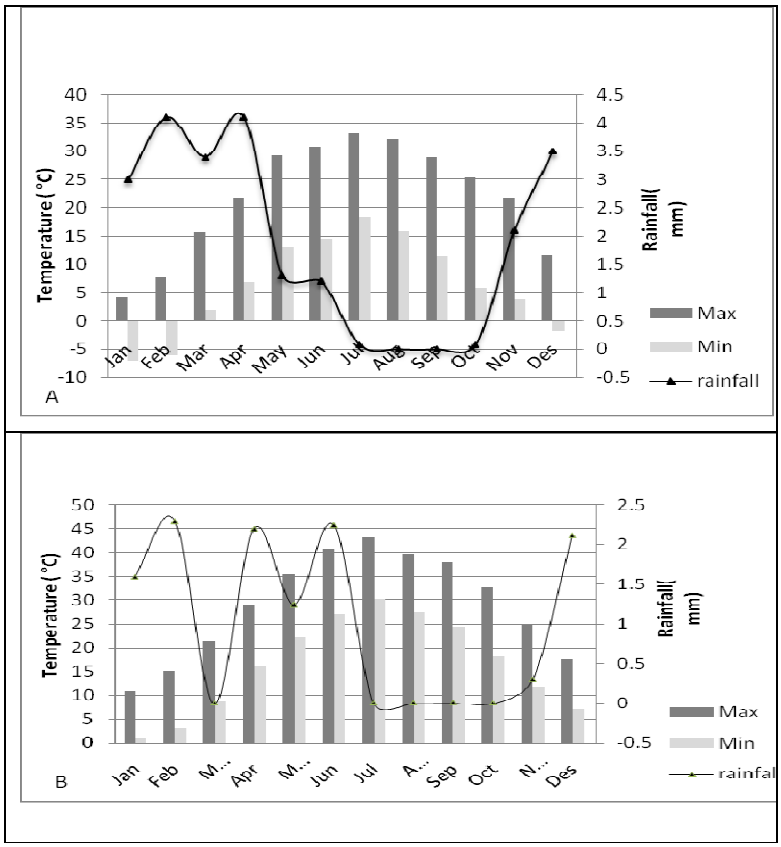

Fig 1. Average of maximum and minimum temperature and total rainfall A, 1400m; B, 700m; average (2007-2008).

such as age of the plant, season, microbial attack, grazing, radiation, competition, and nutritional status, have been proven to have an impact on the secondary metabolite profile in higher plants (Harborne and Williams, 1988). Carotenoid content is influenced by different environmental conditions like drought and temperature (Bouvier *et al*., 1998). As was just discussed above, environmental conditions have an effect on anatomy and secondary metabolites of plants. In the present research, we have investigated the effect of environmental conditions (altitude) on anatomy and quality of saffron. Saffron's quality depends on the concentration of its three major metabolites (crocin, picrocrocin, and safranal) providing the unique color and flavor to the stigmas (Lage and Cantrell, 2009). The method used in this work for the quality determination of saffron is high-performance liquid chromatography (HPLC). This method is the most efficient analytical technique for the analysis of sensitive compounds in complex extracts of natural products (Alonso *et al*., 2007). So objectives of this work are to study the effect of altitude on anatomy and quantity of three major metabolites of *Crocus sativus*.

Results

Anatomical Study

Scanning electron microscopic observation of stigmas in the two altitudes, i.e. 1400 m and 700 m, showed that stigmas were covered by glandular trichoms cuticle wrinkles and scale wax. Observation of stigma surface in both altitudes showed increase in cuticular wrinkle meanwhile density and size of glandular trichoms decreased with altitude (Fig. 2). Leaf observation in both altitudes showed that stomata were located on the abaxial surface of crypts. Diameters of crypts, cuticle wrinkles and epicuticular wax decreased with altitude (Fig. 3). Transverse section of leaves in both altitudes showed that leaves have a central keel and two lateral arms that curve towards the keel. Epidermis is single-layered on both surfaces of the leaf. Epidermal cells are slightly papillae especially on groove parts of the arms. Epidermis has a thick outer wall and is covered by cuticle. On the abaxial surface, leaves have two crypts on which there are stomata. Also, simple unicellular trichoms in different sizes are located in crypts. Mesophyll is isobilateral and includes 2-3 layers of

Table 2. Comparison of leaf character in both altitudes

Crocus sativus L.	Ghaen(1400m)	Tabas(700m)
adaxial cuticle	7.36± 1.22	10± 2.38
abaxial cuticle	1.33±0.57	6.76±0.92
Outer wall of adaxial epiderm	7.78±0.2	6.09±0.05
Outer wall of abaxial epiderm	8.43±0.05	7.01±0.09
Upper epidermis	13.8±0.8	12.45±0.4
Lower epidermis	21.13±0.03	13.66±0.5
Upper palisade cell	86±3	74±4
Lower palisade cell	86.8±9	69.8±7
Cortex diameter	686.1±0.1	164.01± 6.55
Trichomes number	4±1	6±1
Stomata density	6±1	13±1
Stomata ostiole	162.75±0.9	104.77±1.2
Stomata chamber	92±3	138.2±4.23
Outer Crypt diameter	281.13±0.5	430±1.2
Inner Crypt diameter	100.47±2.8	190±3
Crypt depth	433±23	490±30
Crypt length	240±5	268±8

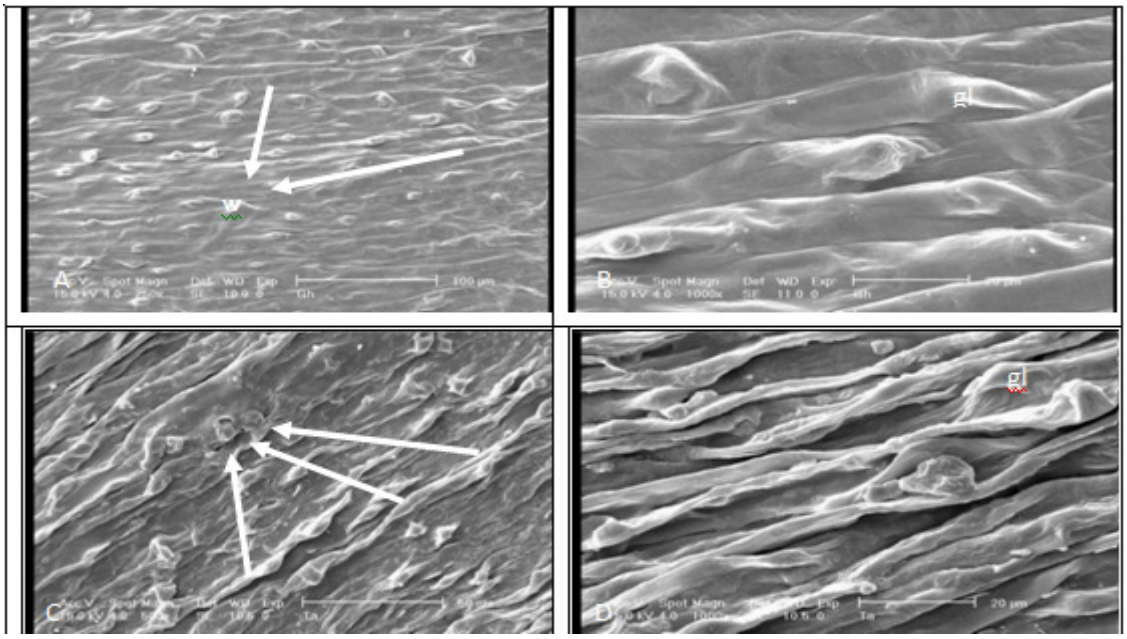

Fig 2. Scanning electron microscope of Crocus *sativus* stigma in both altitude; A-B, 1400m; C-D, 700m, showed decrease deposit of wrinkle wax and size of glandular trichome with altitude.

palisade cells on both sides. Center of leaf consists of parenchyma tissue in the keel which lacks chloroplasts. Vascular bundles are collaterally located in one row and below the abaxial epidermis, which are surrounded by bundle sheaths. The major bundle sheath consists of sclerenchyma cells at the phloem pole (Fig. 4).

ANOVA Result of Leaf Cross-section Studies in the Two Altitudes

Significant differences in some anatomical characteristics were distinct. These differences include trichomes and stomata density, size of stomata chamber and ostiole, leaf thickness (thickness of cuticle, epidermis outer cell wall and palisade cells), and diameter of cortex, crypt, and crypt depth (Table. 2). As the results in Table 2 show, cuticle thickness increases in 700m altitude samples (samples B) compared to samples from 1400m altitude (samples A), while the thickness of epidermis outer cell wall and palisade cells decreases in the former samples. HPLC Quantification of Saffron Metabolites in the Two Mentioned Regions. Three major metabolites of saffron, i.e. crocins (responsible for its color), picrocrocin (responsible for its taste) and safranal (responsible for its odor), were quantified in samples A and B from the two mentioned regions with different environmental conditions. Analyses were done using HPLC method. These constituents were characterized in 3- and 6-year-old saffron plants cultivated in both regions. In each region, 3- and 6-year-old saffron plants were compared to each other. Moreover, saffron plants with the same age were compared to each other in both regions. Concentrations were expressed as the average in the two years 2007 and 2008. Table 3 represents different concentrations of these constituents in these two regions . Statistical analysis showed significant

Table 3. HPLC quantification results of saffron metabolites from different saffron sources

Compound （mg/g of stigmas)	Ghaen 1400m		Tabas 700m	
	3 years	6 years	3 years	6 years
Crocin	29.33±1.09	25.83±1.03	22.97 ±0.1	19.56±1.52
Picrocrocin	7.07±0.16	6.91±0.15	6.06±0.23	5.28±0.18
Safranal	6.17±30.49	15.69±4.03	22.3±1.02	20.09±1.61

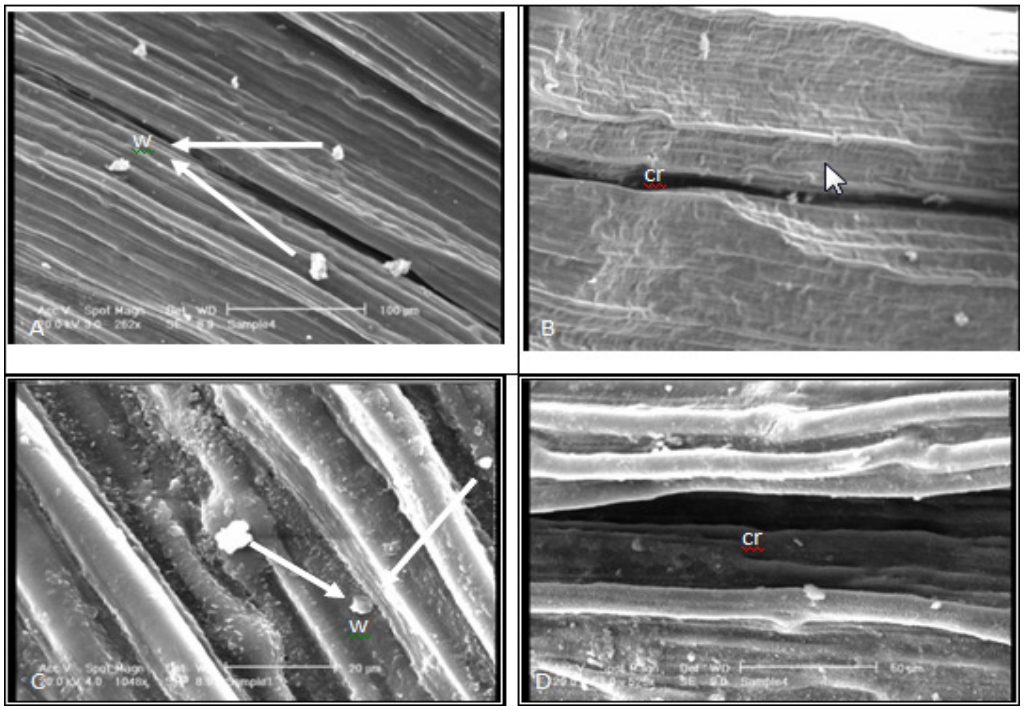

Fig 3. Scanning electron microscope of *Crocus sativus* leaf in both altitude; A-B, 1400m; C-D, 700m, showed decreed diameter of crypt aperture with altitude

differences in the three metabolites crocin, picrocrocin and safranal between 3- and 6-year-old samples in each region. As the results indicate, 3-year-old saffron has more constituents than 6-year-old saffron in each region (Fig. 5). Crocin quantities of 3-year-old and 6-year-old saffron were 29.33 mg/gdw and 25.83 mg/gdw in samples A and 22.97 mg/gdw and 19.56 mg/gdw in samples B, respectively (Table. 3). Comparison of concentrations of crocin between 3-year-old saffron samples A and B indicated that samples A had more crocin than samples B. Similar results were obtaine d by comparison of 6-year-old saffron samples in the two regions (Fig. 5). Picrocrocin concentrations in 3-year-old and 6-year-old saffron samples A were 7.07 and 6.91 mg/gdw, while in samples B they were 6.06 and 5.28 mg/gdw, respectively (Table. 3). As the results show, 3-year-old saffron has more picrocrocin than 6-year-old saffron in both regions. Comparison of picrocrocin content in 3-year-old saffron from the two regions revealed that samples A had more picrocrocin than the other samples. Also, picrocrocin concentration in 6-year-old saffron samples A was higher than that in samples B (Fig. 5). Safranal concentrations in 3-year-old and 6-year-old saffron samples A were 30.49 and 15.69 mg/gdw, whereas the respective values in samples B were 22.3 and 20.09 mg/gdw, respectively (Table. 3). Similar to crocin and picrocrocin, safranal concentration in 3-year-old saffron was higher than 6-year-old saffron in each region. Comparison of safranal concentration in 3-year-old saffron in the two regions showed that samples A had more safranal than samples B while the reverse held for 6-year-old saffron samples (Fig. 5).

Discussion

Study of the wax surface on both leaf sides of samples B by scanning electron microscopy showed an increase in the amount and diversity in forms of wax compared to samples A. This diversity includes crystalline, spherical, scale, and amorphic types. One of the functions of waxes is reducing the water loss caused by transpiration (Li et al., 2006). Physicochemical characteristics of epicuticular waxes differ in response to variable environmental stress; for instance, the increase in crystalline forms in drought conditions can be due to their function for water loss restriction (Dodd and Poveda 2003). Furthermore, the annual rainfall was lower in 700 m altitude compared to 1400 m. Therefore, at the former region with lower altitude, drought conditions are more stressful

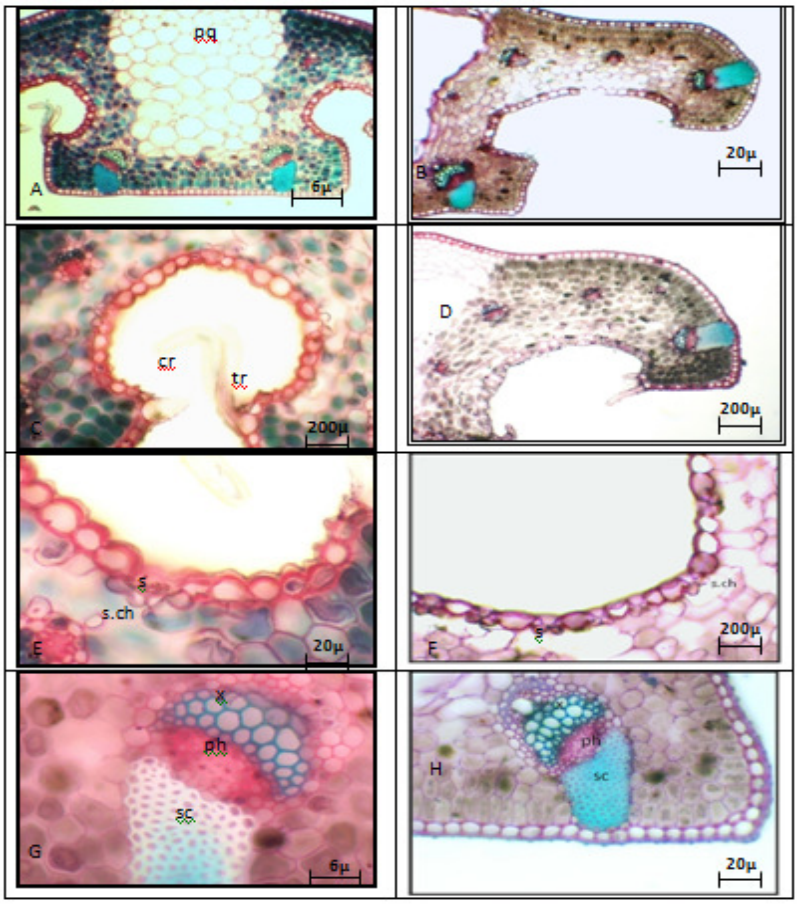

Fig 4. Transversal section of *Crocus sativus* leaf in both altitudes. A, C, E, G, in 1400m; B, D, F, H, in 700m. Showed significant difference in stomata density, size of stomata chamber, leaf thickness (thickness of cuticle, epidermis outer cell wall and palisade cells), and diameter crypt, and crypt depth with altitude

than in the latter region, since these plants have to tolerate the adverse conditions of high temperature and minimal precipitation. So, different anatomical features of Crocus sativus in these regions can be a result of different environmental conditions. In samples from arid regions, the cuticle is the last barrier to restrict water loss, so thick cuticle can reduce leaf transpiration (Dodd and Poveda 2003). Hence, increase in cuticle thickness and decrease in epidermis and palisade cell thickness in leaves of plants from 700 m altitude can be a result of more arid environments compared to 1400 m altitude. Anatomical study of cherimoya trees showed reduction of palisade and spongy cell thickness in high temperatures (Higuchi et al., 1999). Also, drought stress resulted in a decrease in the thickness of epidermal and palisade cells. According to Table 2, the diameter of parenchyma tissue is reduced in 700m altitude (Fig. 4). This could be related to arid condition in this altitude, since under such conditions stomata probably close and photosynthesis is inhibited by reduction in the diffusion of CO2 to chloroplast. The decrease in the number of parenchyma cells in samples B might be due to a decrease in photosynthesis and consequently in the number of consuming cells. Drought stress resulted in a decrease in the size of the epidermal and mesophyll cells with a parallel increase in cell density (Chartzoulakis et al., 2002). Leaf thickness of samples A is much higher than that of samples B. These differences were due to an increase in the size and number of the epidermal and mesophyll cells. Increase in leaf thickness in high altitudes was reported in Populus kang digenesis, Quercus ilex, Populus cathayan, which could be related to increase UV-B in such altitudes (Liu et al., 2005). This could also be a response to low temperature to protect mesophyll cells (Taguchi and Wada, 2001). As Fig 1 shows, the mean maximum and minimum temperatures in altitude of 1400 m were noticeably lower compared to those in altitude of 700 m. Leaves are more pubescent at altitude of 700m compared to those at 1400m (Fig. 4, Table. 2). This could be related to the increase in water stress during the vegetation period at low altitudes. It is also in accordance with the well-known roles of trichomes in blocking transpiration water and in defending against harmful insects (Kofidis et al., 2007). Number of stomata and stoma chamber width increased in samples B, while the stoma ostiole reduced in these samples compared to samples A (Fig. 4, Table. 2). Different information exists concerning the effect of altitude on stomata density. In order to save internal water, xeromorphic plants develop their stomata in local epidermal depressions or in crypts. Arid conditions (high temperature, low water availability) usually lead to higher stomata densities (Kofidis et al., 2007). Rise in density of stomata in arid conditions undoubtedly contributes to a better control of transpiration (Kofidis and Bosabalidis, 2002). Decrease in size of ostiole

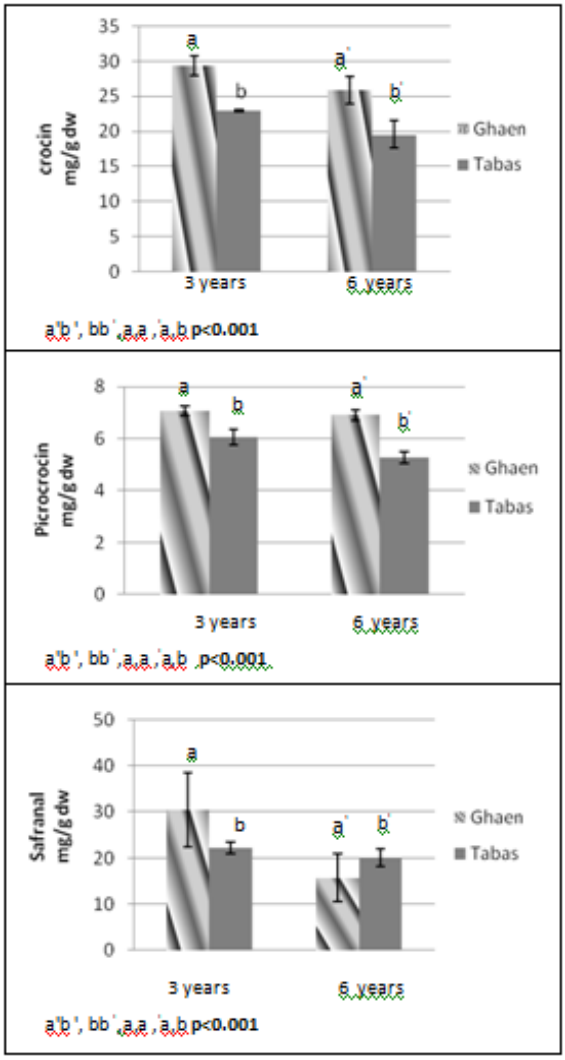

Fig 5. HPLC quantification results of crocin, picrocrocin and safranal constitutes in 3 and 6 years saffrons in Ghaen (1400m) and Tabas (700m). Different letters have significant difference at p<0.001, Error Bars represent standard deviation.

was due to the attempt to restrict water loss in dry conditions in samples B compared to samples A. Increase in stomata density reduces the CO2 diffusion, which in turn leads to resistance against photosynthesis in plant tissues (Prasad and Purohit, 2001). Wider stomata chambers in samples B compared to other samples also increase CO2 conductance diffusion to sites of carboxylation, and thus maintain photosynthetic rates (Chartzoulakis et al., 2002). The values for crypt diameter and depth in samples B were higher than those in samples A. The increased crypt diameter could be due to effective conductance of CO2 toward stomata, and reduces transpiration and water loss. As the results indicate the quantities of crocin, picrocrocin and safranal in saffron samples A were greater than those in saffron samples B (Table. 3, Fig. 5). However, an exception was that safranal concentration in 6-year-old saffron in the latter was higher than that in the former. Crocin and picrocrocin compounds degrade naturally in the cells of stigmas during drying, storage, and extraction. The degree of degradation depends on temperature, humidity, light irradiation and other compounds in the environment. Decomposition of picrocrocin gives rise to safranal during drying and storage processes (Lage and Cantrell, 2009). So, safranal values change by time and during these processes; therefore safranal concentration cannot be determined with certainty. Determination of saffron components from different sources of saffron revealed that this variation could be the result of different drying processes used, or the time and conditions under which the plant product was packed and stored in each country. Also, the values were related to different environmental conditions and cultivation practices in these countries (Abdullaev et al., 2007).

So agronomic and environmental factors affect saffron's quality (Lage and Cantrell, 2009). In this study, we used the same extraction, separation and quantification methods, and the methods used for processing and storing were similar for saffron from the two mentioned regions. Moreover, to control the effect of cultivation practices, sample collection was done from saffron plants of different ages (3-year-old and 6-year-old) with three replications in the two years 2007-2008. In Iran there is no different cultivar of Crocus sativus. Hence, the effect of cultivation practices and farm management are negligible due to the number of replications. So, the higher concentration of the constituents in samples A compared to samples B could be due to the difference in environmental conditions between these two regions. High altitude and low temperature in the region where samples A were cultivated might have affected these constituents. It should be mentioned that, the positive effect of altitude on crocin content has been proven (Lage and Cantrell, 2009). An increase in the content of phenol compounds and carotenoids with rising altitude has been postulated as a response to increasing UV-radiation (Zidorn et al., 2005). Carotenoids biosynthesis is influenced by environmental conditions such as temperature, and quantity of carotenoids increases as temperature decreases (Schonhof et al., 2006). High altitude has a positive effect on phenol compounds (Alonso et al., 2007). Study of the effect of age on saffron metabolites indicated that these constituents are present more in 3-year-old saffron compared to 6-year-old saffron. This could be due to the reduction in corm size, closing of corm to soil surface, low accessibility to mineral elements or reduced enzymatic activity of 6-year-old saffron in comparison with 3-year-old samples. Saffron yield is related to plant age, and saffron production increases from the first to the third year of cultivation (Lage and Cantrell, 2009).

Materials and methods

Plant Materials and Sampling

Samples of *Crocus sativus* L. were collected from Ghaen region (34°12'N/60°57'E, 1400m above sea level) with maximum and minimum temperatures 33 and -7°C, respectively, and Tabas (33°35'N/56°55'E, 690m above sea level) with maximum and minimum temperatures 43 and 1°C, respectively. Samples from these two regions will hereafter be mentioned as samples *A* and *B*, respectively. The total rainfall in the former and latter regions is 1.9 mm and 1 mm, respectively (Fig 1). Meteorological data were collected as average during experiment (2007-2008). Moreover, corms planted in two locations were prepared from same growers in Gonabad Province (Iran).

Also comparison of soil properties in experimental sites showed no significant difference (Table. 1). Sample collection was performed from 3- and 6-year-old saffron plants repeatedly in years 2007-2008. Flowers were picked by hand at the same time of day and then stigmas were separated by hand and dried in oven at 40°C for 24h. Dried stigmas were stored in dark glass jars at 4°C until HPLC analysis was performed.

Anatomical Study

For anatomical observations, leaves and stigmas were fixed in FAA. Cross-sections of the leaves were made with a Reichert sliding microtome and by hand cutting. Sections were cleared in sodium hypochlorite and stained by carmine-vest (1% w/v in 50% ethanol) and methyl green (1% w/v, aqueous), and mounted in gelatin. Epidermal surface was studied by SEM for which the samples were covered by gold. All morpho-anatomical measurements were performed by measurement software with 3 repeats at each part.

Extraction

Saffron stigmas (20 mg) were suspended in 1 ml of methanol-water (50:50, v/v) and magnetically stirred for 24h at 4°C in the dark. After extraction, samples were centrifuged at 30,000g for 20 min to eliminate plant residues, and then the supernatant was collected and filtered through a nylon membrane (Acrodisc 13, 0.45µm pore size, and 13 mm diameter) (Hoshyar and Bathaie, 2007).

HPLC Equipment

HPLC analysis was performed on a multisolvent delivery system (Philips, Germany), a multiple UV wavelength photo-diode array detector. The RP C_{18} column (250 mm length, 4mm internal diameter) was used for analysis.

HPLC Analysis

Metanol and acetonitrile were used as HPLC grade. A linear gradient of methanol (50-50%) in water (15% acetonitrile) was used as a mobile phase with a flow-rate of 1.0 ml/min for a maximum elution time of 30 min at room temperature. Water was distilled, deionized and further purified through an ultra-filtration system. Crocin was detected at 440 nm, picrocrocin at 250 nm and safranal at 310 nm.

Conclusion

The experimental results show that different environmental conditions influence the anatomy and quantity of three major metabolites (crocin, picrocrocin, and safranal) of *Crocus sativus*. Saffron samples *A* (from Ghaen, a region with altitude of 1400 m and lower average temperature) compared to samples *B* (from Tabas, a region with altitude of 700 m and higher average temperature) contain higher concentration of these constituents; so saffron samples *A* from Ghaen region have higher quality in comparison with those in Tabas region. Hence altitude and temperature have an effect on amount of these constituent. Also, study of the effect of age on saffron metabolites indicated that these constituents are present more in 3-year-old saffron compared to 6-year-old saffron. This could be due to the reduction in corm size, closing of corm to soil surface, low accessibility to mineral elements or reduced enzymatic activity of 6-year-old saffron in comparison with 3-year-old samples. However, further research is needed to investigate the effect of different environmental factors, such as temperature, drought, altitude and UV, on anatomy and carotenoids compounds of saffron under experimental conditions.

References

Abdullaev F, Ortega CH, Miranda PR (2007) HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. Food Chemistry, 100: 1126–1131

Alonso-A, Miguel E, Oliveros-BA, Calcagno P, Maria P (2007) Phenolics and condensed tannins of high altitude Pteridium arachnoideum in relation to sunlight exposure, elevation, and rain regime. Biochemical Systematics and Ecology, 35, 1-10

Bouvier F, Backhaus R, Camara B (1998) Induction and control of chromoplast –specific carotenoids genes by oxidative stress. The Journal of Biological Chemistry, 273, 30651-30659

Castillo R, Fernandez AJ, Gomez L (2005) Implications of Carotenoid Biosynthetic Genes inApocarotenoid Formation during the Stigma Development of Crocus sativus and Its Closer Relatives. American Society of Plant Biologists,139: 674-689

Chartzoulakis K, Patakas A, Kofidis G (2002) Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Scientia Horticulturae, 95, 39–50

Dodd RS, Poveda M (2003) Environmental gradients and population divergence contribute to variation in cuticular composition in *Juniperus communis*. Biochemical Systematic and Ecology, 31, 1257–1270

Fernandez J (2004) Biology, biotechnology and biomedicine of saffron. Recent Research Development of Plant Science, 2, 127-159

Harborne JB, Williams CA (1988) Distrbution and evolution of flavonoids in the monocotyledons, Hall Lid. London.

Higuchi H, Sakuratani T, Utsunomiya N (1999) Photosynthesis, leaf morphology and shoot growth as affected by temperatures in cherimoya (*Annona Cherimola* Mill) trees. Scientia Horticulturae, 80, 91-104

Hoshyar R, Bathaie SZ (2007) Quantitative, HPLC analysis of monoterpene aldehydes in different sources of Iranian saffron.Paper presented at the 9th Iranian Congress of Biochemistry and the 2nd International Congress of Biochemistry and Molecular Biology. University of Shiraz, Iran, 29-1 October 2007

Kofidis G, Bosabalidis AM, Moustakas M (2007) Combined effect of altitude and season on leaf characteristics of *clinopodium vulgare* L. (labiatae). Environmental and Experimental Botany. 60- 69

Kofidis G, Bosabalidis A (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Science. 163, 375-379

Krishnan N, Jeyachandran A, Nagendran N (2000) Effect of seasonal and altitudinal variation on growth performance of *Acalypha indica* Linn. In Alagar Hill (Eastern Ghats), South India. Tropical Ecology. 41, 41-45

Lage M, Cantrell C (2009) Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Scientia Horticulturae. 121, 366–373

Li C, Zhang X, Liu X, Luukkanen O, Berninger F (2006) Leaf Morphological and Physiological Responses of *Quercus aquifoliodes* along an Altitudinal Gradient. Silva Fennica. 40, 5-13

Lozano P, Castellar M, Simancas M, Iborra L (1999) Quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. Journal of Chromatography. 830, 477-483

Liu Li, Xu SM, Woo KC (2005) Solar UV-B radiation on growth, photosynthesis and the xanthophylls cycle in tropical acacias and eucalyptus. Environmental and Experimental Botany, 54,121-130

Prasad P, Purohit MC (2001) Altitude acclimatization and concentration of active constituents and calorific value of two medicinal plant species *Rheum emodi* and *R. nobile* (Rhubarb) in Sikkim Himalaya. Current science. 6, 734-735

Schonhof I, Klaring H, Krumbein A, Chuben W, Schrerer M (2006) Effect of temperature increase under radiation condition on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agriculture, Ecosystems and Environment .119,103-111

Sheykhdavodi MJ, Ebrahimi Nik MA, Pourreza Bilondi M, Atashi M, Seyedian M (2010) Mechanization planning for tillage of saffron fields using multiple criteria ecision-making technique as a policy framework in Iran. Australian Journal of Crop Science.4(5):295-300

Taguchi Y, Wada N (2001) Variations of leaf traits of an alpine shrub *Sieversia pentapetala* along an altitudinal gradient and under a stimulated environmental change. Polar Bioscience. 14, 79-87

Zidorn C, Schubert B, Stuppner H (2005) Altitudinal differences in the contents of phenolics in flowering heads of three members of the tribe Lactuceae (Asteraceae) occurring as introduced species in New Zealand. Biochemical Systematics and Ecology.33, 855-872