Biochemical Diversity of Yellow Flag (*Iris pseudacorus* L.) at Various Geographical Locations

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ABSTRACT

Biochemical diversity is a manifestation of geographical area and environmental change. In the present study, 16 ecotypes of yellow flag species were identified and collected through field studies. The ecotypes were identified and then grouped into three populations based on their geographical locations. Using spectrophotometric methods, eight biochemical properties were measured. These were chlorophyll a, chlorophyll b, total chlorophyll, antioxidant capacity, carotenoids, total phenol, flavonoids, and anthocyanin content. The biochemical traits were analyzed in a completely randomized design in three replications using SAS software and principal component analysis via SPSS software. The analysis of variance showed significant differences between the ecotypes in terms of biochemical traits ($p<0.05$; $p<0.01$). Photosyntheti -c pigments occurred maximally in ecotype Q, phenol and flavonoids in ecotype K, antioxidant capacity in ecotype O, and anthocyanin content in ecotype L. The populations were divided into three main groups by cluster analysis. Correlation analysis showed significant, positive correlations between chlorophyll a and total chlorophyll ($r = 90\%$), total chlorophyll and carotenoids ($r = 67\%$), as well as phenol content and flavonoid content ($r = 56 \%$). The results indicated significant bioch -emical diversity in the ecotypes.

Introduction

In ecosystems, plant metabolites are affected by various factors such as species, climate, soil type, altitude, and geographical location. The gene expression and activity of coded proteins in metabolic pathways are reportedly affected by various environmental conditions (Li et al., 2020). Environmental diversity usually prompts an increase in the production of secondary metabolites in plants. Thus, significant differences are usually observed in plants grown in mountainous areas, compared to plants in lowlands, primarily because drought, sunlight, and ultraviolet rays affect plants and cause changes in their active ingredients. Altitude is an influential factor in plant growth and yield. Since environmental temperature usually changes according to altitude, plant height can be affected accordingly. Moreover, factors such as temperature, relative humidity, wind speed, available water, and radiation can induce morphological changes in plants (Fille cache et al., 2012).

In evaluating genetic diversity and characteristics of germplasms in horticultural plants, it is necessary to study cultivars, genotypes, germplasm classification, and breeding potentials. Ultimately, the aim is to formulate strategies for the selection of new cultivars (Naghavi et al., 2007). The first step in breeding

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programs is a comprehensive image of genetic diversity (Jozghasemi, 2016). The two main elements of any breeding program are diversity and selection. Selection can be very efficient if a trait has a good variety in a population. Before plant breeding, preserving plant accessions is crucial to the integrity of ecosystems. To examine biodiversity in breeding programs, it is crucial to understand the nature and extent of diversity in germplasms (Ammarellou et al., 2014). The backbone of plant survival and evolution in nature is genetic diversity. Plants can develop resistance to climate change through genetic diversity in wild genotypes, and using their desirable alleles can significantly assist horticulturalists in breeding climate-resistant varieties (Bhandari et al., 2017). The first step in breeding programs is to know wild species diversity, which is the basis of phenotypic and genotypic selection according to genetic diversity research (Jozghasemi et al., 2016). In studying wild plants, first, detailed studies should be conducted on diversity recognition, domestication, and their improvement. Then, they should be introduced into agriculture and industry. The study of population genetics is a valuable and effective tool for developing strategies for maintaining and increasing the commercial values of plants (Babalar et al., 2013). The genus Iris consists of approximately 300 species divided into six subspecies (Lamote et al., 2002).

Iris is a monocotyledonous plant native to Iran that grows wild in different parts of the country. *Iris* species have great decorative and medicinal value and play a vital role in the ornamental, perfumery, green space, pharmaceutical, and food industries. They also show good resistance to environmental conditions (Azimi et al., 2018; Kemper, 2012). *Iris pseudacorus* is a perennial herbaceous plant of the Iridaceae family. This plant grows in multiples and is easily recognizable by its flowers, as it is the only species of wild *Iris* with yellow flowers (Jaca and Mkhize, 2015). Due to the beautiful form of the flowers and delicate leaves of the yellow flag, this plant is usually planted in gardens. Due to its great popularity, new varieties have been produced in industrial horticulture (Rahimi et al., 2011). *Iris* species contain numerous polyphenolic compounds with antioxidant, antimutagenic, and estrogenic activity (Tarbeeva et al., 2015). Plant metabolites such as phenol and flavonoids have a strong potential to scavenge free radicals in all parts of the plant, such as leaves, fruits, seeds, roots, and skin (Sarker and Oba, 2018).

So far, botanical and biochemical studies have addressed the chemical composition of the roots, leaves, and flowers of different species of irises and their pharmaceutical uses. Studies have shown that irises have a high content of metabolites. Common secondary compounds are flavonoids and isoflavones. Some medical studies suggest that there are positive effects of metabolites of these plants in the treatment of cancers or antibacterial and viral infections. Flavonoids have also been found in the analysis of the leaves and flowers of this plant (Kassak, 2012). The chemical properties of plants can be as widely used in classification as their morphological properties. It has also provided helpful information for understanding the evolutionary relationships of taxa and has helped solve many taxonomic problems (Reynolds, 2007). The system for identifying and using biochemical markers is simple, inexpensive, and more proportionate than morphological markers (Chiara, 2005). These markers contain compounds such as macromolecules.

Macromolecules are biochemical compounds, including phenolic compounds, alkaloids, cyanogens, and non-protein amino acids (Chiara, 2005). The high diversity of natural compounds in plants tends to increase the importance of studying new medicinal compounds. These compounds are obtained through the evolution of the plant defense system, and different types of natural products show the effectiveness of this defense strategy against external factors (Herrmann et al., 2011). In recent years, climate change and human manipulation have affected genetic diversity. High polymorphism rates can be explained by hydrological and geographical segregations (Wróblewska et al., 2003).

Considering the unique properties of the yellow flag in ornamental, medicinal, green space, phytoremediation, and unknown biochemical diversity, studying the variety of its compounds seems necessary. In the present study, an effort was made to analyze the biochemical diversity among *Iris pseudacorus* ecotypes to investigate their relationships. Furthermore, the information on yellow flag biochemical variation would assist in pre-selection criteria for distinguishing potential ecotypes and employing them as parents in breeding programs. Therefore, the present study is the first attempt to determine the diversity of wild *Iris pseudacorus* ecotypes in northern Iran based on biochemical evaluations.

Materials and Methods

Sixteen *Iris pseudacorus* ecotypes were identified based on field studies in two northern provinces of Iran (Guilan and Mazandaran). Their rhizomes

were collected for cultivation (Fig. 1). The rhizomes with a diameter of 4 to 6 cm were randomly collected. The samples were accurately labeled at the end of the flowering season in late July (2018-2020).

The rhizomes were divided into equal parts and planted in pots with clay + peat + sand at the beginning of August. Planting and maintenance operations (including irrigation, weeding, and weeding) regularly appeared. The latitude, longitude, and altitude of their origin from the surface of open waters were determined by GPS. Also, their meteorological statistics were provided by the meteorological departments of Guilan and Mazandaran. The collected ecotypes were grouped according to geographical locations in three natural sites. Ecotype groupings were divided into three populations. The first population included A, B, and C. The second consisted of E, F, G, H, I, J, K, L, and M. The third population comprised N, O, P, and Q ecotypes (Table 1).
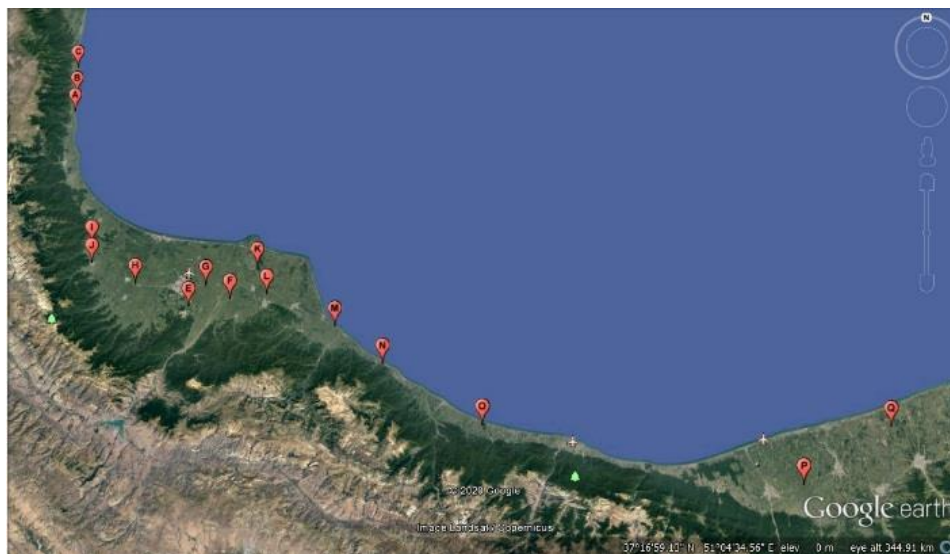

Fig. 1. Ecotype sampling map in northern Iran

Assessment of photosynthetic pigments

Photosynthetic pigments, including chlorophyll a, b, total, and carotenoids, were evaluated (Barnes, 1992). In this method, 0.5 g of fresh tissue was first crushed and poured into a test tube, and then ten ml of pure methyl sulfoxide (DMSO) was added. It was then placed in an oven at 70 °C for three hours until the pigments were extracted and the leaves were entirely colorless. The samples were filtered with Whatman filter paper. After reading their absorption at 663, 645, 480, and 510 nm, the number of photosynthetic pigments was calculated from the following formulae in milligrams per gram of fresh weight:

$$Chla = \frac{(12.7 * OD\ 663) - (2.69 * OD\ 645) * Volume * Dilution\ rate}{1000 * Sample\ weight}$$

$$Chlb = \frac{(22.7 * OD\ 645) - (4.68 * OD\ 663) * Volume * Dilution\ rate}{1000 * Sample\ weight}$$

$$Total\ Chl = \frac{(20.2 * OD\ 645) - (8.02 * OD\ 663) * Volume * Dilution\ rate}{1000 * Sample\ weight}$$

$$Carotenoid = \frac{(7.6 * OD\ 480) - (1.49 * OD\ 510) * Volume * Dilution\ rate}{1000 * Sample\ weight}$$

Measurement of biochemical parameters

To measure the total phenol, flavonoid compounds, and percentage of free radical scavenging, 0.5 g of each sample (fresh leaf) was liquefied in nitrogen powder with 5 ml of 80% methanol (ratio 1 to 10), and the solution was homogenized in closed falcons. The homogenized material was placed on a shaker for 24 hours and then centrifuged at 3000 rpm for 5 minutes. The upper part of the extract was used for measuring the desired biochemical traits.

Measurement of antioxidant capacity by the DPPH method

In this experiment, the percentage of inhibition of DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) was measured using the method proposed by Lee et al. (2003) with a slight modification. First, 1 ml of DPPH (0.1 mM) (4 mg radical to 100 ml of methanol) was added to the test tube, and then 1 ml of methanolic extract was prepared from the yellow flag. The test tubes were then placed in a dark environment for 15 minutes and immediately read with a model spectrophotometer (Model UV-1800) at 517 nm. In addition to the mentioned samples, a test tube

containing only 0.1 mM DPPH was regarded as the control. The spectrophotometer was calibrated with 80% methanol. The values were obtained by the following formula and converted to the percentage of inhibition (sample absorption (As)

and control number (Ac)).

$$\%\text{Free radical scavenging} = \frac{(Ac - As)}{Ac} \times 100$$

Table 1. Geographical and meteorological characteristics of *Iris pseudacorus* ecotypes.

Sample Code	Location	Latitude	Longitude	Accuracy	Altitude	Tmax (°C)	Tmin (°C)	tm (°C)	rrr24 (mm)	um (%)	sshn (h)
A	Khajekari	38° 01′ 45″ N	48° 55′ 05″ E	3 m	-24 m	20.4	13.2	16.6	2.7	78.1	4.7
B	Zoume Mahalleh	38° 06′ 32″ N	48° 54′ 45″ E	3 m	-27 m	20.4	13.2	16.6	2.7	78.1	4.7
C	Moharram Zoume	38° 14′ 3″ N	48° 53′ 34″ E	3 m	-25 m	20.2	12.8	15.9	4.0	79.9	5.4
E	Rasht	37° 11′ 28″ N	49° 38′ 02″ E	9 m	33 m	22.0	12.8	16.7	3.2	83.3	5.4
F	Loulman	37° 13′ 21″ N	49° 49′ 56″ E	3 m	-2 m	21.4	13.2	16.7	4.0	82.7	4.9
G	Jafarabad	37° 16′ 44″ N	49° 42′ 28″ E	3 m	0 m	21.4	13.2	16.7	4.0	82.7	4.9
H	Some′e sara	37° 17′ 8″ N	49° 21′ 29″ E	3 m	-2 m	20.7	15.0	17.3	5.3	82.8	5.5
I	Shanderman	37° 26′ 41″ N	49° 06′ 53″ E	3 m	46 m	20.7	15.0	17.3	5.3	82.8	5.5
J	Masal	37° 22′ 10″ N	49° 07′ 40″ E	3 m	56 m	18.7	11.7	14.5	3.7	81.6	5.0
K	Safrabasteh	37° 21′ 4″ N	49° 57′ 18″ E	3 m	-16 m	21.2	13.9	17.3	3.8	82.4	5.0
L	Lahijan	37° 14′ 26″ N	50° 00′ 36″ E	3 m	-16 m	21.8	12.0	16.7	3.9	81.7	5.6
M	Roudsar	37° 06′ 39″ N	50° 21′ 01″ E	3 m	-25 m	20.5	12.9	17.2	3.2	80.8	5.7
N	Ramsar	36° 57′ 51″ N	50° 35′ 04″ E	3 m	-22 m	20.6	14.3	17.2	3.6	81.7	5.0
O	Nashtaroud	36° 43′ 56″ N	51° 03′ 13″ E	3 m	-23 m	20.7	14.0	17.1	4.1	80.7	5.7
P	Amol	36° 29′ 43″ N	52° 30′ 41″ E	3 m	9 m	22.6	13.4	17.3	1.9	81.2	5.3
Q	Jouybar	36° 41′ 42″ N	52° 57′ 15″ E	3 m	-20 m	23.0	13.0	17.4	1.9	78.5	6.3

rrr24 = 24-hour rainfall, sshn = sunny hours, um = Average relative humidity, tm = Average temperature.

Measurement of total phenol content

Total phenol was also measured using a method proposed by Singleton et al. (1999). Twenty μl of methanolic extract (0.5 g in 5 ml of 80% methanol) was mixed with 100 μl of Folin-ciocalteu and 1.16 ml of distilled water. After 5 to 8 minutes of rest, 300 μl of sodium carbonate M (10.6 g per 100 ml of distilled water) was added. The above solution was placed in a dark steam bath at 40 °C for 30 minutes. For the control, 80% methanol was used instead of the extract. This solution was used for calibrating the spectrophotometer, and then the samples were read at a wavelength of 760 nm. Gallic acid solution (in 80% methanol) was used for drawing the calibration curve of different concentrations of standard micrograms of gallic acid per milliliter (Singleton et al., 1999). Then, the amount of phenol in the extract was obtained using a relevant equation (y = 0.0025 - 0.0024).

Assessment of total flavonoid content

To calculate the flavonoid content by Singleton et al. (1999), the first 0.5 ml of methanolic extract was prepared with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride in ethanol (10 ml) aluminum chloride in 100 ml of distilled water and distilled water, 0.1 ml of 1 M potassium acetate (2.41 g per 10 ml of distilled water) and 2.8 ml of distilled water. Instead of the methanolic extract, only pure methanol was used for preparing the control. The mixture was then placed in the dark for half an hour and read immediately at 415 nm. The values of flavonoids were validated regarding the standard curve. Accordingly, different concentrations were made from the standard quercetin (micrograms per milliliter), and after reading the adsorption value, the standard curve was drawn (Singleton et al., 1999). The total flavonoid concentration (y = 0.0067 + 0.0122) was measured using the line equation obtained from the standard curve.

Anthocyanin measurement

To measure anthocyanin content, 0.5 g of the sample was homogenized with 5 ml of acidic methanol (9 ml of methanol + 1 ml of hydrochloric acid) and placed in the dark at 4 °C for 24 hours. The extract was then centrifuged at 104 g for 10 minutes, and the absorption of the supernatant was read at 520 nm using a spectrophotometer. The amount of anthocyanin was calculated as $A = \varepsilon bc$, in which the value of ε or the extinction coefficient is equal to 3300 mM cm^{-1}, A is the amount of adsorption, b is the width of the cuvette equal to 1 cm, and c is the amount of anthocyanin (M per gram of plant sample) (Wagner, 1979). Biochemical traits were measured at Gorgan University of Agricultural Sciences and Natural Resources laboratories (36° 50′ 36″ N and 54° 23′ 48″ E).

Data processing and analysis

For the analysis of variance and the comparison of mean values, biochemical traits were evaluated in the form of a completely randomized design, with three replications (i.e. three pots per replication) using SAS. The graphs were drawn using Microsoft Excel. The comparison of mean values was carried out using the LSD test (P≤0.05). A cluster analysis was performed to determine the similarity of the 16 ecotypes and their clustering according to the biochemical traits. The data were standardized and then used (Johnson, 1998). A dendrogram was obtained from cluster analysis using Ward's method (Dillon and Goldstein, 1984). In this research, the classification of the ecotypes was shown according to a tree diagram using SAS.V.9.0 and SPSS.V.16 software. Principal component analysis was performed using SPSS software.

Results

The analysis of variance on the biochemical data of 16 yellow flag ecotypes shows that all parameters were significant at one and five percent probability (Table 2). Another considerable value in the analysis of variance is the coefficient of variation. The highest coefficient of variation was related to chlorophyll b leaf (17.4) and showed that it had good diversity. Antioxidant activity was in the second degree, and its coefficient of variation was 13.6, which indicates a high level of variation. Leaf carotenoids with a coefficient of 12.9 suggested a good potential in diversity studies.

Table 2. Analysis of variance (sum of squares) of biochemical traits of Yellow flag (*Iris pseudacorus*) in different ecotypes.

Source of changes	Df	Leaf Chlorophyll a	Leaf Chlorophyll b	Leaf Total Chlorophyll	Leaf Carotenoid	Phenol Content	Flavonoid Content	Antioxidant Activity	Anthocyanin
Ecotypes	15	0.032 **	0.006 **	0.04 **	0.004 *	18275 **	1380 **	115.4 *	0.0007 **
Experimental Error	32	0.004	0.001	0.003	0.001	472	108	51.7	0.00006
% CV	-	10.6	17.4	7	12.9	7	8	13.6	9.8

Respective * and ** at the level of probability of 5, 1%, and ns, respectively, indicate no significant difference.

Data from the mean values of photosynthetic traits (Table 3) showed that in ecotype Q (Jouybar), chlorophyll a, chlorophyll b, and total chlorophyll were at the highest levels. The lowest levels were obtained in ecotypes J and A.

Meteorological data showed that the geographical location of Jouybar has an average temperature that is higher than that of other places, with more sunny hours (Table 1). Among the samples, the most significant carotenoids were found in ecotype F (0.390 mg g^{-1}) and the smallest amount was found in ecotype A (0.255 mg g^{-1}). Total phenol content was highest in ecotype K (490.56 µg g^{-1} gallic acid) and the lowest was observed in ecotype A (165.63 µg g^{-1} gallic acid). Similar to the total phenol content, the total flavonoid content was highest in ecotype K (177.08 µg g^{-1} quercetin) and was lowest in ecotype A (85.59 µg g^{-1} quercetin).

Another critical parameter in the discussion of medicinal plants is the antioxidant capacity of their extracts. The percentage of antioxidant activity of the yellow flag was highest in ecotype O (65.5 %) and was lowest in ecotype K (39.77 %).

The anthocyanin level was highest in ecotype L (0.104 mM cm^{-1}) and lowest in ecotype O (0.0533 mM cm^{-1}).

Table 3. Mean comparison of biochemical traits in *Iris pseudocorus* ecotypes.

Ecotypes	Leaf Chlorophyll a (mg g⁻¹)	Leaf Chlorophyll b (mg g⁻¹)	Leaf Total Chlorophyll (mg g⁻¹)	Leaf Carotenoid (mg g⁻¹)	Phenol Content (µg g⁻¹ gallic acid)	Flavonoid Content (µg g⁻¹ quercetin)	Antioxidant Activity (%)	Anthocyanin (mM cm⁻¹)
A	0.565 ᵉᶠ	0.140 ᵉ	0.708 ᵍ	0.255 ᵉ	165.63 ᵉ	85.59 ʰ	55.39 ᵃᵇᶜ	0.055 ᶠ
B	0.576 ᵉᶠ	0.163 ᵈᵉ	0.743 ᶠᵍ	0.279 ᶜᵈᵉ	274.29 ᵈ	146.43 ᵇᶜ	45.10 ᶜᵈ	0.0701 ᵉ
C	0.673 ᵃᵇᶜᵈᵉ	0.219 ᵇᶜᵈ	0.890 ᶜᵈᵉ	0.343 ᵃᵇᶜ	366.69 ᵇᶜ	157.23 ᵇ	47.69 ᵇᶜᵈ	0.087 ᵇᶜ
E	0.597 ᵈᵉ	0.260 ᵃᵇ	0.862 ᵈᵉ	0.341 ᵃᵇᶜ	381.09 ᵇ	130.36 ᶜᵈᵉᶠ	49.35 ᵇᶜᵈ	0.097 ᵃᵇ
F	0.758 ᵃᵇ	0.261 ᵃᵇ	1.02 ᵃᵇ	0.390 ᵃ	338.83 ᶜ	123.9 ᵈᵉᶠ	57.94 ᵃᵇ	0.0919 ᵃᵇ
G	0.673 ᵃᵇᶜᵈᵉ	0.250 ᵃᵇᶜ	0.92 ᵇᶜᵈᵉ	0.348 ᵃᵇᶜ	350.16 ᵇᶜ	125.34 ᵈᵉᶠ	57.49 ᵃᵇ	0.088 ᵇᶜ
H	0.693 ᵃᵇᶜᵈ	0.267 ᵃᵇ	0.96 ᵇᶜ	0.364 ᵃᵇ	345.36 ᵇᶜ	123.95 ᵈᵉᶠ	52.48 ᵇᶜ	0.084 ᵇᶜᵈ
I	0.615 ᶜᵈᵉ	0.243 ᵃᵇᶜ	0.862 ᵈᵉ	0.332 ᵃᵇᶜᵈ	275.63 ᵈ	113.15 ᶠᵍ	50.11 ᵇᶜᵈ	0.086 ᵇᶜ
J	0.363 ᵍ	0.290 ᵃ	0.658 ᵍ	0.31 ᵇᶜᵈᵉ	270.56 ᵈ	139.67 ᶜᵈ	45.99 ᵇᶜᵈ	0.0762 ᶜᵈᵉ
K	0.724 ᵃᵇᶜ	0.300 ᵃ	1.03 ᵃᵇ	0.355 ᵃᵇ	490.56 ᵃ	177.08 ᵃ	39.77 ᵈ	0.076 ᶜᵈᵉ
L	0.650 ᵇᶜᵈᵉ	0.191 ᶜᵈᵉ	0.845 ᵉᶠ	0.349 ᵃᵇᶜ	274.29 ᵈ	121.01 ᵉᶠ	53.91 ᵃᵇᶜ	0.104 ᵃ
M	0.667 ᵇᶜᵈᵉ	0.208 ᵇᶜᵈᵉ	0.880 ᶜᵈᵉ	0.348 ᵃᵇᶜ	335.89 ᶜ	134.19 ᶜᵈᵉ	53.24 ᵇᶜ	0.0758 ᶜᵈᵉ
N	0.614 ᶜᵈᵉ	0.242 ᵃᵇᶜ	0.860 ᵈᵉ	0.322 ᵃᵇᶜᵈᵉ	296.83 ᵈ	126.33 ᵈᵉᶠ	56.68 ᵃᵇᶜ	0.0722 ᵈᵉ
O	0.481 ᶠ	0.209 ᵇᶜᵈ	0.693 ᵍ	0.267 ᵈᵉ	287.76 ᵈ	96.68 ᵍʰ	65.5 ᵃ	0.0533 ᶠ
P	0.699 ᵃᵇᶜᵈ	0.256 ᵃᵇᶜ	0.960 ᵇᶜᵈ	0.349 ᵃᵇᶜ	274.83 ᵈ	127.43 ᵈᵉᶠ	55.16 ᵃᵇᶜ	0.0537 ᶠ
Q	0.781 ᵃ	0.300 ᵃ	1.08 ᵃ	0.356 ᵃᵇ	174.69 ᵉ	134.54 ᶜᵈᵉ	57.04 ᵃᵇᶜ	0.094 ᵃᵇ

In each column, the averages with common letters are not significantly different.

The data from the correlation table of yellow flag ecotypes, regarding their biochemical traits, showed significant correlations among some of these traits (Table 4). The highest positive correlation (90%) occurred between chlorophyll a and total chlorophyll. Changes in chlorophyll a can strongly affect total chlorophyll content. Then, a positive and significant correlation was obtained between chlorophyll a, carotenoids, and anthocyanins. Chlorophyll b showed positive and significant correlations with total chlorophyll, carotenoids, phenol content, and flavonoids. The correlation of total chlorophyll content had similarities to chlorophyll a and b, along with positive and significant correlations with carotenoids, flavonoid content, and anthocyanins. Carotenoids have a positive and significant correlation with anthocyanins only, and flavonoid content correlated negatively, significantly, with the percentage of antioxidant activity.

The data obtained from biochemical markers were further analyzed by principal component analysis (Table 5). Principal component analysis divided the 16 yellow flag ecotypes in northern Iran into two main components. The principal component analysis showed the first and second principal components, with eigenvalues of 4.07 and 1.76, which explained about 73% of the total variables (Table 5).

Table 4. Correlation coefficients among biochemical traits in *Iris pseudacorus* ecotypes.

Source of changes	Leaf Chlorophyll a	Leaf Chlorophyll b	Leaf Total Chlorophyll	Leaf Carotenoid	Phenol Content	Flavonoid Content	Antioxidant Activity	Anthocyanin
Leaf Chlorophyll a	1							
Leaf Chlorophyll b	0.05 ns	1						
Leaf Total Chlorophyll	0.9 **	0.47 **	1					
Leaf Carotenoid	0.48 **	0.59 **	0.67 **	1				
Phenol Content	0.16 ns	0.34 **	0.29 **	0.32 *	1			
Flavonoid Content	0.23 ns	0.35 **	0.35 **	0.29 *	0.56 **	1		
Antioxidant Activity	0.01 ns	-0.14 ns	-0.04 ns	-0.29 *	-0.27 *	-0.52 **	1	
Anthocyanin	0.34 **	0.22 ns	0.40 **	0.39 **	0.20 ns	0.22 ns	-0.16 ns	1

Respective * and ** at the level of probability of 5, 1%, and ns, respectively, indicate no significant difference.

Table 5. Eigenvalues, total variance, and principal component analysis (PCA) of *Iris pseudacorus* ecotypes.

Traits	1	2
Chl a	0.727 *	0.505 *
Chl b	0.703 *	-0.024
Total Chl	0.870 *	0.413
Carotenoid	0.912 *	0.269
Phenol	0.634 *	-0.467
Flavonoiid	0.712 *	-0.600 *
Antioxidant Capacity	-0.378	0.822 *
Anthocyanin	0.639 *	0.125
Eigenvalues	4.071	1.769
Cumulative variance (%)	50.884	72.992
Total variance (%)	50.884	22.108

Component coefficients are significant > 0.5.

In each principal component, factor coefficients greater than 0.5 were considered significant (Table 5). The first component with 50.88% variance, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, flavonoids, phenol, and anthocyanins, were significant. In the second component, chlorophyll a, flavonoids, and antioxidant capacity were significant. Principal component analysis generally expressed the eight biochemical traits evaluated in the two main components. The first component showed the most significant role of variation. As shown in Table 5, the eigenvalues are more than one, which shows that the measured traits could explain a high percentage of the available diversity. Fig. 2 shows the results of drawing the first and second principal components. According to the first component, ecotypes E, C, K, B, and J were in one group, and the other ecotypes were in another group. Based on the second component, ecotypes K, C, E, M, H, G, F, Q, P, and L were grouped, and other ecotypes were placed in another group (Fig. 2).
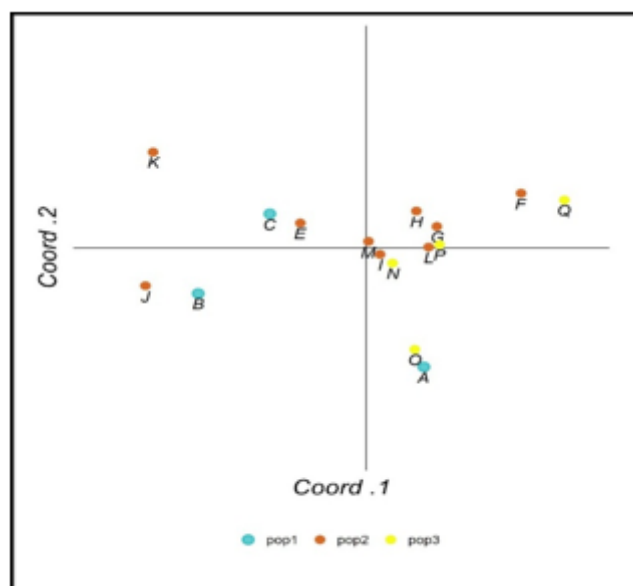
Fig. 2. Principal component analysis (PCA) based on *Iris pseudacorus* accessions and their biochemical traits.

The dendrogram obtained from the cluster analysis (Fig. 3) showed that the populations were grouped into three main physiological and biochemical traits from the cut points acquired in clustering the whole population. The first group consisted of L, P, I, N, O, B, J, G, H, F, M, C, and E ecotypes. The second group was a single member where ecotype K was located. The third group had two members where ecotypes A and Q were located.
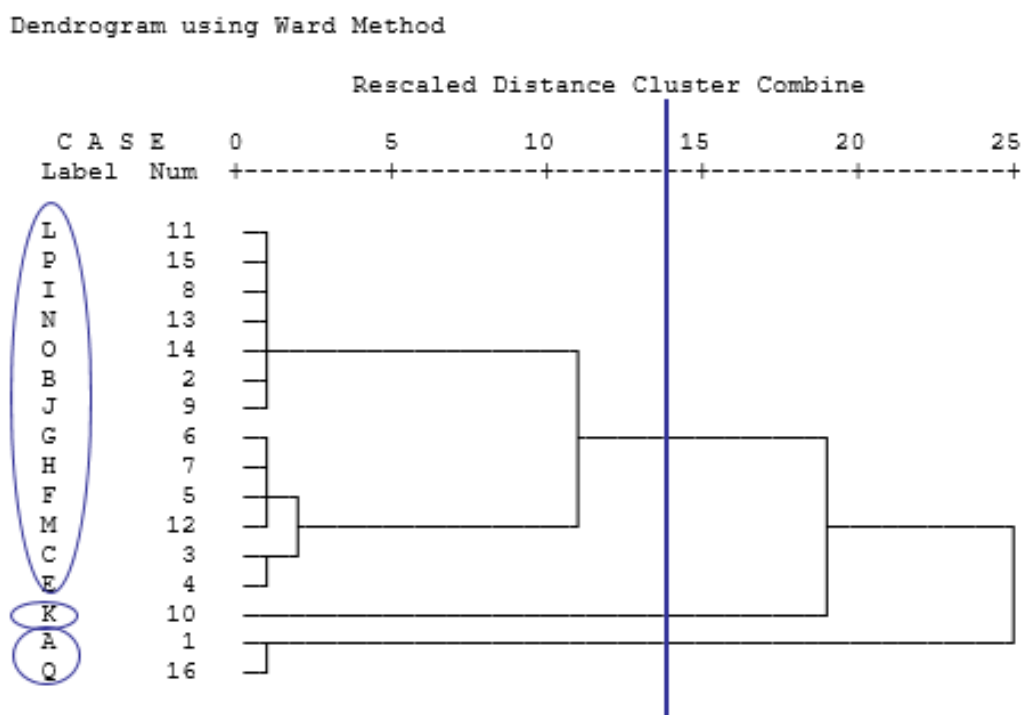

Fig. 3. Dendrogram of *Iris pseudacorus* ecotypes based on biochemical traits assessed by Ward's method.

Discussion

Plant growth cannot be attributed to a specific physiological function or dominant physiological processes in photosynthesis (Parida and Das, 2005). Increased radiation during the plant growing season increases the production of photosynthetic materials and improves the growth of different parts of the plant (Ahmadi et al., 2010). With the increase in light intensity from 100 to 450 µmol m^{-2} in Panax, the chlorophyll content increased from 484 to 515 mg m^{-2} (Chen et al., 2016). Light controls the growth and development of plants through photosynthesis, primarily through the absorption of carbon dioxide. Light intensity affects photosynthesis and varies according to time and location in each habitat, although plants can usually adapt to different light intensities (Zhang et al., 2003). Carotenoids can capture high-energy short wavelengths, convert single oxygen to ternary, and play an antioxidant role by capturing the oxygen radicals produced (Inze and Montago, 2002). Numerous factors can affect the quantity and quality of carotenoids, including climatic factors. Carotenoids can increase the antioxidant capacity of plants and support photosynthesis by producing new proteins and enzymes (Shen et al., 2017). Light is one of the most important factors affecting plant growth that influences changes in radiation, growth, morphology, anatomy, cell physiology, and biochemistry (Deng et al., 2012). The lowest sunshine hours were recorded in ecotype A, which could be the reason for the reduction of carotenoids in this ecotype. In contrast, insufficient ATP is produced under low radiation conditions to stabilize carbon and carbohydrate biosynthesis, thereby reducing plant growth (Shao et al., 2014).

Phenols are involved in developing plant resistance to a wide range of biotic and abiotic stresses (Naikoo et al., 2019). Another valuable parameter in pharmaceutical discussions is the flavonoid content of the plant extract. Flavonoids are tricyclic compounds with many medicinal properties and contain antioxidant, estrogenic, and antitumor activity effects. As the first enzyme in the phenylpropanoid pathway, the Phenylalanine Ammonia-Lyase enzyme converts phenylalanine to 4-Coumaroyl A coenzyme, an active precursor in the production of flavonoids (Jan et al., 2021). Flavonoids and other phenolic compounds are widely distributed in plants, and various biological activities include antioxidant, antimicrobial, and anti-inflammatory, as reported in many studies (Jamshidi, 2010). One of the most critical sources of phytochemical groups of plants is phenolic compounds, which have physiological significance in plants (Mohsenpour et al., 2014). Researchers have found that the amount of phenolic and flavonoid compounds in plants in different regions is influenced by climatic and geographical differences such as altitude in the different areas and genetic differences. This study and other findings showed that the production of biochemical compounds in plants in ecosystems is affected by various factors such as climate, altitude, and geographical location (Mohammad Nezhad Ganji et al., 2017). However, it is also important to note that proving the effect of environmental factors does not detract from genetic factors, and clarifying the role of genetic factors in these compounds would probably not be comprehensive without studying environmental factors (Basey et al., 2015).

Despite physiological protection against intense light in plants, the occurrence of intense light can lead to higher amounts of ROS molecules (Telfer, 2014). As the intensity of light increases, the probability of light oxidation increases due to the saturation of the electron transfer chain capacity. As the light continues to remain intense, ROS synthesis increases in both photosystems. To reduce light inhibition, the plant activates its antioxidant system to inhibit ROS by inhibiting their effect on protein synthesis (Takahashi and Badger, 2011). The plant builds ultraviolet filtering and absorbing molecules, including non-enzymatic or phenolic compounds, in the cytoplasm and accumulates them in a vacuole (Winkel-Shirley, 2001). Since phenolic and flavonoid compounds are part of the plant antioxidant system, reducing the harmful effects of intense light can be achieved through higher amounts of phenolic and flavonoid compounds.

Plants can respond to various stresses by inducing antioxidant defense enzymes that provide excellent protection against damage (Kasote et al., 2015). This concept can be due to ecological differences in plant growth such as climatic, edaphic, and genetic factors (Kavoosi and Rowshan, 2013). Climatic conditions influence the phytochemical properties of medicinal plants. Modeling nature is essential for the cultivation and domestication of medicinal plants. A relevant study aimed to compare and evaluate habitats in terms of phytochemical properties and identified the best growing conditions for plants (Kaghazloo et al., 2017). Identifying different habitats and assessing the impact of environmental factors on the appearance and function of metabolites in medicinal plants is a necessary step for domesticating and maintaining the genetic diversity of these plants (Yavari et al., 2010; Yavari and Shahgolzari, 2016).

Considering the characteristics of the place of growth and the position of the plant in nature, geographical settings comprise a major factor that can significantly affect the number of essential oils and active ingredients in plants. Studies have addressed the effects of habitat conditions on plant chemical composition and the correlations between them (Bertome et al., 2007). The impact of climatic conditions on different plants is different. Future studies need to address the role of climatic factors in the growth of medicinal plants and the production of effective substances. The most important environmental factors for developing medicinal plants that significantly impact the quantity and quality of their active ingredients are light, temperature, rainfall, day length, latitude, altitude, and nutrition. Environmental factors include climatic, topographic, and soil characteristics, each of which affects plant growth, development, yield, and amount of active ingredients (Somjen et al., 2004). Changes in ecological parameters in plant habitats play a vital role in the quantity, quality, and formation of active ingredients of medicinal plants. Achieving the highest yield with the best quality is an important factor determined by plant type, genetic characteristics, metabolite content, and habitat characteristics (Asadi-Samani et al., 2013). In the domestication and cultivation of wild plants, active ingredients may be reduced or not produced by plants in the new site of cultivation. Also, other main active ingredients may be produced that were not produced before (Hosseini et al., 2016). Therefore, the number of compounds may be less than the amount in their original habitats. Differences in the results obtained in different geographical locations can be due to the degradation and instability of anthocyanins. The stability of anthocyanins changes with temperature, oxygen, and light, which is subject to degradation by oxidizing enzymes (Jaiswal et al., 2009).

Conclusion

The results of the present study on diversity, according to the biochemical traits of different yellow flag ecotypes, indicated a good variety among the populations in terms of biochemical characteristics. More diversity was obtained in attributes such as chlorophyll b, antioxidant capacity, and carotenoids. The correlation analysis indicated that the most positive and significant correlations occurred between chlorophyll a - total chlorophyll, total chlorophyll - carotenoids, and phenol - flavonoid content. The existing correlations indicated that the factors that improved each could probably improve the associated characteristics. In cluster analysis, it was found that the populations were divided into three main groups. Different genes probably affected the expression of phenotypic characteristics, and more than one gene may be involved in their production. The expression of phenotypic traits was influenced by factors such as the environment, genotype, and their interactions. This concept indicates an excellent genetic potential among yellow flag populations in the north of Iran as one of its regions of diversity. Therefore, researchers in ornamental plant breeding can collect germplasm by identifying desirable compounds in this ornamental plant.

Conflict of Interest

The authors indicate no conflict of interest for this work.

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