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*Full Length Research Paper*

# Genetic relationships among some *Hesperis* L. (Brassicaceae) species from Turkey assessed by RAPD analysis

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**In this study the phylogenetic relations among infraspecific, specific and supraspecific categories of 6 taxa of the genus *Hesperis* collected from different parts of Turkey were investigated by RAPD analysis. The results of the RAPD analysis support the idea that *H. bicuspidata* (Sect. *Hesperis*), *H. schischkinii* (Sect. *Mediterranea*), *H. pendula* (Sect. *Pachycarpos*), *H. breviscapa*, *H. kotschyi* (Sect. *Cvelevia*) and *H. cappadocica* (Sect. *Contorta*) species need to be placed into different sections according to morphological characters. On the other hand, the phylogenetic order of the sections according to morphological characters and according to molecular data displayed some differences and evolutionary phylogenetic orders of the sections were redesigned. The phylogenetic relations among species were based on the samples *H. breviscapa* and *H. kotschyi* which take place in the same section. The accordance of morphological and molecular similarities was noticed for *H. breviscapa* and *H. kotschyi* species. Besides this, infraspecific taxonomic situations of *H. schischkinii* samples having hairy and glabrous (non-hairy) fruits which show allopatric and sympatric spread were reassessed by RAPD analysis.**

**Key words:** *Hesperis*, RAPD, polymorphism, Turkey.

## INTRODUCTION

The genus *Hesperis* L. (Brassicaceae) is distributed in the temperate-warm climate belt of Eurasia in south and central Europe, southwest Asia, Caucasia, Russia and mountainous regions of western China and Mongolia. This genus has 56 species throughout the world (Tzvelev, 1959; Cullen, 1965; Dvořák, 1980; Davis et al., 1988; Duran et al., 2002, 2003; Duran and Ocak, 2005). Most species in Anatolia are confined to rather restricted areas of distribution. On the other hand, those occurring in moist areas are more widespread, especially in the Euro-Siberian phytogeographic region. Busch (1939) considered the mediterranean area and central Asia as the center of origin of *Hesperis*. However, Dvořák (1973) believed that this genus originated in Anatolia.

Some species of genus *Hesperis* have been used for human benefit throughout history (Duran et al., 2003). For this reason, they were identified and defined by taxonomists at very early ages of human history. Morphological polymorphisms are common in the genus and discrimination of many taxa depends on characters that could be influenced by environmental conditions. But the descriptions of most of the species are generally very short and incomplete. By these descriptions it becomes very difficult or impossible to discriminate similar species (Duran et al., 2003).

In the genus key, as a morphological feature, hair characters were used mostly. Especially in some conditions hair character becomes the only factor in discrimination. But this morphological character could easily be influenced by the changing environmental conditions and appears as a modification type of variation. This type of variations might only be used to support the data obtained from cytogenetic, palynologic and molecular studies.

The development of PCR based molecular marker

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techniques has led to increase in the use of molecular marker technologies in many areas of biology, including systematic studies. In this respect randomly amplified polymorphic DNA (RAPD) technique has drawn much attention in a wide variety of organisms (Veilleux et al., 1995; Hawkins and Harris, 1998; Ergül et al., 2002; Aras, 2003; Aras et al., 2005). RAPD technique provides a simple and convenient method for the detection of polymorphism in the absence of sequence information with a relatively low cost.

In this study 6 different species of *Hesperis,* collected from different parts of Anatolia were studied with RAPD analysis that displays difficulties in discrimination according to their morphological characters. This comprises a preliminary study with molecular markers on *Hesperis* genus and focused on revealing the genetic distances and also defining genotypes of the specimens of 6 species used in the study. According to the results, the phylogenetic relations among infraspecific, specific and supraspecific categories of the 6 taxa, were reassessed. DNA was isolated from fresh and dry leaf tissue according to the availability of the material. For DNA isolation from dry leaf material a previously improved protocol for molecular marker studies was used (Aras et al., 2003). Using dry leaf material provided convenience by avoiding the time consuming and expensive field studies for collecting plant material from their localities.

## MATERIALS AND METHODS

### Plant material

Dry or fresh, leaf or flower (Table 2) tissues of *Hesperis* specimens were used. The 6 different species used in the study were; *H. bicuspidata* (Willd.) Poir., *H. breviscapa* Boiss., *H. kotschyi* Boiss., *H. pendula* DC. subsp. *campicarpa* (Boiss.) Dvořák, *H. schischkinii* Tzvel. and *H. cappadocica* Fourn. 10 samples from the 6 species used in RAPD assay were loaded onto the gels with respect to the order given in Table 1.

### DNA isolation

DNA was isolated from dry leaf material according to an improved protocol given by Aras et al. (2003). Briefly, the extraction protocol was as follows, dry leaf tissue (200 mg) was ground in liquid nitrogen in to a fine powder; 800 µl prewarmed extraction buffer was added to the samples and ground once more in the buffer. 4 µl proteinase K (10 mg/ml) was added to the samples in 1.5 ml eppendorf tube. The samples were incubated at 65°C for 30 min. After the incubation period, samples were cooled to room temperature and 250 µl 5 M potassium acetate was added and incubated on ice for 30 min. Samples were centrifuged at 17.000 g (14,000 rpm) for 15 min and the supernatant was collected to a fresh tube. Samples were extracted with 500 µl phenol : chloroform : isoamylalcohol (25:24:1) gently mixing by inversion 40 - 50 times. After centrifugation for 5 min at 17,000 g supernatant was transferred to a fresh tube and equal volume of isopropanol was added. Samples were ice incubated for 15 min. Then the samples were centrifuged for 5 min at 17,000 g and 76% ethanol was added to the pellet and spun once more for 10 min at 17,000 g. The pellet was washed with 70% ethanol optionally and air dried until all ethanol was removed. The obtained nucleic acid pellet was dissolved in an appropriate amount of TE buffer (30 - 60 µl). The nucleic acids dissolved in TE buffer were treated with ribonuclease (RNase; 10 mg/ml) and stored at -20°C until use. Concentrations of DNA samples were determined spectro-photometrically (Shimadzu UV-260) and also by running on 1% agarose gel with 100 bp DNA ladder (Fermentas) as DNA size marker.

The samples were isolated either from dry tissues or from fresh tissues (leaf or flower) as indicated in Table 2. The only sample that was available in the form of dry leaf tissue and also fresh flower tissue was from *H. schischkinii* species collected from Erzurum province (H1). In 7 PCR amplifications with different primers both samples were used and in duplicate RAPD assays exactly the same band patterns were obtained from the DNAs isolated either from dry or from fresh tissues (Figure 2, lane 1; fresh leaf tissue; lane 4; dry flower tissue). This result confirms that DNA yielded from dry tissues are also suitable for PCR amplification and do not cause any artifacts.

### RAPD assay

PCR reactions were performed as indicated elsewhere (Aras et al., 2005). The 24 primers selected from 48 tested primers were as listed in Table 3. In particular PCR reactions for RAPD analysis were performed in a 25 µl volume containing 200 ng genomic DNA, 2.5 µl 10 X reaction buffer, 3.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTPs, 200 ng primer, 0.5 unit Taq DNA polymerase (Promega). Amplification was performed in a Techne Progene thermal cycler (Techne Cambridge Ltd.) programmed for one cycle of an initial denaturation step at 94°C for 2 min, then subjected to 35 cycles of the following program: 94°C for 30 s, 33°C for 1 min, 72°C for 1 min 45 s. As a final extension step the temperature was held at 72°C for 8 min. Amplification products were electrophoresed in a 1.2% agarose gel containing 0.5 µg/ml ethidium bromide and visualized on UV transilluminator (Figures 1 and 2).

### Data analysis

RAPD assays were repeated twice for each primer and only clear, reproducible bands were scored with specific attention to evaluate the sharp bands, while the faint ones were ignored. RAPD data matrix was used in order to compute the genetic distances of the specimens according to Jaccard's coefficient. The MVSP software package version 3.1 (Kovach, 1999) was used to calculate Jaccard's (1908) similarity coefficients. According to these coefficients a dendogram was constructed by unweighted pair-group method of arithmetic average (UPGMA). Principle coordinate analysis (PCO) was also carried out to show multiple dimensions of the distribution of the genotypes in a scatter-plot.

## RESULTS

To analyze the genetic relationships of the specimens belonging to *Hesperis* genus, out of 48 primers tested, 24 primers which gave clear and reproducible bands were selected (Table 3). The RAPD profiles obtained by the primers OPA 11 and OPC 12 are shown in Figures 1 and 2, respectively. The primers yielded 315 reproducible amplification products of which 248 were polymorphic. In these study, each RAPD band was treated as a separate character and scored as 1 (present)/0(absent) and a rectan-

**Table 1.** Locations of the *Hesperis* samples ordered according to gel loading.

| | |
|---|---|
| **H1. *H. schischkinii* Tzvelev** | B8 Erzurum: Between Ilıca-Aşkale, Kandilli road junction, 1550 m, fallow field.<br>Collector & no.: A.Duran 6177<br>Date: 5.31.2003 |
| **H2. *H. cappadocica* Fourn.** | B6 Sivas: Between Zara-Divriği, 37. km, 1570 m, roadside, stony slopes.<br>Collector & no.: A.Duran 6120<br>Date: 5.29.2003 |
| **H3. *H. cappadocica* Fourn.** | A8 Erzurum: Erzurum-İspir, exit of Gelinkaya village, 1800 m, sandy slopes.<br>Collector & no.: A.Duran 6022.<br>Date: 7.27.2002 |
| **H4. *H. schischkinii* Tzvelev** | A7 Gümüşhane: Şiran-Tersum-Gümüşhane road, 8. km, 1450 m, fallow field.<br>Collector & no.: A.Duran 6192<br>Date: 6.1.2003 |
| **H5. *H. cappadocica* Fourn.** | A8 Gümüşhane: Gümüşhane-Tersum-Şiran road, 16. km, 1140 m, road side, slopes.<br>Collector & no.: A.Duran 6185.<br>Date: 6.1.2003 |
| **H6. *H. schischkinii* Tzvelev** | A7 Gümüşhane: Şiran-Tersum-Gümüşhane road, 8. km, 1450 m, fallow field.<br>Collector & no.: A.Duran 6191<br>Date: 6.1.2003 |
| **H7. *H. breviscapa* Boiss.** | B8 Erzurum: Aşkale-Bayburt road, 16. km, 1750 m, screes.<br>Collector & no.: A.Duran 6175<br>Date: 5.31.2003 |
| **H8. *H. Pendula* DC. subsp. *campicarpa* (Boiss.) Dvorak** | B7 Erzincan: Kemaliye, Taşyol, 1. km, inside the valley, 950 m, limestone rocky.<br>Collector & no.: A.Duran 6152<br>Date: 5.30.2003 |
| **H9. *H. bicuspidata* (Wild.) Poir.** | B6 Sivas: Between Yıldızeli-Akdağmadeni, 49. km, 1240 m, open *Quercus* forest.<br>Collector & no.: A.Duran 6119<br>Date: 5.29.2003 |
| **H10. *H. kotschyi* Boiss.** | B5 Kırşehir: Kervansaray mountain, Hasanpaşa Hill, around transmitter, south, 1650 m, 39º09.22'N, 34º17.51'E, limestone slopes.<br>Collector & no.: A.Duran 6193<br>Date: 6.6.2003 |

**Table 2.** The tissues which the DNA was isolated from *Hesperis* taxa.

| Sample | Tissue |
|---|---|
| 1. *H. schischkinii* | Fresh flower tissue |
| 2. *H. cappadocica* | Dry flower tissue |
| 3. *H. bicuspidata* | Dry leaf tissue |
| 4. *H. schischkinii* | Fresh flower tissue |
| 5. *H. cappadocica* | Fresh leaf tissue |
| 6. *H. schischkinii* | Fresh leaf tissue |
| 7. *H. breviscapa* | Dry flower tissue |
| 8. *H. Pendula* subsp. *campicarpa* | Dry leaf tissue |
| 9. *H. cappadocica* | Fresh leaf tissue |
| 10. *H. kotschyi* | Fresh leaf tissue |

gular binary data matrix was obtained. A similarity matrix was generated by using Jaccard's coefficient (Jaccard, 1908) (Table 4) and converted into distances. The distance matrix was then used in cluster analysis and a dendogram was constructed using UPGMA procedure (Figure 3). According to the similarity index (Table 4) and the cluster analysis (Figure 3), the lowest similarity was found between *H. cappadocica* (H2) and *H. kotschyi* (H10) with the value of 0.423. On the other hand *H. schischkinii* specimens (H4) and (H6) gave the highest similarity ratio with 0.934. In the dendogram, *Hesperis* specimens formed 2 main clusters. *H. bicuspidata* (H9), *H. pendula* subsp. *campicarpa* (H8), *H. kotschyi* (H10) and *H. breviscapa* (H7) formed a group under the first branch of the dendogram. The second branch of the dendogram was formed by 3 samples of *H. cappadocica* (H2, H3 and H5) and 3 samples of *H. schischkinii* (H6, H4 and H1). The genetic similarity ratios, among the different *Hesperis* species and within the same species collected from different localities, varied from 42.3 to 66.9% (except for the samples H4 and H6). According to the results, it may be inferred

**Table 3.** The sequences of the random primers used in the study and total and polymorphic band numbers.

| Primer | Sequence (5'-3') | Total amplified band number | Polymorphic bands | Percent polymorphism (%) |
|---|---|---|---|---|
| OPA-04 | AATCGGGCTG | 15 | 11 | 73.33 |
| OPA-05 | AGGGGTCTTG | 12 | 9 | 75 |
| OPA-06 | GGTCCCTGAC | 13 | 12 | 92.30 |
| OPA-07 | GAAACGGGTG | 10 | 9 | 90 |
| OPA-09 | GGGTAACGCC | 12 | 8 | 66.66 |
| OPA-10 | GTGATCGCAG | 12 | 9 | 75 |
| OPA-11 | CAATCGCCGT | 6 | 5 | 83.33 |
| OPA-13 | CAGCACCCAC | 12 | 10 | 83.33 |
| OPA-15 | TTCCGAACCC | 10 | 6 | 60 |
| OPA-17 | GACCGCTTGT | 21 | 19 | 90.47 |
| OPA-18 | AGGTGACCGT | 25 | 21 | 84 |
| OPC-12 | TGTCATCCCC | 10 | 9 | 90 |
| OPO-04 | AAGTCCGCTC | 8 | 4 | 50 |
| OPO-07 | CAGCACTGAC | 19 | 16 | 84.21 |
| OPO-10 | TGTCTGGGTG | 13 | 10 | 76.92 |
| OPO-19 | GGTGCACGTT | 21 | 19 | 90.47 |
| BC-302 | CGGCCCACGT | 13 | 12 | 92.30 |
| BC-340 | GTTGCCAGCC | 8 | 4 | 50 |
| BC-374 | GGTCAACCCT | 10 | 6 | 60 |
| B-389 | CGCCCGCAGT | 19 | 17 | 89.47 |
| B-379 | GGGCTAGGGT | 12 | 10 | 83.33 |
| UBC-238 | CGGATCGACA | 11 | 8 | 72.72 |
| UBC-251 | CTTGACGGGG | 12 | 9 | 75 |
| OD-08 | GACGGATCAG | 11 | 5 | 45.45 |
| **Total** | | **315** | **248** | **78.73** |

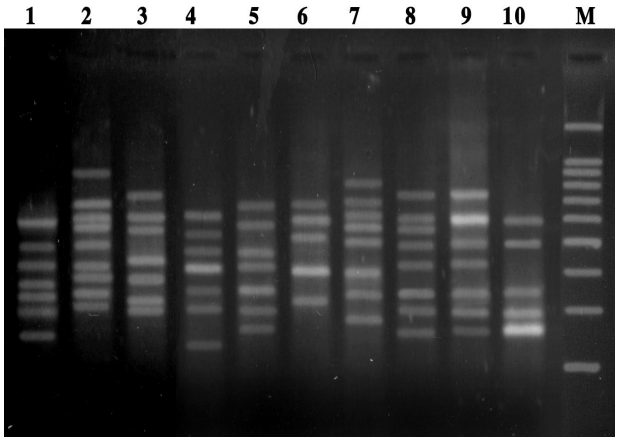


**Figure 1.** RAPD patterns by the primer OPA 11 (5' CAATCGCCGT 3'). Lane M, Molecular weight marker (100 bp. ladder); lane 1, *Hesperis schischkinii* (A.Duran 6177), lane 2, *H. cappadocica* (A.Duran 6120); lane 3, *H. cappadocica* (A.Duran 6022); lane 4, *H. schischkinii* (A.Duran 6192); lane 5, *H. cappadocica* (A.Duran 6185); lane 6, *H. schischkinii* (A.Duran 6191); lane 7, *H. breviscapa* (A.Duran 6175); lane 8, *H. pendula* subsp. *campicarpa* (A.Duran 6152); lane 9, *H. bicuspidata* (A.Duran 6119); lane 10, *H. kotschyi* (A.Duran 6193).

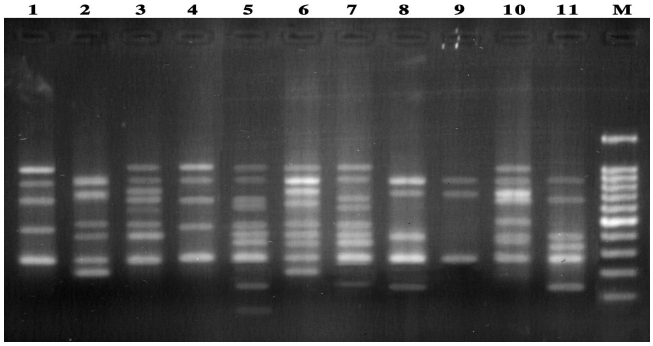


**Figure 2.** Amplification of DNA for RAPD analysis by primer OPC 12 (5' TGTCATCCCC 3'). Lane M, Molecular weight marker (100 bp. ladder); lane 1, *Hesperis schischkinii* (A.Duran 6177); lane 2, *H. cappadocica* (A.Duran 6120); lane 3, *H. cappadocica* (A.Duran 6022); lane 4, *H. schischkinii* (A.Duran 6177); lane 5, *H. schischkinii* (A.Duran 6192); lane 6, *H. cappadocica* (A.Duran 6185); lane 7, *H. schischkinii* (A.Duran 6191); lane 8, *H. breviscapa* (A.Duran 6175); lane 9, *H. pendula* subsp. *campicarpa* (A.Duran 6152); lane 10, *H. bicuspidata* (A.Duran 6119); lane 11, *H. kotschyi* (A.Duran 6193). (H1 and H4 samples are the same samples; H1 is fresh leaf tissue and H4 is dry flower tissue. Both samples yielded the same band patterns implying the high quality of the DNAs from dry tissues).

**Table 4.** Similarity index (Jaccard's coefficient) of the tested accessions.

| | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 |
|---|---|---|---|---|---|---|---|---|---|---|
| H1 | 1.000 | | | | | | | | | |
| H2 | 0.504 | 1.000 | | | | | | | | |
| H3 | 0.536 | 0.597 | 1.000 | | | | | | | |
| H4 | 0.699 | 0.506 | 0.507 | 1.000 | | | | | | |
| H5 | 0.558 | 0.625 | 0.571 | 0.591 | 1.000 | | | | | |
| H6 | 0.699 | 0.520 | 0.507 | 0.934 | 0.605 | 1.000 | | | | |
| H7 | 0.516 | 0.467 | 0.498 | 0.500 | 0.542 | 0.500 | 1.000 | | | |
| H8 | 0.484 | 0.483 | 0.536 | 0.471 | 0.477 | 0.464 | 0.519 | 1.000 | | |
| H9 | 0.480 | 0.558 | 0.524 | 0.487 | 0.588 | 0.494 | 0.511 | 0.589 | 1.000 | |
| H10 | 0.434 | 0.423 | 0.476 | 0.472 | 0.478 | 0.458 | 0.470 | 0.473 | 0.505 | 1.000 |

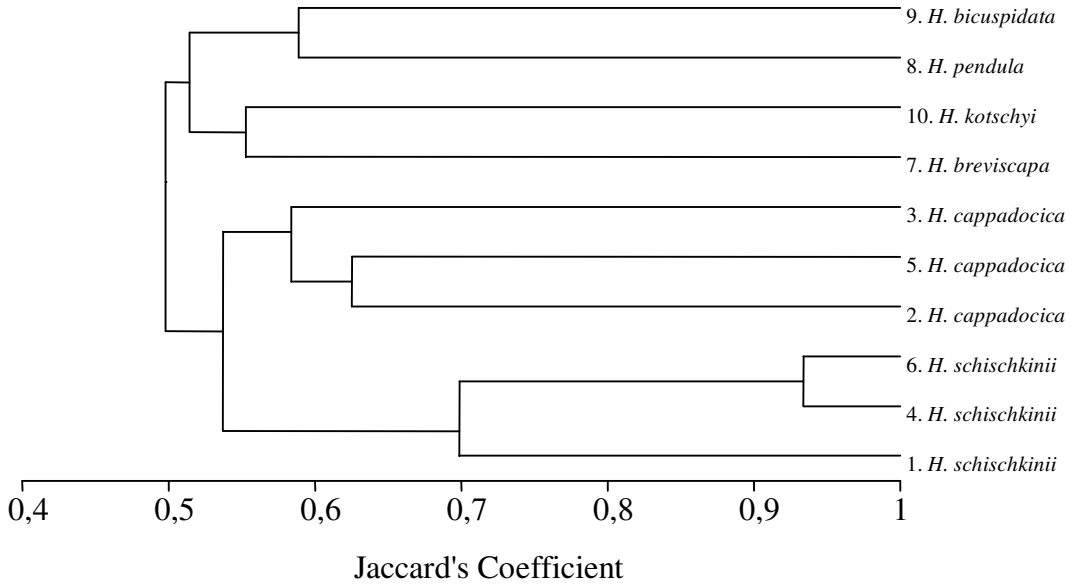


**Figure 3.** Dendogram (UPGMA) showing the genetic relationships among 10 samples from 6 species of *Hesperis* genus.

that the genetic similarities between the specimens of different *Hesperis* species were not high.

In order to determine the ability of the RAPD analysis to display genetic relationships among samples with a minimum distortion, principle co-ordinate analysis (PCO) was carried out (Figure 4). The samples were plotted into a co-ordinate system for the first 3 co-ordinates which accounted for 25.0, 16.5 and 12.1% of the total variation of 53.7%. *H. schischkinii* and *H. cappadocica* samples that were grouped in the dendogram also showed good discrimination with PCO analysis. The 3 samples of *H. schischkinii* (H1, H4 and H6) were grouped on the upper right quarter of the plot and two of the *H. schischkinii* samples (H4 and H6) were placed very close to each other. The 3 samples belonging to *H. cappadocica* (H2, H3 and H5) species formed a set on the lower left quarter of the plot. On the upper left quarter of the plot, the samples from *H. kotschyi* (H10) and *H. breviscapa* (H7) were placed relatively close to each other.

## DISCUSSION

In this study, the phylogenetic relations among infraspecific, specific and supraspecific categories of 6 species of the genus *Hesperis* were investigated by RAPD analysis. All species except *H. breviscapa* and *H. kot-schyi*, take part in different sections. These species and sections are as follows: *H. bicuspidata* is in Sect. *Hes-peris*, *H. schischkinii* in Sect. Mediterranea (Borbás) *A. Duran*, *H. pendula* subsp. *campicarpa* in Sect. *pachycarpos* Fourn., *H. breviscapa* and *H. kotschyi* in Sect. *cvelevia* Dvořák and *H. cappadocica* in Sect. *contorta* (Dvořák) A. Duran.

Results obtained from the RAPD analysis, confirmed

**Table 5.** The old and the new phylogenetic ordering of the sections.

| S/N | Old phylogenetic order | New phylogenetic order |
|---|---|---|
| 1 | Sect. *Cvelevia* | Sect. *Cvelevia* |
| 2 | Sect. *Hesperis* | Sect. *Hesperis* |
| 3 | Sect. *Mediterranea* | Sect. *Pachycarpos* |
| 4 | Sect. *Pachycarpos* | Sect. *Mediterranea* |
| 5 | Sect. *Contorta* | Sect. *Contorta* |

the placement of *H. bicuspidata* (Sect. *Hesperis*), *H. schischkinii* (Sect. *Mediterranea*), *H. pendula* (Sect. *Pachycapos*), *H. breviscapa*, *H. kotschyi* (Sect. *Cvelevia*) and *H. cappadocica* (Sect. *Contorta*) species to different sections according to morphological characters. On the other hand, some differences were noted between the phylogenetic order based on morphological characters and on molecular data. Especially, group formation of Sect. *Cvelevia* (*H. breviscapa*, *H. kotschyi*), Sect. *Hesperis* (*H. bicuspidata*) and Sect. *Pachycarpos* (*H. pendula*) taxa with-in the first branch of the dendogram, was an unexpected result considering fruit character. Although fruits of Sect. *Cvelevia* and Sect. *Hesperis* taxa show erect position, fruits of Sect. *pachycarpos* taxa display pendulose position. A similar situation was noted also for the sections of Sect. mediterranean (*H. schischkinii*) and Sect. *contorta* (*H. cappadocica*) taxa.

While fruits of Sect. Mediterranea taxa are found in erect position, fruits of the species from Sect. *contorta* are in pendulose position. The results of molecular analysis suggest that a linear phylogenetic relation does not exist between the lines of evolutionary development of the taxa and their fruit positions (erect or pendulosa). The evolutionary phylogenetic orders of the sections were redesigned according to the results of the molecular analysis and morphological characters of the taxa. But this new ordering was established without considering fruit positions (Table 5).

*H. breviscapa* and *H. kotschyi* species take part in Sect. *Cvelevia*. The relations between species were based on *H. breviscapa* and *H. kotschyi* samples. The 2 species formed a dual group within the dendogram so the results suggest that their morphological similarities are in accord with the findings obtained from molecular data.

*H. breviscapa* clearly differs from other *Hesperis* species in its habitus, leaf, bracts and fruit and seed characteristics. According to its fruit characteristics this species is closer to *H. kotschyi* species, but with the presence of bracts it could be easily distinguished from *H. kotschyi*. On the other hand with its fruit characteristics *H. kotschyi* resembles *H. breviscapa*, and with its habitus and leaf characteristics it resembles *H. armena* Boiss. and *H. pisidica* Hub.-Mor. *H. kotschyi* which grows especially within marble and limestone cracks and on rocky areas on the north side of the mountains. This species is distributed locally on the similar habitats at Kütahya and Kırşehir provinces except Toros mountains. Although *H. breviscapa* and *H. armena* have bracts, *H. kotschyi* does not have this characteristic. When bracts are considered from taxonomic perspective as a secondary degree character, *H. breviscapa* and *H. kotschyi* species could be regarded as more closely related species (Duran et al., 2003).

An interesting point in the dendogram is the high similarity ratio (0.934) of the *H. schischkinii* samples collected from Gümüşhane province, from the same locality. These 2 samples located just few meters apart from each other and were different in their fruit hair character. H4 sample had hairy fruit and H6 sample had glabrous fruit. One more *H. schischkinii* sample (fruits hairy) collected from around Erzurum (H1) area yielded a ratio of 0.699 with both of the other *H. schischkinii* samples that forms the second highest similarity ratio.

The 3 of the *H. schischkinii* samples formed a subgroup within the second branch of the dendogram. In the morphological revision of the *Hesperis* genus which was completed in 2003, the *H. schischkinii* species was separated into 2 varieties according to its fruit hair character (Duran et al., 2003). But the data from this study do not support recognition of any taxa within *H. shischkinii*. The result obtained by RAPD profiles of the two samples of *H. schischkinii* (H4 and H6) suggests that they are the same taxa and it is unnecessary to separate the varieties of the *H. schischkinii.* Infraspecific taxonomic situations of samples of *H. schischkinii* taxa with hairy and glabrous (non-hairy) fruits were evaluated according to results of RAPD analysis. Results suggest that for samples from *H. schischkinii* taxa, having allopatric and sympatric spread, fruit hair characteristics are not reliable for their infraspecific distinction.

The two *H. cappadocica* samples collected from Sivas (H2) and Gümüşhane (H5) provinces also formed a sister group with the similarity ratio of 0.625. The other *H. cappadocica* sample from Erzurum (H3), displayed a similarity ratio of 0.597 with the sample from Sivas (H2) and 0.571 with the sample from Gümüşhane (H5). With these relatively high similarity ratios, three *H. cappadocica* samples have formed a subgroup within the second branch of the dendogram. *H. cappadocica* species placed apart from the other species of the genus *Hesperis* with full flattenation of its fruit from dorsal, also with its contorted, wide appearance and toothed sides. With respect to its fruit characteristics, *H. cappadocica* is isolated from other species of the genus. Also, fruit hair variations do not compose a significant distinction within population samples of *H. cappadocica* species which has allopatric spread.

Although representatives of the genus *Hesperis* were included in some recent studies of molecular phylogeny of Brassicaceae (Baley et al., 2006; Beilstein et al., 2006) the current study constitutes the first data about the genetic relationships with molecular markers specially focusing

on the genus *Hesperis*. The RAPD technique has been shown to be adequate for the discrimination of the different species of the genus *Hesperis*.

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