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TAXONOMIC SIGNIFICANCE OF POLLEN AND SEED MICROMORPHOLOGY IN THE GENUS HESPERIS L. (BRASSICACEAE)

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Genus *Hesperis* L. belongs to Brassicaceae (Cruciferae) family including 46 species in the world, of which seven taxa are distributed in Iran. Pollen and seed macro- and micromorphological features of the Iranian taxa belonging to three sections, including sects. *Hesperis*, *Diaplictos* and *Pachycarpos* were examined for the first time using light (LM) and scanning electron microscope (SEM). Data analyses were performed using PAST (ver. 3.14) software. The results showed that in *Hesperis* pollen grains are radially symmetrical, monad, isopolar, tricolpate and have a small size. Pollen grain shape is elliptic to circular in equatorial view and circular in polar view. The exine ornamentation is reticulate in all studied specimens. Seed sculpturing pattern revealed two main groups based on the presence or absence of papillae. This study clarified that both pollen and seed macro- and micromorphological characters are significant features in taxonomic classification of the genus *Hesperis*. Unweighted Pair Group Method with arithmetic mean (UPGMA) and Principle Component Analysis (PCA), strongly supported the sectional delimitation of taxa for most characters but not for seed qualitative characters. The results are mostly in agreement with the previous studies.

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Key words: Brassicaceae; *Hesperis*; microsculpturing; pollen; seed; Iran

اهمیّت تاکسونومیک ریزریخت‌شناسی دانه گرده و بذر سرده .Hesperis L (تیره شب بوئیان)

آتنا اسلامی فاروجی: دانشجوی دکتری سیستماتیک گیاهی گروه زیست‌شناسی دانشگاه لرستان

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سرده .Hesperis L متعلّق به تیره شب‌بوئیان (Cruciferae) است و شامل ۴۶ گونه در جهان می‌باشد، از این میان هفت تاکسون آن در ایران پراکنش دارد. صفات ریخت‌شناسی و ریزریخت‌شناسی دانه گرده و دانه تاکسون‌های ایرانی Hesperis متعلّق به سه بخش Hesperis، Diaplictos و Pachycarpos با استفاده از میکروسکوپ نوری (LM) و میکروسکوپ الکترونی نگاره (SEM) برای اوّلین بار مطالعه شد. تحلیل داده‌ها با استفاده از نرم‌افزار PAST (نسخه ۳/۱۴) انجام شد. نتایج نشان داد که دانه‌های گرده Hesperis متقارن شعاعی، موناد، جورقطب، سه شیاری و دارای اندازه کوچک هستند. شکل دانه گرده در نمای استوایی بیضوی تا مدور و در نمای قطبی مدور است. تزئینات اگزین در تمام نمونه‌های مورد مطالعه مشبک است. الگوی تزئینات دانه دو گروه اصلی را بر اساس حضور و عدم حضور زگیل آشکار نمود. این مطالعه روشن نمود که صفات ریختی و ریزریخت‌شناسی دانه گرده و دانه صفات مهمّی در طبقه‌بندی تاکسونومیک سرده Hesperis هستند زیرا تحلیل جفت گروه بدون وزن با میانگین حسابی (UPGMA) و تجزیه مؤلفه اصلی (PCA) به جز در صفات کیفی دانه به شدّت از تعیین حدود بخش‌ها حمایت می‌کند. نتایج عمدتاً با مطالعات پیشین انجام شده مطابقت دارد.

INTRODUCTION

The genus *Hesperis* L. (Brassicaceae) comprises biennial and perennial herbs (Duran & al. 2003) and consists of 46 species worldwide (Al-Shehbaz & al. 2006), mainly occurring in different parts of Europe, Caucasus, Transcaucasia, and to a lesser extent in northern and central Asia, and mostly in Turkey with 28 species (Duran 2008; Aras & al. 2009). The genus is represented by 11 (Dvořák 1968) or six species (Assadi & al. 2017) belonging to sections *Hesperis* Dvořák, *Diaplictos* Dvořák and *Pachycarpos* Fourn. in Iran. The first subgeneric (Dvořák 1973) and sectional (Andrzejowski 1821) studies of *Hesperis* were evaluated from morphological, cytological, and palynological characters, but still taxonomists try to introduce new infrageneric classifications (Duran & al. 2003; Duran, 2016).

The importance of pollen and seed morphology of *Hesperis* was designated by Duran and Ocak (2005), Duran (2009), Pinar & al. (2009), Duran & al. (2011), Duran (2016), Padure & al. (2016) for taxonomic studies. However, the pollen and seed micromorphological characters of the genus *Hesperis* has not been studied on Iranian taxa.

Molecular phylogenetic studies of *Hesperis* have not been yet carried out on a worldwide scale. But in the remaining members of family Brassicaceae, traditional classification systems mostly contradict the modern biosystematics studies due to parallelism and convergence of morphological characters. As a result, the true position of each species will be difficult to understand (Franzke & al. 2011). Despite this fact, previous micromorphological studies such as pollen data demonstrate fascinating perspectives into separating closely related genera and revealed systematic relationships among various taxa of this family (Barthlott 1981, 1984; Bona 2013; Keshavarzi & Esfandani –Bozchaloyi 2014a, 2014b; Bouman 1975; Rollins 1979; Al-Shehbaz 1989; İnceoğlu & Karamustafa 1977; Doğan & İnceoğlu 1990; Brochmann 1992; Buth & Roshan Ara 1987; Koul & al. 2000). The seed studies not only performed on the genus *Hesperis* by recent projects in Turkey and Europe (Duran and Ocak, 2005; Duran, 2009; Pinar & al. 2009; Duran & al. 2011; Padure & al. 2016), but also carried on the other genera especially on *Thlaspi* L. (Meyer, 1973, 1979, 1991). Meyer defined 12 segregates for this taxon, but few of them were accepted by researchers (Mummenhoff and Koch 1994; Zunk & al. 1996; Mummenhoff & al 1997a, b; Esfandani –Bozchaloyi & Keshavarzi 2014; Koch & Mummenhoff 2001). Moreover, Seed surface studies can clarify the natural selection and adaptation processes (Pinar & al. 2009). As a result, it can resolve the taxonomic problems of Brassicaceae at generic and specific levels (Koul & al. 2000). Not only ultrastructural studies considered as a useful instrument to solve taxonomic problems in Brassicaceae (Barthlott 1981; Bouman 1975; Buth & Roshan Ara 1987; Koul & al. 2000; Murley 1951; Vaughan & Whitehouse 1971), but also it recognized as a powerful tool in tribe delimitation since tribe Physarieae was distinguished from the remaining of this family via the presence of more than three colpi in pollen grain (Al-Shehbaz & al. 2006). Seed-coat studies revealed its significance when *Thlaspi* and *Noccaea* were considered as two distinct genera (Meyer 1973, 1979), and Al-Shehbaz & al. (2006) re-evaluated them as belonging to two different tribes.

After Turkey, Iran is the second country in terms of *Hesperis* diversity (Assadi & al. 2017) and despite several infrageneric and infraspecific studies of *Hesperis*, its taxonomic problems have not been resolved yet (Eslami Farouji & al. 2018, unpublished). As pointed out in previous studies (Abdel-Khalik 2002; Bolurian 2009; Dvořák 1965, 1966; Perveen & al. 2004; Pinar & al. 2009 and etc.), the pollen and seed of many *Hesperis* species have been studied by several researchers up to now except the Iranian ones. The micromorphological study of the genus *Hesperis* performed by Duran and Ocak (2005), Duran (2008), Pinar & al. (2009), and Duran & al. (2011) in Turkey. The main aim of the present survey is to estimate the taxonomic value of pollen grain and seed-coat quantitative and qualitative characters in delimitation of Iranian *Hesperis* species, using light and scanning electron microscopy.

MATERIALS AND METHODS

Pollen and seed materials of the present study are provided by different specimens belonging to wild populations, and herbarium materials of Research Institute of Forests and Rangelands (TARI) and Lorestan University herbarium (table 1). The voucher specimens are preserved in TARI. The geographical distribution map is demonstrated in fig. 1.

Seven taxa of Iranian *Hesperis* seed and pollen grain were used in the present survey using light and scanning electron microscope. Therefore, unripe samples were removed from all analyses. In order to examine the stability of seed and pollen grain features, ten accessions were selected per species. Light microscopy (LM) studies performed by acetolysing the pollen grains using Harley method (1992). Moreover, observations carried out using an Olympus BH-2 microscope and pictured by Sony Cyber-shot camera model DSC-WX80.

Table 1. Iranian *Hesperis* species used in the seed and pollen grain study.

Taxa	Locality and collection data
H. hyrcana Bornm. & Gauba	Iran: Azarbayejan, Arasbaran protected region, Veinagh to Ghaghalu, 1000m, 38°54'28"N, 46°51'54"E, 11.07.1976, Assadi and Massoumi, 20521 (TARI); Gilan, Foumen to Masulleh, 200-300m, 37°12'27"N, 49°09'15"E, 18.07.1975, Wendelbo and Assadi, 18563 (TARI); Mazandaran, South of Ramsar, 7 km on road to Javaherdeh, 300m, 36°55'36"N, 50°38'35"E, 27.06.1976, Runemark and Massoumi, 20671 (TARI).
H. luristanica F. Dvořák	Iran: Lorestan, after Nojian, Wark waterfall, 1423m, 33°13'51"N, 48°34'33"E, 02.05.2005, Mehrnia and Karimi, 6493 (Loresten University Herbarium).
H. nivalis Boiss. & Hausskn.	Iran: Chaharmahal Bakhtiari, Shahr-e kord Baba Heydar-Sefid daneh, 2380m, 32°19'47"N, 50°28'15"E, 21.05.1986, Mozaffarian, 54356 (TARI); Hamadan, Avaj, 1600 m, 35°34'45"N, 49°13'27"E, 13.05.1974, Dini and Bazargan, 8573 (TARI).
H. odorata F. Dvořák	Iran: Kermanshah, Parrou Mountain, 1700-2100 m, 34°25'05"N, 47°14'34"E, 17.05.1975, Wendelbo and Assadi, 16732 (TARI); Kordestan, Dasht-e Zaghe on road from Hamadan to Sanandaj 40 km. East of Sanandaj, 2000m, 35°19'18"N, 46°59'10"E, 11.05.1975, Wendelbo and Assadi, 16900 (TARI).
H. persica Boiss. subsp. *persica*	Iran: Azarbayejan, 25 km SE of Jolfa, Kiamaki Protected area, Gheshlagh village, Ghelenj mountain (NH3), 2100-2700m, 38°48'19"N, 45°48'32"E, 19.06.1988, Assadi and Shahsavari, 65716 (TARI); Chaharmahal Bakhtiari, Shahr-e Kurd, tang-e Sayyad protected area, Pir kuh mountain, 2500m, 32°19'41"N, 50°52'36"E, 13.05.1987, Mozaffarian, 59845 (TARI); Fars, S. of Estahbanat, kuh-e Bah (BT2), 1700-2200 m, 29°07'44"N, 54°02'08"E, 07.06.1983, Mozaffarian, 47024 (TARI). Khorasan-e Shomali, ca. 45 km N. of Shirvan, Golool Sarani Protected area (EG3), 1600-2300m, 37°24'33"N, 57°55'43"E, 26.05.1984, Assadi and Massoumi, 50467 (TARI). Kohgiluye and Boirahmad, Yasuj, Sisakht, Bijan neck, 2700-3200m, 30°51'36"N, 51°27'16"E, 23.06.1998, Mozaffarian and Massoumi, 77981 (TARI); Semnan, ca. 50 km N of Semnan, between Sheli and Hikuh villages (YV1), 2400 m, 35°34'43"N, 53°22'47"E, 28.07.1982, Assadi and Mozaffarian, 40593 (TARI); Tehran, SW of Kalan Lavasan, 2200 m, 35°49'13"N, 51°37'31"E, 25.06.1973, Arazm and Bazargan, 6822 (TARI).
H. persica subsp. *kurdica* (F. Dvořák & Hadac) F. Dvořák	Iran: Azarbayejan, Khoy, Belahzuk, 1160m, 38°33'12"N, 44°56'22"E, 06.06.1974, Siami, 956 (TARI); Fars, 25 km S. E. of Fasa, Salou village, kuh-e Raz (YN4), 1600-2200 m, 28°56'38"N, 53°38'02"E, 05.06.1983, Mozaffarian, 46808 (TARI); Kordestan, 61 km from Marivan on road to Paveh (between Daraki and Nowsud), 2500m, 35°02'34"N, 46°21'18"E, 31.05.1978, Runemark and Mozaffarian, 27408 (TARI); Mazandaran, 30 km S. of Ramsar between Kash-e Chal mountain and Miankuh (VA4), 3100m, 36°04'57"N, 50°29'48"E, 12.07.1984, Assadi and Massoumi, 51242 (TARI); Tehran, between Karaj and Chalus, Kandavan, 2600-3050 m, 37°47'41"N, 46°14'55"E, 23.06.1979, Assadi and Mozaffarian, 32871 (TARI); Zanjan, From Zanjan to Mahneshan, 12 km after andabad 2200 m, 36°45'52"N, 48°05'38"E, 24.05.1987, Massoumi, 64795 (TARI).
H. straussii Bornm.	Iran: Chaharmahal Bakhtiari, Lordegan, Rig mountain from South of Rig village, 2295 m, 31°22'57"N, 51°00'11"E, 03.06.2009, Mozaffarian, 97118 (TARI); Kermanshah, Kuh-e Bimar near Hukani village, Kerend, 1500 m, 34°16'50"N, 46°14'70"E, 08.05.1975, Wendelbo and Assadi, 16754 (TARI); Kohgilouye-Boirahmad, Fahlian, 19 km, Yasuj road, 1800m, E, 30°40'06"N, 51°35'15"E, 26.06.1973, Rowshan, 9357 (TARI).

In order to perform Scanning Electron Microscope (SEM) studies, the surface of mature seeds and pollen grains were cleaned using different concentrations of alcohol and formaldehyde-acetic acid. Solid and dried specimens were directly organized on an aluminum stub with an adhesive tape and coated with gold for 30 min in a sputtering chamber (Sputter coater) at the voltage of 15 kV. The micrographs photographed by scanning electron microscope model MIRA3-LMU (Czech Republic, TESCAN Company) with different

magnifications. The pollen terminology follows the recommendations of Erdtman (1952), Faegri and Iversen (1975), Brochmann (1992) and Punt & al. (2007). Additionally, seed studies follow Murley (1951) and Koul & al. (2000) methods.

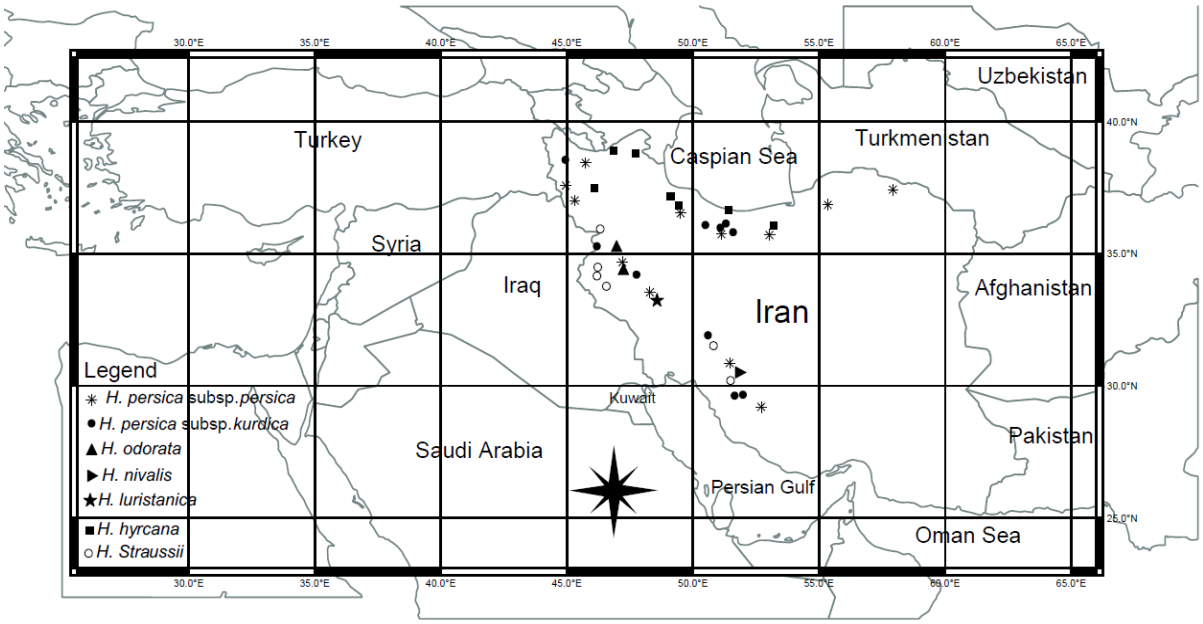

Fig. 1. Geographical distribution map of *Hesperis* species in Iran. Shapes are defined as *H. persica* subsp. *persica* (∗); *H. persica* subsp. *kurdica* (●); *H. odorata* (▲); *H. nivalis* (►); *H. luristanica* (⋆); *H. hyrcana* (■); *H. straussii* (○).

Iranian *Hesperis* specimens considered as Operational Taxonomic Units (OTUs) in order to carry out taxonomic numerical analyses using standardized mean variables. Generally, 14 quantitative and 11 qualitative macro- and micromorphological features were carefully listed and evaluated (table 2-3). Eventually, UPGMA and PCA multivariate analyses of different populations of Iranian *Hesperis* species constructed using PAST (ver. 3.14, Hammer and Harper 2006) with Gower coefficient (1971).

RESULTS

The results obtained from light microscope, SEM studies and statistical numerical analyses well-defined the pollen and seed characters of Iranian *Hesperis* specimens.

General features of pollen grains

The main pollen grain features of the studied specimens are summarized and illustrated in figs. 2-3, and table 2.

The Iranian *Hesperis* pollen grains are radially symmetrical, monad, isopolar and tricolpate. The outline is elliptic to circular in equatorial view and circular in polar view (figs. 2-3). The shape of pollen grains ranges from prolate-spheroidal (0.97-1.15) to subprolate (1.07-1.16). The minimum and maximum polar axis (P) varies from 16.7 µm (*H. straussii* Bornm.) to 25.8 µm (*H. persica* Boiss. subsp. *persica*). Furthermore, the minimum and maximum equatorial axis (E) presented in *H. straussii* (15.8 µm) and *H. persica* subsp. *persica* (21.8 µm), respectively.

Based upon Erdtman terminology (1952), Iranian *Hesperis* pollen grains are small in size (<25 µm). Among the studied populations, the largest and the smallest pollen grains are belonging to *H. nivalis* Boiss. and Hausskn. and *H. straussii*. The ratio of the distance between the apices of two ectocolpi (d/D ratio or average of apocolpium index) (Punt & al. 2007) estimated. *H. nivalis* (0.48 µm) and *H. hyrcana* Bornm. & Gauba (0.35 µm) has the largest and the lowest apocolpium indices. The mesocolpium thickness varies from 14.45 µm in *H. nivalis* to 10.9 µm in *H. straussii*. The maximum and the minimum ratio of colpus length to polar axis were 0.98 µm in *H. nivalis* and 0.92 µm in *H. straussii*.

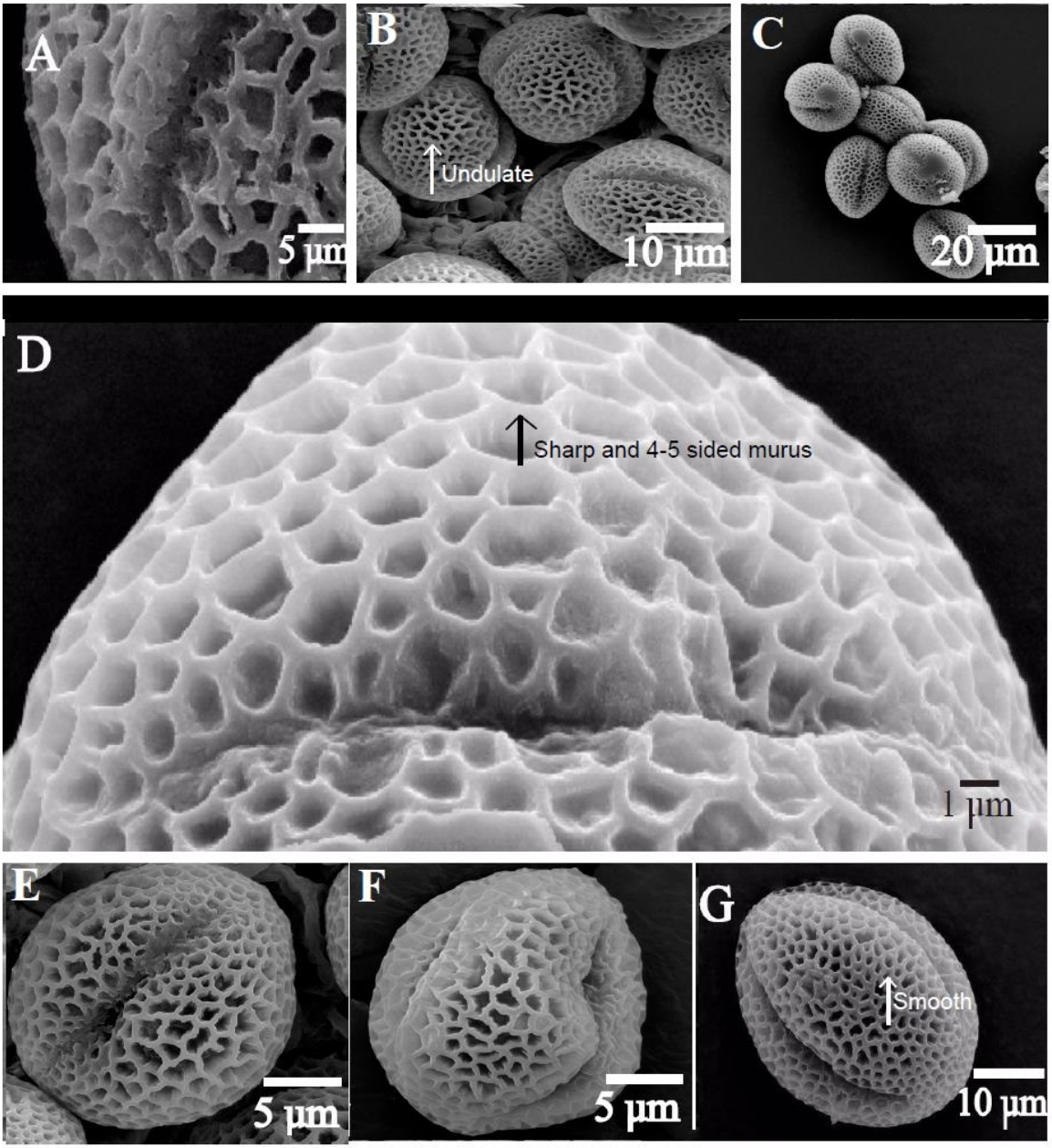

Fig. 2. Pollen grain surface of Iranian *Hesperis* species. A, *H. persica* subsp. *kurdica*; B-C, general view of pollen grains; B, *H. persica* subsp. *persica*; C, *H. luristanica*; D, *H. nivalis*; E, *H. odorata*; F, *H. hyrcana*; G, *H. straussii*; A-B, species of Sect. *Pachycarpos*; C-E, species of Sect. *Diaplictos*; F-G, species of Sect. *Hesperis.*

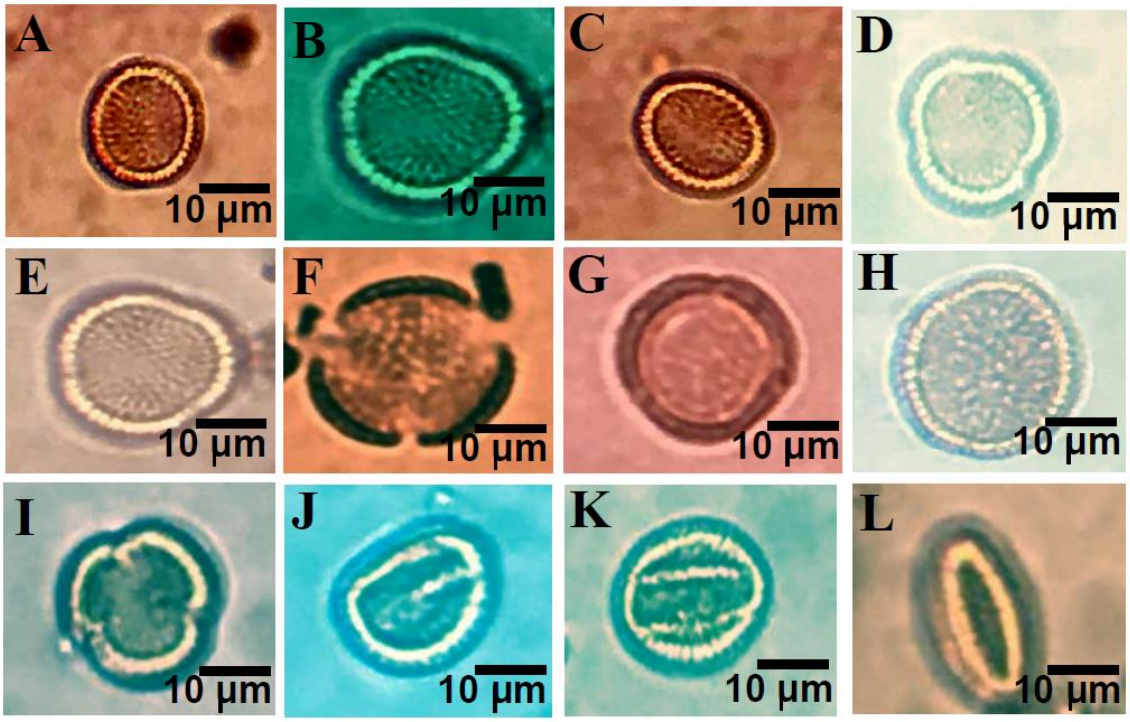

Fig. 3. The LM micrographs of Iranian *Hesperis* pollen grains in the genus *Hesperis* L. A-B, *H. persica* subsp. *kurdica*; C-D, *H. persica* subsp. *persica*; E, *H. luristanica*; F-G, *H. nivalis*; H, *H. odorata*; I-J, *H. hyrcana*; K-L, *H. straussii*; A-I, Polar view; J-L, equatorial view. A-D, Sect. *Pachycarpos*; E-H, Sect. *Diaplictos*; I-L, Sect. *Hesperis*.

Among the studied species, protruded apertures were absent. Based upon measurement of exine thickness, *H. nivalis* (1.71 µm) and *H. straussii* (1.21 µm) demonstrated the maximum and minimum thickness along the polar and equatorial axes.

Microsculpturing features of exine

Reticulate ornamentation observed among all Iranian *Hesperis* species, but they were different in shape, lumen and murus width. The lumen and murus width varies from 1.23- 2.3 to 0.3-0.69 µm. *Hesperis nivalis* demonstrated the highest lumen width, while *H. persica* subsp. *persica* exhibit the lowest lumen width. Furthermore, *H. nivalis* and *H. straussii* have the largest and lowest murus width, respectively. Lumen shape was either regular polygonal in sections *Hesperis* and *Diaplictos* or irregular amorphous in section *Pachycarpos.* Murus shape was various in studied accessions, therefore it was defined as 4-5 sided, sharply angled, smooth, and undulate forms based upon Pinar & al. (2009) (table 2).

Seed macro- and micro morphological features

The seed macro- and micromorphological data are indicated and illustrated in fig. 4 and table 3. Generally, Iranian *Hesperis* species are wingless and seed outline are not symmetrical in the genus except *H. persica* subsp. *persica* and *H. persica* subsp. *kurdica* (F. Dvořák & Hadac) F. Dvořák. Furthermore, they demonstrated dark brown and brown colors with oblong and ellipsoid shapes (table 3). The seed length varies from 2.3 mm in *H. straussii* to 2.9 mm in *H. nivalis* and *H. luristanica* F. Dvořák. The maximum and minimum seed width belongs to *H. hyrcana* and *H. persica* subsp. *kurdica*, respectively.

Based upon seed surface, two main groups of seed sculpturing were discovered according to presence or absence of papillae. Those without papillae have undulate (*H. persica* subsp. *kurdica*) and reticulate walls (*H. luristanica* and *H. straussii*) whereas the remaining species characterized by papillae and reticulate wall surface (*H. hyrcana*, *H. nivalis*, *H. odorata* F. Dvořák, and *H. persica* subsp. *persica*) (fig. 4).

Statistical multivariate analysis

Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Principle Component Analysis (PCA) revealed Iranian *Hesperis* species similarities based upon various quantitative and qualitative characters. The first principal component (PC1) scoring system demonstrated the most variation (82.6%) while PC2 indicated the 13.59% variation.

Table 2. Pollen micromorphological features of Iranian *Hesperis* species (values in µm). Numbers defined as (minimum-) mean ± standard deviation (-maximum). Section (Sect.), Polar axis (P), Equatorial axis (E), Polar axis/Equatorial axis (P/E) ratio, Pollen shape (Ps) and Size (Si), Distance between the apices of two ectocolpi/equatorial diameter (d/D)???, Mesocolpium (Meso); Colpus length/polar axis (Cl/P); Colpus length (Cl); Number of Colpi (NC); Exine thickness (ET); Ornamentation type (OrT); Prolate-spheroidal (PS); Subprolate (SP); Small (S); Reticulate (R); Exine lumen width (ELW); Exine murus width (EMW); Lumen shape (Ls); Regular polygonal (RP); irregular amorphous (IA); Murus shape (MS); 4-5 sided (Si); Sharply angled (Sa); Smooth (S); Undulate (U). Qualitative characters are PS, Si, NC, OrT, Ls, Ms and other characters are regarded as quantitative characters.

Taxa	P	E	P/E	Ps	d/D	Si	CL	ET
Sect. *Hesperis*								
H. hyrcana	17.5-20 (18.6±1.25)	18.4-19.5 (18.7±0.55)	0.97-1.05 (1.05±0.04)	PS	0.28-0.42 (0.35±0.07)	S	16.8-19.7 (18.25±1.45)	1.33-1.43 (1.38±0.05)
H. straussii	16.7-18.3 (17.3±0.8)	15.8-16.6 (16.2±0.4)	1.02-1.15 (1.07±0.06)	PS	0.31-0.41 (0.36±0.05)	S	15.5-17.1 (16.3±0.8)	1.21-1.32 (1.26±0.05)
Sect. *Diaplictos*								
H. nivalis	22.1-25.2 (23.6±1.55)	20.7-21.8 (21.3±0.55)	1.04-1.14 (1.08±0.05)	PS	0.37-0.60 (0.48±0.11)	S	21.5-24.7 (23.1±1.6)	1.71-1.84 (1.77±0.06)
H. odorata	21.3-24.8 (23.1±1.75)	20.2-21.7 (20.9±0.75)	1.03-1.12 (1.07±0.04)	PS	0.41-0.54 (0.47±0.06)	S	20.1-23.7 (21.9±1.8)	1.59-1.68 (1.63±0.04)
H. luristanica	19.9-24.5 (22.2±2.3)	20.1-21.5 (20.7±0.7)	1.01-1.12 (1.06±0.05)	PS	0.35-0.53 (0.44±0.09)	S	18.7-23.1 (20.9±2.2)	1.53-1.66 (1.59±0.06)
Sect. *Pachycarpos*								
H. persica subsp. *persica*	20.1-25.8 (22.9±2.85)	19.2-22.7 (20.2±1.75)	1.12-1.16 (1.15±0.02)	SP	0.33-0.51 (0.42±0.09)	S	19.2-24.8 (22±2.8)	1.57-1.73 (1.65±0.08)
H. persica subsp. *kurdica*	19.5-25.2 (22.4±2.85)	18.8-22.5 (20.15±1.85)	1.07-1.13 (1.14±0.07)	SP	0.28-0.50 (0.39±0.11)	S	18.2-24.5 (21.35±3.15)	1.51-1.69 (1.6±0.09)

Table 2. Continued.

Taxa	CL/P	Meso	NC	OrT	ELW	EMW	Ls	Ms			
								Si	Sa	S	U
Sect. *Hesperis*											
H. hyrcana	0.96-098 (0.97±0.01)	10.4-11.6 (11±0.6)	3	R	1.2-2.5 (1.8±0.65)	0.1-0.65 (0.37±0.27)	RP	+	+	+	+
H. straussii	0.92-0.93 (0.92±0.005)	10.2-11.5 (10.9±0.65)	3	R	0.7-2.4 (1.6±0.85)	0.12-0.5 (0.3±0.19)	RP	+	+	+	+
Sect. *Diaplictos*											
H. nivalis	0.97-0.98 (0.98±0.005)	13.1-15.8 (14.45±1.35)	3	R	1.75-2.7 (2.3±0.47)	0.47-0.89 (0.69±0.2)	RP	+	+	+	+
H. odorata	0.94-0.95 (0.94±0.005)	12.3-13.9 (13.1±0.8)	3	R	1.7-2.5 (2.12±0.4)	0.12-0.55 (0.4±0.21)	RP	+	+	+	+
H. luristanica	0.93-0.94 (0.93±0.005)	12.1-13.6 (12.9±0.75)	3	R	1.67-2.5 (2.10±0.41)	0.12-0.50 (0.35±0.19)	RP	+	+	+	+
Sect. *Pachycarpos*											
H. persica subsp. *persica*	0.95-0.96 (0.95±0.005)	10.7-11.5 (11.1±0.4)	3	R	0.75-1.5 (1.23±0.37)	0.4-0.99 (0.68±0.29)	IA	-	-	+	+
H. persica subsp. *kurdica*	0.93-0.97 (0.95±0.02)	10.1-11.8 (10.95±0.85)	3	R	0.73-1.5 (1.25±0.38)	0.35-0.75 (0.6±0.2)	IA	-	-	+	+

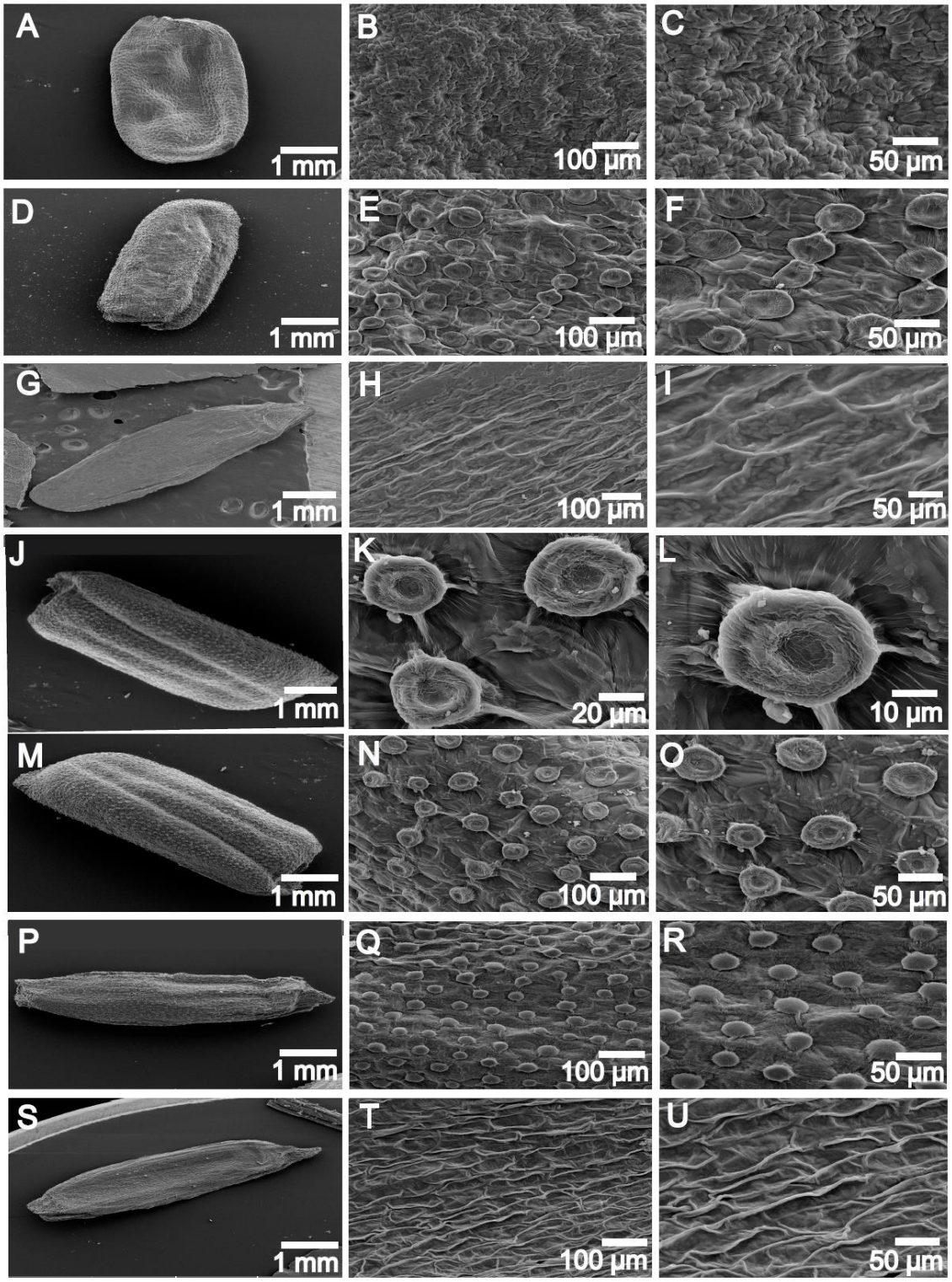

Fig. 4. Seed surface sculpturing of the Iranian *Hesperis* species: A-C, *H. persica* subsp. *kurdica*; D-F, *H. persica* subsp. *persica*; G-I, *H. luristanica*; J-L, *H. nivalis*; M-O, *H. odorata*; P-R, *H. hyrcana*; S-U, *H. straussii.* A, D, G, J, M, P, S are indicating the seed shape. A-F. Sect. *Pachycarpos*; G-O. Sect. *Diaplictos*; P-U. Sect. *Hesperis*.

Equatorial axis (E), Exine lumen width (ELW) and Mesocolpium (MS) characters separate the members of section *Diaplictos* from the remaining taxa (fig. 5). Section *Pachycarpos* excluded from the other sections by Murus shape (Si and Sa), Pollen shape (PS), Seed sculpturing (SSC), Polar axis (P), Colpus length (CL), and Seed color (SC) features (tables 2-3).

Based upon figs. 5-6, all constructed UPGMA dendrograms and PCA graphs supported our idea about the significance of micromorphological characters in delimitation of taxa from the sectional view except dendrogram Part E.

Table 3. Seed SEM micromorphological features of Iranian *Hesperis* species. Numbers defined as (minimum-) mean ± standard deviation (-maximum). Seed size parameters (mm) are based upon mean values and section is abbreviated as sect., Seed shape: (SS), Ellipsoid (E), Oblong (O), Seed length (SL), Seed width (SW), Seed Length/Seed Width (SL/SW), Seed color (SC), Seed sculpturing (SSC). Qualitative characters are SS, SC and SSC; remaining characters are considered as quantitative characters.

Taxa	SS	SL	SW	SL/SW	SC	SSC
Sect. *Hesperis*						
H. hyrcana	E	1.8-2.9 (2.4±0.55)	1.42-1.7 (1.57 ±0.22)	1.24-1.7 (1.47±0.49)	Dark Brown	Reticulate, papillae-unpitted
H. straussii	E	1.7-2.7 (2.3±0.4)	1.33-1.6 (1.5±0.2)	1.28-1.68 (1.48±0.05)	Dark Brown	Reticulate
Sect. *Diaplictos*						
H. nivalis	O	2.45-3.5 (2.9±0.52)	1.45-1.6 (1.52±0.07)	1.68-2.18 (1.93±0.25)	Brown	Reticulate, papillae-pitted
H. odorata	O	2.35-3.3 (2.8±0.47)	1.4-1.5 (1.5±0.05)	1.67-2.2 (1.93±0.26)	Brown	Reticulate, papillae-pitted
H. luristanica	O	2.5-3.3 (2.9±0.4)	1.41-1.5 (1.5±0.04)	1.7-2.2 (1.95±0.25)	Brown	Reticulate
Sect. *Pachycarpos*						
H. persica subsp. *persica*	O	2.1-3 (2.6±0.45)	1.3-1.6 (1.43±0.2)	1.6-1.76 (1.58±0.08)	Brown	Reticulate, papillae-pitted
H. persica subsp. *kurdica*	O	2-2.8 (2.5±0.4)	1.2-1.6 (1.4±0.2)	1.6-1.75 (1.57±0.07)	Brown	Undulate

DISCUSSION

The results of this study are mostly in accordance with previous studies. The pollen ornamentation type is similar among studied taxa. Therefore, it is not taxonomically informative, but size parameters of seed and pollen grains are the most important characters among studied specimens and this statement is well-supported by Pinar & al. (2009) in delimitation of Turkish *Hesperis* species. Pollen shape has taxonomic value since it separates section *Pachycarpos* (fig. 2, A-B) from sections *Hesperis* and *Diaplictos* (fig. 2, C-G). Lumen shape was regular polygonal in all studied sections except section *Pachycarpos* with irregular amorphous shape (fig. 2, table 2). This character considered as a distinguishing feature among sections. Abdel-Khalik (2002) stated that Brassicaceae has tricolpate grains and reticulate exine, which is already supported in the current study (table 2). According to the previous studies (Pinar & al. 2009), symmetrical and isopolar pollen grains are mostly recognized in Turkish *Hesperis* species. This statement is in agreement with Iranian *Hesperis* pollen grains. Bolurian (2009) mentioned that subprolate, prolate-spheroidal and prolate, with reticulate exine ornamentation is common in most genera of Brassicaceae family and the present study provided the same result.

Exine ornamentation did not show any variation among the examined taxa and based on previous surveys exine sculpturing pattern is identical with the other members of family Brassicaceae (Duran & al., 2011; Keshavarzi & al. 2012; Mutlu and Erik, 2012; Tekin and Martin, 2017). According to table 2, the surface of murus is smooth to undulate in all studied species (fig. 2, A-G) while 4-5 sided and sharp angle muri are mostly occurring in sections *Diaplictos* and *Hesperis* (fig. 2, C-G).

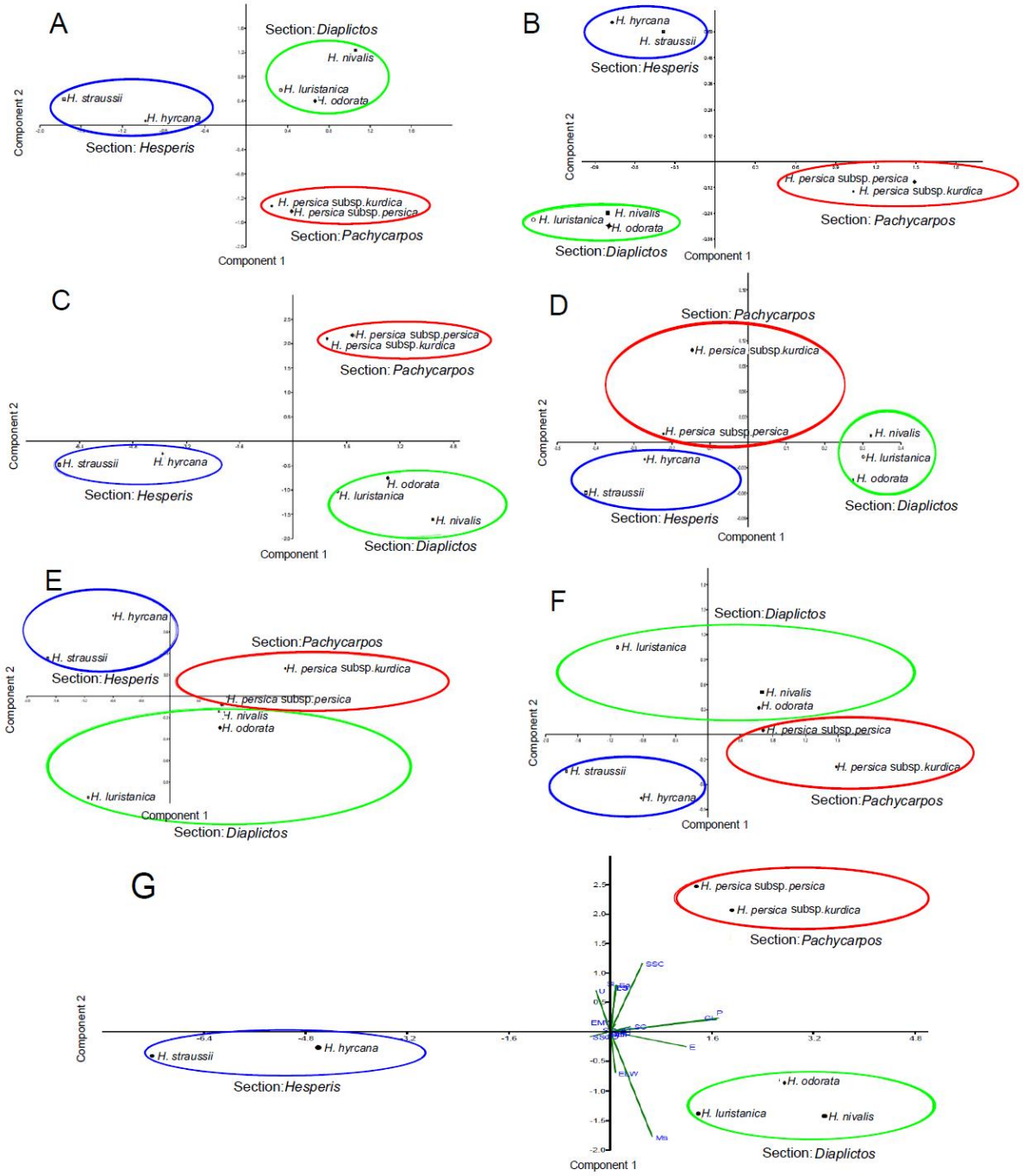

Fig. 5. Principle Component Analysis (PCA) obtained by statistical multivariate analysis of seed and pollen grain quantitative and qualitative characters of Iranian *Hesperis* species. Graphs A, B & C belong to pollen grain quantitative, qualitative and both quantitative and qualitative data, respectively. Dendrograms D, E & F are referring to seed quantitative, qualitative and both quantitative and qualitative data, orderly. Dendrogram G is defined as all quantitative and qualitative features of seed and pollen grain.

Based on Pinar & al. (2009) studies 4-5 sided muri were observed in species of sections *Hesperis* and *Mediterranea*. Pollen shape is subprolate in section *Pachycarpos*, whereas the remaining sections have a spheroidal-prolate shape (fig. 2, table 2). Brochmann (1992) considered muri and lumina shapes as important characters in order to classify the genus *Draba* into four types, these characters showed to de diagnostic in our study as well.

All studied seed characters (shape, size, length, width, color, and sculpture) are taxonomically informative. According to Pinar & al. (2009) seed sculpturing pattern in section *Cvelevia* including *H. breviscapa* Boiss. and *H. kotschyi* Boiss. was considered as a primitive character in the genus *Hesperis* with ocellate seed ornamentation (Type I), whereas remaining sections discussed as advanced groups with reticulate (Type II) and tuberculate (section *contorta*) ornamentations (Type III). In the current survey, the Iranian *Hesperis* species were defined as one type (Type II) (figs. 2-3). Furthermore, scanning electron microscopy demonstrated two main groups in *Hesperis* species based on presence or absence of papillae, which is in accordance with Özüdoğru & al. (2016) who studied *Ricotia* seed, and defined two main groups in *Ricotia*. Furthermore, this kind of classification is thoroughly in accordance with Koul & al. (2000) that classified subtribes Brassicinae, Raphaninae, and Moricandiinae based on presence and absence of papillae. Additionally, seed surface with papillae are classified into two groups (pitted and unpitted). *Hesperis hyrcana* is unpitted, whereas all the other papillate seeds of this genus are clearly pitted. In other words, *H. hyrcana* has circular unique papillae, while three other taxa (*H. nivalis*, *H. odorata* and *H. persica* subsp. *persica*) have flattened papillae (table 3). Papillate seed surface was common in Iranian samples while undulating ornamentation (*H. persica* subsp. *kurdica*) was rarely observed in the studied specimens.

Seed shape is elliptic in section *Hesperis* (*H. hyrcana* and *H. straussii*), while it is oblong in remaining sections (*Pachycarpos* and *Diaplictos*) (fig. 4). The result of this study showed that seed dimensions (seed length and width) are particularly useful to separate taxa from each other. The smallest seed length and seed width belong to the species of section *Hesperis* (*H. hyrcana* and *H. straussii*) and section *Pachycarpos* (*H. persica* subsp. *kurdica* and *H. persica* subsp. *persica*), respectively. The largest seeds observed in section *Diaplictos* (average 2.9 mm in length and 1.52 mm in width), (fig. 4, table 3). Pinar & al. (2009) introduced *H. syriaca* (DC.) Dvořák and *H. kuerschneri* Parolly & Kit Tan as the largest seeds in Turkish *Hesperis* species and *H. matronalis* L. as the smallest ones. In contrast with Turkish samples, ovate and circular seeds are absent in Iranian species. All Iranian *Hesperis* species have brown seed color unless section *Hesperis* which has dark brown color. The seed color was more homogenous (brown and dark brown) in Iranian samples other than their Turkish relatives with brown, light brown, greenish to brown and dark brown colors.

Iranian *Hesperis* seeds are wingless and homogenous in seed size and shape parameters (table 3), and these are in agreement with Pinar & al. (2009) on Turkish *Hesperis* seed size measurements. Özüdoğru & al. (2016) studied the presence or absence of wing in different species of *Ricotia*. Phylogenetically, Huang & al. (2016) clarified that wingless seeds, like what we have observed in *Hesperis* species, can be inherited from an ancestor, and the presence of wing can be because of an evolution during time in order to increase the ability of seed dispersal from one place to far distances. However, such kinds of morphological characters are strongly flexible and recognized in numerous genera (Al-Shehbaz 2012, Huang & al. 2016), which reveal the significance of phylogenetic studies. As a result, molecular studies can disclose the interspecific relationship among different species and we strongly suggest molecular studies to clarify correct taxonomic delimitation of each species.

Ecologically, Pinar & al. (2009) mentioned that there is a close relationship between seed shape and its habitat for instance, ellipsoid seeds grow on mountain slopes while oblong ones grow on rocky places. This statement is totally in agreement with Iranian *Hesperis* species. Considering the ecological condition, species of section *Hesperis* have ellipsoid seeds, while species in remaining sections are characterized by oblong seeds.

The results of our study are in agree with previous sectional classification by Dvořák (1968), (fig. 5). The present study totally in agreement with Duran (2016) and Assadi & al (2017), that treated *H. persica* subsp. *kurdica* and *H. persica* subsp. *persica* as synonyms. Furthermore, PCA and UPGMA dendrograms of seed and pollen grain clearly proved the prior studies by different taxonomists such as Dvořák (1968), Duran & al. (2003) and Duran (2016), (fig. 5-6). In general, macro- and micromorphological features of seed and pollen grain are considered as significant characters in taxa delimitation of Brassicaceae family as stated by Koul & al. (2000).

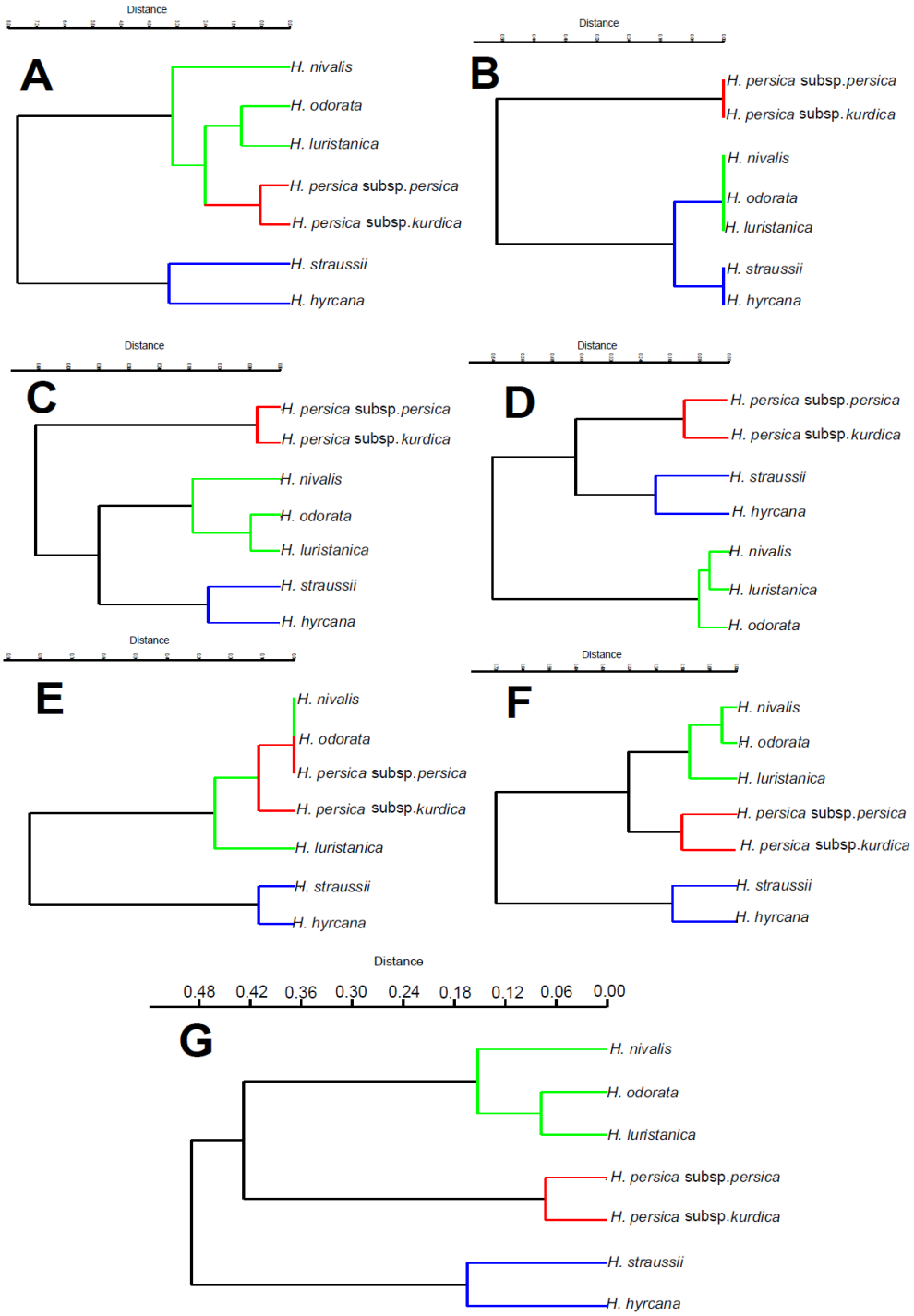

Fig. 6. Dendrograms obtained by UPGMA analysis of seed and pollen grain quantitative and qualitative characters of Iranian *Hesperis* species. Dendrograms A, B & C are belonging to pollen grain quantitative, qualitative and both quantitative and qualitative data, respectively. Dendrograms D, E & F are referring to seed quantitative, qualitative and both quantitative and qualitative data, orderly. Dendrogram G is defined as all quantitative and qualitative features of seed and pollen grain.

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