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Pollen Morphology of some *Onobrychys* Mill. (Fabaceae) Taxa

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ABSTRACT

In this study, the pollen morphology of 13 taxa of the genus *Onobrychis* Mill. (*Onobrychis arenaria* (Kit.) DC. subsp. *arenaria*, *O. inermis* Steven, *O. petraea* (M.Bieb.ex Willd.) Fisch., *O. alba* (Waldst. & Kit.) Desv. subsp. *laconica* (Boiss.) Hayek, *O. megataphros* Boiss., *O. oxyodonta* Boiss., *O. cyri* Grossh., *O. biebersteinii* Širj., *O. vassilczenkoi* Grossh., *O. grandis* Lipsky, *O. hypargyrea* Boiss., *O. kemulariae* Chinth. and *O. caput-galli* (L.) Lam.) grown in the experimental field was examined by light microscopy (LM). Slides were prepared according to the glycerin-jelly method of Wodehouse. Pollen grains were trizonocolpate, radially symmetrical, and isopolar in all taxa. The pollen shape was prolate. The amb was almost circular. All the pollen grains were medium in size, with the largest belonging to *O. cyri* (P × E = 36.55 × 21.60) and the smallest belonging to *O. vassilczenkoi* (P × E = 28.67 × 18.48). The colpus was in the form of a long, narrow slit. Exine ornamentation was reticulate. Based on quantitative data, the species were categorized and separated using principle component analysis (PCA).

1. Introduction

Onobrychis Mill. comprises about 170 species, mainly distributed in southwest Asia, the Mediterranean region, temperate Europe, and Asia [1]. Some of the species are among the finest cultivated forage plants, and some are ornamentals [2]. In the regional taxonomical works, it has been noted that *Onobrychis* is an extremely difficult genus [2, 3].

Studies on the pollen morphology of many genera of the Fabaceae family are of great taxonomic importance [4-9]. Palynological observations on *Onobrychis* to date have remained regional. Pavlova and Manova [10] presented the pollen morphology of 12 taxa from the genus in Bulgaria. Ghanavati and Amirabadizadeh [11] examined palynologically nine *Onobrychis* taxa of the Hedysareae tribe distributed throughout Iran and identified major taxonomical characteristics of their pollen grains. A detailed study on 20 *Onobrychis* taxa from 5 sections occurring in Turkey was carried out by Avcı, et al. [12], and the taxonomic value of the pollen size, structure of the exine, and the sculpture has been revealed. Semnani, et al. [13] investigated the pollen morphology of 17 taxa distributed in Iran and concluded that there was a close relationship between the species of the genus, supported by the pollen morphology. Amirabadizadeh, et al. [14] used palynological as well as morphological and anatomical features in a taxonomic evaluation of eight perennial taxa of *Onobrychis* from northeastern Iran. Based on the pollen morphology of 36 Iranian *Onobrychis* taxa belonging to two subgenera and six sections, Talebi, et al. [15] revealed the taxonomic importance of pollen grain characteristics.

The pollen grain characteristics of 13 *Onobrychis* taxa from various origins were investigated in this study. Three of the species were from Turkey; the others were from Russia, Bulgaria, Australia, and Iran. In addition, the pollen morphology of seven species was investigated for the first time. The aim of this work was to reveal the morphological characteristics of pollen grains in *Onobrychis* taxa and show their taxonomic relationships.

2. Material and Method

The plant material was obtained from the cultivated 13 Onobrychis species in Tekirdağ Namık Kemal University Faculty of Agriculture on trial field (Fig. 1). The studied species were of different origins. The seeds were obtained from the USDA-GRIN gene bank in the United States with the following accession numbers: O. arenaria (Kit.) DC. subsp. arenaria (PI 312954), O. inermis Steven (PI 312943), O. petraea (M. Bieb. Ex Willd.) Fisch. (PI 312946), O. alba (Waldst. & Kit.) Desv. subsp. laconica (Boiss.) Hayek (PI 642147), O. megataphros Boiss.(PI 301107), O. oxyodonta Boiss. (PI 312945), O. cyri Grossh. (PI 314468), O. biebersteinii Širj. (PI 227377), O. vassilczenkoi Grossh. (PI 300580), O. grandis Lipsky (PI 297923), O. hypargyrea Boiss. (PI 383719), O. kemulariae Chinth. (PI 312464), O. caput-galli (L.) Lam. (PI

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Table 1. Pollen measurements of the studied *Onobrychis* taxa (in µm).

	P	E	P/E and shape	Clg	Clt	Ex	In
O. arenaria* subsp. *arenaria	37.65±1.63	23.35±0.93	1.61 prolate	30.95±1.50	3.99±0.44	1.25±0.09	0.54±0.08
O. inermis	38.00±1.17	23.30±0.80	1.63 prolate	31.65±1.14	3.74±0.33	1.17±0.09	0.55±0.06
O. petraea	38.55±1.32	23.05±1.23	1.67 prolate	33.95±1.90	3.80±0.45	1.42±0.13	0.55±0.06
O.alba* subsp. *laconica	39.90±1.21	20.95±0.69	1.90 prolate	35.10±1.48	3.11±1.48	1.19±0.15	0.54±0.06
O. megataphros	34.85±2.25	19.85±1.09	1.76 prolate	28.95±2.14	2.98±0.44	1.11±0.08	0.52±0.10
O. oxyodonta	38.10±1.71	23.65±0.99	1.61 prolate	32.35±1.69	3.43±0.47	1.25±0.14	0.58±0.06
O. cyri	39.55±1.50	22.20±1.15	1.78 prolate	32.85±1.50	3.89±0.49	1.27±0.07	0.61±0.09
O. biebersteinii	37.20±1.32	23.85±0.99	1.56 prolate	31.80±1.01	4.65±0.58	1.20±0.11	0.59±0.07
O. vassilczenkoi	33.10±1.41	20.30±0.86	1.63 prolate	28.15±1.69	3.61±0.33	1.03±0.12	0.55±0.04
O. grandis	34.50±2.42	19.75±1.21	2.00 prolate	29.25±2.29	3.04±0.53	1.14±0.10	0.57±0.07
O. hypargyrea	35.60±0.94	22.65±0.59	1.57 prolate	29.60±1.19	3.35±0.29	1.29±0.13	0.62±0.08
O. kemularie	36.70±1.30	23.30±0.66	1.58 prolate	31.05±1.23	3.46±0.40	1.23±0.11	0.57±0.07
O. caput-galli	38.50±1.36	23.25±0.64	1.66 prolate	32.90±1.37	3.48±0.43	1.12±0.10	0.56±0.08

205304). Slides were prepared according to the glycerin-jelly method of Wodehouse [16]. Measurements and examination of the characteristic features of the pollen grains and their imaging were performed by an Olympus CX41 trinocular light microscope, an Olympus SC30 digital camera, and Cell software at 100× magnification (Figs 2 and 3). In the morphometric evaluation of pollen, the polar axis (P), equatorial diameter (E), colpus length (Clg), colpus width (Clt), exine (Ex), and intine (In) were used. The pollen shape was determined according to the P/E ratio in equatorial view. For each character, 30-50 measurements were taken, and the arithmetic mean and standard deviation were determined (Table 1). The terminology used for pollen descriptions follows Erdtman [17] and Punt, et al. [18].

Principal component analysis (PCA) was used to evaluate which pollen features reflected the greatest proportion of variability and whether these pollen features might be utilized to differentiate species. Statistical analyses were carried out with RStudio Desktop 4.0.4 [19]. FactoMineR [20] and Factoextra [21] packages were used to show loadings and scores. The results were displayed in the form of two-dimensional graph of the first and second principal components (PC1 and PC2, respectively) (Table 2).

Fig. 1 General habit of *O.oxyodonta* in the trial field.

Fig. 2 Light microscope (LM) micrographs of examined *Onobrychis* taxa. a, b *O. biebersteinii*, c, d *O. inermis*, e, f *O. petraea*, g, h *O.alba* subsp. *laconica*, i, j *O. megataphros*, k, l *O. oxyodonta*, m, n *O. cyri*. a, c, e, g, i, k, m equatorial view, b, d, f, h, j, l, n polar view. Scale bar= 20µm.

Fig. 3 Light microscope (LM) micrographs of examined *Onobrychis* taxa. a, b *O. arenaria* subsp. *arenaria*, c, d *O. vassilczenkoi*, e, f *O. grandis*, g, h *O. hypargyrea*, i, j *O. kemularie*, k, l *O. caput-galli*. a, c, e, g, i, k equatorial view, b, d, f, h, j, l polar view. Scale bar= 20µm.

3. Results and Discussion

The pollen grains of the studied *Onobrychis* taxa were similar in terms of type, shape, and exine ornamentation. The pollen grains were trizonocolpate, prolate-shaped, and medium-sized in all the studied taxa. In palynological studies on *Onobrychis* species carried out to date, pollen grains have been determined to be trizonocolpate [10, 12-14]; however, Ghanavati and Amirabadizadeh [11] reported the presence of trizonocolporate pollen in *O. crista-galli* Lam. in addition to trizonocolpate ones.

In 13 taxa, pollen shape was determined as prolate from the ratio of the polar axis to the equatorial diameter (P/E). This ratio varied between 1.55 and 1.89. This pollen shape was similar to previous studies [12-14]. In addition to prolate, perprolate pollen grains were also recorded in the genus [10, 11]. The outline in polar view (amb) was almost circular in all species.

The pollen grains of the studied taxa were medium in size. In previous investigations, the pollen grains were generally recorded as being medium-sized [12-14], however, Pavlova and Manova [10] reported large in addition to medium-sized pollen grains.

Pavlova and Manova [10] determined the pollen sizes for *O. alba* subsp. *laconica* and *O. caput-galli* to be P × E = 36.2 × 18.7 and P × E = 36.9 × 21.2, respectively. In this study, pollen sizes for these two taxa were measured as P × E = 36.10 × 19.10 and P × E = 33.98 × 21.51, respectively. The size of *O. caput-galli* pollen was recorded as P × E = 33.5 × 16.2 by Semnani, et al. [13]. The pollen size of *O. oxyodonta* was reported as P × E = 32.4 × 18.2 and that of *O. hypargyrea* as P × E = 29.00 × 17.8 by Avcı, et al. [12]. In this study, the pollen size of these two species was measured as P × E = 35.30 × 21.80 and P × E = 33.15 × 21.01, respectively. Semnani, et al. [13] reported *O. cyri* pollen dimensions as P × E = 24.70 × 15.2; however, the dimensions of this species in this study were P × E = 36.55 × 21.60.

The pollen traits that indicated the greatest proportion of variability were identified using principal component analysis (PCA), and the species were grouped based on these pollen characteristics. The first two components, PC1 and PC2, accounted for 42.1% and 18.6% of the variance, respectively (Table 2). The individuals (species) and explanatory variables as vectors were shown in the PCA biplot in Fig. 4. The contribution of the characteristic to the first two components of the PCA was indicated by the varying color intensities and lengths of the vectors. The most significant and positively correlated components to the first principal component were P, E, and Clg, which were depicted as green and long vectors. The species with the highest values for these contributors were *O. cyri*, *O. petraea*, *O. inermis*, *O. oxyodonta*, and *O. alba* subsp. *laconica*, which occupied close spaces in the PCA biplot (Table 1, Fig. 4). The most significant components of the second principal component were In, Clt, and Clg. In and Clt (blue vectors) are positively correlated, while Clg (green vector) is

Table 2. Cumulative variance and eigenvectors of principal component analysis (PCA) of the palynological characters of the studied *Onobrychis* taxa.

Principal component axis	1	2
Cumulative variance (%)	42.0506	60.6088
Characters	*Eigenvectors*	
Polar axis (P)	0.8315	-0.3709
Equatorial diameter (E)	0.7648	0.3017
Colpus length (Clg)	0.7853	-0.4331
Colpus width (Clt)	0.5700	0.4674
Exine (Ex)	0.5239	0.0586
Intine (In)	0.1750	0.6895

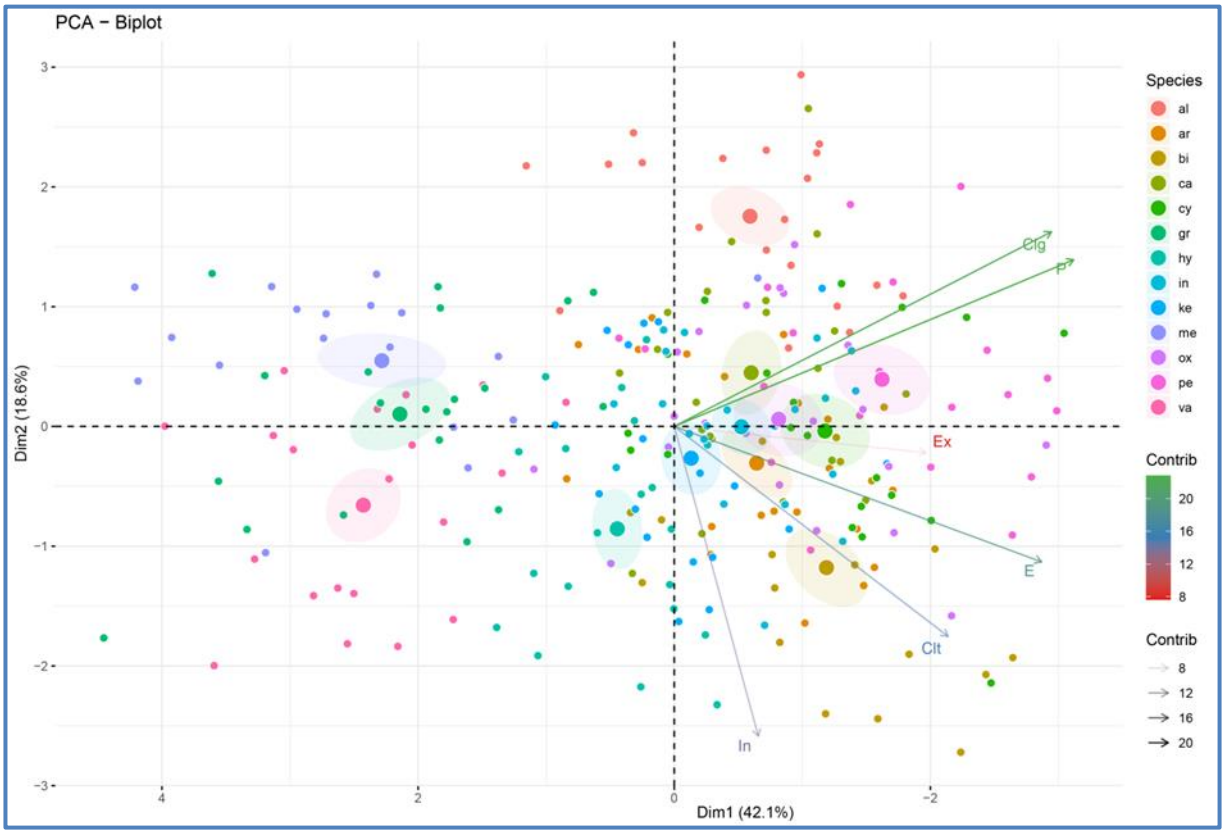

Fig. 4 Biplot of principal component analysis (PCA) of the 13 *Onobrychis* species analyzed based on the variance found for six morphometric characters. The first two components (PC1 and PC2) accounted for 42.1% and 18.6%, respectively, of the variations. The contribution of the characteristic to the first two components of the PCA is indicated by the varying color intensities and lengths of the vectors. The variable vectors are presented in the order in which they contribute to the principal components (gradient colors and vector transparency), with green and long representing high contributions, green-blue or blue representing intermediate contributions, and red and short representing very low contributions. *O. arenaria* subsp. *arenaria* (ar), *O. inermis* (in), *O. petraea* (pe), *O. alba* subsp. *laconica* (al), *O. megataphros* (me), *O. oxyodonta* (ox), *O. cyri* (cy), *O. biebersteinii* (bi), *O. vassilczenkoi* (va), *O. grandis* (gr), *O. hypargyrea* (hy), *O. kemulariae* (ke), and *O. caput-galli* (ca).

negatively correlated to these two (Table 1, Fig. 4). O. vassilczenkoi has the smallest pollen and occupies a space in the opposite direction of P, E, and Clg (Table 1, Fig. 4, lower left quadrant). Having small pollen grains and the smallest values for Clt, O megataphros, and O. grandis occupy close spaces and are close to O. vassilczenkoi in the biplot (Table 1, Fig. 4, upper left quadrant).

4. Conclusion

This study on the pollen morphology of Onobrychis taxa revealed that although the general pollen grain characteristics such as pollen type, pollen shape, and exine ornamentation are similar, the quantitative data could be useful in distinguishing species.

Declaration

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