

SEED MORPHOLOGY IN *Onobrychis* MILLER SECTION HYMENOBRYCHIS DC. FROM TURKEY¹

Morfologia de Sementes em Onobrychis Miller da Seção Hymenobrychis DC. da Turquia

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ABSTRACT - The seed (nutlet) morphology of four *Onobrychis* Miller (Fabaceae: subfamily Papilionoideae, Section Hymenobrychis DC.) taxa from Turkey, including three endemic taxa, was examined using scanning electron microscopy. *Onobrychis tournefortii*, *O. galegifolia*, *O. cappadocica*, *O. albiflora*. The seed examined exhibited variation in size, shape, colour, and surface sculpturing. Seed size ranged between 4.0-5.2 mm length and 2.0-3.6 mm width. Observed shapes included; ellipticus anguste asymmetricus reniformis, Ovatus anguste asymmetricus reniformis and ellipticus reniformis. Seed surface sculpturing revealed two distinct types: reticulate and rugulate. Species of *Onobrychis* are generally similar and confused with those of *Hedysarum* during the identification process. Seed surface micromorphology can suggest taxonomical diagnostic characters for distinguishing species. Many of these characteristics are diagnostic at both the generic and specific levels.

Keywords: seed, morphology, Fabaceae, *Onobrychis*, Turkey.

RESUMO - A morfologia de semente (nutlet) de quatro *Onobrychis* Miller (Fabaceae: subfamília Papilionoideae, seção Hymenobrychis DC.) taxa da Turquia, incluindo três táxons endêmicos, foi examinada usando microscopia eletrônica de varredura: *Onobrychis tournefortii*, *O. galegifolia*, *O. cappadocica* e *O. albiflora*. As sementes examinadas apresentaram variação no tamanho, forma, cor e escultura da superfície. O tamanho da semente variou entre 4,0 e 5,2 mm de comprimento, e a largura, de 2,0 a 3,6 mm. As formas observadas incluíram: *ellipticus anguste asymmetricus reniformis*, *Ovatus anguste asymmetricus reniformis* e *Ellipticus reniformis*. A escultura da superfície da semente revelou dois tipos distintos: *reticulada* e *rugulada*. Espécies de *Onobrychis* são geralmente semelhantes e confundidas com as de *Hedysarum* durante o processo de identificação. A micromorfologia da superfície da semente pode sugerir caracteres diagnósticos taxonômicos para distinguir espécies. Muitas dessas características são diagnósticas em nível tanto genérico quanto específico.

Palavras-chave: semente, morfologia, Fabaceae, *Onobrychis*, Turquia.

INTRODUCTION

Leguminosae comprises three subfamilies namely; Caesalpinioideae, Mimosoideae and Papilionoideae (Willis 1976). However, Hutchinson (1948) promoted the rank of Leguminosae to that of the "Leguminales" order, which in turn comprises the three

families: Caesalpiniaceae, Mimosaceae and Fabaceae (Papilionaceae). *Onobrychis* (sainfoin) is a genus of the Papilionoideae subfamily, *Hedysareae* tribe (Leguminosae) with about 150 species in 2 subgenera and 9 sections, including annual or perennial herbs or shrubs in the world (Rield, 1978). The genus is mainly distributed in northern temperate regions;

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however, centers of its genetic diversity are in the eastern Mediterranean area and south-west Asia (Lock & Simpson 1991). *Onobrychis* is in the area extending from the mediterranean region to Caucasia, the Zagros Mountains and Central Asia. This genus is represented by approximately 342 species throughout the World and by fifty-two species, two sub-genus and five sections in Turkey (Hesamzadeh Hejazi & Ziacy Nasab, 2010). Taxonomically, *Onobrychis* is an extremely problematic genus. The worst problems of which are experienced in Anatolia, one of the main centers of the genus (Dural & Citak, 2015). Genus taxonomy continues to be a subject of discussion, mainly because of the different approaches adopted towards species delimitation, which results in varying numbers of recognized species (Davis et al., 1988, Güner et al., 2000). Species of *Onobrychis* are generally confused with those of *Hedysarum* during the identification process (Hosgoren, 2006). Although seed characters, particularly exomorphic features, are used in many taxonomic treatments, they are far from being fully exploited. Seldom does one find keys or synopses for seed identification and understanding microcharacters requires detailed laboratory work. It has been pointed out that this is partly due to the rather scanty literature relating to seeds and lack of a suitable descriptive terminology which can be universally applied (Rejdali, 1990). Thus, while considerable literature is available on the seeds of some plant families, such as the Umbelliferae and Cyperaceae, little, other than incidental notes in manuals or species descriptions, has been published for many other families. Most of the groups dealt with are of economic importance, particularly the weedy and cultivated species. Wild plants have been relatively ignored. Some authors have produced family synopses or even keys to species based solely on seed characteristics (Sparke & Williams, 1983). The legume is the standard fruit type for the Fabaceae family but there is a wide diversity of subtypes present (Noori, 2002). Some pod and seed characters in some Papilionoideae, such as existing and durability of sepal, fruit and seed size, shape, color, dorsal and ventral ornamentation, wall thickness, pod dehiscing, legume seed number, and etc. have

an important role in seed dispersion and taxonomy. Studies of these characters has revealed the relationship between the taxa and has been used for identification and numerical taxonomy of the legume species (Noori et al., 2005). Taia (2004) has referred that the SEM helps to detect minute taxonomically significant structures in seed coat patterns which might enable us to define species characteristics. Seed morphology and structure in the Leguminosae have been the subject of numerous studies by Corner (1976), Gunn (1981), Lersten et al. (1992), Jones & Geneve (1995), Lu-Ayaz et al. (2000), Hussein et al. (2002) and Salimpour et al. (2007).

The purpose of this study was to describe and investigate the seed micromorphological properties of *Onobrychis tournefortii*, *O. galegifolia*, *O. cappadocia*, *O. albiflora* and to evaluate the diagnostic value of this character in terms of systematic implications.

Detailed studies on the seeds of the investigated taxa here have not been reported in the literature.

MATERIAL AND METHODS

Material used for this study was collected from wild populations in Turkey (Table 1). The plant samples were dried as herbarium sample and stored in the Ahi Evran University Herbarium. In order to assess infraspecific variation, 9-10 seeds from each taxon were measured. For SEM studies, seeds were directly mounted on stubs and coated with gold. Micrographs were taken with a JEOL 5600 SEM. All the specimens were examined, but only the clearest photographs representing each seed sculpturing type were selected and illustrated. Terminology for descriptions of morphological characteristics of the seeds follows Stearn (1978) and Bojòanski and Fargašová (2007).

RESULT AND DISCUSSION

Seed coat characters are successfully employed in the identification and classification of taxa by Erol et al. (2006), Kaplan et al. (2007) and Fawzi et al. (2010). Taia (2004) has stated that the SEM helps to detect minute taxonomically significant

structures in seed coat patterns, which might enable us to define species characteristics. The characteristics of seed morphology (i.e., size, shape, surface sculpturing and colour) for the investigated taxa are given (Table 2).

The studied *Onobrychis* seeds ranged in length from 4.00 mm to 5.20 mm, with the minimum value in *O. tournefortii* and the maximum value in *O. albiflora*, and in width from 2.00 mm to 3.60 mm, with the minimum value in *O. tournefortii* and the maximum value

Table 1 - Data on the collection of the *Onobrychis* Miller section *Hymenobrychis* DC. taxa examined in the present study

Taxon	Voucher locality	Collection Number
<i>O. tournefortii</i> (Willd.) Desv.	A7 Gümüşhane: Kelkit, Deredolu village area, step, 1550 m, 29.07.1993, Ir.-Tur. El. End.	Aktoklu-2225 & Yıldız
<i>O. galegifolia</i> Boiss	B7 Erzincan: Avcılar, step and slopes, 1200 m, 28 vii 1993, Ir.-Tur. El.	Aktoklu-2219 & Yıldız
<i>O. cappadocica</i> Boiss.	B7 Malatya: 2 km to Gürkaynağ, <i>Quercus</i> , 1200 m, 16.07. 1993, Ir.-Tur. El. End.	Yıldız-1971
<i>O. albiflora</i> Hub.-Mor.	B6 Sivas: Sincan-Karaman 9. km, volcanic bedrock, roadside slope, 1150-1200 m, 18.07.1991, Ir.-Tur. El. End	Aktoklu-1839

El: Element, End: Endemic for Turkey.

Table 2 - Comparison of seed shape, size, sculpturing and color in examined *Onobrychis* taxa

Taxon	Shape	Length (mm)			Width (mm)			L/W ratio			Sculpturing	Colour
		M	SD	Range	M	SD	Range	M	SD	Range		
<i>O. tournefortii</i>	<i>Ellipticus anguste</i>	4.04	±0.17	4.0–4.1	2.40	±0.13	2.0–2.1	1.6	±0.10	1.2–1.7	R	light brown with yellow
	<i>asymmetricus</i>											
	<i>reniformis</i>											
<i>O. galegifolia</i>	<i>Ovatus anguste</i>	4.53	±0.07	4.3–4.7	3.31	±0.12	3.2–3.6	1.3	±0.08	1.2–1.4	R	agate-like light brown
	<i>asymmetricus</i>											
	<i>reniformis</i>											
<i>O. cappadocia</i>	<i>Ellipticus reniformis</i>	4.56	±0.12	4.3–4.6	3.11	±0.11	3.0–3.2	1.4	±0.11	1.2–1.5	R	light yellowish brown
<i>O. albiflora</i>	<i>Ellipticus anguste</i>	5.12	±0.07	5.0–5.2	3.17	±0.14	3.0–3.2	1.6	±0.13	1.3–1.7	C	light chestnut-brown
	<i>asymmetricus</i>											
	<i>reniformis</i>											

M: Mean; SD: Standard Deviation; L: Length; W: Width; R: Reticulate; C: Colliculate.

in *O. albiflora*. Hussein et al. (2002) have adopted the size of seed in some delimitation in Caealpinioideae. On the other hand, Thompson (1981) has illustrated that the seed size was regarded as being of minor taxonomic value and such attribute is subjected to ecological and physiological variations.

The general shape of the seeds in the four taxon are different. Seeds of *O. tournefortii* have an ellipsoid narrowly asymmetric kidney-shape (*ellipticus anguste asymmetricus reniformis*); *O. galegifolia* has an ovate asymmetric kidney-shape (*ovatus anguste asymmetricus reniformis*); *O. galegifolia*



has an ellipsoid kidney-shape (ellipticus reniformis); *O. albiflora* has an ellipsoid narrowly asymmetric kidney-shape (ellipticus anguste asymmetricus reniformis) (Figures 1, 2: A-B, C, D). According to Gunn (1981), the Legume testa in Fabaceae is usually

monochrome brown to black, rarely red, cream or white, or occasionally dichrome as mottling or two distinct coloured areas. According to this study, the seeds of *O. tournefortii* and *O. cappadocica* are light brown with yellow white, those of *O. galegifolia* are agate-like

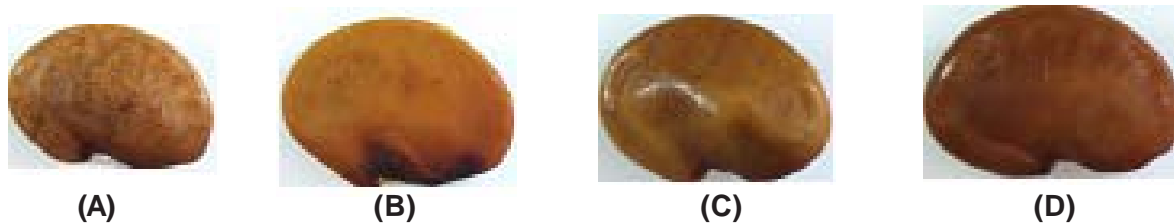


Figure 1 - General view of the seeds investigated *Onobrychis* taxa (A) *O. tournefortii*; (B) *O. galegifolia*; (C) *O. cappadocica*; (D) *O. albiflora*.

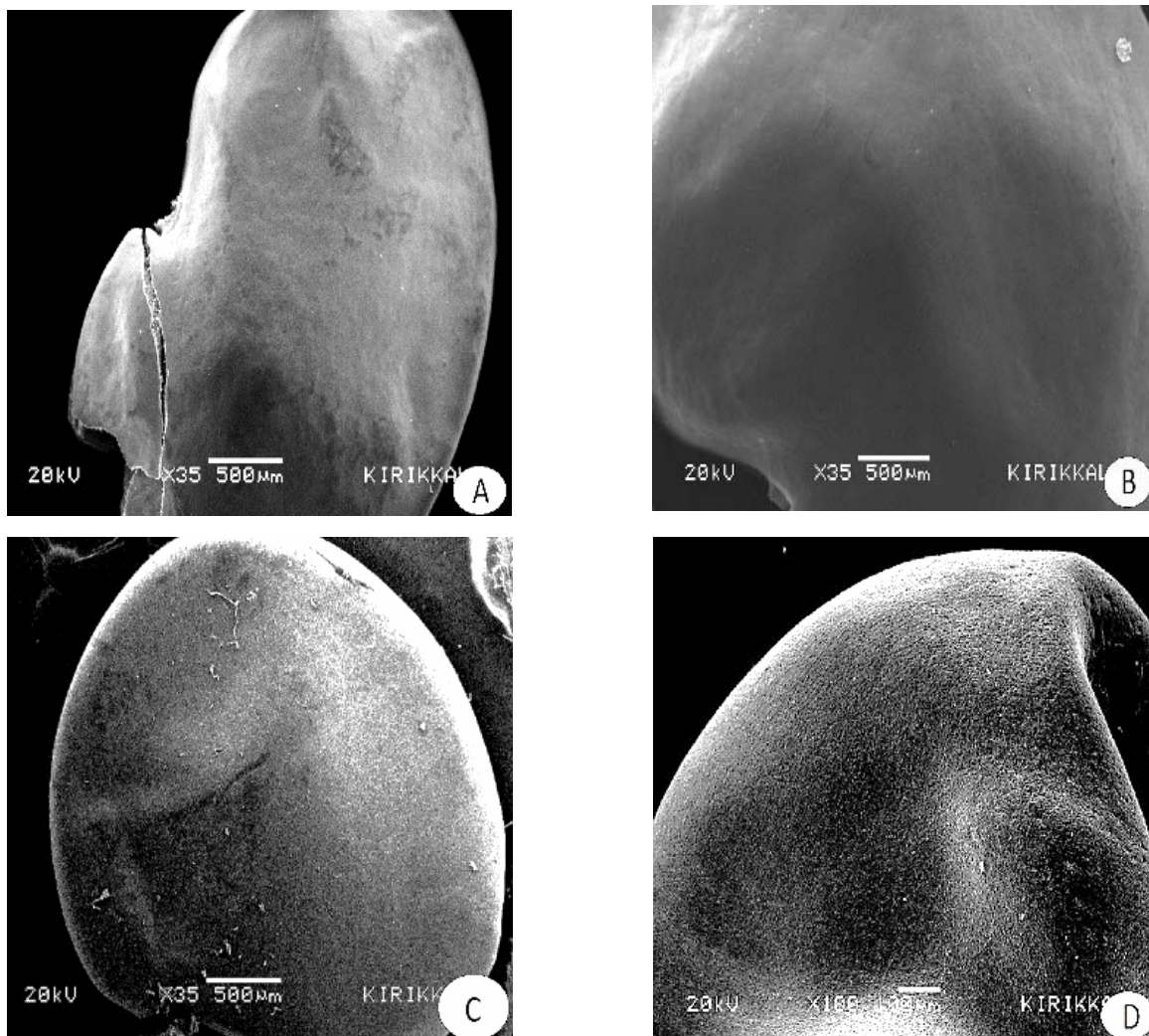


Figure 2 - Scanning electron micrographs of seed type of *Onobrychis* taxa. (A) *O. tournefortii*; (B) *O. galegifolia*; (C) *O. cappadocica*; (D) *O. albiflora* X35.

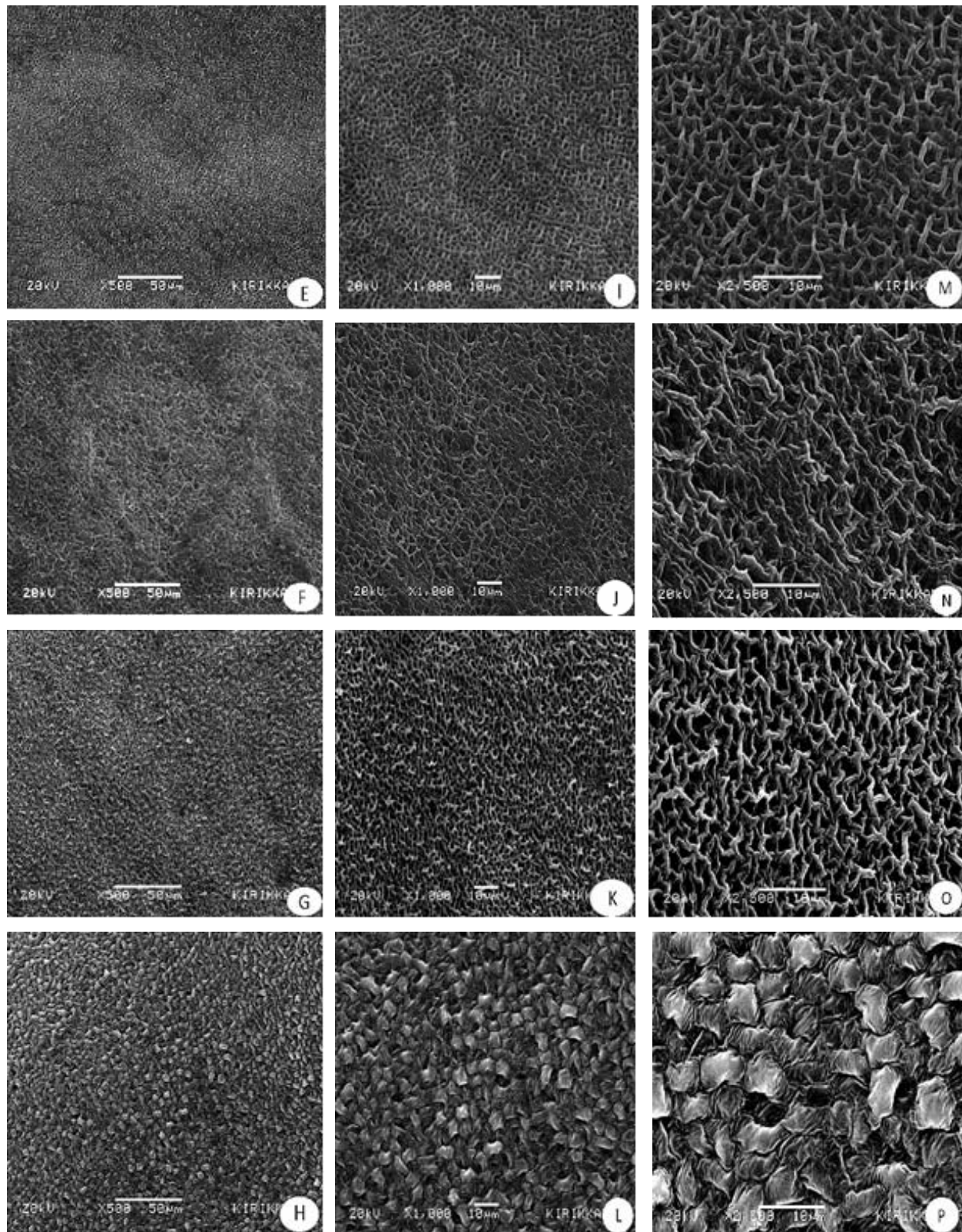


Figure 3 - Scanning electron micrographs of seed surface ornamentation of *Onobrychis* taxa. (E), (I), (M): *O. tournefortii*; (F), (J), (N): *O. galegifolia*; (G), (K), (O): *O. cappadocia*; (H), (L), (P): *O. albiflora*. EFGH: X500; IJKL: X1000; MNOP: X2500.



light brown, and *O. albiflora* are light yellowish brown. This agrees with the results reported by Shyam & Vartak (1985). They have used the color of seeds with other features to describe the seeds of genus *Cassia* and to produce a key for their identification. Conversely, Husain (2000) has regarded this character to be of limited taxonomic value for its possible fluctuation within the same taxon at the different durations.

Seeds of investigated taxa have exhibited two types of surface sculpturing, and are present in the following species.

Reticulate pattern with intergraded forms has occurred in *O. tournefortii* (irregularly reticulate) *O. galegifolia*, and *O. cappadocia* (reticulate-foveolate with ridges of the reticulate being very thick). (Figures 3: E, I, M, F, J, N, G, K, O).

Our seed surface ornamentation findings were concordant with the *Hedysarum* findings by Civelek et al., 1999, Dural & Citak, 2015. The researchers have indicated that seed surface ornamentation of *Hedysarum pannosum*, *H. gmelinii*, *H. dahuricum* and *H. setigerum* were reticulate. Moreover, *H. brachypterum* and *H. dasycarpum* seeds ornamentation were reticulate (Sa et al., 2010). Also in this study it was indicated that *O. tournefortii*, *O. galegifolia* and *O. cappadocia* were reticulate. But it occurred that *O. albiflora*, *H. splendens* seeds were Colliculate pattern. Hence our seed findings support Hosgoren's statement (2006) that species of *Onobrychis* are generally similar and confused with those of *Hedysarum* during the identification process. Herein all terminology used for the description of the testa sculpturing patterns are that by Lersten (1981). In this study, the investigated taxon have different features of seed surface. Hence the variability in seed surface micromorphology is seemingly very useful in the recognition of the species studied. In conclusion, it could be declared that valuable taxonomic evidence has been obtained from studying seed characteristics in some species of *Onobrychis*. Hence these features of seed surface micromorphology can suggest taxonomical diagnostic characters for distinguishing species. Many of these characteristics are diagnostic at both the generic and specific levels.

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