

STYLE MICROMORPHOLOGY IN THE TRIBE FABEAE (FABACEAE) WITH EMPHASIS ON LATHYRUS IN IRAN AND TURKEY

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Stylar features of 35 taxa including *Lathyrus* (26 species), *Pisum* (3 species), *Vicia* (4 species) and *Lens* (2 species) were examined using electron microscopy (SEM). The results show that four genera *Lathyrus*, *Lens*, *Pisum* and *Vicia* are different in terms of stylar features. At least eight stylar types can be recognized in the tribe *Fabeae*. *Lathyrus* is the most variable genus in this regard showing four different stylar types: (1) straight, linear and evenly hairy styles (Sle-type), (2) straight, spatulate and evenly hairy styles (Sse-type), (3) contorted, linear and evenly hairy styles (Cle-type) and (4) contorted, spatulate and evenly hairy styles (Cse-type). Furthermore, the styles are dorsiventrally compressed and adaxially evenly hairy in this genus. Two stylar types: (5) dorsiventrally compressed and evenly hairy all round (De-type) and (6) dorsiventrally compressed and abaxially tufted (Dabt-type) were recognized in *Vicia*. Other two types: (7) cylinder, non-compressed and adaxially evenly hairy style (Cne-type) and (8) longitudinally folded, canaliculated, laterally compressed and adaxially evenly hairy style (Fce-type) were recognized in *Lens* and *Pisum*, respectively. Special focus on *Lathyrus* revealed that stylar features do not corroborate the current sectional classification of the genus. None of the recognized stylar types are characteristic for any recognized sections of *Lathyrus*.

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میکرومورفولوژی خامه در قبیله *Fabeae* (*Fabaceae*) با تاکید بر جنس *Lathyrus*

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خصوصیات خامه ۳۵ گونه شامل ۲۶ گونه از *Lathyrus*، ۳ گونه از *Pisum*، ۴ گونه از *Vicia* و ۲ گونه از *Lens* با استفاده از میکروسکوپ الکترونی (SEM) بررسی شدند. نتیجه بررسی میکرومورفولوژی نشان داد که چهار جنس *Lathyrus*، *Lens*، *Vicia* و *Pisum* در ویژگی‌های خامه‌ای، متفاوت هستند. حداقل هشت نوع خامه در قبیله *Fabeae* حضور دارد. چهار نوع از این خامه‌ها (۱) خامه صاف، خطی و کرکهای یکنواخت (Sle-type) (۲) خامه صاف، قاشقی و کرکهای یکنواخت (Sse-type) (۳) خامه پیچ خورده، خطی و کرکهای یکنواخت (Cle-type) (۴) خامه پیچ خورده، قاشقی و کرکهای یکنواخت (Cse-type) همگی با فشردگی پشتی-شکمی و کرکهای در سطح رو به محور در *Lathyrus* تشخیص داده شدند. دو نوع (۵) خامه با فشردگی پشتی-شکمی و کرکهای یکنواخت در هر دو سطح (De-type) و (۶) خامه با فشردگی پشتی-شکمی و کرکهای کاکل مانند در سطح دور از محور (Dabt-type) در *Vicia*، (۷) خامه استوانه‌ای، بدون فشردگی و کرکهای یکنواخت در سطح رو به محور (Cne-type) در *Lens*، (۸) خامه در طول تاخورده، نودانی با فشردگی جانبی و کرکهای یکنواخت در سطح رو به محور (Fce-type) در *Pisum* یافت شدند. یافته‌های میکرومورفولوژی در مطالعه اخیر نشان می‌دهد که خصوصیات خامه‌ای با طبقه بندی بخش‌های *Lathyrus* هماهنگی ندارد. هیچ کدام از انواع مختلف خامه‌ها، منحصر به بخش‌های مشخص شده در *Lathyrus* نیستند.

INTRODUCTION

The tribe *Fabeae* is a part of the vicioid subclade of the inverted repeat lacking clade of papilionoid legumes (IRLC, Wojciechowski et al., 2000, 2004; Kazempour Osaloo 2007). Morphological and molecular evidence strongly support the monophyly of *Fabeae* (Steele and Wojciechowski 2003; Lock and Maxted 2005; Oskoueian et al., 2010). According to the recent work (Oskoueian et al., 2010), the tribe contains four genera, of which *Lathyrus* and *Vicia*, are large but *Lens* and *Pisum* (including *Vavilovia formosa*) are relatively small genera.

Lathyrus with ca 160 species are distributed throughout the Northern Hemisphere, tropical East Africa and temperate South America. Its main center of diversity is in the Mediterranean and Irano-Turanian regions, with smaller centers in N and S America (Kupicha 1983; Asmussen & Liston 1998; Kenicer 2005). Kupicha (1983) classified species of the genus into 13 sections. Twenty-three species of *Lathyrus* are growing in Iran (Mozaffarian et al., 2008). In Flora of Iran 23 species recognized (Neamati 2000; Mozaffarian et al., 2008). Rechinger (1979) in Flora Iranica grouped those Iranian species into 10 sections. Davis (1970) considered 57 species of *Lathyrus* in Flora of Turkey. Dogan et al. (1992) examined 52 species of Turkish *Lathyrus* based on 40 external vegetative and floral morphological characters. Three characters, style length, style shape and style twister or straight were among them.

Vicia with 40 species is the genus of *Fabeae* in Iran, *Pisum* and *Lens* have two (*P. sativum* and *P. formosum*) and three (*L. culinaris*, *L. orientalis* and *L. cyanea*) species in Flora of Iran, respectively (Pakravan 2000).

Many authors believed that stylar shapes and stylar hair patterns are useful for generic delimitation in the tribe *Fabeae* and to find subgroups within genera (Endo 1994; Kupich 1981; Choi et al., 2006). The dorsiventrally compressed and adaxially hairy style (Dad-type) are diagnostic characters of *Lathyrus* and *Lens*, and longitudinally folded and adaxially hairy styles (Fad-type) are diagnostic characters of *Pisum* (including *Vavilovia*) (Kupich 1981). Kupicha (1983) indicated that all members of *Lathyrus* have a dorsally compressed style, which is pubescent on the adaxial side, and many species have a twisted style, which is a particularly valuable character. She found that stylar shape in the Old World species of *Lathyrus* have been heavily weighted. Kupicha (1976) reported three stylar shapes (laterally compressed, terete and dorsiventrally compressed) and two stylar indumentum (evenly hairy and tufted abaxially hairy) in *Vicia*. Species of *Vicia* has been classified into four stylar types: (1)

dorsiventrally compressed and evenly hairy all round (De-type), (2) terete and evenly hairy all round (Te-type), (3) laterally compressed and evenly hairy all round (Le-type), (4) Dorsiventrally compressed and abaxially tufted hairy (Dabt-type) (Endo et al., 2008).

Choi et al. (2006) based on nrDNA ITS phylogeny of the Old World *Vicia*, showed that the laterally compressed stylar shape and the abaxially tufted stylar indumentum are derived characters. Furthermore, the dorsiventrally compressed stylar shape and the even stylar pubescence are plesiomorphic (Choi et al., 2006).

In this study, we report the stylar features for the Tribe *Fabeae* and in particular to *Lathyrus* using SEM.

MATERIAL AND METHODS

Plant material

Herbarium samples of 35 taxa including *Lathyrus* (26 species), *Pisum* (3 species), *Vicia* (4 species) and *Lens* (2 species) were included in this study. We paid particular attention in sampling to 7 sections (according to Kupicha 1983) of *Lathyrus* (Tab. 1). Because the flowers of herbarium specimens may be influenced by pressing, we examined styles of flowers that set in FAA (formaldehyde: acetic acid: aqueous ethanol = 5:5:90).

Scanning Electron Microscopy

For observation with SEM (scanning electron microscopy), the styles were dissected, suspended in FAA and air dried from FAA onto aluminum stubs. These samples were coated with gold in a Sputter coater, BAL-TEC SCDOOS, and examined with a Philips XL30 microscope at the Tarbiat Modares University.

RESULTS

Stylar shapes. SEM micrographs of the styles in selected studied taxa are presented in Figs. 1 and 2. They show that *Lathyrus* has straight, not contorted style (*L. vernus*, *L. alamutensis*, *L. boissieri*, *L. brachypterus*: Fig. 1B, *L. cyaneus*, *L. digitatus*, *L. karsianus*, *L. gorgoni*: Fig. 1F, *L. aureus*, *L. incurvus*, *L. pratensis*, *L. laxiflorus*: Fig. 1C, *L. czechottianus*, *L. aphaca*: Fig. 1A, *L. sphaericus*: Fig. 1E, *L. vinealis*, *L. inconspicua*) or contorted style (*L. roseus*, *L. cassius*, *L. chloranthus*, *L. cicera*: Fig. 1G, *L. sativus*, *L. tuberosus*, *L. variabilis*: Fig. 1I, *L. rotundifolius*, *L. armenus*), all dorsiventrally compressed. In some species of *Lathyrus* styles are spatulate at the apex (*L. alamutensis*, *L. digitatus*: Fig. 1D), *L. karsianus*, *L. gorgoni* (Fig. 1F), *L. sphaericus* (Fig. 1E), *L. tuberosus*, *L. variabilis* (Fig. 1I), *L. armenus* (Fig. 1H)), the other species of this genus and the other genus examined of *Fabeae* are linear. *Lens* species

Table 1. List of taxa examined for Micromorphological study.

Taxon	Source and voucher	Style type
Lathyrus section Orobos <i>Lathyrus vernus</i> (L.) Bernh. <i>Lathyrus aureus</i> (Steven) Brandza <i>Lathyrus incurvus</i> (Roth) Willd.	Iran: Runemark & Mozaffarian 28061 (TARI) Turkey: O. Eyüboğlu 1661 (GAZI) Iran: Azerbaijan: Kazempour 2008-3 (TMUH)	Sle-type Sle-type Sle-type
section Lathyrostylis <i>Lathyrus cyaneus</i> (Stev.) C. Koch. <i>Lathyrus digitatus</i> (M. Bieb) Fior <i>Lathyrus variabilis</i> (Boiss & Ky.) Maly <i>Lathyrus armenus</i> (Boiss. & Huet) Sirj. <i>Lathyrus alamutensis</i> Mozaff. <i>Lathyrus brachypterus</i> Cel. <i>Lathyrus karsianus</i> Davis <i>Lathyrus boissieri</i> Sirj.	Turkey: Siami 3815 (GAZI) Turkey: M. Vural 4033(GAZI) Turkey : Z. Aytaç & H. Duman 4599 (GAZI) Turkey: Aytaç 8317(GAZI) Iran: Mozaffarian, Ahvazi & Charkhchian 88388(TARI) Turkey: Günes 4688(GAZI) Turkey: Davis & Hedge 30762 (GAZI) Turkey: Bani 1944 (GAZI)	Sle-type Sse-type Cse-type Cse-type Sse-type Sle-type Sse-type Sle-type
section Pratensis <i>Lathyrus pratensis</i> L. <i>Lathyrus czechottianus</i> Bassler <i>Lathyrus laxiflorus</i> (Desf.) Kuntze	Iran: Heidari et al. 1803 (WANRC) Turkey: O. Eyüboğlu 1308(GAZI) Iran: Faghihnia, Rafeiee & Zangooei 25516(FUMH)	Sle-type Sle-type Sle-type
section Aphaca <i>Lathyrus aphaca</i> L.	Iran: Faghihnia & Zangooei 25442 (FUMH)	Sle-type
section Linearicarpus <i>Lathyrus sphaericus</i> Retz. <i>Lathyrus vinealis</i> Boiss. & Nöe in Boiss. <i>Lathyrus inconspicus</i> L.	Iran: Joharchi & Zangooei 33235 (FUMH) Iran: Runemark & Foroghi 19640 (TARI) Iran: Faghihnia & Zangooei 34027 (FUMH)	Sse-type Sle-type Sle-type
section Orobon <i>Lathyrus roseus</i> Stev.	Turkey: Aytaç 8157 (GAZI)	Cle-type
section Lathyrus <i>Lathyrus tuberosus</i> L. <i>Lathyrus rotundifolius</i> subsp. <i>miniatum</i> Willd. <i>Lathyrus gorgoni</i> Parl. <i>Lathyrus cicera</i> L. <i>Lathyrus sativus</i> L. <i>Lathyrus chloranthus</i> Boiss. <i>Lathyrus cassius</i> Boiss.	Iran: Alizadeh et al. 5118 (WANRC) Iran: Alizadeh & Ghasempour 4771 (WANRC) Iran: Neamati & Ghaderi 4427(TARI) Iran: Alizadeh et al., 494(WANRC) Iran: Alizadeh & Zangooei 15589 (FUMH) Iran: Kazempour 2008-5 (TMUH) Iran: Neamati & Ghaderi 4542(TARI)	Cse-type Cle-type Sse-type Cle-type Cle-type Cle-type Cle-type
Pisum <i>Pisum sativum</i> L. <i>Pisum fulvum</i> Sibth & Sm. <i>Pisum formosum</i> (Stev.) Alef.	Iran: Faghihnia, Rafeiee & Zangooei 25498(FUMH) Turkey: Adiguzel & Aytaç 1896 (GAZI) Iran, Dizin : Sonboli et al., 2008-1 (TMUH)	Fce-type Fce-type Fce-type
Lens <i>Lens orientalis</i> (Boiss) Hand-Mzt. <i>Lens cyanea</i> (Boiss & Hohen.) Alef.	Iran: Emadzadeh, Memariani & Zangooei 36153 (FUMH) Iran: Joharchi 34755-a (FUMH)	Cne-type Cne-type
Vicia <i>Vicia ervilia</i> (L.) Willd. <i>Vicia hyrcanica</i> Fisch. & Mey. <i>Vicia peregrina</i> L. <i>Vicia sativa</i> L.	Iran: Emadzadeh, Memariani & Zangooei 36169 (FUMH) Iran: Memariani & Zangooei 38986 (FUMH) Iran: Joharchi & Zangooei 16508 (FUMH) Iran: Kazempour 2008-6 (TMUH)	De-type Dabt-type Dabt-type Dabt-type

WANRC: Herbarium of West Azerbaijan Agricultural and Natural Resource Research Center. -FUMH: Ferdowsi University of Mashhad, Herbarium, Mashhad, Iran. -GAZI: GAZI University Herbarium, Turkey. -TMUH: Tarbiat Modares University Herbarium, Tehran, Iran. -TUH: Tehran University Herbarium, Tehran, Iran. -TARI: Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran

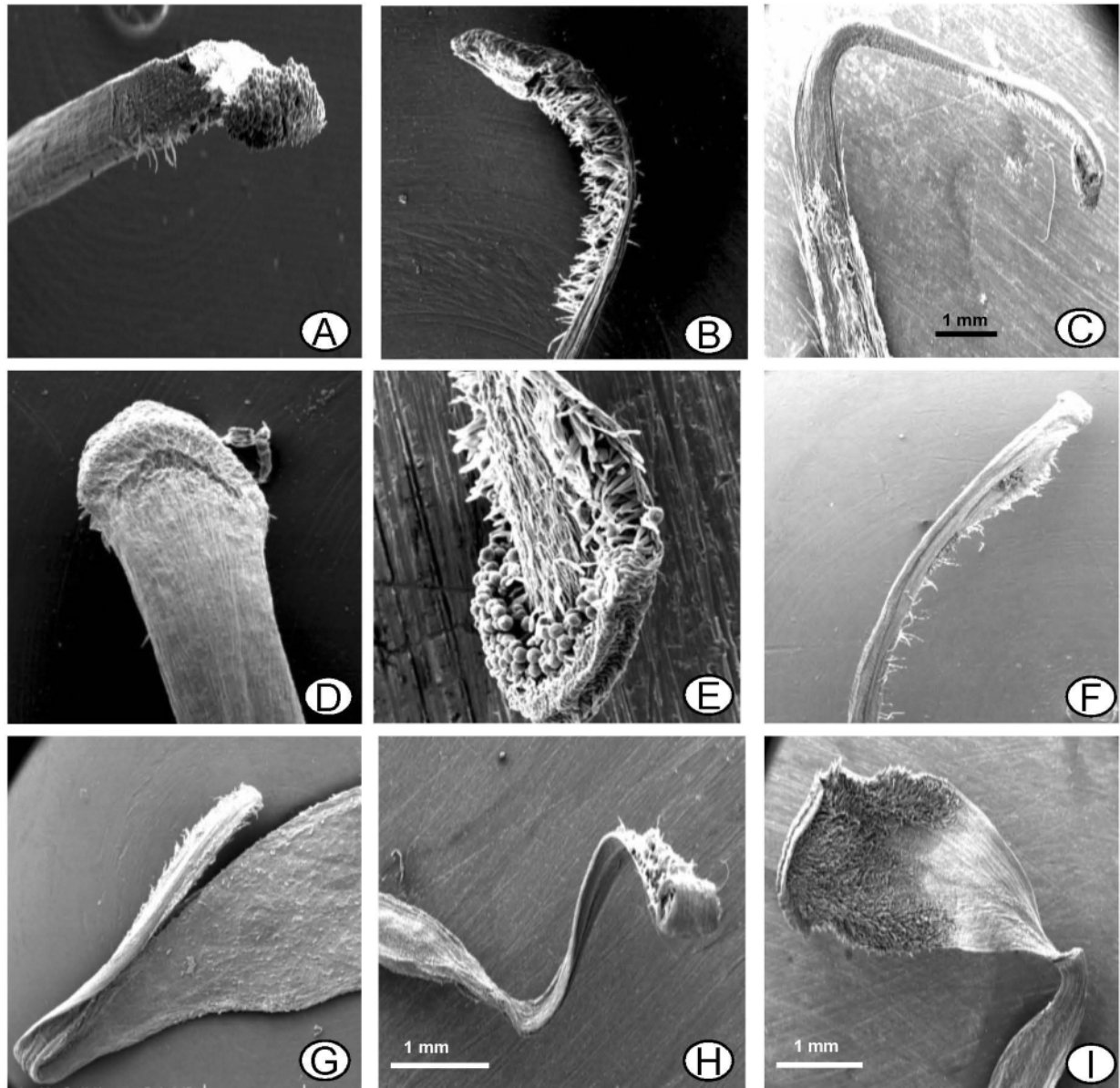


Fig. 1. Scanning Electron micrographs of style features (style shape and style hair pattern) of *Lathyrus*. A, *L. aphaca*. B, *L. brachypterus*. C, *L. laxiflorus*. D, *L. digitatus*. E, *L. sphericus*. F, *L. gorgoni*. G, *L. cicera*. H, *L. armenus*. I, *L. variabilis*.

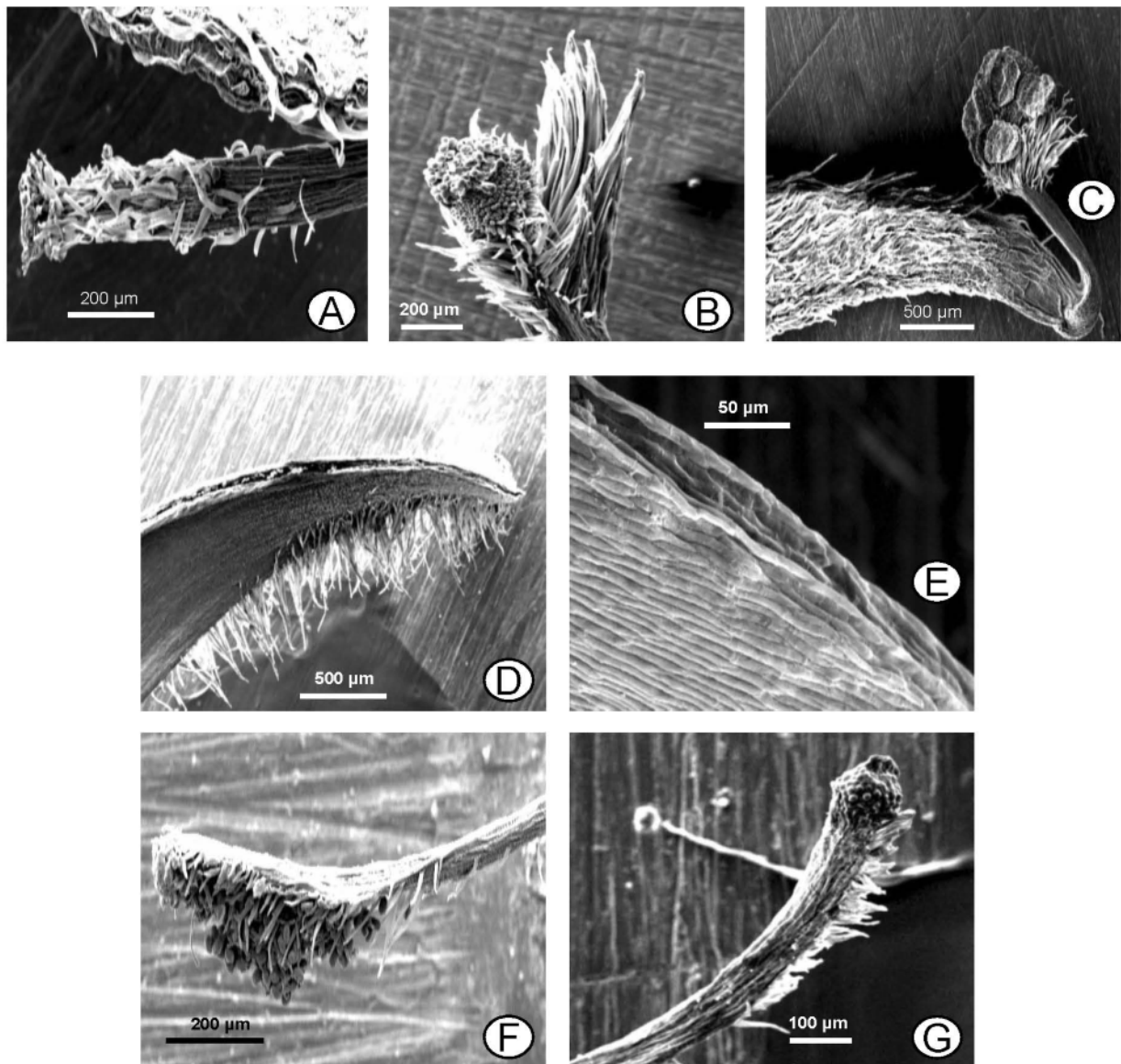


Fig. 2. Scanning Electron micrographs of style features (style shape and style hair pattern) of *Vicia* (A-C), *Pisum* (D-E), *Lens* (F-G). A, *V. ervilia*. B, *V. sativa*. C, *V. peregrina*. D, *P. sativum*. E, *P. formosum*. F, *L. cyanea*. G, *L. orientalis*.

show straight, cylinder and non-compressed style (style in *Lens cyanea* is spatulate at the apex) (Fig. 2F-G). *Vicia* species show dorsiventrally compressed and straight styles (Fig. 2A-C). Longitudinally folded, canaliculate, laterally compressed and straight styles are diagnostic characters of *Pisum* (Fig. 2D-E).

Stylar indumentums. *Pisum* and *Lathyrus* have adaxially evenly hairy styles, but *L. aphaca* and *L. sativus* have slightly hairy style. *Vicia* species have dense ring of stylar hairs (pollen brush) near the stigma.

Two kinds of stylar indumentum were found in *Vicia*: (1) evenly hairy in *V. ervilia* and (2) tufted abaxially hairy in *V. hyrcanica*, *V. sativa* and *V. peregrina*. In *Lens* the examined species possess styles which are evenly hairy at adaxial side.

DISCUSSION

This survey highlights the diversity of style shape and indumentum among genera of tribe *Fabaeae*. *Vicia* with

hairs in both abaxial and adaxial sides (abaxial and adaxial) of styles, *Lathyrus* with dorsiventrally compressed and adaxially hairy styles, *Lens* with non-compressed, cylindrical styles and *Pisum* with longitudinally folded, canaliculated and laterally compressed styles can be distinguished from each other.

In *Lathyrus*, contorted styles are typical in sect. *Lathyrus* (except *L. gorgoni* that style is straight and widened slightly below the stigma) and sect. *Orobon*. This kind of style also occurs in some species of sect. *Lathyrostylis* (*L. variabilis* and *L. armenus*). Our results are in agreement with Kupicha (1983), but as Bassler (1981) mentioned, contorted styles found only in species with broadened styles and is due to lack of space within the keel. The direction of twist is not constant and different specimens of the same species can show contorted or straight styles (Kupicha 1983). All members of the sections *Aphaca*, *Linearicarpus* (except *L. sphaericus* with spatulate style), *Orobus* and *Pratensis* have linear, straight and non contorted styles. Except three species *L. brachypterus*, *L. boissieri* and *L. cyaneus* of sect. *Lathyrostylis*, other species examined of this section as well as two species *L. tuberosus* and *L. gorgoni* (sect. *Lathyrus*) and *L. sphaericus* (sect. *Linearicarpus*) have spatulate styles. The results of this study indicate that, the styler features of *Lathyrus* are diverse and can be classified into four different groups, (1) straight, linear and evenly hairy (Sle-type) (Fig. 1A-C), (2) straight, spatulate and evenly hairy (Sse-type) (Fig. 1D -F), (3) contorted, linear and evenly hairy (Cle-type) (Fig. 1G) and (4) contorted and spatulate and evenly hairy (Cse-type) (Fig. 1H - I). Based on the results presented, the styler features are not in accordance with current sectional classification of *Lathyrus* (Kupicha 1983; Rechinger, 1979). Style characters were not unique in any section of *Lathyrus*, although they seem to provide useful tools at the generic level in the tribe. Endo et al. (2008) and Choi et al., (2006) examined 27 New World and 14 species of *Vicia* regarding style structure, respectively. These species were classified into four styler types. In the present work, two of these four types were surveyed again: (1) dorsiventrally compressed and evenly hairy all round (De-type) (Fig. 2A), (2) dorsiventrally compressed and abaxially tufted (Dabt-type) (Fig. 2B-C).

Lens species examined have straight, cylinder and non-compressed, adaxially evenly hairy style (Cne-type). Apex of style is spatulate in *Lens cyanea* (Fig. 2F) and conical in *Lens orientalis* (Fig. 2G).

Pisum has straight, longitudinally folded, canaliculate, laterally compressed and adaxially evenly hairy style (Fce-type) (Fig. 2D-E). This kind of style is

unique within genus *Pisum* and tribe *Fabeae*. This unique character was recognized as an important synapomorphy for both *Pisum* and *Vavilovia*, and supports their union in one genus (Oskoueian et al. 2010).

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