Comparative seed and leaf micromorphology of *Colutea* species (Fabaceae) from Iran

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Abstract

Light and scanning electron microscopical studies were carried out on seed and leaf surface morphological and micromorphological features of seven legume species belonging to genus *Colutea* L. The different seed types were described, illustrated, compared and their taxonomic importance is discussed. Seed characteristics, particularly exomorphic features, which were shown by scanning electron microscopy, can be used to resolve problems of systematics of species. Considerable variability of seed coats morphology was observed and its implication for species taxonomy is discussed. Within the genus *Colutea* L., species showed different characteristics for seed size and surface, hilum as well as differences in leaf surface characteristics. The study revealed that seed coat ornamentation within the genus shows considerable variation and can be helpful for taxonomic purposes.

Key words: *Colutea*, legume, micromorphology, morphology, scanning microscopy, seed surface.
Abbreviations: SEM, scanning electron microscopy.

Introduction

The Fabaceae is the third largest family of flowering plants with 650 genera including about 18 thousand species. Many of these species are important as food, fodder, wood, ornamentals, and raw materials for industry. A characteristic feature of Fabaceae is capacity to fix the atmospheric nitrogen (N_2) by means of symbiosis with the nitrogen fixing bacteria. Therefore, these plants play an important ecological role. Woody legume seeds often poses impermeable seed coats which require treatment before germination can take place, as it prevents water uptake, gaseous exchange and radicle emergence (Aguinagalde et al. 1990; Baskin, Baskin 1998).

The genus *Colutea* L. (Fabaceae), commonly known as bladder senna, consists of nearly 30 deciduous species found in southern Europe, northeastern and eastern Africa, and western and Central Asia (Browicz 1963; Krussmann 1984; Pijut 2008). *Colutea* includes shrubs and small trees with inflated fruits. There are nine *Colutea* species in Iran; five of them are endemic to the country (Boissier 1872; Ghahremaninejad et al. 2008; Kazempour et al. 2006). The identification and classification of *Colutea* species have always been challenging and difficult, as the species tend to be very similar, especially those with the same flower colour (Pooyan et al. 2014). Micromorphological features of seeds have long been employed as important tools in various scientific studies (Heywood 1971). However, most of the light microscopic features used are concerned with general shape and size rather than details of surface ornamentation (Dhara et al. 2011).

Scanning electron microscopy is a powerful tool to achieve more accurate seed identification, and could be used as a routine technique in study of the spermoderm morphology (Brisson, Peterson 1976). Data on the seed micromorphology of genus *Colutea* are rather limited and mostly confined to papers on other genera of the Fabaceae. The objective of this paper was to provide a detailed account of the seed coat and leaf micro- and macro-morphological features of seven *Colutea* species using light microscopy and scanning electron microscopy (SEM) and to determine the extent to which these seed morphological data can be used as taxonomic characteristics of the species.

Materials and methods

Mature seeds from ripe fruit and young leaves from seven *Colutea* L. species were collected from healthy plants growing in different parts of Iran (Table 1). About 10 leaflets, legums and mature seeds of each species were randomly selected and used for measurements. Mature seeds were collected from dehiscent legums, cleaned with ethylalcohol and dried.

The macromorphological characteristics of the studied seeds were carried out using an Olympus model BX15 light microscope and seed dimensions were measurements and

Table 1. List of the *Colutea* taxa investigated in the present study and herbaria where the vouchers are present (TARI, herbarium of Research Institute of Forests and Rangelands; IAUH, herbarium of Islamic Azad University Avicennia)

Species	Origin, voucher
C. buhsei (Boiss.) Shapar.	Iran: prov. N: Gorgan, 1400m (3871) TARI
C. buhsei (Boiss.) Shapar.	Iran: prov. E: Khorasan, 1550 m, Foroghi (50312) TARI
C. buhsei (Boiss.) Shapar	Iran: prov. S: Ardebil, Khalkhal to Chuli, 1000 m (1), Ferguson, Mirzaei (0000136119) IAUH
C. buhsei (Boiss.) Shapar	Iran: prov. Tehran, 1800 m. Trott, Mirzaei (0000136114) IAUH
C. buhsei (Boiss.) Shapar	Iran: prov. Gorgan, Aliabad, 600 m, Gauba(88858) TARI
C. cilicica Boiss. & Balansa.	Iran: prov. Azerbaijan, Kaleibar, Vinag, 1000 m, Assadi &Wdb, Mirzaei (000013620) IAUH
C. gifana Parsa	Iran: prov. E: Khorasan , Gifan,1300 m, Parsa, Mirzaei (000013628) IAUH
C. gracilis Fryen & Sint.ex Fryen	Iran: prov. N: Gorgan, 2800 m, Mirzaei (0000136111) IAUH
C. persica Boiss.	Iran: prov. Kerman, 2300 m, Mussavi and Tehrani (16256) TARI
C. persica Boiss.	Iran: prov. Fars, Dashte Arzhan, 2200 m, Foroghi (45755) TARI
C. porphyrogramma Rech.f.	Iran: prov. Khorasam, Bojnord, 1350 m, Resh, Mirzaei (000013617) IAUH
C. uniflora G. Beck. ex Stap f.	Iran: prov. Gazvin, 1600 m, Mirzaei (000013621) IAUH

shapes, colours and the positions of hilum were recorded. Density of trichomes on leaf surface, legume size and seed micromorphological features were determined using a SEM model LEO 440i. Measurements with three replications for each of the fruits, seeds and leaves were performed. Each sample was coated with a 550 Å-thick layer of gold in a Polaron SC7610 vacuum coating apparatus for 180 s. Three seeds of each species were measured in average using a Carnoy digital measurement tool (Scholes et al. 2002).

Results

Morphological characteristics and SEM patterns of seed coat and leaf were described in seven taxa of genus *Colutea*. Sunnary of the obtained data are presented in Table 2. It was found that the discriminating seed characters by taxonomic entities were seed size, general shape, location of hilum, legume size and also leaf surface and microscopic texture of seed coat.

Fruit length of the taxa ranged between 20.06 and 50.02 mm, the largest legume was observed in *C. buhsei* and the smallest in *C. porphyrogramma*. The number of seeds varied significantly among the examined species, the highest number of seeds was 24 in *C. buhsei* and the lowest number was seven seeds in *C. porphyrogramma*, while the number of seeds for the other studied species ranged from 10 to 22 (Table 2).

The smallest seeds were found for *C. porphyrogramma* (0.87 mm) and the largest for *C. buhsei* (4.25 mm). Seeds were more or less similar in shapes. Both the colour and shape of seeds were not important in separating different *Colutea* species.

Seed texture characteristics determined using electron microscope images were classified as previously done for other genera of the Fabaceae (Zoric et al. 2010; Gunes, Cirpici 2011). The texture of seeds surface varied between rugosity, foveate and loculate (Table 2). There are four forms of rugosity: open, compact, pitted or coarse (Gontcharova et al. 2009; Al Ghamdi 2011; Teixeira et al. 2013). An opened reticulate surface was observed in *C. gracilis* (Fig. 1 D2), compact seed surface texture in *C. uniflora* (Fig. 1 F2), coarse surface in *C. persica* (Fig. 1 E2), and pitted surface in *C. buhsei* (Fig. 1 A2). Loculated surface texture also has two forms, tabulated as in *C. cilicica* (Fig. 1 B2) and laureated as in *C. gifana* (Fig. 1 C2). A foveated seed surface texture was observed only in *C. porphyrogramma* (Fig. 1 G2).

Leaflet surface in respect to trichomes was classified as downy, floccus, or glabreate. A floccus surface was characteristic for *C. porphyrogramma* (Fig. 1 G3), *C. gifana* had a glabrated surface lacking any trichomes (Fig. 1 C3), and the leaf surface of the other species was downy (Table 2).

Discussion

Seed characteristics, particularly exomorphic features revealed by means of scanning electron microscopy (SEM), have been used in resolving problems of systematics of species (Tobe et al. 1987; Karihaloo, Malik 1994; Koul et al. 2000; Yoshizaki 2003; Javadi 2004) and evolutionary relationships (Heywood et al. 1971; Barthlott 1984; Segarra, Mateu 2002). As the previous studies on other genera of Fabaceae family have shown (Skvortsov, Rusanovitch 1974; Mallick et al. 2003; Johnson et al. 2004; Salimpour et al. 2007; Hosseinzadeh et al. 2008; Al-Ghamdi, Al-Zahrani, 2010; Ozbek et al. 2014; Kahraman et al. 2014) the spermoderm characteristics are genetically determined and are the main source of intra- or interspecific variation.

The present paper is the first report on seed characteristics of genus *Colutea* L. Variation in *Colutea* seed morphology was manifested mainly in seed size, shape, hilum location, as well as seed coat ornamentation.

Table 2. Characteristic features of seed, legum and leaf trichomes of *Colutea* species. R, rugosity; L, loculate; F, foveate; Sub, sub center; Co, cordate; Or, orbicular

Species	Number of seeds	Seed size		Seed shape	Seed colour	Texture	Surface features	Hillum sit	Legume size		Leaflet trichomes
		Length	Width						Length	Width	
C. buhsei	24 ± 3	4.25 ± 0.14	1.70 ± 0.08	co	Dark brown	R	R. pitted	Sub	50.41 ± 3.13	10.48 ± 0.72	Downy
C. cilicica	22 ± 2	4.10 ± 0.33	1.13 ± 0.38	or	Dark and light brown	L	Tabulate	Sub or center	50.02 ± 3.83	20.54 ± 3.88	Downy
C. gifana	12 ± 3	4.11 ± 0.19	1.50 ± 0.12	or	Dark brown	L	Laureate	Sub	50.63 ± 3.36	10.84 ± 1.66	Glabrate
C. gracilis	10 ± 2	4.29 ± 0.25	1.22 ± 0.09	co	Light brown	R	R. opened	Sub	30.56 ± 4.57	10.71 ± 0.88	Downy
C. persica	20 ± 2	3.75 ± 0.43	1.91 ± 0.26	co	Dark brown	R	R. coarsed	Sub	40.81 ± 1.54	10.90 ± 1.16	Downy
C. porphyrogramma	7 ± 2	3.13 ±0.26	0.87 ± 0.07	co	Dark brown	F	Foveate	Sub	20.06 ± 1.45	10.74 ± 0.79	Floccus
C. uniflora	14 ± 3	3.99 ± 0.172	1.46 ± 0.35	or	Dark brown	R	R. compact	Sub	30.85 ± 2.30	10.35 ± 0.29	Downy

Quantitative characteristics associated with seed size contributed most to the total variation. The seed coat patterns were more variable, and six different patterns were distinguished. The seed coat pattern together with seed coat morphological and micromorphological characteristics permitted easy identification of *Colutea* species. The seeds of *Colutea* species showed great variation in testa ornamentations. Seed shape for *C. cilicica* and *C. uniflora* was orbicular, while cordiform in other species. Seed colour had less diagnostic value.

Both fruit (legum) and seed size were important to separating species in the genus *Colutea* (Table 2). Variations in the seed coat patterns at high magnification were generally species-specific. In other words, legum and seed morphological characteristics were helpful in distinguishing various species (Table 2). The results can be used to reveal taxonomic relationships between species of *Colutea*. However, based on the prersent results, the examined characteristics do not provide sufficient information that could be used to distinguish sections of this genus. Seed features do not support the infrageneric classification.

This study on *Colutea* L. seeds showed diversity in shape, dimensions, seed coat surface and leaf surfaces. The SEM study showed high topographic diversity among different species, with unique characteristic for each species (Esau 1977; Moazzeni et al. 2007). This type of study with more species may help to add new information to the knowledge about interspecific relationships in this genus (Werker 1997; Attar et al. 2007).

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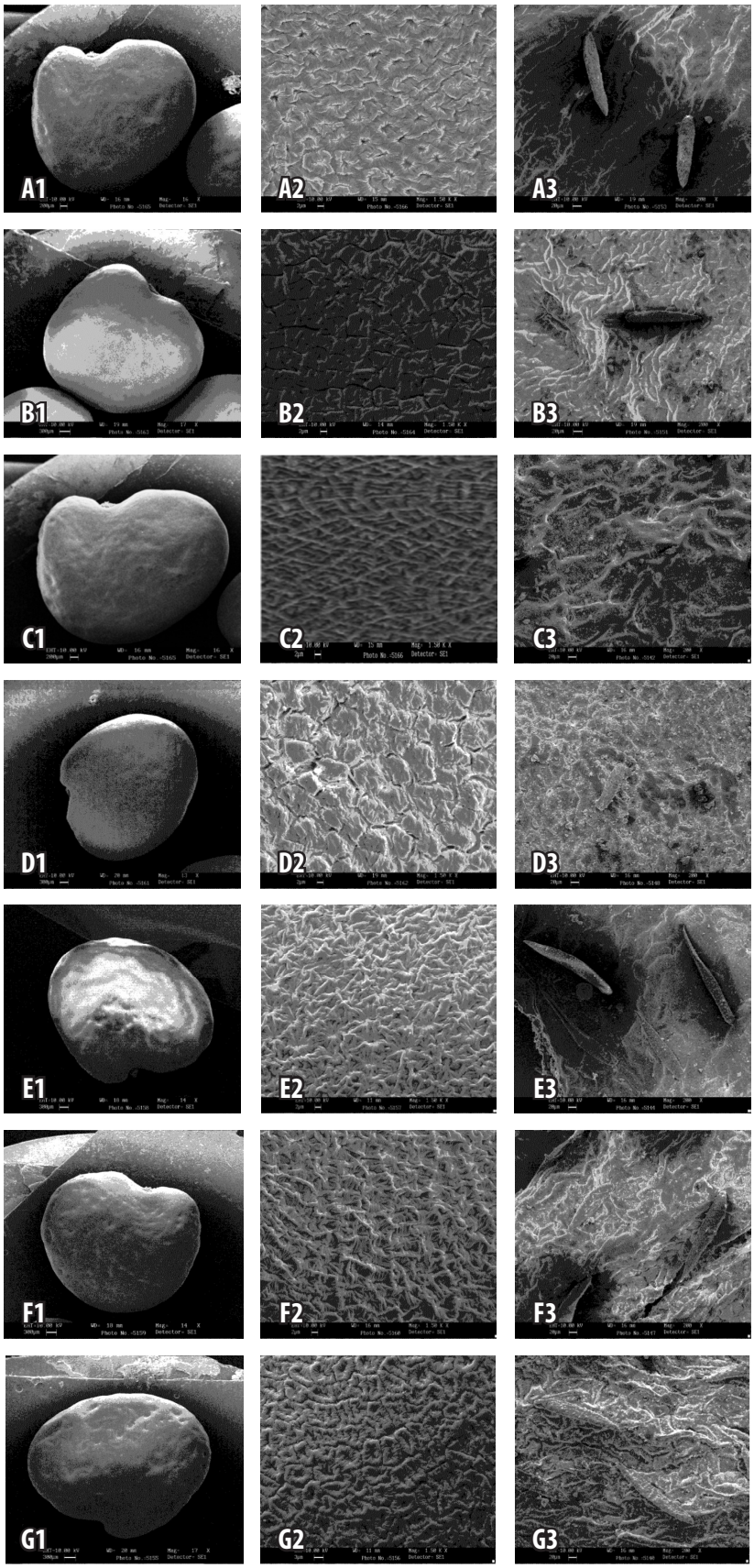

Fig. 1. Scanning electron micrographs of *Colutea* species. Left to right: seeds, details of seed coat surface and leaf surface. A, *C. buhsei*; B, *C. cilicica*; C, *C. gifana*; D, *C. gracilis*, E, *C. persica*; F, *C. uniflora*; G, *C. porphyrogramma*.

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