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ORIGINAL ARTICLE

Seed surface Morphology in some Species of the *Colutea* L. (Fabaceae) in IRAN

Leila Mirzaei*[1], Mostafa Assadi , Taher Nejadsatari [1], Iraj Mehregan
[1] Department of Biology of Basic Science, Tehran Science and Research Branch, Islamic Azad University, Iran
Email: l.mirzaei_2009@yahoo.com
2-Research Institute of Forest and Ranglands, Tehran, Iran

ABSTRACT
The light and scanning electron microscopical studies on seed and leaf surface morphological and micromorphological features of 7 legume species belonging to colutea L. genus of Fabaceae were examined and described in this study. Seed characteristics, particularly exomorphic features, which are revealed through scanning electron microscopy, can be used to resolving problems of systematic of species. Considerable variability of seed coats morphology is reported in the latter species and theirs possible implication for species taxonomy is discussed. However within the Colutea L. genus, the species differ in size, surface seed or leaf and hilum characteristics. The study showed that the seed coat ornamentation، spermoderm pattern can be helpful in identification of species.
Key words :*legume, micromorphology, morphology, scanning microscopy, seed surface.*

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INTRODUCTION

The Fabaceae is one of the third large families within flowering plants and is constituted of 650 genera that include about 18 thousand species. This family includes many important species grown for food, fodder, wood, ornamentals, and raw materials for industry and is characterized by its capacity to actively fix the atmospheric nitrogen (N2) by means of symbiosis with the nitrogen fixing bacterium Rhizobium For this reason, they play important role in nature. Besides, woody legume seed often poses impermeable seed coats which require treatment before germination can take place so their seeds are characterized by extremely tough and impermeable seed coats that hinder germination, since they prevent water entering, gaseous exchanges and radical emission [1, 2] .The genus *Colutea* L. (Fabaceae), commonly known as bladder senna, is composed of near 30 deciduous species and found in southern Europe, northeastern and eastern Africa, and western and Central Asia [3,4,5,6].

Colutea L. is a small genus that includes shrubs or small trees with inflated fruits. The genus includes nine species in Iran; five of them are endemic to the country [7, 8, 9, 10] .The identification, classification and subdivision of *Colutea* species have always been challenging and difficult as the species tend to be very similar, especially those with the same flower colour [11]. Micromorphological features of seeds have long been employed as important tools in various scientific studies. However, most of the light microscopic features used are concerned with general shape and size rather than details of surface ornamentation [12]. Brisson and Peterson [13] mentioned that the Scanning electron microscopy provides great tool to achieve more accurate seed identification, which could be used as a routine technique in study of the Spermoderm morphology.

Data on the seed micromorphology of *Colute*a genus are rather limited and mostly confined to papers on other genus of fabaceae. The aim of the present study is identifying characterize seed coat structure in some species for first time that *Colutea* genus distributed in the Iran and search for micromorphological diagnostic characters that may help to elucidate species relationships in the plant sections.

MATERIAL AND METHODS

Seeds were obtained from collected mature fruits in Iran. The fields, Seed characters of 7 species belonging to *Colutea* L. genus were studied using freshly collected mature seeds (Table 1). Observations were made on thirty randomly selected seeds of each species . Mature seeds were collected from dehiscent legumes, then cleaned with alcohol and kept to drying. The macro morphological characters of the studied seeds were carried out using seed dimensions measured by using an Olympus (model BX15) light microscope and measurements, shapes ,colours and the positions of hilum were recorded. In order to observe the density of trichome on the leaf surface**,** Leaflet number, legume size, and micro-morphological data were obtained using SEM (LEO 440i). Each sample was coated with 550Å-thick layer of gold in a Polaron SC7610 vacuum coating apparatus for 180s. Three seeds of each species were measured in average using Carnoy ,a digital measurement tool [14].

RESULTS AND DISCUSSION

Morphological characters and SEM patterns of seed coat were analyzed in 7 taxa of *Colutea*. Some of the obtained data are presented in Table 2. It was found that the discriminating seed characters by taxonomic entities can be defined as: seed size and general shape, location of hilum, legume size and also leaf surface and microscopic texture of coat seed. Fruits length of other taxa ranged between (1.5cm -7.5 cm) the largest legume in *C.buhsei* and the smallest legume in *C. porphyrogramma* (1.5 cm). The number of seeds varies greatly among examined taxa, the highest number of seeds is 24 seeds in *C.buhsei* and the lowest number is 7 seeds in *C. porphyrogramma* while the numbers of seeds in the rest of the studied species are ranged from 10- 22 seeds (Table 2).

The character of Seeds surface texture and Seeds ornamentation can be considerable diagnostic and systematic value. The texture of seeds surface varies from Rugosity, Foveate and Loculate (Table 2). There are four forms of Rugosity, it may be opened, compoact , pitted or coarsed. Opened reticulate surface presents in *C.gracilis* (Fig 1- D_2), compact seeds surface texture exists in *C.uniflora* (Fig 2- F_2), coarsed surface appeared in *C.persica* (Fig 2- E_2), and pitted surface presents in *C.buhsei* (Fig 1- A_2).The Loculated surface texture also has two forms, it may Tabulated form in *C.cilicica* (Fig 1- B_2) and Laureated that exists in *C.gifana* (Fig 1- C_2) .The Foveated seed surface texture also is appeared only in *C.porphyrogramma* (Fig 2- G_2).The smallest seed is those of *C.porphyrogramma* [3.23 mm] and the largest are those of *C.buhsei and C.persica* (4.25 mm). Seeds are more or less similar in shapes. The colour and Shape of fruits are unimportant to separate among *Colutea* L. species. In this study leaflet surface trichomes was observed that the 3 case include: 1-Downy, 2-Floccus, 3-Glabreate that floccus exists in *C.porphyrogramma* (Fig2- G_3), *C.gifana* was glabrate (Fig1-C_3) and in other species surface were downy. Fainally in more species the number of leaflet trichomes were sparsity and only *C.gifana* miss any trichomes on leaflet that supports with morphology traits (Table 2). According to Skvortsov and Rusanovitch [15] the spermaoderm characteristics are genetically determined and are the main source of intra- or interspecific variation. Lersten [16] stated that the spermaoderm pattern reflects epidermal configuration and cuticular deposition as influenced by seed expansion. Gutterman and Heydecker [17] demonstrated that day length affects seed coat structure while Sharma et al. [18] concluded that edaphic factors are responsible for that difference.

Fruit size lather than Seed sizes are important to separate among species in the genus *Colutea* L. From the above it can be seen that a clear cut distinction can be made among taxa based on the main external seed morphology (Table 2). The variations observed in the seed coat patterns at high magnification were generally species-specific. In other word, Legum and Seed morphological characters were helpful in distinguishing various species. According the present study supports the use of seed morphological characters as a parameter for species identification. The results suggest both a close relationship between different species of *Colutea* L. because there are different types of seed surfaces, seed shapes and size and morphology similar each other from traditional species.

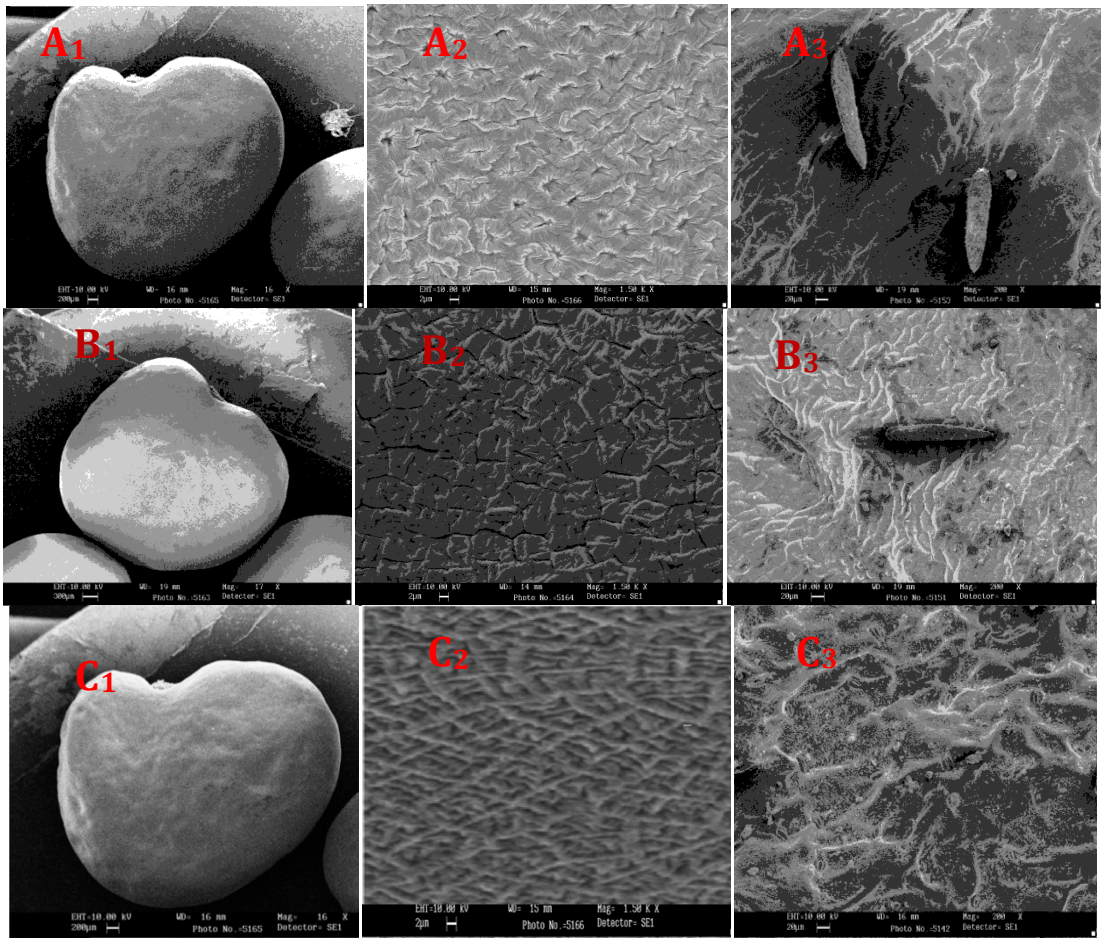
Various seed morphological studies of leguminous taxa have been performed from time to time [19,20,21,22,23,24,25].The this study supports the *Colutea* L. seeds display diversity in shape, dimensions and seed coat surface and leaf surfaces and the SEM study revealed seed coat remarkable topographic diversity among different species, to be characteristic of each species[26,27]. This kind of study with more species may help to open a frame work of our knowledge about interspecific relationships in the genus [28, 29]. The present study provided some useful characters of seed for infrageneric classification and also for delimiting species. Light microscopic features supplemented with SEM proved to be a great tool to achieve more accurate seed identification, as previously suggested by Brisson and Peterson [13]. This method can be used as a routine technique in the study of spermoderm morphology [30, 31].

Table1 .List of taxa investigated in our analysis and herbaria where the vouchers (TARI= herbarium of Research Institute of Forests and Rangelands, IAUH= Islamic Azad University Avicennia herbarium).

Species	**Origin, voucher**
Colutea buhsei (Boiss.) Shapar.	Iran: prov. N : Gorgan, 1400m, (3871 TARI).
Colutea buhsei (Boiss.) Shapar.	Iran: prov. E : Khorasan, 1550 m, Foroghi (50312 TARI) .
Colutea buhsei (Boiss.) Shapar	Iran: prov. S : Ardebil, Khalkhal to chuli, 1000m (1), Ferguson , Mirzaei (0000136119) IAUH.
Colutea buhsei (Boiss.) Shapar	Iran : prov. Tehran, 1800 m. Trott, mirzaei (0000136114)IAUH .
Colutea 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, 20800 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.

Table 2. Characteristic features of the seeds

Species	Seed shape	Seed size (mm) T W L	Texture	Surface features	Seed colur	Hillum Situation	Number seed	Leaflet trichom	Legume size (µm) W L
Colutea persica	cordate	1.91 3.78 4.25	Rugosity	R. coarsed	Dark brown	Sub center	20	downy	9.09 38.15
Colutea cilicica.	orbicular	1.13 3.57 4.13	Loculate	Tabulate	Dark and light brown	Sub center or center	22	downy	15.43 43.21
Colutea buhsei.	cordate	1.7 3.83 4.25	Rugosity	R. pitted	Dark brown	Sub center	24	downy	14.89 54.18
Colutea uniflora.	orbicular	1.46 3.92 3.99	Rugosity	R. compact	Dark brown	Sub center	14	downy	13.59 51.55
Colutea porphyrogramma	cordate	0.87 1.64 3.11	Foveate	Foveate	Dark brown	Sub center	7	floccus	17.42 53.60
Colutea gracilis.	cordate	4.29 1.22 3.30	Rugosity	R.opened	Light brown	Sub center	10	downy	17.18 35.68
Colutea gifana.	orbicular	4.11 1.50 3.53	Loculate	Laureate	Dark brown	Sub center	12	glabrate	18.12 42.33



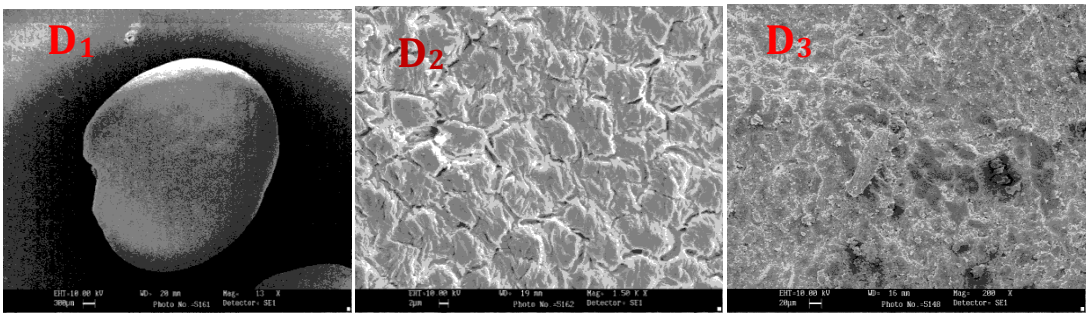


Figure 1. Scanning electron micrographs of *Colutea* L. of left to right seeds, details of seed coat surface and leaf surface respectively. A :*C.buhsei* ; B: *C.cilicica*; C:*C.gifana*; D:*C.gracilis*.

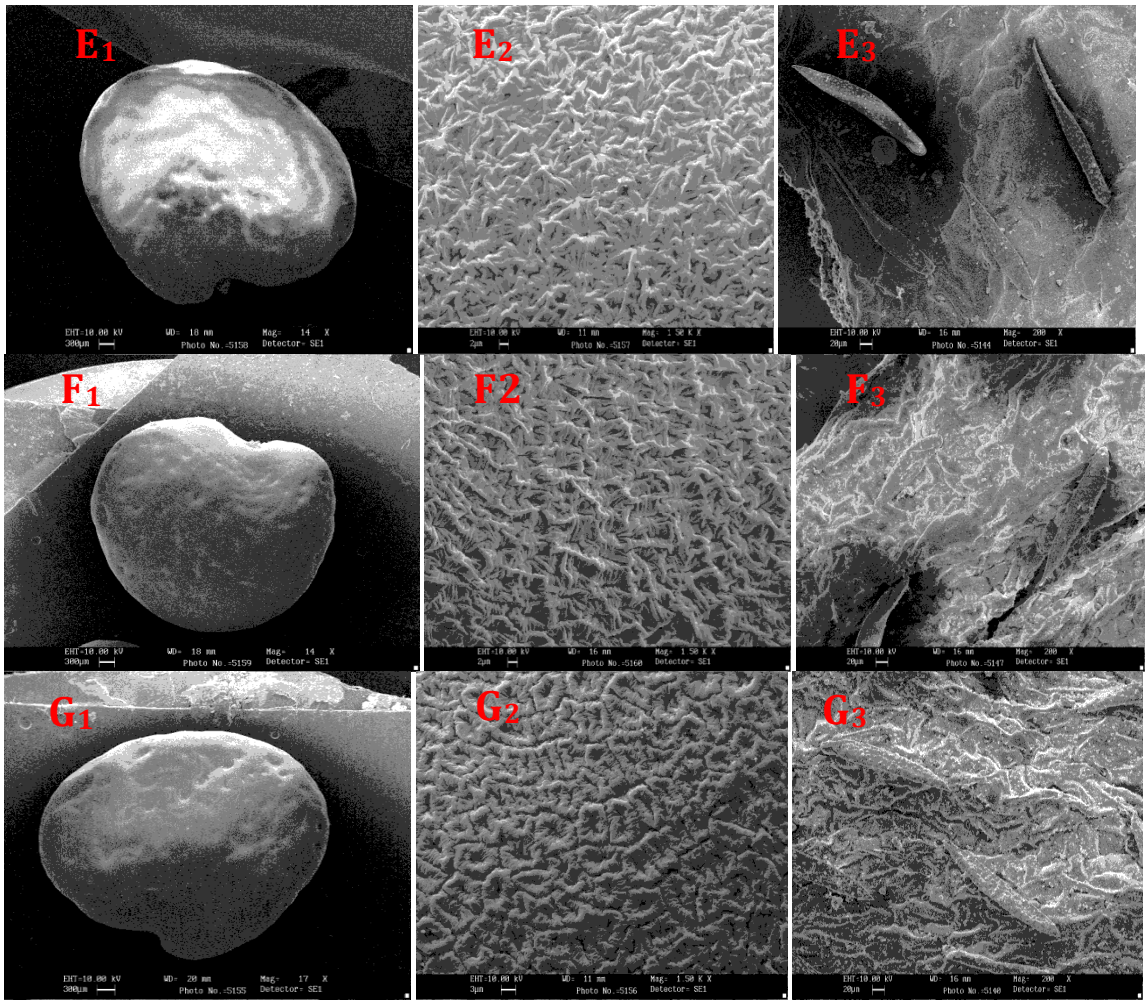


Figure2. Scanning electron micrographs of *Colutea L.* seeds and details of seed coat surface and leaf *surface receptively. E: C.persica ;* F: C. *uniflora;* G:*C.porphyrogramma.*

Results suggest both a close relationship between different species of *Colutea* L. because there are different types of seed surfaces, seed shapes each other from traditional species in this study, and seed morphology and micromorphology were studied in order to verify relationships within species groups.

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