A note on seed coat and plumule morphological variation in the genus Cicer L. (Fabaceae)

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A note on seed coat and plumule morphological variation in the genus *Cicer* L. (Fabaceae)

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Abstract

Seed coat morphology and plumule shape of dormant stage in *Cicer* species, involving wild annuals and perennials, were studied using scanning electron and stereoscopic microscopy. Three types of basic ornamentation patterns in seed coat surface, reticulate, tuberculate and granulate-papillate, were recognized in 13 species of *Cicer*. Six plumular types, PI, PII, PIII, PIV, PV and PVI, were determined among 17 species of *Cicer*. Character state analysis on molecular tree provides not clear-cut relationship among species for two traits, however, commonly sharing character states were partly found. Seed coat and plumular features were suggested to have taxonomic value for this genus. These analyses provide information for understanding of the close relationship among particular species of the genus *Cicer*.

Key Words: *Cicer* L., Fabaceae, morphology, plumule, seed coat.

Introduction

Cicer L. (Fabaceae) consists of about 43 species which are distributed in central and western Asia, Europe, and isolated areas of north, northwest Africa (Van der Maesen, 1987). The annual chickpea, *C. arietinum* L., is a well-known cultivated species and is the third most important legume crop. Other species are important sources of animal feed and traditional human usage as well as agents of soil fertility. Conventionally, cultivated chickpea is divided into two types, 'Kabuli' and 'Desi.' 'Kabuli' is predominantly distributed in Mediterranean countries and the Near East. 'Desi' prevails in the eastern and southern parts of the distribution area of the crop (Van der Maesen, 1972; Zohary and Hoph, 1993). Phylogenetic analysis on wild relatives of crops is fundamental for understanding of their systematic usage as well as plant genetic resources of crop improvement.

The genus has been traditionally classified into four sections: *Cicer* (=*Monocicer* M.G. Popov), *Chamaecicer*, *Polycicer*, and *Acanthocicer* (Van der Maesen, 1972, 1987). However, there are discrepancies on the intraspecific classification of the genus *Cicer* (Javadi and Yamaguchi, 2004a). In order to resolve phylogenetic relationship of the genus *Cicer* at intra- and interspecific relationship, deep evaluation of sufficient traits is necessary.

The importance of ultrastructural pattern analysis of the seed coat observed under the scanning electron microscope (SEM) and visible morphological comparison of seed plumule features at dormant stage has been well recognized as a reliable approach for assessing phenetic relationship and identification of species or taxa (Heywood, 1971; Barthlott, 1981; Tobe et al., 1987; Yoshizaki, 2003). The SEM studies of the seed coat in the *Brassica*, *Linaria* and *Cyanea* assist to resolve phylogenetic relationship obtained by the other methods (Koul et al., 2000; Buss et al., 2001; Sergarra and Mateu, 2001). In the genus *Cicer*, although the seed features have been studied for transferring *Cicer* from the tribe Vicieae to the monogeneric

tribe Cicereae (Kupicha, 1977; Lersten and Gunn, 1981, 1982) and for understanding the interspecific relationship among wild species and cultivars (De Leonardis et al., 1996; Javadi and Yamaguchi, 2004a), very few annual and perennial species have been examined in such studies. On the other hand, morphological studies of plumule feature, which may provides information for understanding species relationship, have not been studied in the genus *Cicer*.

This work was initiated with the objectives of using seed coat pattern and plumule feature to identify taxa and to resolve their phylogenetic relationship.

Materials and Methods

Seed samples from a total of thirteen and seventeen species belonging to four sections were examined in seed coat pattern and plumule variation, respectively (Table 1). Since very few seeds were available for some species, they examined just for scanning electron microscopy. All the examined seeds were harvested from plants which were grown in a green house, the introduced materials from the international gene banks or herbarium specimens (Table 1)

For SEM studies, dry mature seeds were mounted on aluminum stubs using silver paste and coated with Pt-Pd using a sputter coater (Hitachi E102 Ion Sputter). Morphological observations were carried out using a scanning electron microscope (Hitachi S-800) and photographed at different magnifications. For evaluation of uniformity, the ventral and dorsal sides of seed were scanned. Each side of two seeds was examined in species and cultivars.

For observation of plumule features, seeds were scarified and soaked in water for about 3 hours at room temperature, the seed coat were removed, and then two cotyledons were separated carefully. Morphological features of undamaged complete plumule were examined under

Table 1. List of taxa examined.

Taxon / cultivar group	Section	Life cycle	Accession No.	Origin	Remarks
C. arietinum/Kabuli *	*Cicer*	Annual	ICC 11234	Afghanistan	P
			ICC 10350	Turkey	P
C. arietinum/ Desi*	*Cicer*	Annual	PI 595983	Turkey	P
			PI 315796	India	P
*C. reticulatum**	*Cicer*	Annual	PI 572537	Turkey	P
C. echinospermum	*Cicer*	Annual	PI 527930	Turkey	P
C. pinnatifidum	*Cicer*	Annual	PI 599109	Turkey	P,S
*C. judaicum**	*Cicer*	Annual	PI 599104	Lebanon	P,S
C. bijugum	*Cicer*	Annual	PI 599037	Syria	P
C. yamashitae	*Cicer*	Annual	PI 510664	Afghanistan	P,S
*C. cuneatum**	*Cicer*	Annual	PI 458554	Ethiopia	P,S
C. chorassanicum	*Chamaecicer*	Annual	PI 458553	Afghanistan	P
C. kermanense	*Polycicer*	Perennial	NPGBI	Iran	P,S
C. oxyodon	*Polycicer*	Perennial	PI 561103	Turkey	P
C. subaphyllum	*Polycicer*	Perennial	TARI	Iran	S
C. anatolicum	*Polycicer*	Perennial	PI 563626	Turkey	P
C. songaricum	*Polycicer*	Perennial	PI 599035	Uzbekistan	P,S
C. nuristanicum	*Polycicer*	Perennial	PI 604497	Pakistan	S
C. canariense	*Polycicer*	Perennial	PI 557453	Spain (Canary Island)	P
C. microphyllum	*Polycicer*	Perennial	PI 599093	India	P,S
			PI 532928	Pakistan	P,S
C. multijugum	*Polycicer*	Perennial	PI 599085	Uzbekistan	P,S
C. montbretii	*Polycicer*	Perennial	PI 599091	Turkey	P
			PI 599090	Turkey	S
C. tragacanthoides	*Acanthocicer*	Perennial	TN 41-5261	Iran	S
C. stapfianum	*Acanthocicer*	Perennial	TARI	Iran	S

ICC, Internaional Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India; PI, United States Department of Agriculture (USDA), Plant Introduction Station, USA;TARI, Central Herbarium of IRAN. TN, National Plant Genebank (NPGBI), Iran.
*, Harvested seeds were used in analyses. P, Specimen used for plumule features; S, Specimens used for scanning electron microscopy.

Table 2. Terminology in order to describe the seed surfcae patterns (after Murley 1951).

Term	Description
Reticulate	Having a raised network of narrow and sharply angled lines frequently presenting a geometric appearance, each area outlined by a reticulum being an interspace
Tuberculate	With small smooth rounded projections or knobs
Aculeate	Bristly with small pointed projections
Granulate	Knob-like elevations on the surface, grain-like in appearence

photo stereoscope (Nikon SMZ1500). The terminology of seed-coat surface sculpturing and morphological features of seed plumule in the present study follows Murley (1951) (Table 2), Jackson (1971), Barthlott (1981), and Stearn (1983).

Results and Discussion

The salient features of the ventral features and testa topographies of seed in *Cicer* species examined are presented in Tables 3 and 4. Among the thirteen species of *Cicer* investigated, seeds were triangular, ovate, heart-shaped, globular and cordate in outline. The seed size varied from 1.19 mm in *C. montbretii* to 6.19 mm in *C. songaricum*. The lack of seed beak was only observed in *C. cuneatum* which is not common in the genus *Cicer*. The variation in shape of hilum and lens was observed among *Cicer* species, i.e. the shape of lens is cordate, circular, subcircular and subovoid. (Fig. 1, Table 3).

The seed surface sculpture could be divided into three different patterns: reticulate, tuberculate, and granulate-papillate (Fig. 2, Tables 4, 5). Most species represented the reticulate type, five species tuberculate, and two species granulate-papillate (Tables 4, 5). The twelve taxa with the two basic types, i.e. reticulate and tuberculate types, were further characterized and distinguished by the generally species-specific variation in microsculpturing features (Tables 4, 5). A network of raised lines was a common feature in the reticulate type, however, the species varied with regard to the alignment and shape of network, and the architecture of interspace enclosed by raised lines (Fig. 2, Table 4). For instance, among the species with reticulate pattern, *C. subaphyllum* indicated spiny junction of cell wall (Fig. 2 d); *C. kermanense* with tubercle or flat junction (Fig. 2 f). Furthermore, distinct differences were existed in the nature of undulation on the seed coats of *C. stapfianum* and *C. kermanense*. Unlike *C. stapfianum*, the undulations in *C. kermanense* appeared in much elevated (Fig. 2 e, f). On the other hand, *C. judaicum* has foveate secondary sculpture of cell walls which is distinctly different from other species with reticulate testa pattern (Fig. 2 b). Similarly, in the species representing tuberculated, species-specific variation existed in density and type of projections, i.e., *C. songaricum* has high density of acuminate projections, while *C. nursitanicum* indicated low density of acuminate projections (Fig. 2 i, j). *C. cuneatum* showed density of conical projections which has different pattern among the tubercualted species (Fig. 2 h, Table 5). *C. montbretii* indicated a garnulate-papillate pattern (Fig. 2 m).

The plumule variation among the seventeen examined species could be divided into six major types (Fig. 3, Table 6). Type PI has widely spiral form of compound leaf and two adnate or separated stipular parts in plumule. Type PII, narrowly spiral form of compound leaf and two relatively close stipular parts in plumule. Type PIII, short dentate form of compound leaf and two adnate stipular parts in plumule. Type PIV, long dentate form of compound leaf and two adnate stipular parts in plumule. Type PV, bitten form of compound leaf and two adnate stipular parts in plumule. Type PVI, two short stipular parts surrounded the compound leaf in plumule.

Three different types of seed coat patterns and seven types of plumule feature were observed for 21 species (13 and 17 species, respectively) as well as seed coat texture of 9 examined species from previous study (Javadi and Yamaguchi, 2004a). The species included under a particular seed coat group or plumule type shared the same basic pattern. However, most could be distinguished easily on the basis of variation in microsculpturing and feature details.

Table 3. A comparison of ventral side of seed features in *Cicer* species.

Taxa	Section	Seed* size (LxW)	Outline	Seed base	Beak	Hilum flush	Hilum shape	Lens	Lens shape	Ridge on lens
C. judaicum	*Cicer*	3.20 x 2.79	Triangular	Bilobate	Straight beak	Nearly surface	Circular	Raised	Cordae	Present
C. pinnatifidum	*Cicer*	3.62 x 3.52	Ovoid	Bilobate	Straight beak	Nearly surface	Circular	Raised	Circular	Absent
C. yamashitae	*Cicer*	4.20 x 3.70	Heart-shaped	Bilobate	Incurved beak	Sunken	Circular	Raised	Cordate	Absent
C. cuneatum	*Cicer*	3.36 x 3.50	Globular	Nobilobate	Without beak	Sunken	Circular	Raised	Circular	Present
C. kermanense	*Polycicer*	5.01 x 4.59	Obovate	Nobilobate	Incurved beak	Sunken	Ovoid	Raised	Subovoid	Present
C. songaricum	*Polycicer*	6.19 x 4.83	Obovate	Subbilobate	Incurved beak	Sunken	Elliptic	Flat	Subcircular	Present
*C. subaphyllum***	*Polycicer*	5.00 x 4.00	Obovate	Nobilobate	Incurved beak	Sunken	Elliptic	Raised	Subcordate	Present
C. multijugum	*Polycicer*	5.11 x 4.60	Triangular	Subbilobate	Incurved beak	Sunken	Elliptic	Raised	Subcircular	Present
C. microphyllum	*Polycicer*	4.56 x 3.79	Subglobular to ovate	Subbilobate	Incurved beak	Nearly surface	Circular	Flat	Subcircular	Present
C. montbretii	*Polycicer*	1.19 x 1.23	Nearly globular	Subbilobate	Straight beak	Sunken	Circular	Raised	Subovoid	Absent
*C. nuristanicum***	*Polycicer*	5.50 x 5.50	Ovate-cordae	Subbilobate	Incurved beak	Nearly surface	Elliptic	Raised	Circular	Present
C. macracanthum	*Acanthocicer*	4.40 x 3.16	Elongated-ovate	Nobilobate	Incurved beak	Nearly surface	Circular	Flat	Subcircular	Present
C. stapfianum	*Acanthocicer*	5.00 x 3.00	Obovate	Nobilobate	Incurved beak	Sunken	Circular	Flat	Subcircular	Present
C. tragacanthoides	*Acanthocicer*	3.67 x 3.03	Globular	Nobilobate	Incurved beak	Nearly surface	Circular	Raised	Subcircular	Present

*, Average of five seeds (mm); **, seed size from monograph of Van der Maesen (1972).

Plumule type (PVI) of *C. bijugum* not share by the other taxa. Such species-specific pattern helped their identification.

The character state of seed coat pattern and plumule features present on phylogenetic tree of cpDNA *trn*T-F sequences (Javadi and Yamaguchi, 2004b) provides evolutionary implications (Fig. 4). Although present materials included restricted number of species, character state of plumule features reveals a specific pattern on the phylogenetic tree. For example, Type PIV and Type PV are synapomorphy among the members of the African clade and among the members of the annual and perennial clade, respectively. Type PI is in common among the subclade II (*C. arietinum*, *C. reticulatum* and *C. echinospermum*) and Type PIII in two members of subclade I (*C. pinnatifidum* and *C. judaicum*) (Fig. 4)

The two types of domesticated species, 'Desi' and 'Kabuli,' share the plumule Type PI, however they indicated different texture patterns of the seeds (Javadi and Yamaguchi, 2004a). Since *trn*T-F sequences data revealed no variation between 'Desi' and 'Kabuli' types (Javadi and Yamaguchi, 2004b), it seems that plastome evolves too slowly to expect any divergence over the time frame of domestication.

Among the wild species, *C. reticulatum* and *C. echinospermum* indicated similar plumule type with cultivated species, *C. arietinum*. Our data support the previous hypothesis (i.e. Ladizinsky and Adler, 1975; Tayyar and Waines, 1996; Sudupak et al., 2002; Javadi and Yamaguchi, 2004a) in which *C. reticulatum* and *C. echinospermum* were thought to be the closest wild annual species to *C. arietinum*.

It is noteworthy that the papillate pattern of seed texture which is not common in the genus *Cicer* apparently occurs in *C. montbretii*. Papilate pattern of seed coat is typical in Vicieae. It is therefore thought that there is linkage relationship between the members of the genus *Cicer* and Vicieae. On the other hand, beakless seed was observed in *C. cuneatum*. However, the prominent beak is one of the characteristics of the genus *Cicer* (Lersten and Gunn, 1981). Therefore, we suggest that reconsideration of morphological features and taxonomical classification is necessary in the genus *Cicer*.

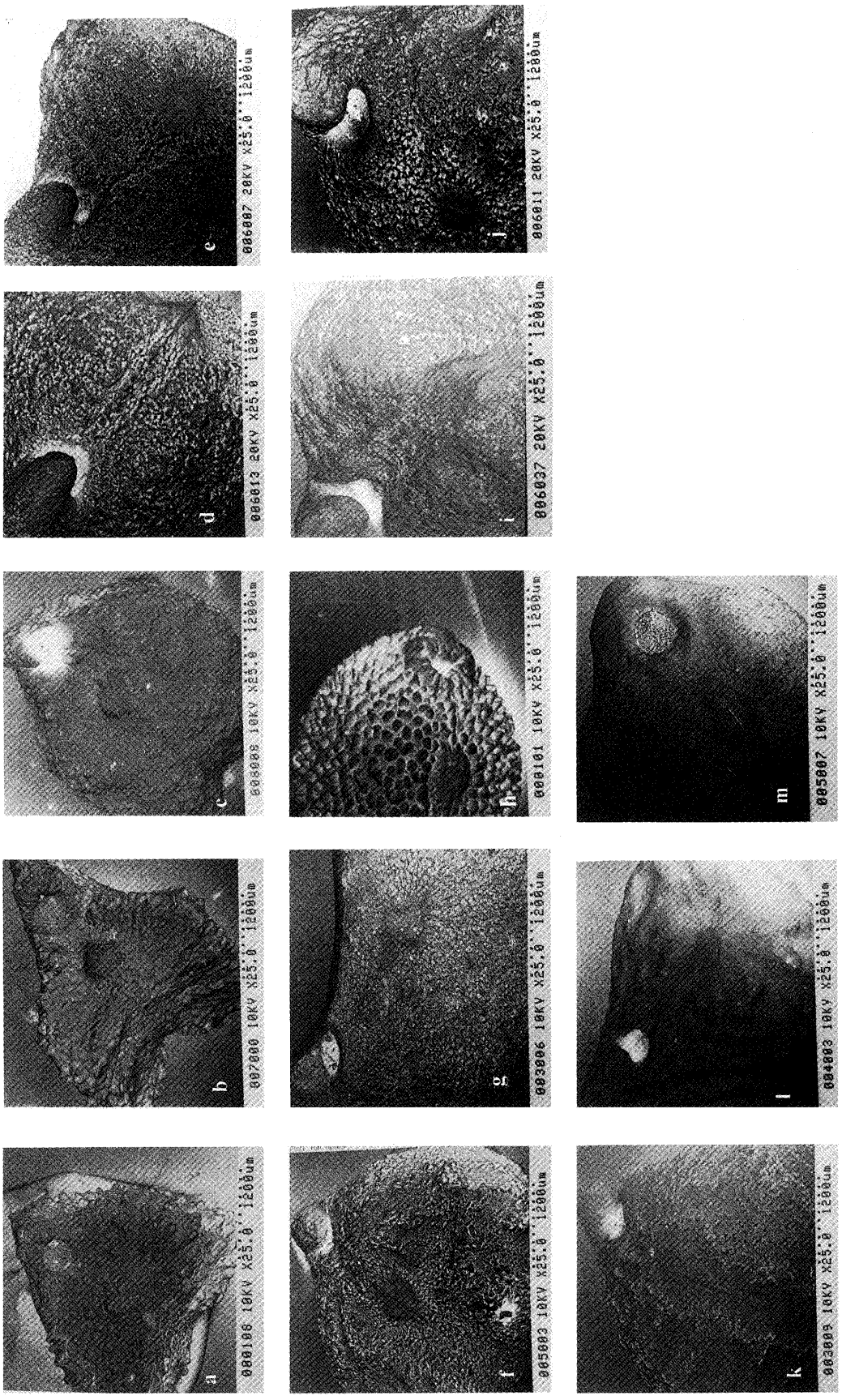

Fig. 1. SEM micrographs of seed ventral side
a, *C. pinnatifidum*; b, *C. judaicum*; c, *C. yamashitae*; d, *C. subaphyllum*; e, *C. stapfianum*; f, *C. kermanense*; g, *C. tragacanthoides*; h, *C. cuneatum*; i, *C. songaricum*; j, *C. nuristanicum*; k, *C. multijugum*; l, *C. microphyllum*; m, *C. montbretii*.

Table 4. Microsculpturing features in taxa showing reticulate type of seed coat pattern.

Taxa	Microsculpturing features												
	Reticulum			Reticulum wall					Interspace				
	Compact	Lax	5-6 sided	Reticulate	Smooth	Undulated	Broken	Raised	Lineolate	Channel	Sunken	Spiny junction	Tubercle junction
C. judaicum	-	-	+	-	+	-	+	-	-	+	-	-	-
C. pinnatifidum	-	+	-	+	+	-	-		+	-	+	-	-
C. yamashitae	+	-	-	-	+	-	-	-	+	-	-	-	-
C. kermanense	-	+	-	-	-	+	+	+	+	-	-	-	+
C. subaphyllum	-	+	-	-	-	-	+	+	+	-	-	+	-
C. stapfianum	-	+	-	-	-	+	+	-	-	-	-	-	+
C. tragacanthoides	-	+	-	-	+	-	+	+	+	-	-	-	+

Table 5. Microsculpturing features in taxa with tuberculate type of seed coat pattern.

Taxon	Basic pattern	Microsculpturing						
		Projections						
		Density			Shape			
		Without projection	Low	High	Aculeate	Conical	Wrinkle	Pitted
C. nursiatnicum	Tuberculate	-	+	-	+	-	-	-
C. songaricum	Tuberculate	-	-	+	+	+	-	-
C. cuneatum	Tuberculate	-	-	+	-	+	-	-
C. microphyllum	Tuberculate	+	-	-	-	-	+	-
C. multijugum	Tuberculate	+	-	-	-	-	-	+

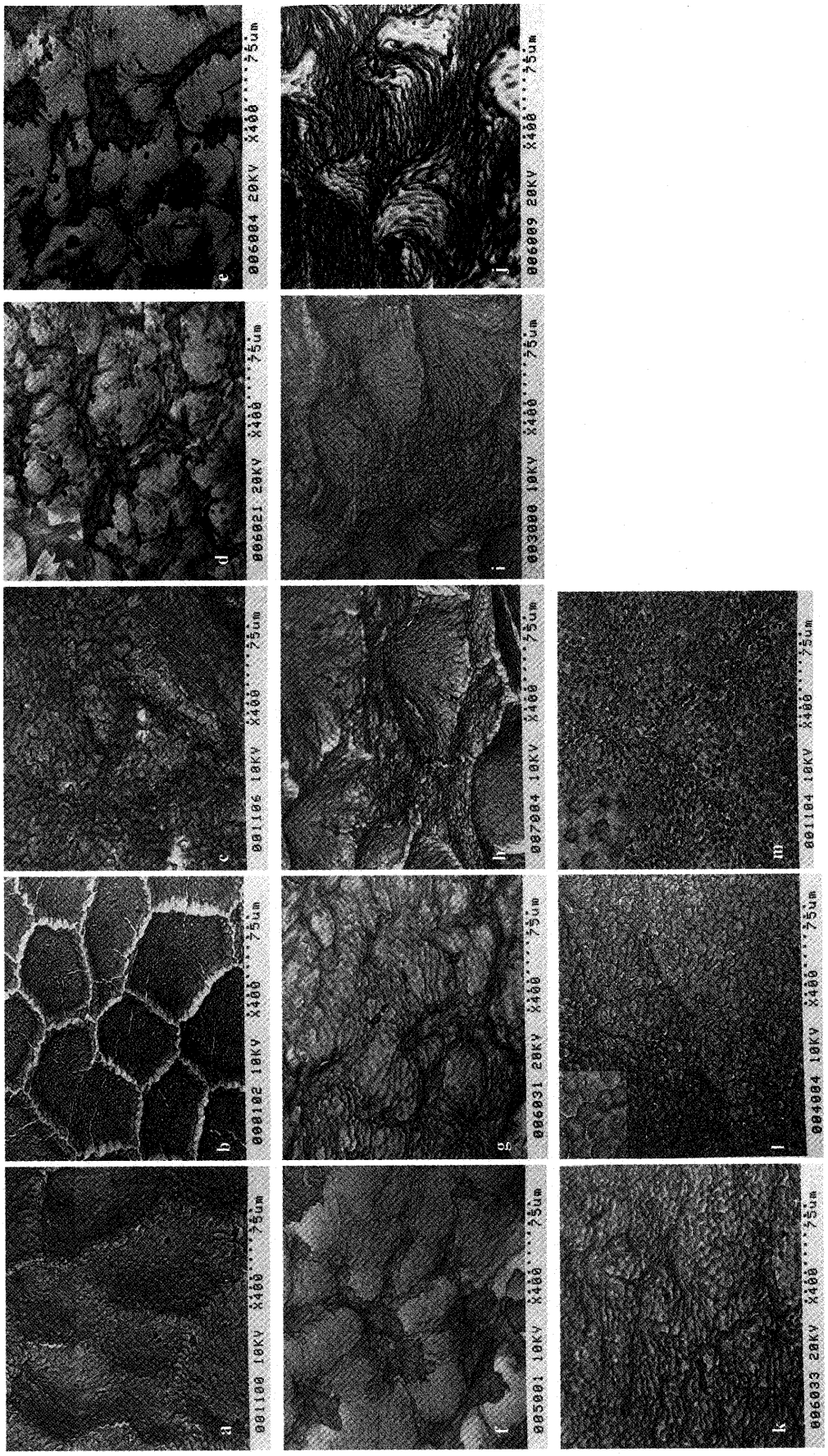

Fig. 2. SEM micrographs of seed dorsal side

a, *C. pinnatifidum*; b, *C. judaicum*; c, *C. yamashitae*; d, *C. subaphyllum*; e, *C. stapfianum*; f, *C. kermanense*; g, *C. tragacanthoides*; h, *C. cuneatum*; i, *C. songaricum*; j, *C. nuristanicum*; k, *C. multijugum*; l, *C. microphyllum*; m, *C. montbretii*.

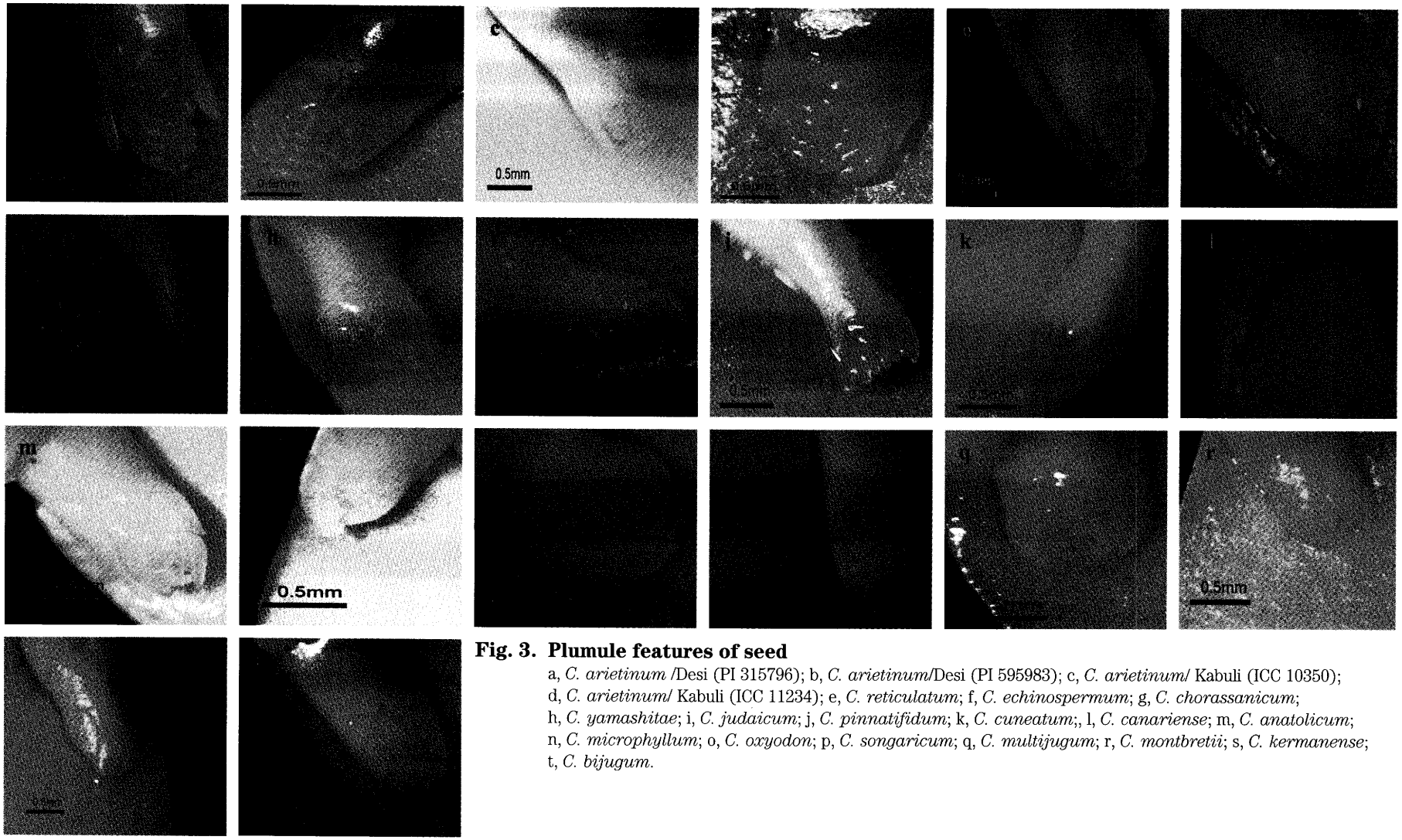

Fig. 3. Plumule features of seed

a, *C. arietinum* /Desi (PI 315796); b, *C. arietinum*/Desi (PI 595983); c, *C. arietinum*/ Kabuli (ICC 10350); d, *C. arietinum*/ Kabuli (ICC 11234); e, *C. reticulatum*; f, *C. echinospermum*; g, *C. chorassanicum*; h, *C. yamashitae*; i, *C. judaicum*; j, *C. pinnatifidum*; k, *C. cuneatum*;, l, *C. canariense*; m, *C. anatolicum*; n, *C. microphyllum*; o, *C. oxyodon*; p, *C. songaricum*; q, *C. multijugum*; r, *C. montbretii*; s, *C. kermanense*; t, *C. bijugum*.

Table 6. Distribution of different plumule features in *Cicer* species.

Type					
PI	PII	PIII	PIV	PV	PVI
C. arietinum	*C. yamashitae*	*C. pinnatifidum*	*C. cuneatum*	*C. anatolicum*	*C. bijugum*
C. reticulatum	*C.chorassanicum*	*C. judaicum*	*C. canariense*	*C. kermanense*	
C. echinospermum				*C. oxyodon*	
				C. microphyllum	
				C. multijugum	
				C. songaricum	
				C. montbretii	

The present study supports the use of seed coat patterns and plumule features as parameters for species identification and resolution of species-relationship. Therefore, this kind of study with more species may help to open a framework of our knowledge about interspecific relationship in the genus *Cicer*.

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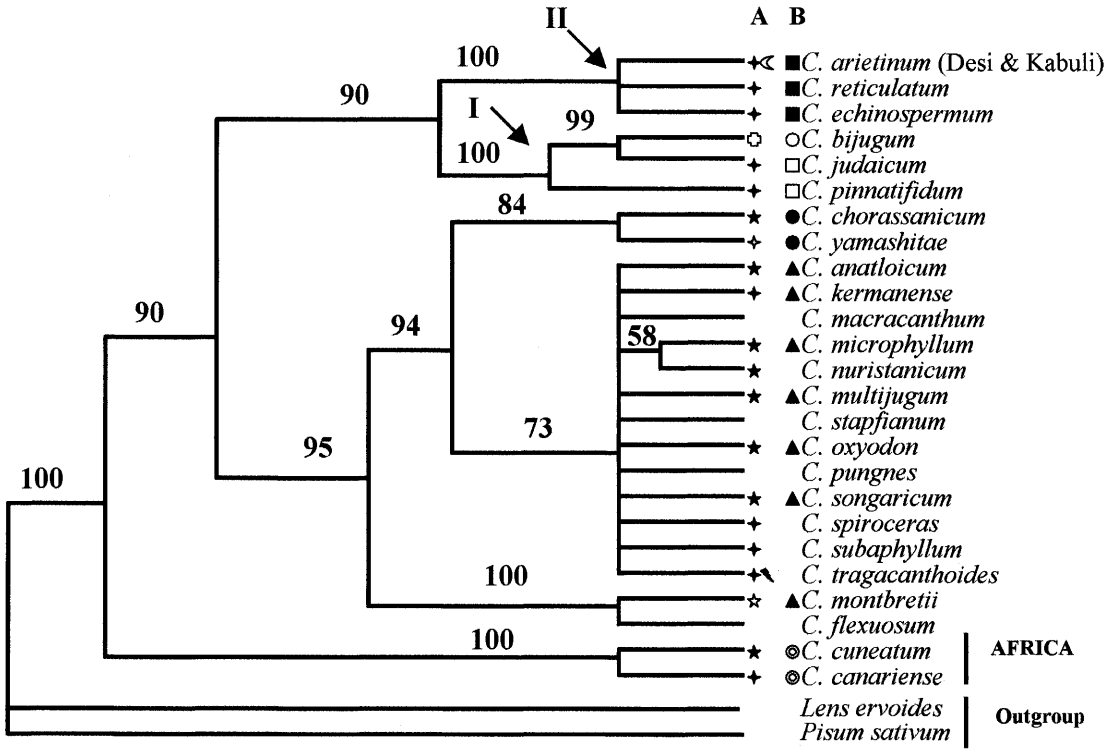

Fig. 4. Character state on the strict consensus phylogenetic tree of *trn*T-F sequences (Javadi and Yamaguchi, 2004b). Numbers above branch indicate bootstrap value (%). A, seed coat pattern (partly included in Javadi and Yamaguchi, 2004a): ✦, reticulate; ★, tuberculate; ☆, granulate-papillate; ᗧ, mammelonate-plate. ○, plumule type: ■, PI; ●, PII; □, PIII; ◎, PIV; ▲, PV; ○, PVI.

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