Seed and pollen study of taxa belonging to the genus *Eruca* Miller in relation to some characters present in species of the genus *Diplotaxis* DC.

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The authors analyzed the seeds and pollen of three taxa belonging to the genus *Eruca* Miller (*E. pinnatifida* (Desf.) Pomel f. *aurea* (Batt.) Maire, *E. sativa* Miller, *E. vesicaria* (L.) Cav. using OM and SEM. The study of morphobiometric data identified three morphotypes and an identification key was elaborated. The length of the polar axis of the pollen grains and the length of the seeds represent the primary characters for distinguishing the taxa of *Eruca* and those belonging to the genus *Diplotaxis* observed in a previous study (De Leonardis *et al*., 2002). Further characters are the thickness of the sexine (more accentuated in *Diplotaxis*) and the different disposition of the seed coat reticulum. Moreover, based on the palynological and seminal characters found the close affinity between *Eruca vesicaria* and *E. sativa* confirmed the systematic placing proposed by Tutin *et al*. (1964) who placed them in *E. vesicaria* (L.) Cav. ssp. *vesicaria* and *E. vesicaria* (L.) Cav. ssp. *sativa* (Miller) Thell. respectively. The two taxa had a greater affinity with respect to *E. pinnatifida* f. *aurea* that is nearer to the group of taxa belonging to the genus *Diplotaxis* as can be seen from the examination of the dendrogram.

Key words**:** pollen, seed, *Eruca*, *Diplotaxis*.

Introduction

The name "rocket" is normally used to indicate botanical species belonging to the genera *Eruca* Mill. and *Diplotaxis* DC. of the Brassicaceae. These are annual and perennial herbs that were known during the Roman period for their aphrodisiac properties (Fernald, 1993) and whose leaves have been traditionally used both in cooking and in pharmacopoeia due to their antiphlogistic, astringent, depurative, diuretic digestive, emollient, tonic, and laxative properties (Tonzig, 1941; Beijerink, 1947; Garnier, 1961; Arietti, 1965; Uphorf, 1968; Ellison *et al*., 1980; Maugini, 1973; Tomaselli, 1974; Balbé, 1978; De Capite, 1984; Gastaldo, 1987; Mascagno, 1987; Anonymous, 1988 and 1991; Biagi & Sperone, 1988; Anzalone, 1989; De Feo & Senatore, 1993).

Over the last few years studies have investigated the vitality and germinability of their seeds, gametophytic selection, etc. which could be useful to improve and/or safeguard the genetic diversity of numerous species belonging to the family of *Brassicaceae* that, for a long time, have been underused (Morinaga, 1934; Labana *et al*., 1977; Gorini, 1979; Arora &

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Lamba, 1980; Goth & Webb, 1980; Lamba & Arora, 1981; Des & Lal, 1982; Matsuzawa & Sarashima, 1986; Mascagno, 1987; Kanthaliya *et al*., 1990; Hammer et al., 1992; Anonymous, 1993). Various contributions were presented at the workshop in Padova (A.A.V.V., 1996) on the "rocket" species examining genetic resources, cross-pollination, cultivation and the world status of the "rocket" species. Genebank collections were also made (Gatersleben (IPK), Braunschweig (FAL) in Germany; IdG in Italy; Wellsbourne (HRI) in UK; Iowa (USDA) in USA to safeguard the species belonging to the two genera.

In this study the authors examined the seeds and pollens from three taxa of the genus *Eruca*, viz. *E. pinnatifida* (Desf.) Pomel f. *aurea* (Batt.) Maire, *E. sativa* Miller, and *E. vesicaria* (L.) Cav.) to characterize the morphology and biometry, as well as to correlate these data with those already available relevant to the genus *Diplotaxis* (De Leonardis *et al*., 2002) so as to identify further diacritic characters between the two genera.

Materials and Methods

The anthers were acetolysed according to Erdtman (1960) and conserved in a glycerine: water solution (1:1). Pollen and seeds were observed using a Zeiss standard LM with 100x objective (N.A. 1.30) equipped with 10x eyepieces and a stereomicroscope Galileo equipped with an ocular micrometer respectively.

For the observations by SEM (JEOL-JSM 35) pollens and seeds were washed, dehydrated, dried by critical point, affixed to alluminium stubs with double stick and coated with gold (100Å) using an Emiscope SC 500.

Pollens grains were designated using the terminology of Erdtman (1969 and 1971) and De Leonardis *et al*. (1986), while seeds were designated following the works of Stearn (19739, Berggren (1981). The seed colour was scored according to Kornerup & Wanscher'manual (1978). Seed material of the 3 taxa belonging to the genus *Eruca* was provided by Professors Gómez-Campo and Martínez Laborde of the Polytechnic University of Madrid, Spain. Full names of species used in the text are those adopted by the institutions providing the germplam. The material (seed and pollen) is preserved at the Department of Botany, University of Catania, Italy.

Abbreviations adopted in the text:

P = polar axis; **E** = equatorial axis; **Pc** = measure colpus in polar direction; **Ec** = measure colpus in equatorial direction; **Psex** = sexine thickness in polar view; **Pnex** = nexine thickness in polar view; **Esex** = sexine thickness in equatorial view; **Enex** = nexine thickness in equatorial view; **Mes** = mesocolpium; **LTP** = polar triangular side; **Plum** = diameter lumen in polar view; **Elum** = diameter lumen in equatorial view; **Pmu** = polar muri thickness; **Emu** = equatorial muri thickness; **L** = lenght; **W** = width; **T** = thickness.

Population codes reported in the dendrogram: *Eruca pinnatifida* f. *aurea*: P01, P02, P03, P04; *E. vesicaria*: V05, V06, V07, V08; *E. sativa*: S09, S10, S11, S12.

As concerns the correlation with the taxa belonging to the genus *Diplotaxis* the species that had the widest biometric variability intervals were considered: *Diplotaxis muralis* (L.) DC. (D13); *D. catholica* (L.) DC. (D14); *D. brachycarpa* Godr. (D15) and *D. viminea* (L.) DC. (D16).

Results

The pollen was characterized by isopolar radiosymmetric monads, with three longitudinal colpal apertures and with the thickness of the sexine and nexine constant. According to the typing present in the Brassicaceae described by De Leonardis *et al*. (1989) the polar lumina are of the finely reticulate-mediumreticulate type while the equatorial ones include the medium reticulate-reticulated types in *E. pinnatifida* f. *aurea*, finely reticulate-medium reticulate in *E. vesicaria*, finely reticulate-reticulate in *E. sativa*.

The seeds showed a tegument characterized by a reticulum with poorly prominent walls in *E. pinnatifida* f. *aurea* and prominent in *E. sativa* and *E. vesicaria*.

Based on the morphological and biometric characters, both palynological and seminal, it is possible to define three morphotypes.

1. *Eruca pinnatifida* f. *aurea* type

Pollen (μm)	Seed (mm)
P = 18(19)20	Shape: ovate
E = 18(20)21	Side outline: elliptical
Pc = 12(14)16	Transversal outline: tightly elliptical
Ec = 2.0(2.6)3.0	Radicular shape: 0.25 wide
Psex = 0.67(0.86)0.90	Radicular extremity: as long as the cotyledoonous, subobtuse
Pnex = 0.67(0.70)0.90	Cotyledonous extremity: subobtuse
Esex = 0.6780.88)0.90	Basal cut: very evident
Enex = 0.67(0.85)0.90	Radicular and cotyledonous furrow: very evident
Mes = 11(12)13	Hilum and micropile: covered with a wide wing of funicular tissue
LTP = 3.6(4.0)4.5 circular lumina	Tegument: regular reticulum, poco prominente with
Plum = 0.45(0.50)0.67	Colour: egg yellow
Elum = 0.67(0.80)1.3	L = 1.40(1.47)1.55
Pmu = 0.45	W = 0.90(0.99)1.05
Emu = 0.45	T = 0.50(0.55)0.60

2. *Eruca sativa* type

P = 16(18)20	Shape: from ovate to widely elliptical
E = 17(18)19	Side outline: widely elliptical
Pc = 11(12)13	Transversal outline: from subcircular to subrhombic
Ec = 1.8(2.3)2.7	Radicular shape: 0.30 wide
Psex = 0.67(0.76)0.90	Radicular extremity: as long as the cotyledoonous, curved, subacute
Pnex = 0.67(0.73)0.90	Cotyledonous extremity: subobtuse
Esex = 0.90	Basal cut: very evident
Enex = 0.90	Radicular and cotyledonous furrow: very evident
Mes = 10(10.3)11	Hilum and micropile: covered with funicular tissue
LTP = 3.6(4.5)5.4 lumina	Tegument: regular reticulum, prominente with subcircular
Plum = 0.45(0.50)0.67	Colour: from yellow brown to green olive
Elum = 0.45(0.60)1.10	L = 1.35(1.44)1.50
Pmu = 0.45	W = 0.95(1.04)1.10
Emu = 0.45	T = 0.75(0.80)0.85

3. *Eruca vesicaria* type

P = 17(19)20	Shape: from widely elliptical to widely ovate
E = 16(19)20	Side outline: widely elliptical
Pc = 12(13)15	Transversal outline: subcircular
Ec = 1.8(2.2)2.7	Radicular shape: 0.30 wide
Psex = 0.67(0.73)0.90	Radicular extremity: as long as the cotyledoonous, subacute
Pnex = 0.67(0.73)0.90	Cotyledonous extremity: subobtuse
Esex = 0.67(0.73)0.90	Basal cut: very evident
Enex = 0.67(0.72)0.90	Radicular and cotyledonous furrow: very evident
Mes = 9(10.7)11	Hilum and micropile: covered with funicular tissue
LTP = 3.6(4.2)4.5 lumina	Tegument: irregular reticulum, prominente with irregular
Plum = 0.45	Colour: from yellow brown to green olive
Elum = 0.45(0.50)0.67	L = 1.30(1.35)1.45
Pmu = 0.45	W = 0.80(0.83)0.90
Emu = 0.45	T = 0.70(0.77)0.85

Key for pollen and seed (including *Diplotaxis* speciea):

1.a. Pollen with a polar axis >20 μm, equatorial sexine thickness > 1.3 μm, seed length ≤ 1.20 mm ..... *Diplotaxis* spp

b. Pollen with a polar axis ≤ 20 μm, equatorial sexine thickness ≤ 0.90 μm, seed length > 1.30 mm 2

2.a. Pollen with a mesocolpium width11-13 μm, seeds with transversal outline tightly elliptical, tegument with poorly prominent reticulum walls ..... *E. pinnatifida* f. *aurea* type

b. Pollen with a mesocolpium width119-11 μm, seeds with different tran-sversal outline, tegument with prominent reticulum walls 3

3.a. Pollen with equatorial thickness of the sexine and nexine 0.67-0.90 μm, seed width 0.80-0.90 mm, subcircular transversal outline, irregular lumina outline ..... *E. vesicaria*

b. Pollen with equatorial thickness of the sexine and nexine (0.9 μm), seed width 0.95-1.10 mm, from subcircular to subrhombic transversal outline, with subcircular lumina ..... *E. sativa*

Discussion

From the palynological point of view there are many studies on the *Brassicaceae* family but these refer only to the taxa isolated without an exhaustive palynological reading with respect to that of other families (e.g. Kapp, 1969; Arobba, 1976; Aytug *et al*., 1971; Faegri & Iversen, 1975; Perez De Paz, 1977; Moore & Webb, 1978; Rollins & Banerjee, 1979; De Leonardis *et al*., 1984, 1986a, 1989, 1989a and 1990; Lahham & Al-Eisawi, 1987).

In particular, as regards the genus *Eruca*, Díez (1987) included in the type *Raphanus raphanistrum* the pollen of *E. sativa* characterized by a lumina of the reticulate type (1.0-1.5 μm) and by polar and equatorial axes of 15-22 μm and 15-19 μm respectively. El Naggar (1988) on samples of *E. sativa* from Egypt reported axes, polar and equatorial, of 33 μm and 17 μm respectively, reticulum of the microreticulate type with a lumina < 1.0 μm and wall

thickness of 0.7 μm. Our data confirm, in part, those of the two above mentioned authors though with notably different values, presumably average, of the polar axis reported by El Naggar. If we compare, palynologically, the biometric and morphological data of the three taxa of *Eruca* with those observed in different species of the genus *Diplotaxis* (De Leonardis *et al*., 2002) the primary character in the distinction of the two genera is the length of the polar axis that in *Eruca* is in the range 16-20 μm with an average value of 19 μm, while in *Diplotaxis* it is 20-31.5 μm with average values in the range 22-27 μm. Really the distinguishability would be greater if we took into consideration *D. muralis* with polar axes of 31-38 μm. This species (n=21 e n=22 in Pignone & Galasso, 1995), considered a hallopolyploid among *D. tenuifolia* (n= 11) and *D. viminea* (n=10) (Harberd & McArthur, 1972), has a greater dimensional increase with respect to the parent species as well as a dimensional dimorphism, presence of tetracolpate granules, tendancy of an irregular distribution of the colpi, and aborted granules.

Persano Oddo & Ricciardelli D'Albore (1989) defined 450 melissopalynological types belonging to 133 families and, limited to only Cruciferae, they distinguished *Brassica* f. < 20 μm, *Brassica* f. 20-25 μm, *Brassica* f. > 25 μm, *Sinapis* f. and *Matthiola*. For the values of the polar axis that we observed in taxa belonging to the genus *Eruca* (16-20 μm) it would be opportune to correct the scale of the intervals.

Furthermore, the thickness of the sexine (1.3 –2.2 μm) more accentuated in *Diplotaxis* represents a further character for distinguishing between the two genera.

If we consider the evolutive tendency that is the most accepted in palynology (Punt, 1967, 1971 and 1976; Walker & Doyle, 1975; De Leonardis *et al.* 1999) we can hypothesize a more evolved status in *Diplotaxis* with respect to the genus *Eruca*, this event is due to the increased dimensions of the pollen grains, the greater length of the colpi, the thickness of the exine, the dimensions of the lumina and the thickness of the muri. However, Blangiforti & Venora (1996), from the karyotypic investigation of *E. vesicaria* ssp. *sativa* (Mill.) Thell., *E. vesicaria* ssp. *pinnatifida* (Desf.) Emb. and Maire and *Diplotaxis tenuifolia* (L.) DC. (all with n=22 in agreement with Warwick and Anderson, 1993), hypothesize a status relatively more evolved for the two taxa of *Eruca* with respect to *D. tenuifolia*.

Little has been done relative to the study of the ornamentation of the tegument of the seeds belonging to the genus *Eruca*. Tegument of Brassicaceae, has been found to be a useful intergenus and/or infraspecies diacritic character with a evolutive tendency. In one study on the genus *Sinapis* and *Brassica nigra* De Leonardis & Fichera (1994) showed how this character is a useful element both for the evaluation of the taxonomic placing of the species and for the diagnosis for the sophistication of the seeds.

From the morphological and anatomical examination of 90 genera and 200 species of this family Vaughan & Whitehouse (1971) observed in *E. vesicaria* ssp. *vesicaria* and *E. vesicaria* ssp. *sativa*, a seed length of 2.0 mm and a slightly reticulate tegument in the former and a length of 2.2 mm and a smooth tegument in the latter. However, if the biometeric data reported by Vaughan & Whitehouse (1971) are slightly greater than those observed by us, the observation of the morphology of the tegument that we carried out by SEM is more detailed showing a common reticulate model in the three taxa. In particular, in *E. pinnatifida* f. *aurea* it is regular and poorly prominent with a lumina clearly circular, in *E. vesicaria* it is irregular and prominent with an irregular lumina and in *E. sativa* it is regular and prominent with a subcircular lumina.

From the comparison of the seeds of *Eruca* and *Diplotaxis* we found a greater seed length in *Eruca* (1.30-1.55 mm) that clearly allows it to be distinguished from those belonging to the genus *Diplotaxis* (0.60-1.20 mm). Furthermore, the seeds of *Diplotaxis* have a tegument

with a reticulum that ranges from irregular to rugulate with muri from subexagonal to very irregular and clearly distinguishable at the base of the reticulum. Between the three taxa of *Eruca* that we observed that the irregular reticulum *E. vesicaria* has the most affinity for the group of *Diplotaxis*. *Eruca vesicaria* and *E. sativa* have a clear morphostructural affinity both for pollen and seeds with respect to *E. pinnatifida* f. *aurea*, confirming the systematic placing proposed by Tutin *et al.* (1964) with *E. vesicaria* (L.) Cav. ssp. *vesicaria* and *E. vesicaria* (L.) Cav. ssp. *sativa* (Miller) Thell.

If we observe the dendrogram (Fig. 1) elaborated on the basis of 16 samples (4 for each taxon), it appears clear both the morphobiometric uniformity of each taxon in the 4 populations and the greater palynological and seminal affinity between *E. vesicaria* and *E. sativa* with respect to *E. pinnatifida* f. *aurea* and, at the same time, the great affinity of the latter for the taxa belonging to the genus *Diplotaxis*.

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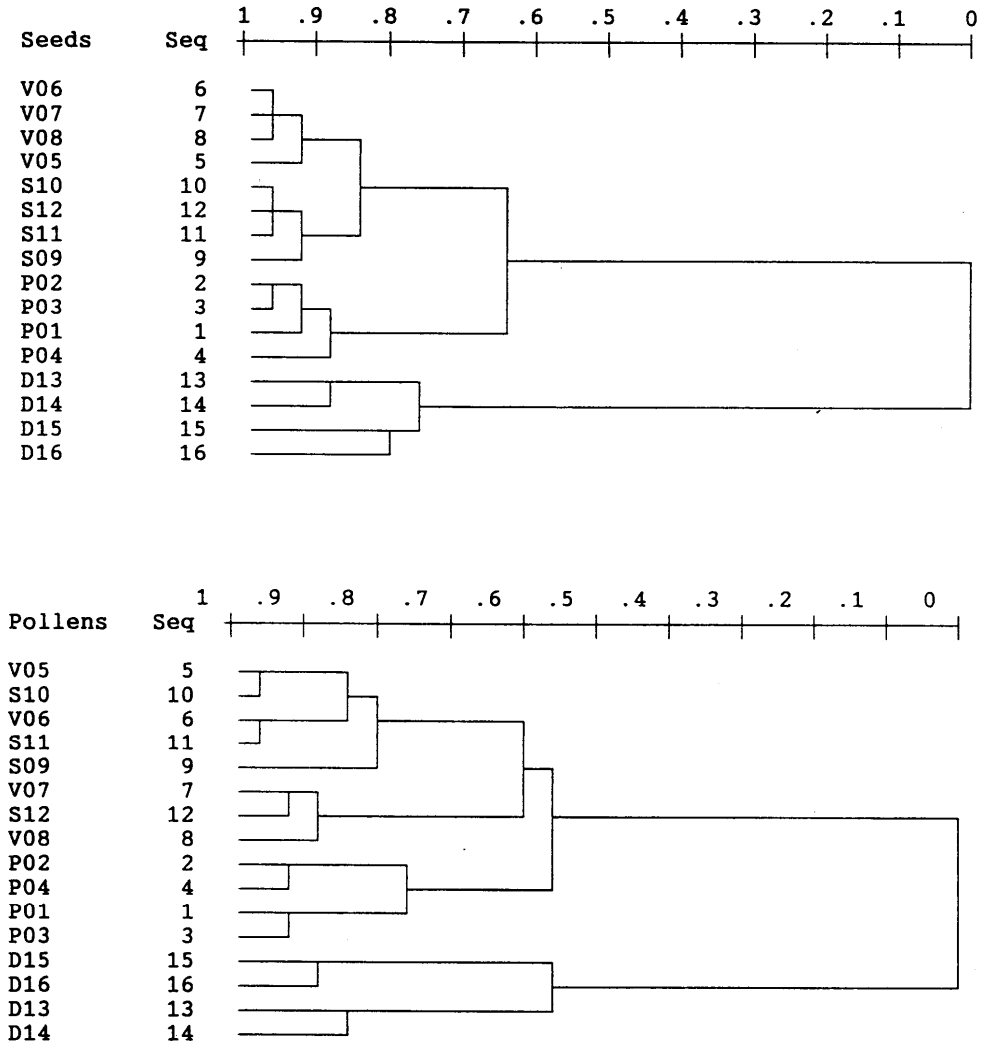

Fig. 1. Dendograms based on hierarchical cluster analysis of pollens and seeds belonging to the genus *Eruca* and *Diplotaxis* .

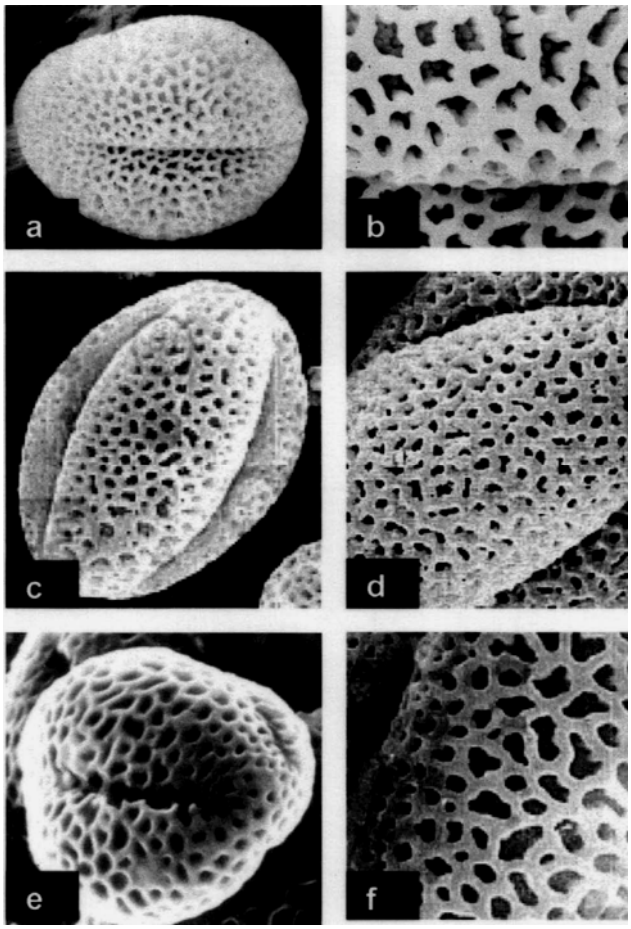

Plate 1: SEM micrographs of pollen grains
a-*Eruca pinnatifida* f. *aurea*: equatorial view x 1500;
b- *E. pinnatifida* f. *aurea*: reticulum x 10000;
c- *E. sativa* : equatorial view x 1400;
d- *E. sativa* : reticulum x 8000;
e- *E. vesicaria:* polar equatorial view x 1600;
f- *E. vesicaria:* reticulum x 10000

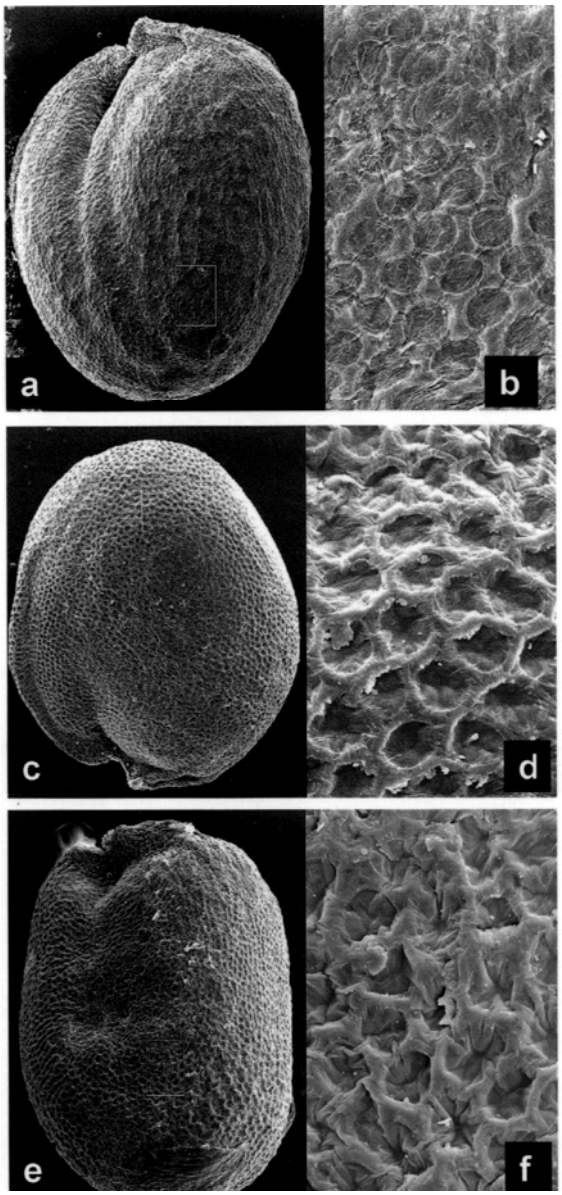

Plate 2: SEM micrographs of seeds
a-*Eruca pinnatifida* f. *aurea*: seed x 55;
b- *E. pinnatifida* f. *aurea*: regular reticulum with circular lumina x 450;
c- *E. sativa* : seed x 55;
d- *E. sativa* : regular reticulum with subcircular lumina x 450;
e- *E. vesicaria:* seed x 56;
f- *E. vesicaria:* irregular reticulum and lumina x 520