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# Micromorphologic Study of the Seed of the Genus *Trifolium*, Section *Lotoidea*, in Iran

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**Abstract:** The characteristics of the seeds of 7 annual and perennial species of *Trifolium*, *Lotoidea* section, in Iran were investigated by means of Scanning Electron Microscopy (SEM) and a stereomicroscope. Contrary to Zohary's belief who maintained that the micromorphology of the *Trifolium's* seed played no role in taxonomy, the results revealed that such characteristics can play a determining role in differentiating such species and distinguishing them from one another. The results of the micromorphologic study of the seed confirmed the morphological results of the species of the *Lotoidea* section to a great extent, but was involved in the separation and differentiation of the two species of *T. repens* and *T. nigrescens*. In general, the characteristics of the species' seeds proved quite effective in determining their phenetic relationship. Among the characteristics studied in the present research, mention can be made of seed shape, hilum shape, seed sculpture and seed length and width (L/W ratio). The results demonstrated that the characteristics of seed length and hilum shape showed the greatest diversity in the species. The longest seed length was that of the species *T. radicosum* and the shortest that of *T. repens*. The spindle shape of the hilum was an exclusive characteristic of *T. radicosum* which distinguished it more than any other characteristic from all the other species of this section. The difference in hilum shape in the two species of *T. repens* and *T. nigrescens* was an important distinctive characteristic.

**Key words:** Seed, *Trifolium*, micromorphology, Iran, *Lotoidea* section

## INTRODUCTION

The genus, *Trifolium* L., is one of the most important genera of the Fabaceae (*papilionaceae*) familym which consists of 52 annual and perennial forage species in Iran. After *Medicago*, this species is regarded as the second valuable forage plant in Iran, of which certain species such as *T. alexandrium* and *T. repens* have a high agronomic value.

*Trifolium* L. was investigated in the form of 6 sections, i.e., *Vesicaria*, *Trifolium*, *Lotoidea*, *Chronosemium*, *Mistyllus*, *Trichocephalum*.

The *Lotoidea* section (Heller, 1971-72) comprises 5 perennial species, namely *T. radicosum*, *T. hybridum*, *T. montanum*, *T. ambiguum* and *T. repens* and 2 annual species, i.e., *T. nigrescens* and *T. glomeratum* in Iran. This section is considered as the most complex section of this genus in term of taxonomy (Zohary and Heller, 1984).

Among the perennial species, the two species of *T. ambiguum* and *T. montanum* have great similarities in reproductive organs and the calyx characteristics, making their taxonomic identification a hard job. Moreover, there are many similarities between the perennial species of *T. repens* and the annual species of *T. nigrescens*, particularly concerning the shapes of calyx, standard, legume, corolla and leaflets, whereby morphological characteristics are not capable of distinguishing these species from one another accurately.

*T. radicosum*, the endemic species of Iran, has not undergone any biosystematic study. The geographical dispersion of this endemic species is in the provinces of Mazandaran and Tehran. With regards to seeds, there are several studies that analyse different aspects such as chemical compositions (Kakea and Esen, 1993), observations through fluorescence microscopy techniques or genetic variation (Smith *et al*., 1995).On the other hand, to use of SEM allows for the observation of structures which would be difficult by other means and some authors have highlighted the importance of this technique for the study of seed-coats specially for those genus or species in which identification is complicated, such as the *Trifolium* (Zohary and Heller, 1984) or *Astragalus* (Karamian and Ranjbar, 2005) in Fabaceae family, where features of seed morphology have been

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widely used to distinguish the different taxa or to find affinities between them. The aim of present study was to illustrate the role of seed features in the identification of species of *Trifolium* (*Lotoidae* section) in Iran and to relate such characters to the systematics of this section. The size, weight, dimensions and shape of the *Trifolium* seed are different in its various species and are therefore of taxonomic value. In fact, most of the information on seed micromorphology in the genus *Trifolium* is related to the works of Zohary and Heller (1984).

Seed shapes in the species of this genus include ovoid, elliptic and oblong-elliptic. Seed surface may be smooth, scabrate, tuberculate or rugulate. In the species relating to three subspecies of the *Lotoidea* section, including *Oxaliodea*, *Ochreata* and *Lotoidea*, the hilum shape is apical (Zohary and Heller, 1984). In the present study, the seed characteristics of the *Lotoidea* section in Iran were analyzed for the purpose of studying the phenetic relationship of the species with one another and for using such characteristics in systematics studies.

## MATERIALS AND METHODS

Seed morphology was studied in 7 taxa of the *Trifolium*, *Lotoidea* section, including *T. repens*, *T. radicosum*, *T. nigrescens*, *T. hybridum*, *T. glomeratum*, *T. montanum* and *T. ambiguum* from Iran. The study was based on dry herbarium specimens from the Department of Plant Genetics and Genetic Resource, Seed and Plant Improvement Institute of Karaj. To observe the seeds under the Scanning Electron Microscope (SEM) they were covered by a thin layer of platinum. The measurements are based on the SEM data obtained.

The SEM images included those with a magnification of 95x to observe the general view of the seeds and those with a magnification of 5500x to view the seed sculpture. In addition, a stereomicroscope was used to study hilum shape, which was drawn with a magnification of 40x. Qualitative characteristics including length, width and L/W ratio of the seed as well as the qualitative characteristics of seed sculpture and hilum shape were measured and investigated on each of the samples. The terminology of seed-coat surface sculpturing basically follows Steran (1992) and Font Quer (1993).

## RESULTS AND DISCUSSION

The qualitative and quantitative characteristics investigated in the present study included length, width and L/W ratio of the seed, seed shape, hilum shape, seed sculpture and seed color (Aghabaighi, 1998). The size, weight, dimensions and shape of the *Trifolium* seed are different in its various species and are therefore of taxonomic value. There are wide variety shapes (Lenticular, oblong-ovoide, reniform and ovoid). The seed coat is wrinkled tubercle, reticulate or regulate. Hillum shape is circular to square, elliptic or spindle.

The smallest length and width were those of the species *T. repens* and the largest, *T. radicosum*. In the two species of *T. ambiguum* and *T. montanum* the seed shape is different despite close morphological characteristics. Seed shape in *T. ambiguum* was lenticular and in *T. montanum*, oblong-ovoid, which conforms to the results obtained by Zohary and Heller (1984). In *T. radicosum*, which is morphologically different from the other species of this section, the seed shape was also different and was elliptic. As with *T. radicosum*, the *T. glomeratum* species showed significant differences with the other species with regard to certain morphological characteristics, seed shape is different from the other species and is reniform. The ovoid shape is the dominant shape in the species of this section, which was observed in the species of *T. repens*, *T. nigrescens* and *T. hybridum*. Seed color did not play any particular role in differentiating the species of the *Lotoidea* section. The seeds were mostly brown to light brown in color.

Hilum shape was different in the two species of *T. repens* and its ancestral species, i.e., *T. nigrescens* (Fig. 1). Hilum shape in *T. repens* was rounded while in *T. nigrescens* it was elliptic. Thus, hilum shape played a decisive role in differentiating these two species (the results has not shown). Hilum shape in the two species of *T. ambiguum* and *T. montanum* was similar and of the four-sided type The spindle-shaped hilum of *T. radicosum* was considered as another differentiating characteristic of this species. In the two species of *T. glomeratum* and *T. hybridum*, which showed little morphological similarities with respect to leaflet shape, inflorescence shape, standard shape and absence of tricome in the calyx tube and tooth, the hilum shape was physical and stood in an elliptic shape.

Figure 2 shows the seed sculptures of the species of this section. The investigations revealed that seed sculptures in the species of this section have variations. In the two very close species, i.e., *T. nigrescens* and *T. repens*, the difference in seed sculpture is quite evident (Fig. 2e and g). In *T. repens*, the seed surface reticulate, while in *T. nigrescens* the sculpture is of wrinkled tubercle type.

Tubercular sculptures in the two shapes of smooth tubercles and wrinkled tubercles were seen in the four species of *T. nigrescens*, *T. hybridum*, *T. radicosum* and *T. glomeratum* (Fig. 2b-c and e-f). Seeds with smooth tubercle (star-shaped) were only seen in *T. glomeratum*

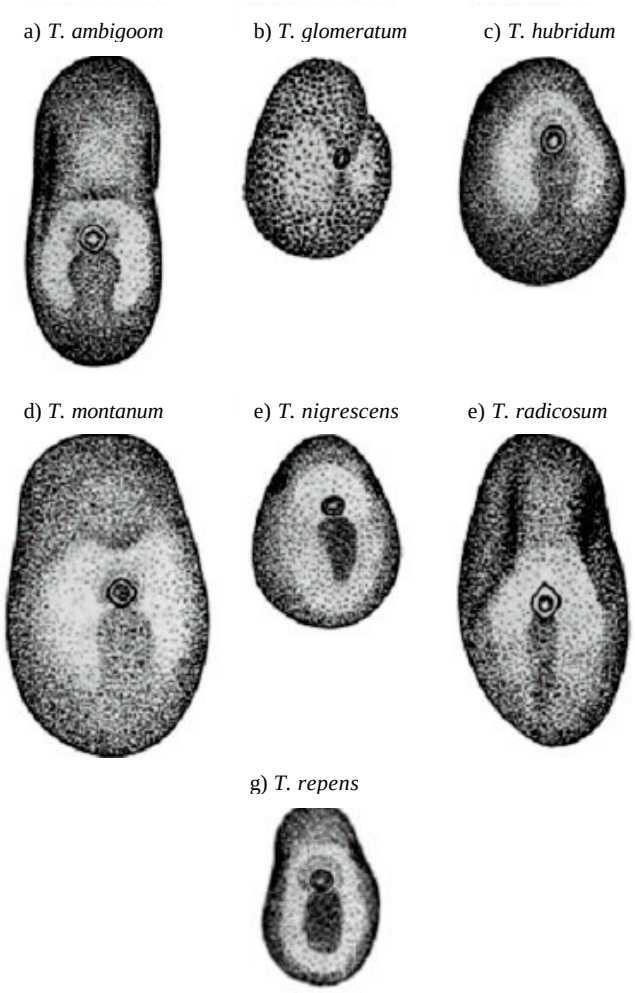


Fig. 1: Hillum shape of *Lotoidae* species

(Fig. 2b). Based on the above characteristics, a cluster dendrogram were drawn for the species of the *Lotoidea* section through the UPGMA and Ward methods as shown in the (Fig. 3). The dendrogram was itself divided into 4 main clusters. The first cluster included *T. repens* only. This result showed that *T. repens* can be separated from its highly similar species, i.e., *T. nigrescens* based on seed characteristics. Thus, the micromorphological study of the seed played an important role in differentiating these two species which are hardly differentiable in terms of such morphological characteristics as leaflet shape, inflorescence shape, corolla color, standard shape, calyx shape and legume shape.

The second cluster included three species: the two species of *T. nigrescens* and *T. hybridum* under one sub-cluster and *T. glomeratum* alone under another sub-cluster and situated near *T. hybridum* with a similarity level of 3.67. An investigation of these three species showed that morphologically they are close to one another and the findings of the seed micromorphologic study also confirmed these results.

There were two species in the third cluster, i.e., *T. montanum* and *T. ambiguum* with a similarity level of 4.2. These species are highly similar to each other morphologically and the results of the seed micromorphologic study very well demonstrate such similarity and places them in a separate cluster.

The fourth cluster included *T. radicosum* alone which showed the greatest difference with the other species. The morphological characteristics of this species, including purple corolla, oblong standard and elliptic seeds, the pollen grain features and the chromosome morphology strengthen the probability that this species is separate from the *Lotoidea* section.

The cluster dendrogram showed that the results of the seed micromorphologic study highly confirm to the results of morphological studies of the above species.

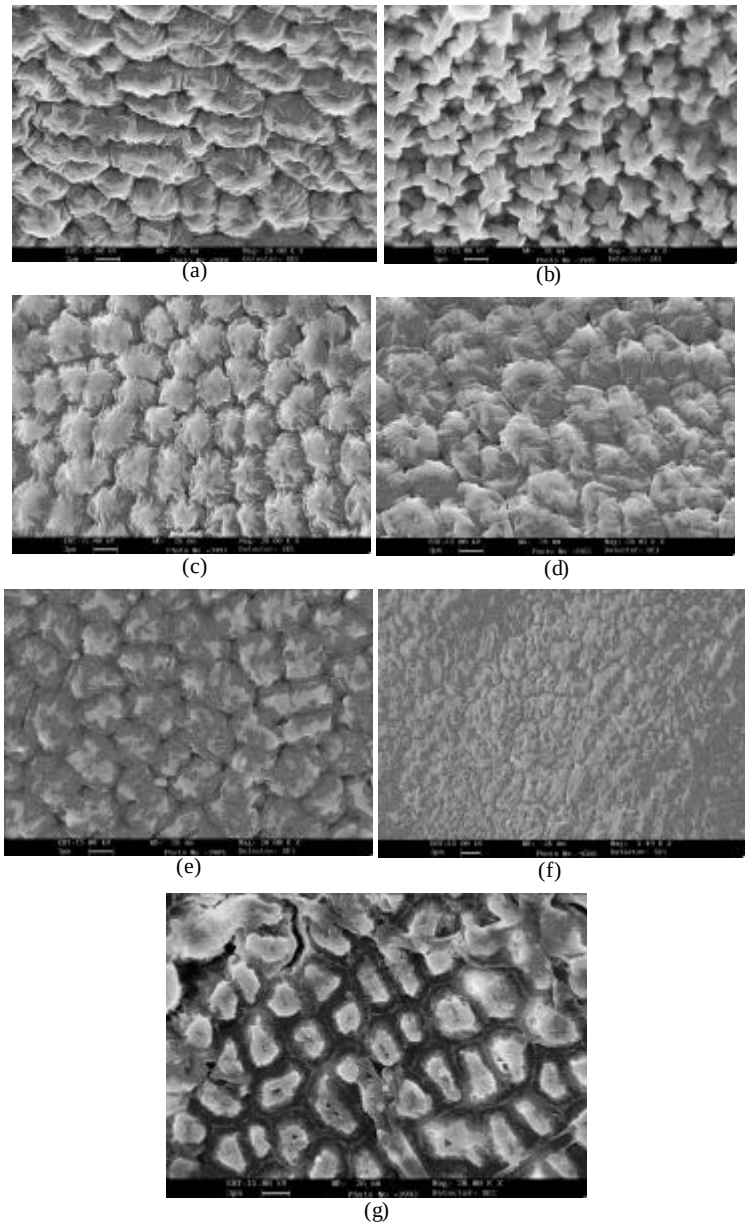


Fig. 2: Seed sculpture: a) *T. ambiguum* b) *T. glomeratum* c) *T. hybridum* d) *T. montanum* e) *T. nigrescens* f) *T. radicosum* g) *T. repens*

The results obtained confirm the usefulness of seed characters for the identification of the species and highlight the division of near species in this section. Zohary and Heller (1984) divided this section into 3 sub section based on seed morphology. In the species relating to three subspecies of the *Lotoidea* section, including *Oxaliodea*.

*Ochreata* and *Lotoidea*, the hilum shape is apical. In the other species of the *Lotoidea* section and in almost all the other sections of this genus, the hilum situation of the seeds have either a marginal or central position.

Watson *et al*. (2000) by using ITS marker support that sect. *Lotoidae* species form the basal grade in the molecular phylogenies. In their cluster *T. montanum* and *T. ambigum* near each other with high parsimony. Present results correspond with their work. On the other hand, based on morphological, palynology and chromosome number in sect. *Lotoidae* in Iran, *T. radicosum*, the endemic species in Iran, has different characters in pollen shape, sculpture and karyotype with other species in this section. Micromorphological studies support these results.

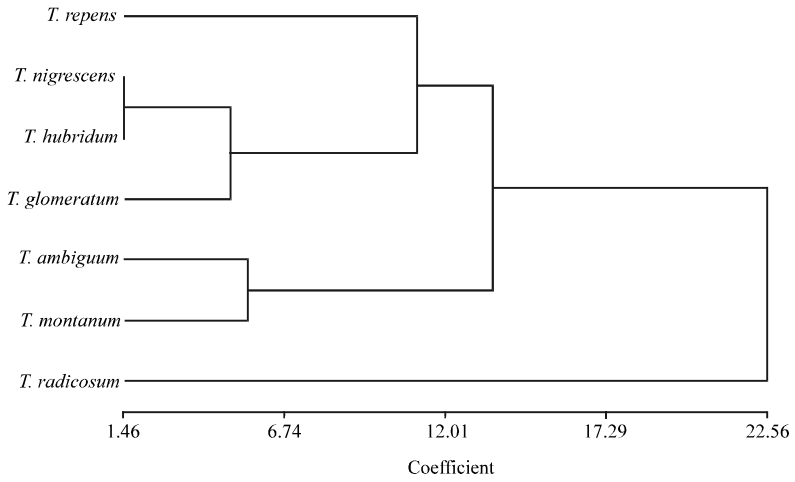


Fig. 3: Cluster dendrogram of *Lotoidea* species through UPGMA and Ward method

The following shows the identification key of the species of the *Lotoidea* section based on seed characteristics:

1a- Seed length and width more than 2×1.5 μm, seed shape elliptic.....*T. radicosum*
1b- Seed length and width less than 2×1.5 μm, seed shape not elliptic.....2
2a- Seed shape ovoid.....3
2b- Seed shape not ovoid.....5
3a- Seed sculpture scale-like, hilum rounded, .....*T. repens*
3b- Seed sculpture wrinkled tubercle, hilum elliptic, .....4
4a- L/W ratio more than 1.1 μm, seed color dark brown, seed length more than 1.1 μm.....*T. nigrescens*
4b- L/W ratio less than 1.1 μm, seed color light brown, seed length less than or equal to 1.1 μm....*T. hybridum*
5a- Hilum shape four-sided.....6
5b- Hilum shape elliptic.....*T. glomeratum*
6a- Seed shape lenticular.....*T. ambiguum*
6b-Seed shape oblong-ovoid …… *T. montanum*

Based on the results, micromorphologic characteristics of the seed in the species of the *Lotoidea* section had effective role in determining the phenetic relationship of these species.

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