

Nutlet Micromorphology Study of Some Species of *Thymus* L. (Lamiaceae) in NE Iran

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Abstract

In the present study, nutlet micromorphological features of five *Thymus* species growing in NE Iran were examined. For this purpose, nutlet shape and ornamentation were assessed using SEM. The results indicated that the P/E of nutlet, abscission scar position, cell shape, anticlinal wall and periclinal surface varied among the studied species. Finally an identification key was prepared basis on the mentioned characteristics for species under study.

Keywords: Lamiaceae, micromorphology, NE Iran, nutlet, SEM, *Thymus*

Introduction

Thymus L. (Lamiaceae: Nepetoideae) is one of the largest genus which comprises 215 species distributed throughout the Mediterranean region, Asia, the Balkan peninsula, Micronesian areas, Europe and SE Saudi Arabia [14]. The number of Iranian *Thymus* species is reported 18, of which 10 species and 3 varieties grow in NE Iran [16, 9]. Morphologically, identification of *Thymus* species is difficult due to having high hybridization level, particularly between the three species including *T. trautvetter* Klokov, *T. transcausicus* Ronniger and *T. transcaspicus* Klokov [17, 20]. Several studies tried to explain the importance of nutlet micromorphological characteristics in species identification [2, 10, 11, 15, 12, 19]. Moon *et al.* (2009) showed the importance of nutlet micromorphology in character Evolution of the Tribe Mentheae (Nepetoideae, Lamiaceae). This type of research was carried out on allied genera such as *Salvia* L. in Turkey [15] and in NE Iran [7]. *Nepeta* L. in Turkey [10] and in Kashmir Himalaya [19] and *Mentha* L. in Turkey [18]. Based on the results of above-mentioned studies, the networking pattern or cell shape of nutlet surface is one of the useful characteristics in identification at the generic and specific level of Lamiaceae [19, 10, 1, 18]. The aim of the present research is to focus on the shape and ornamentation of nutlet of *Thymus* species distributed in NE Iran. Finally, an identification key is provided based on the characters used in this work.

Materials and methods

Herbarium and field-collected specimens sampled from North Khorassan and Khorassan Razavi provinces (NE Iran) were identified using Flora Iranica [16], Flora of Russia, Flora of Turkey, [8] and Flora of Pakistan [3, 4]. All field-collected voucher specimens were preserved in FUMH (Ferdowsi University of Mashhad Herbarium). Nutlet features such shape, colour, abscission scar position and ornamentation were assessed using Stereo microscope and SEM in 200 and 1000 magnification. The list of *Thymus* species in the current study was presented in Table 1. Seed terminology was adapted from Hoen *et al.* and Hesse *et al.* 2009 [6,5].

Results

The results showed that the abscission scar is often located either at the center of the basal end or slightly shifted towards the ventral side with extended round abscissi-

on scar area. Moreover, we observed variation in the nutlet ornamentation, shape of anticlinal wall and periclinal surface (Fig. 1). *Thymus transcaucasicus* is characterized by having slightly irregular rounded cell arrangement and thick ridged anticlinal wall with wrinkled periclinal surface (Fig. 1A- C). *Thymustrans caspicus* is distinguished by its flower -shaped cell with deep ridged anticlinal wall and concave periclinal surface (Fig.1D-F). *Thymus trautvetteri*s differentiated with the rectangular or pentagonal cells and slightly wrinkled periclinal surface (Fig. 1G-I). Furthermore, *T. kotschyanus* and *T. pubescens* are characterized by rounded cell, deep but weakly ridged anticlinal wall including concentric periclinal surface and flower-shaped cell, ridged anticlinal wall, and psilate periclinal surface, respectively (Fig. 1J-N). Psilate is located at the externally smooth surface lacking any particular ornamentation (Fig. 1).

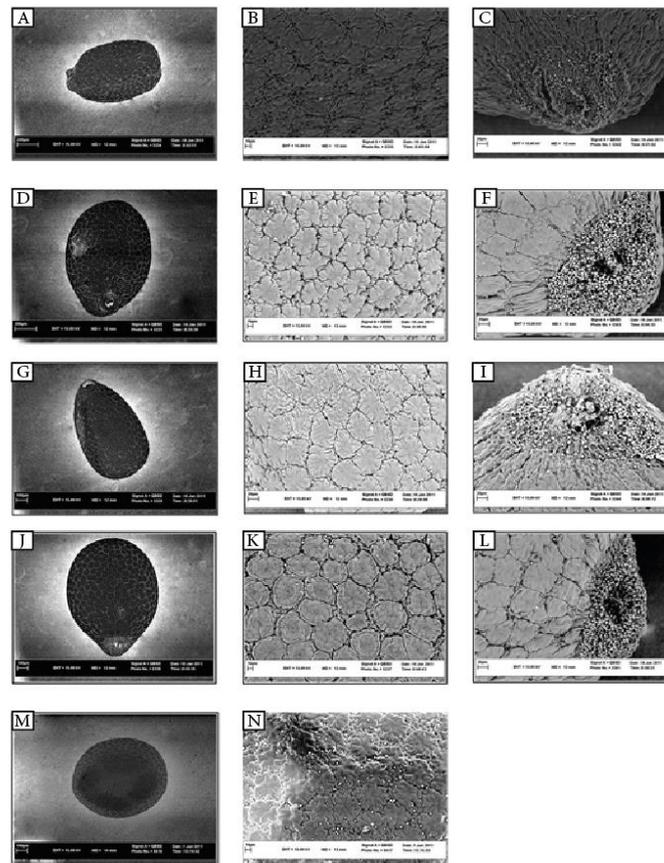


Fig. 1. Electro-micrographs of nutlet shape and ornamentation in A, B, C: *T. transcaucasicus* ×200, 1000. D, E, F: *T. transcaspicus* ×200, 1000. G, H, I: *T. trautvetteri* ×200, 1000. J, K, L: *T. kotschyanus* ×200, 1000. M, N: *T. pubescens* ×200, 1000.

Table 2. Nutlet characteristics of studied *Thymus* species.

Species	P/E	nutlet shape	Nutlet colour	Abscission scar Position	Cell shape	Periclinal surface
<i>T. transcaucasicus</i>	2	prolate	Light brown	Basal toward ventral axis	irregular slightly Rounded	Wrinkled
<i>T. transcaspicus</i>	1.33	prolate	Light brown	Basal toward ventral axis	flower shaped	Concave
<i>T. trautvetteri</i>	1.8	prolate	Light brown	basal	rectangular or pentagonal	Weakly wrinkled
<i>T. kotschyanus</i>	1.33	prolate	Light brown	basal	rounded	Concentric Surface
<i>T. pubescens</i>	1.15	spheroid	Black	basal	flower shaped cell	Psilate

P/E: polar axis length/equatorial axis length

Discussion

According to the results obtained from the current study, two types of nutlet shape were observed including prolate and spheroid. The colour of the nutlets is light brown except for *T. pubescens* in which the colour of the nutlet is black (Fig. 1). The last species is morphologically characterized by having basal branch and two pairs of lateral veins [17]. The periclinal surface is varied from wrinkled, psilate, concave to concentric. *Thymus trautvettri* is the unique species with rectangular-pentagonal cell shape. Identification of this species is difficult due to having high levels of hybridization. Moreover, this species has basal branch, two pairs of prominent semi-lateral vein in mature and hairy cordate leaves [17]. The nutlets of *T. kotschyanus* are characterized by the concentric periclinal surface. However, this species with three pairs of lateral vein in mature leaves and arista and ciliate of upper lobe of calyx has no basal branches [17]. The most nutlet characteristics of *T. transcaspicus* and *T. transcaucasicus* were similar. Nevertheless, the former species differed from the latter by having irregular slightly rounded cell shape and wrinkled periclinal surface. Morphologically, both

species have two pairs of prominent semi-lateral vein. *Tymustrans caspicus* has narrow leaves and basal branch [17].

Regarding to previous works, the networking pattern of the nutlets is a useful characteristic for distinguishing the genera of the family Lamiaceae [19, 10, 1, 18]. Ozkan *et al.* (2009) identified Turkish *Salvia* species based on the middle lobe around the network. .Moreover, Moon *et al.* (2009) mentioned that the nutlet shape, morphology of the abscission scar, distribution of trichomes and surface sculpture can be used for species identification in Lamiaceae. Also, Ling *et al.* (2010) explained that the seed-coat micromorphology can be applied as a criterion for the separation of *Thymus* species. Also, they showed that ventral surface of *T. przewalskii* nutlets has reticulate ornamentation, while that of *T. mandshuricus* nutlets has poltophagy ornamentation. Moreover, mesh shape of negative reticulate ornamentation in *T. nerveless* is nearly irregular square or irregular pentagon, while that of *T. dahuricus* is subcircular [12]. Identification key to the genus *Thymus* among the species under study based on the nutlet characteristics:

- 1) Seed shape spheroid *T. pubescens* Boiss. & Kotschy ex Celak
-) Seed shape prolate2
- 2) Cell arrangement round3
-) Cell arrangement non-round4
- 3) Periclinal wall concentric*T. kotschyanus* Boiss.
-) Periclinal wall wrinkle*T. transcausicus* Ronniger.
- 4) Cell flower-shaped *T. transcaspicus* Klokov
-) Cell rectangular or pentagonal-shaped..... *T. trautvetteri* Klokov

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