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Seed macro and micro morphology of the selected *Nigella* (Ranunculaceae) taxa from Turkey and their systematic significance

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Abstract: Macro and micromorphological properties of intact and mature seeds of 12 taxa (species and varieties) belonging to *Nigella* L. (Ranunculaceae) was investigated using light and scanning electron microscopy. Material studied covers 11 species of 15 Turkish *Nigella*. Studied taxa were divided into two types. Type I has ovate to orbicular seeds that includes four species. Type II has triquedrous seeds and includes seven species. Type II was subdivided into two. Type IIa has triquedrous to subpyramidal seeds (five species) and Type IIb has triquedrous to subglobose seeds (two species). Further segregation was performed micromorphologically and an identification key of studied *Nigella* taxa was given. Studied *Nigella* taxa have a diverse macro and micromorphological characters that utilize to separate them from each other to assess the systematics of *Nigella*.

Key words: *Nigella*; Ranunculaceae; seed morphology; SEM

Introduction

The nigellas are a genus of about 20 species of annuals from the Mediterranean countries and western Asia (Hegnauer 1973; Bown 2002). There are 12 species and one doubtful record in Flora of Turkey (Davis 1965). After the revision of the genus with a new record and two new species (Davis et al. 1988; Dönmez & Mutlu 2004), the total number reached to 15 species in Turkey.

The genus *Nigella* includes some important species with aromatic and medicinal properties. *N. sativa* L. (Black cumin) is one of the most admired medicinal seed in history and also mentioned in Bible and in the words of Prophet Mohammed (Ramadan 2007). It is known as "Çörekotu" in Turkey and its seeds are used as seasoning for foodstuffs like bread and pickles among Turkish people (Baytop 1999) and also traditionally used in Arabian countries, Indian subcontinent and Europe for medicinal and culinary purposes (Bown 2002; Ramadan 2007). *N. damascena* L., *N. arvensis* L. and *N. glandulifera* Freyn & Sint. ex Freyn follow *N. sativa* with respect to the traditional usage. Their usage is very old. Carbonized *N. damascena* seeds that were found in an excellent state in a slag-washing site of cupper ore were dated middle to late Bronze Age (Heiss & Oegol 2005). *Nigella* species have been used traditionally as a condiment and healing herb in southern Europe (Heiss & Oegol 2005) at least since Bronze Age.

A diverse traditional usage on a great geography stimulate researchers to work on *Nigella* in all aspects such as composition of seed oils (Havlik et al. 2006), antibacterial (Landa et al. 2006), antifungal (Landa et al. 2006), antiinflammatorial (Landa et al. 2007), anticancer (Gali-Muhtasib et al. 2006), antitumor (Kumara & Huat 2001), cytotoxic (Thabrew et al. 2005), antiulcer (Arslan et al. 2005), analgesic (Al-Ghamdi 2001), antihypertensive (Zaoui et al. 2000), anti-diabetic (El-Dakhakhny et al. 2002), anticestode and antinematode (Akhtar & Riffat 1991), antioxidative (Kökdil et al. 2006b), immunomodulator (Nazrul Islam et al. 2004) and respiratory stimulation (El Tahir et al. 1993) features of *Nigella* species were studied.

Recently, micromorphology is frequently used to differentiate plant taxa in different systematic ranks in different families. Micromorphology of fruit (Akçın 2007; Guerin 2005; Özcan 2002), especially seed coat (Akbari & Azizian 2006; Fagundez & Izco 2003; Hassan et al. 2005; Johnson et al. 2004; Khalik & van der Maesen 2002; Moazzeni et al. 2002; Moro et al. 2001; Plaza et al. 2004; Shehata & Loutf 2006; Song et al. 2005; Xu 2003; Yıldız 2002) or combination (Juan et al. 2000) of those study results assess their phlogenetic and/or systematic positions. Authors who studied micromorphology of seeds of different genera in Ranunculaceae family emphasize the taxonomic value of seed characters (Constantinidis et al. 2001; İlarslan et al. 1997; Karcz & Tomzcok 1987; Cappelletti & Poldini 1984). Whereas

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Nigella species have been extensively investigated phytochemically and pharmacologically, macro, micro morphological and anatomical studies of this genus are limited (Kökdil et al. 2006a). Karcz & Tomzcok (1987) studied micromorphology of six species of *Nigella* and they stated that the seeds which they studied exhibit a great diversity in outer structures and seed surface features are diagnostic to studied taxa. Constantinidis et al. (2001) studied *Consolida* (DC.) Gray and related genera *Delphinium* L. and *Aconitum* L. and they concluded that seed morphology alone gives good support of the six sections of *Consolida* but in a whole it could be used in conjunction with the other morphological characters of those plants. İlarslan et al. (1997) studied the Turkish *Delphinium* species and emphasized that there are a great diversity of seed types both within and among groups of species but these diversity fits consistently with the systematic approaches followed previously in this genus. Cappelletti and Poldini (1984) worked on European *Aconitum* L. species and they grouped the taxa depending on absence, existence or reduction properties of longitudinal wings of the seeds.

Usually morphological characters well separated Turkish *Nigella* species but some species show a great variation such as *N. sativa* and *N. arvensis*. Most of the studied nigellas are widespread. S. Europe, N.W. Africa, Caucasia, Cyprus, Syria, Iran, Iraq, Crimea, Aegean islands, Lebanon are the distribution area together with Anatolia (Davis 1965). *N. lancifolia* Hub.-Mor. is endemic to Turkey and its threat category is vulnerable (Ekim et. al. 2000). Varieties of *N. arvensis* are listed in the threat categories in the Red Data Book of Turkish Plants such as; var. *caudata* Boiss., var. *mutica* Bornm., var. *palaestina* (Zohary) Zohary & Feinbrun and var. *tauricola* P.H. Davis are vulnerable; var. *oblanceolata* P.H. Davis is critically endangered and var. *anatolica* Zohary has lower risk (Ekim et. al. 2000). The last three ones are endemic to Anatolia (Davis 1965, 1988). *N. icarica* Strid is endemic to Ikaria island (Davis 1965) and the thread category is data deficient (Ekim et. al. 2000). *N. fumariifolia* Kotschy is distributed in Cyprus and East Aegean islands (Davis 1965). *N. turcica* Donmez & Mutlu is a new species described from Iğdır-Tuzluca (Armenian border of Turkey) that is known only from the type locality and its thread category is critically endangered (Donmez & Mutlu 2004). *N. sativa* (cultivated species in Turkey), *N. turcica* (new species), *N. fumariifolia* and *N. icarica* (distributed outside of the Turkey) couldn't be collected.

The main objective of this study is to investigate the seed morphology of Turkish *Nigella* species to find out the evidence of possible taxonomic significance. If macro and/or micromorphological seed characters without any of the vegetative one are sufficient to determine its taxon, that would be a good tool to identify ancient seed of *Nigella* found in archeological studies and also today's specimens that lack diagnos-

Table 1. Information on the plant materials used in this study.

Taxa	Locality and Voucher (fl: in flowering, sd: in seedling stages)
N. oxypetala	B7 Malatya: around Inonü University Campus, fallow fields, 1500 m, 08 vi 2003 (fl.), 28 vi 2002 (sd.) G. Kökdil & D. Kara. AEF 23130 and A. İlçim 1212 KSUH
N. latisecta	C6 Kahramanmaraş : Afşin, Yazı village, fallow fields, 1100 m, 13 vi 2002 (fl.), 8 vii 2002 (sd.), A. İlçim & G. Kökdil. AEF 23126 and A. İlçim 1286 KSUH.
N. orientalis	C6 Kahramanmaraş: Çağlayancerit, Başdervişli village, ruderal fields, 1280 m, 14 vi 2002 (fl.), 08 vii 2002 (sd.), A. İlçim & G. Kökdil. AEF 23131 and A. İlçim 1242 KSUH.
N. lancifolia	B5 Aksaray: Ortaköy, fallow fields, 900 m, 29.v.2003 (fl.), 13 vii 2002 (sd.), D. Kara & G. Kökdil. AEF 23133 and A. İlçim 1288 KSUH
N. segetalis	B5 Aksaray: Ortaköy, field edges, 900 m, 30 vi 2002 (fl.), 01 viii 2002 (sd.), D. Kara & G. Kökdil. AEF 23134 and A. İlçim 1228 KSUH
N. stellaris	C5 Mersin: Çiftlikköy kampüsü, 5 m. 15.v.2002 (fl.), 9 vi 2002 (sd.), G. Kökdil & H. Kılıç. AEF 23129 and A. İlçim 1251 KSUH
N. arvensis var. *glauca*	B5 Aksaray: Ortaköy, fields, 900 m, 07 vi 2002 (fl.), 30 vii 2002 (sd.), D. Kara & G. Kökdil. AEF 23135 and A. İlçim 1287 KSUH
N. arvensis var. *caudata*	C8 Şanlıurfa: Ceylanpınar, Gürgür Baba, around Police Station, 27 vi 2002 (fl. & sd.), D. Kara & G. Kökdil and A. İlçim 1402 KSUH.
N. damascena	C6 Gaziantep: Botanic Garden of the Gaziantep University, 450 m. 10 vii 2002 (fl. & sd.), A. İlçim & G. Kökdil. AEF 23127 and A. İlçim 1270 KSUH.
N. elata	A2 Bursa: Campus of Uludağ University, bushy places, 1100 m, 26 vii 2002 (fl.), 25 viii 2002 (sd.), G. Kökdil & D. Kara. AEF 23128 and A. İlçim 1278 KSUH
N. unguicularis	C6 Kahramanmaraş: Çağlayancerit, Erince mountain, waste places, 1153 m, 14 vi 2002 (fl.), 9 vii 2002 (sd.), A. İlçim & G. Kökdil. AEF 23136 and A. İlçim 1217 KSUH.
N. nigellastrum	C6 Kahramanmaraş: Başdervişli village, hillsides, 1400 m, 14 vi 2002 (fl.), 09 vii 2002 (sd.), A. İlçim & G. Kökdil. AEF 23132 and A. İlçim 1281 KSUH.

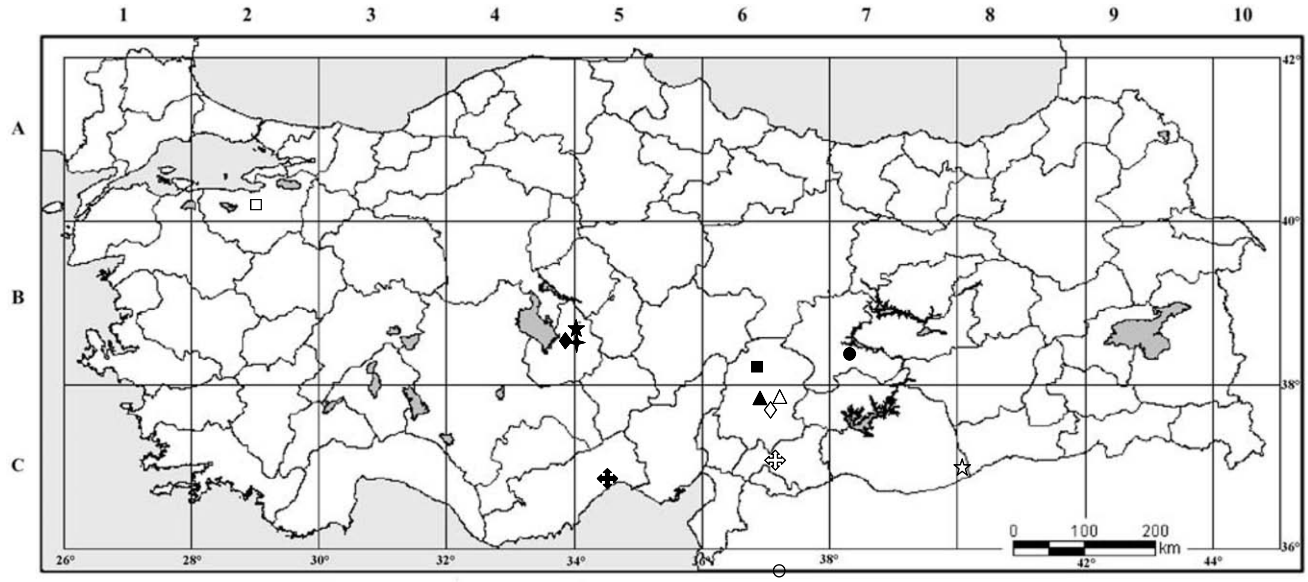


Fig. 1. Collection sites of studied *Nigella* taxa. *N. oxypetala* (●), *N. latisecta* (■), *N. orientalis* (▲), *N. lancifolia* (♦), *N. segetalis* (✦), *N. stellaris* (✚), *N. arvensis* var. *glauca* (★), *N. arvensis* var. *caudata* (☆), *N. damascena* (✥), *N. elata* (□), *N. unguicularis* (△) and *N. nigellastrum* (◊)

tic parts such as petals. To the best of our knowledge, this is the first report on seed coat micromorphology of Turkish *Nigella* that concerns its systematic implications.

Material and methods

Mature seeds of the *Nigella* species were obtained mainly from wild localities and collected within the years 2002–2003. Voucher specimens were collected during the flowering and seedling periods and identified. Herbarium vouchers kept in AEF (Herbarium of the Faculty of Pharmacy, Ankara University) and KSUH (Herbarium of the Faculty of Arts and Science, Kahramanmaraş Sütçü İmam University). Origin of seeds, collecting times of voucher specimens, collectors and voucher numbers are listed in Table 1, where the code of the Davis's (1965) Flora of Turkey grid system and the corresponding province are given. Localities of the plant material are shown on a map (Fig. 1). Seed dimensions were measured at least from 20 intact and mature seeds. The significance of the difference between mean values of the measurements was determined by one way analysis of variance at 95% confidence interval by using SPSS program. Duncan test, which is one of the Post Hoc multiple comparisons of one-way ANOVA of SPSS 13 (Statistical Package for Social Sciences, SPSS Inc., Illinois) was used to detect mean differences in seed dimensions. For scanning electron microscopy, four-seven dry mature seeds from each population were mounted on stubs, using double-sided adhesive tape and were coated with gold with a Polaron SC7620 sputter coater. Coated seeds were examined and photographed with LEO 440 scanning electron microscope with an accelerating voltage of 15 kV at Erciyes University Technology Research and Developing Centre.

The terminology of Stearn (1992), Punt (1994) and Bartlott (1981) was adopted to describe morphology and the SEM aspects of the *Nigella* seed coat. The abbreviations of the authorities of plant names follow the Standard of Brummit and Powell (1992).

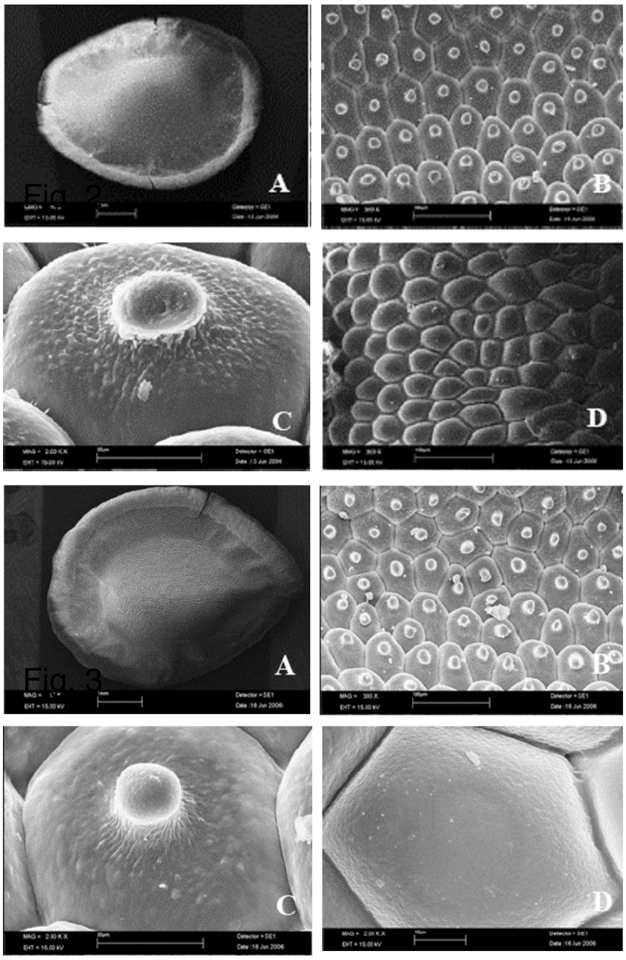
Results

We observed variations in shape, colour and dimensions in *Nigella* seeds and they are given in Table 2 and Table 3. Outer periclinal walls are persistent in all of the studied taxa. As a result of this study, *Nigella* seeds were mainly divided into two types morphologically.

Type I includes *N. orientalis* L., *N. oxypetala* L., *N. latisecta* P.H. Davis and *N. lancifolia* Hub.-Mor. General seed characteristics of Type I are ovate to orbicular, emarginate, valves ±equally inflated. Seeds are bilateral. Centre of the seed is swollen into two sides to compose a disc shape. There is a wing that surrounds the disc (Figs 2A, 3A, 4A, 5A). Micromorphology of disc and wing cells is different from each other. There is a transition region between disc and wing area. Colour is black-yellow, mostly consist of a mosaic of black and yellow testa cells. Detailed seed characteristics other than general ones of each species that belongs to Type I are given below.

N. oxypetala

Disc consists of pentagonal-hexagonal or cylindrical cells (Fig. 2B) which have an appendage like a nipple at the centre of periclinal walls (Fig. 2C). There is a rugulate sculpturing concentrated around the nipple but sometimes this rugulation is not obvious. Periclinal walls of disc cells are slightly convex. Anticlinal walls of the disc cells are straight and depressed. Transition cells occupy a narrow area and characterized by absence of nipple. There is a small hole instead of the nipple in those cells. Wing cells are hexagonal to rounded to form a dome shape. Wing cells have a wide (2–7 μm) and straight anticlinal walls (Fig. 2D). Periclinal walls of these cells are smooth and convex or sometimes straight in unrounded cells.



Figs 2–3. 2 – *Nigella oxypetala*; 3 – *Nigella latisecta* (A – General view of the seed; B, C – Disc cells; D – Wing cells).

N. latisecta
Disc, transition and wing cell characteristics are similar to *N. oxypetala* (Figs. 3B, C). Anticlinal walls of the wing cells that have mostly narrow walls (0.5–1.2 μm) in *N. latisecta* (Fig. 3D), that is the only difference from *N. oxypetala*.

N. orientalis
Disc usually consists of hexagonal, sometimes tetra-polygonal cells whose periclinal walls are straight and rugulate with irregular projections (Figs. 4B, C). Anticlinal walls of the disc cells are straight and depressed. Transition cells between disc and wing are tetra to polygonal and more elongated compared to the disc cells (Fig. 4D), occupy a wider area than *N. oxypetala* and *N. latisecta*. Periclinal walls of transition cells are straight or slightly convex, ±smooth to inconspicuously fimbrillate. There are two straight ridges at the boundary of transition cells, one belongs to each transition cell and there is a canal between the ridges. Wing cells are tetra-hexagonal or elongated with rounded corners. Periclinal walls of wing cells are convex and they have mostly longitudinally fimbrilliar and rarely reticular sculpturing. Anticlinal walls of wing cells composed of fimbrillation (Fig. 4E). Secondary sculpturing of some wing cells on periclinal wall is interrupted by one to three small or large concave zones.

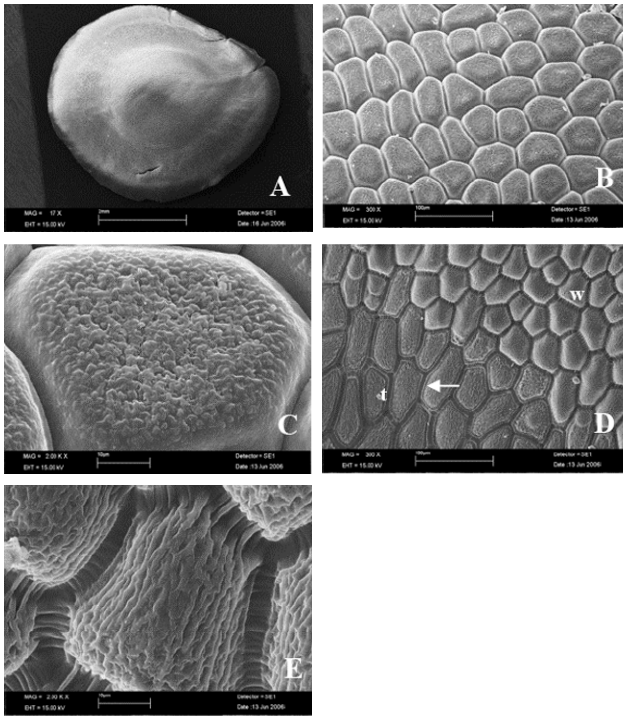


Fig. 4. *Nigella orientalis.* A – General view of the seed. B, C – Disc cells. D – Transition and wing cells. E – A wing cell with longitudinally fimbrillated sculpturing and fimbrillated boundary (t – transition cells, w – wing cells). Arrow points the ridges of transition cells on the boundary.

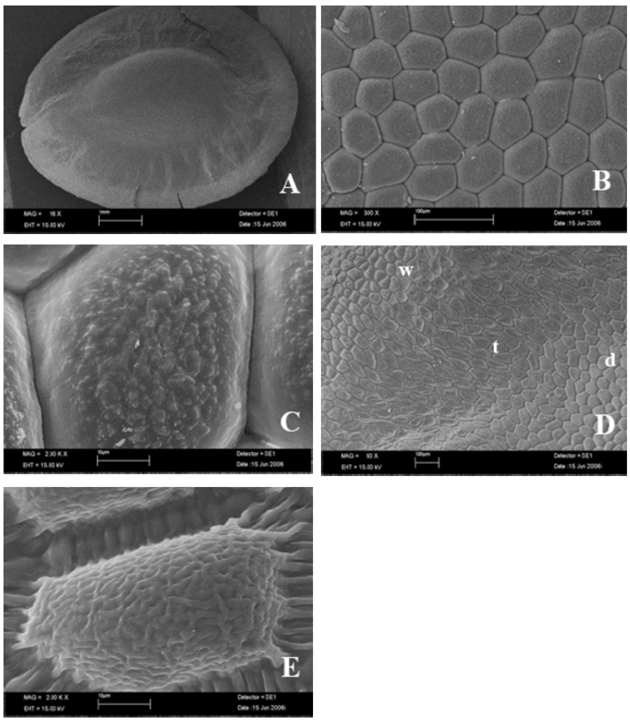
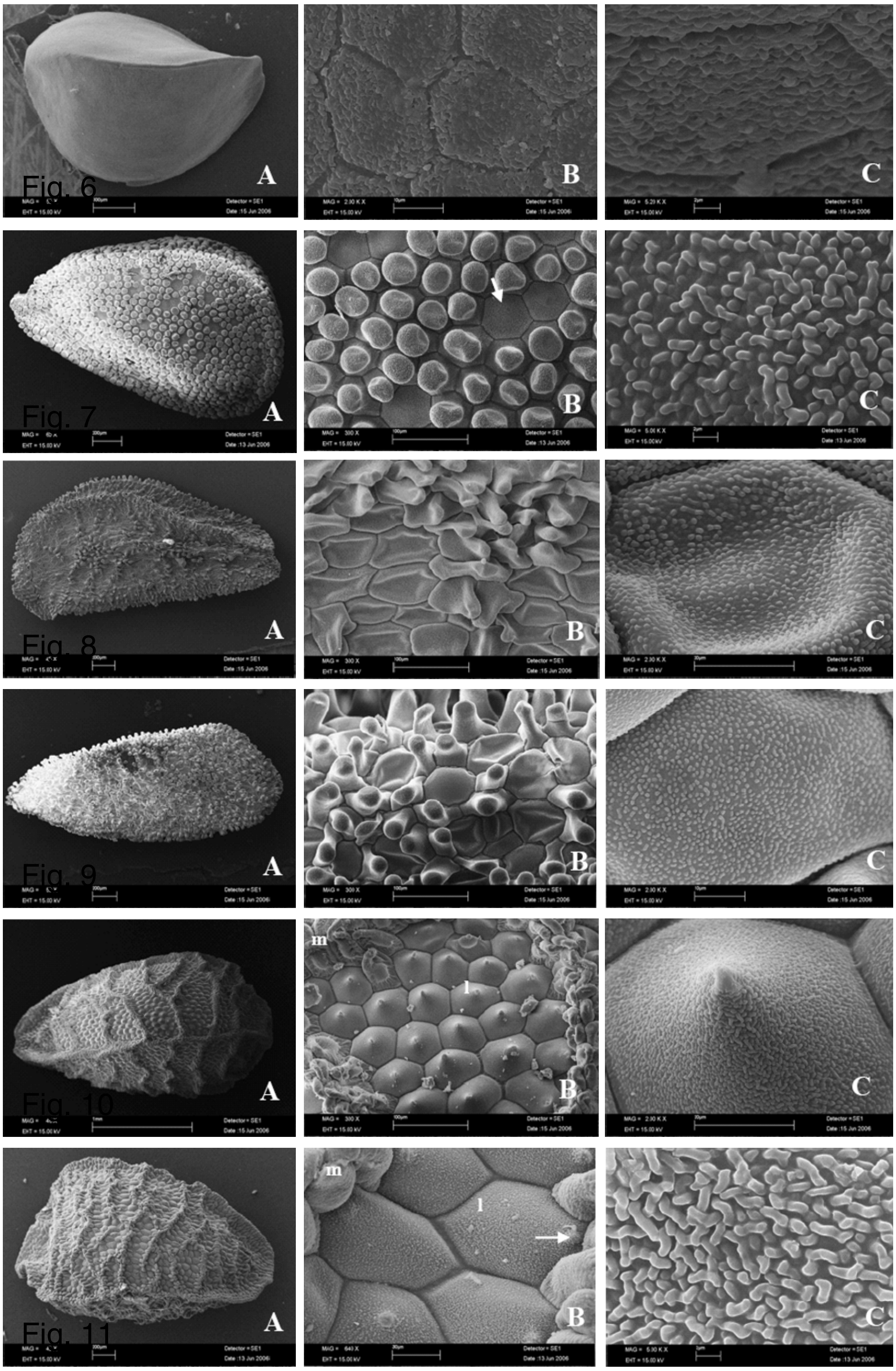


Fig. 5. *Nigella lancifolia.* A – General view of the seed. B, C – Disc cells. D – Disc, transition and wing cells. E – Wing cell with reticulate sculpturing and fimbrillated boundary (d – disc cells, t – transition cells, w – wing cells).



Figs 6–11. 6 – *Nigella segetalis (*A – General view of the seed; B – Testa cells; C – Rugulate sculpturing of testa cells with irregular projections); 7 – *Nigella stellaris* (A – General view of the seed; B – Gemmae of the testa cells; C – Rugulate sculpturing of testa cells with regular projections. Arrow points ungemmated cells); 8 –*Nigella arvensis* var. *glauca (*A – General view of the seed; B – Columellated and uncolumellated testa cells; C – Rugulate sculpturing of uncolumellated testa cells with regular projections); 9 – *Nigella arvensis* var. *caudata (*A – General view of the seed; B – Columellated and uncolumellated testa cells; C – Rugulate sculpturing of uncolumellated testa cells with regular projections); 10 – *Nigella damascena* [A – General view of the seed; B – Cells of lumen and muri; C – A lumen cell with rugulate sculpturing and having a tapering mucro at the centre of the cell (m – muri cells, l – lumen cells)]; 11 – *Nigella elata* [A – General view of the seed; B – Cells of lumen and muri; C – A lumen cell with rugulate sculpturing (m – muri cells, l – lumen cells). Arrow points blunt mucro on the periphery of the lumen cell].

N. lancifolia
All disc, transition and wing cell characteristics are similar to *N. orientalis* (Figs. 5A–D) but periclinal walls of the wing cells are reticulate (Fig. 5E).

Type II consist of triquedrous seeds and subdivided into two groups according to seed shape. Seeds of Type IIa are triquedrous to subpyramidal and this subgroup includes *N. segetalis* M. Bieb., *N. stellaris* Boiss., *N. arvensis* L. var. *glauca* Boiss., *N. arvensis* L. var. *caudate* Boiss., *N. damascena* L. and *N. elata* Boiss.

Type IIb includes *N. unguicularis* (Lam.) Spenn. and *N. nigellastrum* (L.) Willk. and they are characterized by triquedrous to subglobose seeds. Seeds of Type IIa are smooth, gemmate, columellate or reticulate. *N. segetalis* is smooth (Fig. 6A); *N. stellaris* is gemmated (Fig. 7A); taxa of *N. arvensis* are columellated (Figs. 8A, 9A); *N. damascena* and *N. elata* are reticulated (Figs. 10A, 11A). Members of Type IIb seeds have reticulate sculpturing (Figs. 12A, 13A). Microreticulate, pitted, rugulate and fimbrillate seconder sculpturing were observed on the outer periclinal walls of Type II seed testa cells. Detailed seed characteristics of each taxa that belongs to Type II are given below;

N. segetalis
At the first glance seed surface appears smooth. At the detailed picture, it consists of pentagonal-hexagonal cells (Fig. 6B) whose periclinal walls are straight and rugulate with irregular projections (Fig. 6C) like *N. orientalis* and *N. lancifolia*. Anticlinal walls are straight and depressed. *N. segetalis* is the only species whose seeds do not have any striations even at the edges of the seeds.

N. stellaris
Seeds are striated lengthwise at each of three ridges (Fig. 7A). Some seeds have an apical mucro. Seed surface is composed of hexagonal cells which are covered by gemmae located on the centre of periclinal walls of each cell (Fig. 7B). There are some ungemmated cells among these gemmated cells. Periclinal walls of these cells are straight. Both periclinal walls and gemmae surfaces are rugulate (Fig. 7C). Anticlinal walls of testa cells are straight and depressed.

N. arvensis var. *glauca*
Seeds have three high longitudinal ridges on each angle of the seed. In general view, seed surface is columellated (Fig. 8A). Testa is composed of columellated and uncolumellated cells. Columellae are especially crowded on edges to form the ridges with uniform arrangement. There is no transversal ridge between the longitudinal ones. Uncolumellated cells are concave (Fig. 8B) and their periclinal walls are rugulate with regular shaped projections (Fig. 8C). Anticlinal walls of the testa cells are straight and depressed.

N. arvensis var. *caudata*
Seed characteristics are very similar to *N. arvensis* var. *glauca*. But *N. arvensis* var. *caudata* has dense columellae than var. *glauca* (Figs. 9A–C).

N. damascena
A reticulate structure is composed of three prominent lengthwise striations on each edge and cross continuous striations that connected with them and each other in *N. damascena* seeds (Fig. 10A). Hexagonal cells are located inside of those reticulation is characterized by the consistent presence of a tapering mucro at the centre of cells (Fig. 10B). Periclinal walls of these cells are convex and rugulate with regular shaped projections (Fig. 10C). Anticlinal walls are straight and depressed. Striations (muri) were composed of rounded, sometimes a little bit elongated cells, compressed from both sides that look like erythrocyte. They are piled up irregularly and they are smaller than the lumen cells.

N. elata
In general view, seeds are similar to *N. damascena* (Fig. 11A). A reticulate structure is composed of three prominent lengthwise striations on each edge of seeds and cross continuous striations connected with them and each other. Lumen mostly composed of hexagonal, sometimes elongated hexagonal cells whose corners are somewhat rounded (Fig. 11B). Periclinal walls of these cells are convex, straight or rarely slightly concave and rugulate with regular shaped of projections (Fig. 11C). There is an inconspicuous blunt mucro that is mostly located on cell periphery that next to the muri, rarely they are located at the centre of periclinal walls of lumen cells (Fig. 11B). Anticlinal walls of lumen cells are straight and depressed. Muri cells are mostly cylindrical, sometimes rounded and compressed from both sides that similar to muri cells of *N. damascena*.

N. unguicularis
Seeds are reticulate (Fig. 12A). Lumina are large and have (3–)5–8(–11) hexagonal or elongated cells whose periclinal walls fimbrillated lengthwise (Fig. 12B). Muri cells are orbicular, depressed from both sides which located next to lumen cells or hexagonal to polymorphic whose periclinal walls slightly convex or mostly concave and furnished with microreticulate or pitted sculpturing. Anticlinal walls of muri cells are straight. There is an obvious ridge that composes the border between hexagonal cells of the muri (Fig. 12C).

N. nigellastrum
Seeds are reticulate (Fig. 13A). Lumina are narrow and have 1–4 elongated cells or none whose periclinal walls fimbrillated lengthwise (Fig. 13B). Muri cells are orbicular to polygonal whose periclinal walls slightly convex or mostly concave and furnished with rugulate sculpturing. Periclinal walls of some muri cells have a cone shape seconder sculpturing consist of accumulated rugulate projections usually at the centre of cells. Anticlinal walls of muri cells are straight and they have two obvious ridges that each belongs to other cells. A straight canal composes the border of muri cells (Fig. 13C).

Discussion

The morphological properties of seeds of *Nigella* species were exhibited some similarities and differences compared with each other. The present study showed that two major seed types were determined morphologically. Type I includes *N. oxypetala, N. latisecta, N. orientalis* and *N. lancifolia* that have seeds of the same shape and colour but the dimensions are slightly different. Mean difference of the disc width among the Type I species

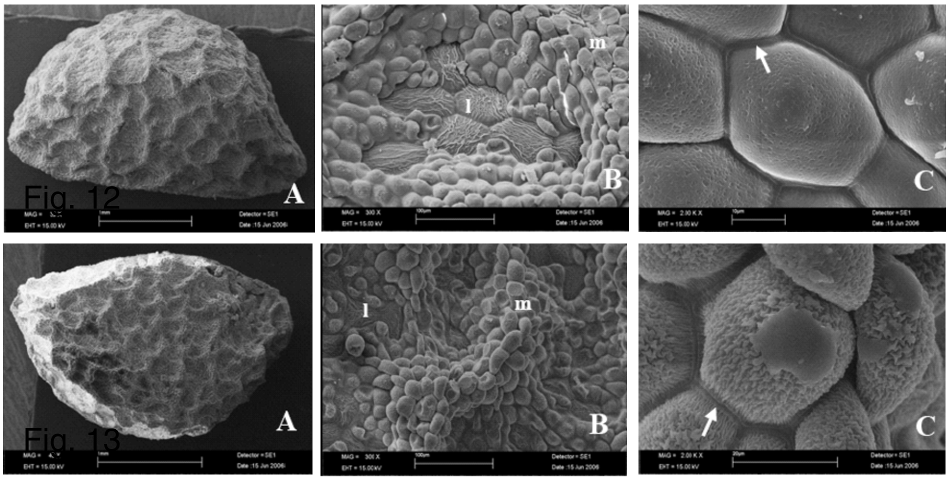


Fig. 12–13. 12 – *Nigella unguicularis* [A – General view of the seed; B – Muri and fimbrillated lumen cells; C – Muri cells with a conspicuous ridge on the boundary (m – muri cells, l – lumen cells). Arrow points the ridge on the boundary of muri cells]. 13 – *Nigella nigellastrum*; [A – General view of the seed; B – Muri and fimbrillated lumen cells; C – Muri cells with two ridges next to a canal on the boundary and a cone at the centre of periclinal wall (m – muri cells, l – lumen cells). Arrow points the ridges on the boundary of muri cells].

Table 2. Comparison the seed characteristics of *Nigella* whose seeds are ovate to orbicular.

Taxa	Shape	Colour	Seed length (mm) (min-max) mean ± SE	Seed width (mm) (min-max) mean ± SE	Seed height (mm) (min-max) mean ± SE	Disc length (mm); (min-max) mean ±SE	Disc width (mm); (min-max) mean ±SE	Emargination depth (mm); (min-max) mean ±SE	Wing width (mm); (min-max) mean ±SE
N. orientalis	ovorb	blye	(4.09–6.2) 5.247a*±0.108	(4.08–5.79) 4.821a±0.090	(0.65–1.01) 0.823a±0.018	(2.41–4) 3.129a±0.089	(1.72–3.08) 2.380a±0.065	(0.18–0.52) 0.370a±0.0235	(0.65–2.01) 1.120a±0.0485
N. oxypetala	ovorb	blye	(4.92–6.77) 5.771b±0.100	(3.94–5.87) 4.861a±0.113	(0.58–1.22) 0.829a±0.033	(2.69–4.65) 3.748b±0.113	(1.57–3.03) 2.447a±0.076	(0.3–0.7) 0.474b±0.021	(0.56–1.7) 1.113a±0.055
N. latisecta	ovorb	blye	(5.35–7.25) 6.123c±0.096	(4.59–6.34) 5.310b±0.086	(0.65–1.14) 0.854a±0.0262	(2.93–3.88) 3.493b±0.0564	(2.01–2.91) 2.438a±0.044	(0.36–0.74) 0.544c±0.0243	(0.71–2.05) 1.328b±0.0526
N. lancifolia	ovorb	blye	(5–6.51) 5.822b±0.079	(4.56–5.38) 4.998a±0.044	(0.65–1.1) 0.959b±0.024	(1.42–3.92) 3.303a±0.100	(0.98–2.76) 2.331a±0.070	(0.22–0.58) 0.407a±0.020	(0.7–1.67) 1.167a±0.0346

ovorb – ovate to orbicular; blye – black-yellow, mostly mosaic of black and yellow *Different letters in the columns indicates statistical significance of the characters among the species ($P < 0.05$).

was found to be statistically not significant. Other than seed width, rest of the seed dimensions showed statistically significant mean differences among some of the Type I species (Table 2). Disc cells of *N. oxypetala* and *N. latisecta* have nipple like projections at the centre of each cells that exactly differentiate from *N. orientalis* and *N. lancifolia* lacking such kind of projection.

Wing cells of both *N. oxypetala* and *N. latisecta* are convex and smooth, but boundaries of wing cells of *N. oxypetala* is wider than *N. latisecta*, 2–7 μm and 0.5–1.2 μm respectively. In fact, *N. latisecta* have some wing cells whose boundaries are as wide as *N. oxypetala*, but these cells are not conspicuous as much as in *N. oxypetala*. Disc cell characteristics of *N. orientalis* and *N. lancifolia* are similar. Majority of wing cells of *N. orientalis* have fimbrilliar secondary sculpturing that elongated lengthwise and a small number of cells have reticulate sculpturing, whereas periclinal walls of wing cells are only reticulate in *N. lancifolia*.

Type II taxa have triquedrous seeds and more heterogenous sculpturing compared to Type I. Morphological characters such as seed shape, colour and dimensions are very diverse. There are statistically significant mean differences among most of the taxa for all of the seed size characters of Type II, especially for *N. nigellastrum* and *N. unguicularis* (Table 3). Type II is subdivided into two groups depending on seed shape. Seeds of Type IIa are triquedrous to subpyramidal and it includes *N. segetalis*, *N. stellaris*, *N. arvensis* var. *glauca*, *N. arvensis* var. *caudata*, *N. damascena* and *N. elata*. Seed shape is a valuable character to differentiate *Nigella* taxa practically but the seed dimensions are not useful practically as much as seed shapes. *N. segetalis* is the only species whose seeds are smooth in Type II. *N. stellaris* is the only species whose seeds are gemmated in studied taxa. *N. damascena* and *N. elata* are the species that have reticulated seeds in Type IIa. Secondary sculpturing of lumen cells differentiates *N.*

Table 3. Comparison the seed characteristics of *Nigella* whose seeds are triquedrous.

Taxa	Shape	Colour	Seed length (mm) (min-max) mean ± SE	Seed width (mm) (min-max) mean ± SE	Seed height (mm) (min-max) mean ± SE
N. segetalis	trisbpyr	blye	(1.51–2.12) 1.727a* ± 0.0257	(0.91–1.62) 1.275a ± 0.0300	(0.94–1.47) 1.153a ± 0.0239
N. stellaris	trisbpyr	blye	(0.87–2.19) 1.833b ± 0.0388	(0.65–1.78) 1.050b ± 0.0464	(0.67–1.3) 1.047b ± 0.0268
N. arvensis var. *glauca*	trisbpyr	brye	(1.78–2.52) 2.226c ± 0.0348	(0.31–0.96) 0.63c ± 0.0308	(0.41–1.42) 1.001c ± 0.0355
N. arvensis var. *caudata*	trisbpyr	brye	(1.45–2.47) 2.163c ± 0.0498	(0.41–1.3) 0.765d ± 0.0538	(0.65–1.24) 0.928c ± 0.0360
N. damascena	trisbpyr	black	(1.94–2.82) 2.428d ± 0.0330	(0.97–181) 1.241a ± 0.0301	(1.11–1.77) 1.458d ± 0.0203
N. elata	trisbpyr	black	(1.76–2.6) 2.241c ± 0.035	(0.82–1.67) 1.255a ± 0.0410	(1.07–1.76) 1.368d ± 0.032
N. nigellastrum	trisbgl	brye	(2.15–2.84) 2.471d ± 0.0387	(1.01–1.96) 1.474e ± 0.0682	(0.98–1.75) 1.474e ± 0.0391
N. unguicularis	trisbgl	brye	(2.52–3.33) 2.895e ± 0.0324	(1.25–2.34) 1.856f ± 0.0685	(1.2–2.04) 1.615f ± 0.0465

Trisbpyr – triquetrous to subpyramidal, adaxial face convex; trisbgl – triquetrous to subglobose, adaxial face convex; blye – black-yellow, mostly mosaic of black and yellow; brye – brown-yellow.
*Different letters in the columns indicate statistical significance of the characters among the taxa ($P < 0.05$).

damascena from *N. elata*. Lumen cells of *N. damascena* are characterized by the consistent presence of a tapering mucro at the centre of each cell while lumen cells of *N. elata* have an indistinct blunt mucro usually located peripherally next to the muri on the periclinal walls. *N. arvensis* is the only species whose seeds are columellated. Both *N. arvensis* taxa studied have the similar seed characteristics. Columellae number is the only difference between the varieties of *N. arvensis*. Variety *caudata* has denser columellae than variety *glauca*. Type IIb have triquedrous to subglobose seeds and it includes *N. unguicularis* and *N. nigellastrum* whose seeds are reticulate. Muri and lumen cells are different and periclinal walls of lumen cells are fimbrillated lengthwise in both species. Lumen is larger and bears more lumen cells in *N. unguicularis* than in *N. nigellastrum*. Muri cell boundaries also differentiate those two species. A ridge compose the boundary of two muri cells in *N. unguicularis*, while there is a canal between two ridges that each belongs to one of the muri cells. Periclinal walls of muri cells are pitted or microreticulated in *N. unguicularis*, while it is rugulate in *N. nigellastrum*.

Results of this study allow us to prepare an identification key of studied *Nigella* taxa by using seed characters that is given below;

Identification key of studied *Nigella* taxa depends on seed characters

1. Seeds are ovate to orbicular
  2. All of the disc cells have a mucro like nipple at the centre of the cell
    3. Wing cells are convex and have a broader anticlinal walls (2–7 μm) .... ***oxypetala***
    3. Wing cells are convex and most of them have thin anticlinal walls (0.5–1.2 μm) .... ***latisecta***
  2. Disc cells do not have a mucro like nipple, surfaces of all disc cells are straight and rugulate
    4. Periclinal walls of wing cells are fimbrillated lengthwise or rarely reticulated .... ***orientalis***
    4. Periclinal walls of wing cells have reticulate sculpturing .... ***lancifolia***
1. Seeds are triquedrous to subpyramidal or triquedrous to subglobose
  5. Seed surface is smooth .... ***segetalis***
  5. Seed surface is gemmated, reticulated or collumelated
    6. Surface cells have a gemma at the centre of each cell. Some surface cells do not have a gemma .... ***stellaris***
    6. Any of the surface cells do not have a gemma
      7. Seed surface is collumelated
        8. Columellae are scattered on the surface of the seed .... ***arvensis* var. *glauca***
        8. Columellae are dense on the surface of the seed .... ***arvensis* var. *caudata***
      7. Seed surface is reticulate, not columellated
        9. Seeds are triquedrous to subpyramidal and reticulated with prominent 3 striations lengthwise at the each edge and cross continuous striations

connected with them and each other

10. Periclinal walls of cells which located between the striations are convex and have a distinct tapering mucro at the centre.... ***damascene***
10. Periclinal walls of cells which located between the striations have an indistinct blunt mucro at the cell periphery that next to muri .... ***elata***

9. Seeds are triquedrous to subglobose and reticulate. Reticulation does not have a prominent striation even on the edges of the seed

11. Reticulation is wide and there are a group of cells (3–)5–8(-11) whose periclinal walls fimbrillated lengthwise in lumina. Periclinal wall of muri cells are pitted or microreticulate. There is a ridge that compose the border of muri cells..................... ***unguicularis***
11. Reticulation is narrow, there are 1–4 cells or sometimes none whose periclinal walls fimbrillated lengthwise in lumen. Periclinal wall of muri cells are rugulate. There is a canal that composes the border of muri cells. There are two ridges at the each sides of the canal on the boundary of the muri cell............... .... ***nigellastrum***

Bilateral seeds of Type I are exactly different from *Consolida*, *Delphinium*, *Aconitum* and Type II seeds of *Nigella*. Except *D. staphisagria* L., *Consolida* and *Delphinium* seeds have lamellae or scales (Constantinidis et al. 2001; İlarslan et al. 1997) but none of the studied *Nigella* taxa bear such a structure.

N. orientalis, *N. damascena* and *N. arvensis* studied by Karcz and Tomczok (1987). Our observations are mostly similar to their data although we used different terminology for the same structures, but there are some differences. They separated *N. orientalis* seeds into four regions. One of these regions is peripheral sites of seeds which we did not study. They observed a conical projection in central portion of the outer periclinal walls of wing cells but we did not notice a projection on wing cells. They observed depressed boundaries with cuticular striations in transition cells, but we observed a canal between two ridges which belong to neighbouring testa cells. In our finding hexagonal lumen cells of *N. damascena* have convex periclinal walls with a tapering mucro at the centre but they observed lumen cells expended especially in tangential direction. They also observed convex and concave periclinal walls of lumen cells with a conical projection. Those conical projections are not seen in our findings. Colours of *N. orientalis* and *N. arvensis* are different in our and their studies. This could be due to variations or different developmental stages of studied seeds.

Absence, existence or the reduction of existing wings are another striking characters of the seeds other than their morphology. Type I seeds have one wing that surrounds throughout the bilateral seeds. Type II taxa exhibit a reduction of longitudinal wings. *N. stellaris* and taxa of *N. arvensis* have prominent longitudinal wings at all three edges. *N. damascena* and *N. elata* have prominent longitudinal wings at all three edges but they are not conspicuous as much as wings of *N. stellaris* and taxa of *N. arvensis* due to having additional cross striations that compose a reticulate structure. Those wings are higher than cross striations especially at the tapering site of the seeds. Striations of seed edges are the same height with cross striations in *N. unguicularis* and *N. nigellastrum*. Therefore, those seeds are reticulated without a conspicuous wing. Finally, *N. segetalis* is the only species whose seeds are wingless and smooth. Having longitudinal three wings and reduction of those wings of Type II seeds of *Nigella* resemble the *Aconitum* seeds more than *Consolida* and *Delphinium*. Wing reduction in *Aconitum* was suggested that an ecological adaptation (Cappelletti & Poldini 1984). Differential wing pattern and reduction do not fit the geographical distribution and their habitats in *Nigella*. *N. segetalis*, *N. nigellastrum*, *N. damascena* share the distribution site with *N. orientalis*, *N. oxypetala* and *N. latisecta* and they have similar habitats such as fallow fields, vineyards and hillsides.

Seeds of Type I species are exactly different from other *Nigella* taxa and can be replaced in a separate group. This systematic arrangement agrees with the results of the Kökdil et al. (2006a) based on stem anatomy and morphology and previous systematic approaches (Boissier 1867, Post 1932).

As a conclusion, shape, colours, dimensions, primary and secondary sculpturing features of seeds exhibit a great diversity. Our results show that seed characters are taxonomically important and could be used as descriptive and/or diagnostic characters of *Nigella*. *Nigella* seeds alone, without the vegetative parts of the plant could be used to identify *Nigella* taxa macro and micromorphologically. A supplemental study is needed to complete the features of deficient taxa of *Nigella* that also made a contribution to phylogeny of the genus.

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