Morphological and palynological features of the genus *Dasypyrum* (*Poaceae*) in Turkey

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Abstract. This article presents a revision of the genus *Dasypyrum* in Turkey, based on 25 populations gathered during field trips between 2006 and 2008. These populations were subjected to examination on the basis of their morphological and palynological characteristics. A corrected and updated description, as well as a distribution map of *D. villosum* are provided. Furthermore, the relationship between *Dasypyrum* and two other genera included in the tribe *Triticeae* (*Secale* and *Triticum*) is discussed on the basis of palynological features. The palynological investigations showed that the genus *Dasypyrum* is close to *Secale* rather than to *Triticum.*

Key words: *Dasypyrum*, distribution, pollen morphology, *Triticeae*, Turkey

Introduction

Genus *Dasypyrum* (Coss. & Durieu) T. Durand (*Poaceae*) is considered a very important genetic source in breeding experiments, due to its close phylogenetic relationship with *Triticum* L. and *Secale* L., and particularly their cultivated species. The genus is represented with two species: *D. villosum* (L.) P. Candargy and *D. breviaristatum* (H. Lindb.) Fred. The former is an annual diploid species distributed naturally in Turkey. The latter perennial species consists of diploid and tetraploid cytotypes and is distributed only in a restricted area over 1000 m a.s.l. in Morocco and Greece. Morphologically, this genus is quite distinct from the other genera within the tribe *Triticeae* by its two-keeled glumes (Frederiksen 1991; Galasso & al. 1997; Ohta & Morishita 2001).

Dasypyrum villosum was originally named *Secale villosum* by Linnaeus (1753). Later on it was referred to various genera, such as *Agropyron* Gaertn., *Haynaldia* Schur and *Triticum*.

On the basis of morphological similarities, many researchers refer to *Dasypyrum* is closer to the *Triticum*/*Agropyron* complex and *Secale* (Sakamoto 1973; West & al. 1988). According to Kellogg (1989), *Dasypyrum* was close to *Agropyron* Gaertn., *Crithodium* Link. and *Eremopyrum* (Ledeb.) Jaub. & Spach, because they all have keeled glumes. On the other hand, Kellogg (1992) showed that **V** genome of *D. villosum* was closely related to **S** genome of *Pseudoroegneria* (Nevski) Á. Löve and *Elymus repens* (L.) Gould. on the basis of cpDNA. Also, on the basis of the chloroplast DNA data, Mason Gamer (2004) showed that *D. villosum* is closely associated with *Pseudoroegneria*, *Elymus* L. and *Thinopyrum* Á. Löve.

This phylogenetic view was equally supported by Hsiao & al. (1995) on the basis of the sequences of an internal transcribed spacer (ITS) region of the nuclear ribosomal DNAs of *D. villosum* and *Pseudoroegneria*.

The relationship between the genera *Dasypyrum* and *Triticum* has been investigated in various ways, such as polymorphism of storage proteins (Montebove & al. 1987; Shewry & al. 1987), isozymes points (Montebove & al. 1987; Liu & al. 1995) and crossing efforts (Blanco & al. 1988; Bothmer & Claesson 1990).

The above-mentioned studies suggested a closer relationship with *Triticum*. On the other hand, morphological (Baum 1977, 1978a, b), biochemical (Baum 1983), cytological, and molecular DNA analyses (Linde-Laursen & al. 1992; Vershinin & Heslop-Harrison 1998) and DNA/DNA hybridization experiments (Lucas & Jahier 1988; Uslu & al. 1999) suggested a closer relationship with *Secale*.

Several phylogenetic analyses of the genus *Dasypyrum* have been reported so far on the basis of morphology, cytology and molecular biology (Gradzielewska 2006). Relatively few studies have been conducted on the genus *Dasypyrum* and its morphologically close genera *Triticum* and *Secale* regarding their pollen morphology (Fritsch & al. 1977; Kruse 1980).

In this paper, in an attempt to revise the genus *Dasypyrum* in Turkey and to contribute to the phylogeny of this genus in the tribe, palynological features of *D. villosum* and its morphologically close species *T. monococum* L. and *S. cereale* L. are compared.

Material and methods

The specimens used in both morphological and palynological parts of our study are collected from natural habitats of the species in Turkey. Voucher specimens are kept in the GAZI herbarium.

The description of *D. villosum* is based on measurements of about 130 specimens representing 25 *D. villosum* populations collected from Turkey (Table 1). Morphological measurements were made with Leica L2 Stereomicroscope and Leica Application Suite software package. Sterile parts of the specimens were measured directly on pressed material and the results were given in a range in the description; fertile parts are measured after boiling in tap water. The measurements of fertile parts of *D. villosum* include: spike length (SpL), spikelet length (SpkL), glume length (GL), lemma length (LL), palea length (PL) awn length of glumes and awn length of lemmas. Five randomly taken spikes from each population were measured. Means and standard deviations (SD) were computed for each population by means of Microsoft Excel (Table 2).

Table 1. List of studied populations of *D. villosum*.

No	*Collector / Number*	Location	Northing/ Easting
1	*E. Cabi* 3183	**A1: Edirne:** Keşan to Enez, 30 km to Enez, 132 m, 03 June 2008.	40°40.571'N 26°20.785'E
2	*E. Cabi* 3173	**A1: Çanakkale**: Gelibolu to Keşan, 8 km to Keşan, roadsides, 50 m, 03 June 2008.	40°45.978'N 26°42.213'E
3	*E. Cabi* 3199a	**A1 Edirne**: Enez to İpsala, 2 km N. of Enez towards the border with Greece, 31 m, 03 June 2008.	40°44.033'N 26°07.435'E
4	*E. Cabi* 783	**A1: Kırklareli**: Hasköy to Kırklareli, 167 m, roadsides, 29 May 2006.	41°40.829'N 27°06.208'E
5	*E.Cabi* 747	**A1: Tekirdağ**: Gaziköy to Uçmakdere arası, 127 m, 28 May 2006.	40°47.402'N 27°21.997'E
6	*E.Cabi* 752	**A1 Tekirdağ**: Uçmakdere to Kumbağ, 276 m, roadsides, 28 May 2006.	40°48.788'N 27°23.387'E
7	*E.Cabi* 763	**A1 Tekirdağ**: Gaziköy to Kumbağ, 5 km to Kumbağ, 234 m, open sides of *Quercus*, *Pinus*, 28 May 2006.	40°50.472'N 27°26.101'E
8	*E. Cabi* 801	**A1 Kırklareli**:Vize to Saray, Çakıllı köyü yakınları, 184 m, roadsides, 29 May 2006.	41°28.571'N 27°53.249'E
9	*E. Cabi* 848	**A1 Tekirdağ**: Şarköy to Gelibolu, 91 m, 30 May 2006.	40°34.301'N 26°56.103'E
10	*E. Cabi* 774	**A1 Tekirdağ**: Tekirdağ to Silivri, 20 km from Tekirdağ, 7 m,	41°00.787'N 27°44.278'E
11	*E. Cabi* 3162	**A1(E) Çanakkale:** Eceabat, 36 km to Gelibolu, next to beach under Kavak ağacı, 0-5 m, 03 June 2008.	40°12'56"N 26°22'81"E
12	*E .Cabi* 722	**A1(E) Çanakkale** Eceabat-Gelibolu peninsula, near Anzac Newzealand outpast, 8 m, maritime sands, 28 May 2006.	40°15.210'N 26°16.819'E
13	*E Cabi* 857	**A1(A) Çanakkale**: İntepe, 127 m., roadsides, 31 May 2006.	40°00.534'N 26°19.534'E
14	*E. Cabi* 817	**A2(E) İstanbul**: Silivri to Tekirdağ, around German sitesi, 23 m, dry pastures.	41°00.171'N 27°58.388'E
15	*E. Cabi* 3204	**A1(E) İstanbul:** Silivri, s.l., 04 June 2008.	41°04.128'N 28°08.468'E
16	*E. Cabi* 3210	**A2 (E)** Belgrad forest, 05 June 2008.	
17	*E. Cabi* 805	**A1 (E)** Halkalı train station environs, 21 m, 30 May 2006.	41°01.112'N 28°046.081'E
18	*E. Cabi* 515	**B1 İzmir**: Tire to Selçuk, 1.5 km to Belevine, 12 May 2006.	38°01.004'N 27°28.304'E
19	*E. Cabi* 525	**C1 Aydın** Selçuk to Şirince, 2 km to Şirince, 250 m, under olive trees, 12 May 2006.	
20	*E. Cabi* 304	**C1 Muğla**: Milas to Akbüke, before 7 km to Kızılağaç village, 160 m, 04 May 2006	37°19.418'N 27°36.410'E
21	*E. Cabi* 516	**C1 Aydın** Selçuk to Efes, 7 m, roadsides, 12 May 2006.	37°56.856'N 27°20.541'E
22	*E. Cabi* 521	**C1 Aydın** Efes next to historical sites of Efes, 8 m, 12 May 2006.	37°56.690'N 27°20.514'E
23	*E. Cabi* 533	**C1 Aydın**: Selçuk to Kuşadası, 89 m, 13 May 2006.	37°55.034'N 27°16.788'E
24	*E. Cabi* 537	**C1 Aydın**: Kuşadası Güzel Çamlı, National Park of Dilek peninsula, 65 m, 13 May 2006.	37°42.401'N 27°12.119'E
25	*E. Cabi* 3824	**C2 Denizli**: NW of Denizli, Üçler, 31 May 2008.	37°47.706'N 29°01.064'E

Table 2. Morphological measurements of *D. villosum* accessions gathered during field studies (mean + standard deviation) (*n* = 5).

No	*Collector / Number*	SpL (cm)	SpkL (mm)	GL (mm)	LL (mm)	PL (mm)	AofG (mm)	AofL (mm)
1.	*E .Cabi* 3183	6.2±0.57	14.2±1.09	5.5±0.50	10.9±0.74	11.2±0.83	3.74±0.25	4.04±0.36
2.	*E. Cabi* 3173	7.2±0.57	20.2±0.83	6.2±0.44	11.6±1.14	11.3±0.83	3.06±0.56	3.68±0.21
3.	*E. Cabi* 3199a	5.0±0.35	20.4±1.14	7.2±0.27	12.0±0.70	11.4±0.41	4.54±0.36	5.8±0.27
4.	*E. Cabi* 783	4.7±0.25	16.5±1.22	6.0±0.21	13.0±0.70	12.1±0.41	4.86±0.21	5.4±0.41
5.	*E. Cabi* 747	5.2±075	15.4±0.54	5.9±0.22	11.3±0.44	11.0±0.35	4.5±0.35	5.1±0.74
6.	*E. Cabi* 752	6.9±0.74	15.0±0.70	6.4±0.54	11.0±0.70	10.5±0.50	6.02±0.31	6.6±0.41
7.	*E. Cabi* 763	6.4±0.41	16.0±0.70	6.2±0.57	10.6±0.54	10.4±0.41	4.4±0.65	5.1±0.54
8.	*E. Cabi* 801	7.4±0.89	14.0±0.70	6.0±0.36	12.2±0.44	12.0±0.70	4.06±0.56	4.5±0.35
9.	*E. Cabi* 848	6.2±0.57	17.1±0.54	5.9±0.54	11.8±0.83	11.1±0.54	4.1±0.41	4.5±0.25
10.	*E. Cabi* 774	7.3±1.20	18.6±1.14	6.5±0.35	14.0±0.70	13.3±0.44	3.6±0.41	5.2±0.57
11.	*E. Cabi* 3162	5.5±1.11	15.6±1.14	5.9±0.21	11.5±0.5	11.3±0.57	4.2±0.23	4.42±0.42
12.	*E. Cabi* 722	5.5±0.50	15.2±1.30	5.8±0.27	11.3±0.44	11.2±0.44	5.0±0.79	4.4±0.41
13.	*E. Cabi* 857	5.7±1.20	19.2±0.83	5.7±0.44	12.8±0.83	12.0±0.70	4.1±0.56	4.50±0.35
14.	*E. Cabi* 817	6.1±0.89	19.7±0.44	6.7±0.44	12.9±0.5	12.5±0.5	6.1±0.41	5.9±0.54
15.	*E. Cabi* 3204	5.7±0.65	20.4±1.14	6.5±0.35	12.0±0.70	11.1±0.54	5.5±0.5	5.1±0.54
16.	*E. Cabi* 3210	3.5±0.39	18.4±0.89	6.2±0.57	11.0±0.70	10.5±0.50	5.2±0.57	5.4±0.41
17.	*E. Cabi* 805	7.4±0.89	18.4±0.89	5.8±0.27	12.5±0.5	12.0±0.70	5.5±0.5	5.9±0.54
18.	*E. Cabi* 515	5.5±1.65	17.1±0.54	5.5±0.35	11.5±0.5	11.2±0.44	4.4±1.08	5.2±0.57
19.	*E. Cabi* 525	4.7±0.97	20.4±1.14	5.5±0.35	12.0±0.70	11.2±0.44	3.4±1.14	5.4±0.41
20.	*E.Cabi* 304	4.0±0.70	16.3±0.83	5.9±0.21	9.6±0.65	9.5±0.5	4.7±1.64	5.9±0.54
21.	*E. Cabi* 516	8.2±0.54	19.3±0.44	6.5±0.35	13.0±0.70	12.7±0.44	3.7±0.25	3.7±0.25
22.	*E. Cabi* 521	3.8±0.23	17.1±0.54	6.7±0.44	12.0±0.70	11.1±0.54	3.1±0.56	3.9±1.24
23.	*E. Cabi* 533	4.5±0.35	18.4±0.89	6.7±0.44	11.5±0.5	11.2±0.44	4.1±0.41	4.7±1.64
24.	*E. Cabi* 537	6.1±0.89	20.2±0.83	5.9±0.54	13.5±0.5	12.7±0.44	4.7±1.64	5.2±0.57
25.	*E. Cabi* 3824	5.5±0.50	17.1±0.54	6.2±0.57	13.5±0.5	12.5±0.50	3.4±1.14	4.5±0.25

SpL: spike length; **SpkL**: spikelet length; **GL**: glume length; **LL**: lemma length (mm); **PL**: palea length (mm); **AofG**: awn length of glume (mm); **AofL**: awn length of lemma.

Pollen samples of each studied taxon were obtained from the herbarium species listed in Table 3. For palynological investigations, pollen slides for morphological examination by light microscopy were prepared according to Wodehouse (1935) and Erdtman (1952), and the measurements were made with a Leica DM1000 microscope and Leica Application Suite software package. Measurements include the following parameters: long axis of spheroidal pollen grains (A), short axis of spheroidal pollen grains (B), long axis of elipsoid porus (pa), short axis of elipsoid porus (pb), exine thickness, annulus diameter (An), A/B ratio, operculum diameter (Op.), thickest part of intine (I), and intine thickness (i) (Table 4).

Table 3. List of palynologicaly studied accessions.

No	*Collector / Number*	Taxa	Locality	Northing / Easting
1.	*E.Cabi* 516	*Dasypyrum villosum*	**C1 Aydın:** Selçuk to Efes, 7 m, roadsides, 12 May 2006.	37°56.856'N 27°20.541'E
2.	*E. Cabi* 2244	*Secale cereale* var. *cereale*	**B2 Uşak**: Karahallı to Ulubey, 1 km to Ulubey, edge of cultivated fields; 712m. 11 May 2006.	38°24.304'N 29°17.394'E
3.	*E. Cabi* 2545	*S. cereale* var. *vavilovii*	**B10 Ağrı:** Doğubeyazıt to Iğdır, 9 km to Iğdır, roadsides; 1589 m, 08 July 2007.	39°38.554'N 44°02.178'E
4.	*E. Cabi* 2374	*Triticum monococcum*	**A4 Kastamonu**: İhsangazi, Kuşçalar district, Sekicek street, limestone slopes, 900 m, 13 June 2007.	41°14'29.8"N 33°31'39.1"E

In order to study the pollen texture ornamentation, dry pollen grains were first mounted on double-sided carbon tape affixed to aluminum stubs. Grains were coated with gold with a Hummle VII sputter coater and observations were made using a Jeol 6060 Scanning Electron Microscope (SEM) at the Department of Biology, Gazi University. SEM images were used to describe surface texture of the pollen. Scabrae density per 1 μm², as well as the height and width of scabrae are measured directly on the SEM images, using the facilities of image processing software Adobe Photoshop CS2. Ten randomly chosen 1 μm² areas and scabrae on the image were measured. The descriptive terminologies of Faegri & Iversen (1975) and Punt & al. (2007) were followed.

Table 4. Morphology of pollen parameters (μm) of the three investigated taxa (mean + standard deviation) (n = 30).

Taxon	Preparation	A	B	A/B	Pa	Pb	I	i	Exine	Op	An
Daspyrum villosum	W	39.39±1.86	33.67±2.17	1.19	3.76±0.64	3.22±0.36	3.74±0.51	0.56±0.12	1.34±0.31	2.60±0.61	8.36±0.27
	E	52.19±3.76	40.32±2.76	1.29	4.45±0.50	3.99±0.68			1.80±0.30		10.05±0.36
Secale cereale. var. *cereale*	W	42.30±4.07	33.72±3.46	1.25	4.25±0.38	3.62±0.27	3.62±1.06	0.87±0.33	1.24±0.21	1.77±0.19	7.70±0.57
	E	40.17±1.86	30.03±1.90	1.33	3.48±0.26	3.15±0.29			1.34±0.17		8.16±0.58
Triticum monococcum	W	35.34±1.83	35.34±1.17	1.00	3.69±0.33	3.45±0.34	3.41±1.21	0.82±0.12	1.59±0.16	1.99±0.27	8.89±0.80
	E	32.72±1.99	32.38±2.03	1.01	3.27±0.20	2.92±0.21			1.55±0.18		8.84±0.54

W: non-acetolyzed pollen grains; **E**: acetolysed pollen grains; **A**: long axis of spheroidal pollen grains; **B**: short axis of spheroidal pollen grains; **A/B**: shape of pollen grains; **Pa**: long axis of ellipsoid porus; **Pb**: short axis of ellipsoid porus; **I**: thickest part of the intine; **i**: intine thickness; **Op**: operculum diameter; **An**: annulus thickness.

Results and discussion

Dasypyrum villosum

Plant morphology (Plate I, Figs 1–6)

Tufted annual. Stems 25–100 cm with 2–5 nodes, usually decumbent or geniculate-ascending, glabrous. Leaf blades 7–22 cm × 2–4 mm, flat, sparsely scabrous to densely pilose on the upper surface, glabrous beneath. Spike 4–10 cm; rachis densely barbate on margins. Spikelets 7–20 mm (excl. awn), compressed. Glumes 5–7.5 mm long, with small fascicles of rigid hairs 2–4 mm long on keels, with 2.0–5 cm scabrid awn. Lower lemma 10–16 mm, glabrous below, sparsely pilose above, with fascicles of rigid hairs below awn; awn 2–6 cm, scabrid. Palea 10–15 mm. long, membranous, anthers 5–8 mm long, caryopsis 4–5 mm long.

Habitat and ecology. The species grows in sandy arid areas, on rocky limestone slopes. Its altitudinal range varies from sea level to 200 m. Other species growing in the area with *D. villosum* include: *Cynosurus cristatus* L., *Secale cereale* L., *Taeniatherum caput-medusae* (L.) Nevski., *Hordeum murinum* Huds., *Hordeum bulbosum* L., *H. murinum* L., *Elymus elongatus* (Host) Runemark, *Quercus coccifera* L., *Cistus creticus* L., *Stachys cretica* subsp. *lesbiaca* Rech. f., *Bromus sterilis* L., *B. tectorum* L., *Aegilops geniculata* Roth, *A. triuncialis* L., *Psoralea bituminosa* L., *Trifolium campestre* Schreb., *T. arvense* L., *Ononis viscosa* L., *Onobrychis caput-galli* Lam., *Thymbra spicata* L., *Coridothymus capitatus* (L.) Rchb. f., *Sonchus oleraceus* L., *Juniperus oxycedrus* L., *Salvia viridis* L., *S. virgata* Jacq., *Hymenocarpus circinnatus* Savi, *Sarcopoterium spinosum* Spach, *Avena fatua* L.

Phenology. Flowering in May to June, fruiting from June to July.

General distribution. European Turkey, W. Anatolia, S. European islands, S.W. Russia, Crimea, Caucasia. A Mediterranean element. The species is confined from A1 (A1 sensu Davis 1965) to C2 squares in Turkey (Fig. 1).

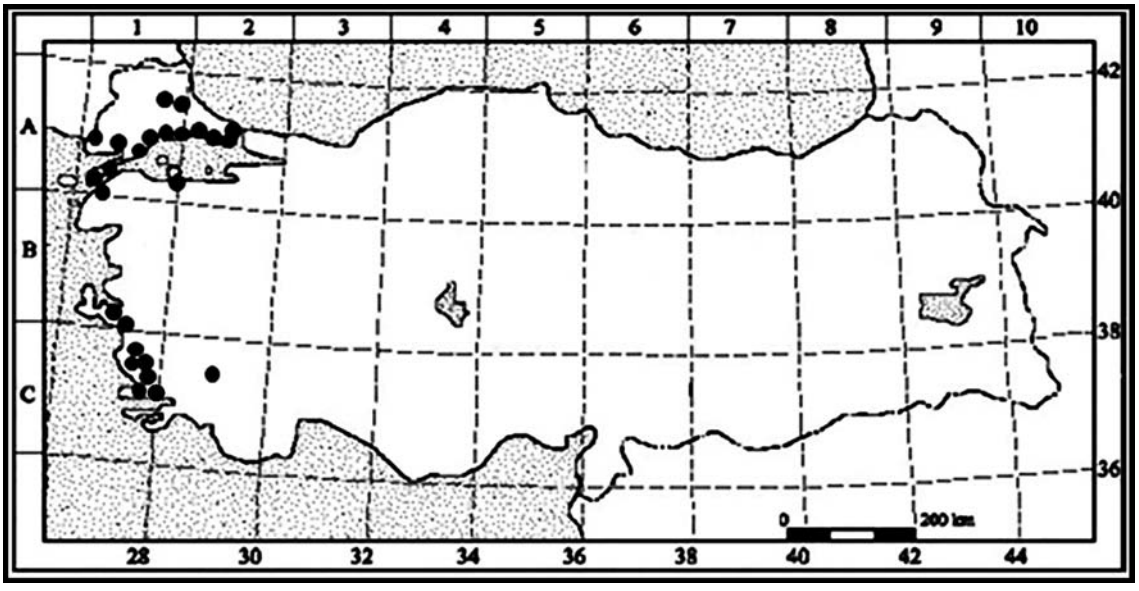
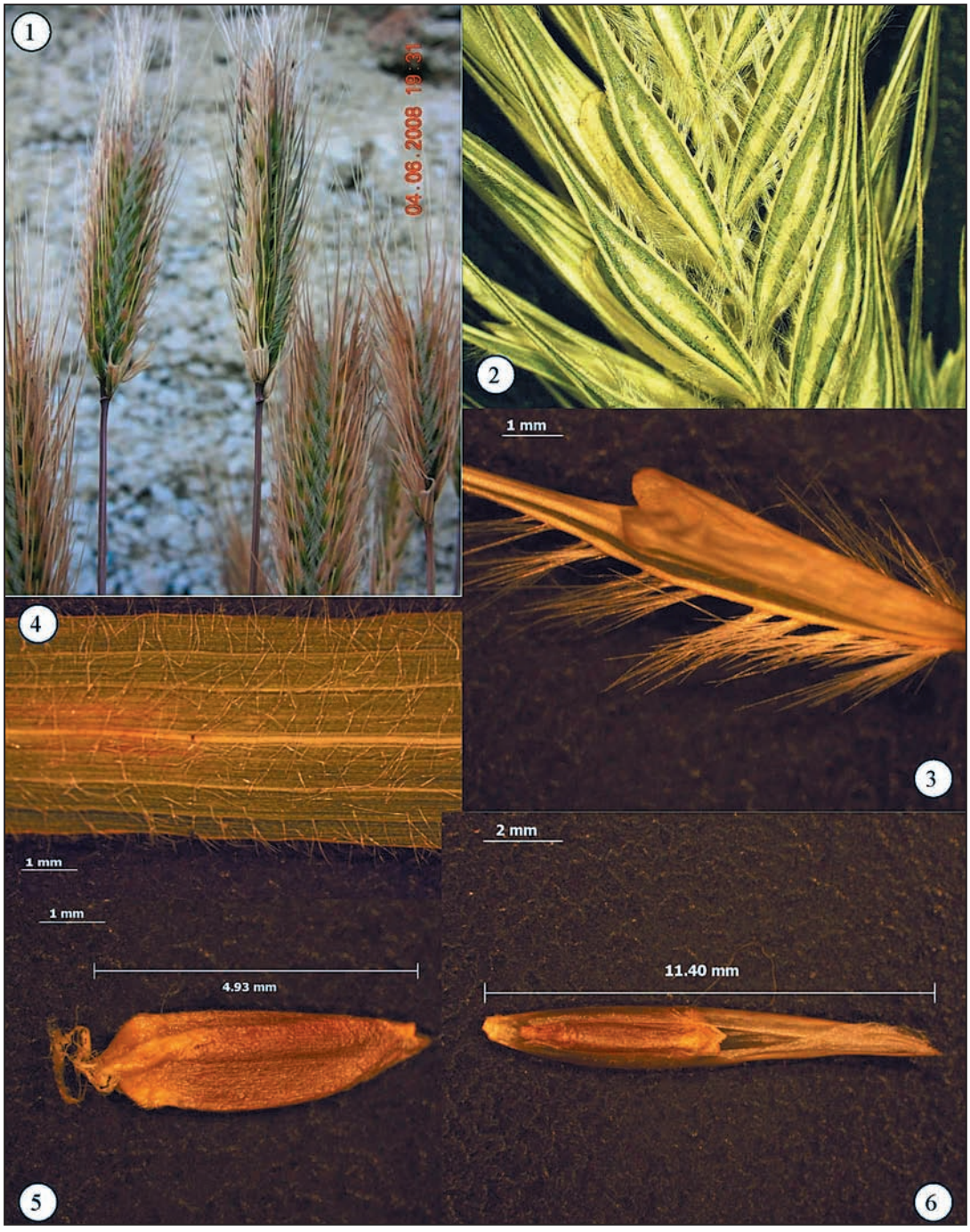


Fig. 1. Distribution of *D. villosum* in Turkey.

Palynological features (Plates II & III)

The results of palynological investigations have shown that all pollen grains of *D. villosum* and its morphologically similar species *S. cereale* (wild and cultivated varieties) and *T. monococum* are monoporate and have scabrate exine surfaces. The pollen shape and scabra density per 1 μm², as well as the height and width of scabrae are specific features for differentiation of the taxa. The mean and standard deviations of the measured pollen parameters of taxa are given in Table 4. All investigated taxa had monoporate and prolate, subprolate or prolate spheroidal pollen grains. The pore was surrounded by an annulus and was partly covered by an operculum. While in *D. villosum* and *S. cereale* the pollen shape is subprolate (1.14–1.33 μm) or prolate (1.33–2.00 μm), in *T. monococum* the pollens are prolate spheroidal (1.00–1.14 μm) (Plate II, Figs 1–4; Table 4).

Plate I



Figs 1–6. Morphological features of *D. villosum*: **1,** Spike; **2,** Close view of spike; **3,** Glume; **4,** Indumentum of leaf blades, **5,** Seed; **6,** Palea and seed.

Pollen grains of *Poaceae* were classified as annulate or nonannulate, and operculate or nonoperculate by Salgado-Labouriau & al. (1990), Chaturvedi & al. (1998) and Perveen (2000, 2006). In this study, we have observed that all examined taxa were annulate and operculate. The exine ornamentation types in this family were defined as insular, granulose, spinulose, verrucose, and brevicerebro ornate (Chaturvedi & al. 1994, 1998; Liu & al. 2004). Erdtman (1969), Moore & al. (1978, 1991), Başer & al. (2009), and Özler & al. (2009) used the term scabrate for exine sculpture covered with small (<1 μm) elements, equivalent to granulate exine ornamentations. According to our SEM investigations, exine sculpture is scabrate in all examined taxa (Plate III, Figs 1–3). Kohler & Lange (1979) suggested that the number of spinules may be used for identification.

In the present study, we have found that the investigated taxa could be differentiated on the basis of the exine parameters, such as the number and distance between the scabrae. In *S. cereale* var. *cereale,* the number of scabrae in 1 μm² varies from 5 to 10 (8.8±2.18); the width of scabrae is 0.40±0.12 μm and the height of scabrae is 0.50 ± 0.16 μm. In *T. monococum,* the number of scabrae in 1 μm² is 12–15 (13.5±1.15); the width of scabrae is 0.30±0.08 μm and the height of scabrae is 0.30±0.09 μm. In *D. villosum*, the number of scabrae in 1 μm² is 3–5 (4.5±0.5); the width of scabrae is 0.30±0.08 μm and the height of scabrae is 0.1±0.06 μm (Plate III, Figs 1–3). The potential usefulness of the pollen exine features of the Gramineae as markers of gene expression and inheritance was shown mainly in intergeneric hybrids, where the influence of one or both parents on the exine sculpture of the resulted hybrids was recorded (Grant 1972; Rajendra & al. 1978).

The pollen shape and density of scabrae no/μm² on the exine surface can be used as the most functional differentiating characters. On the basis of pollen shape and the number of scabrae *Dasypyrum* is closer to *Secale* rather than to *Triticum*.

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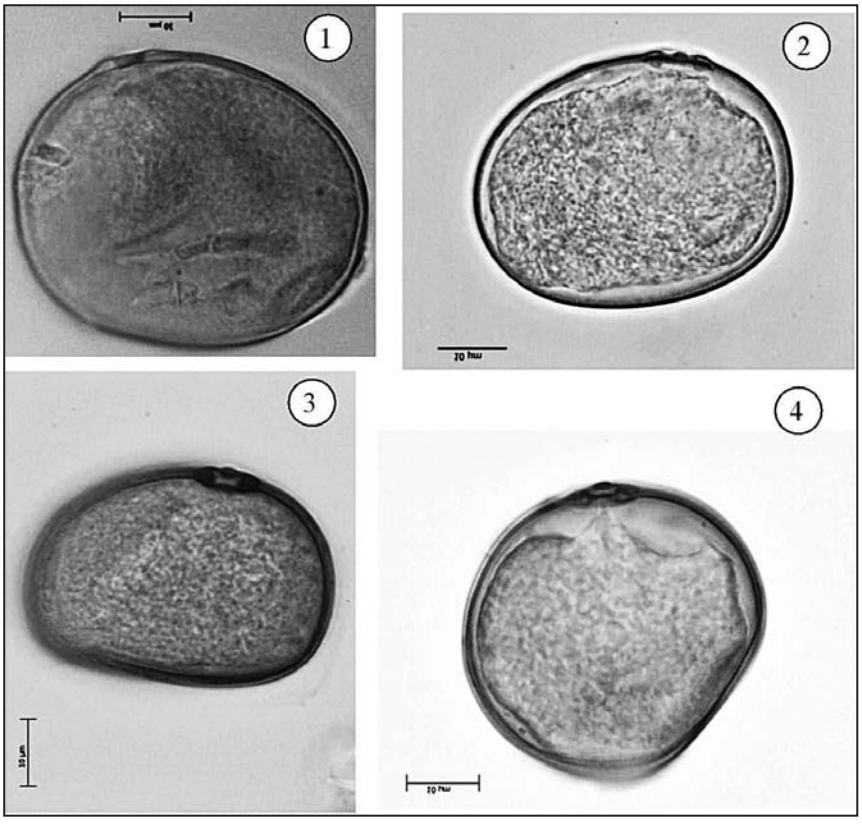
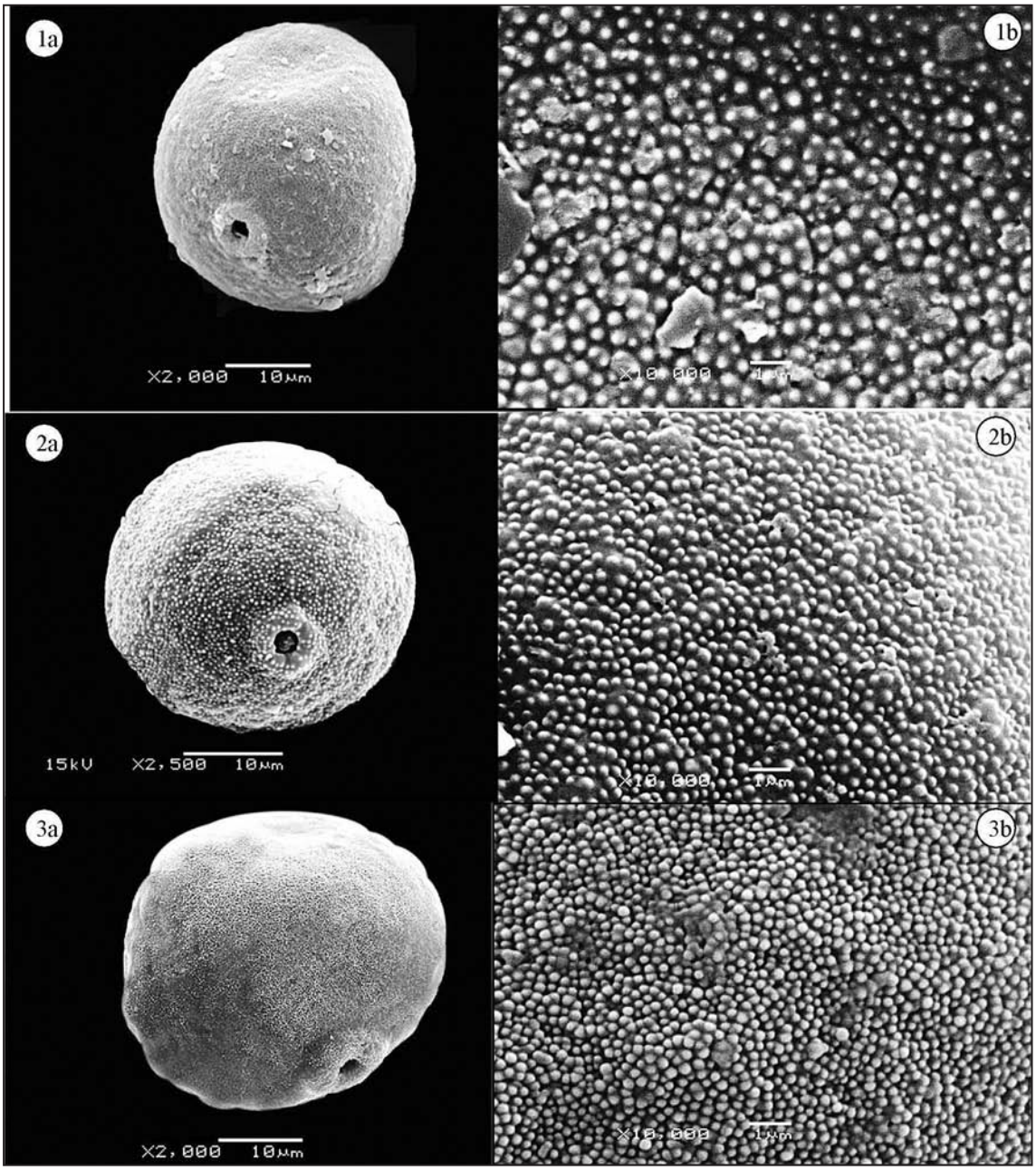
Plate II



Figs 1–4. Light micrographs of pollen grains of: **1,** *D. villosum*; **2,** *S. cereale*; **3**. *S. cereale* var. *vavilovi*; **4**, *T. monococum*.

Plate III



Figs 1–3. SEM observations of: **1,** *D. villosum*, **a –** general view; **b –** exine ornamentation; **2,** *S. cereale*, **a –** general view; **b –** exine ornamentation; **3**, *T. monococum*, **a –** general view; **b –** exine ornamentation.

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