

Achene morphology of Turkish *Carthamus* species

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Abstract

The present paper represents the macro and micro-characters of the achenes of *Carthamus* (Asteraceae) species naturally growing in Turkey. A diagnostic identification key, descriptions and images of achenes were given. SEM investigation of seed coat sculpturing revealed 3 distinct types of surface structure: I: Striate, II: Irregularly striate and III: Finely striate. Our research shows that achene morphology is of great value for taxonomist and for plant breeders for identification of Turkish *Carthamus* species without the need of herbarium specimen.

Keywords: *Carthamus*; achene; morphology; achene coat surface.

Abbreviations: SEM-scanning electron microscope, Syn-synonym, CD-*Carthamus dentatus*, CG-*Carthamus glaucus*, CL-*Carthamus lanatus*, CP-*Carthamus persicus*, CT-*Carthamus tenuis*.

Introduction

There are approximately 25 species and subspecies belonging to *Carthamus*. The centre of origin of the genus is regarded as the eastern part of the Mediterranean region (Ashri and Knowles, 1960; Weiss, 1971). In Flora of Turkey there are 10 taxa (6 species, 4 subspecies) belonging to this genus (Kupicha, 1975; Güner et al. 2000). Six out of 10 taxa naturally grow in Turkey, the rest distributed in the East Aegean islands. Within these, *Carthamus tinctorius* L. is an alien species for the Turkish flora, cultivated for oil extraction (Hanelt, 1963) and as a saffron substitute. Several researchers proposed different taxonomic classifications in and under genus level (summarised in Table 1). On the basis of some differences in vegetative characters and in the cypselas, Cassini (1819) and De Candolle (1838) suggested that *Carthamus* should include only the *Carthamus tinctorius* group, and the remaining species should be classified in a separate genus *Kentrophyllum* (Vilatersana et al., 2005): including *Kentrophyllum dentatum* DC., *K. glaucum* Tausch and *K. lanatum* (L.) DC. ex Duby from Turkey. Ashri (1957; Ashri and Knowles 1960), Knowles (1958) and Estilai (1977) based their classification on chromosome numbers and morphological characteristics, according to their classification Turkish *Carthamus* species grouped under section I (n=12), II (n=10) and III (n=22). Hanelt (1961, 1963) proposed five sections and Turkish *Carthamus* were grouped under Section *Carthamus*, *Odontagnathius*, *Lepidopappus* and *Atractylis*. Moreover according to Vilatersana et al. (2000, 2005), Sehgal et al. (2009) and Bowles et al. (2010) classification system, Turkish *Carthamus* grouped under two sections: *Carthamus* and *Atractylis*. In all proposed classifications mentioned above

(De Candolle, 1838; Cassini, 1819; Ashri, 1957; Knowles 1958; Ashri and Knowles 1960; Hanelt, 1961, 1963; Estilai 1977; Vilatersana et al. 2000, 2005; Sehgal, 2009; Bowles et al., 2010), *Carthamus persicus* was reported as the closest relative of cultivated safflower, *C. tinctorius*. Also, Konar et al. (2010) reported that the seed fatty acid composition of *Carthamus persicus* have many resemblances with the fatty acid composition of *Carthamus tinctorius*. The aim of this research is to provide information about achenes of wild Turkish *Carthamus* species. We believe that the information about the achenes of Turkish *Carthamus* species, given in this paper would be very informative for plant taxonomists and for agriculturists working on improving safflower types.

Results

The common features of *Carthamus* achenes are having obpyramidal oblique shape with 4 angles and the color of upper part of achene is darker than below. Pappus-palea many, scabrous. Hilum shape is obpyramidal or obovate, sculpture ornamentation is striate to finely and irregularly striate.

Key of the Carthamus species from Turkey, based on the achene morphology

1-All achenes epappose; surface of achene finely striate.... *C. tinctorius*

1- All achenes pappose or at least inner achenes pappose; surface of achene striate or irregularly striate.... 2 *striate*

Table 1. The taxonomic classification of Turkish *Carthamus* taxa proposed by researchers (modified from Sehgal et al., 2009).

De Candolle (1838), Cassini (1819)	Ashri (1957), Knowles (1958), Ashri and Knowles (1960), Estilai (1977)	Hanelt (1961, 1963)	Vilatersana et al. (2000, 2005), Bowles et al. (2010)	Sehgal et al. (2009)
Genus <i>Carthamus</i> (x = 12)	Section I (n = 12)	Section <i>Carthamus</i>	Section <i>Carthamus</i>	Section <i>Carthamus</i>
<i>C. tinctorius</i>	<i>C. tinctorius</i>	<i>C. tinctorius</i>	<i>C. tinctorius</i>	<i>C. tinctorius</i>
<i>C. palaestinus</i> (Sin. <i>C. persicus</i> Desf. ex Willd.)	<i>C. palaestinus</i> (Sin. <i>C. persicus</i>)	<i>C. persicus</i>	<i>C. persicus</i>	<i>C. palaestinus</i> (Sin. <i>C. persicus</i>) <i>C. glaucus</i> subsp. <i>glaucus</i>
Genus <i>Kentrophyllum</i> (x = 10, 11 and 12)	Section II (n = 10)	Section <i>Odontagnathius</i>	Section <i>Atractylis</i>	Section <i>Atractylis</i>
<i>K. dentatum</i>	<i>C. glaucus</i>	<i>C. dentatus</i>	<i>C. lanatus</i>	<i>C. lanatus</i>
<i>K. glaucum</i>	<i>C. tenuis</i>		<i>C. dentatus</i>	
<i>K. lanatum</i>		Section <i>Lepidopappus</i>	<i>C. glaucus</i>	
	Section III (n = 22)	Ser. <i>Lepidopappi</i>	<i>C. tenuis</i> subsp. <i>tenuis</i>	
	<i>C. lanatus</i>	<i>C. glaucus</i>	<i>C. tenuis</i> subsp. <i>gracillimus</i>	
		<i>C. tenuis</i> subsp. <i>tenuis</i>		
		<i>C. tenuis</i> subsp. <i>gracillimus</i>		
		Section <i>Atractylis</i>		
		<i>C. lanatus</i>		

2- The length of pappus at least three times longer than achene length.....*C. dentatus*

2- The length of pappus is two times longer than achene length....3

3- Inner pappus is distinctly shorter than outer.....4

4- The largest part of achene is apex; surface of achene irregularly striate.....*C. tenuis*

4- The largest part of achene is median; surface of achene striate.....5

5- Pappus 6-11 mm; palea of inner pappus spatulate; hilum obovate.....*C. glaucus*

5- Pappus longer, to 18 mm; palea of inner pappus linear; hilum obpyramidal.....*C. lanatus*

3- The length of inner pappus equal to the outer....*C. persicus*

***Carthamus dentatus* (Forssk.) Vahl.**

Achenes broadly obpyramidal, oblique, 4 angled, light brown, upper part darker, 2.5-7x2.5-6 mm. Pappus 6-11 mm, brown, inner pappus shorter than outer. Palea scabrous. Hilum obpyramidal, 0.5-1 x 0.8-1.5 mm. Sculpture ornamentation of achene surface striate (Figure 1A, B, Figure 2 A, G, L).

***Carthamus glaucus* M. Bieb. subsp. *glaucus*.**

Achenes broadly obpyramidal or inverted dome-shaped, oblique, 4 angled, light brown, upper part darker, 3-5.5x2.5-5 mm. Pappus 6-11 mm, brown, inner pappus shorter than outer, palea of inner pappus spatulate. Palea scabrous. Hilum obovate, 1.2-1.5 x 0.8-1 mm. Sculpture ornamentation of achene surface striate (Figure 1C, D, Figure 2 B, H, M).

***Carthamus lanatus* L.**

Achenes broadly obpyramidal, oblique, 4 angled, light brown to brown, upper part darker, 3-7x2.5-5.5 mm. Pappus 6-18

mm, straw coloured to dark brown, inner pappus shorter than outer. Palea scabrous. Hilum obpyramidal, 1-1.5 x 0.6-0.8 mm. Sculpture ornamentation of achene surface striate (Figure 1E, F, Figure 2 C, I, N).

Carthamus tenuis* (Boiss. & Blanche) Bornm. subsp. *tenuis

Achenes broadly obpyramidal, oblique, 4 angled, light brown, upper part darker, 3-4x3 mm. Pappus 7.5-9 mm, light to dark brown, inner pappus shorter than outer. Palea scabrous. Hilum obpyramidal, 0.75-0.9 x 1.2-1.3 mm. Sculpture ornamentation of achene surface finely and irregularly striate (Figure 1G, H, Figure 2 D, J, O).

subsp. *gracillimus* (Rech. f.) Hanelt

Achenes broadly obpyramidal, oblique, 4 angled, light brown to brown, upper part darker, 3-6.5x2.5-5 mm. Pappus 7-11 mm, straw coloured to brown, inner pappus shorter than outer. Palea scabrous. Hilum obpyramidal, 0.7-0.9 x 1.2-1.4 mm. Sculpture ornamentation of achene surface irregularly striate.

***Carthamus persicus* Desf. ex Willd.**

Achenes broadly obpyramidal, oblique, 4 angled, light brown, upper part darker, 3-6x3.5-4.5 mm. Pappus 8.5-12.5 mm, light brown, inner pappus \pm equal length with outer. Palea scabrous. Hilum obovate, 0.5-0.8 x 0.4-0.6 mm. Sculpture ornamentation of achene surface striate (Figure 1I, J, Figure 2 E, K, P).

***Carthamus tinctorius* L.**

Achenes obpyramidal, oblique, 4 angled, shiny white, upper part darker, 3-6.5x2.5-5 mm. Pappus absent or rarely the inner achenes with short narrow scales. Hilum obovate, 1-1.5

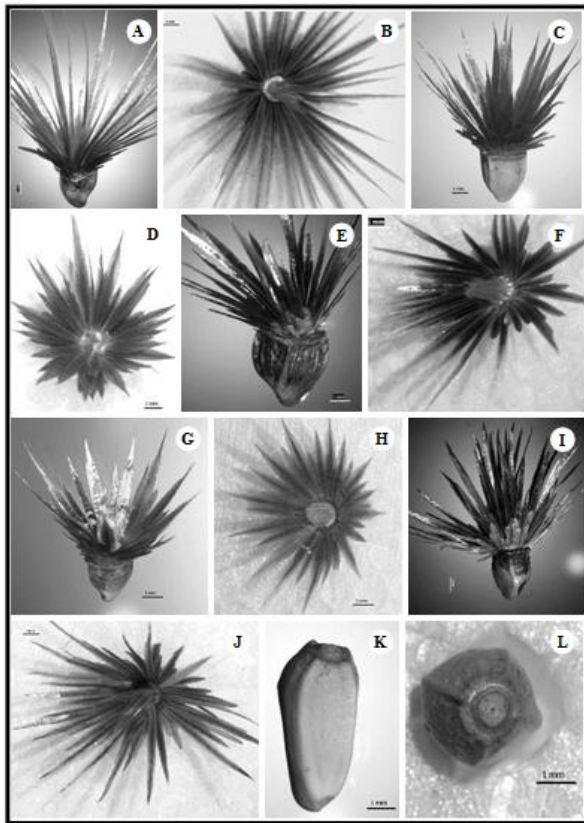


Fig 1. A: Lateral achene view of *C. dentatus* (CD32) B: Apical achene view of *C. dentatus* (CD32) C: Lateral achene view of *C. glaucus* subsp. *glaucus* (CG5) D: Apical achene view of *C. glaucus* subsp. *glaucus* E: Lateral achene view of *C. lanatus* (CL14) F: Apical achene view of *C. lanatus* (CL14) G: Lateral achene view of *C. tenuis* subsp. *tenuis* (CT3) H: Apical achene view of *C. tenuis* subsp. *tenuis* (CT3) I: Lateral achene view of *C. persicus* (CP 1) J: Apical achene view of *C. persicus* (CP 1) K: Lateral achene view of *C. tinctorius* (cultivar Dincer, gathered from Eskişehir Transitional Zone Agricultural Research Institute) L: Apical achene view of *C. tinctorius*.

x 0.6-0.9 mm. Sculpture ornamentation of achene surface finely striate (Figure 1K, L, Figure 2 F, Q).

Discussion

There is difference among taxa in means of achene length, width and pappus length (Table 2). Among all taxa *C. lanatus* has the longest achene. *C. dentatus* has the longest pappus length, while *C. glaucus* has shortest (Table 3). *C. dentatus* and *C. tenuis* are the most dissimilar taxa, *C. persicus* and *C. lanatus* are the least dissimilar taxa on the bases of achene morphology (Table 4). Hilum shape is obpyramidal in *C. dentatus*, *C. lanatus* and *C. tenuis*, while obovate in *C. glaucus*, *C. persicus* and *C. tinctorius*. Sehgal et al. (2009) used molecular data to reconstruct phylogeny of *Carthamus* in their research. The species with obovate hilum in our findings are in correlated with the Sehgal et al.'s (2009) *Carthamus* section.

Hilum shape similarity can be considered as another morphological support for *C. persicus* and *C. tinctorius* close relationship, beside other morphological and molecular similarities. Other than this grouping, our findings do not fit

any proposed subgeneric classifications (De Candolle, 1838; Cassini, 1819; Ashri, 1957; Knowles 1958; Ashri and Knowles 1960; Hanelt, 1961, 1963; Estilai 1977; Vilatersana et al, 2000, 2005; Sehgal, 2009; Bowles et al., 2010). SEM investigation of seed coat sculpture revealed 3 distinct types of surface structure: I: Striate in *C. dentatus*, *C. glaucus*, *C. lanatus* and *C. persicus*, II: Irregularly striate in *C. tenuis*, and III: Finely striate in *C. tinctorius*. Within Asteraceae, the sculpture ornamentation of *Carthamus tinctorius* resembles to *Cirsium aytachii* H.Duman & R.R.Mill (Bani and Adigüzel, 2010). The Flora of Turkey (Kupicha, 1975) does not involve the information about the achene morphology in descriptions and identification key of *Carthamus* species. Therefore, identification of species is not possible for a researcher only with achenes. Our research is the first to represent achene morphology of the Turkish *Carthamus* species. Using morphological characters of fruits as taxonomical markers is very common for Asteraceae, for example Abid and Qaiser (2009) used micromorphological characters of cypselas for taxonomic delimitation of 44 species included in 15 genera of the tribe Anthemideae (Asteraceae) from Pakistan and Kashmir. Moreover, Bhar and Mukherjee (2004) investigated macro and micro-characters of cypselas of 7 species belonging to 5 genera (*Achillea*, *Anthemis*, *Leucanthemum*, *Matricaria* and *Tanacetum*) of the tribe Anthemideae (Asteraceae) and claimed exomorphic characters of cypselas serve as reliable taxonomic marker in systematic study. Like our research, this kind of investigations give information on micro and macro-morphological characters of fruits and provide an identification key basing on these characters. Results of our study show that the achene morphology alone is not enough to solve taxonomical situation, but is a powerful tool with other characters and is of great value for taxonomist and for plant breeders for identification of species without the need of herbarium specimen.

Materials and methods

Plant material

Specimens were collected in 2011 during the excursions for the General Directorate of Agricultural Research and Policy project "The genetic and morphological characteristics of *Carthamus* L. species found in Anatolian natural flora and their facilities to be improvement materials". Seventy-four populations belonging to 5 species were collected in Turkey: 36 populations from *C. dentatus*, 28 populations from *C. lanatus*, 6 populations from *C. glaucus*, 1 population from *C. persicus*, 1 population from *C. tenuis* subsp. *tenuis* and 2 populations from *C. tenuis* subsp. *gracillimus* (Supplementary data). *C. tinctorius* cultivar Dincer, gathered from Eskişehir Transitional Zone Agricultural Research Institute.

Statistical analysis

Length, width and pappus length of achenes were measured from 5 individuals per each population (Supplementary data). Summarized metric data were given for each species by evaluating populations (JMP 5.0.1a, SAS Institute, 1989-2002). The NTSYS program written for the IBM PC by Rohlf (2000) was used to calculate symmetric (dissimilarity) matrix. The SIMINT similarity with Euclidian distance combined with unweighted pair group average linking was used for this purpose.

Table 2. ANOVA of the achene length, width and pappus length of populations using Sum of squares (DF: degrees of freedom; MS: mean squares). Also given is the coefficient of variation (CV)^a as a measure of the unpredictability of the means.

SOURCE	DF	MS	CV
Length	4	4.04	16,15
Width	4	3.05	15,64
Pappus Length	4	787,84	20,66

P < 0.001. *CV = (standard deviation x 100) / mean (thus expressed as a percentage).

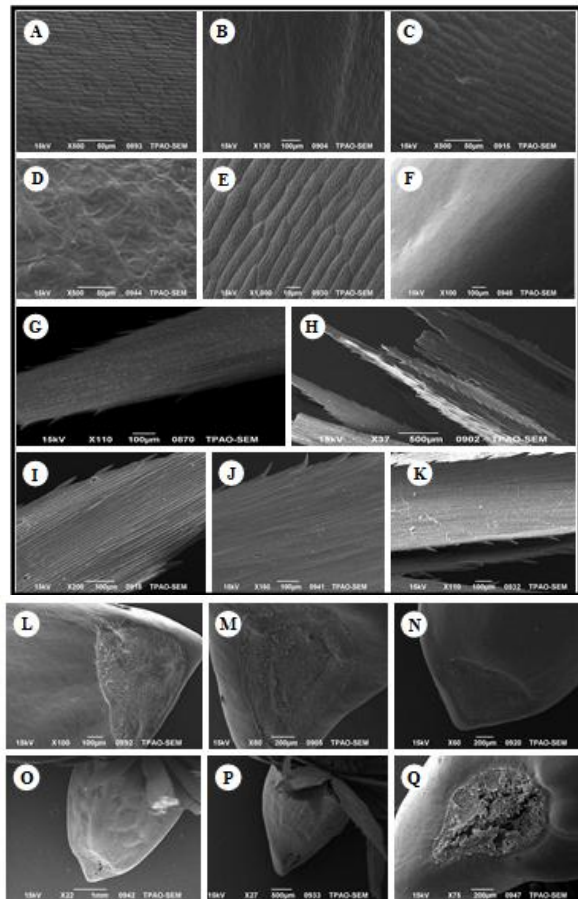


Fig 2. A: Striate surface view of *C. dentatus* (CD32) B: Striate surface view of *C. glaucus* subsp. *glaucus* (CG5) C: Striate surface view of *C. lanatus* (CL14) D: Striate surface view of *C. tenuis* subsp. *tenuis* (CT3) E: Striate surface view of *C. persicus* (CP 1) F: Striate surface view of *C. tinctorius* (cultivar Dincer, gathered from Eskişehir Transitional Zone Agricultural Research Institute) G: Palea of *C. dentatus* (CD32) H: Palea of *C. glaucus* subsp. *glaucus* (CG5) I: Palea of *C. lanatus* (CL14) J: Palea of *C. tenuis* subsp. *tenuis* (CT3) K: Palea of *C. persicus* (CP 1) L: Obpyramidal hilum of *C. dentatus* (CD32) M: Obovate hilum of *C. glaucus* subsp. *glaucus* (CG5) N: Obpyramidal hilum of *C. lanatus* (CL14) O: Obpyramidal hilum of *C. tenuis* subsp. *tenuis* (CT3) P: Obovate hilum of *C. persicus* (CP 1) Q: Obovate hilum of *C. tinctorius* (cultivar Dincer, gathered from Eskişehir Transitional Zone Agricultural Research Institute).

Table 3. General statistics of *Carthamus* achenes of collected populations

		CD	CG	CL	CP	CT
Mean	Length	4.82	4.42	5.06	4.80	4.27
	Width	4.20	3.86	4.20	4.00	3.37
	Pappus length	15.52	8.40	10.11	10.60	8.90
Min	Length	2.50	3.00	3.00	3.00	3.00
	Width	2.50	2.50	2.50	3.50	2.50
	Pappus Length	9.00	6.00	6.00	8.50	7.00
Max	Length	7.00	5.50	7.00	6.00	6.50
	Width	6.00	5.00	5.50	4.50	5.00
	Pappus Length	23.00	11.00	18.00	12.50	11.00
Std Err	Length	0.06	0.13	0.06	0.49	0.33
	Width	0.05	0.12	0.05	0.22	0.19
	Pappus Length	0.22	0.25	0.19	0.80	0.34
Variance	Length	0.59	0.41	0.57	1.20	1.60
	Width	0.46	0.34	0.37	0.25	0.55
	Pappus Length	9.55	1.52	4.79	3.18	1.76

Table 4. Symmetric (dissimilarity) matrix of *Carthamus* species.

	CD	CG	CL	CP	CT
CD	0.0000000	2.9686961	2.0554966	1.8340521	3.7781909
CG	2.9686961	0.0000000	2.2954830	1.4688595	1.5225903
CL	2.0554966	2.2954830	0.0000000	1.0053146	3.4749674
CP	1.8340521	1.4688595	1.0053146	0.0000000	2.5479745
CT	3.7781909	1.5225903	3.4749674	2.5479745	0.0000000

Microscopy

Achenes were observed and photographed with Leica S6D stereomicroscope. For scanning electron microscopy (SEM), seeds were directly mounted on stubs and covered with gold and images were taken by using the model of Jeol JSM-6060 scanning electron microscope.

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