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Carpological and Karyological Characteristics of Endemic *Tripleurospermum callosum* in Turkey

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Abstract: Morphology of achene (fruit, cypsela) and chromosome in *T. callosum* (Boiss. et Heldr.) E. Hossain an endemic member of the genus *Tripleurospermum* Sch. Bip. (Anthemideae, Asteraceae) in Turkey were studied using morphological, micromorphological and karyological techniques. The micromorphological characteristics of the achenes are reported here for the first time. Scanning electron microscopic (SEM) observations revealed that the achenes have had cell junctions, longitidunal striations and network in epicarp ornamentation. Some correlations between carpological and karyological characters were noted and systematic significance of them was discussed.

Key words: Achene · chromosome · morphology · SEM

INTRODUCTION

The genus *Tripleurospermum* belongs to tribe Anthemideae which is in the Asteraceae (Compositae) family and comprises about 38 species distributed mainly Europe and temperate Asia, a few species also North America and North Africa [1]. It is also difficult to name exact number of species, without monographical treatment of the whole genus, since not rarely the species of *Tripleurospermum* have been referred to genera *Pyrethrum*, *Chrysanthemum*, *Matricaria*, *Chamaemelum* and others [2].

The problems that the genus has in terms of taxonomy can not be ignored. *Tripleurospermum* is very similar to *Matricaria* in terms of its morphological characteristics. It is also similar to many other Anthemideae genera in habits. Therefore, it has been confused both taxonomically and nomenclaturally with the genus *Matricaria* and other Anthemideae genera [2-5].

Tripleurospermum species have achene type fruits (cypsela). Ripe achenes are generally necessary for identification in this very critical genus. *Tripleurospermum* species are rather outlined morphologically and they are easily recognised by the peculiar structure of the achenes. But the study of the species only the herbarium materials is very difficult [2].

A part of *Tripleurospermum* species distributed in the flora of other countries [2, 6-8]. It is represented by 26 taxa in the level of species and variety in Flora of Turkey and the East Aegean Islands [9]. Most of these taxa are distributed in northeast Anatolia. More recently, the number of *Tripleurospermum* taxa known from Turkey reached 27 [10].

Studies on carpology and karyology of this species are limited. The micromorphological structure of the achenes has not been studied so far. Therefore, the purpose of this paper is to investigate the achene and chromosome characteristics of *T. callosum*. This paper is also an introduction to micromorphological studies on the genus. The karyological data, when considered with carpological data, might be heplful in establishing systematic relationships within the genus.

MATERIALS AND METHODS

Plant material: Specimens were collected from north-east Anatolia, Turkey. Localities of the its are listed below. They are deposited in the herbarium at the Karadeniz Technical University, Department of Biology (KTUB).

A8 Gümüþhane: Kop Dağı pass, meadows, damp alpine, 2200 m, 14.vii.2000, Inceer 69.

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A7 Gümüshane: Tekke köyü, rood- sides, 1100 m, 21.vi.2001, Inceer 120.

A8 Rize: Ikizdere, Anzer village, meadows, road sides, 2200 m, 12.vii.2001, Inceer 137.

A8 Rize: Ikizdere, between Ikizdere and Anzer village, rood-sides, stream-sides, 1800 m, 13.vii.2002, Inceer 155.

A9 Artvin: Savsat, above Sahra National Park, damp subalpine pastures, roadsides, grassy slopes, 1900 m, 8.vi.2002, Inceer 149.

Carpological analyses: The achenes were obtained from living or herbarium specimens. Stereo-binoculer microscope was used to morphometric analyses of the achenes. Measurements were made of 20 achenes from different specimens.

SEM observations were made of eight to ten achenes from different specimens. The achenes were treated with 1/9 (v/v) mixture of concentrated sulfuric acid: acetic anhydride and sonicated in an ultrasonic cleaner in 50 Hz for 10 min to remove the some particles such as dustiues and pollens. Then they were rinsed in distiled water and oven-dried for 24 h at 60°C [11]. Dried achenes were spulter-coated with 200-300°A of gold before observation with JSM - 6400 Scanning Electron Microscopy. The achenes were photographed at an accelerating voltage of 5 KV. Micrographs were obtained at a magnification of X300-500.

Karyotype analyses: Root tip meristems only belonging to the specimens of Inceer 137 obtained from natural populations were used for chromosome analysis. The tips of roots, cleaned of soil particles, were cut off and pre-treated with 0.05% colchicine for 2.5 h. The root tips were then fixed in ethanol-acetic acid (3:1) for at least 24 h at 4°C, hydrolyzed in 1 N HCl at 60°C for 12-13 min and then rinsed with tap water for a minimum of 2-3 min. Staining was carried out in Feulgen for 1 h and squash preparations were made [12].

The best metaphase plates of each specimen, normally at least three, were photographed and idiogram was prepared from enlarged prints by measuring the total length of the chromosomes and of their arms and satellites. The centromeric index (r) was then calculated as the long : short arm ratio and chromosomes classified according to Levan *et al.* [13]. The intrachromosomal asymmetry index (A_1) was calculated according to the formula proposed by Romero Zarco [14], while the interchromosomal asymmetry index (A_2) was measured as the ratio standard devision of chromosome length/mean chromosome length.

RESULTS AND DISCUSSION

Carpology: Shape of the achenes are more or less obpyramidal. They are 1.6-1.9 mm in length and 0.4-0.6 mm in width. Surface of its is tuberculate-rugulose in dorsal side. The achenes is colored as dark brown or blackish at maturity. They are non-mucilaginous and 3-ribbed in ventral side. The ribs are white, thin, acute and fissures are broad, rugulose or tuberculate. Corona is marginiform, white, crenulate. It is 1/8-1/6 as long as achene (Table 1, Fig. 1). Carpological data of *T. callosum* were compared with data in the "Flora of Turkey and the East Aegean Islands" [9]. They are basically consistent with Grierson [9]. However, there are some differences in the measurements of the achenes (Table 2).

Table 1: Carpological characters and measurements in *T. callosum*

Achene length (cm)			Achene width (cm)			
Min	Max	Mean ± SE	Min	Max	Mean ± SE	Epicarp ornamentation
0.16	0.19	0.18±0.05	0.04	0.06	0.05±0.05	Striations, cell junctions, reticulate

Table 2: A comparison of some characters of *T. callosum*, *T. sevanense* and *T. transcaucasicum*

	Characters	*T. callosum* (Present study)	*T. callosum*[a]	*T. sevanense*[a]	*T. transcaucasicum*[a]
Achene	Shape	± obpyramidal	± obpyramidal	obpyramidal	obpyramidal
	Length	1.6-1.9 mm	1.5-1.8 mm	1-1.5 mm	up to 1.5 mm
	Width	0.4-0.6 mm	0.4-0.6 mm	0.4-0.5 mm	0.5-0.8 mm
	Mucilaginous	no	no	no	yes
	Corona	1/8-1/6 as long as achene	1/8-1/6 (1/5) as long as achene	1/8-1/6 as long as achene	1/8-1/6 (1/5) as long as achene
	Total karyotype length (TKL)	31.99 μm	31.43 μm[b]	31.05 μm[b]	13.48 μm[b]
Chromosome	Ploidy level	4x	4x[b]	4x[b]	2x[b]
	A_1	0.38	0.38[b]	0.35[b]	0.47[b]
	A_2	0.19	0.18[b]	0.19[b]	0.18[b]

[a]: Grierson [9] [b]: Inceer and Beyazoglu [12]

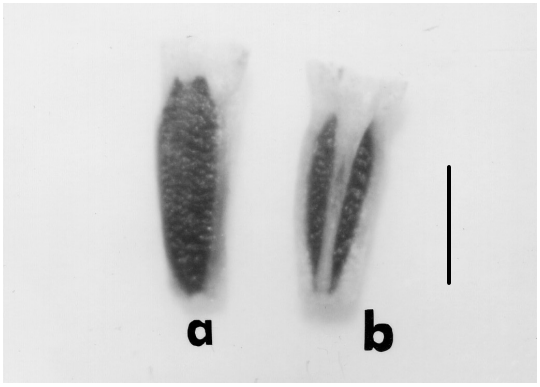
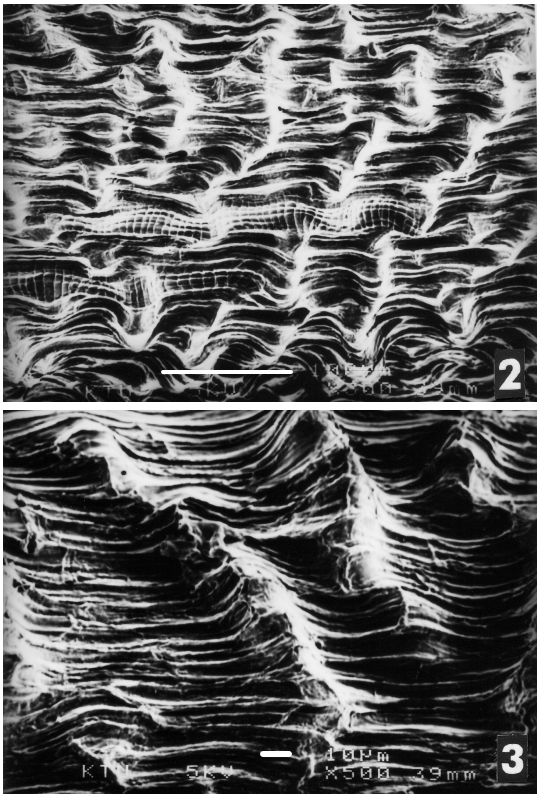


Fig. 1: General view of the achene, a: dorsal side, b: ventral side, Scale bar: 1 mm



Figs. 2 & 3: SEM micrographs of the achenes, Fig. 2: Dorsal surface (X300), Fig. 3: Ventral surface (X500)

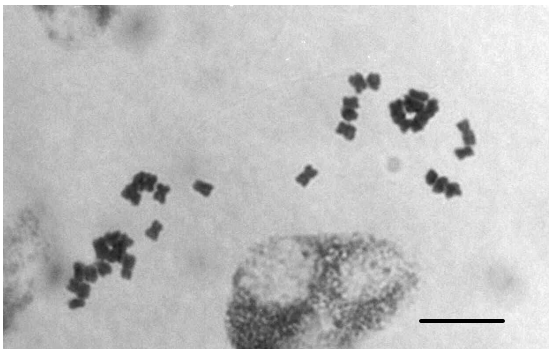


Fig. 4: Somatic metaphase (2n = 36), Scale bar = 10 µm

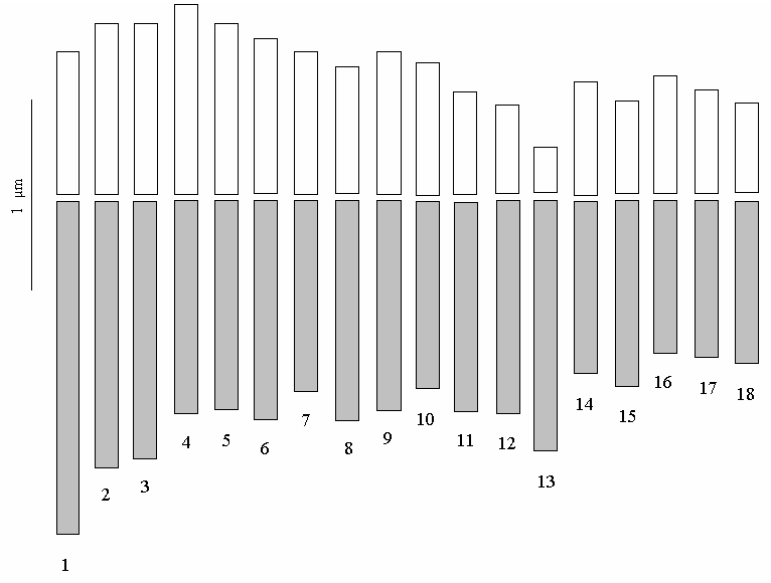


Fig. 5: Haploid idiogram

Table 3: Karyotype of *T. callosum* (Chromosomal formula: 2n = 4x = 36 = 22m + 12sm + 2st)

Pairs	L	S	L+S	L:S	Type	CI
1	1.75±0.04	0.75±0.04	2.50±0.07	2.33	sm	30.00
2	1.40±0.03	0.90±0.04	2.30±0.07	1.56	m	39.13
3	1.35±0.05	0.90±0.04	2.25±0.09	1.50	m	40.00
4	1.12±0.10	1.00±0.09	2.12±0.18	1.12	m	47.17
5	1.10±0.08	0.90±0.09	2.00±0.17	1.22	m	45.00
6	1.15±0.05	0.82±0.05	1.97±0.10	1.40	m	41.62
7	1.00±0.03	0.75±0.10	1.75±0.12	1.33	m	42.86
8	1.15±0.07	0.67±0.05	1.82±0.11	1.72	sm	36.81
9	1.00±0.08	0.75±0.08	1.75±0.16	1.33	m	42.86
10	0.98±0.10	0.70±0.06	1.68±0.15	1.40	m	41.67
11	1.10±0.04	0.54±0.05	1.64±0.11	2.04	sm	32.93
12	1.12±0.06	0.47±0.05	1.59±0.11	2.38	sm	29.56
13	1.31±0.03	0.24±0.04	1.55±0.05	5.46	st	15.48
14	0.90±0.04	0.60±0.07	1.50±0.05	1.50	m	40.00
15	0.97±0.04	0.49±0.09	1.46±0.08	1.98	sm	33.56
16	0.80±0.05	0.62±0.05	1.42±0.10	1.29	m	43.66
17	0.82±0.07	0.55±0.05	1.37±0.12	1.49	m	40.15
18	0.85±0.09	0.47±0.05	1.32±0.13	1.81	sm	35.61

Note: Abbreviations are as follows: L; length of the long arm of the chromosome, i.e., mean value ± SE (μm), S; length of the short arm of the chromosome, i.e., mean value ± SE (μm), L+S; total length of the chromosome, i.e., mean value ± SE (μm), L:S; length ratio of long and short arms of the chromosome, CI; centromeric index

The micromorphological characters of the achenes are reported here for the first time. As could be seen Figs. 2-3, epicarpic cells in the achenes are rather elongate with longitudinal striations and cell junctions. Wall of these cells are thin and conspicuously white. In addition, the achenes have network with more or less equal longitudinal and transversal folds (reticulate epicarp) and grooves in some area of dorsal epicarp ornamentation. ventral surface. Similar observations were found in the genus *Artemisia* L. (Anthemideae, Asteraceae) [15]. Structually, these surface characters of the achenes can result from very different portions of the cell wall. Cutler and Brandham [16] and Stace [17] reported that these characters was a strong genetic control. Thus, the variations in epicarpic cells may be used as a taxonomic criterion at specific level for this genus, but it should be combined with other morphological characters.

Karyology: *T. callosum* is tetraploid species with 2n = 4x = 36 chromosomes (Figs. 4 & 5). In the karyotype of this species, the following chromosome types could be distingushed: 22 metacentric, 12 submetacentric and 2 subacrocentric chromosomes (Table 3). No satelites were observed. Total karyotype length, roughly indicative of the DNA content, is 31.99 μm. Symmetry class is 2A. The value for A_1 or intrachromosomal asymmetry index is 0.38. For the A_2 or interchromosomal asymmetry index is 0.19 (Table 2). Present counts confirm the tetraploid level (2n = 4x = 36) previously established [12, 18]. The chromosome data obtained from present study are basically similar to that described by Inceer and Beyazoglu [12]. Slight karyological differences are found between two populations of *T. callosum* (Table 2). These minor changes may represent geographical variation within the species.

Tetraploid *T. callosum* is closely allied to tetraploid *T. sevanense* and diploid *T. transcausicum* [12]; from the former it differs in having a characteristic branching pattern (i.e. from base upwards), very laxly corymbose inflorescence and ovoid-conical receptacle; from the later it is distinguished habit and glandular disc flowers [9]. These closely related species have also some different carpological and karyological properties (Table 2). There is a good correlation between ploidy level and mucilaginous. Total karyotype length is also correlated with achene length. It is suggested that carpological and karyological characters can be used as additional data to support the morphological separation of the species.

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