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Karyological, morphological and palynological studies of the populations of tanacetum pinnatum boiss (asteraceae) in hamedan (Iran)

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ABSTRACT

In this study, chromosome number and some morphological features of 6 populations of *Tanacetum pinnatum* Boiss. collected from different localities in Hamedan province, Iran, are reported. In this species, five populations with diploid chromosome number (2n=2x=18) and one population with tetraploid chromosome number (2n=4x=36) were found. Some vegetative and reproductive features were measured morphometricaly. Pollen grain's characteristics were also studied using light microscopy. Morphological and palynological data were analyzed by MVSP software with UPGMA method. Analysis indicated significant differences among the populations and showed that tetraploid population is quit deferent from diploid ones. It seems that there are significant differences depending on ploidy level, morphological features and pollen characteristic in tetraploid plant and diploids.

KEYWORDS

Chromosome;
Palynology;
Variation;
Tanacetum pinnatum.

INTRODUCTION

The genus Tanacetum L. from Asteraceae, tribe Anthemideae, comprising about 160 species, distributed in Europe, Asia, Northern Africa and North America[1], and constitutes one of the most problematic genera in the terms of taxonomic complexity, especially concerning genus delimitation against closely related entities[2]. This polymorphic genus is characterized by a considerable variation, between its species, concerning flowers, inflorescence morphology, and achene's properties[3]. Tanacetum contained 54 species in Flora Iranica[4] and some of them are endemic to Iran[5].

T. pinnatum is a perennial plant that exhibits divergency morphologically: its height varies between 20 and 60 cm, basal leaves between 5 and 17 cm. Its inflorescence is corymb, with numerous capitule or rarely 3 to 8 dense capitule, ovate to cylindrical involucre and leaves are pinnatisect[6]. The major constituents in the essential oil of *T. pinnatum* are camphor, α-pinene and camphene[7]. Data for the species are scarce and according to our bibliographical studies, only few studies have been performed about this species in Iran[7,8].

In Anthemideae, polyploidy is very common, particularly in the some genera such as Chrysanthemum[9], Ajania[10] and Artemisia[11]. The genus Tanacetum shows ploidy levels variation upon species, even in different populations of the same species. For example, there are different ploydies, from diploid (2X) to decaploid

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(10X) in *T. polycephalum*[8,12,13,14] and diploid (2X) to tetraploid (4X) in *T. parthenium*[15,16]. Chromosome number was reported only as 2n= 18 for *Tanacetum pinnatum*[8].

One of the main aims in this paper was to document the chromosome numbers and karyological information of the populations of *T. pinnatum*, collected from different localities in Hamedan province, in order to obtain information about chromosome number that could be useful in their taxonomical application. Some morphological features and palynological characteristics of each population were also investigated in order to assess the existence the correlation between morphology and level of ploidy, as the second goal in this paper.

MATERIAL AND METHODS

Plant materials

Herbarium vouchers of the all studied species are deposited in the Herbarium of Department of Biology, Faculty of Science, Bu-Ali Sina University, Hamedan, Iran (BHU). The locations, collectors and dates are shown in the TABLE 1.

Karyological studies

Chromosome counts were made on somatic metaphases using standard squash techniques. Seeds collected from naturally growing plants were used in the present study. Root-tip meristems were obtained by germinating seeds on the wet filter paper in Petri dishes at approximately 20°C. Samples were pretreated with 0.05% colchicines for 2 h 30 min at room temperature. The material was fixed in 3:1 v/v absolute ethanol: glacial acetic acid for a minimum of 24 h at 4°C.

Meristems were hydrolysed in 1 M hydrogen chloride (HCl) for 30 min at room temperature. They were then stained in 2% acetic orcein for a minimum of 3 h at 4°C[17]. Squashes were made in 45% acetic acid. Photographs were taken through a Zeiss Axiostra Plus microscope (Germany) with a Canon G11 digital camera (Japan).

Morphological studied

Twelve morphological characters were assessed by numerical analysis (TABLE 2). Cluster analysis (CA) and principal components analysis (PCA) were performed by SYN-TAX PC 5.0 (Podani 1993). For CA, a pair-wise matrix of resemblance values was calculated from raw standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets[18]. A dendrogram was generated by the unweighted pair-group method by using arithmetic averages (UPGMA). Also, cophenetic correlation coefficient (rcs) was calculated[18]. For PCA, the raw data were used

TABLE 1 : The studied populations of T. pinnatum

Populations	Locations	Geographical characters	Altitude (m)	Collector	Chromosome number	Date of collection
a	Hamedan, mountains Kabudrahang, Goliabad	N: 35°14.641' E: 048° 50. 904'	1910	Ahmadi	2n=18	1 June 2011
B	Hamedan, mountains Kabudrahang, Goliabad	N: 35°14. 649' E: 048° 50. 524'	1870	Ahmadi	2n=18	1 June 2011
C	Hamedan, mountains Kabudrahang, Goliabad	N: 35°14. 583' E: 048° 50. 767'	1840	Ahmadi	2n=18	1June 2011
d	Hamedan, from Lalejin to Taherloo	N: 35°05.959' E: 048° 26. 270'	2019	Ahmadi	2n=18	30 May 2011
e	Hamedan, mountains Kabudrahang, Goliabad	N: 34°14. 560' E: 048° 50. 509'	1840	Ahmadi	2n=18	1 June 2011
F	Hamedan, Nahavand, Gamasiab	N: 34°02. 632' E: 048° 22. 982'	1883	Ahmadi	2n=36	29 May 2011

TABLE 2 : Morphological characteristics in the six populations of T. pinnatum

Populations Characters	1	2	3	4	5	6
Height of plant (cm)	36.4	35	40	32	40.8	45.5
Basal leaf length (cm)	5.1	9.5	5	5.2	6.6	9.8
Basal leaf width (mm)	8	10	15	6	14	8
Stem leaf length (cm)	2.7	3.8	2.3	3.2	3.3	1.7
Stem leaf width (mm)	4	6	7	7	6	8
Size of stomata (µm)	3.24- 2.62	3.5- 2.8	3.3- 2.9	3.45- 2.9	3.25-2.6	4.4- 3
Size of achene (mm)	2- 0.7	1.8- 1.2	1.2- 0.8	2.2- 0.6	1.7- 0.6	3- 1
Size of the Involucre (mm)	4.5- 5	5- 5.5	4- 4.3	4- 4.6	6- 4.5	5.5- 2.5
Size of the Pappus (mm)	0.5- 1	0.3- 1.1	0.2- 0.6	0.7- 1	0.6- 1	0.8- 1.1
No. of ribs	6	6	6	6	6	6
Shape of Involucre	Ovate	Ovate	Ovate	Ovate	Ovate	Cylindrical
Length of Peduncle (mm)	7	13	14	11	12	31

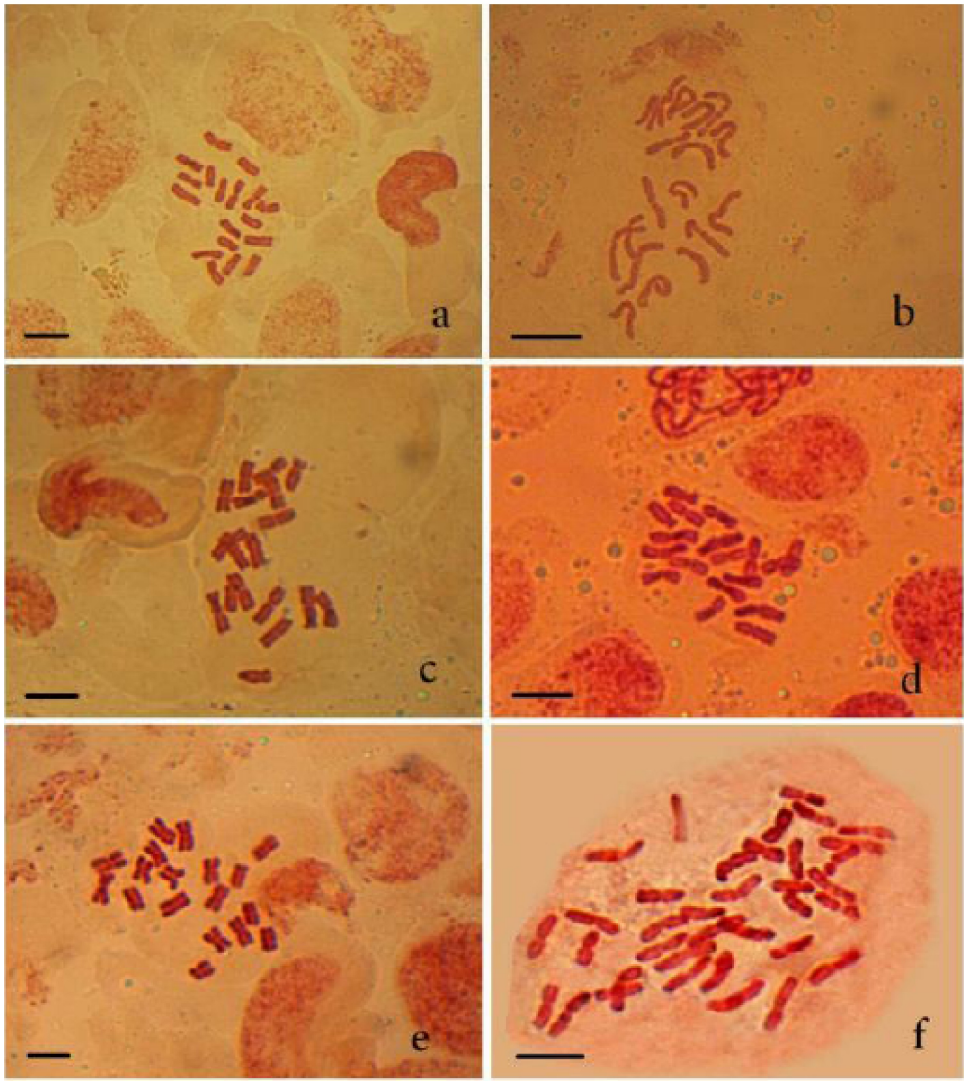

Figure 1 : Somatic metaphases of different populations of Tanacetum pinnatum. a-e) Five populations with chromosome number 2n= 18. F) A population with chromosome number 2n=36. Scale bars in all figures = 5µm

to create a correlation matrix, and two eigenvectors were extracted, providing two axes onto which the raw

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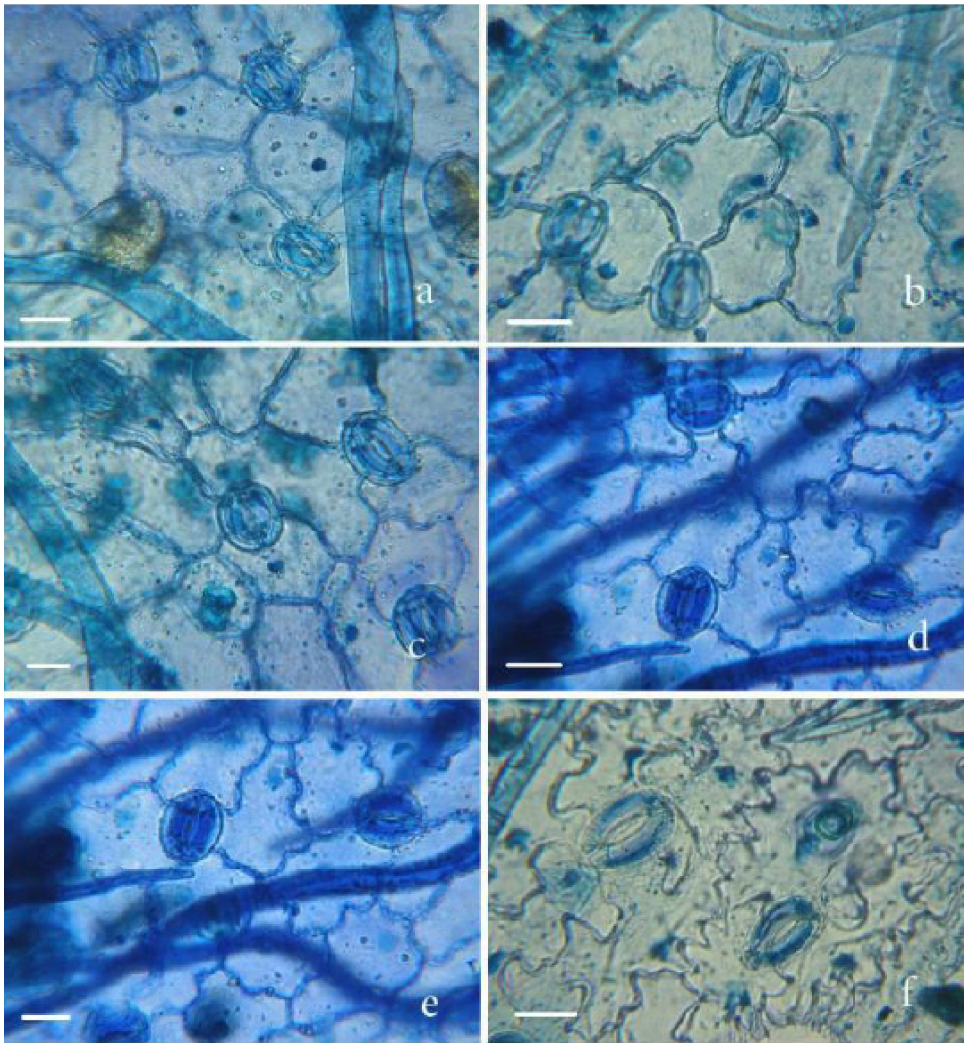

Figure 2 : Stomata and epidermal cell of different studied populations of T. pinnatum. Scale bars in all figures = 10 µm

data were projected to give a two-dimensional plot of the taxa and characters.

Palynological studies

Pollen grains were removed from herbarium sheets. General preparation consisted of acetolyzing mature pollen grains as described by Erdtman[19]. Finally, samples were examined using light microscope. Polar diameter (P), equatorial diameter (E), Spine Length (L), thickness (A), exine thickness (Ex) and distance between two spines (De), P/E and L/A were measured.

RESULTS AND DISCUSSION

Chromosome number

Most of the studied populations of *T. pinnatum* had the chromosome number 2n=18 (TABLE 1, Figures 1) and our count confirms the existence of the diploid cytotype of this species, reported by Chehregani and Mehanfar[8]. However, one population was found with tetraploid chromosome number 2n=4x=36 (Figure 1). This is the first report about its tetraploidy as new ploidy level for this species (TABLE1).

Morphological results

Morphometric measurement indicated that plant height varied from 32 to 45.5 cm, basal leaf length and width ranged from 5.1 to 9.8 cm and from 6 to 15 mm respectively. Stem leaf length and width ranged from 1.7 to 3.88 cm and from 4 to 8, respectively. It was also determined that in diploid plants stomatal cell length varied from 3.24 to 3.5 µm, while in tetraploid plant was 4.4 µm (Figure 2). Furthermore, it was found that involucre length varied from 4 to 6 mm, achene length ranged from 1.2 to 3 mm and length of Peduncle varied

from 7 to 13 mm in diploid populations but they are different from tetraplod population (TABLE 2).

In the tetraploid population it was found that Involucre length varied from and 2.5 to 5.5 mm, achene length ranged from 1 to 3 mm and length of Peduncle was 31 mm. The diploid populations exhibited ovate Involucre whereas the tetraploid population had cylindrical Involucre.

Analysis of Morphological data by MVSP software with UPGMA method showed two distinctive different groups in the populations of *T. pinnatum* (Figure 3).

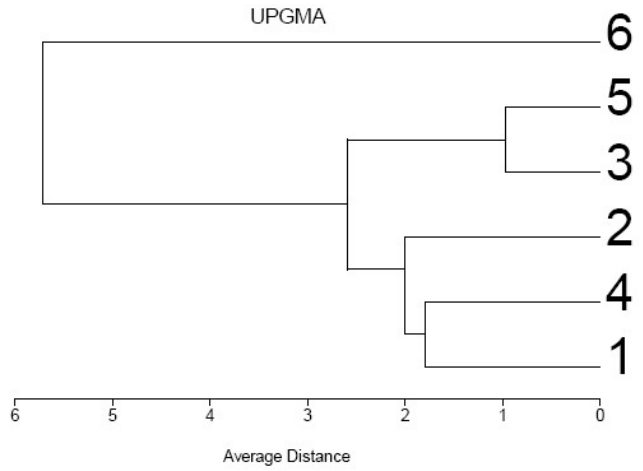

Figure 3 : Dendrogram of the six populations of T. pinnatum obtained by analysis of 12 morphological characters. The tetraploid population was separated carefully regarding morphological characteristics

Tanacetum pinnatun was investigated as term of palynology and the results showed that the pollen grains are belonging to the Anthemis-type that is accordance with some prior results[20,21], which is characterized by long spines as exine ornamental elements. Polar diameter ranged from 27.2 to 35.2 µm. P/E ratio varies from 0.92 to 0.97. The exine thickness ranged from 1.14 µm to 1.66 µm. Spine length and thickness ranged from 2.3 to 4 µm and from 3.08 to 3.9 µm. Distance between to spine varies between 4.4 to 7 µm and L/A ratio was varied from 0.64 to 1.05 (TABLE 3 and Figure 4). Results indicated that the tetraploid population has larger pollen grains than the diploid ones (TABLE 3).Palynological data were analyzed by MVSP software with UPGMA method and results showed that the diploid populations are similar regarding palynological characteristics but are different from the tetraploid population of *T. pinnatum* and the population was

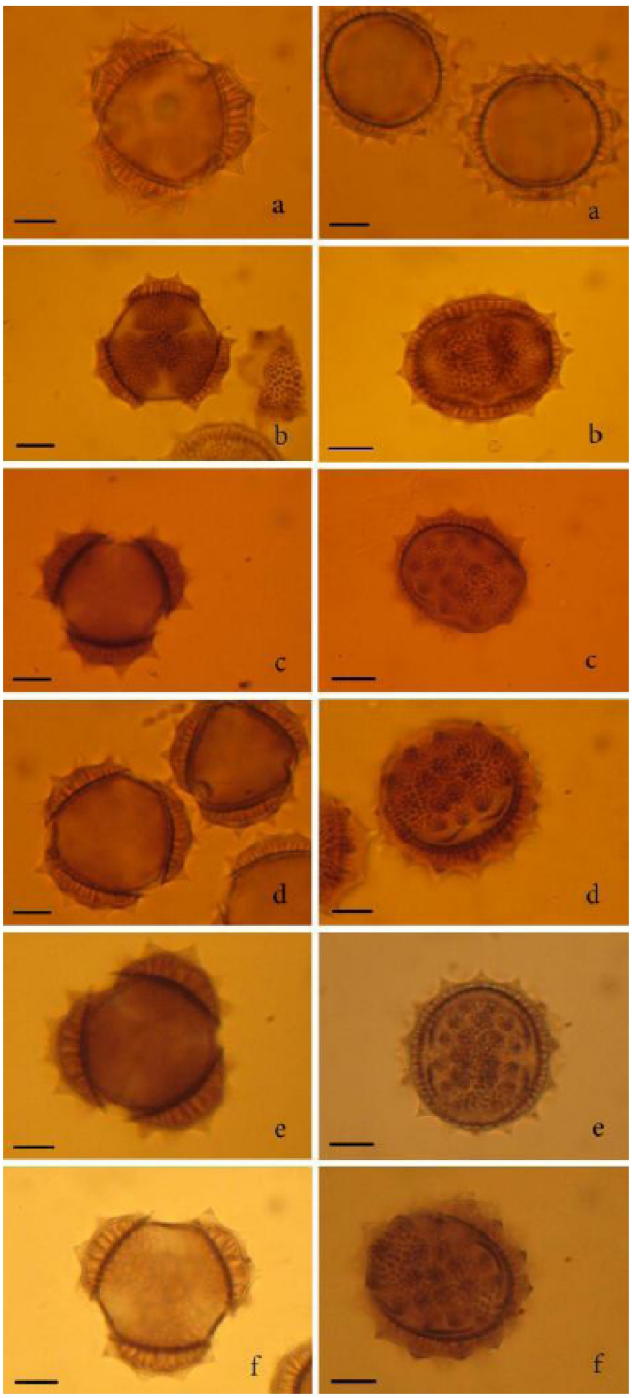

Figure 4 : Pollen grains of the studied populations of T. pinnatum. Left, polar view and right, equatorial view. Scale bars in all figures = 10 µm

located in a single branch in the resulted cluster (Figure 5).

Polyploid populations show considerable ecological, morphological and genetic differences compared to their corresponding diploids[22]. According to Oliveira[23] in some strains of *Stevia rebaudiana* there is a positive correlation between the size of the pollen

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TABLE 3 : Pollen measurements of the different populations of tanacetum pinnatun

Characters Popultions	Polar dameter (µm)	Equatorial diameter (µm)	(P/E) (µm)	Spine Length (µm)	Spine thickness (µm)	(L/A) (µm)	Exine Thickness (µm)	Ditance Between two spines (µm)
1	29.8	30.8	0.97	2.7	3.6	0.75	1.2	4.9
2	29.4	31.8	0.92	2.3	3.6	0.64	1.36	5.2
3	27.2	29.4	0.92	3.5	3.32	1.05	1.6	7
4	30.8	32	0.96	2.7	3.5	0.77	1.66	4.4
5	28.4	30.2	0.94	3.14	3.16	0.99	1.14	5.4
6	35.2	36.6	0.96	2.7	3.08	0.88	1.58	6.02

grains or stomata and the level of ploidy that is accordance with our results. The results of this research work showed that the size of the pollen, that are reflected in the P and E values among the populations, height of plant, achene length, length of Peduncle and size of stomata in teteaploid plant were larger than populations with diploid level. Compared to diploids, the tetraploid population had larger stem leaf width and lower Spine thickness, but these variations are minor and do not reveal significant differences between the tetraploid and diploids. Morphological differences were also observed between diploid plants of *T.pinnatum.*

The obtained groups of morphological data had a good correlation with palynological groups that confirmed a positive correlation between the level of ploidy, morphological features and pollen characteristic in tetraploid plant and diploids. This is the first report about the presence of teraploidy in this species and it is recommended for further studies regarding its taxonomical position, because its characteristics is significantly different from diploid populations. Its ploidy level can be regarded as the sign of new species formation[8].

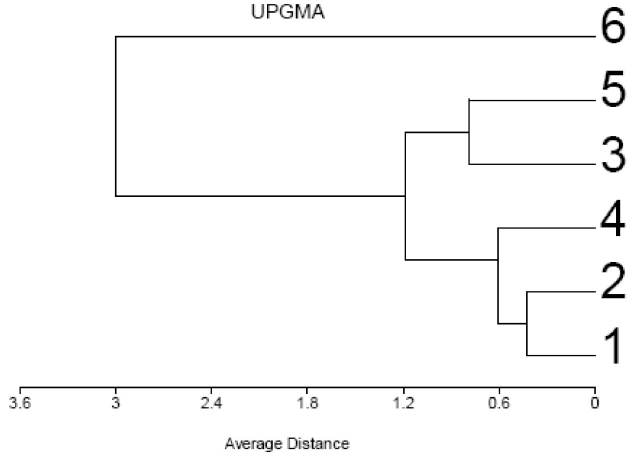

Figure 5 : Dendrogram of T. pinnatum populations obtained by analysis of palynological characters

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