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Karyotype Analysis of *Tulipa pulchella* (Liliaceae) (Fenzl ex Regel) Baker

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Abstract

Objective: In this study the somatic chromosome numbers and detailed morphometric properties of *Tulipa pulchella* (Fenzl ex Regel) Baker was examined.

Material and Methods: The seeds were germinated on moist filter paper in petri dishes at 25°C. Actively growing root tips were pretreated with 0.05 M colchicine for 6 h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 N HCl at 60°C for 1-2 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope X40 objective and were recorded with an Olympus Camedia C-4000 digital camera.

Results: The chromosome number of *Tulipa pulchella* is determined as 2n = 24 for this taxon. The karyotype consists of 8 submedian region (sm) and 4 subterminal (st) chromosomes. The metaphase chromosome length ranges from 9.35 to 16.48 µm, longest to shortest chromosome ratio was 1.9:4.1, total karyotype length (TKL) was 150.99 um and the karyotype symmetry was type 1A.

Conclusion: The basic chromosome number of *Tulipa pulchella* is x=12. The majority of tulip species and cultivars is diploid (2n=2x=24), however, encounters to triploids (2n=3x=36), tetraploids (2n=4x=48) and even some pentaploids (2n=5x=60) and hexaploid (2n=6x=72). Identifying the chromosome number of this species in this study would provide a base for biosystematic studies.

Keywords: *Tulipa pulchella*; Chromosome Number; Karyotype Analysis

Introduction

Tulipa L. is a member of Liliaceae, which was formerly treated as a large family, Liliaceae sensu lato. According to the recent phylogenetic studies, Liliaceae includes 15 genera (1-4). The highest diversity of the genus *Tulipa* is found in the Tien Shan and Pamir Alai mountain ranges in Central Asia. The genus extends from western China, Inner Mongolia and the western Himalaya throughout Central Asia and Iran to the Caucasus, the Balkans, Greece, Cyprus and Turkey, south to the Arabian Peninsula and southeastern Pakistan, and north to the Ukraine and southern Siberia. It extends westwards throughout North Africa to Morocco and the Iberian Peninsula (5). Although the genus is also found throughout the Mediterranean and most other parts of Europe, these regions are not part of the natural distribution of the genus. These tulip species were brought by merchants and travelers from Anatolia and Central Asia to Europe and escaped from cultivation into the wild where they naturalized (5-11).

Tulipa comprises about 40 or more than 100 species throughout the world, and is widely used as an ornamental plant because of its very attractive flowers. Although European people first introduced *Tulipa* species with the gift from Kanuni Sultan Süleyman, a sultan of Ottoman Empire, to Emperor Ferdinand I in 1554–1562 today about 65% of the world's cultivated tulips are produced in the Netherlands and the tulip has become a national symbol of this country (12-14). The genus has historically been subdivided into two subgenera, *Tulipa* (*Leiostemones* Boiss.) and *Eriostemones* Boiss., which are clearly distinguished by morphological characteristics (15). *Tulipa* is represented by 18 taxa in Anatolia and these occur mostly in the Irano-Turanian floristic region of Turkey (16-18).

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The total number of tulip species is not known exactly, but according to various researchers the genus *Tulipa* is represented by 40–100 species. In the World checklist of selected plant families (19) 503 names have been listed for *Tulipa*, but only 121 taxa have been accepted. Most recently Christenhusz et al. accepted 76 species. Most species are distributed in Central Asia and the Caucasus (65 species), Iran and adjoining regions (36 species) and Turkey (18 species) (4,5).

The genus *Tulipa* is currently the most important bulbous geophyte in the World and contains around 35 species in the subg. *Tulipa* with glabrous stamens, and 20 species in the subg. *Eriostemones* with bossed, usually hairy stamens (5,11,14). *Tulipa* was revised for the "Flora of Turkey and the east Aegean Islands" by Marais, accepting 14 species and one infaspecific taxon. Since then, three new species have been described from Turkey (17,18,20,21). In Turkey, Eker et al. revised the genus *Tulipa*. Due to detailed morphologic, geographic and cytotaxonomic studies of the taxa, the genus *Tulipa* was divided into two subgenera and they represented 17 species, two subspecies and two varieties (in total 19 taxa) (4). Marais reduced *T. pulchella* (Fenzl ex Regel) Baker to a synonym of *T. humilis* Herbert, and similarly Van Raamsdonk and De Vries accepted this species as a form of *T. humilis* (15,18). Christenhusz et al. treated it as a natural variety of *T. humilis* (5). Although both species show large variation in flower colour, there is consistency of how this varies; mauve in *T. humilis* and pinkish-purple to magenta colours in *T. pulchella* are dominant and typical. *T. pulchella* differs also from *T. humilis* in having blackish-brown bulb tunics and a bicolored floral blotch. Additionally, these species have an allopatric distribution, at least in Turkey (4). Eker et. al. reported as chromosome numbers of *T. pulchella* and synonym *T. humilis* 2n=2x=24 (4).

Material and Methods

Plant material was collected from natural habitats during the fruiting season in Mersin in 2015. Voucher specimen was deposited at the Firat University Herbarium (FUH). Karyological studies were conducted on meristematic cells obtained from the root tips.

The seeds were germinated on moist filter paper in petri dishes at 25°C. Actively growing root tips were pretreated with 0.05 M colchisin for 6 h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 N HCl at 60°C for 1-2 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope X40 objective (Olympus Optical Co. Ltd., Tokyo, Japan), and were recorded with an Olympus Camedia C-4000 digital camera (Olympus Optical Co. Ltd., Tokyo, Japan). The short arm (S), long arm (L) and total lengths of each chromosome were measured and the relative lengths, arm ratios, and centromeric indices were determined from images of selected cells. Chromosomes were classified according to the nomenclature of Levan et al. (22). The intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2) followed those of Romero-Zarco (23). The karyotype symmetry nomenclature followed Stebbins (24). Also, relevant literature the online chromosome number databases, Index to Plant Chromosome Numbers (IPCN) (25).

Figure 1: Habit of *T. pulchella* in the wild.

Results

The results of this study showed that the chromosome number of *T. pulchella* is 2n=24. Karyotype analysis of this species to reveal the many values were calculated. The number of somatic chromosome, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), Stebbins and asymmetry indexes (A1, A2) are presented in Table 1; relative length, arm ration, centromeric index, type, in Table 2. Metaphase chromosomes in Fig. 2 shown and haploid idiograms of *T. pulchella* in Fig. 3. The basic chromosome number determined as x = 12 for

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this taxon. The karyotype consists of 8 submedian region (sm) and 4 subterminal (st) chromosomes. The metaphase chromosome length ranges from 9.35 to 16.48 µm, longest to shortest chromosome ratio is 1.9:4.1, total karyotype length (TKL) 150.99 µm and the karyotype symmetry is type 1A.

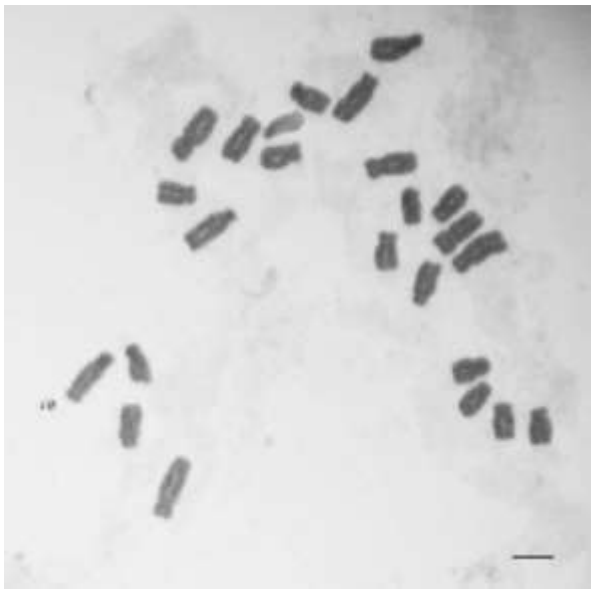

Figure 2. Somatic metaphases of *T. pulchella.* Scale bar 10 µm.

Chromosomes of tulips have been studied since the early twentieth century. Guignard, who first determined the chromosome complement in tulips, found three species to be diploids with 24 pairs of chromosomes (26). Examining varieties of garden tulips, De Mol found a basic number of 12 (27). Also, he discovered one triploid, 'Pink Beauty', with 36 chromosomes. Newton published two papers in which he recorded triploids, tetraploids and one pentaploid (28,29). Morever, Kroon and Jongerius first discovered a hexaploid origin for *T. polychroma* Stapf (= *T. biflora* Stapf) (30). Although many tulips have 24 pairs of chromosomes, some have more than the basic number of 2n=24, including triploids (2n=36), tetraploids (2n=48), pentaploids (2n=60) and exceptionally hexaploids (2n= 72) (10,29,31).

Chromosome numbers have been reported for 63 *Tulipa* species (30) and over 600 varieties (32). The basic chromosome number is x=12. The majority of tulip species and cultivars is diploid (2n=2x=24), however, triploids (2n=3x=36), tetraploids (2n=4x=48) and even some pentaploids (2n=5x=60) and hexaploid (2n=6x=72) ocur (30,32-36).

Table 1. Somatic chromosome number, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), asymmetry indexes (A1, A2) of Romero Zarco (1986) and symmetry classes (SC) of Stebbins (1971) of ***T. Pulchella.***

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A1	A2	SC
Tulipa pulchella	24	2x	8sm+4st	9.35-16.48	150.99	0.61	0.18	1A

Table 2. Karyo-morphological parameters of *T. pulchella.*

Chromosome Pair No	Total Length µm)	Long Arm(L) µm)	Short Arm(S) µm)	Arm Ration (L/S)	Centromeric Index (CI=S/CL)	Relative Length RL%=(CL/ΣCL)x100	Chromosome Type
1	16.48	13.26	3.22	4.11	0.19	10.92	st
2	15.67	10.62	5.04	2.10	0.32	10.37	sm
3	15.30	12.17	3.13	3.88	0.20	10.13	st
4	13.44	10.53	2.90	3.62	0.21	8.90	st
5	14.03	9.26	4.76	1.94	0.33	9.29	sm
6	12.62	8.99	3.63	2.47	0.28	8.36	sm
7	11.12	8.67	2.45	3.53	0.22	7.37	st
8	11.58	8.35	3.22	2.59	0.27	7.67	sm
9	10.99	8.08	2.90	2.78	0.26	7.28	sm
10	10.35	7.49	2.86	2.61	0.27	6.85	sm
11	9.99	6.67	3.31	2.01	0.33	6.61	sm
12	9.35	6.22	3.13	1.98	0.33	6.19	sm

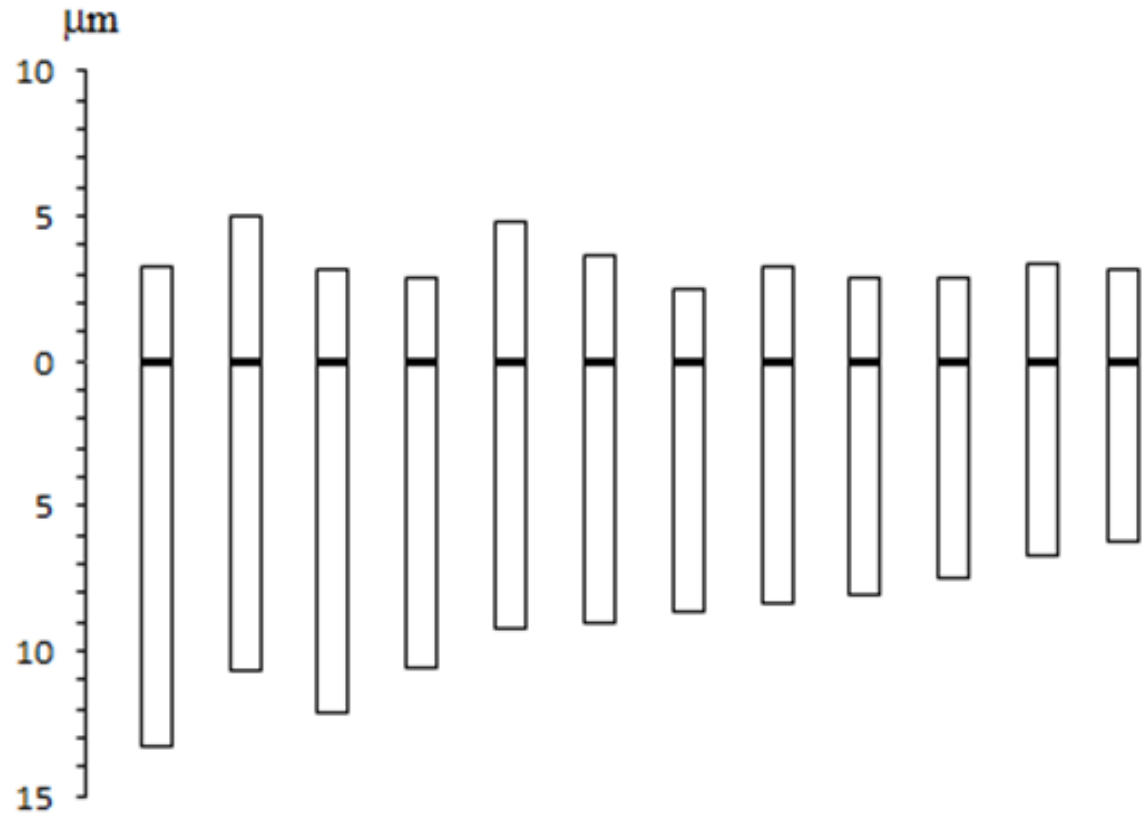

Figure 3. Haploid idiogram of *T. pulchella.*

Abedi et al. were examined chromosomal and karyotype parameters and genome size in 22 Iranian populations of nine different *Tulipa* species. Most species were diploid 2n = 2x = 24 (37). 2n=24 chromosomes of this species was reported in the literature (15,38,39). In cytotaxonomic analyses, the basic chromosome number was found to be 2n=24 (x=12) (*T. humilis, T. pulchella*). Polyploidy was observed in some taxa, such as 2n=3x=36 (*T. aleppensis* Boiss. ex Regel*, T. orphanidea* Boiss. ex Heldr), 2n=4x=48 (*T. sylvestris* subsp. *sylvestris* L.) and 2n=5x=60 (*T. clusiana* Red) (4). Therefore, the present count confirmed the earlier reports on 2n chromosomes number.

Conclusion

According to our knowledge, chromosome number and morphology report for *T. pulchella*. Identifying the chromosome number of this species in this study provides a base for biosystematic studies.

Conflict of interest: The authors declare they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article, and declare study has ethical permissions if required.

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