Karyotype Analysis of the Fiddleneck (*Phacelia tanacetifolia* Benth.)

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Abstract: Two varieties of fiddleneck (*Phacelia tanacetifolia* Benth.) plant were determined for visualizing somatic chromosomes. The 4-5 days old root tips were pre-treated in 6% α -monobromonaphthalene in +4 °C for 7.5 h, then fixed in glacial acetic acid for 30 min and transferred to 70% ethanol for long storage. When the root tips were analyzed, they were hydrolyzed with 1 N HCl for 13 min at room temperature (25 °C). After hydrolyzing, root tips were stained with 2% aceto orcein in darkness for 2.5 h. The squash method for preparation was used for chromosomal investigations. The chromosome length (C), relative length (RL), the long arm (L) and short arm (S) lengths, arm ratio (AR; L/S) and centromeric index (S/C) were calculated for caryologic parameters. The ideograms and detailed chromosome morphology measurements of the species were performed by the use of MicroMeasure 3.3. According to results, fiddleneck (*Phacelia tanacetifolia* Benth.) has $2n = 22$ chromosomes and the karyotype formulas of two varieties of fiddleneck were 16 median and 6 submedian (16 m + 6 sm).

Key words: Fiddleneck, cytogenetic, karyotype, micro measure, chromosome number, *Phacelia tanacetifolia*.

1. Introduction

Fiddleneck (*Phacelia tanacetifolia* Benth.) is a genus of about 200 species of annual or perennial herbaceous plants, which are members of the family Boraginaceae and are native to Southwestern United States and Mexico [1]. Many species can be cultivated as honey plants and garden plants due to its aesthetic appearance. Fiddleneck (*Phacelia tanacetifolia* Benth.) is an annual plant. Moreover, the genus is one of the top 20 most common bee plants as a rich source of nectar and pollen, most preferred by honey bees [2]. It is particularly an important source of nectar for wild bees, such as *Bombus* [3]. Since fiddleneck (*Phacelia tanacetifolia* Benth.) is attractive to a large number of insects, it can be used as a trap plant in the field as a biological preventive against the harms of crop plants [4]. Thus, it can also help the environment by minimizing the application of pesticides [5]. It is also attractive to hoverflies (family Syrphidae), which are useful as biological pest control agents, because they

feed on aphids and other pests [6]. The photodormant seeds of fiddleneck (*Phacelia tanacetifolia* Benth.) can only germinate in the dark [7].

Two varieties of fiddleneck (*Phacelia tanacetifolia* Benth.), which is used as materials in this study, is registered officially in Turkey. Also, these varieties are very important for bee forage and beekeeping. In addition, these two varieties of fiddleneck are thought to use as parental plant in breeding programmes. After determining chromosome numbers and caryological features of these varieties, it is planned to intercross with their relatives which have same ploidy level. Although, there has been a lack of cytological information in the relevant literature in regard to the subject of the study—fiddleneck (*Phacelia tanacetifolia* Benth.) and no caryological studies have been reported. Therefore, the study aimed to determine the number of chromosomes, cytological characteristics and the methods that can be utilized to characterize the genus of fiddleneck (*Phacelia tanacetifolia* Benth.).

2. Materials and Methods

The seeds of fiddleneck (*Phacelia tanacetifolia*

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Benth.) in the karyotype analysis were obtained from Saglamtimur Aegean Agricultural Research Institute, and the type of Enton was provided by the gene bank.

For visualizing somatic chromosomes, root tips were obtained from germinated fiddleneck (*Phacelia tanacetifolia* Benth.) seeds germinated in petri dishes at room temperature (25 °C). The 4-5 days old root tips were pre-treated in 6% α -monobromonaphtalane in +4 °C for 7.5 h, then fixed in glacial acetic acid for 30 min and transferred to 70% ethanol for long storage. When the root tips were analyzed, they were hydrolyzed with 1 N HCl for 13 min at room temperature (25 °C). After hydrolyzing, root tips stained with 2% aceto orcein in darkness for 2.5 h. Then, finally they were squashed in 45% acetic acid. Slides were observed with Olympus BX-51 microscope, photographs were taken with Olympus

BX-51 camera at room temperature (25 °C) and the magnification was 8,000 $\times$. Six chromosomal parameters were measured by MicroMeasure 3.3 program [8], i.e., chromosome length (C), relative length (RL), the long arm (L) and short arm (S) lengths, arm ratio (AR; L/S), centromeric index (S/C). Ideograms were drawn based on long arm length/short arm length. Karyotype formulas of two varieties of fiddleneck were determined by the methods in Ref. [9].

3. Results and Discussion

In this study, the somatic chromosomes number and cytological features of two varieties of fiddleneck (*Phacelia tanacetifolia* Benth.) were determined. Table 1 presents the caryological characteristics on the cultivars of Enton and Saglamtimur. The images of two fiddleneck (*Phacelia tanacetifolia* Benth.) varieties and

Table 1 Caryological features of Enton and Saglamtimur cultivars.

Number	Type	Chromosome length (μm)	Relative length (%)	Long arm length (μm)	Short arm length (μm)	Arm ratio	Centromeric index
Enton cultivar							
1	m	4.73	11.42	2.80	1.93	1.45	0.81
2	sm	4.26	10.27	2.87	1.39	2.06	0.65
3	m	4.13	9.97	2.48	1.65	1.50	0.80
4	m	3.97	9.59	2.36	1.62	1.46	0.81
5	sm	3.85	9.30	2.45	1.40	1.75	0.73
6	m	3.70	8.93	1.92	1.78	1.08	0.96
7	m	3.56	8.59	2.20	1.36	1.62	0.76
8	m	3.49	8.43	2.07	1.43	1.45	0.82
9	m	3.43	8.27	1.94	1.49	1.30	0.87
10	sm	3.36	8.12	2.27	1.10	2.50	0.64
11	m	2.94	7.11	1.75	1.19	1.47	0.80
Total		41.42					
Saglamtimur cultivar							
1	sm	5.79	13.15	3.73	2.06	1.81	0.72
2	sm	4.81	10.92	3.08	1.73	1.78	0.72
3	sm	4.65	10.56	3.00	1.65	1.82	0.71
4	m	4.50	10.22	2.77	1.73	1.60	0.77
5	m	4.29	9.75	2.59	1.71	1.51	0.80
6	m	3.98	9.04	2.13	1.84	1.16	0.93
7	m	3.51	7.97	2.01	1.42	1.42	0.81
8	m	3.37	7.66	1.99	1.39	1.43	0.82
9	m	3.26	7.41	1.77	1.49	1.19	0.91
10	m	3.12	7.10	1.76	1.36	1.29	0.87
11	m	2.74	6.23	1.85	0.89	2.08	0.65
Total		44.01					

m: median; sm: sub-median.

Fig. 1 Somatic metaphases and ideogram of Enton cultivar.

Fig. 2 Somatic metaphases and ideogram of Saglamtimur cultivar.

ideograms were shown in Figs. 1 and 2. The results indicated that the chromosome number of fiddleneck (*Phacelia tanacetifolia* Benth.) is $2n = 22$. These results are consistent with the results obtained by Sugiura [10] and Cave and Constance [11].

Saglamtimur cultivar has the maximum and minimum values for six parameters. There are the smallest and biggest chromosome length (2.74 μm and 5.79 μm , respectively), the lowest and highest relative length (6.23% and 13.15%, respectively), the maximum long arm length (3.73 μm) and the minimum short arm length (0.89 μm). Enton cultivar has the smallest and biggest arm ratio (1.08 and 2.50, respectively). In terms of the centromeric index, Enton cultivar has the smallest and biggest centromeric index (0.65 and 0.96, respectively).

The karyotype formulas of the species of fiddleneck (*Phacelia tanacetifolia* Benth.), on which the karyotype analysis was carried out, were found identical $2n = 22$ (16 median + 6 submedian).

3.1 Enton Cultivar

According to results of Enton cultivar (Table 1), the biggest chromosome length was calculated 4.73 μm , and the smallest chromosome length was calculated 2.94 μm . The highest relative length value was measured 11.42% and the smallest relative value was measured 7.11%. Long arm length was measured between 2.87 μm and 1.75 μm , while short arm length was measured between 1.93 μm and 1.10 μm . Chromosomes arm ratios were calculated between 1.08 and 2.50. The centromeric index was measured

between 0.96 and 0.64.

3.2 *Saglamtimur* Cultivar

As a results of *Saglamtimur* cultivar (Table 1), the largest chromosome length was calculated 5.79 μm , while the smallest chromosome length was calculated 2.74 μm . The largest relative length value was measured 13.15% and the smallest relative value was measured 6.23%. While long arm length was measured between 3.73 μm and 1.76 μm , short arm length was measured between 2.06 μm and 0.89 μm . Chromosomes arm ratios were between 2.08 and 1.16. The centromeric index varies between 0.93 and 0.65.

4. Conclusions

Turkey has the remarkable place for bee culture in the world. The plants like fiddleneck (*Phacelia tanacetifolia* Benth.), which has better quality, should evaluate more in bee forage for making better situation about bee industry in Turkey. As a result in this research, the varieties of fiddleneck has $2n = 22$ chromosomes and the karyotype formulas is 16 median and 6 submedian, so they can be utilized as parental plant, because of determined chromosome numbers, caryomorphological features and ploidy level. Additionally, this research will make opportunity to investigate the origin of varieties of fiddleneck (*Phacelia tanacetifolia* Benth.) and then new varieties of fiddleneck are came into use for bee forage farmers in Turkey and the world.

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