

NUMERICAL AND MORPHOSTRUCTURAL CHARACTERISTICS OF TRIGONELLA FOENUM-GRAECUM (2N=16) MITOTIC CHROMOSOMES

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INTRODUCTION

Trigonella foenum-graecum L. (Fabaceae) species is very interesting one, for its pharmaceutical, industrial and alimentary valences (Pârvu, 2005).

Foenum-graecum name means Greek Hay, the plant being used to scent inferior hay. The name of the genus, *Trigonella*, is derived from the old Greek name, denoting “three-angled” (the trait of its corolla). The seeds of fenugreek have been used all through the ages and were held in high repute among the Egyptians, Greeks and Romans for medicinal and culinary purposes. Compounds extracted from the plant have shown cardiogenic, hypoglycemic, diuretic, antiphlogistic and hypotensive activity. One of its alkaloids, called “trigonelline”, has shown potential for use in cancer therapy.

The cytogenetic analysis of this species is a part of complex investigations about the chromosomal features of medicinal and aromatic plants, made in our laboratory (Băra et al., 1986, 1987; Lu and Băra, 1993; Căpraru et al., 2004a, b; Cîmpeanu et al., 2004). The cytogenetic data, and, especially, those regarding the satellites chromosomes number, are different from one author to another. For this reason we have proposed to establish some morphostructural characteristics of the chromosomes at this species.

MATERIAL AND METHODS

The study was carried out using seeds of *Trigonella foenum graecum* L., from 2005 harvest. For germination the seeds were placed in Petri dishes on filter paper moistened with distilled water, at thermostat (22°C). The root tips were collected when had about 10-20 mm length, and pre-treatment was assured placing them in 0.2% colchicines, for 2 hours, in the dark, at room temperature. After that, the seeds were washed with distilled water and then fixed in fresh Carnoy solution, for 24 hours at room temperature. Until analyse, the roots were stored in 70% Ethanol, at 4°C, in the refrigerator.

Before squashing, root tips were softened in

HCl 50%, at room temperature. The most part of root tips have cut and squashed in 45% acetic acid. The staining was performed with Carr solution (Cîmpeanu et al., 2002). Images have taken with Nikon Eclipse 600 bright field microscope 100x objective in oil immersion, with a digital camera Cool Pix Nikon.

RESULTS AND DISCUSSIONS

The metaphases (with well-spread chromosomes - with less than two overlapping) study, revealed 2n=16, situation which is in conformity with literature data (Floria et al., 1997; Tiță and Pădureanu, 2003, Dundas et al., 2006).

Establishing of eight homologous pairs had made using the arms ratio (q/p), the centromeric index, the difference between long arm and short arm (q-p) and the relative length.

The arms length it was established by Adobe Photoshop 8.0 programme. Therefore, the total length of each chromosome is represented by the sum of two arms (including the centromere length).

Analyzing the results (table 1), in the metaphase with highly degree of condensation chromosomes (fig. 1), the total length of chromosomes is between 2.79 µm (first pair) and 2.18 µm (last pair), and the decreasing rate of the total length, from first to last pair, is varying from 0.14 µm (between first and second pair) to 0.01 µm (between sixth and seventh pairs).

The relative length of the eight pairs of chromosomes, established according formula: (TL/HSL) x 100, it was between 14.18 and 11.09.

About the arms ratio, the minimum value it was recorded at first pair (1.02µm), and the maximum (2.56µm) at second pair, and the difference from the long arm to the short arm it was 0.03µm (first pair), and 1.16µm (second pair).

The centromeric index was between 49.46 (first pair) and 28.11 (second pair).

According to arms ratio, centromeric index, and relative length we established two morphological types of chromosomes, namely: **m** (I, VI and VIII pairs) with arms ratio between 1.02 and 1.59, and also the type **sm** (II, III, IV, V, VII pairs), which has arms ratio varying from 1.78 to 2.56.

Table 1. The chromosome traits - *Trigonella foenum-graecum* late metaphase

Chromosomes		Total length		Long arms		Short arm		Arm ratio (q/p)	Arm difference (q-p)	Centromeric index	Relative length
Pair	Type	μm	Limits of variability	μm	Limits of variability	μm	Limits of variability				
I	m	2.79	2.71-2.87	1.41	1.38-1.44	1.38	1.33-1.44	1.02	0.03	49.46	14.18
II	sm	2.65	2.60-2.71	1.91	1.88-1.93	0.75	0.72-0.72	2.56	1.16	28.11	13.47
III	sm	2.56	2.54-2.57	1.77	1.77-1.77	0.79	0.77-0.80	2.25	0.99	30.72	12.98
IV	sm	2.54	2.54-2.54	1.66	1.66-1.66	0.88	0.88-0.88	1.89	0.78	34.65	12.91
V	sm	2.38	2.32-2.43	1.52	1.49-1.55	0.86	0.083-0.88	1.78	0.67	36.00	12.07
VI	m	2.30	2.21-2.38	1.41	1.38-1.44	0.89	0.83-0.94	1.59	0.53	38.56	11.66
VII	sm	2.29	2.27-2.32	1.49	1.49-1.49	0.80	0.77-0.83	1.86	0.69	34.93	11.64
VIII	m	2.18	2.15-2.21	1.19	1.16-1.22	0.99	0.99-0.99	1.20	0.20	45.41	11.08
HSL		19.68									

HSL – haploid set length

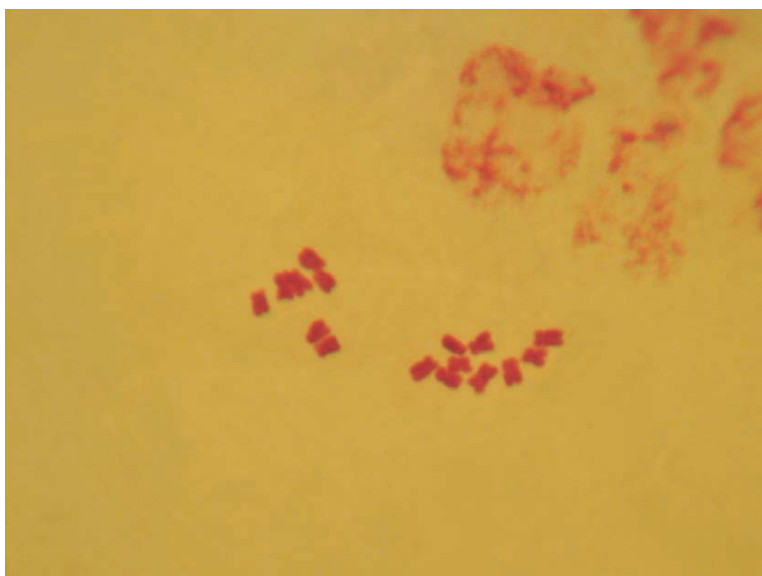
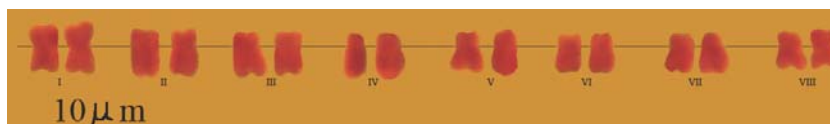
Fig. 1. *Trigonella foenum-graecum* late metaphase

Fig. 2. The karyotype of late metaphase

In table 2 there are data for the homologous pairs from the metaphase in which the chromosomes had a small degree of condensation (fig. 3). The total length was between 4.31μm (first pair) and 2.85 μm (eighth pair) and the relative length was between 14.50μm and 9.78μm. The decreasing chromosomes length rate was little, because the 5th and 6th pairs had the same length, the decreasing rate being assured by the rest of chromosomes, the biggest one being between 7th and 8th (0.46μm).

Arms ratio was between 1.08 (2nd pair) and 3.50 (5th pair), and the arms difference, for the same chromosomes pairs, it was 0.17μm and 1.93μm. The centromeric index had values between 48.00 (2nd pair) and 22.22 (5th pair).

Unlike the anterior metaphase, in this case it was possible to identify three morphological types of chromosomes: three **m** (II, VI, VII) pairs with $r=1.08-1.65$, four pairs **sm** (I, III, V, VIII) with $r=1.91-2.60$ and one pair of type **st** (V) which had $r=3.50$.

In both analysed metaphases, shown as karyotypes (figures 2 and 4), we did not identified chromosomes with satellites, in the spite of the fact that Tiță and Padureanu (2003), have specified the presence of secondary constriction at the long arm of chromosomes of first and second pairs. Martin (www.bab.com.tr) emphasized both metaphases with chromosomes without satellites and metaphases in which chromosomes have secondary constriction (first pair), but at the short arm.

Table 2. The chromosome traits - *Trigonella foenum-graecum* early metaphase

Chromosomes		Total length		Long arms		Short arm		Arm ratio (q/p)	Arm difference (q-p)	Centromeric index	Relative length
Pair	Type	µm	Limits of variability	µm	Limits of variability	µm	Limits of variability				
I	sm	4.31	4.25-4.36	2.98	2.93-3.04	1.33	1.33-1.33	2.25	1.66	30.77	14.81
II	m	4.14	4.09-4.14	2.15	2.10-2.15	1.99	1.99-1.99	1.08	0.17	48.00	14.25
III	sm	3.98	3.92-4.06	2.87	2.90-2.93	1.10	1.02-1.13	2.60	1.77	27.78	13.68
IV	sm	3.54	3.37-3.65	2.32	2.21-2.38	1.22	1.16-1.27	1.91	1.10	34.38	12.16
V	st	3.48	3.43-3.54	2.71	2.65-2.76	0.77	0.77-0.77	3.50	1.93	22.22	11.97
VI	m	3.48	3.37-3.54	2.15	2.10-2.15	1.33	1.27-1.38	1.63	0.83	38.10	11.97
VII	m	3.31	3.15-3.43	1.99	1.93-2.04	1.33	1.22-1.38	1.50	0.66	40.00	11.40
VIII	sm	2.85	2.80-2.89	1.93	1.88-1.93	0.92	0.91-0.93	2.09	1.01	32.04	9.78
HSL		29.09									

HSL – haploid set length

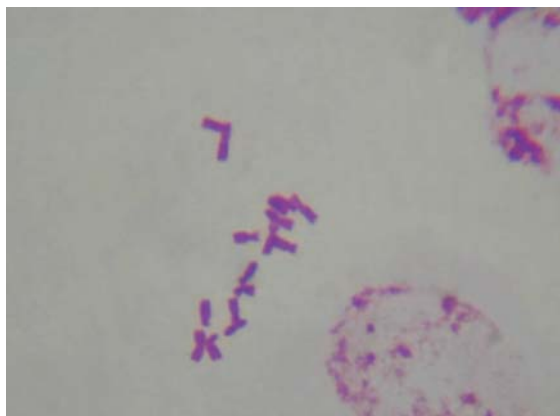
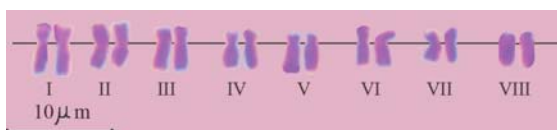
Fig. 3. *Trigonella foenum-graecum* early metaphase

Fig. 4. The karyotype of early metaphase

SUMMARY

Trigonella foenum-graecum L. is an important medicinal species. The karyological study have showed that $2n=16$. We identified following types of chromosomes: **m**, **sm** and, sometimes, **st** (only in early metaphase). So, we can appreciate that the karyotype of this species is enough symmetric and less evolved.

CONCLUSIONS

The number of chromosomes from the root apex cells of *Trigonella foenum-graecum* L. is $2n=16$.

In both analysed metaphases we have identified the following types of chromosomes: **m** (median), **sm** (submedian) and **st** (subtelocentric), this last type being signalised only in early metaphase.

The karyotype of this species is fairly symmetric being less evolved.

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