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Karyotype Characterisation of Reputed Variety of Fenugreek (*Trigonella foenum-graecum*) in West Azerbaijan-Iran

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Abstract

Fenugreek (*Trigonella foenum-graecum*) of family Leguminosae and subfamily Papilionaceae is extensively grown in West Azerbaijan-Iran as forage and vegetable. It has more economical and medicinal benefits as well. Cytogenetical studies are necessary to improve the economical value and hybridization between close related species and varieties. In order to analyze karyotype of *Trigonella foenum-graecum* and identification chromosomes type by using improved methods, plants and seeds specimens were collected from different farmlands of West Azerbaijan. Root tip meristems obtained from germination of seeds were pretreated with saturated solution of ά-bromonaphthalene and 8-Hydroxiquinolin then fixed in Lewitsky () solution and stained in Aceto-Iron - Hematoxylin. Ten metaphasic plates were used to analyze karyotype parameters. The karyotype of *Trigonella foenum-graecum* consists of 2n=2x=16 chromosomes. Size of chromosomes changes from 6.698 ±0.238 µm (the largest chromosome) to 5.063 ± 0.162 µm (the smallest chromosome). All of them are submetacentrics except the chromosome 8, which is metacentric. Pairs 1 and 4 are satellited chromosomes as a chromosome marker. Sizes of satellites are 1.978 and 1.364 µm respectively. Karyotype details were illustrated in complete article.

Keywords: Fenugreek**,** *Trigonella foenum-graecum*, Karyotype, Chromosome

INTRODUCTION

Fenugreek *(Trigonello foenum-graecum)* is an annual legume distributed in the Mediterranean region, Europe, Asia, South Africa and Australia. Fenugreek is widely cultivated as a leafy vegetable; seed is used for medicinal purpose and as a condiment for flavoring food preparations [1]. Ground seeds are mixed with wheat-flour for making bread in Egypt and in Switzerland for flavoring cheese. Roasted seeds are used as a substitute for coffee in some parts of Africa.

Cytological studies including karyotype analysis have been reported in different species and cultivars of fenugreek with the somatic chromosome number 2n = 16 chromosomes [2-7]. The six species of the section *Foenum-graecum* of *Trigonella* have the same chromosome number, 2n=2x=16. *T. gladiata* and *T. cariensis* have fairly symmetrical karyotypes, while those of *T. foenum-graecum, T. berythea, T. macrorrhyncha* and *T. cassia* are asymmetrical. C-bands are present in all six species but the number of bands and their positive vary considerably among the species. The karyotype evidence suggests that none of the available species of the *Foenum-graecum* section can be considered as the wild progenitor of fenugreek [8].

Chromosome number determination and karyotype analysis is the prerequisite to assess the genomic status of the species for various levels of taxonomic grouping of the plants and genetic and breeding studies. The present work was carried out to describe the somatic karyotype of *Trigonello foenum-graecum.*

We have elaborated an improved routine technique for staining plant chromosomes and preparing squashes. Application of this technique in present work made it possible to study the karyotype of fenugreek in more detail than different workers who performed it applying the traditional routine cytological methods.

MATERIALS AND METHODS

Chromosome studies were carried out in meristematic cells of root tips (1 cm in length) of germinated seeds from fenugreek (*Trigonello foenum-graecum*) localities in West Azerbaijan province in Iran. Seeds were germinated in darkness 25˚C on moist filter paper in petri dishes.

Table 1. Numerical data concerning the karyotype of *Trigonello foenum-graecum* L. (N=10).

No. Pair	Total (L+S) µm	Long arm(L) µm	Short arm(S) µm	Arm ratio(L/S)	CI (S*100/(L+S))	sat	Type	L%	S%	RL%
1	7.028 ± 0.238	3.598 ± 0.106	1.452 ± 0.102	2.665 ± 0.278	21.842	1.978	**sm**	7.990	3.224	14.87
2	6.671 ± 0.184	4.356 ± 0.289	2.315 ± 0.134	2.040 ± 0.256	35.417		**sm**	9.673	5.140	14.81
3	6.246 ± 0.189	4.493 ± 0.131	1.753 ± 0.121	2.707 ± 0.217	27.908		**sm**	9.977	3.893	13.87
4	6.198 ± 0.282	3.554 ± 0.120	1.280 ± 0.125	2.995 ± 0.218	20.267	1.364	**sm**	7.892	2.842	13.76
5	6.043 ± 0.184	4.478 ± 0.203	1.565 ± 0.041	2.903 ± 0.185	26.264		**sm**	9.945	3.476	13.42
6	5.761 ± 0.161	4.267 ± 0.155	1.494 ± 0.061	2.918 ± 0.176	26.063		**sm**	9.477	3.319	12.80
7	5.361 ± 0.136	3.777 ± 0.106	1.584 ± 0.061	2.423 ± 0.114	29.540		**sm**	8.389	3.518	11.91
8	5.064 ± 0.162	2.924 ± 0.082	2.140 ± 0.106	1.396 ± 0.065	42.068		**m**	6.493	4.752	11.24
Mean	6.005 ± 0.087	3.931 ± 0.077	1.698 ± 0.048	2.506 ± 0.086						
Total	45.029							69.835	30.164	

Chromosomal formula is 2n = 2x = 16 = 10sm + 4sm^{sat} + 2m ; SC= 3A ; A1= 0.56 , A2= 0.10

L: Length of the long arm of the chromosome (µm) , S: Length of the short arm of the chromosome (µm) , CI: Centromer index , (m: Metacentric , sm : Submetacentric) ; Centromere type according to Levan et al .(1964) , sat: Satellites , SC= Symmetry class is 2A, Asymmetry indices : A1= 0.56 and A2=0.10 , according to Romero-Zarco ,1986 ,N: Number of mitotic metaphases used to determine the karyotype , L% and S% : Indices that express the contribution of each arm of each chromosome to the total length of karyotype .

Preparation of samples

Root tips were excised and pretreated in 2mM solution of 8 hydroxyquinoline at about 4°C for 3-3.5 h, washed in distilled water for 10-15 min.

Fixation of samples

Root tips fixed in Lewitsky fixative at 4°C for 30-36h. The fixative was prepared just before use, by mixing equal parts (in volume) of 1% chromic acid and 4% formaldehyde (10% formalin). Then the roots were washed under tap water for 3h and transferred into 70% ethanol [9-12].

Hydrolysis and Staining

Roots were rinsed in distilled water, before treating in 1N NaOH at 60°C for 10 min, then washed in distilled water for about 30min and followed by staining in aceto-iron-hematoxylin at 30-34°C for 15-20h [9-12].

Observation of samples

Stained roots were washed in distilled water for at least 30 min. Then the root tips were cut in 1.0-1.2 mm length and macerated in cytase for at least 2 h (can be kept in a refrigerator overnight). Each of the root tips was carefully transferred onto a drop of 45% acetic acid on a slide for 3-5 min, divided into small pieces, covered by a cover slip and gently squashed.

Squashes were examined under Olympus BH-2 microscopes and chromosomes photographed using digital camera system. Chromosome measurements were made on 10 metaphase plates by application of MicroMeasure 3.3 software [13]. Arm rations, average lengths, relative lengths and standard errors were calculated using Excel 2003 (Microsoft) and SPSS v13. The chromosomes were classified according to Levan *et al.* [14] considering their centromere position.

RESULTS AND DISCUSSION

Present investigation showed that Azerbaijan's fenugreek has 2n=2x=16 chromosomes in a somatic cell and it supports the conclusions of others [2-7]. This investigation, show clearly that using an improved routing techniques (Fixative of Lewitsky and aceto-iron-hematoxylin as stain) for karyotype analyzing in combination with computer image analysis is a reliable and efficient method for karyotyping the chromosomes of fenugreek [9-12]. The individual chromosomes can be identified directly by morphological characteristics (Fig. 1; Table 1). Preparation without cytoplasm and cell walls was necessary to reveal chromosomes in more contrast and clearly, we macerated root tips in cytase (Authors extracted the enzyme from the stomach of snail *Helix lucorum*) for at least 2 h. It was found that the *Trigonello foenum-graecum* L. genome was composed of seven submetacentric pairs (chromosome 1, 2, 3, 4, 5, 6, 7) and one metacentric pairs (chromosome 8) (Table 1, Fig 2). The characteristics of the eight somatic chromosome pairs are described as follows: Chromosome types are numbered according to their sizes (Fig. 1, Table 1). Chromosome 1 is the biggest and is submetacentric with an arm ratio (r) of 2.665 ± 0.278. It has a large satellite on the short arm. Chromosome 2 is submetacentric and with an arm ratio (r) of 2.040 ± 0.256. Chromosome 3 is submetacentric and with an arm ratio (r) of 2.707 ± 0.217. Chromosome 4 is submetacentric, has satellite on the short arm too and with an arm ratio (r) of 2.995 ± 0.218. Chromosomes

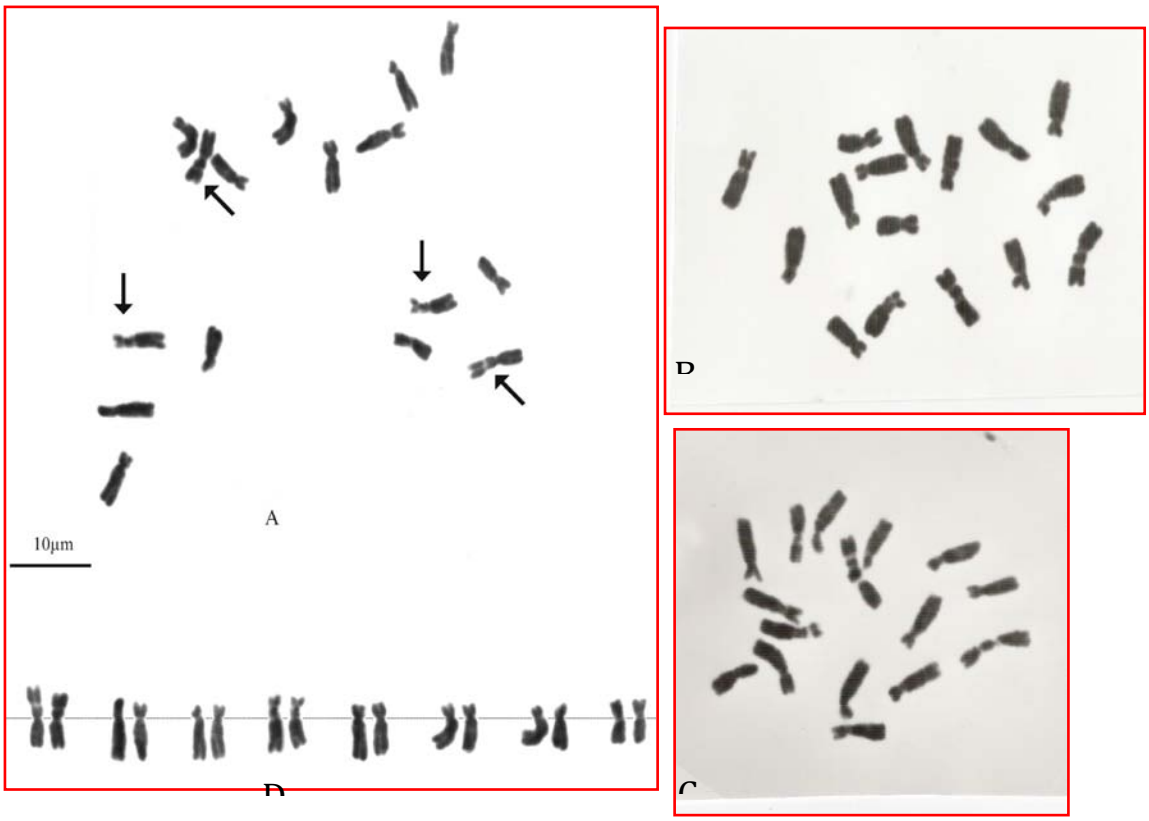

Figure 1 (A, B, C). Mitotic metaphase plates of *Trigonello foenum-graecum* L., D: karyogram , constructed from chromosomes of A plate (2n= 2x = 16).

5, 6 and 7 are submetacentric but the chromosome 8 is the only metacentrics and smallest pairs with an arm ratio(r) 1.396 ± 0.06 and 5.063 ± 0.162 µm for its size.

Pairs 1 and 4 are satellited chromosomes as a chromosome marker that were not reported before but by application of this routine squash techniques were established clearly in all metaphase plate (Fig.1). Sizes of satellites are 1.978 and 1.364 µm respectively. Total length of chromosomes in one metaphase, on an average from 10 metaphase plates, was 2n=2x=16= 45.029 µm. The average length of one chromosome was 6.005 ± 0.087 µm and the average arm ratio for total karyotype was 2.506 ± 0.086.

The karyotype and idiogram (Figures 1 and 2) representing the basic genome shows that chromosomes 1 and 4 can be easily recognized by their size and secondary construction and chromosome 8 as well can be recognized by especial characteristics; metacentrics and its size. The other chromosomes show only small differences in size but can be distinguished by characteristic arm ratio.

Thus, the present technique can provide a method for investigating the phylogenetic relationship between *Trigonella* species and for detecting structural re-arrangements in the chromosomes.

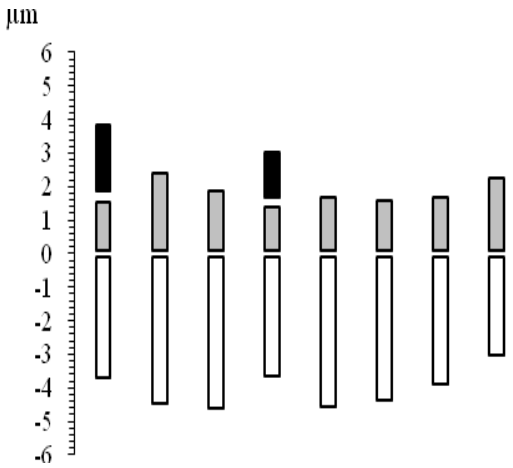

Figure 2. Haploid ideogram of *Trigonello foenum-graecum* L.

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