CYTOPHOTOMETRIC ESTIMATION OF 4C DNA AND KARYOTYPE ANALYSIS IN TEN CULTIVARS OF TRIGONELLA FOENUM-GRAECUM - II.

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Cytophotodensitometric estimation of 4C DNA content in root tip cells and karyotype analysis were carried out in ten cultivars of fenugreek (*Trigonella foenum-graecum*). Somatic chromosome number was 2n=16 in all the cultivars. Detailed karyotype analysis revealed cultivar specific chromosomal characteristics and minute structural alterations in chromosomes of the genome. The 4C DNA content varied significantly from 9.116pg in the cultivar TG-41 to 12.420 pg in TG-03. Significant variations in the chromosome length and volume and total form percentage (TF%) were noted at cultivar level. Correlation coefficient studies revealed interdependence between the chromosome volume and nuclear DNA content of the varieties. Variations in chromosome structure and the nuclear DNA content despite the same somatic chromosome number suggest micro-evolution leading to the development of new cultivars.

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Key words: fenugreek, genome size, karyotype, *Trigonella.*

تخمین سیتوفتومتری 4C DNA و تجزیه کاریوتیپ ده رقم شنبلیله II

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تخمین سیتوفتومتری 4C DNA سلولهای نوک ریشه و تجزیه کاریوتیپ ده رقم شنبلیله (*Trigonella foenum-graecum*) انجام شده است. تعداد کروموزومهای سوماتیک۱۶= ۲n برای همه رقمها ذکر می‌شود. تجزیه جزیی کاریوتیپ نشان‌دهنده ویژگیهای کروموزومی اختصاصی ارقام و جابجایی ساختاری کمی در کروموزومهای ژنوم است. محتوای 4 C DNA از ۹/۱۱pg در رقم TG-41 تا ۱۲/۴۲۰pg در رقم TG-03 تغییر نمود. دگرگونی معنی‌داری در طول، حجم و درصد شکل کلی (TF%) در سطح رقم دیده شد. مطالعات ضریب همبستگی نشان دهنده ارتباط بین حجم کروموزوم و محتوای DNA هسته در واریته ها است. دگرگونی ساختار کروموزوم و محتوای DNA علیرغم تعداد کروموزومهای برابر نشان دهنده وجود ریزتکامل در جهت توسعه ارقام جدید است.

Introduction

Fenugreek (*Trigonella 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 flavouring food preparations (Anonymous, 1976). Ground seeds are mixed with wheat-flour for making bread in Egypt and in Switzerland for flavouring 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 (Frahm-Leliveld, 1957; Singh & Roy, 1970; Raghuvanshi & Singh, 1974 a,b; Lavania & Sharma, 1980; Bir & Kumari, 1980; Das & al. 1997). 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. Some members of *Leguminosae* like *Glycine* (Hammatt & al. ,1991), *Vigna* (Parida & al., 1990), *Cicer arietinum* (Mukherjee & Sharma, 1986), *Cassia* (Ohri & al., 1986, Das & Chatterjee, 1994), *Vicia* (Raina & Bisht, 1988; Maxted & al., 1991), mung bean (Ignacimuthu and Babu, 1988) and *Arachis hypogaea* (Dhillon and Miksche, 1982) were studied for their DNA content. We reported the inter-cultivar level differences of 4C DNA content in fenugreek (Das & al., 1997, 2001). This work is the continuation of our earlier DNA estimates and chromosome analysis on different cultivars of *Trigonella* (Das & al, 1997; 2001). The present investigation deals with the karyotype analysis and estimation of 4C DNA content to establish the differences in genomic constituents in ten cultivars of fenugreek with the correlation of different cytological and cytochemical parameters to delineate the affinity between the cultivars during micro-evolution.

Materials and methods

Seeds of ten cultivars of fenugreek (*Trigonella foenum-graecum* L.) (TG-03, TG-12, TG-18, TG-22, TG-23, TG-25, TG-41, TG-63, TG-77 and TG-196) were obtained from the Department of Spice and Plantation Crops, Tamil Nadu Agricultural University, Coimbatore, and grown in the experimental gardens of the Regional Plant Resource Centre, Bhubaneswar .

Healthy root-tips were pretreated in saturated *para*-dichlorobenzene and aesculine mixture (1:1) for 3.5 h at 14°C followed by overnight fixation in 1:3 propionic ethanol. Staining of chromosomes was done in 2% propionic orcein. Chromosome squash preparations were carried out in 45% propionic acid. Total chromosome length was estimated by adding the length of all chromosomes in the karyotype. The volume was obtained by applying the formula $\pi r^2 h$, where 'r' is the radius and 'h' is the length of the chromosome.

For Feulgen cytophotometric estimation of 4C DNA, ten fixed root-tips from each species were hydrolysed in I N HCI for 12 min. at 60°C, washed in distilled water and stained in Schiff's reagent for 2 h at 14°C; each root-tip squash was prepared in 45% acetic acid. Ten scorings were made from each slide and 4C DNA was estimated from metaphase chromosomes using a Nikon Optiphot microscope with micro spectrophotometer following the method of Sharma & Sharrna (1980) and applying monochromatic light at 550 nm. *In situ* DNA values were obtained on the basis of optical density which were then converted to picograms (pg) by using Van't Hofs (1965) 4C nuclear DNA value of 67. 1 pg for *Allium cepa* cv. Deshi as standard. In order to assess the significant differences of the 4C DNA content among the cultivars of fenugreek, if any, an ANOVA test (Sokal & Rohlf, 1973) was performed.

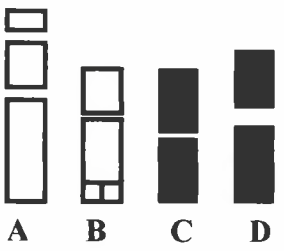


Fig. 1. Standard karyotype of fenugreek (*Trigonella foenum-graecum*).

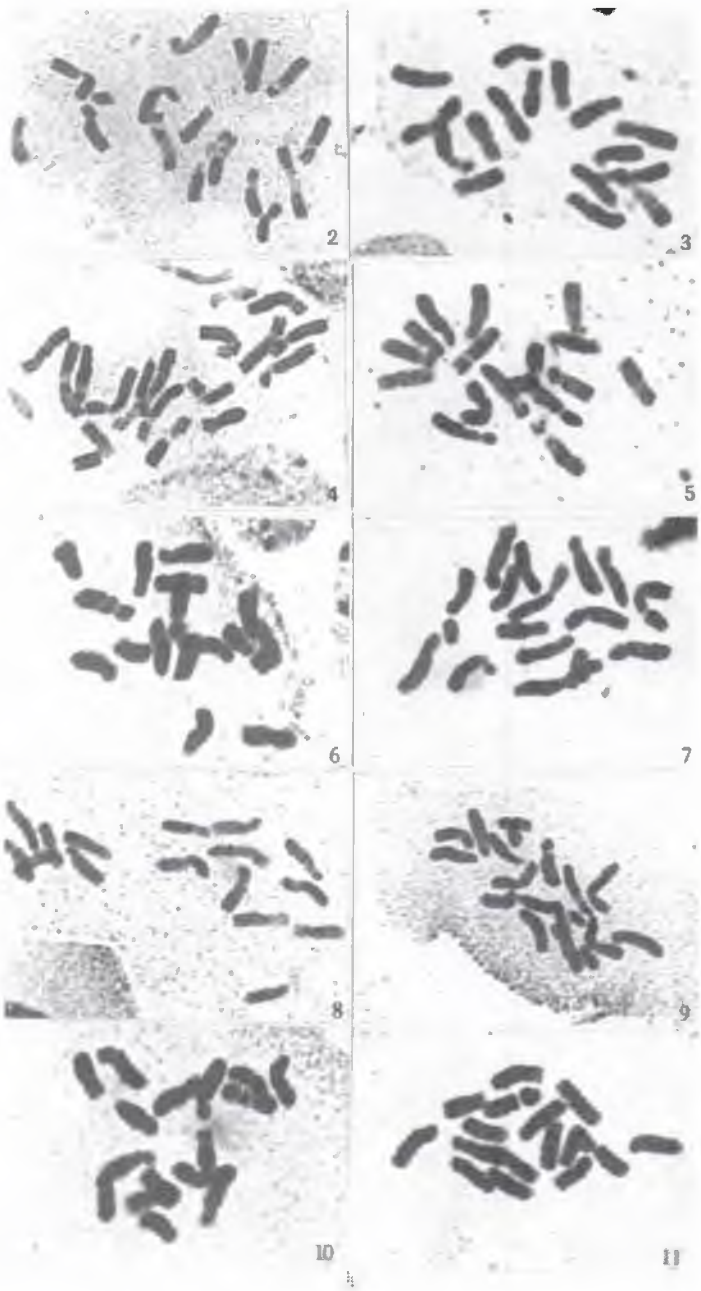
Results

The somatic chromosome 2n = 16 was observed in all the cultivars of fenugreek (Table 1). A number of common chromosome types based on their size and the position of the constrictions, though they differed from one another in minute details of the karyotype, were observed. Different types of chromosomes which were noted are presented in Figure 1. Type A chromosomes were comparatively large having two constrictions; one nearly terminal to subterminal and the other nearly median to median. Type B chromosomes showed two constrictions, primary constriction was in median position and secondary constriction was on nearly terminal position. Type C chromosomes had median to nearly median primary constrictions. Type D chromosome had sub-terminal or nearly sub- terminal primary constrictions. On the basis of the chromosome types, karyotype formulae revealed clear differences in minute structural details of the chromosomes in ten cultivars of fenugreek (Table 1; Figures 2 to 11). Type D chromosomes were noted in all the cultivars. All the four types of chromosomes were present in TG-12, TG-25 and TG-77. Remarkably, type C chromosomes were absent in TG-23 and TG-63. The number of type D chromosomes were more in all the cultivars and the maximum number of type D chromosomes was noted in TG-63. The

Table 1. Cytological parameters in ten cultivars of fenugreek (*Trigonella foenum -graecum*).

Cultivars	2n	Karyotype formula	Chromosome length (μm±SE)	Chromosome volume (μm^3±SE)	4C DNA content (pg±SE)	CL per chromosome (μm)	CV per chromosome (μm^3)	4C DNA per chromosome (pg)	TF % (±SE)
TG 03	16	4B+6C+6D	65.53±0.33	61.55±0.12	12.420±0.03	4.09	3.84	0.776	35.22±0.20
TG-12	16	2A+2B+2C+10D	48.74±0.25	51.96±0.65	11.334±0.02	3.04	3.24	0.708	28.41±0.34
TG-18	16	2B+8C+6D	38.63±0.44	53.17±0.17	11.868±0.08	2.41	3.32	0.742	31.74±0.25
TG-22	16	2A+2C+12D	46.33±0.29	38.60±0.41	9.116±0.11	2.89	2.41	0.569	32.51±0.10
TG-23	16	2A+14D	40.66±0.08	41.14±0.53	11.756±0.08	2.54	2.57	0.735	30.82±0.31
TG-25	16	2A+2B+6C+6D	54.44±0.44	44.79±0.32	10.062±0.09	3.40	2.79	0.629	34.88±0.23
TG-41	16	2B+4C+10D	44.81±0.26	37.31±0.28	9.212±0.07	2.80	2.33	0.576	33.01±0.22
TG-63	16	2B+14D	42.72±0.15	48.44±0.55	10.246±0.07	2.67	3.02	0.625	25.72±0.14
TG-77	16	2A+2B+4C+8D	50.45±0.11	48.41±0.31	10.466±0.06	3.15	3.02	0.654	30.82±0.19
TG-196	16	4A+4C+8D	50.98±0.34	42.46±0.45	9.521±0.05	3.18	2.65	0.595	36.50±0.27

2n= somatic chromosome number, CL= Chromosome Length, CV= Chromosome volume, TF%= Total form percentage



Figs. 2-11. somatic metaphase plates showing 2n=16 chromosomes in different cultivars of fenugreek.

Table 2. Analysis of variance (ANOVA) of 4C DNA content among the different cultivars of fenugreek.

Surce	DF	SS	MS	F
Between cultivars	9	78.435	8.7154	0.724
Within cultivars	90	19.243	0.214	-
Total	99			

* Significant at P≥0.01; DF: degree of freedom; SS: sum of squares; MS: mean squares; F: variance ratio.

chromosome length and chromosome volume varied from 38.63μm-65.53μm in TG-18 and TG-03 and 37.31μm^3-61.66μm^3 in TG-41 and TG-03 respectively (Table 1). The TF% (total form percentage) also varied significantly from 25.72% in TG-63 to 36.50% in TG-196. Chromosome analysis showed symmetric karyotypes having nearly median to nearly sub-median chromosomes in the genomes. The 4C DNA content differed significantly (Tables 1 and 2) among the different cultivars. The nuclear DNA content significantly varied from 9.116 pg in TG-22 to 12.420 pg in TG-3. ANOVA tests revealed significant variations among the cultivars in the 4C DNA content (Table 2). The critical difference (CD) values at 1% and 5% levels were 0.955 and 0.121 respectively. The CD values between the means of 4C DNA following Duncan's multiple range tests showed significant differences among the cultivars (Table 3). A positive correlation was noted with regard to the chromosome volume and nuclear DNA content from the 'r' values of different cytological parameters (Table 4).

Table 3: Critical difference of the 4C DNA content (pg) among the different cultivars of fenugreek.

	TG-22	TG-41	TG-196	TG-25	TG-63	TG-77	TG-12	TG-23	TG-18
TG-41	0.105ns								
TG-196	0.405*	0.309*							
TG-25	0.946*	0.850*	0.541*						
TG-63	1.130**	1.034**	0.725*	0.184*					
TG-77	1.350**	1.254**	0.945*	0.404*	0.220*				
TG-12	2.218**	2.122**	1.813**	1.272**	1.088**	0.871*			
TG-23	2.640**	2.544**	2.235**	1.694**	1.510**	1.290**	0.422*		
TG-18	2.752**	2.656**	2.347**	1.806**	1.622**	1.402**	0.534*	0.112ns	
TG-03	3.304**	3.208**	2.899**	2.358**	2.174**	1.954**	1.086**	0.664*	0.552*

CD (Critical difference) at 1% level = 0.995, CD at 5% level = 0.121; ns= not significant; *= significant at 5% level; **= significant at 1% level. CD was according to Duncan's multiple range test.

Discussion

Karyotype analysis in ten cultivars of fenugreek revealed some interesting features at the cultivar level. Structural alteration of somatic chromosomes was noted; all the cultivars of fenugreek showed 2n = 16 chromosomes. Although the types of chromosomes were common in all the members, those variations of the C and D types

Table 4. Correlation coefficient (r) values of different genomic parameters and corresponding 't' values in different cultivars of fenugreek.

Cytological parameters	r values	t values
chromosome length vs Chromosome volume	0.492	6.75*
Chromosome length vs 4C DNA content	0.167	1.24ns
Chromosome volume vs 4C DNA content	0.787	11.12*

* Highly significant at 5% level, ns=not significant.

of chromosomes were the most striking features. Furthermore, in respect of karyotype formula there were minute differences between TG-23 and TG-63 and TG-25 and TG-77, but genomic chromosome length and volume varied significantly. The numerical variations in types C and D chromosomes in different cultivars suggest a gradual alteration of chromosomes during micro-evolution. The gradual alterations and shifting of TF% values (Table 1) might be due to the chromosomal alteration in the genome. The structural alterations in the chromosome morphology as well as variation of secondary constricted chromosomes in the cultivars might be due to duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution (Das & al. 1997).

Total chromosome length and volume differed markedly among the cultivars of fenugreek (Table 1). A proportional increase was observed in chromosome length with an increase in chromosome volume and 4C DNA content among the cultivars. Average chromosome volume and 4C DNA content varied significantly. These facts indicate the predetermined genetic control of chromosome coiling. Evidently differences in chromosome length or chromosome volume were due to differential condensation and spiralization of the chromosome arms. In addition, the species-specific compaction of DNA threads along with nucleosomes, or the additional gene sequences with altered non-histone proteins (Das & Mallick, 1989a), play an important role for chromosomal architecture of the varieties and cultivars.

Investigations of the 4C DNA amount showed significant variation between different cultivars of *T. foenum-graecum* (Tables I to 3). The maximum (12.421 pg) 4C DNA content was noted in TG-3 and the minimum (9.116 pg) in TG-22. The average DNA amount per chromosome varied markedly (Table 1). The chromosome volume, however, showed a higher correlation with DNA content (0.787) than the chromosome length (0.492). Intervarietal and interspecific variations were also noticed in several other taxa (Price, 1976; Mukherjee & Sharma, 1986; Chattopadhyay & Sharma, 1990; Das & Mallick, 1993a,b, Das & Das 1994, 1997). The variability in the stable DNA content at the varietal/cultivar level might be attributed to the loss or addition of many repeats in the micro- and macro-environment of the genome during evolution (Price & al., 1980).

The significant correlation confirmed that the nuclear DNA content had a direct influence on the chromosomal volume (Table 4). Perhaps the maximum correlation of these genomic characteristics (t =11.12) leads to differential genetic interaction during the process of micro- and macro-evolution (Yamaguchi & Tsunoda, 1969; Das & Mallick, 1989a,b) through selection.

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