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

A. B. Das, S. Mohanty & P. Das

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Estimation of nuclear DNA content through cytophoto-densitometer in somatic cells and karyotype analysis were carried out for the first time in ten cultivars of fenugreek (*Trigonella foenum-graecum*). Somatic chromosome number is reported 2n=16 in all the cultivars. Detailed karyotype analysis revealed cultivar specific chromosomal characteristics and minute structural alterations in chromosomes. The 4C DNA content varied significantly from 7.23 pg in the cultivar TG-103 to 9.12 pg in TG-09. Significant variations in the genome length, volume and total form percentage were noted at cultivar level. A positive significant correlation coefficient revealed interdependence between the chromosome volume and nuclear DNA content of the cultivars. Significant variations in 4C nuclear DNA content among different cultivars despite the same somatic chromosome number suggest a changes in the repetitive DNA sequences of the genomes.

A. B. Das, S. Mohanty and P. Das, Cytogenetics Laboratory, Regional Plant Resource Centre, Bhubaneswar 751015, Orissa, India

Key words. fenugreek, nuclear DNA, karyotype , *Trigonella*

تخمین سیتوفتومتری4C DNA و تجزیه کاریوتیپ ده رقم شنبلیله

آنات باندهو داس، موهانتی و پرماناندا داس

تخمین محتوای DNA هسته سلولهای سوماتیک از طریق سیتوفتومتری و تجزیه کاریوتیپ ده رقم شنبلیله *(Trigonella foenum-graecum)* برای اولین بار انجام شد. تعداد کروموزومهای سوماتیک ۲n=۱۶ برای همه رقمها گزارش می‌شود. جزییات کاریوتیپ در ارقام مختلف، تغییرات ساختاری کمی را نشان می‌دهد. محتوی 4C DNA از ۷/۲۳ pg در یکی از ارقام تا ۹/۱۲ pg در رقم دیگری تفاوت می‌کند. تغییرات معنی‌داری در طول و حجم ژنوم و همچنین درصد شکل کل (TF%) در سطح ارقام دیده شد. ضریب همبستگی مثبت بین حجم کروموزوم و محتوای DNA هسته در ارقام مشاهده گردید. گوناگونی معنی‌دار در مقدار 4C DNA بین ارقام مختلف، علیرغم برابری تعداد کروموزومهای ثابت، نشان دهنده تغییر در توالی DNA ژنوم است.

Introduction

Trigonella foenum-graecum L. (fenugreek) is an annual legume distributed in the Mediterranean region, Europe, Asia, South Africa and Australia. Fenugreek is widely cultivated as a leafy vegetable and its 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 and Roy, 1970; Raghuvanshi & Singh 1974 a,b; Lavania & Sharma 1980; Bir & Kumari 1980, Das & *al.* 1997). Chromosome number determination and karyotype analysis is the preliminary requisite to assess the genomic status of the species for various levels of taxonomic grouping of the plants. Some members of *Leguminocae* 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 & Babu 1988) and *Arachis hypogaea* (Dhillon & Miksche, 1982) were studied for their DNA content. Recently, we reported the inter-cultivar differences of 4C DNA content in fenugreek (Das & *al.* 1997). This work is the continuation of our earlier DNA estimates and chromosome anlysis on different cultivars of *Trigonella.* The present investigation deals with the determination of somatic chromosome number, 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

Young root-tips of ten cultivars of fenugreek *i.e.* (TG-9, TG-45, TG-68, TG-73, TG-78, TG-92, TG-103, TG-127, TG-129 and TG-130) 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 $\pi r^2 h$, respectively, 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 1 N HCl 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 microspectrophotometer following the method of Sharma & Sharma (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 Hof's (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.

Observations

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 nearly subterminal in position and the other nearly median to median in position. Type B chromosomes showed two constrictions, primary constriction was in median position and secondary constriction was on nearly terminal position. Type C chromosomes were median to nearly median primary constrictions. Type D chromosome were 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 chromosome in ten cultivars of fenugreek (Table 1; Figures 2 to 11).

Type C chromosomes were observed in all the studied cultivars. All the four types of chromosomes were present in TG-73, TG-92 and TG-129. Remarkably, type D chromosomes were absent in TG-127. The number of type D chromosomes were numerous in all the cultivars and the maximum number of type D chromosomes was noted in TG-45.

The chromosome length and chromosome volume varied from 63.82μm in TG-73 to 102.98μm in TG-92 and 53.18μm^3 in TG-127 to 136.80μm^3 in TG-09 (Table 1). The TF% (total form percentage) also varied significantly from 26.02% in TG-78 to 39.72% in TG-127.

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

Cultivars	2n	Karyotype Formula	Genomic chromosome length (μm±SE)	Genomic chromosome Volume (μm^3±SE)	4C DNA content	GCL per Chromosome (μm)	GCV per chromosome (μm^3)	4CDNA per chromosome (pg)	TF %(±SE)
TG-09	16	2B+6C+8D	73.10±0.13	136.80±0.19	9.12±0.10	4.56	8.55	0.57	32.88±0.23
TG-45	16	2A+2C+12D	95.74±0.22	91.44±0.35	8.65±0.05	5.98	5.71	0.54	31.26±0.31
TG-68	16	2A+6C+8D	73.11±0.24	60.04±0.22	8.10±0.11	4.56	3.75	0.50	33.22±0.26
TG-73	16	2A+2B+10C+2D	63.82±0.19	58.16±0.41	7.69±0.12	3.98	3.63	0.48	36.19±0.14
TG-78	16	4B+4C+14D	86.82±0.28	90.21±0.33	8.50±0.07	5.42	5.63	0.53	26.02±0.11
TG-92	16	2A+2B+4C+8D	102.98±0.14	85.70±0.39	8.75±0.13	6.43	5.35	0.54	29.32±0.23
TG-103	16	2A+6C+8D	87.50±0.22	72.48±0.20	7.23±0.09	5.46	4.53	0.45	33.84±0.29
TG-127	16	4A+12C	72.04±0.18	53.18±0.15	7.45±0.08	4.50	3.32	0.46	39.72±0.24
TG-129	16	2A+2B+6C+2D	68.96±0.18	57.15±0.37	7.60±0.10	4.31	3.37	0.48	32.16±0.13
TG-130	16	2A+10C+4D	79.27±0.24	66.05±0.25	7.56±0.09	4.95	4.12	0.47	37.50±0.21

2n=somatic chromosome number, GCL=genomic chromosome length, GCV=genomic chromosome volume.

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

Source	DF	SS	MS	F
Between cultivars	9	54.225	6.025	24.004*
Within cultivars	90	22.564	0.251	-
Total	99			

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

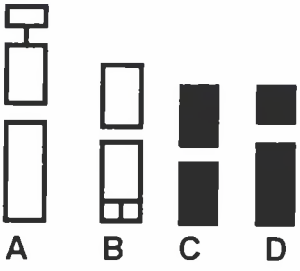

Fig. 1. Standard karyotype of fenugreek (*Trigonella foenum-graecum).*

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 7.23 pg inTG-103 to 9.12 pg inTG-09. 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 1.16 and 0.27, 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 genome chromosome volume and nuclear DNA content from the 'r' values of different cytological parameters (Table 4).

Discussion

Karyotype analysis in ten cultivars of fenugreek revealed some interesting facts at the cultivar level. Structural alteration of somatic chromosomes was noted; all the cultivars of fenugreek showed 2n = 16 chromosomes. Although the type of chromosomes were common in all the members, variation in the number of C and D types were the most striking feature. Furthermore, karyotype formulae minutely varied between TG-73 and TG-92 and TG-103 and TG-130. A similar karyotype was observed in TG-68 and TG-103 (Table 1), but 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, 1999, 2000).

Total chromosome length and volume differed markedly among the cultivars of fenugreek (Table 1). A proportional increase in chromosome length with an increase in chromosome volume and 4C DNA content was

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

	TG-103	TG-127	TG-130	TG-129	TG-73	TG-68	TG-78	TG-45	TG-92
TG-127	0.22ns								
TG-130	0.33*	0.11ns							
TG-129	0.37*	0.15ns	0.04ns						
TG-73	0.46*	0.24ns	0.13ns	0.09ns					
TG-68	0.87*	0.65*	0.54*	0.50*	0.41*				
TG-78	1.27**	1.05*	0.94*	0.90*	0.81*	0.40*			
TG-45	1.42**	1.20**	1.09*	1.05*	0.96*	0.55*	0.15ns		
TG-92	1.52**	1.30**	1.19**	1.15*	1.06*	0.65*	0.25ns	0.10ns	
TG-09	1.89**	1.67**	1.56**	1.52**	1.43**	1.02*	0.62*	0.47*	0.37ns

CD (critical difference) at 1% level = 1.16, CD at 5% level =0.27; ns=not significance, *=significant at 5% level, **=significant at 1% level.

observed in 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), might have played a 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 1 to 3). The maximum (9.12 pg) 4C DNA content was noted in TG-09 and the minimum (7.23 pg) in TG-103. The average DNA amount per chromosome varied markedly (Table 1). The genomic chromosome volume, however, showed a higher correlation with genome size i.e. DNA content (r=0.0.829) than the genome length (r=0.317). Although the nuclear DNA content in all the studied cultivars of fenugreek is reported here for the first time, intervarietal and interspecific variations were 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 't' values between different parameters confirmed that only nuclear DNA had a direct influence on chromosomal volume to a great extent (Table 4). Perhaps the maximum correlation of these genomic characteristics (t =8.23) 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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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	0.317	5.24*
Chromosome length *vs* 4C DNA content	0.416	1.12ns
Chromosome volume *vs* 4C DNA content	0.829	8.23*

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

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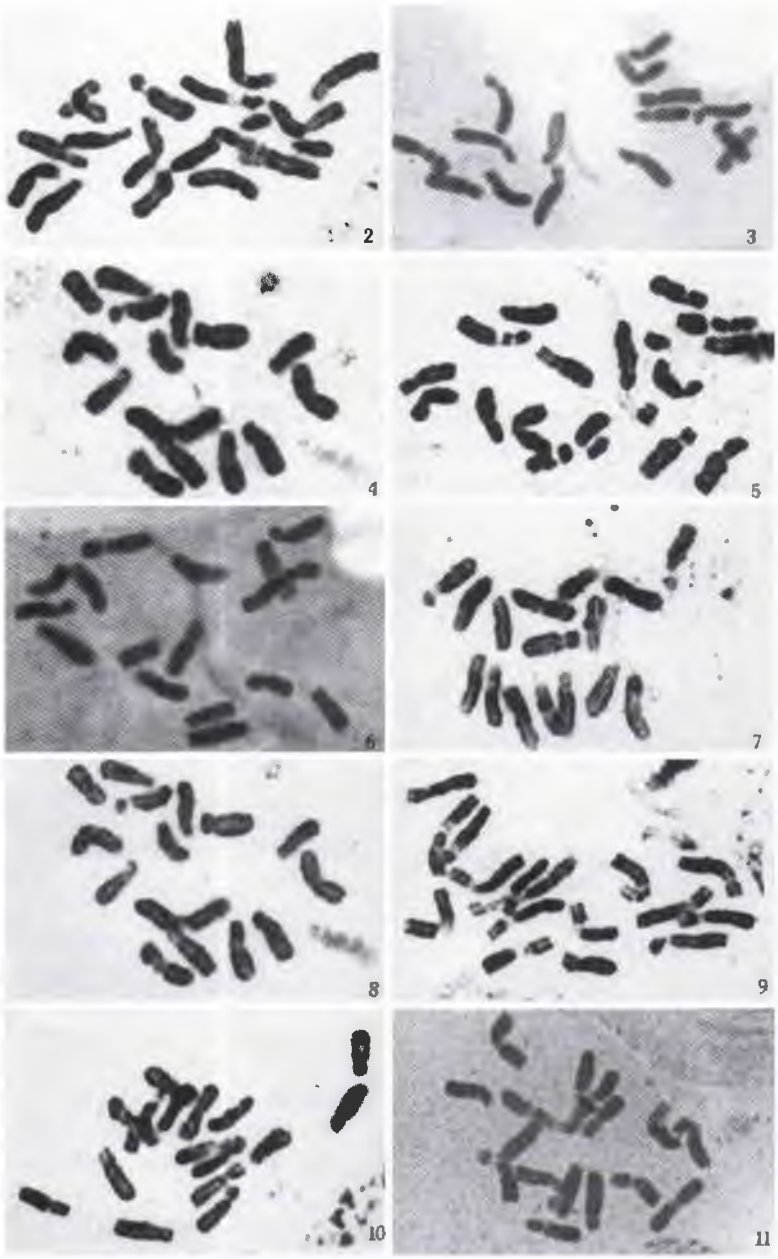

Figs. 2-11. Somatic metaphase plates showing 2n=16 chromosomes in different cultivars of fenugreek (x2068). 2. TG-09; 3. TG-127; 4. TG-45;5. TG-92;6. TG-78; 7. TG-103; 8. TG-130; 9. TG-68; 10. TG-73; 11. TG-129.

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