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Flow cytometric genome size estimation of major seed spice grown in India

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Abstract
Seed spices crops mainly cumin, coriander, fennel and fenugreek are predominantly cultivated in Indian sub continent to meet the domestic as well as international demand. The genomic information for genome size and genomic diversity is meagre on these crops, in the present investigation flow cytometer estimates were obtained for the 2C DNA content in term of picogram (pg) in these crops. Among the studied crops only fenugreek is from Fabaceae family rest are from the Apiaceae family; the 2C DNA content in Apiaceae crops ranged from 1.30 pg in coriander to 2.66 pg in cumin, whereas it was 7.37 pg in fenugreek. The information on genome size is useful in studying the whole genome sequence, and also to characterise the ploidy levels in the genetic resources. To our knowledge the genome size of fennel, coriander and fenugreek estimated in the present study is the first report using flow cytometry on Indian cultivars.

Keywords: C-value, flow cytometry, seed spices, cumin, coriander, fennel, fenugreek

Introduction
India is leader in seed spice production and trade, among the seed spices cumin, coriander, fennel, fenugreek are cultivated in large. In the country more than 90 lakh tones of seed spice is produced annually from an area of around 93 lakh hectares. More than 90 percent area under seed spice crops is occupied by these four crops. National and international demand is increasing for seed spices; cumin alone is having an export market of worth 4251 corers (Spice Board, India). These crops are grown in the western arid and semi arid parts of India, wherein productivity levels are drastically in high-range depending upon the local resources and climatic conditions. In the past four decades more than 110 high yielding varieties has been developed under the All India Coordinated Programme on Spices (AICRP Spices). The genetic improvement programme has been carried out by following conventional methods and in few instances its by use of mutagens (Singh and Solanki, 2015) [14]. In the national gene bank more than 5000 accessions are maintained by various centres of AICRP on Spices (Kakani *et al*., 2022) [7]. Numerous studied has been carried out to characterise the genetic resources at phenotypic level, but information on genomic diversity is scanty (Singh and Solanki, 2015; Solanki *et al*., 2017a,b; Parthasarathy *et al*., 2017; Parameguru P *et al*., 2017) [15, 16, 14, 11]. In the recent years research reports are there on assessment of genetic diversity using PCR based markers [Tulsani *et al*., (2020) [19]; Tomar *et al*., (2014a) [17]; Tomar *et al*., (2014b) [18]; Subha and Tomar (2019)] but basic information on genome size is very limited or not available.
Flow cytometry-based plant genome estimation started way back in 1980's, DNA content estimation helps in understanding the crop at cellular level for its further improvement using advanced tools and techniques (Dolezel and Bartos, 2005) [2] and is also helpful in deciphering the plant whole genome sequence. Flow cytometry is a very robust technique for estimating DNA amounts and for comparative analysis among genetic resources to know the ploidy level variation (Lysák *et al.*, 2000; Emshwiller, 2002) [9] considering the karyotype information in general (Bennett *et al*. 1995). In flow cytometer estimation sample preparation is convenient and is rapid, there is no need of dividing cells. Therefore, this method offers varied application in life sciences research (Kheiria *et al*., 2012) [8]. In the present investigation flow cytometer-based estimation of the 2C DNA content value was done in cumin, coriander, fennel and fenugreek. To our knowledge this is the first time the genome size of fennel, coriander and fenugreek is estimated and reported (Table 1) using flow cytometry on Indian cultivars.

Materials and Method
Seed spices crops *i.e.*, cumin, fennel, coriander, fenugreek, cultivars *viz.*, Gujarat Cumin 4,

Gujarat Fennel 1, Gujarat Coriander 1, Gujarat Methi 1 respectively were studied. Cultivar seeds were germinated and maintained in Germination chamber (Hi-point), 50-60 mg leaf samples from 2-week-old plants were collected for flow cytometry analysis. Each sample was chopped with in single edge razor blade for about 1 minute in 1 ml of chopping buffer having PVP40 (polyvinylpyrrolidone) to reduce noise in the measuring event as per Galbraith *et al.* (1983) [4]. The homogenate was filtered through nylon filter (80-um) to remove large debris. Filtered nuclei were stained with 50 mg ml^{-1} propidium iodide (PI; Himedia-IN), and 50 mg ml^{-1} RNase (Himedia-IN) was added to nuclear suspension to prevent staining of double-stranded RNA. Samples were incubated on ice and analysed within 10 to 15 min of preparation.

The sample fluorescence was estimated to know the DNA content on a BD Accuri® C6 flow cytometer. Several plant samples were measured, each with 5000-6000 nuclei and evaluated the coefficient of variation (where CV = standard deviation/mean channel number (Ormerod, 2008) [10]. Each estimate was repeated three times. Repeated analysis was done till the CV was observed below 5%. The DNA content for each sample was estimated using the following formula:

$$\text{DNA content (pg)} = \frac{\text{mean of sample peak position}}{\text{mean of standard peak pick position}} \times \text{known 2C Value (pg) of the standard}$$

Further, The DNA content were converted to Megabase pairs (Mbp) by the following the standard relationship of 1pg=978 Mbp. *Catharanthus roseus* (L.) G. Don. 2C value of 1.51 pg available in Plant DNA C-values Database data.kew.org/cvalues/ was used as internal standard to measure the genome size of cumin, fennel, coriander and fenugreek.

Result and Discussion

The assay result was obtained in histogram showing variable fluorescence intensities corresponding to the 2C DNA contents. The single prominent DNA peak represents nuclei in G1 phase of the cell cycle and a minor, or sometimes undetectable, peak represents G2-phase nuclei (Fig. 1). The seed spices crops were analysed with *Catharanthus roseus* (L.) genome size as internal reference standard having known 2C value of 1.51 pg. The genome size of diploid species *Cuminum cyminum* (cumin) cv. GC-4 was 1.76-fold that of the standard and its 2C-value was estimated to be 2.66 pg DNA. (Fig. 1, Table 1). The genome size of diploid species *Foeniculum vulgare* (fennel) was 1.529-fold that of the internal standard and its 2C- value was estimated to be 2.31pg DNA (Fig 1.b, Table 1). The genome size of diploid species *Coriandrum sativum* (Coriander) cv. GCo-1 was 0.860-fold of the internal standard and its 2c value was estimated to be 1.30 pg DNA. Thus, the genome size of three Apiaceae family seeds spice lies within the range of 1.30 pg to 2.66 pg DNA. There was drastic increase in 2C content on diploid Fabaceae species *Trigonella foenum-graecum* (Fenugreek) cv. GM-1; genome size was 4.880-fold higher than internal standard showing 2C value of 7.37 pg DNA (Fig 2).

To our knowledge this is the first time the genome size of fennel, coriander and fenugreek is estimated and reported (Table 1) using flow cytometry on Indian cultivars. Genome size variation has been studied by Hasan *et al*., (2016) [5] in cumin cultivars, high range of variation was observed among cultivars ranging from 1.74 pg in germplasm accession 184-J-2002-15 to 2.66 pg in RZ 209, whereas in GC-4 cultivar it was 2.51 pg near to the estimate of 2.66 in present investigation. The information will help to enrich plant genome database. Genome size has important practical implication in the modern genomics. The small genome size of coriander offers ample opportunity for rapid sequencing of the whole genome to dig out the molecular information for further improvement. Advance studies taking diverse genetic resources for estimating the 2C content can also help in knowing the 2C content variation in the genus. Carrot, a model crop of Apiaceae family genome size is estimate to be around 480Mb (Iorizzo *et al*., 2011) [6], whereas, genome size estimated in cumin, fennel and coriander is higher showing high diversity in the family. Hence, the information obtained is going to be useful for studying the genomic diversity in the available genetic resources in these most important seed spice crops having very high commercial value.

Table 1: 2C DNA content estimates in Seed spice crops (cumin, fennel, fenugreek and fenugreek)

SN	Seed spices	Family	Ploidy Level	Cultivars	DNA Content	
					2C DNA (pg)	Mbp
1.	*Cuminum cyminum* (Cumin)	Apiaceae	2n = 2x = 24	GC 4	2.66	2601.48
2.	*Foeniculum vulgare* (Fennel)	Apiaceae	2n = 2x = 22	GF 1	2.31	2259.18
3.	*Coriandrum sativum* (Coriander)	Apiaceae	2n = 2x = 22	GCo-1	1.30	1271.4
4.	*Trigonella foenum-graecum* (Fenugreek)	Fabaceae	2n = 2x = 16	GM 1	7.37	7207.86

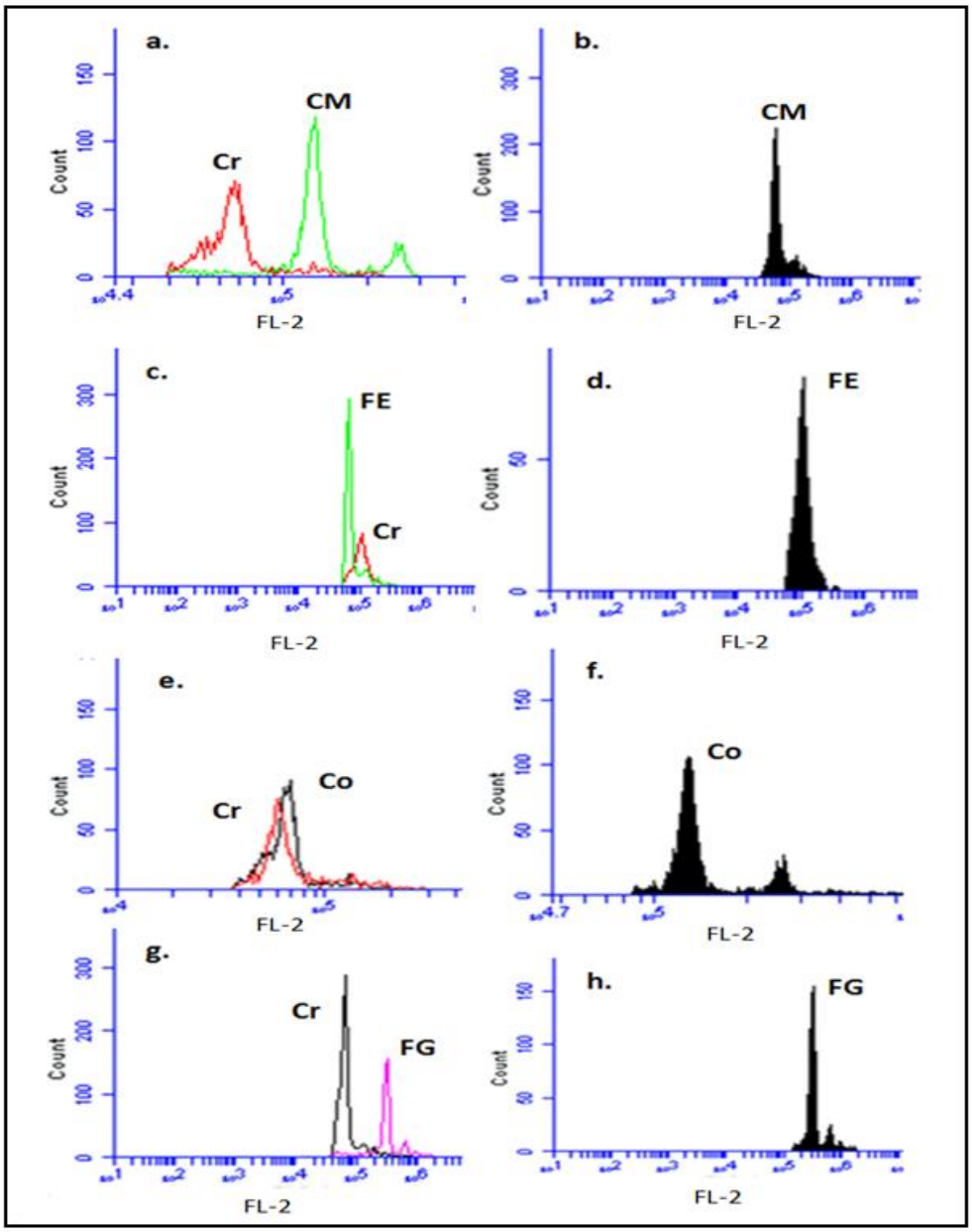

Fig 1: Line graphs and histograms of relative PI fluorescence intensities (FI-2) obtained after analysis of nuclei isolated from Cumin (CM-a,b), Fennel (FE- c,d), Coriander (Co- e,f)) and Fenugreek (FG- g, h) and internal standard *C. roseus* (Cr) shown in each graph.

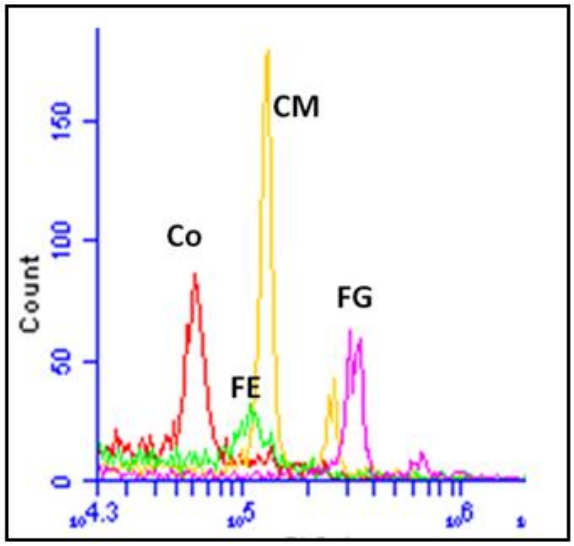

Fig 2: Comparative line graph of Cumin (CM), Fennel (FE), Coriander (Co) and Fenugreek (FG) showing 2C DNA content

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