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Estimation of nuclear DNA content of cumin

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Abstract

Cumin (*Cuminum cyminum* L.) is an economically and pharmaceutically crop of family Apiaceae. The information on genome size of cumin is still unavailable. Flow cytometry is an important tool for determination of the DNA nuclear content. Nuclear DNA size of six cumin genotype was measured using flow cytometry. The relative nuclear DNA content of all varieties ranged from 1.74 (184-JC-2002-15) to 2.66 (RZ-209) with a mean of 2.29+0.31. Flow cytometry is shown here to be a useful tool to indicate genome size estimate of cumin genotypes. Determining DNA content will useful in the verification of the efficiency of manipulation techniques of the ploidy level, mainly in works related to chromosomal duplication induction.

Cumin (*Cuminum cyminum*), native to the Eastern Mediterranean region and Southwest Asia, is a diploid (2n = 14) plant in of Apiaceae family. It is the second most popular spice in the world after black pepper. Its seeds have been commonly used for culinary and flavouring purposes due to strong and aromatic flavour (Deepak, 2013) and also in ethanomedical therapy since ancient times in various countries. Globally, India is largest producer (70%) as well as consumer of cumin (Sastry and Anandraj, 2013). The cumin is cultivated primarily in Asia, Europe, Middle East and North Africa (Lim, 2013).

Although a number of potential species (ca 18) have been described based on morphological differences, merely four species names (C. borszczowii, C. cyminum, C. setifolium and C. sudanenseare) botanically acceptable (http://www.theplantlist. org/browse/A/Apiaceae/

Cuminum/). Thus, phenotypically low variability has observed in cumin. Currently the 2C DNA content of cumin is unknown, therefore, the objective of this research was to use flow cytometry to determine the 2C DNA content in picograms (pg). Knowledge of genome size is critical for a variety of research including taxonomy and evolutionary studies, for gene cloning and genome sequencing projects, as well as for the discrimination of morphologically similar species (Morozowska et al., 2010). To the best of our knowledge, this is the first report on genome size estimation of cumin using flow cytometry.

The cytometric survey was conducted on five popularly grown cumin varieties. The varieties were germinated on filter-paper bridges in glass culture tube on ¼ MS media in a walk-in-chamber. Young leaves and roots are the best choice for isolation of nuclei because of low polyphenolic compound oxidizing agents, chlorophyll pigments etc. The material for cytological survey was comprised the healthy and fresh roots of 10 plants. Diploid maize (2C=2x=5.43 pg; Dolež˘el et al., 2007) were used as internal standards for flow cytometry.

Flow cytometry was performed on approximately 20-30 mg freshly harvested root tissue. For this, actively growing 4-8 mm root tips were excised from the 7 days old seedlings. Root tissues were finely chopped with a sharp razor blade in the centre of a plastic Petri dish containing one ml of ice-cold nuclei isolation buffer [Galbraith's buffer: 45mM $MgCl_2$; 30mM sodium citrate; 20mM MOPS (N-morpholino propanesulphonic acid); 0.1% (w/v) Triton X-100; pH 7.0). Then, homogenate was filtered through a 50-µm mesh nylon filter into a labelled sample tube and stained with 50 µg mL^{-1} propidium iodide (PI: Sigma-Aldrich, France). RNase, at a concentration of 50 mg mL-1 (Roche, France), was added to the homogenate to avoid

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staining of double-stranded RNA by propidium iodide. The sample was then incubated on ice before analysis (a few minutes to 1 h) with occasional shaking.

The nuclear homogenate were analyzed with a flow cytometer (BD FACSJazz™ Cell sorter, USA). For each variety, 5000 -6000 nuclei were measured in three replications. Before, any measurement of nuclei, instrument was aligned by adjusting fluidic FSC (Forward scattered), SSC (Side scattered) and nob. Samples were run until empty at a speed of 0.5psi. Instrument linearity was set by using Rainbow Fluorescence QC particle and set to the fine adjustment until CV (coefficient of variation) become 3.0 for 488 m laser beam. A linear scale was used and histograms were analysed using the BD FACSJazz software. The linear relationship between the ratio of target species and the internal standard 2C peak positions on the histograms was used for nuclear genome size estimation. Relative DNA content of individual plant was expressed using a DNA Index (DI) calculated according to the formula:

Sample 2C value (DNA pg) =

$$\text{Reference 2C value} \times \frac{\text{Sample 2C mean peak position}}{\text{Reference 2C mean peak position}}$$

The DNA content (pg) was converted to megabase pairs of nucleotides (Mbp) using the relationship 1pg = 978 Mbp (Dolezel *et al.*, 2007).

Nuclear DNA content, a quantitative character of an organism, should remain constant among individuals of a species as long as there is no interbreeding to mix up the gene pool (Murovec *et al.*, 2009). To quantify the genomic DNA content, flow cytometry is a reliable and efficient method (Tatum *et al.*, 2005). The applied cytometric procedure was appropriate for the material analyzed in this study and no technical problem was encountered during the analysis of the samples. In present study, homogenate of isolated nuclei gave characteristic histograms (Fig. 1). Overall, the trustworthiness in the results of the DNA content estimates by flow cytometry is mentioned by the coefficients of variation attributed to the histograms generated from the readings (Timbó *et al.*, 2012). The CV can vary according, for example, to the buffers and standards used in the evaluations and to the vegetal species studied (Doležel *et al.*, 1989; Loureiro *et al.*, 2006). With a mean of 2.7%, the coefficient of variation (CV) of the 2C peaks for the cumin and standard species (maize) was between 1 to 5%. The low CV value of the 2C peaks indicated that a measurement in present work was accurate. Flow cytometric analysis of nuclei isolated from roots shows single peak corresponding to 2C level (G0+G1 phase) and no peak corresponding to 4C level (G2+M phase) was not detected. The relative nuclear DNA content of 60 individual seedlings from all varieties ranged from 1.74 (184-JC-2002-15) to 2.66 (RZ-209); this is 1.5-fold variation. The mean DNA content was 2.29$\pm$0.31. Table 1 is showing the variability of relative nuclear DNA content among cultivars. Differences in genome size may be largely caused by different amounts of noncoding repetitive DNA, to which transposable elements, satellite DNA, introns and pseudogenes can contribute (Bennett and Leitch, 2005). Moreover, ecological factors such as temperature may also explain the genome size variation (Bennett *et al.*, 2000). The nuclear DNA content in the present report was lower than Capsicum species reported by Moscone *et al.*, (2003). The stained nuclei generated histograms of the relative DNA contents of sample and comparative primary standard as shown in Fig. 1. Overall, very low variations in DNA content were observed among varieties. This was expected as breeding in cumin is cumbersome and most varieties were derived from selection and had a narrow genetic base.

The results of present study showed that flow cytometry gave a quick and very reliable determination of the ploidy level and is a relatively convenient and rapid method compared with chromosome counts. Flow cytometry is shown here to be a useful tool to indicate genome size estimate of cumin genotypes. Determining DNA content will support to establish natural diploid mapping populations to analyze genetic diversity and population structure necessary for marker trait association analysis. Besides, it can also be useful in the verification of the efficiency of manipulation techniques of the ploidy level, mainly in works related to chromosomal duplication induction.

Table 1. Genome content of cumin genotypes

Sr. No.	Genotype	2C DNA Content	
		Pg[1]	Mbp[2]
1	GC-2	2.47	2423
2	GC-4	2.51	2459
3	184-JC-2002-15	1.74	1702
4	RZ-209	2.66	2609
5	RZ-223	2.24	2193
6	RZ-341	2.13	2088

Pg[1]=Pico Gram and Mbp[2]= Mega base pair. The DNA content (pg) was converted to mega base pairs of nucleotides (Mbp) using the relationship 1pg = 978 Mbp (Dolezel et al., 2007).

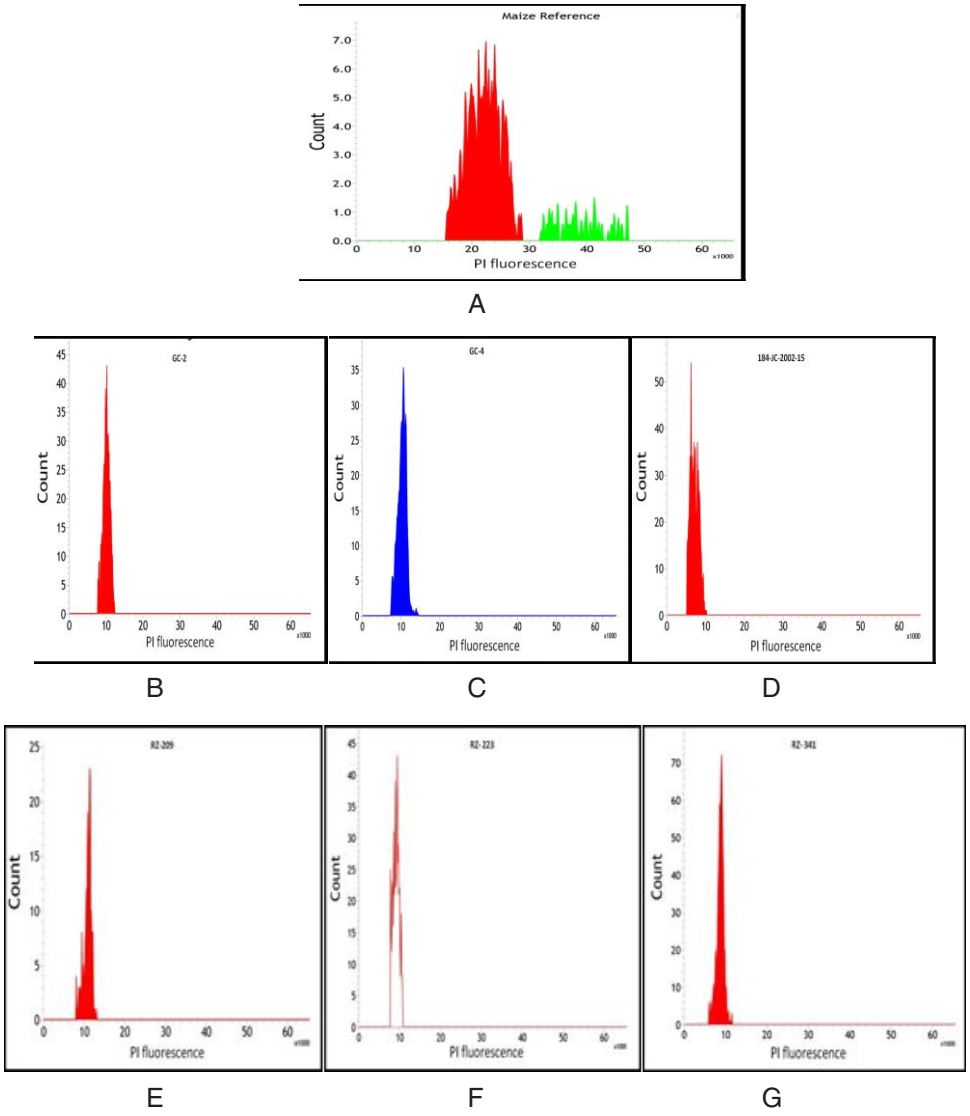

Fig. 1. Histograms of relative PI fluorescence intensities obtained after simultaneous analysis of nuclei isolated from: (A) Maize- internal standard (B) GC-2 © GC-4 (D) 184-JC-2002-15 (E) RZ-209 (F) RZ-223 (G) RZ-341.

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