ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2521-0858 (Print)
ISSN: 2521-0866 (Online)
CODEN: SHJCAS

Science Heritage Journal (GWS)

DOI: http://doi.org/10.26480/gws.01.2021.14.16

RESEARCH ARTICLE

GENOME SIZE DETERMINATION OF CUCUMBER (*CUCUMIS SATIVUS*), HONEYDEW (*CUCUMIS MELO* INODORUS) AND ROCK MELON (*CUCUMIS MELO* CANTALUPENSIS) VIA FLOW CYTOMETRY

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ARTICLE DETAILS

Article History:

Received 25 July 2021
Accepted 29 August 2021
Available online 14 September 2021

ABSTRACT

The family of Cucurbitaceae consists of species with economical and nutritional value. Morphologically, there are only few differences between *Cucumis* species. The interspecific and intraspecific variation in the genome size of the *Cucumis* species are not discovered yet. Due to this, this study aims to determine the genome size of *C. sativus, C. melo* inodorus and *C. melo* cantalupensis using flow cytometry (FCM) method. Nuclei suspension of selected *Cucumis* species were extracted using LBO1 lysis buffer by manual chopping technique and stained by propidium iodide priot to FCM analysis. Genome size of *C. sativus*, *C. melo* inodorus (Honeydew) and *C. melo* cantalupensis (Rockmelon) were determined by using *Glycine max* (Soybean) as an external reference standard (2C = 2.5 pg). This study found that the genome size of *C. sativus*, *C. melo* inodorus and *C. melo* cantalupensis estimated to be 2.83 pg, 3.00 pg and 3.47 pg respectively. The genome size data obtained from this study can be used in future genome studies as well as species characterization.

KEYWORDS

Cucumis species, flow cytometry, genome size.

1. INTRODUCTION

The family of Cucurbitaceae is described as a plant that show specific characteristics includes pollen grains and ovules produced from different flower either within the same plant or different plant. Morphologically, members of Cucurbitaceae commonly have rather roughly hairy, toothed leaves with palmate venation but no stipules. The tendrils which are believed by some botanist to be modified stem often coil like watch springs when attaching to a support (Berry, 2019). The family Cucurbitaceae has been subdivided by their economical important which are Zanonioideae as low economic impact species and Cucurbitoideae as high economic impact species group (Kristkoval et al., 2003). It is known that genus *Cucumis* is one of the 118 generas within the family of Cucurbitaceae that is popular for cultivation by the human for the years. Thus, the taxonomist classified the genus *Cucumis* into approximately 30 different species based on minute differences between herbarium specimens and their natural habitats.

Cucumis species emerge to be among the important crops commercially all over the world. They are highlighted for highly resistance toward various climates, pests and diseases. However, the exact and accurate description of most *Cucumis* species is still debatable and in ambiguous state. The information on the relationship between *Cucumis* species in term of genome size has not been discovered yet (Cheng et al., 2020). Regarding this, cytogenetics analysis along with morphological approaches could be used to develop genomic resources for *Cucumis* species as a crucial basic intragenic information for further study.

A group researchers claimed that FCM is the best method to analyze the fluorescence microscopic particles in liquid suspension as well as genome size of plant species (Dolezel et al., 2007). Vast population can be measured in the short time. The interception between light beam and suspension produces scattered light in which will be captured and converted by the optical sensor into electrical signal. The advantages of FCM are convenient, fast and reliable analysis. In addition, the sample preparation only takes few minutes using inexpensive reagents and the device is available at affordable prices (Dolezel et al., 2007; Dolezel and Batos, 2005). The purpose of this study is to provide genome size data of three *Cucumis* species which are *C. sativus* (cucumber), *C. melo* indorus group (honeydew) and *C. melo* cantalupensis (rockmelon) via FCM method. The data obtained is important in future research including genome sequencing, species relationship as well as plant breeding.

2. MATERIAL AND METHODS

2.1 Plant material

C. sativus, *C. melo* inodorus and *C. melo* cantalupensis seeds were planted under favorable conditions. For nuclei preparation, the cotyledons of *C. sativus*, *C. melo* inodorus and *C. melo* cantalupensis were used in the FCM

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Cite the Article: Raden Muhamad Imaduddin Yumni, Mohd Fauzihan Karim, Mohd Razik Midin (2021). Genome Size Determination of Cucumber (*Cucumis Sativus*), Honeydew (*Cucumis Melo* Inodorus) And Rock Melon (*Cucumis Melo* Cantalupensis) Via Flow Cytometry. *Science Heritage Journal, 5(1): 14-16.*

analysis as they easier to process due to soft structure with less secondary metabolites component (Jedzejczyk and Sliwinska, 2010).

2.2 Nuclei extraction and staining

The cotyledons of *Cucumis* species were cut into small pieces using sharp razor blade within the petri dish containing LBO1 lysis buffer supplemented with mercaptoethanol and RNaseA. Two cotyledons were used for every species (five replicates for each species). The samples were then filtered using 50 µm nylon mesh (42-µm pore size) and put into a 10 ml falcon tubes. Propidium iodide (PI) was added into the nuclei suspensions and incubated for 10 minutes at room temperature to allow optimum PI intercalation with the DNA strands. PI fluorochrome was selected for this analysis because it has been proven to produce more precise fluorescent intensity histogram peak with lower CV and able to intercalate the entire DNA region (Midin et al., 2013).

2.3 FCM measurement

FACScalibur flow cytometer (Becton Dickinson, San Jose, CA.) equipped with argon ion laser at 488-nm wavelength was used to measure fluorescence intensity peaks of each sample and reference standard. Histograms were collected for 5000 events for each sample. The system integrated with CellQuest software was used to generate the values of fluorescent intensity peaks as well as CV values.

2.4 Data analysis

The genome size was determined based on the values of the G1 peak means using formula:

$$\frac{G1\ peak\ mean\ of\ sample \times 2C\ DNA\ content\ of\ reference\ standard}{G1\ peak\ mean\ of\ standard}$$

In this study, *Glycine max* cv. Polanka (soybean) was used as an external reference standard as its genome size was well established and stable. Thus, by computing the G1 peak mean of all samples used into the formula above, the genome size of samples can be obtained in the unit of picogram (pg). Genome size among *Cucumis* species was then compared using one-way Analysis of variance (ANOVA) for statistical significance.

3. Results and Discussion

3.1 Histogram DNA peak analysis

A group researchers reported that manual chopping resulted considerable amount of debris and low debris background in the histogram DNA analysis ((Midin et al., 2013). This is due to reduce uncontrolled chopping and excessive nuclei breakage. In addition, manual chopping provides unbiased or random selection of nuclei in tissue zone for FCM analysis (Galbraith et al., 1983). Due to this, manual chopping was adopted in this experiment. DNA histograms with low debris background and sharp DNA peak were successfully generated by all *Cucumis* species. Other researcher also suggested a combination of electronic thresholding and right gating strategies to eliminate the debris from the histogram (Midin et al., 2018). In order to facilitate the release of nuclei suspension, LBO1 lysis was selected for nuclei preparation of *C. sativus*, *C. melo* inodorus and *C. melo* cantalupensis cotyledons. As a result, DNA histograms with distinct and sharp peak were generated for these three species as shown in the Figure 1.

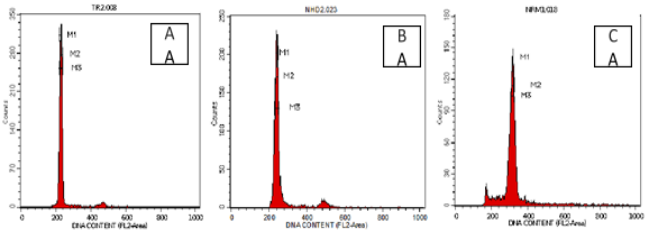

Figure 1: Histograms of nuclei fluorescence intensity: (A) *C. sativus* (B) *C. melo* inodorus, (C) *C. melo* centalupensis

The lysis buffer used in this study is to ensure the stability of nuclei, protect DNA and facilitate stoichiometric staining (Loureire et al., 2006). LBO1 lysis buffer consists of chromatin stabilizer, chelator agents, inorganic salt, organic buffer and non-ionic detergents. The chromatin stabilizer such as $MgCl_2$ and Mg_2SO_4 is important for nuclei stability. Meanwhile, chelator agent like ethylenediaminetetraacetic acid (EDTA) is crucial for divalent cation binding and nuclease factor. The inorganic salts commonly used for strengthening ionic bond and the present of organic buffer as pH stabilizer. The most important component in lysis buffer is non-ionic detergent including Triton X-100 and Tween 20 which disrupt chloroplast and release the nuclei into suspension (Loureire et al., 2006). Loureiro et al. [10] preferred the usage of LBO1 lysis buffer as it generates good results as compared to other lysis buffer, Galbraith's and Tris-$MgCl_2$ (Loureire et al., 2006). A group researcher also reported that LBO1 lysis buffer allows high efficiency of PI to intercalate with the DNA (Madon et al., 2008). As for now, there are about 25 lysis buffers developed and 8 often and popularly used on 30 plant species (Loureiro et al., 2006). Different plant species composed different chemical composition and tissues that influence how the type of buffer works. Briefly, the cytosolic compounds released during cell lysis process interact with nuclear DNA and fluorochrome, hence affect the sample quality and possible stoichiometric errors in staining process. The DNA histograms indicates the quality of nuclei suspension. The good DNA histogram possess minimal amount of background debris, symmetrical peak of G_0/G_1 and low coefficient of variation (CV) value. The CV value which less than 3% is acceptable for most species (Dolezel and Bartos, 2005).

3.2 Genome size determination

The external DNA reference standard was adopted in this experiment in order to calculate the unknown genome size of the samples. The selection of soybean as an external DNA reference standard is due to its genetic stability and consist of well-established genome size which is 2C = 2.5 pg (Loureiro et al., 2006; Hendrix and Stewart, 2005). The DNA histogram of soybean also fulfilled the requirements of FCM histogram DNA peak as low CV values (<3%) and less debris background produced. Table 1 presents the estimated genome size of *C. sativus*, *C, melo* inodorus and *C. melo* var cantalupensis. Based on the result, the genome size of *C. sativus* was found to be the lowest genome size as compared to other *Cucumis* species, with an average 2.83 pg. Meanwhile, *C. melo* illustrate higher genome size with the average of 3.00 pg and 3.47 for inodorus and cantalupensis respectively.

Table 1: Genome size of *C. sativus*, *C. melo* inodorus and *C. melo* centalupensis

Species	Replicate	Nuclear genome size(pg)	Nuclear genome size (pg) *average
C. sativus (cucumber)	1	3.32	2.83
	2	2.90	
	3	2.71	
	4	2.66	
	5	2.55	
C. melo inodorus (honeydew)	1	3.07	3.00
	2	3.05	
	3	2.97	
	4	3.04	
	5	2.87	
C. melo centalupensis (rockmelon)	1	4.02	3.47
	2	3.73	
	3	3.24	
	4	3.10	
		3.24	

3.3 Interspecific and intraspecific variation in genome size

The genome size variation information is vital for evolution and species relationship studies. A few changes in DNA content in intraspecific level can be demonstrated by phenotypic difference (Murray, 2005). The variation is existing even within the same species (Madon et al., 2008;

Bennett and Leitch, 2005; Smarda and Bures, 2010). Based on the statistical analysis conducted on the genome size of three *Cucumis* species used, there is no significant difference between *C. sativus* and *C. melo* inodorus but *C. sativus* with *C. melo* cantalupensis is significantly different (Figure 2). On the other hand, there is no significant difference at intraspecific level of *C. melo*. Hypothetically, the genome size between difference species *C. sativus* (Cucumber) and *C. melo* inodorus (Honeydew) can be the same for two reasons. The first reason is they might have same chromosome number. In contrary, they might have different chromosome number but the same DNA content or value for the whole genome. Due to this, further study on the genome of *Cucumis* species are required by using other cytogenetics approaches including chromosome counting, karyotyping and Fluorescence *in situ* hybridization (FISH).

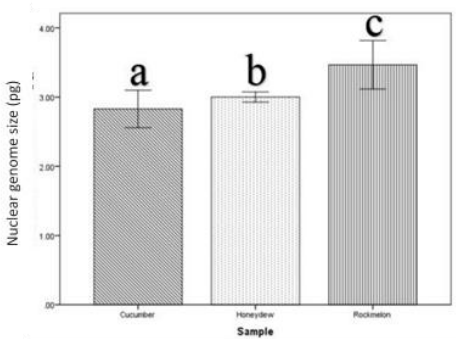

Figure 2: Average mean of nuclear genome size of three *Cucumis* species

4. Conclusion

This study revealed that the genome size of *C. sativus*, *C. melo* inodorus and *C. melo* var cantulpensis were found to be 2.83 pg, 3.00 pg and 3.47 pg respectively. Based on the statistical analysis, no significant variation was found in term of genome size between *C. sativus* with *C. melo* inodorus (interspecific variation) and *C. melo* inodorus with *C. melo* cantalupensis. Nevertheless, significant interspecific genome size variation was found between *C. sativus* with *C. melo* cantalupensis. The genome size information provided in this study is important for further researches including breeding and genomics of *Cucumis* species for crop improvement. The data can be used by researcher in the future genome sequencing project. The information can be also used in order to categorize species instead of analyzing phenotypic characteristics.

References

Bennett, M.D., Leitch, I.J., 2005. Plant Genome Size Research: A Field In Focus. Annals of Botany, 95, Pp. 1-6.

Berry, P.E. 2019. Cucurbitales. Retrieved from: https://www.britannica.com/plant/Cucurbitales

Cheng, H., Kong, W.P., Zhang, M.M., Hou, D., 2020. The complete chloroplast genome of Cucumis melo L. "Shengkaihua" (Cucurbitaceae) and its phylogenetic implication. Mitochondrial DNA Part B, 5(2), Pp. 1253–1254.

Doležel, J., Bartoš, J.A.N., 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Annals of botany, 95 (1), Pp. 99-110.

Doležel, J., Greilhuber, J., Suda, J., 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols, 2 (9), Pp. 2233-2244.

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P., Firoozabady, E., 1983. Rapid Flow Cytometric Analysis of the Cell Cycle in Intact Plant Tissues. Science, 220 (4601), Pp. 1049–1051.

Hendrix, B., Stewart, J.M., 2005. Estimation of the nuclear DNA content of Gossypium species. Annals of Botany, 95, Pp. 789-797.

Jedrzejczyk, I., Sliwinska, E., 2010. Leaves and seeds as materials for flow cytometric estimation of the genome size of 11 rosaceae woody species containing DNA-Staining inhibitors. Journal of Botany, Pp. 1–9.

Křístková1, E., Lebeda, A., Vinter, V., Blahoušek, O., 2003. Genetic resources of the genus Cucumis and their morphological description. Horticultural Science (Prague), 30 (1), Pp. 14–42.

Loureiro, J., Rodriguez, E., Doležel, J., Santos, C., 2006. Comparison of Four Nuclear Isolation Buffers for Plant DNA Flow Cytometry. Annals of Botany, 98 (3), Pp.679–689.

Madon, M., Phoon, L. Q., Clyde, M.M., Mohd, D.A., 2008. Application of flow cytometry for estimation of nuclear DNA content in Elaies. Journal of Oil Palm Research, 20, Pp. 447-452.

Midin, M.R., Nordin, M.S., Madon, M., Saleh, M.N., Goh, H.H., and Normah, M.N., 2018. Determination of the chromosome number and genome size of Garcinia mangostana L. via cytogenetics, flow cytometry and k-mer analyses. Caryologia, 71, Pp. 35-44.

Midin, M.R., Samsul Kamal, R., Tarmizi, A.H., Nulit, R., Madon, M., 2013. Analysis of oil palm clones, their suspension calli and regenerants via flow cytometry (FCM) and rDNA-fluorescence in situ hybridisation (rDNA-FISH). Journal of Oil Palm Research, 25 (3), Pp. 357-367.

Murray, B.G., 2005. When does Intraspecific C-value Variation become Taxonomically Significant? Annals of Botany, 95 (1), Pp. 119–125.

Šmarda, P., Bureš, P., 2010. Understanding intraspecific variation in genome size in plants. Preslia, 82 (1), Pp. 41-61.