FLOW CYTOMETRIC ANALYSIS OF NUCLEAR DNA BETWEEN OKRA LANDRACES (ABELMOSCHUS ESCULENTUS L.)

Naser M. Salameh
Department of Plant Production, Faculty of Agriculture, Mutah University, Al Karak, Jordan

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ABSTRACT

Okra Abelmachus esculentus L. (Moench), is an economically important vegetable crop grown in tropical and sub-tropical as well as Mediterranean countries. The genome size of the Abelmoschus genus species is still rare. Ploidy determinations have traditionally been done by counting chromosomes of stained root tips, but this method is laborious and often difficult with species which have small chromosomes and high ploidy levels and can lead to misclassified. Flow cytometry has been widely used in various aspects of plant research, such as the determination of the DNA nuclear content. Nuclear DNA size of fifteen okra genotype was measured using flow cytometry. The data showed variation between Turkish genotype and Jordanian genotype. Genome size of okra ranged from 3.98 pg 2C−1 in Jordanian landrace to 17.67pg 2C−1 in Turkish landrace. The 2C genome size in Mbp ranged from 3897-17321 among Abelmoschus esculentus genotypes. Further studies is recommended to accurate chromosome count to be linked with the genome size.

Keywords: Okra, Genome Size, Flow Cytometry, Relative DNA Content

1. INTRODUCTION

Okra Abelmachus esculentus L. (Moench), is an economically important vegetable crop grown in tropical and sub-tropical as well as Mediterranean countries (Duzyaman and Vural, 2003). This crop is suitable for cultivation as a garden crop as well as on large commercial farms. Okra is cultivated for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable. Okra fruit can be cooked in a variety of ways. Despite being a minor crop okra has gained considerable interest as an alternative to traditional vegetables in many countries throughout the world. This vegetable provides an important input of vitamins and mineral salts, including calcium; which are often lacking in diet of developing countries (Omonhinmin and Osawaru, 1991). Okra seeds have also gained much interest as a new oil and protein source (Duzyaman, 1997).

The origin of okra remains unclear, but center of genetic diversity include West Africa, India and Southern Asia (Hamon and Van Sloten, 1998). There are significant variations in the chromosome numbers and ploidy levels of different species in the genus Abelmachus (Charrier, 1984). The chromosome number and ploidy levels of different species are given in Table 1. Jordanian landraces have diverse characters (Salameh and Kasrawi, 2007). Its red fruit color is dominant over green and this trait is controlled by two independent genes. The length of fruits, their diameter and number of ridges are quantitatively inherited traits (Salameh and Kasrawi, 2011). The difference in gene numbers controlling these characters may be due to the presence or absence of genes or the presence or absence of entire chromosomes. Mahadeen (2014) showed that there is an increase in okra growing in Jordan.

However very little information is available about cytogenetics and reproductive biology of this very important vegetable crop.

As the accurate amount of nuclear DNA content is extremely important to understand the hereditary constituent of an organism, flow cytometry, originally developed for medical studies, is an easy, rapid, accurate and convenient tool for estimating plant genome size, ploidy level, assessing DNA content and analyzing the cell cycle (Greilhuber, 1998; Winkelmann et al., 1998).
Table 1. Variation of chromosome number in okra (*Abelmoschus esculentus* L.) (Charrier, 1984)

| Species                      | Number (2n) | Authors                         |
|------------------------------|-------------|---------------------------------|
| *(Abelmoschus esculentus* L.)| ±66         | Ford (1938)                     |
|                             | 72          | Kamalova (1977)                 |
|                             | 108         | Datta and Naug (1968)           |
|                             | 118         | Krenke (In: Tischler, 1937)     |
|                             | 120         | Purewal and Randhawa (1947)     |
|                             | 122         | Krenke (In: Tischler, 1937)     |
|                             | 124         | Kuwada (1961; 1966)             |
|                             | 126-134     | Chizaki (1934)                  |
|                             | 130         | Skovsted (1935); Gadwal *et al.* (1968) |
|                             | 131-143     | Siemonsma (1981)                |
|                             | 132         | Medvedeva (1936); Roy and Jha (1958) |
|                             | ±132        | Breslave *et al.* (1934)        |
|                             | 144         | Datta and Naug (1968)           |

Voluminous information on the nuclear DNA contents in plants is being published by Bennett *et al.* (2000) and Hanson *et al.* (2001).

In addition to determining the nuclear DNA content, flow cytometry in higher plants is used for studying plant protoplast (protoplast size, cell wall synthesis, chlorophyll content, alkaloid content, RNA content, protein content, protoplast-microbe interaction, sorting of protoplast fusion products) and chromosomes (chromosome size, centromeric index, sorting of large quantities of chromosomes of single type for gene isolation and mapping) (Lucretti *et al.*, 1999).

Flow cytometry allows reliable and rapid estimation of nuclear DNA content. This method finds numerous applications in plant taxonomy and breeding. The prominent applications have been ploidy level determination, analysis of nuclear DNA content and genome size estimation. Moreover flow cytometry has become increasingly popular and has been used for genome analysis in a variety of species (Palomino *et al.*, 2003).

Genome size of different animals e.g Dairy goat (Fletcher *et al.*, 2013) as well as different, plants has been estimated by flow cytometry like Pinus (O’Brien *et al.*, 1996), Lemon (Iannelli *et al.*, 1998), Arachis hypogea (Temsch and Greilhuber, 2000), Musa (Roux *et al.*, 2003), Atriplex halimus (Walker *et al.*, 2005), Consolea (Negron-Ortiz, 2007), Vicia faba (Kovarova *et al.*, 2007) Cactus (Lema-Ruminska, 2011), Coffea (Clarindo *et al.*, 2012), Calendula species (Nora *et al.*, 2013), Eryngium (Tavares *et al.*, 2013), Lathyrus (Ochatt *et al.*, 2013) and Phragmites australis (Nakagawa *et al.*, 2013).

However, regardless of the increasing importance of nuclear DNA content, to our knowledge, there are no genome size estimations available for *Abelmoschus* available. Therefore, the present study is the first attempt at defining the size of the genome of okra by measuring the nuclear DNA content using Flow Cytometry.

2. MATERIALS AND METHODS

2.1. Plant Materials

Different landraces have been collected from different parts of Jordan in addition to some landraces from the neighboring countries (Table 2).

2.2. Flow Cytometry

Preliminary attempts to use leaves of okra plants did not produce any results, presumably due to abundant mucilage and acidity of the tissue. Subsequently, a suspension of intact nuclei of landrace root tissue was analyzed by flow cytometry using the kit with Cystain UV precise P kit from Partec. Three individuals per accession were chopped in 400 µL extraction buffer, then after addition of 1600 µL staining buffer the tissues were mixed well. The suspension was filtered with Celltrics-filter (~ 50 µm) in a cuvette. The DNA content of each sample was measured using the Partec Cell Analyzer II. As a quality control, nuclear DNA content estimates were only considered when the coefficient of variation of G₀/G₁ peaks (CV peaks) was below 5%. Samples with higher CV peaks values were discarded and new sample was prepared. Maize KYS nuclei were used as the internal reference standard. Relative DNA content of individual plant was expressed using a DNA Index (DI) calculated according to the formula:

\[
\text{Sample 2C DNA content} = \frac{\text{Sample } G_0 \text{ peak mean}}{\text{standard } 2C \text{ DNA content (pg DNA)}} \times \text{standard } G_0 \text{ peak mean}
\]
Flow cytometric histogram of relative fluorescence intensity obtained after simultaneous analyses of nuclei isolated from roots of *Abelmoschus esculentus*.

**Table 2.** Nuclear DNA content estimations in *Abelmoschus esculentus*

| Landrace | Origin | 2C value (pg). FC | No. of Mbp |
|----------|--------|------------------|------------|
| Okra 12  | Turkey | 17.67            | 17321      |
| Okra 19  | Egypt  | 4.82             | 4720       |
| Okra 20  | Egypt  | 5.66             | 5543       |
| Okra 21  | Jordan | 4.46             | 4374       |
| Okra 22  | Jordan | 5.57             | 5456       |
| Okra 23  | Jordan | 3.98             | 3897       |
| Okra 24  | Jordan | 5.04             | 4936       |
| Okra 26  | Jordan | 4.51             | 4417       |
| Okra 27  | Jordan | 5.83             | 5716       |
| Okra 28  | Jordan | 4.55             | 4460       |
| Okra 29  | Jordan | 6.67             | 6539       |
| Okra 30  | Jordan | 6.62             | 6149       |
| Okra 34  | Jordan | 4.55             | 4460       |
| Okra 35  | Jordan | 5.08             | 4980       |
| Okra 38  | Jordan | 4.73             | 4633       |

The DNA content (pg) was converted to megabase pairs of nucleotides (Mbp) using the relationship 1pg = 978 Mbp (Dolezel et al., 2003).

**3. RESULTS**

Flow Cytometry histograms presented two dominant peaks, corresponding to the G₀/G₁ nuclei of the sample and the primary reference standard. The stained nuclei generated histograms of the relative DNA contents of sample and comparative primary standard as shown in Fig. 1. The 2C nuclear DNA content of *Abelmoschus esculentus* was determined, for the first time, using flow cytometry (Table 2). Genome size of okra ranged from 3.98 pg 2C⁻¹ in landrace 23 (Jordanian landrace) to 17.67 pg 2C⁻¹ in landrace 12 (Turkish landrace). The 2C genome size in Mbp ranged from 3897-17321 among *Abelmoschus esculentus* genotypes corresponding to 4.4% of variation between them.

**4. DISCUSSION**

The amount of DNA per chromosome set is known to be a fairly constant characteristic of a species. Therefore, in addition to the number of chromosomes, which are since long regarded as an important tool for delimiting species, in the past two decades an increasing interest on genome size studies and its significance has been observed, with many studies aiming at using genome size as a taxonomic marker (Kron et al., 2007). Despite this increase, the complex genus *Abelmoschus* has been completely neglected in the literature and the genome size values presented in this study are the first estimates for the genus.

The relative nuclear DNA content was estimated in fifteen genotypes of *Abelmoschus esculentus*. Ploidy level has traditionally been determined based on chromosome counts. Recent studies have estimated ploidy level based on assessments of nuclear DNA content using flow cytometry (Marhold et al., 2010). Flow cytometry is a relatively convenient and rapid method compared with chromosome counts (Suda et al., 2007).
There was a variation in nuclear DNA content among *Abelmoschus esculentus* genotypes. Differences in genome size are known to 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).

The variation in nuclear DNA content could be shown between the Turkish genotype in one side and the other genotypes in the other side. This variation has been in accordance with (Rayburn and Auger, 1990) who found a significant positive correlation between genome size of corn and altitude, suggesting that corn follows the trend of increasing DNA content with increasing altitude.

Kron *et al.* (2007) revealed that the individuals of *Eryngium duriaei* from higher altitudes presented a significantly higher genome size than those belonging to populations from lower altitudes.

5. CONCLUSION

In conclusion, this work contributed with important basic scientific knowledge on genome size in the cytologically complex genus *Abelmoschus*. Indeed further research should be conducted to find the accurate chromosome count and to link the chromosome number with the nuclear DNA content as a first step for starting breeding program of okra.

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