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RESEARCH ARTICLE

DNA content, ploidy level determination and genetic variations of okra (*Abelmoschus esculentus* L.) genotypes

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Abstract

Okra (*Abelmoschus esculentus* L.) is a minor small crop in Jordan; it has attracted a lot of attention as a substitute for conventional vegetables throughout the world. There are conflicting reports about chromosome numbers in this species. To determine the ploidy level of different okra genotypes, okra root tips were treated with HCl maceration, enzymatic maceration, and Carmine acid squashing. Treating cells with HCl didn't macerate the cell in a way that enables chromosome count. The enzymatic treatment combination showed no significant effect on cell maceration. Carmine's acetic acid squashing method was able to digest the cells but in a way that all chromosomes from neighboring cells gathered, making it difficult to count them from each cell. Flow cytometry as an alternative way to assess okra ploidy, was considered as an option. The genome size of okra ranged from 4.11 pg 2C in genotype 43 to 6.27 pg 2C in genotype 30.

Keywords: Okra, landraces, chromosome preparation, ploidy level, flow cytometry

Introduction

Cultivated okra [*Abelmoschus esculentus* (L.) Moench], is an important vegetable crop cultivated in tropical, subtropical and mild temperate parts of the world. Major okra-growing regions are part of temperate Asia, southern Europe, Northern Africa, the United States and all parts of the tropics. Okra is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus and the Southern United States (FAOSTAT 2008). Okra fruits are frequently eaten as vegetables in many cultures and are valued for their palatable green seedpods (Huang et al. 2017). In tropical and subtropical regions of the world, it is considered a significant summer and rainy season vegetable crop (Mishra et al. 2015). The okra is usually consumed as a vegetable crop but in countries like Iran, Egypt, Lebanon, Israel, Jordan, Iraq, Greece, Turkey and other parts of the eastern Mediterranean, it is widely used in a thick stew made with vegetables and meat.

Okra is also used in folk medicine, especially because of its diabetes anti-hyperglycemic influence. However, several cell and animal studies have demonstrated that it is quite difficult to separate, evaluate and further test the active components due to the considerable mucilage content (Minh 2023; Huang et al. 2017). Many studies showed significant differences in the number of chromosomes and the degree of ploidy in multiple species within the *Abelmoschus* genus (Kumari et al. 2017; Charrier 1984). The plant's phenotypic expression is regulated primarily by its genetic makeup and association with the environment. In addition, multiple gene behavior or dominance and epistasis (non-allelic interaction) regulate the genotype of the plant (Mishra et al. 2015). *A. angulosus* possesses the fewest chromosomes, 2n = 56, in the okra genus (Ford 1938), while *A. caillie* has the largest number of chromosomes in the genus 2n = 200 (Singh and Bhatnagar 1975; Siemonsma 1982). The cultivated okra species have 130 somatic chromosome numbers (Joshi and Hardas 1956; Datta and Nuag 1968). Knowledge of genetic heterogeneity and interrelationships between traits is expected to enhance yield and other characteristics. Any crop improvement is proportional to the extent of the genetic diversity in the

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germplasm(Mishra et al. 2015). In plant breeding, the most crucial factor is genetic variability/diversity, which is a main method used to cope with the ever-growing demand on food supply from an increasing world population (Kumari et al. 2017). Breeders must be knowledgeable about the kind and degree of genetic divergence in order to select potential parents for deliberate hybridization in heterosis breeding and for the advantage of transgressive segregations (Kumari et al. 2017).

Significant features that contribute to understanding the link between genotypes with tight genetic relationships are the number of chromosomes and the degree of ploidy. Recent stats suggest that 25 to 30% of flowering plants are polyploids (Lomonosova et al. 2020). The range in taxonomic distance between different okra genotypes was found to vary from 0.23 to 1.0. Each cluster had accessions and genotypes from several geographic locations with varying climatic conditions and occasionally even from other continents (Salameh 2014b). The assessment of okra ploidy by flow cytometry may be an option (Li et al. 2020). Attempts have been made to determine the level of ploidy by morphological features because the development habits of plants with different levels of ploidy can vary. However, this approach has not always proved effective (Yan et al. 2016).

The size of the genome (GS) is an organism's basic biological function and is often calculated by the C value (Li et al. 2020). The C value is a measurement of the amount of DNA in a nuclear genome that has not been duplicated; it is comparable to the number of picograms of DNA in a haploid nucleus (Bolin et al. 2018). C values can be associated with the degree of ploidy and are crucial diagnostic traits of populations with various degrees of ploidy (Bolin et al. 2018). The difference in the size of the intraspecies genome is particularly apparent when the geographical gap is comparatively long or when the climate conditions vary greatly. Techniques for determining genome size, include genome sequencing (Zhang et al. 2012), Flow cytometry (Chu et al. 2018), and Feulgen microdensitometry (Guo et al. 2015). Flow cytometry is a preferred technique to assess the C value of plant DNA because of its ease of use, speed, affordability, and reasonably accurate detection performance (Li et al. 2020). To our knowledge there is scarce information on the okra genome and ploidy level. As a result, the primary goal of the current investigation was to assess the ploidy level of different okra genotypes from Jordan as well as other countries.

Material and methods

To determine the ploidy stage of plant, direct metaphase count on root meristem squash preparations was used. Different landraces have been collected from different parts of Jordan; in addition, some landraces have been kindly supplied by Dr. Duzyaman (More details about the landraces as well as the origin of each landrace are shown in Table 1). For miotic chromosome preparation, different maceration procedures were used.

Enzymatic and HCl maceration

The chromosomal preparation procedure was somewhat enhanced using Sun et al.'s techniques, 2015. Okra seeds were removed from the dried fruits and stored in paper bags, seeds then were soaked in distilled water for 24 hours. The seeds were then rolled in wet paper towels (6 seeds each) and placed upright in plastic container containing water the container was covered loosely and incubated for germination in the dark at 28°C for 5 days. Afterward, root tips (2 cm) for chromosome analysis were collected. Root tips were incubated for 5 to 6 hours at 20 to 25°C in 0.04%, 8-Hydroxychinolin and DMS to enhance the numbers of visible mitotic chromosomes O. Root tips were washed 3-4 times in dd.H_2O for 5 minutes each. Afterward, the roots were fixed in fixative solution (3 parts methyl alcohol and 1 part acetic acid) (Carnoy fluid) and stored at -20°C. The samples were then rinsed three times with dd. H_2O for 5 min each. Washed root tips then treated with enzyme solution consisting of (2.5% cellulose + 2.5% pectolase in 7.5 mM EDTA, 75 mM KCl, pH 4) in a plastic multi-well plate. Water was added to one of the wells to generate a humid atmosphere. Then samples were incubated at 37°C for 50 minutes. With tweezers, the apical meristem was correctly crushed, reduced to about 1 mm, and spread uniformly across the slide. The slides were left to dry, then observed

Table 1. Origin and source of the okra genotypes under study

Genotype	Origin	Source
43	Malaysia	WVC
42	Malaysia	WVC
34	Philippines	WVC
38	USA	WVC
41	Thailand	WVC
40	Yugoslavia	WVC
39	Guatemala	WVC
1	India	WVC
35	USA	WVC
44	Myanmar	WVC
22	Jordan	NARC
27	Jordan	NARC
30	Jordan	NARC

WVC = World Vegetable Centre; + NARC = National Agricultural Research Centre; #The genotype names is unknown as these were supplied as numbers only from World Vegetable Centre and National Agricultural Research centre.

under a microscope, photographed, and counted with digital camera. Roots have been taken from the fixative solution (Carnoy fluid), then washed three times with dd. H_2O and then treated with I N HCl at 60°C for different times (1, 5, 15, 30 minutes).

Carmine acid squashing

Roots were put in a small cup, afterwards the fixative solution was replaced by Carmine acetic acid stain. The samples were stained at room temperature overnight. In the next day, the roots were incubated for at least 5 minutes in 45% acetic acid; the root tips were placed on a clean slide and cut into small pieces. A 45% acetic acid drop was added before covering the sample with a cover slip.

Flow cytometry

The method of flow cytometry preparation was slightly improved by referring to the method of (Salameh, 2014b). The kit with Cystain UV precise P kit from Partec, was used to perform flow cytometric analysis on a suspension of intact nuclei from landrace root tissue. Per accession, three individuals were chopped in 400 µL extraction buffer, then the tissues were well mixed with the addition of 1600 L of staining buffer. The suspension was processed through Celltrics-filter (~ 50 µm) in a cuvette. Partec Cell Analyzer II was used to measure the DNA content of each sample. Estimates of nuclear DNA content were only taken into consideration as a quality check when the coefficient of variance of G_0/G_1 peaks (CV_{peaks}) was below 5%. Samples with greater CV_{peaks} values were deleted, and a fresh sample was created. The internal reference standard was created using maize KYS nuclei. A DNA Index (DI) was used to express each plant's relative DNA content according to the formula:

$$\text{Sample 2C DNA content} = \frac{\text{Sample G1 peak mean}}{\text{Standard G1 peak mean}} \times \text{standard 2C DNA content (pg DNA)}$$

Results and discussion

Treating the roots with 1 N HCl did not macerate the tissue to facilitate chromosome counting (Fig. 1). Incubating the roots in HCl for 1 minute failed to macerate the root cells. Increasing the incubation period to 5, 15 and 30 minutes showed no effect on root cell maceration (Fig. 1).

Enzymatic maceration

Different enzyme combinations have been tested (2.5% cellulose + 2.5% pectolase), and (2.5% cellulose + 2.5% pectolase + 2.5% cytohelicase) but neither enzyme combination nor the incubation period was able to macerate the roots of okra (Fig. 2).

Using Carmine acetic acid stain squashing method, we could see okra chromosomes outside the cell (Fig. 3). Unfortunately, this method macerated many cells simultaneously, making it difficult to count the chromosome numbers of individual cells.

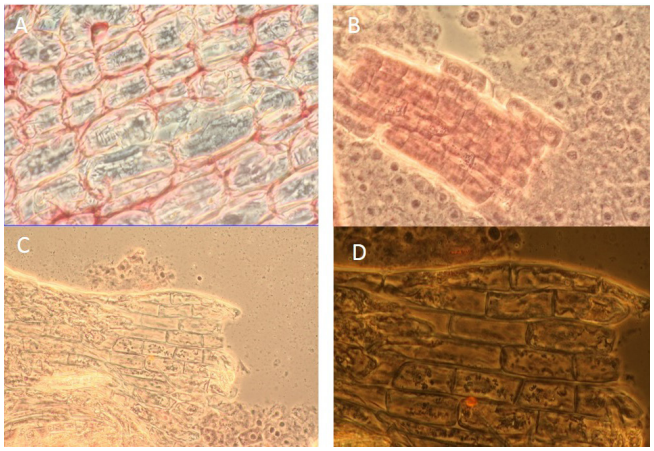

Fig. 1. Okra root tips maceration with HCl for 1 minute (A), 5 minutes (B), 15 minutes (C), and 30 minutes (D)

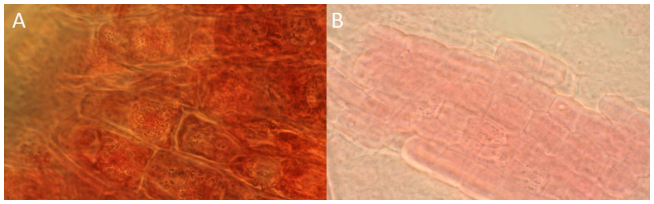

Fig. 2. Enzymatic maceration using (A): 2.5% cellulose + 2.5% pectolase and (B): 2.5% cellulose + 2.5% pectolase + 2.5% cytohelicase

Ploidy determination by direct count chromosomes in metaphase on squash preparations of root meristem is a labor-intensive method requiring the availability of samples collected in the fruiting stage (Lomonosova et al. 2020). Furthermore, it is not always possible to germinate seeds and "pick up" the ideal meristem metaphase stage appropriate for counting chromosomes and estimating the ploid level (Lomonosova et al. 2020). Therefore, karyological data are often restricted by chromosome numbers obtained in a few species' samples, which do not allow the true value of polyploidy in taxa evolution to be estimated. Because it is difficult and time-consuming, traditional chromosomal preparation of okra was not suited for large volumes of material to establish the chromosome number and ploidy level (Lomonosova et al. 2020).

Flow cytometry

It is apparent from Fig. 4 that the tested okra genotypes prevail significant variation in genome size regardless of the morphological similarity. These variations could be

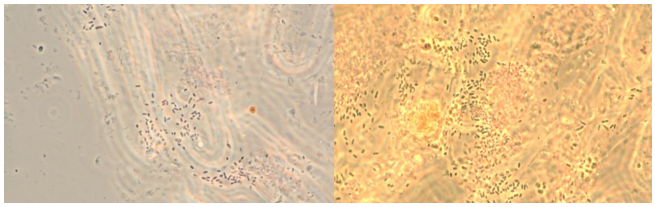

Fig. 3. Okra chromosomes after being macerated with Carmine acetic aid

Table 2. Nuclear DNA content estimates in Abelmoschus esculentus from Jordan and different geographical regions

Genotype	Origin	Ploidy level	2C value (pg), FC	No. of Mbp
43	Malaysia	2x	4.11	4019
42	Malaysia	2x	4.46	4365
34	Philippines	2x	4.55	4451
38	USA	2x	4.73	4624
41	Thailand	2x	4.73	4624
40	Yugoslavia	2x	4.77	4667
39	Guatemala	2x	4.9	4797
1	India	2x	5.04	4926
35	USA	2x	5.08	4970
44	Myanmar	2x	5.13	5013
22	Jordan	2x	5.61	5488
KYS	Maize genotype	2x	5.7	5575
27	Jordan	2x	5.83	5704
30	Jordan	2x	6.27	6136

attributed to the different geographical regions. In the present study, the genome size of okra genotypes ranged from 4.11 pg 2C-1 in genotype 43 (Malaysian origin) to 6.27 pg 2C-1 in genotype 30 (Jordanian genotype). In contrast, the 2C genome size in Mbp ranged from 4019 to 5704 Mbp in genotypes 43 and 30, respectively (Table 2). Fig. 5 shows that okra one peak indicating that it has 2n ploidy level. In a comparative genomic analysis using chromosome-scale, Wang et al. (2023) presented that okra genome size to be 1.19 Gb.

Canonical variable analysis

The canonical variable identifier classified the tested genotypes into 3 groups, the first group includes the genotypes (39, 41, 38, 40, 1, 35 and 44), while group 2 includes (43, 42 and 34), group 3 includes (22, KYS, 27 and 30). It was interesting to note that the Jordanian genotypes gathered in one group while the other genotypes from different continents were distributed into the other groups. It is also worthy to note that group one included genotypes from different continents while group 2 includes genotypes

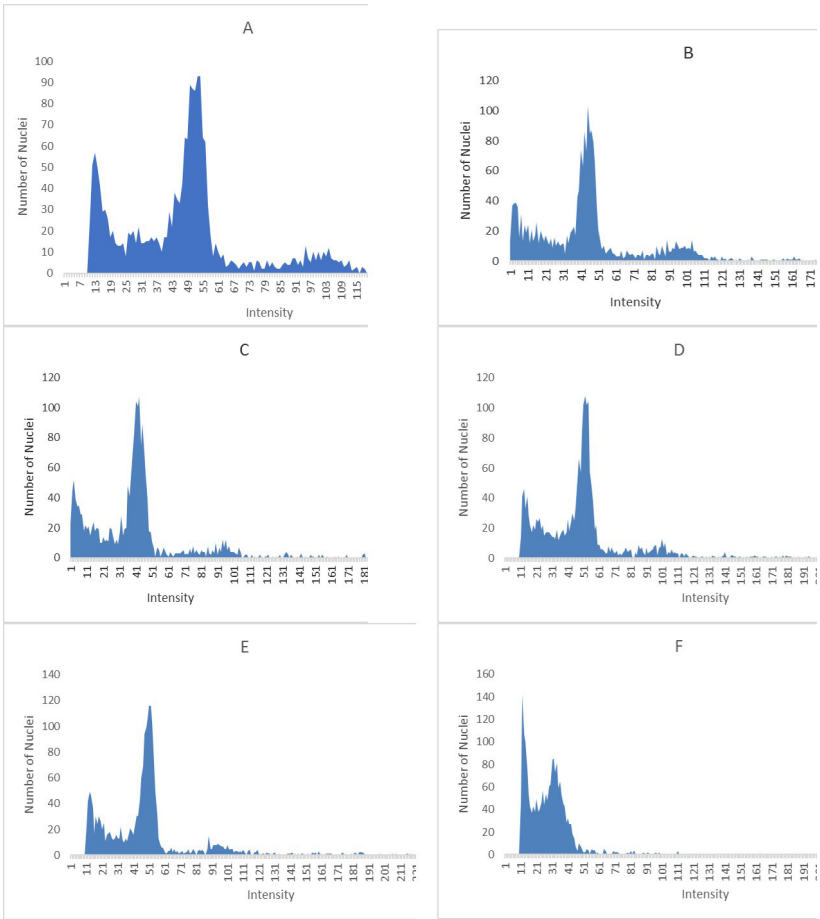

Fig. 4. Different types of inflorescence developmental variation are displayed in a histogram of the relative nuclear DNA content of the okra genotypes. The vertical axis displays the number of nuclei, while the fluoresce represents the relative DNA concentration. (a) genotype numer1 (Malaysia), (b) genotype number 6 (Philippines), (c) genotype number 11 (USA), (d) genotype 9 (Yugoslavia), (e) genotype number 20 (Myanmar), and (f) genotype number 5 (Jordan)

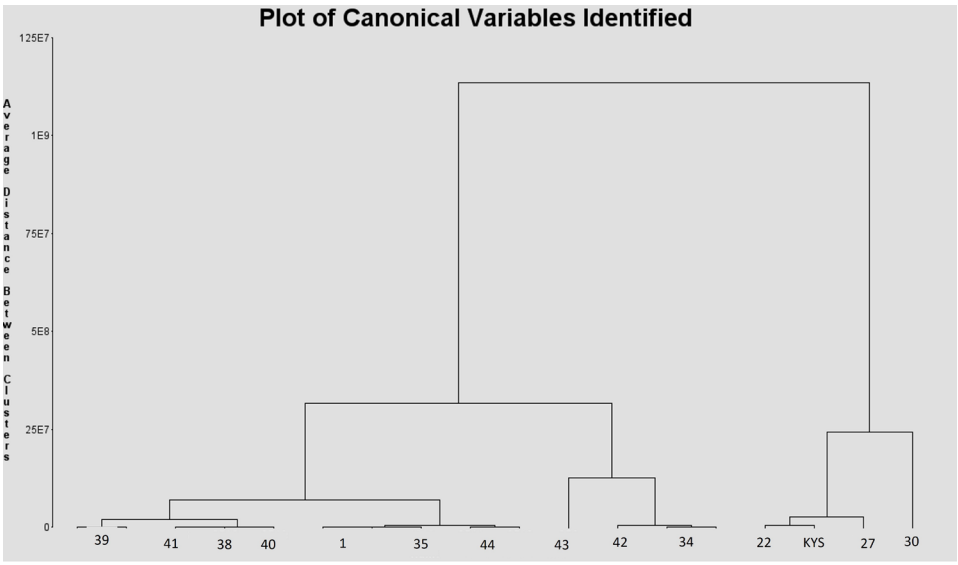

Fig. 5. Plot canonical variable analysis of 14 okra genotypes from different geographical regions

from the same continent and are from two neighboring countries.

The assessment of a species' DNA content offers valuable understanding for cell and molecular genetics research, as well as fundamental evidence for plant genomics research and genetic evolution (Yan et al. 2016). The standard approach for determining the degree of ploidy is to count the number of chromosomes that have proved reliable in a single plant cell and have been used for various plants (Wang et al. 2009). Chromosome counting, however, is a time-consuming and labor-intensive operation, particularly when analyzing a large collection of samples. In addition to that, maceration technique needs to be optimized for either enzymatic maceration or Carmine acidic acid to a point at which chromosomes could be counted easily for each separate, single cell. Flow cytometry is the newest method for determining the level of ploidy, which appears to be a powerful technique. This approach combines the benefits of microscopy and biochemical analysis (Wang et al. 2009). Conventional chromosome preparation was not ideal for large volumes of material since it is difficult and time-consuming, making it unsuitable for determining the chromosomal number and ploidy level of okra accessions. As a result, we used flow cytometry to assess the ploidy of *Abelomoschus esculentus* (Li et al. 2020).

The variation in DNA content between the different genotypes was also reported by (Salameh 2014a) in which he indicated that individuals at high elevations showed a slightly higher genome size than those belonging to individuals from different elevations. In another study, Salameh (2014b) indicated six similarly clusters in okra genotypes using AFLP markers in which the genotypes from the Philippines, Malaysia, USA and Thailand were in a same cluster were some other genotypes from Yugoslavia, Jordan, Guatemala and India were in a different cluster. These results indicate the great variation within okra genotypes around the world which could be a powerful tool for breeders and geneticists to use this diversity to improve the different agronomic traits with high economical value in each country. The flow cytometry analysis as well as the plot canonical analysis revealed a great variation between the different okra genotypes. et al. (2018) indicated that breeding strategies require knowledge of genetic similarities and diversity among okra genotypes. In another study, Kumari et al. (2017) found large range of variability for all traits studied in which PCV was greater than the GCV, indicating the importance of environmental influences.

The present results showed that great genetic variation exist between the different genotypes and that the genotypes are related to their geographical area. Group 2 and 3 contain genotypes from the same area (e.g., Southeast Asia and Jordan, respectively), while group 1 includes a wide range of variation as it contains genotypes from different geographical regions (e.g., Central America, North America, South Asia and Southeast and Central Europe).

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References

Anchor J. F., Hartwig C. L., Schafran,P., Komarnytsky S., Carolina N. and Carolina N. 2018. Application of DNA Flow Cytometry to Aid Species Delimitation in Isoetes. Castanea, **83**(1): 38–47. https://doi.org/10.2179/16-120

Charrier A. 1984. Genetic Resources of the Genus *Abelmoschus*

Med. (Okra). 1st Edn., IBPGR, Rome, pp: 61.

Chu Z., Wen J., Yang Y., Nie Z. and Meng, Y. 2018. Genome size variation and evolution in the grape family Vitaceae. J. Syst. Evol., **56**(4): 273–282. https://doi.org/10.1111/jse.12310

Datta P.C and Naug A. 1968. A few strains of *Abelmoschus esculentus* (L) Moench. Their karyological in relation to phylogeny and organ development, Beitr, Biol, Pflanzen., 45. pp 113-126.

G. A., Khaled A. G. A., Obiadalla-Ali H. A. and Ahmed, A. Y. M. 2018. Issr markers linked to agronomic traits in okra. Int. J. Mod. Agric., **7**(1): 9–15.

Ford C.E. 1938. A contribution to a cytogenetical survey of the Malvaceae. Genetica, **20**: 431-452.FAOSTAT. 2008. (http://fao.org).

Huang C., Wang C., Lee, Y. and Peng C. 2017. Active subfractions of *Abelmoschus esculentus* substantially prevent free fatty acid-induced β cell apoptosis via inhibiting dipeptidyl peptidase-4. PLoS ONE, **12**(7): 1–17.

Joshi A.B. and Hardas M.W. 1956. Alloploid nature of Okra, *Abelmoschus esculentus* (L.) Moench. Nature, **178:** 1190.

Kumari M., Solankey S. S., Akhtar S. and Neha P. 2017. Assessment of genetic variability and character association in okra genotypes for yield and contributing characters. J. Appl. Nat. Sci., **9**(3): 1825–1830.

Li W., Liu L., Wang Y., Fan G., Zhang S. and Wang Y. 2020. Determination of genome size and chromosome ploidy of selected taxa from *Prunus armeniaca* by flow cytometry. Sci. Hortic, **261**: 108987.

Lomonosova M.N., An'kova T. V. , Voronkova M. S., Korolyuk E. A., Banaev E. V. and Skaptsov M. V. 2020. Ploidy level of the representatives of Chenopodiaceae based on genome size and chromosome numbers. Turczaninowia, **23**(1): 24–31. https://doi.org/10.14258/turczaninowia.23.1.3

Minh N. N. 2023. Investigation and prediction of the extensional viscosity of okra mucilage using the Giesekus model. Biorheology, **59**(1–2): 29–42.

Mishra A., Mishra H., Senapati N. and Tripathy P.2015. Genetic variability and correlation studies in Okra (*Abelmoschus esculentus* (L.) Monech). EJPB, **6**(3): 866–869.

Salameh N. M. 2014a. Flow cytometric analysis of Nuclear DNA between okra landraces (*Abelmoschus esculentus* L.). Am. J. Agric. Biol. Sci., **9**(2): 245–250. https://doi.org/10.3844/ajabssp.2014.245.250

Salameh N. M. 2014b. Genetic diversity of okra (*Abelmoschus esculentus* L.) landraces from different agro-ecological regions revealed by amplified fragment length polymorphism analysis. Am. J. Appl. Sci., **11**(7): 1157–1163. https://doi.org/10.3844/ajassp.2014.1157.1163

Siemonsmo J.S. 1982. West African okra. morphological and cytological indications for the existence of a natural amphiploid of *Abelmoschus esculentus* (L.) Moench and *A. Manihot* (L.) Medikus. Euphytica, **31**(1): 241-52.

Singh H.B. and Bhatnagar A. 1975. Chromosome number in an okra from Ghana. Indian J. Genet. Plant Breed., **36**: 26-27.

Wang Y., Bigelow C. A. and Jiang Y. 2009. Ploidy level and DNA content of perennial ryegrass germplasm as determined by flow cytometry. Hortic. Sci., **44**(7): 2049–2052.

Wang R., Li W., He Q., Zhang H., Wang M., Zheng X., Liu Z., Wang Y., Du C., Du H. and Xing L.2023. The genome of okra (*Abelmoschus esculentus*) provides insights into its genome evolution and high nutrient content. Hortic Res., **2:** 10(8): uhad120. doi: 10.1093/hr/uhad120. PMID: 37554345; PMCID: PMC10405168.

Yan J., Zhang J., Sun K., Chang D., Bai S. and Shen Y. 2016. Ploidy level and DNA content of *Erianthus arundinaceus* as determined by flow cytometry and the association with biological characteristics. PLoS ONE, **11**(3): 1–15. https://doi.org/10.1371/journal.pone.0151948

Zhang Q., Chen W., Sun L., Zhao F., Huang B., Yang W., Tao Y., Wang J., Yuan Z., Fan G., Xing Z., Han C., Pan H., Zhong X., Shi W., Liang X., Du D., Sun F., Xu Z. and Zheng Z. 2012. The genome of *Prunus mume*. Nat. Commun., **3:** 1318. https://doi.org/10.1038/ncomms2290