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Nuclear DNA Content, Ploidy Level, Chromosome Number in Turkish Okra Landraces

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ABSTRACT

Molecular studies show that okra germplasm harbour narrow genetic diversity despite certain level of phenotypic variation in Türkiye. However, there is a gap in the information on the cytogenetics of Turkish okra genotypes. Studies on the cytogenetics and ploidy level may provide further information on the genetic diversity of Turkish okra germplasm. This study, therefore, investigated nuclear DNA content, ploidy level and chromosome number of 26 okra landraces and 3 commercial cultivars (Akköy-41, Kabaklı-11 and Marmara-1). 2C nuclear DNA content varied from 3.05 to 3.20 pg with mean 2C values ranging between 3.11 and 3.18. The variation in nuclear DNA content was, however, statistically insignificant. Okra had a high number of chromosomes with very small sizes. The chromosome number of the plants investigated in the study was determined to be 2n (10x)= 128±2. Based on these results, the genotypes investigated are probably allodehaploid with some extra chromosomes and B chromosomes. In conclusion, the Okra germplasm has, therefore, a narrow base of genetic diversity in the germplasm pool, which may limit the success of future breeding programs. Suggestions were discussed to enhance genetic diversity in the germplasm for more effective breeding programs.

Key Words: *Germplasm, Diversity, Cytogenetic, Flow cytometry, Abelmoschus esculentus.*

INTRODUCTION

Okra [(*Abelmoschus esculentus* (L.) Moench.)] is cultivated in tropical, subtropical, and warm temperature regions of the world (Kumar *et al*., 2013). Okra is grown on 2.8 million ha and produced around 11 million tons globally, most of the production being in Asia and

Africa (FAO, 2024). In Türkiye, okra is grown on 4554 ha with an annual production of 30,484 tons (FAO, 2024). The crop is mainly grown for its fresh and dried fruits as a vegetable, which is rich in cellulose, hemicellulose, proteins, vitamins, and minerals (Kumar *et al.*, 2013). The low levels of carbohydrates, calories and fat in the fruits make okra an ideal diet food (Stawski *et al.*, 2021). Its stems are also a rich source of valuable fibers for textile industry (Stawski *et al.*, 2021). Seeds contain 20-24% proteins and 13-22% good quality edible oil having high levels of unsaturated linoleic acid (Kumar *et al.*, 2013; Anwar et al., 2020). Global warming conditions experienced currently necessitate tolerant cultivars to abiotic stress factors. Okra has a robust plant structure and greater tolerance to drought and high temperatures (Benchasri, 2012; Dhankar *et al.*, 2013; Singh *et al.*, 2023). Development of climate resilient cultivars may lessen the effects of climate change and compensate projected yield losses (Onyeneke *et al.*, 2023).

Successful breeding requires a rich variation in the gene pool available. Okra is a largely self-pollinated species, but variation is present due to variable level of cross pollination depending on bee presence (Singh *et al.*, 2023). Wild species of okra are present in Nile Valley and Ethiopia (Yıldız *et al.*, 2015). This species is regarded as an allopolyploid derived from the regular polyploid series (Nieuwenhuis *et al.*, 2023). With chromosome numbers ranging between 72 and 144, diploid and tetraploid genotypes are reported (Nieuwenhuis *et al.*, 2023).

Morphological similarities between Turkish Okra accessions and African okra germplasm indicate that Turkish okra landraces were most likely derived from African Continent (Düzyaman, 2009). Okra genotypes in Greece were exclusively derived from Turkish landraces (Kyriakopoulou *et al.*, 2014; Koutsos *et al.*, 2000). Evaluation of genetic diversity shows that there is phenotypic and agro-morphological variation in the germplasm of landraces grown in Anatolia (Düzyaman, 2005; Yıldız *et al.*, 2015; Yıldız *et al.*, 2016; Örkçü, 2016; Kantar *et al.*, 2021). Molecular studies with DNA markers indicate, however, that Turkish okra germplasm base contains narrow genetic diversity at the molecular level (Vural *et al.*, 2000; Düzyaman, 2005; Gulsen *et al.*, 2007; Kyriakopoulou *et al.*, 2014; Yıldız *et al.*, 2015; Kantar *et al.*, 2021). Selection pressure over a long period of time probably led to narrow genetic background in Turkish okra germplasm (Yıldız *et al.*, 2015). There are several studies investigating morphological and molecular diversity in Turkish okra germplasm. However, no detailed information is available on the variation in nuclear DNA content, ploidy level and chromosome number. This study, therefore, investigated nuclear

DNA content, chromosome number and ploidy level in common okra landraces and cultivars grown in Türkiye with flow cytometry.

MATERIALS AND METHODS

Materials

A collection of 26 genotypes were evaluated for nuclear DNA content and ploidy level. Of the accessions, 20 okra genotypes were locally grown ecotypes, which were previously collected from farmers from different locations in mostly Western Türkiye (Table 1). Two accessions were breeders lines acquired from Ataturk Central Horticultural Research Institute (ACHRS), Yalova, Türkiye. Nationally registered cultivars (Akköy-41, Kabaklı-11 and Marmara-1) obtained from ACHRS, and one standard commercial type (STD-20) were also included in the experiment. Seeds derived from a single fruit from each genotype were sown in 6 m rows with 1 m inter and 20 cm intra row spacing in soil on 06.03.2019 under greenhouse conditions at the Experimental Farm of Faculty of Agriculture, Akdeniz University, Türkiye. The experimental soil of clay loam texture was a slightly alkali (pH= 7.6) with a lime content of 17.7% and organic matter content of 2.1%. Total N content was 0.09% with P_2O_5 content of 0.0013%, K_2O content of 0.19%, $CaCO_3$ content of 0.4% and content of 0.09%. Mn, Zn, Cu and Fe contents were 2.67, 0.47, 0.25 and 1.2 mg/kg respectively.

Table 1. Okra genotypes and cultivars investigated in the flow cytometry studies.

Lines	Name	Plant degree of branching	Plant height (cm)	Stem Diameter (mm)	Stem colour	Fruit Diameter (mm)	Fruit Colour
STD-20[a]	Sultani	weak	65	7.6	green	14.3	green
MGL-10	Landrace	medium	90	7.1	green	13.9	green
GAN-21	Landrace	weak	57	6.9	green	16.7	green
AYD-13	Landrace	weak	56	4.6	green	18.8	green
MGL-7	Landrace	weak	40	5.4	green	15.0	green
MGL-6	Local Mixed	weak	59	5.5	green	12.0	red
GAN-19	Landrace	weak	40	6.9	green	16.1	green
MGL-3	Landrace	weak	60	6.4	green	15.5	red
MGL-4	Local red	weak	70	6.2	red	13.9	red
MGL-2	Landrace	weak	60	7.4	green	16.4	green
USK-17	Sultani	weak	90	9.4	green	15.0	green
UİS-15	Landrace	weak	105	7.9	green	12.2	green
UİS-16	Landrace	weak	94	8.0	green	30.4	green
MGL-5	Local yellow	weak	87	9.3	green	15.4	green
AYD-11	Landrace	weak	64	6.6	green	18.1	green
AYD-12	Landrace	weak	73	6.5	green	19.3	green
AYD-18	Tastaban	weak	56	7.2	green	26.7	green

Marmara 1	ACHRS[c]	medium	75	7.3	green	25.0	green
MGL-9	Landrace	medium	120	9.4	red	15.1	green
Kabaklı-11	ACHRS[c]	medium	107	8.1	green	17.1	green
YLV-22	BAL[b]	medium	64	7.1	green	32.9	green
YLV-23	BAL[b]	weak	74	4.7	green	16.5	green
BKL-1	Local	weak	100	7.6	green	10.6	green
MGL-14	Endeze	weak	56	8.9	green	15.6	green
Akköy-41	ACHRS[c]	weak	50	5.9	green	15.8	green
MGL-8	Landrace	weak	60	6.2	green	15.7	green

[a] Sunagri, [b] BAL Breeders Advanced Line. C Registered cultivars from ACHRS Ataturk Central Horticultural Research Institute, Yalova, Türkiye.

Method

Nuclear DNA content analysis by flow cytometry

Fresh leaf tissues of young and healthy plants grown in greenhouse were used for nuclear DNA content analysis. Three individual plants were analysed for each land race, genotype, and cultivar. DAPI (4'-6-diamidino-2-phenylindole) was used as fluorochrome. Okra samples and leaf sections of tomato (Lycopersicon esculentum Mill cv. H-2274) (Arumuganathan and Earle, 1991) as an internal standard were simultaneously chopped, vortexed and stained using the 'CyStain UV Precise P' nuclei extraction and staining kit (Partec GmbH, Munster) according to the manufacturer's instructions. Samples were analysed using a Partec CyFlow Space flow cytometer (Munster, Germany). The absolute DNA contents of okra landraces were calculated based on the ratios of the G1 peak means of sample and tomato standard (nuclear DNA content of 2 pg/2C) (Figure 1).

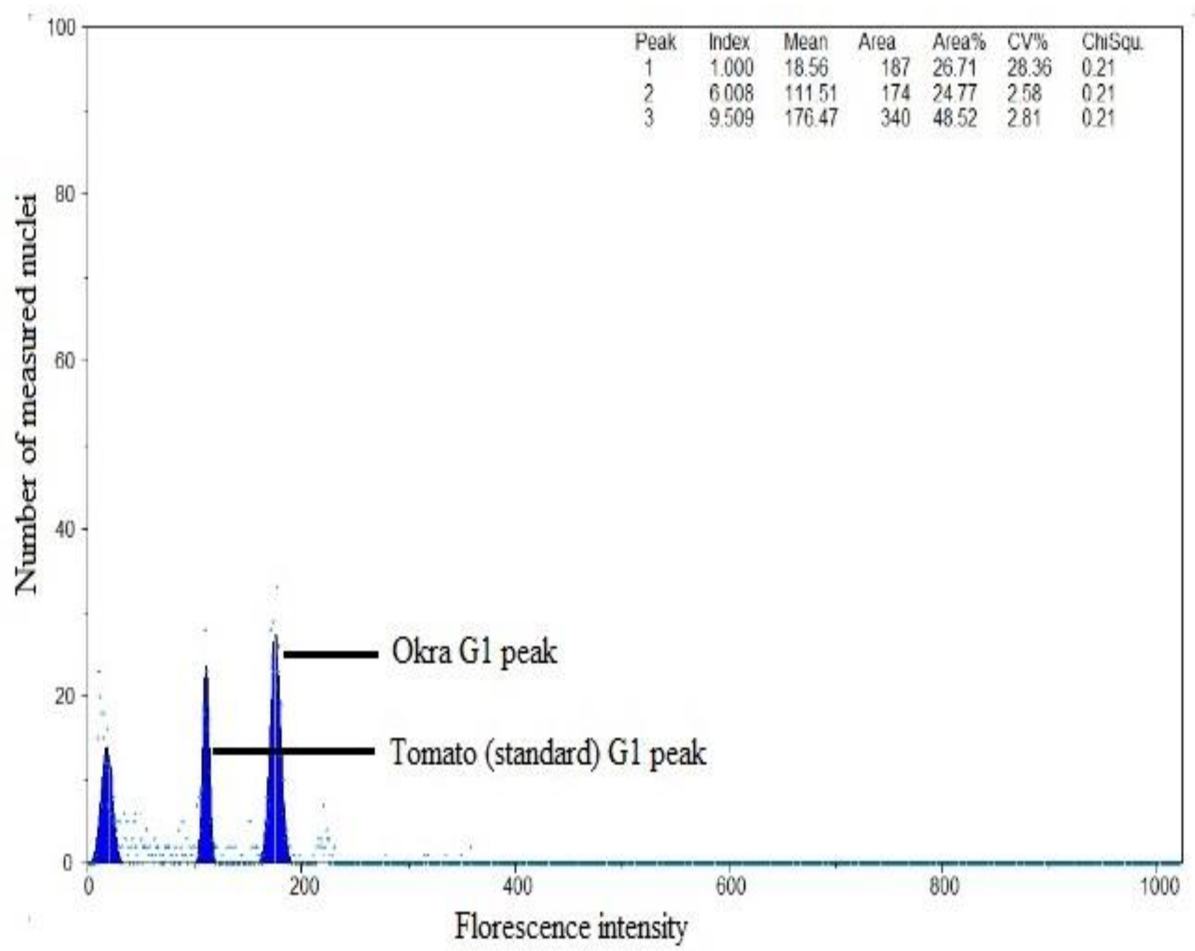

Figure 1. Relative florescence intensity of the G1 peaks of tomato (left) and okra (right) plants in flow cytometry analysis.

Chromosome counting

Cells with good chromosome distribution were selected to determine chromosome numbers. Cytological preparations were made using meristem tissues from actively growing root tips. Root tips were harvested from a few weeks old seedlings grown in pots and treated in 0.05% colchicine at room temperature for 3 h followed by fixation in ethanol:acetic acid (3:1, v/v). Cytological preparations were performed as described by Jenkins & Hasterok (2007). Firstly, the fixed roots were washed in 0.01M citric acid-sodium citrate buffer (pH 4.8, 5 min., 4 times) and then fragmentized enzymatically at 37 °C in a mixture comprising 20% (v/v) pectinase (Sigma), 1% (w/v) cellulase (Calbiochem), and 1% (w/v) cellulase 'Onozuka R-10' (Serva) for 2 hours. After this process, the meristem was transferred to a slide in a drop of 45% acetic acid; then, a coverslip was placed on the slide and squashed. The coverslips were removed from the slides by a razor after storing them in the freezer (-80 °C) for a few hours.

The slides were air-dried and stained by DAPI. Images were captured by using a fluorescence microscope with CCD digital camera (SPOT RT). Chromosome number of only two plants were counted in the study since all the landraces had similar DNA content.

Data Analysis

Data were analysed using SPSS statistical package. A simple statistical procedure of confidence intervals was used to compare mean DNA content of the strains (Steel and Torrie, 1960). A confidence interval was calculated for each mean by the following equation: P (X1-t 0.05 Sx< μ< X1+ t 0.05Sx)= 0.95 where t 0.05 is the "t" statistic and s= s/n1/2 where n is the number of plants analysed for a strain and s is their standard deviation. Accession means with overlapping confidence intervals were assumed to be similar. This is equivalent to conducting a simple *t* test to compare specific means (Steel and Torrie, 1960).

RESULTS

A rare problem was encountered in the isolation of the nucleus from okra leaves during sample preparation for nuclear DNA content analysis by flow cytometry. The viscosity of the homogenate increased enormously after chopping the leaf tissues in the nucleus isolation buffer, which was difficult to pipet and filter. Vortex application of viscose jelly like homogenate simply produced easy- to-work liquid. The rest of the protocol was the same as explained in the manual of the kit. This minor change made it possible to determine nuclear DNA content of okra plants by flow cytometer. Based on those results, 2C nuclear DNA content of the okra landraces and cultivars analysed in the study varied from 3.05 to 3.20 pg while their mean 2C values, as an average of 3 individuals, varied between 3.11 and 3.18 (Table 2). The differences were not statistically significant (Table 2). Based on the results obtained from this study, all the landraces and cultivars had very similar nuclear DNA content indicating that they had same ploidy level. Okra had a high number of chromosomes with very small sizes (Figure 2), which made it impossible to determine their exact number. Therefore, the chromosome number of the plants investigated in the study was determined as around 128±2 (Figure 2).

Table 2. Nuclear DNA content of the okra landraces and cultivars investigated (2C/pg).

Lines	Plant 1	Plant 2	Plant 3	Mean	SD	$T*S_x$	Confidence	Interval
							lower	upper
BLK-1	3.12	3.19	3.09	3.13	0.051	0.042	3.09	3.17
MGL-2	3.17	3.20	3.13	3.16	0.035	0.029	3.13	3.19
MGL-3	3.20	3.13	3.15	3.16	0.036	0.029	3.13	3.18
MGL-4	3.14	3.10	3.16	3.13	0.031	0.025	3.10	3.15
MGL-5	3.13	3.21	3.16	3.16	0.040	0.033	3.13	3.20
MGL-6	3.13	3.10	3.19	3.14	0.046	0.037	3.10	3.17
MGL-7	3.10	3.17	3.12	3.12	0.025	0.021	3.10	3.15
MGL-8	3.05	3.13	3.17	3.11	0.061	0.050	3.06	3.16
MGL-9	3.17	3.13	3.12	3.14	0.026	0.022	3.11	3.16
MGL-10	3.17	3.20	3.11	3.16	0.046	0.037	3.12	3.19
AYD-11	3.14	3.18	3.08	3.13	0.050	0.041	3.09	3.17
AYD-12	3.19	3.14	3.16	3.16	0.025	0.021	3.14	3.18
AYD-13	3.13	3.17	3.14	3.14	0.021	0.017	3.13	3.16
MGL-14	3.11	3.13	3.18	3.14	0.036	0.029	3.11	3.16
UIS-15	3.17	3.20	3.12	3.16	0.040	0.033	3.13	3.19
UIS-16	3.19	3.11	3.16	3.15	0.040	0.033	3.12	3.18
USK-17	3.16	3.21	3.10	3.15	0.055	0.045	3.11	3.20
AYD-18	3.19	3.15	3.18	3.17	0.021	0.017	3.15	3.19
GAN-19	3.09	3.16	3.17	3.14	0.044	0.036	3.10	3.17
STD-20	3.18	3.20	3.12	3.16	0.042	0.034	3.13	3.20
GAN-21	3.15	3.12	3.19	3.15	0.035	0.029	3.12	3.18
YLV-22	3.16	3.18	3.13	3.15	0.025	0.021	3.13	3.17
YLV-23	3.20	3.14	3.21	3.18	0.038	0.031	3.15	3.21
Akköy-41	3.19	3.17	3.19	3.18	0.012	0.009	3.17	3.19
Kabaklı-11	3.13	3.14	3.11	3.13	0.020	0.016	3.11	3.15
Marmara 1	3.18	3.11	3.22	3.17	0.056	0.046	3.12	3.21

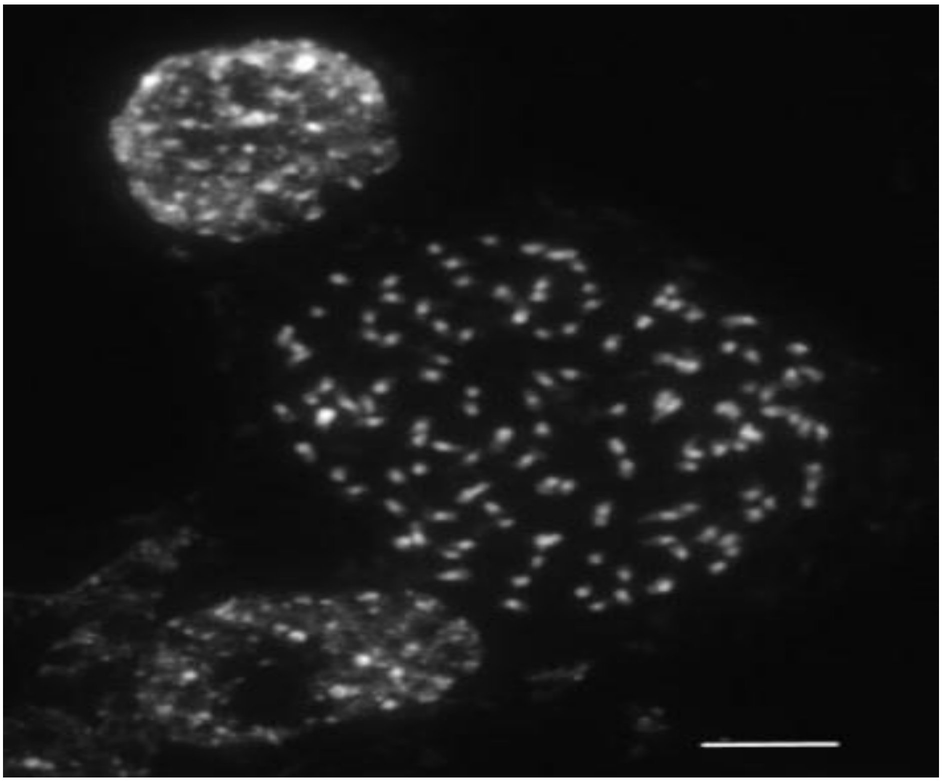

Figure 2. Images of mitotic chromosomes of the okra investigated (Bar is 5 µm).

DISCUSSION

Data presented here show that nuclear DNA content, ploidy level and chromosome number did not vary within the germplasm. All the landraces and cultivars had similar nuclear DNA content and chromosome number as they were off the same ploidy level. Previous studies employing molecular techniques with DNA markers concluded that Turkish okra germplasm had a narrow genetical base and low levels of diversity harboured (Gulsen *et al.*, 2007; Yıldız *et al.*, 2015) in spite of relatively greater morphological diversity (Düzyaman, 2005; Yıldız *et al.*, 2016; Örkçü, 2016; Kantar *et al.*, 2021). Our study filled this information gap by investigating nuclear DNA content and ploidy level. Narrow genetic base in the germplasm pool may decrease the effectiveness of okra breeding programs. Genetic diversity should be increased, therefore, in the germplasm pool to increase success in breeding programs. Employing larger germplasm collections using lines and genotypes with greater genetic distance, avoiding those with common background in hybridisation programs (Yıldız *et al.*, 2015), introducing genotypes from foreign genetic sources in to breeding pool (Yıldız *et al.*, 2015) and enhancing variation by inter-specific hybridisations (Benchasri, 2012; Seth et al., 2016) were suggested for more successful breeding programs. Reciprocal hybridisation of (*Abelmoschus esculentus* (L.) Moench) with other wild okra species produced fertile vigorous plants, which may offer new opportunities for creating recombination and diversity (Benchasri, 2012). Successful attempts were also reported for heterosis breeding in okra (Dhankhar, 2016; Bhatt *et al.*, 2016).

Information on nuclear DNA content of Turkish okra germplasm is quite scarce. Unfortunately, we were able to find only two studies on okra nuclear DNA content (Salameh, 2014; Örkçü, 2016). Örkçü (2016) studied nuclear DNA content of 20 okra landraces including 3 commercial okra cultivars of Akköy-41, Kabaklı-11, and Marmara-1 in Türkiye. Mean 2C nuclear DNA content varied from 2.86 to 3.18 pg among 20 landraces in this study (Örkçü, 2016). The results of this earlier study were quite comparable with the results (3.11 pg-3.18 pg) obtained in our study (Table 1). The small differences between the two studies could be attributed to the different fluorochromes and internal standards used. DAPI was the fluorochrome in the current study while PI was used as fluorochrome by Örkçü (2016). These two fluorochromes had different binding modes to the DNA and therefore they could cause differences to some extent up to %15 (Doležel and Bartos, 2005). The differences in chromatin structure of the standard and sample nuclei can also cause differences in nuclear DNA content measurements (Doležel and Bartos, 2005). Although DAPI binding preferentially to AT rich regions of DNA it was the choice of the fluorochromes since it provided DNA content histograms with much higher resolution than propidium iodide in this specific study.

In the second study, Salameh (2014) investigated nuclear DNA content of 15 Jordanian okra accessions including one accession from Türkiye and reported that the mean 2C nuclear DNA content of okra showed very high variation. Based on the results of this study, the 2C nuclear DNA content of the Jordanian landraces varied from 3.98 to 6.67 pg as the 2C nuclear DNA content of the Turkish landrace (Okra 12) was 17.67 pg. The nuclear DNA content of the okra plants reported in this study was far higher than the results of the current study. In addition, the variation was also too high even if we consider only Jordanian landraces excluding the Turkish landrace (Okra 12). This made us suspect on the quality of the data obtained in the previous study. Unfortunately, demonstrably wrong data have been accumulating in this area especially on genome size variation or genome plasticity in literature. Therefore, we consider the results of this study not reliable. The image of flow histogram presented in the publication also support the low quality of the data. 2C DNA content of maize varies from 5.5 to 6.2 pg due to its special situation (Comertpay, 2019). Based on the results of Salameh, (2014) okra landraces had almost similar DNA content with the maize. But, when we look at the histogram image presented in the publication it looks like the maize had approximately 4 times larger genome than okra. The data presented in the publication could, therefore, not prove unusually high values for okra.

Okra has small but high number of chromosomes. These characteristics make chromosome counting a challenging task and, hence, it was challenging to count the chromosomes precisely. Nevertheless, the chromosome number of okra plants in our study was approximately 2n=128. . Örkçü (2014) also determined the chromosome number of Turkish landraces as 2n=128. Other studies reported chromosome numbers for okra (*A. esculentus*) ranging between 2n=66 and 144 (Kumar *et al.*, 2010; Benchasri, 2012). In a recent study, Nieuwenhuis *et al*., 2023 presented a detailed insight into the complex genome and transcriptome architecture of okra (*A. esculentus* cv. Green Star F1) and its haploid descendant, using cytogenetic characterization of its mitotic cell complements. They reported that 2C DNA amount for the okra plant was at 2.99 pg ±0.01 and chromosomes number was 2n=130. DAPI was also the choice of fluorochrome in their study. They also reported a low genetic diversity in okra after investigating single nucleotide polymorphisms in 11 public okra accessions. Abelmoschus esculentus (usually 2n = 130) is probably an amphidiploids (allotetraploid), derived from *Abelmoschus tuberculatus* Pal & H.B.Singh (2n = 58), a wild species from India, and a species with 2n = 72 chromosomes (possibly *Abelmoschus ficulneus* (L.) Wight & Arn. ex Wight) (Kumar et al., 2013). In the current study, all the material had very similar nuclear DNA content. Considering essential chromosome number of okra as x=12, all the okra plants used in the study can be accepted as allodecaploid with possibility of some extra chromosomes.

CONCLUSIONS

This study investigated nuclear DNA content and ploidy level in Turkish okra germplasm of 26 genotypes. No significant variation was detected in nuclear DNA content, ploidy level and chromosome number. The results presented here confirmed the previous studies that okra germplasm has a narrow base of genetic diversity in Turkish germplasm pool, which may limit the success of breeding programs.

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