

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 $2n (10x) = 128 \pm 2$. 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

37 Africa (FAO, 2024). In Türkiye, okra is grown on 4554 ha with an annual production of
38 30,484 tons (FAO, 2024). The crop is mainly grown for its fresh and dried fruits as a
39 vegetable, which is rich in cellulose, hemicellulose, proteins, vitamins, and minerals (Kumar
40 *et al.*, 2013). The low levels of carbohydrates, calories and fat in the fruits make okra an ideal
41 diet food (Stawski *et al.*, 2021). Its stems are also a rich source of valuable fibers for textile
42 industry (Stawski *et al.*, 2021). Seeds contain 20-24% proteins and 13-22% good quality
43 edible oil having high levels of unsaturated linoleic acid (Kumar *et al.*, 2013; Anwar *et al.*,
44 2020). Global warming conditions experienced currently necessitate tolerant cultivars to
45 abiotic stress factors. Okra has a robust plant structure and greater tolerance to drought and
46 high temperatures (Benchasri, 2012; Dhankar *et al.*, 2013; Singh *et al.*, 2023). Development
47 of climate resilient cultivars may lessen the effects of climate change and compensate
48 projected yield losses (Onyeneke *et al.*, 2023).

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

56 Morphological similarities between Turkish Okra accessions and African okra germplasm
57 indicate that Turkish okra landraces were most likely derived from African Continent
58 (Düzyaman, 2009). Okra genotypes in Greece were exclusively derived from Turkish
59 landraces (Kyriakopoulou *et al.*, 2014; Koutsos *et al.*, 2000). Evaluation of genetic diversity
60 shows that there is phenotypic and agro-morphological variation in the germplasm of
61 landraces grown in Anatolia (Düzyaman, 2005; Yıldız *et al.*, 2015; Yıldız *et al.*, 2016; Örkçü,
62 2016; Kantar *et al.*, 2021). Molecular studies with DNA markers indicate, however, that
63 Turkish okra germplasm base contains narrow genetic diversity at the molecular level (Vural
64 *et al.*, 2000; Düzyaman, 2005; Gulsen *et al.*, 2007; Kyriakopoulou *et al.*, 2014; Yıldız *et al.*,
65 2015; Kantar *et al.*, 2021). Selection pressure over a long period of time probably led to
66 narrow genetic background in Turkish okra germplasm (Yıldız *et al.*, 2015). There are
67 several studies investigating morphological and molecular diversity in Turkish okra
68 germplasm. However, no detailed information is available on the variation in nuclear DNA
69 content, ploidy level and chromosome number. This study, therefore, investigated nuclear

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

72
 73 **MATERIALS AND METHODS**

74 **Materials**

75 A collection of 26 genotypes were evaluated for nuclear DNA content and ploidy level. Of
 76 the accessions, 20 okra genotypes were locally grown ecotypes, which were previously
 77 collected from farmers from different locations in mostly Western Türkiye (Table 1). Two
 78 accessions were breeders lines acquired from Ataturk Central Horticultural Research Institute
 79 (ACHRS), Yalova, Türkiye. Nationally registered cultivars (Akköy-41, Kabaklı-11 and
 80 Marmara-1) obtained from ACHRS, and one standard commercial type (STD-20) were also
 81 included in the experiment. Seeds derived from a single fruit from each genotype were sown
 82 in 6 m rows with 1 m inter and 20 cm intra row spacing in soil on 06.03.2019 under
 83 greenhouse conditions at the Experimental Farm of Faculty of Agriculture, Akdeniz
 84 University, Türkiye. The experimental soil of clay loam texture was a slightly alkali (pH=
 85 7.6) with a lime content of 17.7% and organic matter content of 2.1%. Total N content was
 86 0.09% with P₂O₅ content of 0.0013%, K₂O content of 0.19%, CaCO₃ content of 0.4% and
 87 content of 0.09%. Mn, Zn, Cu and Fe contents were 2.67, 0.47, 0.25 and 1.2 mg/kg
 88 respectively.

89
 90 **Table 1.** Okra genotypes and cultivars investigated in the flow cytometry studies.

Lines	Name	Plant degree of branchin g	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
UIS-15	Landrace	weak	105	7.9	green	12.2	green
UIS-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

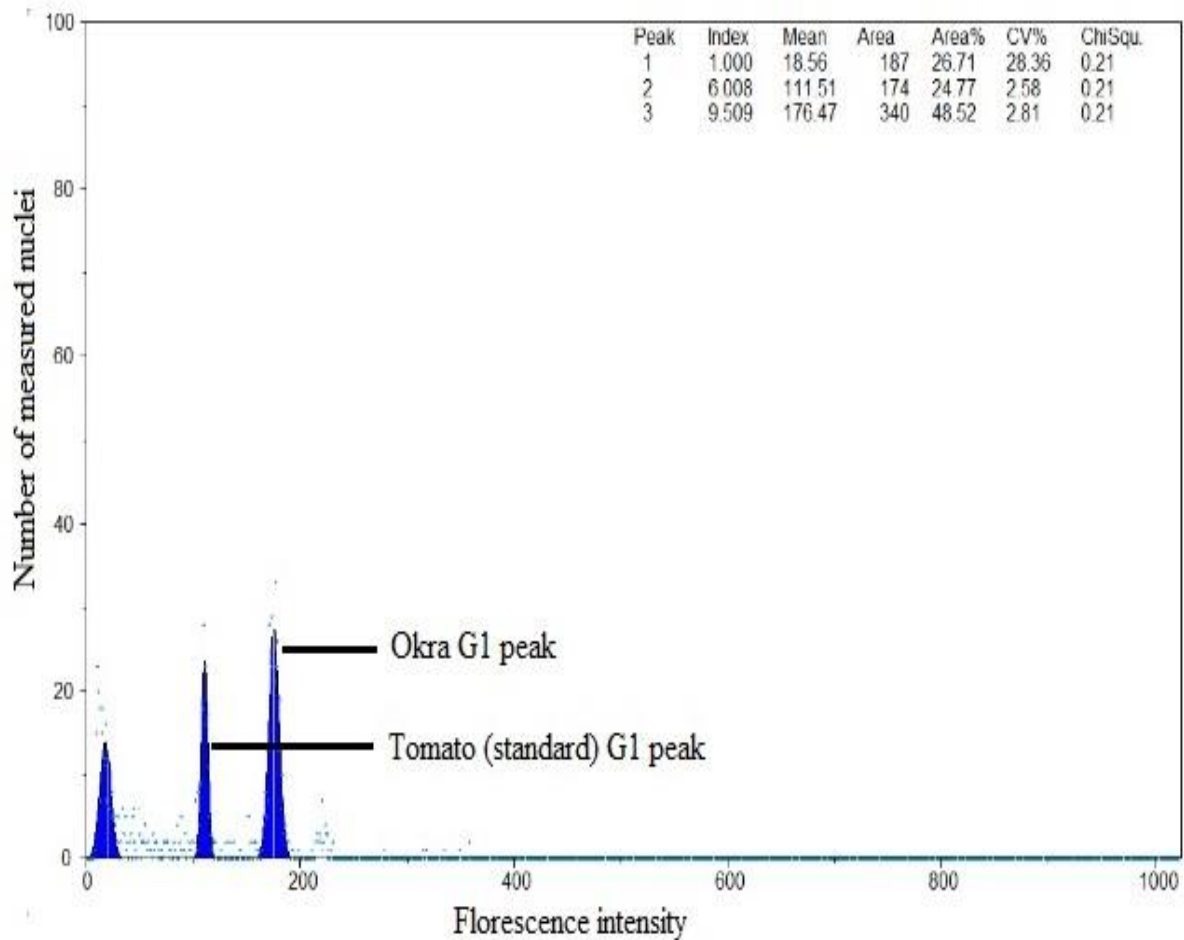
Marmara 1	ACHRS ^c	medium	75	7.3	green	25.0	green
MGL-9	Landrace	medium	120	9.4	red	15.1	green
Kabaklı-11	ACHRS ^c	medium	107	8.1	green	17.1	green
YLV-22	BAL ^b	medium	64	7.1	green	32.9	green
YLV-23	BAL ^b	weak	74	4.7	green	16.5	green
BKL-1	Local	weak	100	7.6	green	10.6	green
MGL-14	Endeze	weak	56	8.9	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

91 ^a Sunagri, ^b BAL Breeders Advanced Line. C Registered cultivars from ACHRS Atatürk Central Horticultural
92 Research Institute, Yalova, Türkiye.

94 Method

95 Nuclear DNA content analysis by flow cytometry

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



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

110 Chromosome counting

111 Cells with good chromosome distribution were selected to determine chromosome numbers.

112 Cytological preparations were made using meristem tissues from actively growing root tips.
113 Root tips were harvested from a few weeks old seedlings grown in pots and treated in 0.05%
114 colchicine at room temperature for 3 h followed by fixation in ethanol:acetic acid (3:1, v/v).
115 Cytological preparations were performed as described by Jenkins & Hasterok (2007). Firstly,
116 the fixed roots were washed in 0.01M citric acid-sodium citrate buffer (pH 4.8, 5 min., 4
117 times) and then fragmented enzymatically at 37 °C in a mixture comprising 20% (v/v)
118 pectinase (Sigma), 1% (w/v) cellulase (Calbiochem), and 1% (w/v) cellulase ‘Onozuka R-10’
119 (Serva) for 2 hours. After this process, the meristem was transferred to a slide in a drop of
120 45% acetic acid; then, a coverslip was placed on the slide and squashed. The coverslips were
121 removed from the slides by a razor after storing them in the freezer (-80 °C) for a few hours.

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

125

126 **Data Analysis**

127 Data were analysed using SPSS statistical package. A simple statistical procedure of
128 confidence intervals was used to compare mean DNA content of the strains (Steel and Torrie,
129 1960). A confidence interval was calculated for each mean by the following equation: $P(X_1 - t$
130 $0.05 S_x < \mu < X_1 + t 0.05 S_x) = 0.95$ where $t 0.05$ is the “ t ” statistic and $s = s/n^{1/2}$ where n is the
131 number of plants analysed for a strain and s is their standard deviation. Accession means with
132 overlapping confidence intervals were assumed to be similar. This is equivalent to conducting
133 a simple t test to compare specific means (Steel and Torrie, 1960).

134

135 **RESULTS**

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

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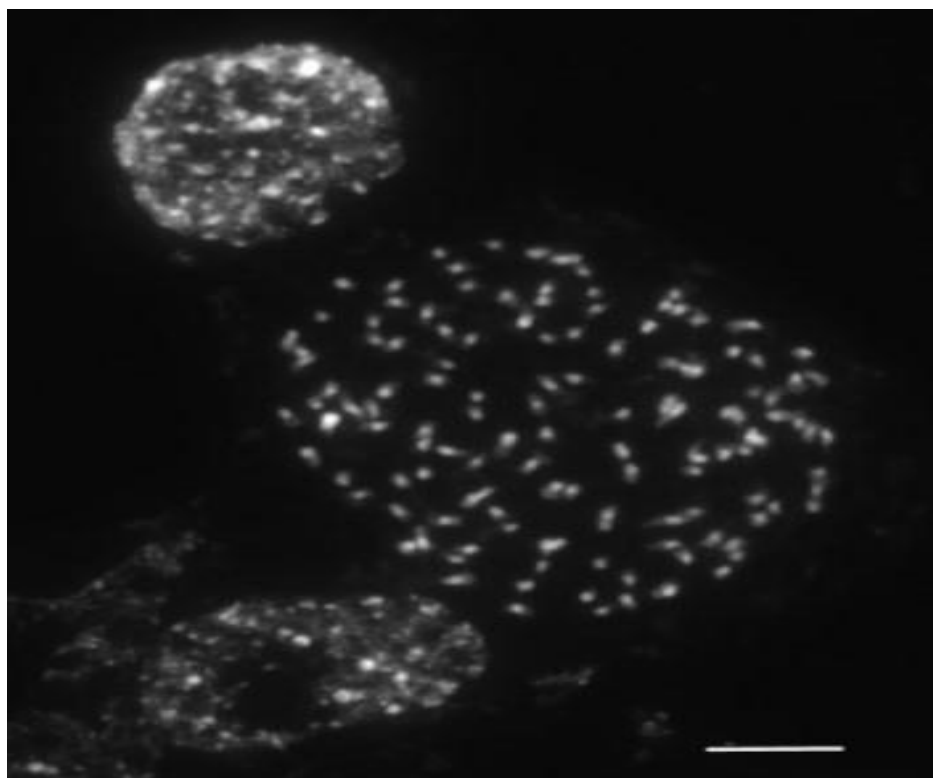
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Table 2. Nuclear DNA content of the okra landraces and cultivars investigated (2C/pg).

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 μ m).

159 DISCUSSION

160 Data presented here show that nuclear DNA content, ploidy level and chromosome number
161 did not vary within the germplasm. All the landraces and cultivars had similar nuclear DNA
162 content and chromosome number as they were off the same ploidy level. Previous studies
163 employing molecular techniques with DNA markers concluded that Turkish okra germplasm
164 had a narrow genetical base and low levels of diversity harboured (Gulsen *et al.*, 2007; Yıldız
165 *et al.*, 2015) in spite of relatively greater morphological diversity (Düzyaman, 2005; Yıldız *et al.*,
166 2016; Örkçü, 2016; Kantar *et al.*, 2021). Our study filled this information gap by
167 investigating nuclear DNA content and ploidy level. Narrow genetic base in the germplasm
168 pool may decrease the effectiveness of okra breeding programs. Genetic diversity should be
169 increased, therefore, in the germplasm pool to increase success in breeding programs.
170 Employing larger germplasm collections using lines and genotypes with greater genetic
171 distance, avoiding those with common background in hybridisation programs (Yıldız *et al.*,
172 2015), introducing genotypes from foreign genetic sources in to breeding pool (Yıldız *et al.*,
173 2015) and enhancing variation by inter-specific hybridisations (Benchasri, 2012; Seth *et al.*,
174 2016) were suggested for more successful breeding programs. Reciprocal hybridisation of
175 (*Abelmoschus esculentus* (L.) Moench) with other wild okra species produced fertile vigorous
176 plants, which may offer new opportunities for creating recombination and diversity
177 (Benchasri, 2012). Successful attempts were also reported for heterosis breeding in okra
178 (Dhankhar, 2016; Bhatt *et al.*, 2016).

179 Information on nuclear DNA content of Turkish okra germplasm is quite scarce.
180 Unfortunately, we were able to find only two studies on okra nuclear DNA content (Salameh,
181 2014; Örkçü, 2016). Örkçü (2016) studied nuclear DNA content of 20 okra landraces
182 including 3 commercial okra cultivars of Akköy-41, Kabaklı-11, and Marmara-1 in Türkiye.
183 Mean 2C nuclear DNA content varied from 2.86 to 3.18 pg among 20 landraces in this study
184 (Örkçü, 2016). The results of this earlier study were quite comparable with the results (3.11
185 pg-3.18 pg) obtained in our study (Table 1). The small differences between the two studies
186 could be attributed to the different fluorochromes and internal standards used. DAPI was the
187 fluorochrome in the current study while PI was used as fluorochrome by Örkçü (2016). These
188 two fluorochromes had different binding modes to the DNA and therefore they could cause
189 differences to some extent up to %15 (Doležel and Bartos, 2005). The differences in
190 chromatin structure of the standard and sample nuclei can also cause differences in nuclear
191 DNA content measurements (Doležel and Bartos, 2005). Although DAPI binding

192 preferentially to AT rich regions of DNA it was the choice of the fluorochromes since it
193 provided DNA content histograms with much higher resolution than propidium iodide in this
194 specific study.

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

212 Okra has small but high number of chromosomes. These characteristics make chromosome
213 counting a challenging task and, hence, it was challenging to count the chromosomes
214 precisely. Nevertheless, the chromosome number of okra plants in our study was
215 approximately $2n=128$. . Örkçü (2014) also determined the chromosome number of Turkish
216 landraces as $2n=128$. Other studies reported chromosome numbers for okra (*A. esculentus*)
217 ranging between $2n=66$ and 144 (Kumar *et al.*, 2010; Benchasri, 2012). In a recent study,
218 Nieuwenhuis *et al.*, 2023 presented a detailed insight into the complex genome and
219 transcriptome architecture of okra (*A. esculentus* cv. Green Star F1) and its haploid
220 descendant, using cytogenetic characterization of its mitotic cell complements. They reported
221 that 2C DNA amount for the okra plant was at 2.99 pg ± 0.01 and chromosomes number was
222 $2n=130$. DAPI was also the choice of fluorochrome in their study. They also reported a low
223 genetic diversity in okra after investigating single nucleotide polymorphisms in 11 public okra
224 accessions. *Abelmoschus esculentus* (usually $2n = 130$) is probably an amphidiploids

225 (allotetraploid), derived from *Abelmoschus tuberculatus* Pal & H.B.Singh ($2n = 58$), a wild
226 species from India, and a species with $2n = 72$ chromosomes (possibly *Abelmoschus ficulneus*
227 (L.) Wight & Arn. ex Wight) (Kumar et al., 2013). In the current study, all the material had
228 very similar nuclear DNA content. Considering essential chromosome number of okra as
229 $x=12$, all the okra plants used in the study can be accepted as allodecaploid with possibility of
230 some extra chromosomes.

231

232 CONCLUSIONS

233 This study investigated nuclear DNA content and ploidy level in Turkish okra germplasm of
234 26 genotypes. No significant variation was detected in nuclear DNA content, ploidy level and
235 chromosome number. The results presented here confirmed the previous studies that okra
236 germplasm has a narrow base of genetic diversity in Turkish germplasm pool, which may
237 limit the success of breeding programs.

238

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241

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