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RESEARCH ARTICLE

Elucidating karyological and agro-morphological characteristics of *Vicia cassia* boiss. and *V. aintabensis* Boiss. & Hausskn

Hüseyin Keleş · Iskender Tiryaki

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Abstract This study aimed to determine the karyological characteristics, DNA content, and agro-morphological plant characteristics of *Vicia cassia* Boiss. and *Vicia aintabensis* Boiss. & Hausskn. collected from natural flora of Turkiye. The results were also compared with those of *Vicia sativa*. The main stem length, the numbers of main stem branches per plant, the number of leaves of main stem, the number of leaflets per leaf, the number of grains per pod, the pod width and length as well as thousand seed weight characteristics showed significant variation across the species, with the exception of 50% inflorescences ($p < 0.4987$) and natural plant heights ($p < 0.3276$). Karyotype formulas of *Vicia cassia*, *V. aintabensis* and *V. sativa* were determined as $2n = 2x = 14 = 3\ m + 1t^{sat} + 1sm + 2t$, $2n = 2x = 14 = 1sm + 4st + 2t$, and $2n = 2x = 12 = 5st + 1\ m$, respectively. The genome size of *V. aintabensis* (17,227.47 Mbp) was 5.26 and 5.01 times larger than *V. cassia* (3273.85 Mbp) and *V. sativa* (3435.22 Mpb), respectively. The importance of the karyomorphological data was also evaluated with the morphological evidences.

Keywords Karyotype analysis · *Vicia* species · DNA content · Flow cytometry · Chromosome number · Fabaceae

H. Keleş · I. Tiryaki (✉)
Department of Field Crops, Faculty of Agriculture, Kahramanmaraş Sütçü Imam University, 46100 Kahramanmaraş, Türkiye
e-mail: itiryaki@comu.edu.tr; tiryaki46@yahoo.com

I. Tiryaki
Department of Agricultural Biotechnology, Faculty of Agriculture, Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Türkiye

Introduction

The genus *Vicia*, belonging to the legume family Fabaceae, is characterized by its significant biodiversity and ecological adaptability, and comprises around 200 species, 65 subspecies and 55 varieties worldwide (Basbag et al. 2013; Bryant and Hughes 2011). *V. faba* L. (broad bean), *V. narbonensis* L. (narbon vetch), and *V. sativa* L. (common or field vetch) are well-known economically important species of this genus in today's agricultural production (Tiryaki and Tuna 2012) not only for their role in nitrogen fixation in natural ecosystems (Suresh et al. 2015) but also serve as fodder (Espinoza-Montes et al. 2018; Georgieva et al. 2016), food (Zhang et al. 2020), and cover crops (Mukumbareza et al. 2016) in agricultural settings. Although *Vicia* species are found throughout the temperate zones of both hemispheres, they are particularly common in the Mediterranean and Middle East (Bryant and Hughes 2011). The northeastern Mediterranean, which includes southeast part of Turkiye, Iraq, Iran, and the southern Mediterranean, is the center of variation and the most likely genetic origin of subgenus *Vicia*. Turkiye has a

rich diversity of *Vicia* genus grown naturally in grassland and natural vegetation (Basbag et al. 2013).

Even though they require specific taxonomic expertise, plant agro-morphological aspects like leaf and seed structure, pollen morphology, floral characteristics, and legume shape are crucial taxonomic traits for reliable species identification in the genus (Bosmali et al. 2022; Nam et al. 2012). In addition, recent karyological research has also been enriched by in situ hybridization with repetitive DNA sequences (Navratilova et al. 2003; Tiryaki et al. 2021), while flow cytometer analysis further helped to determine ploidy and genome sizes of referred plant species (Dolezel et al. 2012; Metcalfe et al. 2019; Molnar et al. 2014; Vrana et al. 2012). Genome size has considered a species-specific constant, and variation in genome size among related species has doubled the C-value (Praca-Fontes et al. 2011). The C stands for the consistency of DNA amount of an individual's unreplicated haploid genome, to reflect genome size fluctuation regardless of organism complexity (Praca-Fontes et al. 2011). Therefore, the comparison of C-values of different plant species is a natural technique to understand evolutionary relationships and the systematics of small taxonomic groups (Tiryaki and Tuna 2012).

Despite the significance of *Vicia* species, comprehensive karyological research is uncommon and normally focus on a limited number of species, usually those that have direct agricultural value. This has severely hampered our understanding of the genus's speciation strategies as well as the evolution of karyotypic and genomic size. Thus, the goal of this study was to identify the karyological, DNA content, and some plant characteristics of two species of *Vicia* that are possibly less well-known in the broader agricultural context: *Vicia cassia* and *Vicia aintabensis*. The outcomes were also compared with one of the most well-known forage species in the genus, *Vicia sativa*.

Material and method

Material

The vetch species collected from the natural flora of Sanliurfa and Adıyaman were taxonomically classified as *Vicia cassia* Boiss. and *V. aintabensis* Boiss., respectively, by the Biology Department of Kahramanmaras Sutcu Imam University, Turkiye. The seeds of species were propagated and used in the field experiment to determine some agro-morphologic properties.

Method

Field experiment

Agro-morphological characterizations were done using a completely randomized block design with three replications in a field located at East Mediterranean Transitional Zone Agricultural Research Institute, Kahramanmaras, Turkiye, in the 2008–2009 growing season. The latitude and longitude were 37°38' North and 36°37' East, respectively, and the altitude was 474 m. The growing season, which ran from November 2008 to June 2009, saw an average temperature of 12.8°C. The average annual minimum and maximum temperatures were 4.5 and 26.3°C, respectively. The study soil is categorized as loam soil with a pH of 7.55, mean annual precipitation of 818.4 mm during the growth season, and values of $CaCO_3$ of 26.92%, P_2O_5 of 0.48 kg/ha, K_2O of 0.41 kg/ha, and 1.85% organic content. Using a seed planter, four rows of seeds 4 m in length and 30 cm apart (3.6 m2) were planted at a depth of 3 cm. The weeds were controlled mechanically and neither fertilizer nor pesticides were applied. The outer rows and 50 cm of row heads were removed, and data from 10 randomly selected plants was collected. The 50% of inflorescences in days, the main stem length, the main stem thickness, natural plant height, the number of main stem branches per plant, the number of leaves per plant, the number of leaflets per leaf, the pod length and width, the number of grains per pod and 1000 seed weight as well as flower color have been determined.

Cytophotometric analysis

Seeds were germinated in Petri dishes at room temperature (22–23 °C and 55–65% humidity), with the interior faces covered with moistened paper. The roots elongated to 0.5–1.0 cm length of *Vicia* species were removed by bistoury and washed off for 1 min under topwater after they were pretreated with saturated solution of 8-hidroxquonalin 0.02 M to block mitosis for 3 h at 4 °C. The samples were then fixed

with Carnoy (ethanol: acetic acid, 3:1 (v/v)) for 24 h at 4 °C and kept in 70% ethyl alcohol at 4 °C until required. The slides were created as described before (Nazırdeh et al 2009). The roots were hydrolyzed with 1 N HCl for 11–12 min at 60 °C. The HCl residue of roots was removed by washing topwater and dried up with filter paper before the tips of the roots were stained with the aceto-iron-hematoxylien reagent for 20 h at 30 °C. The roots were then treated with 2% pectinase and used for karyotype analysis. For karyotype analysis of each species, the best 10 metaphase images viewed under a photomicroscope (Olympus BX51) were photographed and used for image analysis (Nazırdeh et al. 2009).

Karyomorphometry

Long and short arm lengths, total arm length, arm ratio, centromeric index, and relative length were calculated using quantitative information obtained from chromosomal arms. The position of centromeres of each chromosome and the chromosome types were classified using the nomenclature proposed by (Levan et al. 1964). Karyograms and ideograms were created using Adobe Photoshop (version 7.0) and Corel Draw (version × 3) applications, respectively.

The best visible images of a single cell at the metaphase stage were photographed under a photomicroscope (Olympus BX51). These images were then used in karyotype analysis. Homologous chromosomes were determined and ordered from smallest to largest and placed in order by using Photoshop (version 7.0) program (Nazırdeh et al. 2009). Corel Draw (version × 3) was used to draw ideograms of chromosomes of a given species.

Determination of nuclear DNA content

The nuclear DNA content analysis was performed on four individual plants from each species in the flow cytometry (Partec CyFlowR Space) as described previously (Tiryaki and Tuna 2012). A commercial kit (CyStain PI absolute P) containing propidium iodide as the fluorescent dye was utilized to isolate the nucleus of accessions as the manufacturer recommended. The safflower, cultivar Dincer, (*Carthamus tinctorious* L.) was utilized as an internal standard. To reduce variation in the preparation, fresh healthy leaf tissues from 3 to 4-week-old seedlings of each accession and internal standard were co-chopped into 0.25 to 1 mm segments in 1 ml solution A [24 ml $MgSO_4$ buffer (ice-cold); 25 mg dithiothreitol; 500 µl propidium iodide stock (5.0 mg in 1.0 ml double distilled H_2O); 625 µl Triton X-100 stock (1.0 g in 10 ml double distilled H_2O)]. The fluid and tissue were filtered through a 30 µm nylon mesh into a microcentrifuge tube, and then centrifuged at 13.000 rpm for 20 s. The supernatant was discarded. The pellet was resuspended in 400 µl solution B [7.5 ml solution A; 17.5 µl DNAse-free RNAse] and incubated for 20 min at 37 °C before flow cytometric analysis. PI-stained samples were stimulated with a 15 mW argon ion laser at 488 nm. Red PI fluorescence area signals (FL2A) from nuclei were recorded in the FL2 channel. The mean DNA content per sample was determined by analyzing 10.000 nuclei of each sample. The absolute DNA amount of a sample is determined using the previously reported G1 peak means (Dolezel and Bartos 2005). The genome size was calculated as 1 pg (Tiryaki and Tuna 2012).

Data analysis

One-way ANOVA was used to determine significant levels of agro-morphological and nuclear DNA content data using the SAS package program (SAS 1997). Fisher's LSD test was used to test for mean differences at the $p < 0.05$ level.

Results

Agro-morphological data

The results of the analysis of variances showed that all agro-morphological characteristics examined showed significant variation across genotypes, except for 50% inflorescences ($p < 0.4987$) and natural plant heights ($p < 0.3276$) (Table 1). The natural plant height varied from 49.20 to 54.9 cm, and the days to 50% inflorescences ranged from 173.66 to 176.33 days (Table 1). The petals of *V. cassia*, *V. aintabensis*, and *V. sativa* were determined as red, off-white, and violet, respectively. The average main stem lengths were 84.73 cm, 95.86 cm and 131.33 cm, while the number of branches of the main stem was 5.40, 5.33 and 2.60 for *V. cassia*, *V. sativa*

Table 1 The mean of some agro-morphologic properties of three *Vicia* species determined in the study

Species	*Vicia cassia*	*Vicia aintabensis*	*Vicia sativa*
Days to 50% of inflorescences	173.66 ^{ns}	176.33 ^{ns}	175.66 ^{ns}
Natural plant height (cm)	49.20 ^{ns}	54.90 ^{ns}	54.20 ^{ns}
Main stem length (cm)	84.73^{c}	95.86^{b}	131.33^{a}
Number of main stem branches per plant	5.40^{a}	5.33^{a}	2.60^{b}
Number of leaves of the main stem per plant	9.66^{b}	18.73^{a}	15.73^{a}
Number of leaflets per leaf	8.93^{b}	10.80^{a}	11.86^{a}
Number of grains per pod	4.00^{b}	3.13^{c}	6.03^{a}
Pod width (mm)	6.56^{b}	9.13^{a}	6.50^{b}
Pod length (mm)	31.30^{c}	39.30^{b}	49.53^{a}
1000 seeds weight (gr)	16.29^{b}	51.25^{a}	48.91^{a}
Colour of flower	red	off-white	violet

The same letter in the rows is not significant at $P<0.05$. ns, not significant at $P<0.05$

and *V. aintabensis*, respectively (Table 1). While *V. aintabensis* had the highest (18.73) number of leaves of per main stem of plant, *V. cassia* had the lowest (9.16) (Table 1). The number of leaflets per leaf differed significantly ($p<0.0169$) across the *Vicia* species studied (Table 1). *V. Sativa* had 11.86 leaflets per leaf, while *V. cassia* and *V. aintabensis* had 8.93 and 10.80 leaflets per leaf, respectively (Table 1). There were significant ($p<0.0010$) differences in the number of grains per pod among *Vicia* species. The average number of grains per pod ranged from 3.13 (*V. aintabensis*) to 6.03 (*V. sativa*) (Table 1). The analysis of variance revealed that pod width ($p<0.0075$) and length ($p<0.0078$) were significantly different across the *Vicia* species studied (Table 1). The pod of *V. sativa* was measured 6.50 mm while the pod of *V. aintabensis* was measured 9.13 mm (Table 1). The pod lengths of *V. cassia*, *V. aintabensis*, and *V. cassia* were determined as 31.30 mm, 39.30 mm, and 49.53 mm, respectively (Table 1). The 1000 seed weights were found to be 16.29 gr for *V. cassia*, 48.91gr for *V. sativa* and 51.25 gr for *V. aintabensis* (Table 1).

Karyomorphometry

The chromosomal count of *Vicia cassia* was $2n=2x=14$ (Fig. 1a), indicating diploid. Table 2 displays the measured chromosomal parameters. The formula for karyotype was computed as follows: 1 m $+2$ m $+3t^{sat}+4$sm $+5$ m $+6t+7$ m. There was a satellite on *Vicia cassia*'s third chromosome (Fig. 1b). The measurement of the entire haploid chromosomal set length was 24.25 µm, with chromosome IV

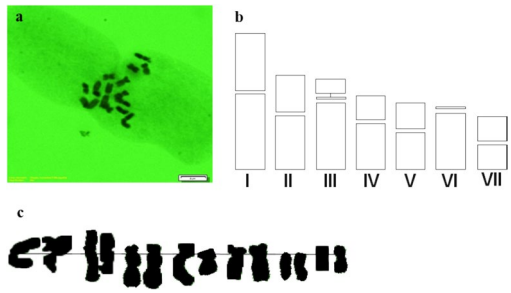

Fig. 1 Microphotograph of mitotic chromosomes (**a**), ideogram (**b**) and karyogram (**c**) of *Vicia cassia*. The bar in the microphotograph represents 5 µm

exhibiting the highest relative arm ratio (r) of 1.91 (Table 2). The relative chromosome length (R) was the highest on chromosome I, while the chromosome arm index (I) ranged from zero (Chrs. III and VI) to 50.0 (Chr. VII) (Table 2).

The microphotograph, ideogram and karyotype of *V. aintabensis* are shown in Fig. 2 and the chromosomal parameters measured are presented on Table 2. The chromosomal number was found to be $2n=2x=14$ (Fig. 2). The total haploid chromosomal set length was measured at 53.19 µm (Table 2). The formula for the karyotype was found to be 1sm $+2$st $+3$st $+4t+5t+6$st $+7$st. The r value was the highest on chromosome VII (6.95), while it was the highest on chromosome II (7.20). The I value ranged from zero (Chrs. IV and V) to 36.38 (Chr. I) (Table 2).

The chromosomal parameters of the *Vicia sativa* were given in Table 2 while the microphotograph, ideogram and karyotype are shown in Fig. 3. It was found that *Vicia sativa* has $2n=2x=12$

Table 2 The chromosomes characteristics of the haploid complement of three *Vicia* species

Species	Chromosome	L (µm)	S (µm)	C = S + L (µm)	r = S/L	I = 100*S/C	R = C*50/ksu	Centromere position	Sat. length (µm)
Vicia cassia	I	3.38	2.57	5.95	1.31	43.19	12.26	M	–
	II	2.41	1.65	4.06	1.46	40.64	8.37	M	–
	III	2.97	0.00	3.59	∞	0.00	7.40	T	0.62
	IV	2.05	1.07	3.12	1.91	34.29	6.43	Sm	–
	V	1.65	1.15	2.8	1.43	41.07	5.77	M	–
	VI	2.53	0.00	2.53	∞	0.00	5.21	T	–
	VII	1.10	1.10	2.20	1	50.00	4.53	M	
Vicia aintabensis	I	6.40	3.66	10.06	1.74	36.38	4.53	Sm	–
	II	6.63	1.03	7.66	6.89	13.44	7.20	St	–
	III	6.47	0.99	7.46	6.43	13.27	7.01	St	–
	IV	7.22	0.00	7.22	∞	0.00	6.78	T	–
	V	7.01	0.00	7.01	∞	0.00	6.74	T	–
	VI	6.10	1.08	7.18	6.62	15.04	6.58	St	–
	VII	5.77	0.83	6.60	6.95	12.57	6.20	St	–
V. *sativa* (cultivar Karaelci)	I	3.43	0.69	4.12	4.97	16.74	10.03	St	–
	II	3.16	0.69	3.85	4.57	17.92	9.37	St	–
	III	2.84	0.64	3.48	4.43	18.39	8.47	St	–
	IV	1.73	1.72	3.45	1.00	49.85	8.40	M	–
	V	2.59	0.85	3.44	3.04	24.70	8.37	St	–
	VI	1.60	0.59	2.19	2.71	26.94	5.33	St	–

L: long arm, **S**: short arm, **C**: total arm length, **Sat**: satellite, **r**: relative arm ratio, **I**: arm index, **R**: relative chromosome length, **ksu**: haploid chromosome set length, **St**: subtelomeric chromosome, **M**: median, **Sm**: submedian, **T**: telomeric

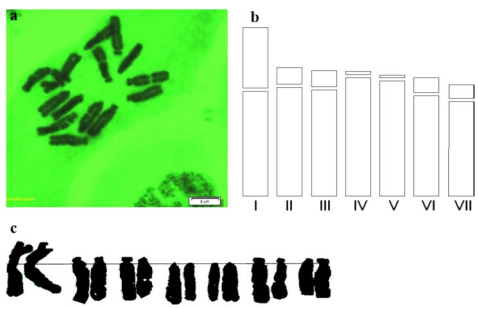

Fig. 2 Microphotograph of mitotic chromosomes (**a**), ideogram (**b**) and karyogram (**c**) of *Vicia aintabensis*. The bar in the microphotograph represents 5 µm

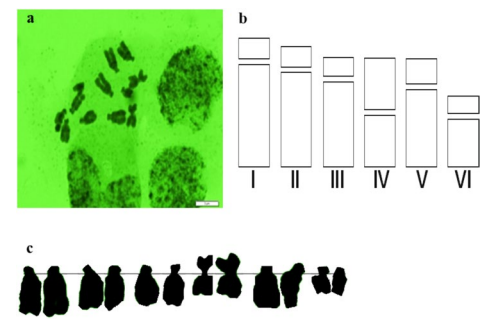

Fig. 3 Microphotograph of mitotic chromosomes (**a**), ideogram (**b**) and karyogram (**c**) of *Vicia sativa*. The bar in the microphotograph represents 5 µm

chromosomes (Fig. 3). One of those was an independent metacentric, while the remaining ones were subterminal (Table 2). The length of the entire haploid chromosomal set was found to be 20.53 µm (Table 2). The chromosomal karyotype formula of *Vicia sativa* was determined as 1st + 2st + 3st + 4 m + 5st + 6st. The chromosome I had the greatest R-value (10.03) and chromosome VI the lowest (5.33) (Table 2). On chromosome I, the r value was highest (4.97), and the I value varied from 16.74 (Chr. I) to 49.85 (Chr. IV) (Table 2).

Nuclear DNA content

A significant ($p < 0.0001$) variance was determined in DNA content of *Vicia* species (Table 3). *Vicia cassia* had the lowest mean 2C value (3.347 pg), whereas *V. aintabensis* had the highest (17.615 pg). The genome size of *V. aintabensis* (17,227.47 Mbp) was found to be 5.26 and 5.01 times larger than *V. cassia* (3273.85 Mbp) and *V. sativa* (3435.22 Mpb), respectively (Table 3).

Discussion

The genetic diversity within *Vicia* has untapped potential for enhancing crop resilience, nutritional characteristics, and environmental sustainability (Ampomah and Huss-Danell 2016; Sanz et al. 2007) because inter- or intra-cross breeding is so prevalent (Basbag et al. 2013; Bryant and Hughes 2011). This offers a new opportunity for the establishment of breeding programs through the adoption of novel genotypes of *Vicia* species following the clarification of plant and karyomorphometric research. Chromosome size variation is considered the primary source of plant diversification in the *Vicia* genus (Storme and Mason 2014; Zhang and Mosjidis 1995, 1998). Numerous noteworthy species of *Vicia* include diploid chromosome numbers of (2n) 10, 12, and 14 (Bryant and Hughes 2011; Navratilova et al. 2003; Zhang and Mosjidis 1998) although diploids ($2n = 2\times = 14$) and autotetraploids ($2n = 4\times = 28$) of *V. cracca* were reported with improved agronomic characteristics (Eliasova and Munzbergova 2017; Eliasova et al. 2014; Storme and Mason 2014). To the best of our knowledge, the agro-morphological, karyotypical, and genome size properties of the *Vicia cassia* are the first time explored while *Vicia aintabensis* was further illuminated by this study. While both *Vicia* species displayed shorter stem lengths than *V. sativa*, there were no discernible variations between them for days to 50% of inflorescences (Table 1). *Vicia cassia* had the smallest seed compared to both *V. aintabensis* and *V. sativa*, but both species had significantly more main stem branches (Table 1). The number of leaves in the main stem and the number of leaflets per leaf of *V. aintabensis* were not significantly different from those of *V. sativa* (Table 1). Although more research is needed to evaluate the forage characteristics of both species, all of the agro-morphological factors measured suggested that *V. aintabensis* can be used for seed production and feed in a manner comparable to *V. sativa*.

Vicia shows high levels of species diversity and numerous *Vicia* species displayed a wide range of karyotype structural variation (Avila Robledillo et al. 2018; Macas et al. 2006, 2003). Three distinct chromosome numbers ($2n = 10$, 12, and 14) have been reported for *V. sativa* (Basbag et al. 2013; Bryant and Hughes 2011; Navratilova et al. 2003; Sevimay et al. 2005) (Ladizinsky 1998; Ladizinsky and Waines 1982). For example, in a previous evaluation (Sevimay et al. 2005), eight Turkish cultivars of *V. sativa*, including cultivar Karaelci, which was also used as a comparison cultivar in this study, were found to have metacentric chromosomes with submedian orientation and $2n = 2x = 12$ chromosomes, which was consistent with the chromosome characteristics reported in this study (Table 2). The current study also revealed that *V. cassia* and *V. aintabensis* both possessed their unique chromosome properties in addition to having the same number of haploid chromosomal sets ($n = 7$) (Table 2). However, *V. aintabensis* (17,227.47 Mbp) provided a genome size 5.26 and 5.01 times larger than *V. cassia* (3273.85 Mbp) and *V. sativa* (3435.22

Table 3. The source of plant material and nuclear DNA content of *Vicia* spp. based on internal standard of safflower (cultivar Dincer) and their genome sizes

Species	Source of material	Mean 2C-value*	2C (Mbp)
V. cassia	$37^0$25′58.21″ N-$38^0$29′12.75" E	3.347 ± 0.032	3273.85 ± 31.31
V. aintabensis	$39^0$49′60.60″ N-$38^0$16′36.61″ E	17.615 ± 0.202	17,227.47 ± 195.84
V. sativa	Cultivar karaelci	3.512 ± 0.022	3435.22 ± 21.68
Coeff. var. (%)	1.363		

*, SE; n = 4.

Mpb), respectively (Tables 2 and 3). Although the total length of the haploid chromosome was shorter (40.57 µm) than our result, a previous study described a *V. aintabensis* karyotype formula that was comparable to this study (Table 2) (Caputo et al. 2006), suggested the existence of genome size variations at the population level of this species. It is quite possible that progressive alterations in karyotypes could result from differential proliferation of repetitive DNA regions in the genome (Neumann et al. 2006, 2001).

Despite having seven pairs of chromosomes ($2n = 14$) and a longer haploid chromosome length (24.25 µm) than *V. sativa* (20.53 µm), *V. cassia* had the lowest 2C DNA content out of the three tested species, suggesting that the 2C values obtained from flow cytometer analysis and the haloid chromosome length measured in karyotype analysis were not be correlated (Tables 2and 3). In contrast to karyological analysis, flow cytometer provides a dependable and highly sensitive approach for identifying extremely small variations in nuclear DNA contents in a wide variety of organisms (Dolezel and Bartos 2005; Dolezel et al. 2012). Therefore, while chromosomal number variations (polyploidy and aneuploidy), structural alterations (translocations, inversions, and deletions), and the physical mapping of genes and markers are all important aspects of understanding the genetic organization and evolution of *Vicia* species (Dolezel and Lucretti 1995; Navratilova et al. 2003), flow cytometer analysis complements karyological analysis by helping to ascertain the ploidy levels and genome sizes of referred plant species studied in this study (Dolezel et al. 2012; Metcalfe et al. 2019; Molnar et al. 2014; Vrana et al. 2012).

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Author contributions IT; conceived the idea, granted financial support, planned the experiments, performed the data analysis, interpreted the data and drafted the manuscript; HK; collected data on agro-morphological parameters, completed Cytophotometric, Karyomorphometry and the nuclear DNA content analysis. All writers have reviewed and approved the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

Ethical Standards. 'Not applicable'.

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