TÜBİTAK

Research Article

Turk J Agric For
36 (2012) 645-653
© TÜBİTAK
doi:10.3906/tar-1201-47

Determination of intraspecific nuclear DNA content variation in common vetch (*Vicia sativa* L.) lines and cultivars based on two distinct internal reference standards

İskender TİRYAKİ[1,*], Metin TUNA[2]

[1]Department of Field Crops, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, 46100 Kahramanmaraş – TURKEY

[2]Department of Field Crops, Faculty of Agriculture, Namık Kemal University, 59030 Tekirdağ – TURKEY

Received: 23.01.2012 ● Accepted: 16.05.2012

Abstract: The 2C nuclear DNA contents of 40 common vetch (*Vicia sativa* L.) lines and cultivars were determined by using both safflower (*Carthamus tinctorius* L.) and barley (*Hordeum vulgare* L.) as internal reference standards in flow cytometry analysis and correlated with plant morphological characters and 1000-seed weight. The t-test results showed a significant difference between the mean DNA values of the barley and safflower standards ($t = -16.74$, $P = 0.0001$). Data analysis also indicated significant ($P < 0.01$) intraspecific nuclear DNA content variation within the common vetch lines and cultivars for both internal standards used. The DNA content values ranged from 3.342 pg to 3.652 pg and from 3.600 pg to 4.002 pg for the internal standards of safflower and barley, respectively. The DNA differences within the internal standards were 0.310 pg and 0.402 pg for safflower and barley, respectively. The internal standard of barley constantly produced higher DNA content values than the values of safflower standard for all vetch lines and cultivars. Nuclear DNA content differences between 2 internal standards for the same plant material reached as high as 15.59% (0.540 pg), which is equivalent to 528.12 Mbp DNA. No significant correlations between 1000-seed weight and the nuclear DNA contents of barley ($P < 0.51$) or safflower ($P < 0.76$) were detected.

Key words: DNA C-value, flow cytometry, intraspecific variation, nuclear genome size, nuclear DNA amount

Introduction

The genus *Vicia* L. comprises annual herbaceous wild and cultivated legumes and includes several economically important species such as *V. faba* L. (broad bean), *V. narbonensis* L. (narbon vetch), and *V. sativa* L. (common or field vetch) (Jaaska 1997). *V. sativa* is considered to be one of the most economically important annual forage species of this genus and has multiple uses, such as for forage, fodder, and green manure (Maxted 1995). This crop is best adapted to the semiarid region of Mediterranean-type environments and has great ecological importance since it is present in the natural plant cover of all continents (Maxted 1995; van de Wouw et al. 2003).

It is crucial to elucidate the nuclear genome content of plant species, not only for the overall understanding of the genomes of related species but also to further the exploitation of the ploidy screening of germplasm, to detect aneuploidy, and to study cell cycle kinetics and reproductive pathways (Doležel and Bartos 2005). The knowledge of DNA C-values has, therefore, contributed to several scientific disciplines

* E-mail: itiryaki@ksu.edu.tr

including systematics, cytogenetics, and evolution (Bennett and Leitch 2005a, 2011). Unfortunately, genome and molecular genetic analysis related to vetch varieties has lagged behind that of other plant species such as corn and alfalfa.

Genome size has been regarded as a species-specific constant (Greilhuber 1998) and genome size variation among similar species has been called the C-value paradox (Thomas 1971). "C" stands for the constancy of an individual's DNA content of unreplicated haploid genomes, which indicates genome size variation irrespective of the complexity of the organism (Swift 1950). The comparison of the C-values of various plant species provides a natural way to explain phylogenetic relationships and systematics of narrow taxonomic groups (Raina 1990; Ohri et al. 2004).

Wide variations in the amount of nuclear DNA are well known to occur within many families or genera. It has been reported that interspecific variation in nuclear DNA content is mostly correlated with such traits as minimum generation time (Bennett 1972), growth in different latitudes and ecological conditions (Bennett 1987; Ohri 2005), seed size (Ohri et al. 1998), physiology (Jasienski and Bazzaz 1995), nuclear volume (Jovtchev et al. 2006), and development (Marciniak and Bilecka 1986; Mohanty et al. 2004).

On the other hand, intraspecific changes in genome size are a current issue of great interest since this variation is naturally related to factors that lead to the divergence and evolution of species (Ohri 1998). It was previously thought that variation in genome size occurred only between species. However, previous reports have revealed a striking range of variation in genome size present within plant species such as soybean (Rayburn et al. 1997; Haun et al. 2011), gloxinia (Zaitlin and Pierce 2010), sunflower (Michaelson et al. 1991), and maize (Rayburn et al. 1989; Vinogradov 1999), and variation could be as high as 48% among the leaves of individual sunflower plants (Michaelson et al. 1991).

Flow cytometry may successfully be used to determine the genome size and the relative DNA content of unknown samples after a process of comparing the data with the relative fluorescence intensity of nuclei of a reference standard whose genome size has been previously determined (Doležel and Bartos 2005; Doležel and Greilhuber 2010). Absolute DNA amounts are traditionally reported in picograms of DNA and, consequently, genome size is reported in base pairs in many plant groups. Even if the instrument settings are not changed, the analysis may be compromised by random instrument drift or by variations in the sample preparation and staining. It has been suggested that these differences can be avoided by the internal standardization of the samples, in which the nuclei of the standard and the sample are isolated, stained, and analyzed simultaneously (Doležel 1991).

To estimate the genome size of a species, several randomly selected plants are analyzed, and each is analyzed several times. While the analysis of several plants permits monitoring of intraspecific variation, the replicated measurements of the same plant facilitate the detection of variations in the procedure. The number of plants and replicated measurements vary among the different studies and are generally lower in large-scale screening experiments (Suda et al. 2003). Nevertheless, it is generally assumed that a minimum of 3 plants should be analyzed (Greilhuber and Obermayer 1997; Lysak et al. 1999) when intraspecific genome size variation is being studied.

The objectives of the present study were to determine nuclear DNA content and to assess the magnitude of intraspecific genomic DNA variation in the common vetch lines provided from various resources, and to reveal the effect of the reference standard in determining the nuclei of the target DNA in flow cytometry analysis.

Materials and methods

Plant materials

Forty common vetch lines from 5 various sources were used as plant material. Seeds from all the lines were grown and selfed under the same field conditions for 2 years, during the plant growing season of 2008 and 2009. The seeds collected from natural flora were also reconfirmed by detailed morphological characterization to assure the correct taxonomic classification. The names, sources, and accession numbers of the plant material are listed in the Table.

Nuclear DNA content determination by flow cytometry

Four individual plants from each line were used in the nuclear DNA content analysis. The DNA content per nucleus was determined as previously described (Arumuganathan and Earle 1991) using the flow cytometer CYTOMICS FC 500 (Beckman Coulter, Inc., Fullerton, CA, USA) at the Central Laboratory of the Medical School of Trakya University, Edirne, Turkey. The procedure consisted of the preparation of suspensions of intact nuclei by chopping plant tissues and lysing protoplasts in a $MgSO_4$ buffer mixed with DNA standards and stained with propidium iodide (PI) in a solution containing DNAse-free RNAse. The fluorescence intensities of the stained nuclei were measured and the values of the nuclear DNA content were estimated by comparing the fluorescence intensities of the nuclei of the test samples with the related internal DNA standards. Either barley (*Hordeum vulgare* L.) cultivar Sladoran or safflower (*Carthamus tinctorius* L.) cultivar Dincer was used as the internal standard. To minimize variation in the sample preparation of the related standards and the samples, the nuclei of both were isolated, stained, and analyzed simultaneously. Nuclear DNA values are expressed in picograms as C values (Bennett and Smith 1976). The letter C stands for 'constant' or the DNA in a haploid nucleus or genome, and the 2C values reported in this paper represent the DNA content of a diploid somatic nucleus. Barley is a diploid (2x = 14) species that has a 2C complement of DNA of 5.325 pg per nucleus while safflower is a diploid (2n = 12) species that has a C complement of DNA of 2.65 pg (Tuna et al. 2001).

Fresh healthy leaf tissues from 3–4 week old seedlings, about 50 mg of target samples, and 20 mg of either barley or safflower internal standards were simultaneously excised and placed on ice in a sterile plastic petri dish for the flow cytometer analysis. Tissue was chopped into segments of 0.25–1 mm in 1 mL of solution A (consisting of 24 mL $MgSO_4$ buffer [ice cold], 25 mg dithiothreitol, 500 µL propidium iodide stock [5.0 mg propidium iodide in 1.0 mL double-distilled H_2O], and 625 µL Triton X-100 stock [1.0 g Triton X-100 in 10 mL double-distilled H_2O]). The solution and tissues were filtered through a 30-µm nylon mesh into a microcentrifuge tube and centrifuged at high speed (13,000 rpm) for about 20 s. The supernatant was then discarded, and the pellet was resuspended in 400 µL of solution B (consisting of 7.5 mL solution A and 17.5 µL DNAse-free RNAse) and incubated for 20 min at 37 °C before flow cytometric analysis. Samples stained with PI were excited with a 15-mW argon ion laser at 488 nm. Red PI fluorescence area signals from the nuclei were collected in the FL2 channel. Mean DNA content per sample was based on the analysis of 10,000 nuclei per sample. The absolute DNA amount of a sample is calculated based on the values of the G1 peak means as previously reported (Doležel and Bartos 2005). Genome size differences between the 2 reference standards were calculated to be 1 pg (Doležel et al. 2003).

Thousand-seed weight

Thousand-seed weight was calculated from an average of four 100-seed weights per line (Fırıncıoğlu et al. 2010).

Statistical analysis

Data were subjected to analysis of variance using SAS (SAS 1997), and the mean separation was performed by Fisher's least significant difference test if the F-test was significant at $P < 0.05$. A t-test was performed to compare the mean DNA values obtained with the barley and safflower standards. A usual pooled t-test was used to compare the mean DNA values of barley and safflower standards based on the equality of variances test results. The univariate procedure was used to calculate the simple statistic values of mean, mode, and median. Pearson's correlation coefficient was used to assess the correlations between the nuclear DNA content and the 1000-seed weight of individual plants.

Results

The mean nuclear DNA contents of 40 common vetch lines from 5 various resources are presented in the Table. The equality of variances test results indicated that the assumption of equal variances was reasonable for the data (folded F-statistic $F' = 1:17$, with $P = 0.6315$). The t-test results showed that the mean DNA values obtained with the barley and safflower standards were significantly different ($t = -16.74$, $P = 0.0001$). The results also revealed

Table. Nuclear DNA content of the lines and varieties based on internal standards of safflower and barley, mean difference in genome size, and 1000-seed weight of common vetch lines.*

			Mean 2C value[¶]		Mean difference between 2 internal standards (pg)	Mean difference between 2 internal standards (%)	2C (Mbp)[†]	1000-seed weight (g)
Register no.	Accession no.	Source[b]	Safflower[+]	Barley				
TA-11	TARM-61724	Ankara Agri. Res. Inst.	3.362 ± 0.068	3.697 ± 0.043	0.335	9.96	327.63	55.33 ± 0.58
TA-12	TARM-61731	Ankara Agri. Res. Inst.	3.347 ± 0.035	3.770 ± 0.052	0.423	12.63	413.69	47.50 ± 1.42
TA-13	TARM-61877	Ankara Agri. Res. Inst.	3.547 ± 0.037	3.780 ± 0.035	0.233	6.56	227.87	52.33 ± 1.89
TA-14	TARM-61938	Ankara Agri. Res. Inst.	3.542 ± 0.055	3.797 ± 0.009	0.255	7.19	249.39	50.83 ± 2.29
TA-15	TARM-61946	Ankara Agri. Res. Inst.	3.485 ± 0.077	3.785 ± 0.091	0.300	8.60	293.40	55.50 ± 1.13
TA-16	TARM-L-292/1	Ankara Agri. Res. Inst.	3.652 ± 0.035	3.755 ± 0.055	0.103	2.82	100.73	51.33 ± 2.20
TA-17	TARM-2639	Ankara Agri. Res. Inst.	3.447 ± 0.062	3.702 ± 0.012	0.255	7.39	249.39	53.08 ± 2.12
TA-18	TARM-2617	Ankara Agri. Res. Inst.	3.375 ± 0.042	3.732 ± 0.073	0.357	10.57	349.15	54.25 ± 3.04
TA-19	TARM-L-581	Ankara Agri. Res. Inst.	3.500 ± 0.033	3.760 ± 0.088	0.260	7.42	254.28	56.83 ± 0.47
DV-11	Natural flora of Şanlıurfa	37°16′20.42″N, 37°50′17.22″E	3.485 ± 0.077	3.800 ± 0.081	0.315	9.03	308.07	46.58 ± 1.02
DV-12	Natural flora of Adıyaman	37°51′30.62″N, 38°19′14.67″E	3.435 ± 0.047	3.687 ± 0.117	0.252	7.33	246.46	41.41 ± 1.44
DV-13	Natural flora of Adıyaman	37°52′30.43″N, 38°23′15.55″E	3.477 ± 0.109	3.785 ± 0.045	0.308	8.85	301.22	41.16 ± 1.38
DV-16	Natural flora of Adana	37°03′46.94″N, 35°21′20.23″E	3.425 ± 0.063	3.780 ± 0.028	0.355	10.36	347.19	54.33 ± 2.60
IC-11	Sel 2709	ICARDA	3.420 ± 0.048	3.762 ± 0.040	0.342	10.23	334.48	59.66 ± 1.95
IC-12	Sel 2714	ICARDA	3.550 ± 0.037	3.827 ± 0.074	0.277	7.80	270.91	57.79 ± 0.75
IC-13	Sel 2717	ICARDA	3.460 ± 0.067	3.600 ± 0.065	0.140	4.04	136.92	49.61 ± 2.67
IC-14	Sel 2721	ICARDA	3.480 ± 0.129	3.810 ± 0.134	0.330	9.48	322.74	51.59 ± 2.10
IC-15	IFVS 505 Sel 2746	ICARDA	3.520 ± 0.060	3.652 ± 0.047	0.132	3.93	129.10	59.71 ± 2.12
GB-17	TR54409	Menemen Agri. Res. Inst.	3.505 ± 0.023	3.637 ± 0.053	0.132	3.76	129.10	61.21 ± 1.76
GB-18	TR57556	Menemen Agri. Res. Inst.	3.390 ± 0.021	3.730 ± 0.031	0.340	10.02	332.52	47.73 ± 3.70
GB-19	TR54404	Menemen Agri. Res. Inst.	3.525 ± 0.088	3.722 ± 0.038	0.197	5.58	192.67	36.33 ± 3.77
GB-20	TR57832	Menemen Agri. Res. Inst.	3.477 ± 0.060	3.672 ± 0.030	0.195	5.60	190.71	63.12 ± 2.68
GB-21	TR63225	Menemen Agri. Res. Inst.	3.545 ± 0.118	3.672 ± 0.017	0.127	3.58	124.21	40.28 ± 0.59
GB-22	TR5441	Menemen Agri. Res. Inst.	3.510 ± 0.055	3.802 ± 0.130	0.292	8.31	285.58	48.62 ± 2.52
GB-23	TR33295	Menemen Agri. Res. Inst.	3.590 ± 0.011	3.707 ± 0.063	0.117	3.25	114.43	50.63 ± 1.54
GB-24	TR33452	Menemen Agri. Res. Inst.	3.435 ± 0.197	3.717 ± 0.044	0.282	8.20	275.80	45.00 ± 0.27
GB-25	TR4392	Menemen Agri. Res. Inst.	3.550 ± 0.014	3.645 ± 0.038	0.095	2.67	92.91	47.98 ± 0.99
GB-26	TR57563	Menemen Agri. Res. Inst.	3.462 ± 0.102	4.002 ± 0.108	0.540	15.59	528.12	46.74 ± 0.62
GB-27	TR51477	Menemen Agri. Res. Inst.	3.445 ± 0.005	3.747 ± 0.033	0.302	8.76	295.36	42.61 ± 1.36
GB-28	TR576564	Menemen Agri. Res. Inst.	3.482 ± 0.062	3.780 ± 0.142	0.298	8.55	291.44	61.56 ± 2.97
GB-29	TR33253	Menemen Agri. Res. Inst.	3.485 ± 0.065	3.707 ± 0.069	0.222	6.37	217.12	41.02 ± 1.80
GB-30	TR54249	Menemen Agri. Res. Inst.	3.517 ± 0.091	3.802 ± 0.022	0.285	8.10	278.73	41.17 ± 1.41
GB-31	TR54261	Menemen Agri. Res. Inst.	3.480 ± 0.055	3.637 ± 0.050	0.157	4.51	153.55	42.57 ± 1.50
GB-32	TR44449	Menemen Agri. Res. Inst.	3.500 ± 0.074	3.730 ± 0.073	0.230	6.57	224.94	28.11 ± 1.39
GB-34	TR33268	Menemen Agri. Res. Inst.	3.502 ± 0.061	3.725 ± 0.070	0.223	6.36	218.09	47.86 ± 1.41
CE-7	Kubilay-82	Variety	3.512 ± 0.057	3.800 ± 0.070	0.288	8.20	281.66	62.91 ± 1.73
CE-8	Nilüfer	Variety	3.500 ± 0.064	3.640 ± 0.073	0.140	4.00	136.92	46.33 ± 1.02
CE-10	Selçuk-99	Variety	3.342 ± 0.085	3.817 ± 0.111	0.475	14.21	464.55	51.58 ± 3.59
CE-11	Uludağ	Variety	3.390 ± 0.035	3.720 ± 0.059	0.330	9.73	322.74	51.62 ± 2.13
CE-12	Ürem-79	Variety	3.580 ± 0.053	3.777 ± 0.053	0.197	5.50	192.67	65.31 ± 0.28
		Mean	**3.481 ± 0.090**	**3.742 ± 0.094**	**0.261**	**7.49**	**255.26**	**50.23 ± 8.11**
		Median	**3.480**	**3.735**	-	-	-	**49.90**
		Mode	**3.520**	**3.720**	-	-	-	**54.25**
		$LSD_{0.01}$	**0.101**	**0.096**	-	-	-	**2.591**
		Coefficient of variance (%)	**2.074**	**1.842**	-	-	-	**3.683**

*The assumption of equal variances was reasonable for the data (folded F-statistic F′ = 1.17, with P = 0.6315). The t-test results showed significant difference between the mean DNA values of the barley and safflower standards (t = −16.74, P = 0.0001).

[b]ICARDA, International Center for Agricultural Research in the Dry Areas, Aleppo, Syria; Ankara Agri. Res. Inst., Ankara Agricultural Research Institute, Ankara, Turkey; Menemen Agri. Res. Inst., Menemen Agricultural Research Institute, Menemen, Turkey; natural flora, seeds were collected locally.

[†]1 pg = 978 Mbp (Doležel et al. 2003). [+]1C nuclear DNA content (mean value ± standard deviation of 4 samples). [¶]Internal standard used in each case.

significant ($P < 0.01$) intraspecific nuclear DNA content variation for both of the internal standards that were used to estimate the DNA content of the unknown plant material. However, data analysis based on the source of the plant material did not show any significant nuclear DNA content variation (data not shown). The values ranged from 3.342 pg to 3.652 pg and from 3.600 pg to 4.002 pg for the internal standards of safflower and barley, respectively. The largest genome size was found in line TARM-L-292/1 (3.652 pg) in the safflower standard, while line TR57563 (4.002 pg) had the largest mean 2C value in the internal standard of barley. The lowest genome size was determined for cultivar Selçuk-99 and line Sel2717 in the internal standards of safflower and barley, respectively. The mean values were 3.481 pg and 3.742 pg while median values were 3.480 pg and 3.735 pg for the internal standards of safflower and barley, respectively. As for the modes, the values that occurred most frequently in the data set of 2C values were 3.520 pg and 3.720 pg for the internal standards of safflower and barley, respectively. The mean nuclear DNA content differences between the 2 internal standards ranged from 0.103 pg (100.73 Mbp DNA) to 0.540 pg (528.12 Mbp DNA) and the mean of the mean differences of the 2 standards was determined to be 7.49% (0.261 pg), which was equivalent to 255.26 Mbp DNA. Line TARM-L-292/1 had the lowest value for the mean difference (0.103 pg), while the largest mean difference based on the internal standards was obtained from line TR57563 (0.540 pg). The internal standard of barley constantly provided a higher DNA content for all samples.

Significant 1000-seed weights were determined among the vetch lines. The 1000-seed weight ranged from 28.11 g (TR44449) to 65.31 g (cultivar Ürem-79). The results of the 1000-seed weights revealed that line TR44449, sourced from the Menemen Agricultural Research Institute, had the smallest seeds, while cultivar Ürem-79 had the biggest seeds. We did not detect any significant correlation between the 1000-seed weight and nuclear DNA contents of barley ($P < 0.51$) or safflower ($P < 0.76$).

Discussion

Changes in genome size within a narrow group of species are believed to be a true indicator of the ongoing processes of speciation or genetic divergence (Price 1976; Murray 2005). The study of DNA amounts has helped to distinguish various taxa in the *Vicia narbonensis* L. and *V. sativa* L. complexes (Raina 1990). Considerable variation in the nuclear DNA content (3.85–27.07 pg) as well as in the basic chromosome number (2n = 10, 12, or 14) has been reported between *Vicia* species (Raina and Narayan 1984; Raina 1988; Maxted 1995; Kahlaoui et al. 2009).

It is now evident that flow cytometry is a reliable and highly sensitive method for detecting even small nuclear DNA amount differences in many living organisms (Rayburn and Wetzel 2002; Doležel and Bartos 2005). However, it is also evident that there is a need for an agreement on internal reference standards for DNA flow cytometry analysis since the relative DNA content of unknown samples can only be determined after a process of comparing data with the relative fluorescence intensity of nuclei of a reference standard whose genome size is previously known. Various reports have used different internal standards, including human (Lysak et al. 2000), domestic chicken (Galbraith et al. 1983), and rainbow trout (Turpeinen et al. 1999), as well as various plant species such as model plant *Arabidopsis* (Bennett et al. 2003), petunia (Marie and Brown 1993), alfalfa (Martel et al. 1997), oat (Morgan et al. 1995), and pea (Baranyi and Greilhuber 1996). Since the internal standards of flow cytometry analysis were not calibrated against each other, most of the intraspecific nuclear DNA amount variation below the species level was attributed either to artifacts in the analysis, including in densitometry and cytofluorometry techniques, or to differences in chromosome number, chromosome size (polyploidy, aneuploidy, B chromosomes, sex chromosomes), and inherent undetected cryptic species (Greilhuber 1998; Gregory 2005; Murray 2005). It was also reported that about 5% of genome size differences may be explained by the use of different instruments and techniques (Feulgen microspectrophotometry versus flow cytometry) and different internal standards (Doležel and Bartos 2005). Furthermore, minor instrumental drifts (e.g., due to slight differences in instrument alignment) may result in very small but statistically significant differences between estimates produced on different days (Doležel and Bartos 2005). More recently, reports have indicated that

the presence of metabolic compounds that interfere with DNA staining, such as tannins, flavonoids, and anthocyanins (Price et al. 2000; Noirot et al. 2005; Walker et al. 2006; Bennett et al. 2008; Smarda and Bures 2010), may also result in very small significant differences between the estimates. It is well known that seeds of the common vetch possess antioxidant activity and contain low-molecular-weight phenolic compounds (Amarowicz et al. 2008; Pastor-Cavada et al. 2008); however, there is no report indicating that seedlings have such compounds that compromise the reliability of estimated DNA content. Therefore, the amount of nuclear DNA variation in the common vetch lines and varieties determined in this study is not likely to be due to the presence of metabolic compounds interfering with DNA staining.

There are also several reports indicating that intraspecific C-value variation can be determined if the appropriate controls and standards have been used (Bennett and Thomas 1991; Hall et al. 2000; Moscone et al. 2003; Smarda and Bures 2006; Smarda et al. 2010). The results of the present study revealed that the internal standards of flow cytometry analysis are important and may provide significant differences in the reliable detection of target nuclear DNA contents for the same plant species even if the same instrument and alignments were used. We used 2 internal standards from the 2 distinct plant families of oat and safflower. Although both of the internal standards were able to detect significant genome size variation in the common vetch lines, the internal standard of barley constantly provided a higher nuclear DNA content in comparison to the internal standard of safflower, suggesting that the reason for the discrepancies in the genome size variation of the same species among the laboratories may mainly be caused by the internal standard differences. The nuclear DNA content differences between the 2 internal standards for the same line reached as high as 0.540 pg, which was equivalent to 528.12 Mbp DNA. Kahlaoui et al. (2009), using soybean as the internal standard, found that the 2C nuclear DNA contents of 3 varieties of *V. sativa* were between 3.69 pg and 3.79 pg, similar to the values obtained in the present work with barley.

Intraspecific genome size variability correlated with ecogeography has been documented for many plant species including *Poa annua* (Grime 1983), *Bulbine bulbosa* (Watson 1987), various species of *Eleusine* (Bennett and Bennett 1992), *Dactylis glomerata* (Reeves et al. 1998), *Zea mays* subsp. *mays* (Poggio et al. 1998), *Ceratonia siliqua* (Bures et al. 2004), and *Festuca pallens* (Smarda and Bures 2006). A previous report indicated that less than 3% of the variation in genome size is weakly associated with variation in microclimatic conditions (Kalendar et al. 2000). The present report proved that there is significant intraspecific genome size variation within the *V. sativa* species, and that this significance is irrespective of the source of the lines, which were obtained from very distinct genetic sources.

The significant interspecific genome size variation is mainly attributed to the variation in noncoding DNA content, differences in transposable element content (Fedoroff 2000; Bennetzen et al. 2005; Paux et al. 2006), or differences in the amounts of repetitive DNA content of the related genome (Bennetzen 2002). Plant species with intraspecific variation in genome size have been reported, including soybean (Graham et al. 1994; Rayburn et al. 1997), sunflower (Michaelson et al. 1991), pea (Arumuganathan and Earle 1991), and maize (Rayburn et al. 1989), and variation as high as 32% was described (Michaelson et al. 1991). Advanced understanding of the nuclear genome and its components provides some clues about the mechanisms that could be responsible for intraspecific variation in genome size, including the activation of Class I retrotransposons (Bennetzen and Kellogg 1997), and for genome decrease/increase by deletions/insertions (Petrov 1997; Gregory 2003; Bennetzen et al. 2005). Recently, a variation in BARE-1 retrotransposon copy number was observed in populations of wild barley in response to differing microclimates (Kalendar et al. 2000). Studies have sometimes led to the assumption that some degree of chromosomal variation within populations due to duplications and deletions, spontaneous aneuploidy and polyploidy, heterochromatic segments, B-chromosomes, and, in special cases, sex chromosomes as well as changes in the copy number of certain DNA sequences causes interindividual DNA content variation (Greilhuber 1998; Bennett and Leitch 2005b).

Vicia sativa has a relatively small genome among the species of the genus, which include *V. grandiflora*, *V. pannonica*, *V. villosa*, and *V. narbonensis* (Navràtilovà et al. 2003; Kahlaoui et al. 2009). Of those 6 pairs of chromosomes, 1 is metacentric, 4 are subacrocentric, and 1 is acrocentric (Navràtilovà et al. 2003). Previous reports have revealed that the common vetch chromosomes differed only in 1 of 2 genus-specific satellite repeats (VicTRs), and specifically in VicTR-B, which are rich in A–T bases, amplified, and present in large clusters with (sub)terminal localization (Navratilova et al. 2003). It is also reported that satellite repeats are to known to undergo rapid changes in copy numbers and the copy numbers of VicTR-A and -B sequences vary. Furthermore, VicTR-B appears to be more conserved than VicTR-A, which occurs almost exclusively at terminal regions of chromosome arms (Macas et al. 2000). These findings suggest that intraspecific genome size differences in the common vetch lines may be attributed to differences in the repetitive DNA content of A–T bases in genus-specific satellite repeats such as VicTR-B.

Both negative and positive correlations were reported between nuclear DNA content and plant morphological characters such as seed size (Chung et al. 1998; Sugiyama et al. 2002). Although significant seed size variation was determined among the common vetch lines tested, this variation was not correlated with nuclear DNA content, suggesting that seed size is not an important source for intraspecific nuclear DNA variation in the common vetch.

Our results lead to the conclusion that differences in genome size within *Vicia sativa* are not due to formation of polyploidy, and therefore suggest that other differential features are present in the genome. Our results have also revealed that the discrepancies in genome size variation in the same species can be due to internal standard differences in addition to methodological and instrumental drifts.

Acknowledgments

Financial support of this work was provided by the Scientific and Technological Research Council of Turkey (TÜBİTAK) with research grant TOVAG–107O012. The efforts of Abdullah Cil, who provided most of the starter plant material, are also greatly appreciated.

References

Amarowicz R, Troszynska A, Pegg RB (2008) Antioxidative and radical scavenging effects of phenolics from *Vicia sativum*. Fitoterapia 79: 121–122.

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9: 208–218.

Baranyi M, Greilhuber J (1996) Flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum*. Theor Appl Gen 92: 297–307.

Bennett MD (1972) Nuclear DNA content and minimum generation time in herbaceous plants. Proc R Soc Lond B Biol Sci 181: 109–135.

Bennett MD (1987) Variation in genomic form in plants and its ecological implications. New Phyt 106: 177–200.

Bennett MD, Leitch IJ (2005a) Nuclear DNA amounts in angiosperms: progress, problems and prospects. Ann Bot 95: 45–90.

Bennett MD, Leitch IJ (2005b) Plant genome size research: a field in focus. Ann Bot 95: 1–6.

Bennett MD, Leitch IJ (2011) Nuclear DNA amounts in angiosperms: targets, trends and tomorrow. Ann Bot 107: 467–590.

Bennett MD, Leitch IJ, Price HJ, Johnston JS (2003) Comparisons with *Caenorhabditis* (100 Mb) and *Drosophila* (175 Mb) using flow cytometry show genome size in *Arabidopsis* to be 157 Mb and thus 25% larger than the *Arabidopsis* genome initiative estimate of 125 Mb. Ann Bot 91: 547–557.

Bennett MD, Price HJ, Johnston JS (2008) Anthocyanin inhibits propidium iodide DNA fluorescence in *Euphorbia pulcherrima*: implications for genome size variation and flow cytometry. Ann Bot 101: 777–790.

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans Royal Soc L Series B: 274: 227–274.

Bennett ST, Bennett MD (1992) Variation in nuclear DNA amount between wild and cultivated populations of *Milium effusum* (2n = 28). Genome 35: 1050–1053.

Bennett ST, Thomas SM (1991) Karyological analysis and genome size in *Milium* (Gramineae) with special reference to polyploidy and chromosomal evolution. Genome 34: 868–878.

Bennetzen JL (2002) Mechanisms and rates of genome expansion and contraction in flowering plants. Genetica 115: 29–36.

Bennetzen JL, Kellogg EA (1997) Do plants have a one-way ticket to genomic obesity? Plant Cell 9: 1509–1514.

Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot (Lond) 95: 127–132.

Bures P, Pavlicek T, Horova L, Nevo E (2004) Microgeographic genome size differentiation of the carob tree, *Ceratonia siliqua*, at 'Evolution Canyon', Israel. Ann Bot 93: 529–535.

Chung J, Lee JH, Arumuganathan K, Graef GL, Specht JE (1998) Relationships between nuclear DNA content and seed and leaf size in soybean. Theor Appl Genet 96: 1064–1068.

Doležel J (1991) Flow cytometric analysis of nuclear DNA content in higher plants. Phyt Anal 2: 143–154.

Doležel J, Bartos J (2005) Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot 95: 99–110.

Doležel J, Bartos J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry A 51: 127–128; author reply: 129.

Doležel J, Greilhuber J (2010) Nuclear genome size: are we getting closer? Cytometry A 77: 635–642.

Fedoroff N (2000) Transposons and genome evolution in plants. Proc Natl Acad Sci USA 97: 7002–7007.

Fırıncıoğlu HK, Ünal S, Erbektaş E, Doğruyol L (2010) Relationships between seed yield and yield components in common vetch (*Vicia sativa* ssp. *sativa*) populations sown in spring and autumn in central Turkey. Field Crops Res 116: 30–37.

Galbraith DW, Harkins KR, Maddox JM, Mayres N, Sharma DP, Firoozabady E (1983) Rapid flow cytophotometric analysis of the cell cycle in intact plant tissues. Science 220: 1049–1051.

Graham MJ, Nivkell CD, Rayburn AL (1994) Relationship between genome size and maturity group in soybean. Theor Appl Genet 88: 429–432.

Gregory TR (2003) Is small indel bias a determinant of genome size? Trends Genet 19: 485–488.

Gregory TR (2005) Genome size evolution in animals. In: The Evolution of the Genome (Ed. TR Gregory). Elsevier, San Diego, pp. 3–87.

Greilhuber J (1998) Intraspecific variation in genome size: a critical reassessment. Annal Bot 82: 27–35.

Greilhuber J, Obermayer R (1997) Genome size and maturity group in *Glycine max* (soybean). Heredity 78: 547–551.

Grime JP (1983) Prediction of weed and crop response to climate based on measurement of DNA content. Asp Appl Biol 4: 87–98.

Hall SE, Dvorak WS, Johnston JS, Price HJ, Williams CG (2000) Flow cytometric analysis of DNA content for tropical and temperate new world pines. Annal Bot 86: 1081–1086.

Haun WJ, Hyten DL, Xu WW, Gerhardt DJ, Albert TJ, Richmond T, Jeddeloh JA, Jia G,

Springer NM, Vance CP, Stupar RM (2011) The composition and origins of genomic variation among individuals of the soybean reference cultivar Williams 82. Plant Physiol 155: 645–655.

Jaaska V (1997) Isoenzyme diversity and phylogenetic affinities in *Vicia* subgenus *Vicia* (*Fabaceae*). Gen Res Crop Evol 44: 557–574.

Jasienski M, Bazzaz FA (1995) Genome size and high CO_2. Nature 376: 559–560.

Jovtchev G, Schubert V, Meister A, Barow M, Schubert I (2006) Nuclear DNA content and nuclear and cell volume are positively correlated in angiosperms. Cesk Otol 114: 77–82.

Kahlaoui S, Walker DJ, Correal E, Martínez-Gómez P, Hassen H, Bouzid S (2009) The morphology, chromosome number and nuclear DNA content of Tunisian populations of three *Vicia* species. Afr J Biotechnol 8: 3184–3191.

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimate divergence. Proc Natl Acad Sci USA 97: 6603–6607.

Lysak MA, Doleželova M, Horry JP, Swennen R, Doležel J (1999) Flow cytometric analysis of nuclear DNA content in *Musa*. Theor Appl Genet 98: 1344–1350.

Lysak MA, Rostkova A, Dixon JM, Rossi G, Doležel J (2000) Limited genome size variation in *Sesleria albicans*. Annal Bot 86: 399–403.

Macas J, Pozarkova D, Navratilova A, Nouzova M, Neumann P (2000) Two new families of tandem repeats isolated from genus *Vicia* using genomic self-priming PCR. Mol Gen Genet 263: 741–751.

Marciniak K, Bilecka A (1986) Changes in nuclear and nucleolar protein content during the growth and differentiation of root parenchyma cells in plant species with different DNA-endoreplication dynamics. Histochemistry 85: 51–56.

Marie D, Brown SC. 1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. Biol Cell 78: 41–51.

Martel E, De Nay D, Siljak-Yakovlev S, Brown S, Sarr A (1997) Genome size variation and basic chromosome number in pearl millet and fourteen related *Pennisetum* species. J Heredity 88: 139–143.

Maxted N (1995) An Ecogeographical Study of *Vicia* Subgenus *Vicia*. Systematic and Ecogeographic Studies on Crop Genepools, 8. International Plant Genetic Resources Institute, Rome.

Michaelson MJ, Price HJ, Johnston JS, Ellison JR (1991) Variation of nuclear DNA content in *Helianthus annuus* (Asteraceae). Amer J Bot 78: 1238–1243.

Mohanty IC, Mahapatra D, Mohanty S, Das AB (2004) Karyotype analyses and studies on the nuclear DNA content in 30 genotypes of potato (*Solanum tuberosum* L.). Cell Biol Int 28: 625–633.

Morgan ER, Burge GK, Seelye JF, Grant JE, Hopping ME (1995) Interspecific hybridization between *Limonium perigrinum* Bergius and *Limonium purpuratum* L. Euphytica 83: 215–24.

Moscone EA, Baryani M, Ebert I, Greilhuber J, Ehrendorfer F, Hunziker AT (2003) Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by flow cytometry and Feulgen densitometry. Ann Bot 92: 21–29.

Murray BG (2005) When does intraspecific C-value variation become taxonomically significant? Ann Bot 95: 119–125.

Navràtilovà A, Neumann P, Macas J (2003) Karyotype analysis of four *Vicia* species using in situ hybridization with repetitive sequences. Ann Bot 91: 921–926.

Noirot M, Barre P, Duperray C, Hamon S, Kochko A (2005) Investigation on the causes of stoichiometric error in genome size estimation using heat experiments: consequences on data interpretation. Ann Bot 95: 111–118.

Ohri D (1998) Genome size variation and plant systematics. Ann Bot 82: 75–83.

Ohri D (2005) Climate and growth form: the consequences for genome size in plants. Plant Biol (Stuttg) 7: 449–458.

Ohri D, Bhargava A, Chatterjee A (2004) Nuclear DNA amounts in 112 species of tropical hardwoods – new estimates. Plant Biol (Stuttg) 6: 555–61

Ohri D, Fritsch RM, Hanelt P (1998) Evolution of genome size in *Allium* (*Alliaceae*). Plant Syst Evol 210: 57–86.

Pastor-Cavada E, Juan R, Pastor JE, Alaiz M, Giron-Calle J, Vioque J (2008) Antioxidative activity in the seeds of 28 *Vicia* species from Southern Spain. J Food Bioch 35: 1373–1380.

Paux E, Roger D, Badaeva E, Gay G, Bernard M, Sourdille P, Feuillet C (2006) Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B. Plant J 48: 463–474.

Petrov D (1997) Slow but steady: reduction of genome size through biased mutation. Plant Cell 9: 1900–1901.

Poggio L, Rosato M, Chiavarino AM, Naranjo CA (1998) Genome size and environmental correlation in maize (*Zea mays* ssp. *mays*, Poaceae). Ann Bot 82 (Suppl. A): 107–115.

Price HJ (1976) Evolution of DNA content in higher plants. Bot Rev 42: 27–52.

Price HJ, Hodnett G, Johnston JS (2000) Sunflower (*Helianthus annuus*) leaves contain compounds that reduce nuclear propidium iodide fluorescence. Ann Bot 86: 929–934.

Raina SN (1990) Genome organization and evolution in the genus *Vicia*. In: Biological Approaches and Evolutionary Trends in Plants (Ed. S Kawano). Academic Press, London, pp. 183–201.

Raina SN, Bisht MS (1988) DNA amounts and chromatin compactness in *Vicia*. Genetica 77: 65–77.

Raina SN, Narayan RKJ (1984) Changes in DNA composition in the evolution of *Vicia* species. Theor Appl Genet 68: 187–192.

Rayburn AL, Auger JA, Benzinger ES, Hepburn AG (1989) Detection of intraspecific DNA content variation in *Zea mays* L. by flow cytometry. J Exp Bot 40: 1179–1183.

Rayburn AL, Biradar DP, Bullock DG, Nelson RL, Gourmet C, Wetzel JB (1997) Nuclear DNA content diversity in Chinese soybean introductions. Ann Bot 80: 321–325.

Rayburn AL, Wetzel JB (2002) Flow cytometric analyses of intraplant nuclear DNA content variation induced by sticky chromosomes. Cytometry 49: 36–41.

Reeves G, Francis D, Davies MS, Rogers HJ, Hodkinson TR (1998) Genome size is negatively correlated with altitude in natural populations of *Dactylis glomerata*. Ann Bot 82 (Suppl. A): 99–105.

SAS (1997) SAS/STAT Software: Changes and Enhancements through Release 6.12. SAS Institute, Cary, North Carolina, USA.

Smarda P, Bures P (2006) Intraspecific DNA content variability in *Festuca pallens* on different geographical scales and ploidy levels. Ann Bot 98: 665–678.

Smarda P, Bures P (2010) Understanding intraspecific variation in genome size in plants. Preslia 82: 41–61.

Smarda P, Horova L, Bures P, Hralova I, Markova M (2010) Stabilizing selection on genome size in a population of *Festuca pallens* under conditions of intensive intraspecific competition. New Phytol 187: 1195–1204.

Suda J, Kyncl T, Freiova R (2003) Nuclear DNA amounts in Macaronesian angiosperms. Ann Bot 92: 153–164.

Sugiyama S, Yamaguchi K, Yamada T (2002) Intraspecific phenotypic variation associated with nuclear DNA content in *Lolium perenne* L. Euphytica 128: 145–151.

Swift HH (1950) The constancy of desoxyribose nucleic acid in plant nuclei. Proc Natl Acad Sci USA 36: 643–654.

Thomas CAJ (1971) The genetic organization of chromosomes. Ann Rev Genet 5: 237–256.

Tuna M, Vogel KP, Arumuganathan K, Gill KS (2001) DNA contents and ploidy determination of bromegrass germplasm accessions by flow cytometry. Crops Sci 41: 1629–1634.

Turpeinen T, Kulmala J, Nevo E (1999) Genome size variation in *Hordeum spontaneum* populations. Genome 42: 1049–1099.

van de Wouw M, Maxted N, Ford-Lloyd BV (2003) Agro-morphological characterisation of common vetch and its close relatives. Euphytica 130: 281–292.

Vinogradov AE (1999) Intron-genome size relationship on a large evolutionary scale. J Mol Evol 49: 376–384.

Walker DJ, Monino I, Correal E (2006) Genome size in *Bituminaria bituminosa* (L.) C.H. Stirton (Fabaceae) populations: separation of "true" differences from environmental effects on DNA determination. Envir Exp Bot 55: 258–265.

Watson EM (1987) Nuclear DNA content in the Australian Bulbine. Genome 29: 225–235.

Zaitlin D, Pierce AJ (2010) Nuclear DNA content in *Sinningia* (Gesneriaceae); intraspecific genome size variation and genome characterization in *S. speciosa*. Genome 53: 1066–1082.