TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2020) 44: 612-630

doi:10.3906/tar-1910-33

Molecular cytogenetic characterization of common bean (*Phaseolus vulgaris* L.) accessions

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Received: 14.10.2019 • **Accepted/Published Online:** 17.06.2020 • **Final Version:** 08.12.2020

Abstract: With an annual global production of approximately 25 million tons, the common bean (*Phaseolus vulgaris* L), a member of the genus *Phaseolus*, is one of the major protein sources used as food for humans. In this study, it was aimed to investigate the genome size of the common bean genetic resource collection (154 common bean accessions) in Turkey by flow cytometry (FCM) and determine whether geographical variables affected the genome size. In addition, the number and distribution of 5S and 45S ribosomal DNA loci were designated by performing a fluorescence in situ hybridization (FISH) analysis in some of the accessions. The FCM analyses revealed that the mean nuclear DNA content of the accessions varied from 1.28 pg2C-1 to 1.55 pg2C-1 (mean 1.35 pg2C-1), and the differences between these accessions were statistically significant ($P < 0.01$). Intraspecific variation in the genome size was determined, and a positive correlation was found between the altitude and genome size. However, latitude and longitude did not have any statistically significant effect on the genome size. In the principal coordinate analysis, the accessions were divided into 3groups. Based on the results of the FISH analysis performed on 5 different accessions with varying genome sizes, using 5S and 45S rDNA genes as probes, the number of 5S rDNA loci was 4 in the common bean and stable among the common bean accessions, while the number of 45S rDNA loci was highly polymorphic, varying between 6 and 16. Consequently, it was determined in the present study that the genetic resource collection of common bean had a wide variation in terms of genome size and genome organization.

Key words: *Phaseolus vulgaris*, genome size, flow cytometry, geographical variables, fluorescence *in situ* hybridization, gene pool

1. Introduction

The common bean (*Phaseolus vulgaris*, 2n = 2x = 22) is one of the most important legumes worldwide, and it is an important source of nutrients, especially in East Africa and Latin America (Blair et al., 2010). The common bean is a member of the genus *Phaseolus*, which consists of approximately 50 species that are classified into 8 clades (Delgado-Salinas et al., 2006). The region encompassing Ecuador and northern Peru is considered to be the origin of the common bean (Kami et al., 1995), and it has subsequently been dispersed both northwards and southwards due to the establishment of the Mesoamerican and Andean gene pools, respectively (Gepts, 1998). The divergence of the gene pools occurred prior to the domestication events within the individual gene pools (Mamidi et al., 2013; Schmutz et al., 2014). After the independent domestication events, local adaptation created diverse landraces (Iwata-Otsubo et al., 2016), which may have possibly caused morphological and genetic variability.

Since the common bean is an important nutrient, it has economic importance; therefore, breeding efforts have focused on the global development of disease-resistant bean species with higher yields (Castro-Guerrero et al., 2016). One of the most important stages of breeding studies is the study of genetic diversity, and molecular markers have been used to contribute to these studies in *P. vulgaris*. To date, 390 SSR markers have been identified in beans, and new SSRs are being developed (Blair et al., 2012, Nadeem et al., 2018). Since the chromosomes of the common bean are small in size and morphologically similar, chromosome studies gained momentum after the development of the fluorescence in situ hybridization (FISH) technique. Two types of ribosomal RNA genes, 5S rDNA and 45S rDNA, encoding 18S–5.8S–25S ribosomal RNAs, are widely used as probes for FISH (Maluszynska, 2002). Studies using these probes and cytogenetic maps showed differences between the Andean and Mesoamerican gene pools. For example, in their study, Pedrosa-Harand et al. (2006)

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explained that the difference between the gene pools was the distribution of 7 45S rDNA loci in the Andean varieties and 3 45S rDNA loci in the Mesoamerican. In later studies, 25-bp oligonucleotide probes (CentPv1 and CentPv2) and their variants (CentPv1_A and CentPv2_A) were designed for use in karyotype maps (Iwata et al., 2013). Furthermore, Iwata-Otsubo et al. (2016) performed FISH with a mixture of oligonucleotide probes (Cy5-CentPv1, TEX615-CentPv2, FAM-CentPv1_A, FAM-CentPv2_A, and TEX615-khipu) and nick-translated a 5S rDNA probe labeled with fluorescein.

Numerous molecular cytogenetic studies have been conducted to investigate the chromosomal structure of the common bean, which included the number of rDNA loci and distribution, mapping of single and repetitive BAC clones, and development of cytogenetic maps (Moscone et al., 1999; Pedrosa-Harand et al., 2006, 2009; Fonsêca et al., 2010; Bonifacio et al., 2012). In addition, the genome size of the accessions is also important in cytogenetic studies since the core DNA content is species-specific (Bennett and Leitch, 1995). Therefore, genome size information is an important issue in ploidy analysis, genome analysis, taxonomy, evolution, and breeding studies (Rees and Walters, 1965; Ohri, 1998; Özkan et al., 2003; Savaş Tuna et al., 2017, 2019). Today, the flow cytometry method is used to determine genome size; however, the genetic resources of the common bean have not been examined in detail using the flow cytometry method (FCM). To date, a very limited number of studies have been conducted, in which only a few common bean accessions coexisted or were analyzed with other species (Ayonoadu, 1974; Bennet et al., 1982; Castagnaro et al., 1990; Arumuganathan and Earle, 1991; Beletti et al., 1997; Mekki et al., 2007; Labotan et al., 2018).

The purposes of this study were to (i) determine the genome size of accessions in the common bean collection from various regions of the world, (ii) examine the effects of altitude, latitude, and longitude on the genome size, and (iii) identify the number and chromosomal distribution of 5S and 45S rDNA loci by performing a FISH analysis in some accessions and determine their gene pools.

2. Material and methods

2.1. Plant material

All of the common bean accessions analyzed in this study are listed in Table 1. Accessions of the common bean obtained from different locations in Turkey, as well as abroad, were used as material.

2.2. Growing of the plant material

The seeds of common bean accessions were grown in rows using the dibbling method in an experimental field at the Agriculture Faculty at Namık Kemal University, Tekirdağ, Turkey. Each row consisted of 6 plants of the same accession, and the distance between and within the rows was 50 × 30 cm for the climbing types and 80 × 40 cm for the dwarf types.

2.3. Determination of the genome size

The genome size of the accessions was determined using the FCM. Suspensions of intact nuclei were prepared using commercial kits manufactured by Sysmex Partec GmbH (Münster, Germany). The fresh leaf tissues of each common bean (20 mg) and a standard leaf tissue (*Lycopersicon esculentum*, 25 mg) were simultaneously chopped in a petri dish with 0.5 mL of extraction buffer. The homogenized solution was transferred into a glass tube through a 30-µm filter, and then, 2 mL of staining buffer (CyStain PI absolute P) was added to each tube. Before the FCM analysis, the samples were incubated at room temperature in the dark for at least 30 min. A total of 5 seedlings were analyzed individually for each accession, and 5000 nuclei were analyzed in each sample. These samples were run through a Partec CyFlow space flow cytometer (Sysmex Partec GmbH), and the results were analyzed by FloMax analysis software specifically dedicated to this cytometer. The genome size of the common bean seedlings was calculated based on the relative positions of the G1 peaks of the sample and standard. Only the results of samples that had a coefficient of variation (CV) that was less than 3% were used in the calculations. The standard deviation was calculated for the genome size of each accession using relevant measurements.

2.4. Chromosome preparation

Root tips were harvested from germinating seeds and treated in 2 mM of 8-hydroxyquinoline at 10 °C for 20–24 h, followed by fixation in ethanol:acetic acid (3:1, v/v) (Pedrosa-Harrand et al., 2006). Somatic chromosome preparations were performed as described by Jenkins and Hasterok (2007). First, the obtained roots were washed in 0.01M of 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-Aldrich Corp., St. Louis, MO, USA), 1% (w/v) cellulase (Calbiochem, San Diego, CA, USA), and 1% (w/v) cellulase Onozuka R-10 (Serva) for 3 h. After this, 1 dissected meristem of each sample was transferred onto a slide in a drop of 45% acetic acid and then, a coverslip was placed on the slide and squashed. The coverslips were removed from the slides, and the preparations were placed in the freezer at −80 °C. The prepared samples were fixed in ethanol:glacial acetic acid (3:1), dehydrated in absolute ethanol, and air-dried.

2.5. DNA probes

In this study, 5S rDNA (pTa794) (Gerlach and Dyer, 1980) and 45S rDNA (Unfried and Gruendler, 1990) were used as probes. The 5S rDNA was labeled using PCR withdigoxigenin-11-dUTP (F. Hoffman-La Roche Ltd.,

Table 1. Accession code number, accession name, location, growing pattern, latitude, longitude, altitude, mean genome size and standard deviation, and significance group of the common bean accessions used as material in the study.

Accession code number	Accession name	Location	Growing pattern	Latitude	Longitude	Altitude (m)	Mean genome size ($pg2C^{-1}$), ± SD	Significance group
70	Bombay	Bolu Mudurnu, Turkey	Climbing	40°43′49.0″	31°37′12.4″	840	1.548 ± 0.014	a
60	Piyazlık	Kırklareli, Turkey	Climbing	41°44′07.4″	27°13′29.7″	203	1.546 ± 0.011	a
134	Limka	Holland seed, Holland	Climbing	-	-	-	1.440 ± 0.018	ab
11	Alacalı Ayşe	Bozdağ-İzmir,Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.434 ± 0.065	abc
5	Sürmeli barbunya	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.408 ± 0.043	bcd
138	E-Z pick	Johnny seed, USA	Dwarf	-	-	-	1.402 ± 0.055	b-e
129	Güz fasulyesi village variety	Trabzon, Turkey	Dwarf	40°44′56.3″	40°00′04.0″	37	1.400 ± 0.047	b-f
100	Sırık fasulye	Turkey	Climbing	-	-	-	1.400 ± 0.030	b-f
141	Maxi bell	Johnny seed, USA	Dwarf	-	-	-	1.400 ± 0.024	b-f
8	Sürmeli	Birgi-İzmir, Turkey	Climbing	38°15′29.2″	28°04′26.1″	326	1.396 ± 0.049	b-g
137	Yerli 23	Antalya, Turkey	Climbing	36°56′04.9″	30°44′07.0″	40	1.396 ± 0.046	b-g
112	TR65047	Manisa (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.396 ± 0.035	b-g
107	TR39074	Aydın (Aegean Agricultural Research Institute),Turkey	Climbing	-	-	-	1.394 ± 0.039	b-h
111	TR33486	Kırklareli (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.394 ± 0.031	b-h
122	Emergo155	Kienpenkerl, Germany	Climbing	-	-	-	1.388 ± 0.044	b-i
106	Sırık fasulye	Isparta, Turkey	Climbing	37°45′34.0″	30°32′39.4″	1035	1.388 ± 0.035	b-i
123	Purple teepe 141	Kienpenkerl, Germany	Dwarf	-	-	-	1.388 ± 0.028	b-i
114	TR43097	Çanakkale (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.384 ± 0.038	b-k
131	Solista	Marshalls, England	Climbing	-	-	-	1.384 ± 0.018	b-k
161	Yunus 90	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Dwarf	-	-	-	1.382 ± 0.043	b-k
98	Fasulye çalı	Torbalı-İzmir, Turkey	Climbing	38°14′46.4″	27°29′17.3″	35	1.380 ± 0.033	b-k
7	Sürmeli-Alacalı	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.378 ± 0.049	b-k
71	Dermason	Erzincan, Turkey	Climbing	37°53′29.1″	32°27′15.4″	1214	1.378 ± 0.039	b-k
140	Dellinel 3155	Twinplus, Holland	Dwarf	-	-	-	1.378 ± 0.037	b-k
177	Terzibaba	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.378 ± 0.032	b-k
30	Yerli Ayşe	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.376 ± 0.015	b-k
155	Alman Ayşe (Commercial)	Altın tohum, Turkey	Climbing	-	-	-	1.374 ± 0.039	b-k
99	Kuru Alman	Torbalı-İzmir,Turkey	Climbing	38°14′46.4″	27°29′17.3″	35	1.374 ± 0.028	b-k
78	Köy Pop 4	Havsa-Edirne, Turkey	Dwarf	41°32′54.8″	26°49′00.1″	26	1.374 ± 0.018	b-k

Table 1. (Continued).

36	Simav fasulye	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.372 ± 0.045	b-l
44	Alman Ayşe tipi	Gölcük, Turkey	Climbing	38°19′26.2″	28°06′08.7″	1050	1.372 ± 0.040	b-l
51	Ayşekadın	Edirne, Turkey	Dwarf	41°40′08.4″	26°33′38.6″	41	1.372 ± 0.030	b-l
33	Ayşe2	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.372 ± 0.028	b-l
95	Sırık fasulye	Turkey	Climbing	-	-	-	1.370 ± 0.047	b-l
96	Sırık fasulye	Torbalı-İzmir, Turkey	Climbing	38°14′46.4″	27°29′17.3″	35	1.370 ± 0.029	b-l
145	Mexican bean	USA	Dwarf	23°53′12″	102°34′07″	1532	1.368 ± 0.053	b-l
121	Helda	Vilmorin, Turkey	Climbing	-	-	-	1.368 ± 0.041	b-l
86	Alacalı fasulye	Isparta, Turkey	Climbing	37°45′34.0″	30°32′39.4″	1035	1.368 ± 0.038	b-l
175	Zülbiye	Black Sea Agricultural Research Institute/ Samsun, Turkey	Dwarf	-	-	-	1.366 ± 0.054	b-m
110	TR28094	Muğla (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.366 ± 0.037	b-m
66	Beyaz renkli	Niğde, Turkey	Climbing	37°58′19.5″	34°39′59.9″	1230	1.366 ± 0.033	b-m
169	Şehriali 90	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Dwarf	-	-	-	1.364 ± 0.046	b-m
108	TR43497	İstanbul (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.364 ± 0.023	b-m
68	Horoz	Antalya, Turkey	Climbing	36°56′04.9″	30°44′07.0″	40	1.362 ± 0.057	b-m
113	TR38090	Balıkesir (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.362 ± 0.050	b-m
81	Köy Pop 3	Havsa-Edirne, Turkey	Dwarf	41°32′54.8″	26°49′00.1″	26	1.362 ± 0.019	b-m
89	İspir fasulye	Karadeniz, Turkey	Climbing	41°10′04.8″	36°25′24.0″	554	1.360 ± 0.035	b-m
150	Serra (Commercial)	May, Turkey	Climbing	-	-	-	1.360 ± 0.020	b-m
118	Volare	May, Turkey	Climbing	-	-	-	1.360 ± 0.007	b-m
104	Sırık fasulye	İzmir, Turkey	Climbing	38°16′23.9″	27°07′51.6″	2	1.358 ± 0.034	b-m
83	Yerli yerel	Ovacık-Lüleburgaz-Kırklareli, Turkey	Climbing	41°23′58.1″	27°21′02.8″	66	1.358 ± 0.023	b-m
174	Göynük 98	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Dwarf	-	-	-	1.358 ± 0.020	b-m
143	Piyazlık	Peru	Climbing	8°00′05″	75°01′10″	165	1.358 ± 0.013	b-m
144	Taze fasulye	Bulgaria	Dwarf	42°57′48″	25°28′37″	179	1.356 ± 0.045	c-m
103	Sırık fasulye	Isparta, Turkey	Climbing	37°45′34.0″	30°32′39.4″	1035	1.356 ± 0.038	c-m
139	Yerli 4	Erzincan, Turkey	Climbing	39°35′32.6″	39°04′56.6″	1274	1.356 ± 0.031	c-m
101	Oturak fasulye	Turkey	Dwarf	-	-	-	1.354 ± 0.043	c-m
109	TR62091	İzmir (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.354 ± 0.037	c-m
73	Küçük dermason	Konya, Turkey	Climbing	37°53′29.1″	32°27′15.4″	1016	1.354 ± 0.023	c-m

Table 1. (Continued).

170	Önceler	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Dwarf	-	-	-	1.354 ± 0.015	c-m
9	Alacalı Ayşe	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.352 ± 0.037	c-m
28	Kumbar	Ödemiş-İzmir, Turkey	Climbing	38°13′42.1″	27°58′27.8″	123	1.352 ± 0.032	c-m
132	Algarve	Marshalls, England	Climbing	-	-	-	1.352 ± 0.025	c-m
42	Kuru fasulye	Gölcük, Turkey	Dwarf	38°19′26.2″	28°06′08.7″	1050	1.352 ± 0.021	c-m
49	Boncuk Ayşe	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.350 ± 0.055	d-m
52	Gino tipi	Bandırma, Turkey	Dwarf	40°20′50.6″	27°57′10.5″	20	1.350 ± 0.055	d-m
117	TR57759	Tekirdağ (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.350 ± 0.043	d-m
105	Oturak fasulye	Isparta, Turkey	Dwarf	37°45′34.0″	30°32′39.4″	1035	1.350 ± 0.040	d-m
148	Gino (Commercial)	May, Turkey	Dwarf	-	-	-	1.348 ± 0.079	d-m
119	Taze fasulye	Korkuteli- Antalya, Turkey	Dwarf	37°11′00.0″	30°02′00.7″	1020	1.348 ± 0.052	d-m
149	Elinda (Commercial)	May, Turkey	Dwarf	-	-	-	1.348 ± 0.032	d-m
40	Horoz fasulye	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.348 ± 0.031	d-m
63	Sırık boncuk	Tokat, Turkey	Climbing	40°15′39.5″	36°15′14.2″	623	1.346 ± 0.059	d-m
164	Mecidiye (barbunya)	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.346 ± 0.048	d-m
55	Sarıkız	Kırklareli, Turkey	Dwarf	41°44′07.4″	27°13′29.7″	203	1.346 ± 0.039	d-m
84	Ballıhoca köyü	Muratlı-Tekirdağ, Turkey	Climbing	40°59′16.1″	27°10′10.0″	92	1.346 ± 0.016	d-m
97	Sırık fasulye	Turkey	Climbing	-	-	-	1.344 ± 0.033	d-m
65	Krem renkli	Niğde, Turkey	Climbing	37°58′19.5″	34°39′59.9″	1230	1.344 ± 0.033	d-m
171	Karacaşehir 90	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Climbing	-	-	-	1.342 ± 0.075	d-m
41	Alman	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.342 ± 0.037	d-m
172	Bulduk	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Climbing	-	-	-	1.342 ± 0.027	d-m
25	Boncuk Ayşe (I)	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.340 ± 0.045	d-m
27	Ayşe kadın	Edirne, Turkey	Dwarf	41°40′34.0″	26°34′05.8″	41	1.340 ± 0.010	d-m
62	Sırık 40 günlük	Tokat, Turkey	Climbing	40°15′39.5″	36°15′14.2″	623	1.338 ± 0.037	d-m
163	Hınıs variety (şeker)	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.336 ± 0.046	d-m
45	Fasulye	Bozdağ-İzmir, Turkey	Dwarf	38°20′25.5″	28°04′36.4″	1135	1.334 ± 0.013	d-m
39	Kaynarca	Kırklareli, Turkey	Climbing	41°44′07.4″	27°13′29.7″	203	1.334 ± 0.011	d-m
26	Sarıkız fasulye	Bandırma, Turkey	Dwarf	40°20′50.6″	27°57′10.5″	20	1.332 ± 0.045	d-m
6	Barbunya	Gölcük, Turkey	Climbing	38°19′26.2″	28°06′08.7″	1050	1.332 ± 0.044	d-m
74	Ebeköy	Bursa, Turkey	Climbing	40°11′07.5″	29°02′45.6″	155	1.332 ± 0.042	d-m

Table 1. (Continued).

94	Oturak fasulye	Turkey	Dwarf	-	-	-	1.332 ± 0.028	d-m
133	Magnum Village Variety	Turkey	Dwarf	-	-	-	1.332 ± 0.026	d-m
22	Krem boncuk	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.332 ± 0.025	d-m
90	Taze fasulye	İzmir, Turkey	Dwarf	38°16′23.9″	27°07′51.6″	2	1.332 ± 0.023	d-m
47	Boncuk Ayşe	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.332 ± 0.019	d-m
4	Alacalı Ayşe	Gölcük, Turkey	Climbing	38°19′26.2″	28°06′08.7″	1050	1.332 ± 0.017	d-m
173	Akın	Geçit Kuşağı TAE/ Eskişehir, Turkey	Dwarf	-	-	-	1.332 ± 0.016	d-m
156	Sarıkız (Commercial)	Neobi	Dwarf	-	-	-	1.332 ± 0.016	d-m
102	Kuru fasulye	Torbalı-İzmir, Turkey	Dwarf	38°14′46.4″	27°29′17.3″	35	1.330 ± 0.044	d-m
35	Sarı şeker	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.330 ± 0.034	d-m
166	Yakutiye 98	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.330 ± 0.031	d-m
56	Horoz	Bandırma, Turkey	Dwarf	40°20′50.6″	27°57′10.5″	20	1.330 ± 0.030	d-m
79	Köy Pop 1	Havsa-Edirne, Turkey	Dwarf	41°32′54.8″	26°49′00.1″	26	1.328 ± 0.037	d-m
69	Şeker fasulye	Turkey	Climbing	-	-	-	1.328 ± 0.032	d-m
82	Horoz-local population	Uzunköprü-Edirne, Turkey	Dwarf	41°16′15.6″	26°41′51.9″	10	1.328 ± 0.004	d-m
64	Sarıkız bodur	Tokat, Turkey	Dwarf	40°15′39.5″	36°15′14.2″	623	1.326 ± 0.041	d-m
46	Horoz fasulye	Bandırma, Turkey	Dwarf	40°20′50.6″	27°57′10.5″	20	1.326 ± 0.031	d-m
19	Barbunya	Kırklareli, Turkey	Climbing	41°44′07.4″	27°13′29.7″	203	1.326 ± 0.027	d-m
67	Alacalı fasulye	Antalya, Turkey	Climbing	36°56′04.9″	30°44′07.0″	40	1.326 ± 0.027	d-m
54	Boncuk Ayşe	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.326 ± 0.024	d-m
1	Barbunya	Gölcük, Turkey	Climbing	38°16′18.6″	28°00′11.4″	1050	1.326 ± 0.023	d-m
147	Sarıkız (Commercial)	Küçükçiftlik, Turkey	Dwarf	-	-	-	1.326 ± 0.011	d-m
57	Çine 1	Aydın-Çine, Turkey	Dwarf	37°36′50.3″	28°03′40.2″	87	1.324 ± 0.052	e-m
24	Boncuk Ayşe (III)	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.324 ± 0.040	e-m
124	Sarıkız Village Variety	Turkey	Dwarf	-	-	-	1.324± 0.030	e-m
77	Bulgaristan fasulyesi	Çorlu-Tekirdağ, Turkey	Dwarf	41°08′58.3″	27°48′29.7″	193	1.324 ± 0.029	e-m
38	Oturak fasulye	Gölcük, Turkey	Dwarf	38°19′26.2″	28°06′08.7″	1050	1.324 ± 0.024	e-m
157	40 günlük Amerikan Atlantis (Commercial)	Arzuman, Turkey	Dwarf	-	-	-	1.324 ± 0.021	e-m
92	Oturak fasulye	Turkey	Dwarf	-	-	-	1.324 ± 0.020	e-m
153	Yalova 17 (Commercial)	Atatürk Horticultural Central Research Instıtute/Yalova,Turkey	Dwarf	-	-	-	1.324 ± 0.016	e-m
12	Barbunya	Gölcük, Turkey	Climbing	38°19′26.2″	28°06′08.7″	1050	1.322 ± 0.053	e-m
142	Provider	Johnny seed, USA	Dwarf	-	-	-	1.322 ± 0.023	e-m
3	Barbunya	Gölcük, Turkey	Climbing	38°16′18.6″	28°00′11.4″	1050	1.322 ± 0.016	e-m
20	Barbunya	Kırklareli, Turkey	Climbing	41°44′07.4″	27°13′29.7″	203	1.320 ± 0.041	e-m

Table 1. (Continued).

91	Sırık fasulye	İzmir, Turkey	Climbing	38°16′23.9″	27°07′51.6″	2	1.320 ± 0.031	e-m
2	Yerli Barbunya	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.320 ± 0.029	e-m
13	Sürmeli Ayşe kadın	Gölcük, Turkey	Dwarf	38°19′26.2″	28°06′08.7″	1050	1.320 ± 0.024	e-m
72	İspir fasulyesi	Karadeniz, Turkey	Climbing	40°29′26.0″	41°00′08.1″	1241	1.320 ± 0.024	e-m
125	Barbunya	Turkey	Climbing	-	-	-	1.318 ± 0.024	f-m
21	Barbunya	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.316 ± 0.058	g-m
14	Barbunya	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.316 ± 0.028	g-m
31	Sarıkız	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.316 ± 0.028	g-m
10	Yerli barbunya	Bozdağ-İzmir, Turkey	Climbing	38°20′25.5″	28°04′36.4″	1135	1.316± 0.024	g-m
162	Village Variety (şeker)	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.314 ± 0.045	g-m
61	Soma kuru fasulye	Soma,Turkey	Dwarf	39°11′17.3″	27°36′33.4″	175	1.312 ± 0.043	h-m
115	TR43574	Sakarya (Aegean Agricultural Research Institute), Turkey	Climbing	-	-	-	1.312 ± 0.029	h-m
88	Alacalı Ayşe	Burdur, Turkey	Climbing	37°43′09.8″	30°15′02.0″	950	1.312 ± 0.023	h-m
120	Yerli2	Bursa, Turkey	Dwarf	40°11′07.5″	29°02′45.6″	155	1.312 ± 0.021	h-m
15	Barbunya	Çine-Aydın, Turkey	Climbing	37°36′50.3″	28°03′40.2″	87	1.310 ± 0.036	i-m
136	Yerli 25 Village Variety	Beypazarı, Turkey	Climbing	40°09′14.0″	31°56′09.8″	700	1.310 ± 0.030	i-m
128	Ayşe kadın Village Variety	Trabzon, Turkey	Climbing	41°00′22″	39°42′59″	37	1.310 ± 0.018	i-m
158	Demir Magnumax (Commercial)	Arzuman, Turkey	Dwarf	-	-	-	1.308 ± 0.071	i-m
159	Simbo Saddle (Commercial)	Arzuman, Turkey	Dwarf	-	-	-	1.308 ± 0.046	i-m
85	Taze fasulye	Havsa-Edirne, Turkey	Dwarf	41°32′54.8″	26°49′00.1″	26	1.308 ± 0.032	i-m
53	Boncuk Ayşe	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.308 ± 0.027	i-m
176	Akdağ	Black Sea Agricultural Research Institute/ Samsun, Turkey	Dwarf	-	-	-	1.308 ± 0.023	i-m
165	Aras 98	East Anatolian Agricultural Research Institute/Erzurum, Turkey	Dwarf	-	-	-	1.306 ± 0.029	i-m
87	Barbunya	Isparta, Turkey	Climbing	37°45′34.0″	30°32′39.4″	1035	1.304 ± 0.025	klm
48	Hatay taze oturak	Bandırma, Turkey	Dwarf	40°20′50.6″	27°57′10.5″	20	1.304 ± 0.021	klm
23	Barbunya	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.304 ± 0.021	klm
168	Eskişehir 855	Transitional Zone Agricultural Institute/ Eskişehir, Turkey	Dwarf	-	-	-	1.302 ± 0.046	klm
18	Barbunya	Aydın-Çine, Turkey	Climbing	37°36′50.3″	28°03′40.2″	87	1.302 ± 0.034	klm
17	Bodur barbunya	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.290 ± 0.052	lm
16	Sırık barbunya	Bandırma, Turkey	Climbing	40°20′50.6″	27°57′10.5″	20	1.284 ± 0.015	m
Mean: 1.348 Max: 1.548 Min: 1.284 HKO:0.002								

Basel, Switzerland). The other probe was a 2.3-kb ClaI subclone of the 25S rDNA coding region of Arabidopsis thaliana (Unfried and Gruendler, 1990), which was labeled by nick translation usingtetramethyl-rhodamine-5-dUTP (F. Hoffman-La Roche Ltd.). This probe was used to detect the localization of 18S–5.8S–25S rRNA genes (45S rDNA) in the chromosomes (Hasterok et al., 2006).

2.6. FISH analysis

The procedure was performed according to that reported by Hasterok et al. (2006) with some modifications. The slides were treated with RNase (100 µg/mL) in 2 × saline sodium citrate (SSC) at 37 °C for 1 h, and then washed in 2 × SSC and dehydrated in ethanol (5 min, 2 times). The hybridization mixture was prepared for the r DNA probes. The hybridization mixture consisted of 50% deionized formamide, 10% dextran sulfate, 2 × SSC, 0.5% sodium dodecyl sulfate, salmon sperm-blocking DNA (50–100 times excess of the labeled probe), and ~3 ng/µL (100–150 ng/slide) of each labeled probe DNA. To decrease the cross-hybridization of the r DNA probes, the sheared and unlabeled total nuclear DNA of the complementary genome was added as a blocking DNA. The hybridization mixture was denatured at 85 °C for 10 min, and then applied to the chromosome preparations. The slides and DNA probes were denatured together at 70 °C for 5 min in an in situ thermal cycler (Hybaid Ltd., London, UK) and subsequently allowed to hybridize overnight in a humid chamber at 37 °C. Following this process, the slides were washed in 10% formamide in 0.1 × SSC (2 × 5 min, 42 °C). The immunodetection of digoxigenated probes was undertaken with fluorescein isothiocyanate-conjugated antidigoxigenin antibodies (F. Hoffman-La Roche Ltd.). The counterstaining and mounting of the dehydrated preparations were made using 2.5 g/mL of DAPI in a Vectashield antifade buffer (Vector Laboratories Inc., Burlingame, CA, USA).

2.7. Image capturing and processing

Preparations were examined under an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan) and images of the cells with well-distributed mitotic chromosomes were taken usinga Spot RT Slider CCD digital camera (SPOT Imaging, Sterling Heights, MI, USA) attached to the microscope. Image processing and superimposition were performed using Wasabi (Hamamatsu Photonics K.K., Shizuoka, Japan).

2.8. Statistical analysis

To test the statistical significance of the differences between the genome sizes of the accessions, the variance analysis and Duncan test were performed. Correlation and regression analyses were conducted in order to determine the relationship between the altitude, latitude, longitude, and the genome size. In addition, principal coordinate analysis (PCoA) was performed considering all of the data. The XLSTAT 2020.1.3.65335 analysis program (Addinsoft Inc., Paris, France) was used in all of the analyses.

3. Results and discussion

As a result of the chromosome analysis, it was determined that all of the accessions had 22 chromosomes (2n = 2x = 22) (Figures 1–3). In this study, the genome size of 154 accessions was determined using the FCM (Table 1). Good-quality G1 peaks were obtained with CVs lower than 3%, indicating the sensitivity of the measurements (Figures 1–3). The mean genome size of the common bean accessions varied between 1.28 and 1.55 pg2C^{-1},while the species mean was calculated as 1.35 pg2C^{-1} (Table 1). Previous studies detected the genome size of the common bean as 3.7 pg2C^{-1} (Ayonoadu, 1974), 2.7 pg2C^{-1}(Bennett et.al, 1982), 1.32 pg2C^{-1} (Arumuganathan and Earle, 1991), 1.40–1.53 pg2C^{-1}(Nagl and Treviranus, 1995), 1.39 pg2C^{-1} (Andean accessions), 1.41 pg2C^{-1} (Mesoamerican accessions) (Beletti et al., 1997), and 1.58 pg2C^{-1} (Barow and Meister, 2003). Castagnaro et al. (1990) reported that the genome size of the wild common bean was 1.71 pg2C^{-1}, whereas the cultivated common bean had different values, such as 1.56, 1.63, 1.69, and 1.79 pg2C^{-1}. In another study involving 9 accessions, it was reported that the genome size of the common bean varied between 2.65 and 4.96 pg2C^{-1} (Mekki et al., 2007). It was clear that the results obtained from the current study were similar to some of the previous studies, while different from others. The reasons for the differences can be attributed to the use of different methods, techniques, internal standards and accessions, or technical problems (Dolezel and Bartos, 2005).

Moreover, the average genome sizes of the climbing- and dwarf-type common bean accessions were very similar; 1.35 and 1.34 pg2C^{-1}, respectively. However, variability was higher in the climbing types (1.55–1.28 pg2C^{-1}) when compared to the dwarf types (1.30–1.40 pg2C^{-1}). Two of the climbing-type accessions (60 and 70) had a significantly higher mean genome size (1.55 pg) when compared to the other accessions examined in the study. Therefore, these 2 accessions were further investigated with the FISH method. The accession (16) with the lowest mean genome size (1.28 pg2C^{-1}) was also a climbing-type. Based on the results from the analyses, intraspecific genome size variations in the common bean were detected. These variations were statistically significant ($P < 0.01$), and the accessions formed different groups based on the Duncan test (Table 1). The causes of the intraspecific variation have been previously investigated by a number of studies. While Bennetzen (2007), Feuillet and Keller (2002), and Sharma and Raina (2005) suggested that the intraspecific variation was caused by the number of repetitive DNA sequences and the accumulation of retrotransposons, Greilhuber

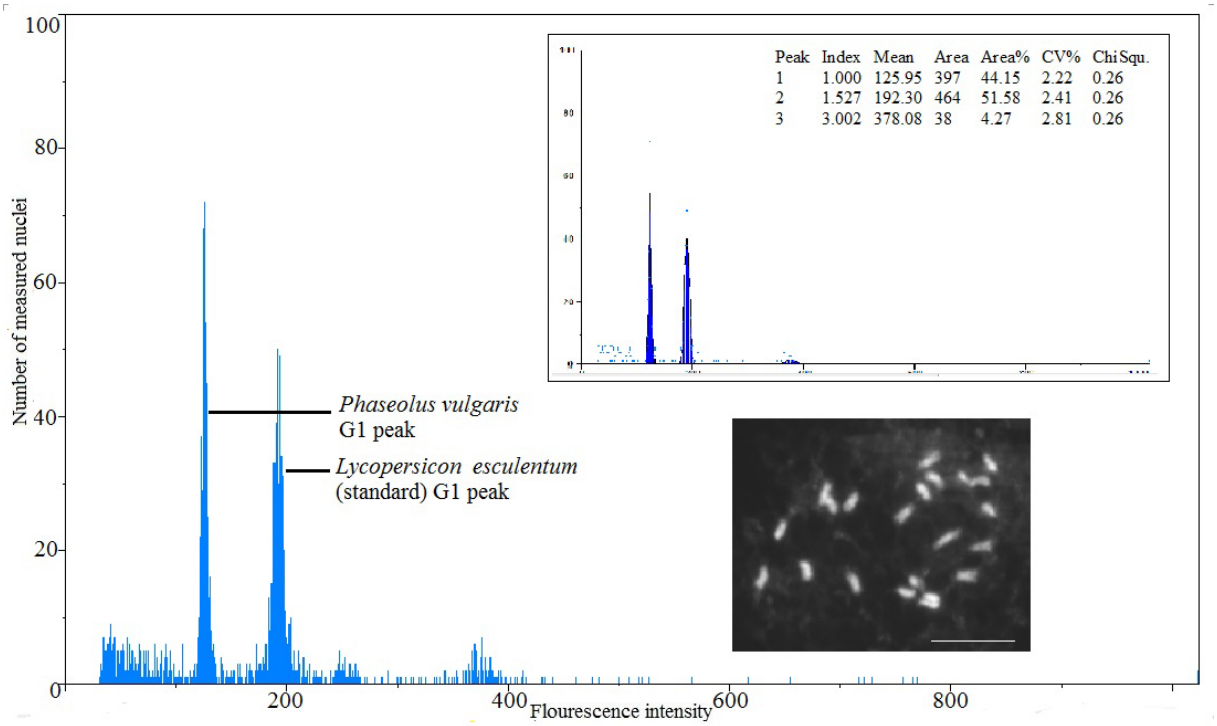

Figure 1. Relative positions of the G1 peaks of *Phaseolus vulgaris* L. (sample number: 91–4) and internal standard (*L. esculentum* Mill.) plants and mitotic chromosomes of the accession (genome size: 1.28 pg2C^{-1}, scale bars = 5 µm).

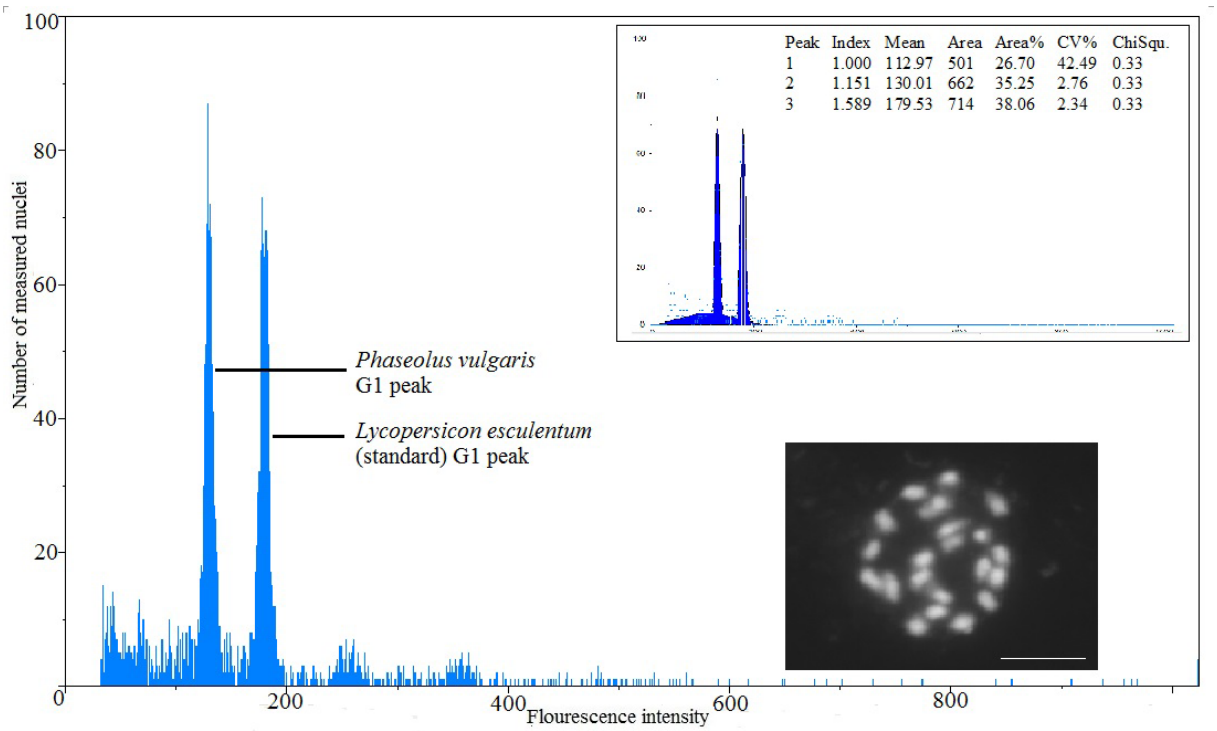

Figure 2. Relative positions of the G1 peaks of *Phaseolus vulgaris* L. (sample number: 109–2) and internal standard (*L. esculentum* Mill.) plants and mitotic chromosomes of the accession (genome size: 1.41pg2C^{-1}, scale bars = 5 µm).

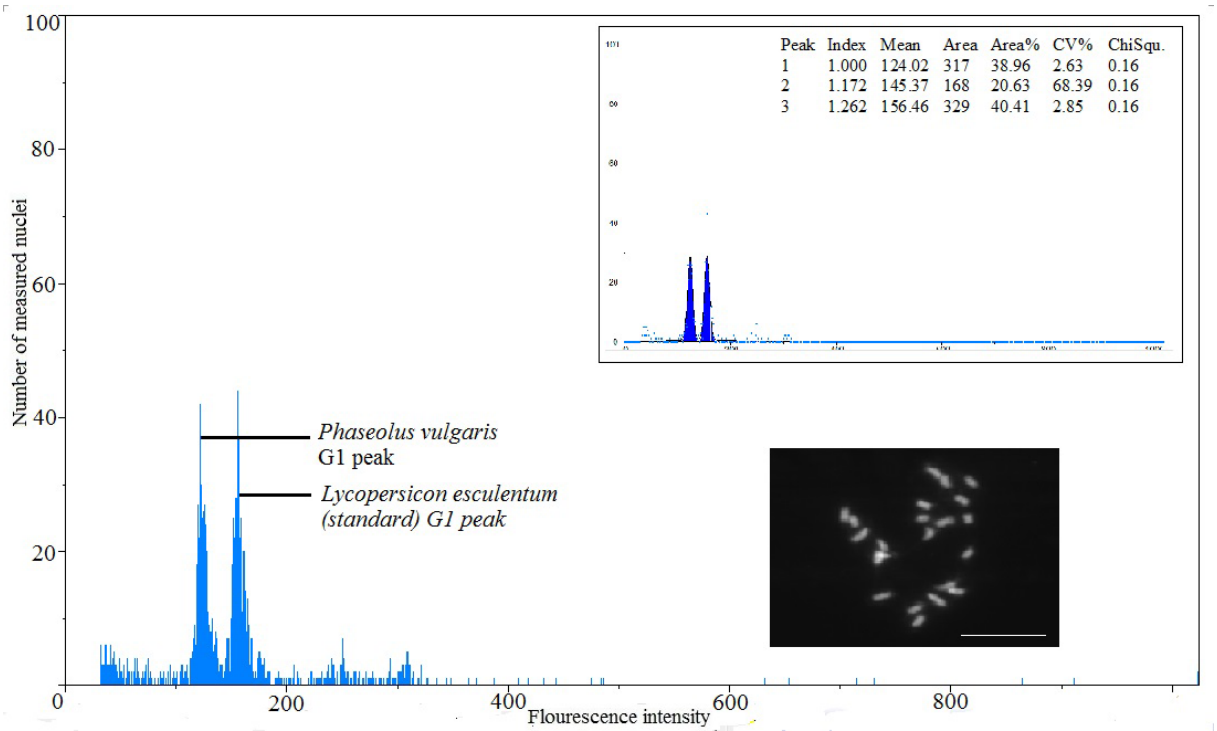

Figure 3. Relative positions of the G1 peaks of *Phaseolus vulgaris* L. (sample number: 60–4) and internal standard (*L. esculentum* Mill.) plants and mitotic chromosomes of the accession (genome size: 1.55 pg2C^{-1}, scale bars = 5 µm).

(2005) and Gregory (2005) considered that it was affected by the chromosome number (polyploidy, aneuploidy), chromosome size, incorrect classification, or insufficient standardization. Knight et al. (2005) found that ecological and geographic changes had an effect. In addition, it has been shown that differences in climate (especially precipitation and temperature) and geography (location, region, altitude, and latitude) are associated with genome size variations and ploidy levels (Manzadena et al., 2012; López-Alvarez et al., 2015; Bareither et al., 2017; Savaş Tuna et al., 2017, 2019; Souza et al., 2019). It has also been explained that geographical isolation (Pecinka et al., 2006; Wang et al., 2013), geographic distance (Savaş Tuna et al., 2019), deletion and duplication (Vlasova et al., 2016), and ecological selection (Wang et al., 2013) were effective in genome size variations.

When the Duncan test results were examined, it was observed that the accessions formed different groups (Table 1). Looking at these groups, it was determined that the samples collected from the same location were in the same group (25, 26, 35, 40, 46, 47, 49, 52, 54, and 56; 3, 12, 13, and 38; and 62–64) or those collected from the same location were in distant groups (7, 9, 10, 14, and 31; 19, 39, 55, and 60; and 78, 79, 81, and 85). However, in some cases, the samples collected from different locations were detected to be in the same group (60 and 70; 100, 129, and 141; and 106, 122,and 123), showing that the genome sizes of the accessions were affected by their locations, but this effect was not statistically significant. For *Helianthus* sp., *H. perforatum*,and *B. hyridum*, it was found that the location, i.e. the area where the plant was collected, had no effect on genome size variations (Sims and Price, 1985; Savaş Tuna et al., 2017, 2019). Turkey is not an origin center for *Helianthus*, and the common bean has been domesticated, has commercial varieties, and is cultivated by farmers for nutritional and commercial purposes. Therefore, the effect of location on genome size variations may not be significant.

In the present study, the effect of altitude on the genome size was investigated using correlation and regression analyses (Figure 4). There was a positive correlation (0.207*) between the altitude and genome size in the common bean ($P = 0.042$), indicating statistical significance ($P < 0.05$). Accordingly, the average genome size increased as the altitude increased. In some previous studies, a positive or negative correlation was reported between the altitude and genome size. For instance, researchers have described that in Indian maize populations (Rayburn and Auger, 1990), *Vicia faba* (Ceccarelli et al., 1995), *Dasypyrum villosum* (Caceres et al., 1998), tetraploid *Festuca pallens* (Smarda and Bures, 2006), *O. pumila* and *A. montbretiana* (Hoffmann et al., 2010), *Pinus yunnanensis* (Wang et al., 2013), *Hypericum perforatum* (Savaş Tuna et al., 2017), *Allium populations* (Guo et al., 2018), *Zea mays* (Bilinski

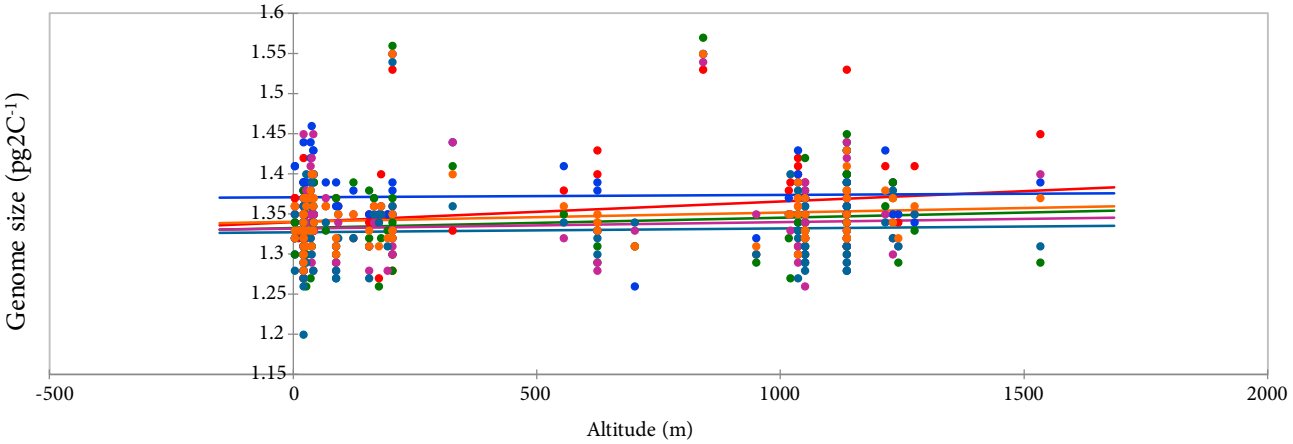

Figure 4. Results of the regression analysis between the altitude and genome size.

et al., 2018), and *Crepis* (İnceer et al., 2018), there was a positive correlation between the altitude and genome size, similar to the results herein. On the other hand, other studies have reported a negative correlation between the altitude and genome size (Creber et al., 1994; Bottini et al., 2000; Chia et al., 2012; Manzaneda et al., 2012; Wang et al., 2013; Akbudak et al., 2018; Savaş Tuna et al., 2019). Some researchers have reported that the genome size was not significantly correlated with altitude in *Vicia faba* (Ceccarelli et al, 1995), neotropical *Lonchocarpus* trees (Palomino and Sauso, 2000), *Sesleria albicans* (Lysaak et al., 2000), *T. boeoticum*, *T. dicoccoides*, and *T. araraticum* (Özkan et al., 2010).

Latitude and longitude are other geographical variables affecting genome size variations. The effect of latitude on the genome size was investigated by correlation and regression analyses (P = 0.347), but the result obtained was not statistically significant (P > 0.05) (Figure 5). In the literature, it was shown that latitude had no effect on genome size variations in *Helianthus* (Sims and Price, 1985), *Dactylis glomerata* (Creber et al, 1994), *Vicia faba* (Ceccarelli et al, 1995), *T. boeoticum* and *T. dicoccoides* (Özkan et al., 2010), *Hordeum marinum*and *H. pubiflorum* (Jakob et al., 2004). However, other researchers have determined a positive (Bottini et al., 2000; Walker et al., 2006; Sheng et al., 2016; Souza et al., 2019) or negative (Rayburn et al., 1985; Smarta and Bures, 2006; Özkan et al., 2010) correlation between latitude and the genome size.

The results were similar for the analysis of the correlation between longitude and the genome size (P = 0.237, P > 0.05) (Figure 6), and they were in agreement with the literature. For instance, it was reported that genome size variations of *Vicia faba* (Ceccarelli et al, 1995), *T. boeoticum*, and *T. dicoccoide s*(Özkan et al., 2010) were not affected by longitude. On the other hand, Pecinka et al. (2006), Smarta and Bures (2006), Walker et al. (2006), Özkan et al. (2010), and Sheng et al. (2016) explained, in their studies, that there was a positive correlation between longitude and the genome size. Bottini et al. (2000) reported a negative correlation in *Berberis* L. In their study, with 3 *Pinus yunnanensis* ecotypes, Wang et al. (2013) found a significant correlation between altitude, latitude, longitude, and genetic diversity, but the negativity or positivity of this correlation varied according to the ecological niches of the ecotypes. The results of the current study can be explained by the lack of information on the altitude, latitude, and longitude, or they may be due to the small number of samples obtained from countries geographically distant to Turkey. For example, there were only 2 samples from Central and South America. If these samples were removed from the analysis, different results could be obtained. However, the aim here was to compare the samples in a collection with no missing data.

PCoA was performed using the genome sizes, altitude, latitude, and longitude data of the accessions (Figure 7). As a result of this analysis, the accessions were divided into 3 groups. It was determined that accessions 60 (1.546 pg2C^{-1}) and 70 (1.548 pg2C^{-1}), whose genome size was larger than the remaining accessions, formed 1group. It was also observed that accessions 143 and 145, from Central and South America, were in another group, probably due to the differences in their latitude and longitude when compared to the other accessions. The accessions other than these 4 were also in the same group. This analysis also allowed for comments to be made on geographic isolation and reproductive isolation.Naturally, there is no gene flow from America to Europe due to geographic isolation (Kwak et al., 2009; Angioi et al., 2010). For this reason, it is normal for accessions from the USA and Peru to form a group together. On the other hand, the existence of high-frequency intergene pool hybridization in Europe indicated that the geographical isolation between the

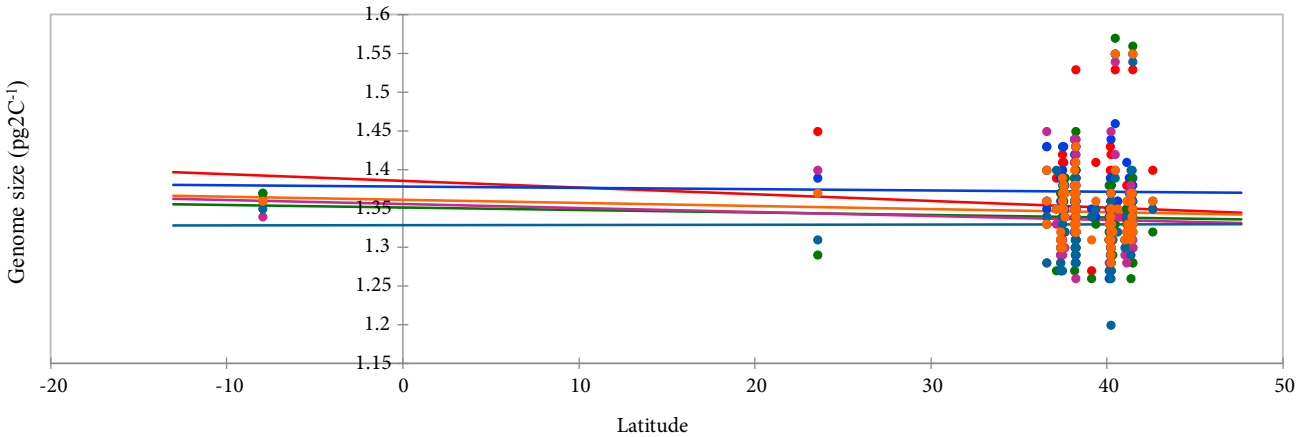

Figure 5. Results of the regression analysis between the latitude and genome size.

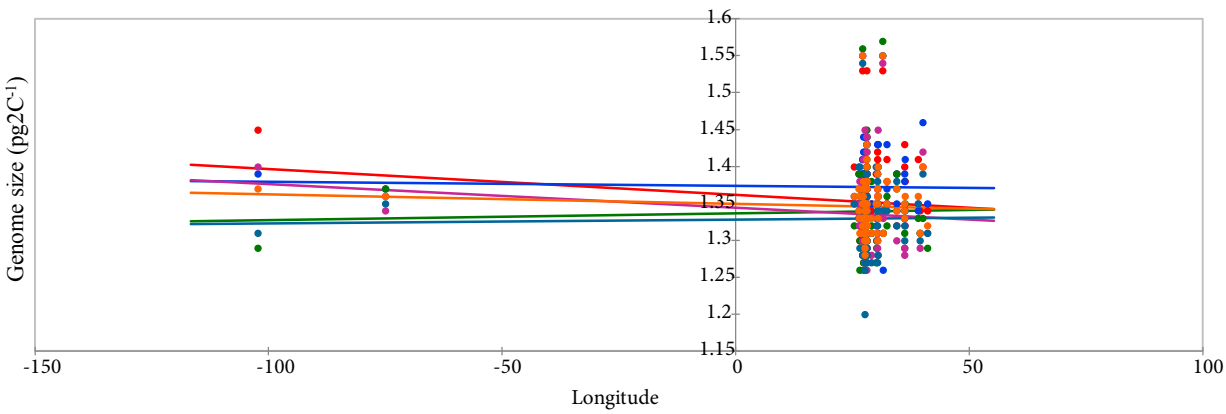

Figure 6. Results of the regression analysis between the longitude and genome size.

2 gene pools were broken down in this area (Angioi et al., 2010). Hybridization has a significant impact on the structure of genetic and genotypic diversity in nuclear genomes. It was explained that hybridization reduces the genetic diversity of gene pools (Rossi et al., 2009; Nanni et al., 2011; Bitocchi et al., 2013). The majority of common beans in the collection herein were provided from Turkey and Europe. In this case, many accessions may be in the same group due to genetic similarity. Moreover, farmers and customers prefer beans with similar characteristics, which can result in a large number of accessions being collected in a group.

Although the chromosome numbers of all of the accessions examined in this study were the same, significant differences were detected in the DNA contents. Therefore, FISH analyses were carried out on some accessions that had a different genome size in order to evaluate the degree of variation in the number and position of the 45S rDNA and 5S rDNA loci. At the same time, it was aimed to determine from which gene pool these accessions originated. The accessions used in the FISH analysis and their 45SrDNA and 5S rDNA loci numbers are summarized in Table 2. Based on the FISH results presented in Table 2, the number of 45S rDNA loci varied between 6 and 16 among the common bean accessions, and the accessions were separated into 2 clear groups based on their 45S rDNA loci numbers. In Group 1, there were 3 accessions (23, 91, 109) with a lower genome size that had a higher number of 45S rDNA loci when compared to the 2 accessions (60, 70) included in Group 2. In Group 1,with a higher number of 45S rDNA loci, the number of 45S rDNA loci was not stable among the cells and varied between 12 and 16, with 14 being the most common number. In the samples of accession 23, there were 12 to 15 45S rDNA loci. Since 1 or 2 of the 45S rDNA loci had signals with very low intensity, they could not be consistently observed in all of the chromosome complements and properly visualized. These samples had 14 45S rDNA loci in general (Figures 8a and 8b). In the samples of accession 91, there were 14 to 16 45S rDNA loci. Some of the FISH signals were very small and poor; therefore, it was difficult to detect them. Accession number 109, a commercial variety developed by the Aegean Agricultural Research Institute, also had FISH results similar to the former 2 accessions (23 and 91). In

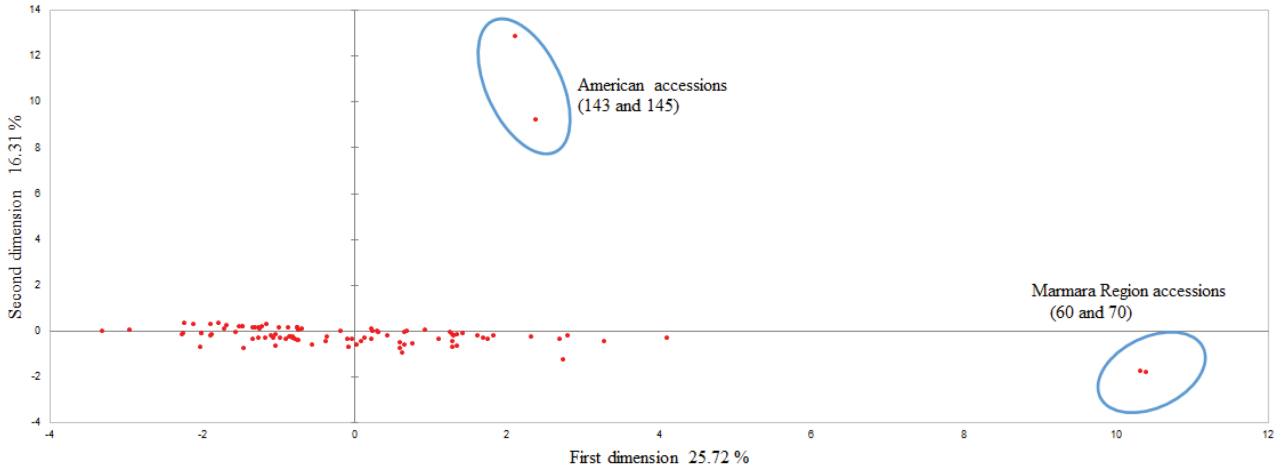

Figure 7. Results of the PCoA based on the genome size.

Table 2. Group number, accession code number, type, ploidy level, mean genome size,and standard deviation of common bean accessions analyzed using FISH. Number and distribution of the 5S and 45S rDNA loci in the genome are provided.

Group No.	Accession code number	Type	Ploidy level	Mean genome size (pg2C^{-1})	Gene Pool	Total number of signals	5S rDNA		45S rDNA
							Location		Location
							P	I	T
1	23	L	Diploid	1.304 ± 0.021	Andean	18	2	2	14
	91	L	Diploid	1.320 ± 0.031	Andean	18	2	2	14
	109	C	Diploid	1.354 ± 0.037	Andean	18	2	2	14
2	60	L	Diploid	1.546 ± 0.011	Mesoamerican	10	2	2	6
	70	L	Diploid	1.548 ± 0.014	Mesoamerican	10	2	2	6
C: commercial variety, L: landrace, P: proximal, I: interstitial, T: terminal.									

this accession, 14 45S rDNA loci were determined. FISH signals observed on 2 chromosomes were very weak, and they could not be detected clearly in each chromosome complement; therefore, 14 was accepted as the average number. Two accessions included in Group 2 had a low number of 45SrDNA loci (mostly 6 loci). In the sample of accession 60, there were 6 45S rDNA loci. In the sample of accession 70, there were 6 to 8 45S rDNA loci (Figures 8c and 8d). Since 1 or 2 of the 45S rDNA loci had very low intensity signals, they could not be consistently observed in all of the chromosome complements. The results of the number of 45S rDNA loci obtained in the current study were similar to those of previous studies. Pedrosa-Harand et al. (2006) performed FISH analyses on some common bean accessions from both the Mesoamerican and Andean gene pools using a 45S rDNA probe, and they reported that the number of 45S rDNA loci varied between 6 and, rarely, 8 in the accessions of the Mesoamerican gene pool, and varied between 12 and 18 in the accessions of the Andean gene pool. In addition, between 6 and 16 loci were observed in accessions resulting from crosses between the Andean and Mesoamerican samples. In another study, the number of 45S rDNA loci varied between 14 and 18 (Almeida and Pedrosa-Harand, 2011). As with the current results, Altrock et al. (2011) also reported 14 45S rDNA loci. The small differences between these studies can mostly be attributed to the different origins of the materials investigated, differences in the technical mechanisms, and sensitivity of the FISH performed by different groups (Snowdon et al., 2000; Hasterok et al., 2001; Labotan et al., 2018).

Unlike the results with the 45S rDNA loci, the number of the 5S rDNA loci was quite stable in the common bean accessions, as all of the accessions investigated in the study had 4 5S rDNA loci (Figures 8a–8d). In previous studies, the same or similar results were obtained. For example, in their study on common bean chromosomes, Moscone et al. (1999) demonstrated the presence of 3 pairs of 5S rDNA loci. Later, Pedrosa-Harand (2006), Almeida and Pedrosa-Harand (2011), Altrock et al. (2011), and Iwata-Otsubo et

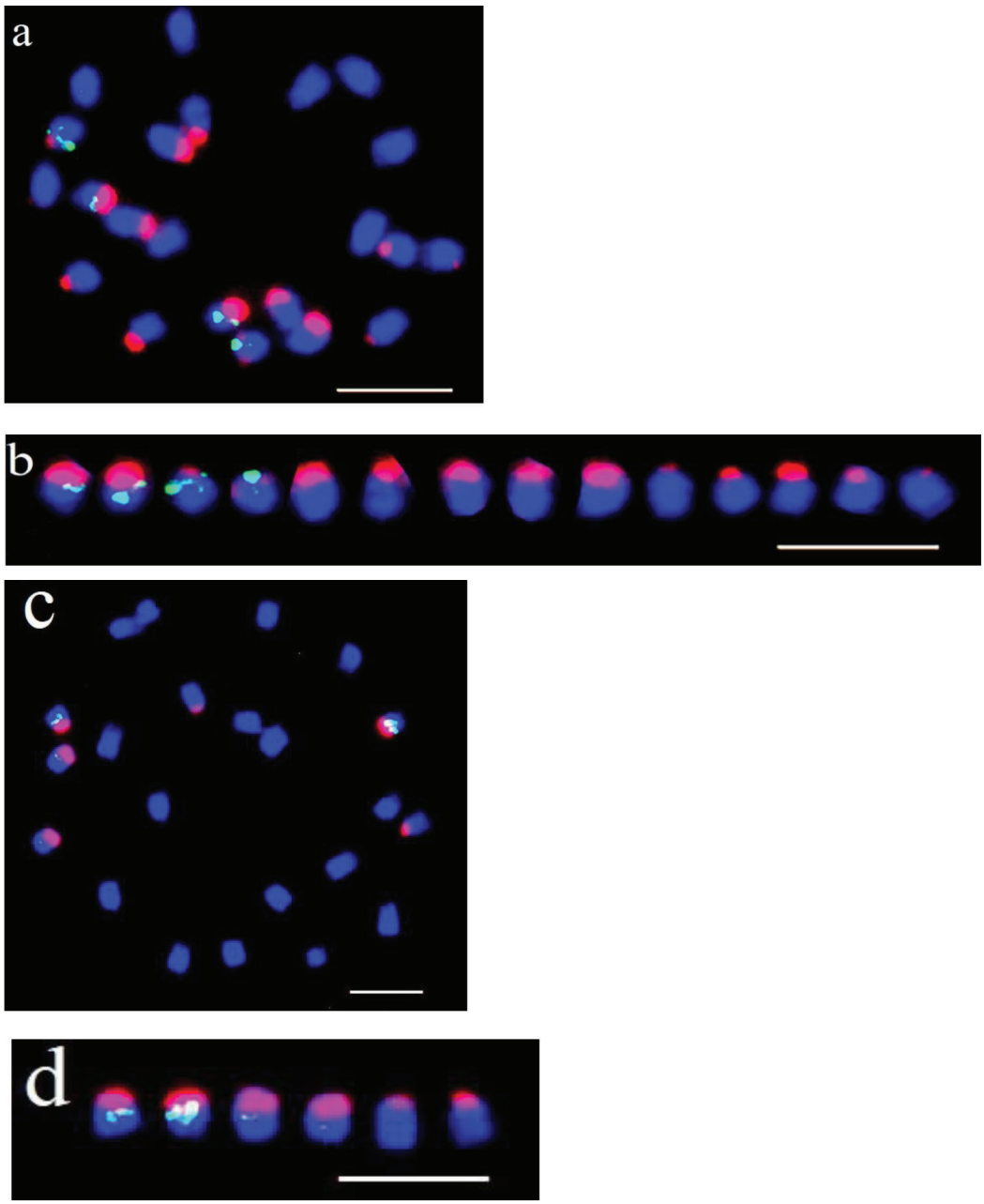

Figure 8. Number and distribution of 5S (green) and 45S (red) rDNA loci on the somatic metaphase chromosomes of *Phaseolus vulgaris* L. accessions.a and b: accession code number 23, c and d: accession code number 70. DAPI counterstaining-blue fluorescence. Scale bars = 5 µm.

al. (2016) observed 4 sites of 5S rDNA loci and determined their localization on chromosomes. In all of the samples of the 5 accessions examined in the present study, the number of 5S rDNA loci was very stable, and 4 FISH signals were always present on the respective chromosomes. However, the number of 45S rDNA loci was polymorphic in the common bean, and it varied between 6 and 16 based on the origin of the accessions. Such a phenomenon is common since the variation in the number of 5S and 45S rDNA loci among the plant species of the same genus was explained in previous studies on rice (Fukui et al., 1994; Chung et al., 2008), *Passiflora* (Melo and Guerra, 2003), *Selaginella* (Marcon et al., 2005), *Brassica* (Hasterok et al., 2006), and *Grapefruit* (De Moraes et al., 2007). However, examples of less variation among different accessions of the same species were seen in *Avena agadiriana* (Hayasaki et al., 2001), *Aegilops speltoides* (Raskina et al., 2004), *Brassica* (Hasterok et al., 2006), *Citrus paradisi* (De Moraes et al.,

2007), coffee (Hamon et al., 2009), and *Arachis* (Robledo and Seijo, 2009). When the locations of the 45S and 5S rDNA loci were examined in the chromosomes, all of the 45S rDNA loci were observed at terminal positions in all of the accessions that were analyzed. In contrast to the 45S rDNA, 4 5S rDNA loci observed in all of the analyzed accessions were in proximal and interstitial positions (Figures 8a–8d). These results were in line with the previous studies (Pedrosa-Harand et al., 2006; Almeida and Pedrosa-Harand, 2011; Altrock et al., 2011).

The intraspecific variation observed in the number of 45S rDNA loci of the common bean is very important and helps determine the gene pool (Andean, Mesoamerican) to which the samples belong. The variation is very pronounced in the Andean lineages of the species, whereas it is relatively limited in the Mesoamerican gene pool. There are some arguments that have attempted to explain why this variation is observed in gene pools; for example, Pedrosa-Harand et al. (2006) reported that differences in variation between the gene pools might occur due to the repetition of rDNA accumulation in the Andean strains. Moreover, it was explained that these repetitions were still active and rapidly developing by increasing/decreasing their copy numbers (Iwata-Otsubo et al., 2016).Therefore, the reason for the differences in variation might be repetitions. In addition, geographic isolation, domestication processes (Bitocchi et al., 2013), gene flow between wild and domesticated subpopulations(Papa and Gepts, 2003; Blair at al., 2012), genetic drift (Chacón et al., 2005; Pedrosa-Harand et al., 2006), and hybridization (Angioi et al., 2010; Gioia et al., 2013) may be among the reasons for this variation. This is an expected result, since events such as natural selection and domestication are closely related to the geographical region, climate, and ecological factors, which all affect the genetic structure (Cortés et al., 2012; López-Alvarez et al., 2015; Souza et al., 2019). Therefore, the variation in the location and number of rDNA loci may be due to differences in the geographical regions (Pedrosa-Harand, 2006), and climatic and ecological (Galeano et al, 2009; Cortés et al., 2012) factors. Another reason for variation is that in recent years, in response to market demands, landraces have been replaced by increasing commercial varieties (Lioi et al., 2005). In this study, the relationship between the genome size and the number of 45SrDNA loci was remarkable; the average number of 45S rDNA loci was determined as 6 in 2 accessions, with a genome size of 1.55 pg2C^{-1}, and 14 in the remaining 3 accessions, with genome sizes varying between 1.30 and 1.35 pg2C^{-1}. In previous studies, it was reported that gene pools also showed differences in the genome size. For instance, Beletti et al. (1997) reported that the genome size of the Andean accessions was 1.39 pg2C^{-1}, while that of the Mesoamerican accessions was 1.41 pg2C^{-1}. It was also determined that the genome size of G19833 (Andean bean reference genome) was 473 Mpb (~1.06pg2C^{-1}) (Schmutz et al., 2014) and that of BAT93 (Mesoamerican bean reference genome) was 549.6 Mpb (~1.23pg 2C^{-1}) (Vlasova et al., 2016). Lobatan et al. (2018) noted that the Mesoamerican types tended to have a larger genome than the Andean types. When the results of flow cytometry and FISH analysis were evaluated together, accessions 60 and 70 that had 6 45S rDNA loci, and a genome size larger than the other accessions, were of the Mesoamerican gene pool origin. In the FISH analysis, other accessions (23, 91, and 109) originated from the Andean gene pool. Similarly, previous studies have suggested that the common beans in Turkey originated from 2 gene pools (Nemli et al., 2017; and Nadeem et al., 2018). In addition, Angioi et al. (2010) reported that the European common bean emerged from both gene pools from the Americas, but the majority (67%) of the analyzed European accessions were attributed to the Andean gene pool. However, in Europe, a high level of variation and the presence of frequent hybridization, which has led to new variants, has been seen (Santalla et al., 2002). It was reported that 44% of the European bean germplasm consisted of hybridizations between the Andean and Mesoamerican gene pools (Santalla et al., 2002; Angioi et al., 2010). In a future study, it will be aimed to comparedthe landraces and commercial varieties grown in different geographical regions of Turkey using different probes and wild forms available from the gene bank.

Consequently, it was determined in the present study that the genetic resource collection of the common bean had a wide variation in terms ofthe genome size and organization. A positive correlation was detected between the altitude and genome size in the intraspecific genome size variation. However, latitude and longitude did not have any statistically significant effect on the genome size. In the PCoA, it was observed that the accessions were divided into 3 groups. This result was expected, considering the differences in the genome size, latitude, and longitude of some of the accessions. Moreover, the intraspecific variation was determined for the 45S rDNA loci in the FISH analysis. Domestication,origin, hybridization, geographical isolation, natural selection, farmer and customer selection, and environmental factors were thought to be effective in the intraspecific variation detected in the FCM and FISH analyses, as explained in previous studies. In the current collection, there were beans that originated from both gene pools, and the accessions belonging to the Mesoamerican gene pool had a larger genome size than those of the Andean gene pool.

It is our belief that the data obtained in this study will be an important guide in other studies with beans.

Acknowledgments
We would like to thank the Scientific and Technological Research Council of Turkey (TÜBİTAK) for providing financial support for this study under project no. 116O057.

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