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CHROMOSOME ANALYSIS OF SOME *PHASEOLUS VULGARIS* L. GENOTYPES IN TURKEY

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Abstract

Karyotypes parameters in four selected Turkish bean genotypes (BT, HK, VN, and EL) were studied. Genotypes (4 levels) and chromosomes (11 levels) were compared in factorial experiment based on completely randomized design with five replications. Genotypes showed significant differences in long arm, short arm and total chromosome length and divided into three separate groups. Genotypes BL and HK were placed together in a separate group, probably showing the weaker kinship with the other genotypes. Also, the significant differences in all recorded parameters were observed among chromosomes of each genotype. All genotypes were diploid 2n = 22 with no satellite and differed significantly in karyotypic parameters. The most and the least chromatin length (34.24 µm and 15.65 µm) were observed in BT and EL genotypes, respectively. Also, all genotypes showed high chromosomal symmetry and categorized in Stebbins's Class 1A. The karyotype of genotype BT was found to be more symmetrical than others.

Introduction

The chromosomes of each genome carry genetic information that causes the phenotypic emergence of traits. Any change in the structure, sequence, and size of chromosomes will entail changes in the genome and genetic diversity. So, the first step to identify the genome of a species is to explore the number, shape, and behavior of its chromosomes. Chromosome characterization is widely used for classification, proper identification and determination of genetic diversity of an organism (Mirzaie-Nodoushan *et al.* 2002, Palmer *et al.* 2003). These research works turn out to be more valuable when they allow comparing species and their populations since plant species show different adaptive features depending on their growth environment. Moreover, new cultivars or species may develop due to differences in adaptation growth (Gupta 1995). Karyotype is an important tool for referring a set of characteristics of chromosomes such as size and type of chromosomes, centromere position, satellited chromosome number and chromosomes secondary constrictions. The karyotypic differences and similarities of different groups are attributed to their evolutionary relationships (Mirzaei-Nodoushan *et al.* 2002). Karyotypic findings have been very useful for phylogenetic analyses of closely related and morphologically similar plants (Quick 1993).

Common bean (*Phaseolus vulgaris* L.) is an annual plant belonging to Fabaceae with 2n = 22 chromosomes. It has the highest acreage and economic value among the beans worldwide (Salehi 2005). This crop is mostly consumed as a source of plant protein in developing countries and as a dietary supplement in developed countries. The first step to consider genetic reserves is to investigate inter-species and intra-species genetic diversity since the key requirement for breeding and introducing plants with higher genetic potential is the availability of genetic diversity in the base population; this is to increase the chance of finding and selecting for more desirable traits (Mohammadi 2006). One of the methods to study

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genetic diversity is the cytological techniques that identify appropriate individuals in a plant population. In this respect, it is of crucial importance to explore the cytological characteristics of different bean cultivars in order to identify more adaptive populations and accomplish higher yields. According to Mihaela Cimpeanu *et al.* (2005) all of the studied cultivars had 2n = 22 submetacentric and morphologically uniform chromosomes without satellite. On the other hand, Mercado-Roaru and Delgado-Salinas (1998) reported metacentric and submetacentric chromosomes in studied 10 karyotypes except *Phaseolus filiformis* and *P. chiapasanus* which possess metacentric chromosomes and sub telocentric chromosomes. Sadeghi and Cheghamirza (2012) studied cytogenetic diversity of some cultivars of beans and reported that among the 13 studied genotypes, the ploidy levels were x=9 (for two diploids), x=10 (for two diploids) and x=11 (for nine diploids).

The present study was conducted to analyze the genomes of four best selected Turkish bean genotypes based on their karyotypic and chromosomal information. It is believed that these selected genotypes have differences based on chromosomal characteristics, which may be a source of extensive genetic diversity and/or chromosomal abnormalities. Also, the expected results can be used in breeding and crossing programs of this crop.

Materials and Methods

Genotypes grown in 10 provinces located in the east and center of Turkey were selected in the 2011 and 2012 years. At the end of the second year followed by harvesting, four superior genotypes were selected out of total 38 genotypes based on seed weight, size, quality and other parameters scores. The least and the highest scores were 346 and 443, respectively. Genotypes with a score of 400 or more were considered as superior genotypes (Table 1).

Table 1. Genotypes, regions and selection codes of studied Turkish *Phaseolus vulgaris*.

Genotype code	Region	Selection code
BT	Bitlis	471
HK	Hakkari	596
VN	Van	387
EL	Elazig	411

The study was carried out at the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University of Turkey. The chromosome number was studied based on the observation of mitosis of end-root meristem cells, which are highly capable of fast division and have a high mitotic index. The seeds of the studied cultivars were sterilized with 2% sodium hypochlorite solution and were culture in sterile filter papers and incubated in growth chamber at 20°C. After germination, roots of 1-2 cm in length were selected and pretreated with α-bromonaphthalene solution (0.5%) for 4 hrs. (Najafi *et al.* 2013). Then, root segments rinsed 30 minutes with water and placed in Lewitsky's solution for 16 hrs. as a fixative for karyotypic studies. The samples rinsed with water (30min) and hydrolyzed with 1N NaOH at 60°C for 10 min in a hot water bath. Then, samples dried, stained by Aceto-Orcein solution (2%) and examined by the squash technique (Agayev 1996) on the slide. The best metaphase plates were captured at 100 X magnification using BX50 Olympus optic microscope for measuring karyotype parameters. Several parameters including long arm length (LAi), short arm length (SAi), total chromosome length (TL), arms ratio (AR), short arm to long arm length ratio (r-value), chromosome form

percentage (%F), total karyotypic form percentage (%TF), total chromatin length (X), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) were calculated (Romero Zarco 1986) (Table 2).

Table 2. Cytogenetic parameters used to analyze *Phaseolus vulgaris* karyotypes.

Parameters	Formula
Short arm (SAi)	Distance between the end of the short arm to centromere (µm)
Long arm (LAi)	Distance between the end of the long arm to centromere (µm)
Total length (TL)	TL=LAi+SAi (µm)
Centromer index (CI)	$CI = \frac{SA_i}{TL_i} \times 100$ (%)
Intrachromosomal Asymmetry Index (A_1)	$A1 = 1 - \frac{\sum_{i=1}^{n} \frac{S_i}{L_i}}{n}$
Interchromosomal Asymmetry Index (A_2)	$A_2 = \frac{Sx}{\overline{X}}$

n: chromosome number

Genotypes (4 levels) and chromosomes (11 levels) were compared in a factorial experiment based on completely randomized design with five replications.

Results and Discussion

Results of karyotypic studies revealed that the ploidy level was diploid in all four studied bean genotypes. The ideograms and somatic chromosomes of studied genotypes presented in Figs 1 to 4. The analysis of variance on chromosomal parameters revealed significant differences among the genotypes in long arm length (L), short arm length (S), chromosome total length (TL), arm ratio (AR), short arm to long arm length ratio (r-value) and chromosome form percentage (%F) at $p < 0.01$ level (Table 3). Also, the chromosomes exhibited significant differences in all the parameters ($p < 0.01$).

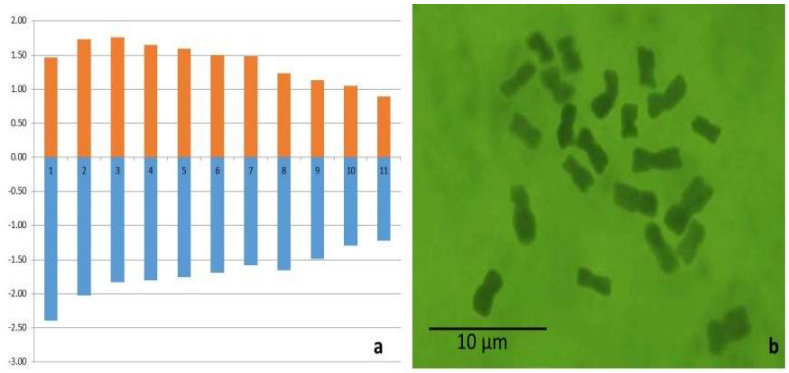

Fig. 1. Ideograms (a) and Somatic chromosomes (b) of *Phaseolus vulgaris* genotype BT.

Based on results presented in Table 4, genotype BT had the longest chromosome (3.11 µm) and the highest mean total chromatin length (34.24 µm). The shortest chromosome length (1.42 µm) and the least mean total chromatin length (15.65µm) were observed on genotype EL. The comparison of karyotypes in terms of their symmetry showed that genotypes HK and EL had the highest and the least karyotype total form percentages (46.97 and 43.40%), respectively.

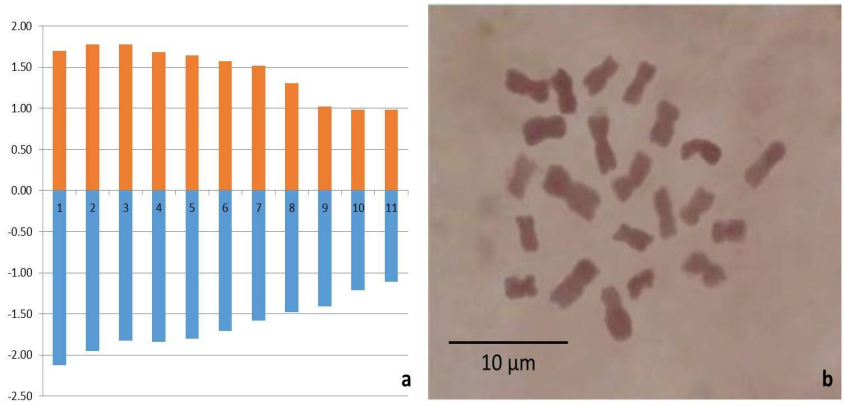

Fig. 2. Ideograms (a) and Somatic chromosomes (b) of *Phaseolus vulgaris* genotype HK.

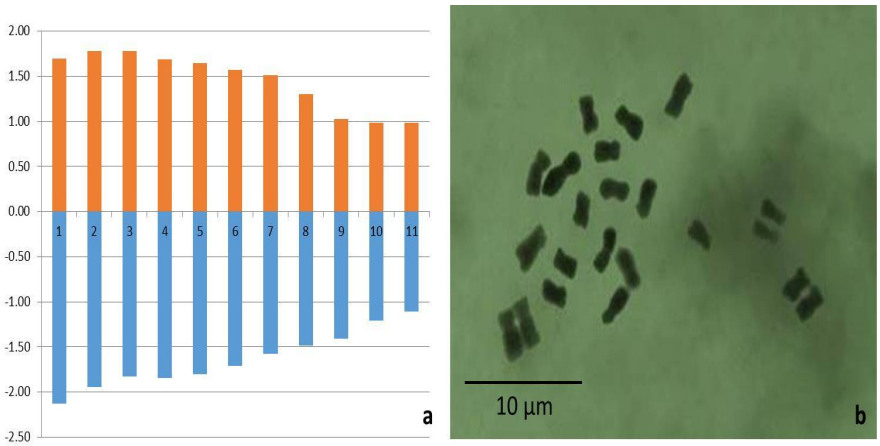

Fig. 3. Ideograms (a) and Somatic chromosomes (b) of *Phaseolus vulgaris* genotype VN.

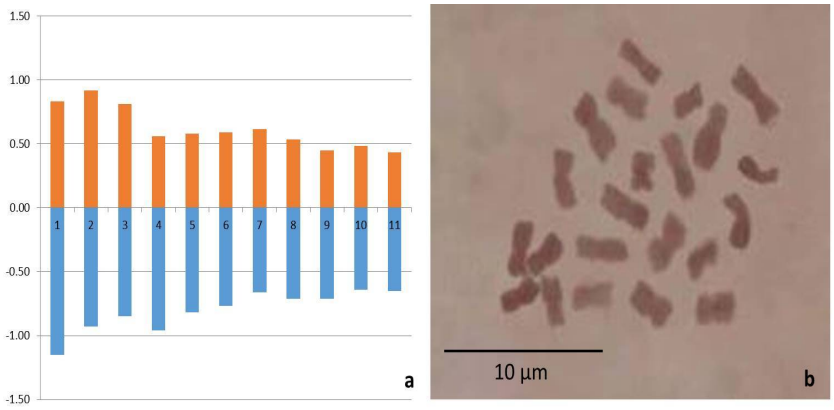

Fig. 4. Ideograms (a) and Somatic chromosomes (b) of *Phaseolus vulgaris* genotype EL.

Table 3. ANOVA for chromosomal parameters in studied *Phaseolus vulgaris* genotypes.

Sources of variance(SOV)	Df	MS					
		S	L	TL	AR	r-value	%F (TL)
Genotypes	10	8.279**	9.419**	35.499**	0.435**	0.118**	0.924**
Chromosome	3	1.108**	1.457**	4.676**	0.279**	0.089**	15.105**
Genotype × Chromosome	30	0.058**	0.046ns	0.144**	0.114ns	0.027**	0.584**
Error	176	0.021	0.029	0.052	0.066	0.014	0.198
Total	219						

S: Short arm, L: Long arm, TL: Total length, AR: Arm ratio. **: Significant at $p < 0.01$, ns: Not significant

Table 4. Karyotype characteristics in four genotypes of *Phaseolus vulgaris.*

KF	ST	SAT	X	% TF	%F ± Se
2x=2n=20m+2sm	1A	-	34.24 ± 0.35	45.22 ± 1.52	4.11 ± 0.91
2x=2n=22m	1A	-	33.98 ± 1.35	46.97 ± 0.70	4.27 ± 0.96
2x=2n=22m	1A	-	24.90 ± 1.12	45.58 ± 1.30	4.14 ± 0.88
2x=2n=22m	1A	-	15.65 ± 0.99	43.40 ± 1.22	3.95 ± 1.12

Table continued right side

r-value±Se	AR ± Se	TL ± Se	S ± Se	L ± Se	Genotype
0.83 ± 0.11	1.25 ± 0.10	3.11 ± 0.32	1.41 ± 0.35	1.70 ± 0.38	BT
0.44 ± 0.16	1.15 ± 0.05	3.09 ± 0.12	1.64 ± 0.33	1.64 ± 0.33	HK
0.43 ± 0.09	1.21 ± 0.08	2.26 ± 0.10	1.03 ± 0.22	1.23 ± 0.28	VN
0.39 ± 0.05	1.36 ± 0.07	1.42 ± 0.10	0.62 ± 0.18	0.80 ± 0.18	EL

Se: Standard error, L: Long Arm, S: Short arm, TL: Total chromosome length, AR: Arm Ratio, %F: Form percentage, %TF: Total form percentage, X: Karyotype total chromatin length(µm), SAT: Satelite, ST: Stebbins classification, KF: Genotype karyotype formula, m: metacentric.

Based on Stebbins's (1971) classification, all the genotypes were placed in class 1A. Based on Levan's (Levan *et al.* 1964), all chromosomes of genotypes VN, HK, and EL were of metacentric (m) type whereas genotype BT had a pair of sub-metacentric (sm) chromosome, and the remains were metacentric (m) type. The results of these two methods are absolutely consistent and showed that the studied bean genotypes have symmetric karyotypes and have primitive evolutionary condition. Results of means comparison among different bean chromosomes by DMRT ($p < 0.01$) showed that the chromosomes are classified into seven groups by the parameter L. Chromosomes 1 and 2 each fall into a separate group (Table 5). The chromosomes are also categorized into six groups based on the parameter S. Based on the parameter TL, the chromosomes were divided into nine overlapping groups. Chromosome 8 falls into a separate group and chromosomes 9, 10 and 11 are grouped together.

The comparison of means among the studied bean genotypes (Table 6) by Duncan's multiple range test ($p < 0.01$) categorized the genotypes in three groups in terms of the parameters L, S, and TL. Genotypes BL and HK were separately placed in a single group, perhaps implying their weaker kinship with the other genotypes.

Table 5. Chromosome parameters means comparison among *Phaseolus vulgaris* chromosomes.

Chr.no.	L	S	TL	AR	r-value	%F
1	1.832±0.062^{a}	1.314±0.022^{ab}	3.146±0.021^{a}	1.447±0.024^{a}	0.742±0.019^{d}	4.931±0.062^{ab}
2	1.689±0.141^{b}	1.416±0.010^{a}	3.033±0.034^{ab}	1.135±0.011^{b}	0.89±0.001^{abc}	5.285±0.062^{a}
3	1.477±0.142^{c}	1.413±0.012^{a}	2.891±0.042^{bc}	1.049±0.004^{b}	0.954±0.003^{a}	5.193±0.058^{a}
4	1.488±0.085^{c}	1.283±0.016^{bc}	2.771±0.012^{cd}	1.269±0.013^{ab}	0.836±0.013^{bcd}	4.576±0.062^{bc}
5	1.431±0.148^{c}	1.224±0.134^{bcd}	2.655±0.020^{de}	1.227±0.012^{ab}	0.837±0.012^{bcd}	4.384±0.054^{cd}
6	1.363±0.019^{cd}	1.164±0.171^{cd}	2.528±0.077^{ef}	1.237±0.009^{ab}	0.847±0.015^{bcd}	4.183±0.049^{d}
7	1.241±0.831^{de}	1.151±0.019^{d}	2.392±0.003^{fg}	1.0915±0.002^{b}	0.924±0.019^{ab}	4.172±0.062^{d}
8	1.232±0.057^{de}	0.993±0.061^{e}	2.225±0.006^{g}	1.284±0.011^{ab}	0.805±0.003^{cd}	3.605±0.029^{e}
9	1.147±0.018^{ef}	0.855±0.073^{f}	2.002±0.001^{h}	1.39±0.002^{a}	0.745±0.012^{d}	3.120±0.036^{f}
10	1.015±0.025^{fg}	0.815±0.002^{f}	1.83±0.119^{hi}	1.264±0.024^{ab}	0.803±0.017^{cd}	3.013±0.039^{f}
11	0.95±0.009^{g}	0.769±0.022^{f}	1.719±0.003^{i}	1.277±0.033^{ab}	0.819±0.024^{bcd}	2.850±0.053^{f}

L: long arm, S: short arm, TL: total length, AR: arm ratio, F: form percentage. Means within each column followed by different lowercase letters are significantly different at the %1 level according to Duncan's multiple test range.

Table 6. Chromosome parameters means comparison among *Phaseolus vulgaris* genotypes.

Genotype	L	S	TL
BL	1.704±0.171^{a}	1.408±0.114^{a}	3.112±0.303^{a}
HK	1.638±0.124^{a}	1.45±0.002^{a}	3.088±0.223^{a}
VN	1.23±0.119^{b}	1.03±0.003^{b}	2.262±0.173^{b}
EL	0.806±0.175^{c}	0.618±0.091^{c}	1.424±0.210^{c}

L: long arm, S: short arm, TL: total length. Means within each column followed by different lowercase letters are significantly different at the %1 level according to Duncan's multiple test range.

The chromosome number in the nine examined genotypes of *Phaseolus vulgaris* L. was found to be diploid with 2n = 22 confirming earlier reports on the chromosome numbers of *Phaseolus vulgaris* (Karpetschenko 1925, Mercado-Ruaro and Delgado Salinas 1996, 1998). The karyotype analysis revealed that the genotypes HK and VN have eleven metacentric chromosomes while genotypes BT and EL have ten metacentric chromosomes plus one submetacentric chromosome. These results disagree with Haq *et al*. (1980) who concluded that the chromosomes of *Phaseolus vulgaris* can arbitrarily be described with median, submedian or subterminal centromeres, but the present findings agree with Mercado-Ruaro and Delgado-Salinas (2000) who reported a predominance of metacentric and submetacentric chromosomes. These variations in the gross morphology of the chromosomes might be due to the occurrence of pericentric and paracentric inversions and translocation which have been regarded as the main factors involved in the karyotypic evolution of the genus *Phaseolus* by some authors (Sarbhoy 1977, 1980, Sinha and Roy 1979a, b). In the present study, the mean chromosome length of the genotypes VN (2.26µm) and EL (1.42 µm) are generally smaller than the size reported by Sarbhoy (1978) regarding the mean length of chromosome for *Phaseolus vulgaris* (3 µm), while in genotypes BT and HK mean length of chromosomes (3.11 µm and 3.09 µm) are near the size which was reported by Sarbhoy

(1978). Present results showed that genotypes BT and HK have the longer chromosomes and VN and EL genotypes have smaller chromosomes while the moderate genotypes have intermediate chromosomes.

Chromosome total length is the first factor reflecting the DNA content of a nucleus and the ratio of DNA content and protein content, which is always constant at all growth stages, shows the amount of genetic material. This ratio is of crucial biological significance. The results showed significant differences in chromosome length of each genotype in all parameters of chromosomes. All four genotypes had no satellite. According to Stebbins' bilateral table, all four genotypes were placed in Class 1A, so they had high chromosomal symmetry. This index, thus, failed to show the diversity of the four genotypes. Genotype BT had more symmetric karyotype than genotypes HK, VN and EL. The results absolutely consistent and showed that the bean genotypes studied here have symmetric karyotype and have primitive evolutionary condition.

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