Cytogenetic Evaluation of Some Genera of Persian *Phaseolus*

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Summary This research was carried out to evaluate the cytogenetic diversity of Persian beans with using an image analysis system. Ploidy levels of genotypes were determined from $x=9$ (2 diploids), $x=10$ (2 diploids) and $x=11$ (9 diploids). The highest chromatin length belonged to genotype 5 ($12.501\mu\text{m}$) and the lowest to genotype 13 ($5.042\mu\text{m}$). With regards to the Stebbins two-way table, I, L, and F genotypes divided to group a 2A; A, B, and N genotypes divided to group 1B; and the rest of the genotypes divided to group 2B. The most asymmetric karyotype between and within chromosomes was genotype N. Five important karyotype indexes that are related to the cytogenetic diversity of Persian beans were studied in Completely Randomized Design (CRD) with 3 replications, at the University of Tehran in 2010. The results of ANOVA showed significant differences among genotypes in all karyotype indexes (A , % S_{yi} , %TF, SC, A_2 and A_1). Principal components analysis classified the 5 karyotype indexes in to 2 new components that explained 96.41% of total variations. The first component explained 65.34% of total variations that mainly included diastatic arm ratio, CI, A_1 , TF, S_{yi} and A . The second component explained 31.28% of total variations that mainly included TL, SA, LA and A_2 . Cluster analysis by the Ward method classified characters in to 3 groups. The results showed that genotypes were significantly different in all characters; therefore analysis of these clusters showed the most genetic distance to be between N and H.

Key words Chromosomes, Karyotype indexes, Bean, Completely Randomized Design.

Cereals play an important role to support both qualitative and quantitative food needs of human societies, especially in developing countries in Asia, Africa and Latin America. Also these plants are important in soil fertilization processes and stabilize significant amounts of atmospheric nitrogen annually. The common bean (*Phaseolus vulgare*) is a diploid plant ($2n=22$) from the Fabaceae family has a genome size of about 600 Mb and its origins can be traced to south America (Arumuganathan *et al.* 1991). The Papilionideae subfamily belongs to the Fabaceae family. This subfamily has important economic genera like *Glysin* (Soybean), *Phaseolus* (American beans) and *Vigna* (Asiatic beans). The common bean is one of the smaller genome-sized species and most karyotype studies so far have related to chromosome number; however, today many genetic maps for the common bean are being presented (Gepts *et al.* 2008). Several BAC (bacterial artificial chromosome) libraries exist or are being developed for various genotypes of *Phaseolus* (Pedrosa *et al.* 2009, Kami *et al.* 2006, Vanhouten and MacKenzie 1999) Karyotype definition is the arrangement of basic chromosomes from long to short size. Some of the symmetric karyotype indexes are:

- 1) TF% (Total form percentage): closer to 50% means higher number of metacentric chromosomes than telocentric and acrocentric chromosomes.

$$\text{TF\%} = \frac{\text{Total sum of short arms lengths}}{\text{Total sum of chromosome lengths}} \times 100$$

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This index has been used by Mercado-Ruaro and Delgado-Salinas (1998) on some bean genotypes.

2) A_1 (Inter chromosomal asymmetry index):

$$A_1 = 1 - \frac{\sum_{i=1}^n \frac{q_i}{p_i}}{n}$$

q = short arm length

p = long arm length

n = number of studied cells

And A_2 (intra chromosomal asymmetry):

S_{CL} = chromosomes length standard deviation

$$A_2 = \frac{S_{CL}}{\bar{X}_{CL}}$$

$\bar{X}_{CL}$ = mean chromosome length

Seijo and Fernandez 2003, used A_2 to analysis some *Lathyrus* species.

3) Sy_i (Karyotype symmetry index) determined by Greilhuber and Speta (1976).

$$Sy_i = \frac{\text{Mean length of short arms}}{\text{Mean length of long arms}} \times 100$$

4) A (Karyotype asymmetry index) presented by Watanabe *et al.* 1999, Paszko 2006

$$A = \frac{\sum \frac{p_i - q_i}{p_i + q_i}}{n}$$

q = short arm length

p = long arm length

n = number of studied cells

Cytogenetic and karyotype studies are one of the methods for diversity evaluation in plants. It is obvious that species with more similarity in chromosomal properties are closer to each other and this can help to select and produce better lines and varieties in breeding programs. The goal of this research is to determine karyotype diversity in some Iranian beans.

Materials and methods

In order to evaluate karyotype diversity in some genera of *Phaseolus*, 13 genotypes provided by the gene bank of Tehran University were studied. After seed disinfection with 5% hypo sodium chloride for 7 min, they were planted in petri dishes under 80% humidity, 23°C and 12 h light period. When seeds germinated and 0.8–1.5 cm radicle the appeared, the apical section was separated. Then pre treatment (0.5% colchicine solution), fixation (acetic acid–ethanol 3 : 1, 18 h), washing (sodium acetate 2.5 M), hydrolysis (chloridric acid 1 N, 10 min, 55°C), washing with distilled water and staining with acetoorcein 1% for 24 h in room temperature were performed. 1–2 mm apical radicle were transferred onto the lam and then squashed with 1 drop of glycerol–acetic acid 45%. A chromosomal study was performed with imaging analysis system with ×1800 enlargement.

Fig. 1. Photos of metaphase root cells of beans with their ideograms

Fig. 2. Dendrogram for karyotype traits of Iranian beans.

Table 1. Karyotype characteristics of studied beans

Genotype	Karyotype formula	A	%Syi	%TF	SC	A_2	A_1	X	$2n$
1	5m+6sm	0.120	78.57	44	1B	0.065	0.214	11	22
2	9m+2sm	0.223	63.27	38.75	1B	0.099	0.365	11	22
3	4m+7sm	0.151	73.66	42.41	2B	0.055	0.263	11	22
4	5m+6sm	0.22	64.16	39.08	2B	0.091	0.36	11	22
5	5m+6sm	0.193	67.47	40.28	2A	0.05	0.323	11	22
6	7m+4sm	0.183	68.81	40.76	2B	0.044	0.31	11	22
7	8m+3sm	0.266	58.22	36.79	2B	0.119	0.42	11	22
8	5m+6sm	0.231	62.02	38.27	2A	0.082	0.375	11	22
9	10m_1sm	0.149	74.40	42.65	2B	0.093	0.258	11	22
10	7m+3sm	0.211	64.41	39.18	2B	0.099	0.346	10	20
11	8m+2sm+1t	0.220	63.09	38.68	2A	0.168	0.36	10	20
12	7m+3sm	0.192	68.46	40.64	2B	0.147	0.32	9	18
13	8m+2sm	0.117	79.02	44.13	1B	0.023	0.209	9	18

Table 2. Variance analysis of karyotype traits

S.O.V	DF	Means of squares				
		CL	TL	AR	SA	LA
Genotype	13	14.55**	16.37**	0.05**	2.45**	6.35**
Error	28	4.04	0.87	0.01	0.18	0.32
CV (%)	—	4.98	11.22	8.67	12.68	11.51

ns: No significance *Significant (5%) **Highly significant (1%).

Karyotypes were prepared by a micro measure software and 3 metaphase cells (replication) were selected from each slide and the following indexes calculated: TL (total chromosome length), S (total sum of short arms length), L (total sum of long arms length), L/S (arm ratio), L-S (d -value), $CI\% = (S/L + s)$ (centromeric index), $RL\% = (\text{Realtive Length})$, $S\% = (\text{shortest chromosome length/longest chromosome length}) \times 100$ (relative length of shortest chromosome), DRL (different relative length of large to short chromosome), A_1 (Inter chromosomal asymmetry index), A_2 (intra chromosomal asymmetry), TF% (Total form percentage), Syi (Karyotype symmetry index), A (Karyotype asymmetry index). The Levan method was used to determine chromosome kind and karyotype formula. PCA analysis with SPSS software was performed to determine the role of each trait in genetic diversity and cluster analysis with Past software performed to grouping genotypes.

Results and discussion

Table 1 shows the characteristics of chromosome numbers, karyotype formula, A_1 and A_2 indexes, Stebbins classes, TF%, Syi and A for each genotype. All genotypes were diploids but the basic chromosome was different ($X=9, 10, 11$) which represents genetic diversity in the material. The Stebbins index for genotypes 6, 9, 12 was 2A, for genotypes 1, 2, 13 was 1B and for the rest of the genotypes was 2B. The highest amount of Syi% (78.57) was related to genotype 1, which means this genotype has the most symmetric karyotype. In comparison with the trend of A_1 and A_2 indices, genotype 8 had the most inter-chromosomal asymmetry and genotype 12 had the most intra-chromosomal asymmetry. Genotypes 1 and 13 had the lowest amount of A_1 and A_2 .

Variance analysis for 13 genotypes based on CRD with 3 replications showed high significant differences between genotypes for all traits which represents a high level of genetic diversity in

Table 3. Mean comparison between genotypes for karyotype traits

Genotype	LA		SA		Arm		TL		CI	
AA	4.58	def	3.6	cde	1.27	d	8.19	d	43.95	a
BB	5.44	cd	3.44	efd	1.58	abc	8.89	cd	38.8	bcd
CC	4.97	de	3.67	bcde	1.35	bcd	8.66	cd	42.41	ab
DD	6.09	bc	3.91	bcd	1.56	abc	10.01	bc	38.98	bcd
EE	7.46	a	5.03	a	1.48	abc	12.5	a	40.32	abcd
FF	5.26	cd	3.62	cde	1.45	bcd	8.89	dc	40.8	abc
GG	4.65	def	2.7	hfg	1.73	a	7.36	de	36.65	d
HH	7.14	a	4.43	ab	1.6	ab	11.58	ab	38.4	cd
II	4.13	gef	3.07	efg	1.35	cd	7.2	de	42.54	ab
JJ	3.8	fgh	2.45	gh	1.54	abc	6.25	fe	39.41	bcd
KK	3.3	gh	2.08	h	1.57	abc	5.38	f	38.97	bcd
LL	3.38	gh	2.32	gh	1.48	abcd	5.71	fe	40.39	abcd
MM	2.81	h	2.22	h	1.26	d	5.04	f	44.13	a

Table 4. Eigenvalues, cumulative variance and variance of 2 PCA

Karyotype traits	PCA1	PCA2
TL	0.30	0.94
arm ratio	0.99	-0.09
SA	0.12	0.98
LA	0.40	0.90
CI	-0.99	0.07
A_1	0.99	-0.05
A_2	0.56	-0.64
TF	-0.99	0.07
Syi	-0.99	0.06
A	0.99	-0.07
Eigenvalues	6.534	3.129
Variance	65.34	31.28
Cumulative variance	65.34	96.63

these genotypes.

Mean comparison by Duncan's method in Table 3 shows there is at least some overlap between genotypes for the whole length of the chromosome. For TL, the lowest and highest amounts related to genotypes were 13 and 6 respectively.

In order to determine the role of each trait in genotype variation, PCA was performed (Table 4). Two primary components represented 96.41% of the total variation. The first component with 65.34% of total variation represented Arm Ratio, CI, A_1 , TF, Syi and A. The second component with 31.28% of total variation represented TL, S, L and A_2 .

Cluster analysis by paired group method was performed to cluster genotypes. With 99% similarity all genotypes divided into 4 clusters. Based on a dendrogram, genotypes (8, 10) which located in the farthest group had the most asymmetric chromosome structure and the most genetic distance. This non-coordination leads to genomic incompatibility such as weakness in the fertility of offspring. Genotypes 10 and 11 ($2n=20$) and 12 ($2n=18$) are grouped in 1 cluster. Also, genotype 13 ($2n=18$) is located in a separated cluster near to genotypes 8, 12 and 10.

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