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GENETIC DIVERSITY OF COMMON BEAN GENOTYPES AS REVEALED BY SEED STORAGE PROTEINS AND SOME AGRONOMIC TRAITS

ABSTRACT

Evaluation of the genetic diversity present within species is essential for conservation, management and utilization of the genetic resources. The objective of this study was to evaluate genetic variability of 70 common bean genotypes for seed storage proteins, grain morphological characteristics and agronomic traits. Two methods of extracting soluble seed proteins in salt were used.

Positive correlations were observed among both seed morphological characters and developmental characters while yield components showed negative correlations with each other. Factor analysis for agronomic and grain morphological traits resulted in three factors were named yield components, seed morphology and phenology, respectively. Most genotypes had lower or medium scores for yield components and phenology factors. Considerable diversity was observed for seed morphology factor among the common bean genotypes.

Nei's diversity coefficient (H_e = 0.4), effective number of alleles (A_e = 1.69) and number of polymorphic loci (N = 17) indicated larger variation in the extraction method of soluble proteins in low salt (0.2 M NaCl) than high salt (1 M NaCl) condition. Considering that the centers of diversity for common bean are different in seed size, the result of G_{ST} statistics showed that bands with relative mobility of 30, 32, 38 and 40 differentiated two weight groups more than other bands. Furthermore, significant differences were observed between these bands for number of pods per plant and number of seeds per plant.

Key words: agronomic traits, genetic diversity, *Phaseolus vulgaris* L., SDS-PAGE, seed storage proteins

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world, representing 50% of the grain legumes for direct human consumption (McClean *et al.*, 2004). The crop was originated and was domesti-

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cated in the new world in two centers of origin (Andes and Mesoamerica), which gave rise to two major gene pools (Andean and Mesoamerican) distinguished by seed size and other characters. Evidence based on seed size (Evans, 1980), morphological traits (Singh *et al.*, 1991b), phaseolin seed proteins (Gepts *et al.*, 1986), allozymes (Santalla *et al.*, 2002) and DNA markers (Beebe *et al.*, 2000; Blair *et al.*, 2006) have confirmed the existence of the two gene pools. Furthermore, the two gene pools were divided into six races, three Andean (Nueva Granada, Peru and Chile) and three Mesoamerican (Mesoamerica, Durango and Jalisco) (Singh *et al.*, 1991a, b), with an additional race reported for Guatemalan climbing beans (Beebe *et al.*, 2000).

Common bean is widely distributed around the world and secondary centers of diversity exist in the Caribbean (Castiñeiras *et al.*, 1991; Durán *et al.*, 2005), South America outside the Andean primary center (Maciel *et al.*, 2003), Europe (Rodiño *et al.*, 2006; Rodiño *et al.*, 2003; Rodino *et al.*, 2001; Santalla *et al.*, 2002), Africa (Khairallah *et al.*, 1990; Martin and Adams, 1987a, b) and Asia (Singh, 1999). Within Asia, collections exist in India (Tiwari *et al.*, 2005), and Iran (Pirbalouti *et al.*, 2006). China, Iran, Turkey and Japan are the most important countries that produce common bean in Asia. Common bean has the highest yield than other food legumes in Iran (FAO, 2003). Three types of white, red and pinto bean are produced in Iran.

The genetic base of most common bean cultivars within a market class is narrow (Voysest *et al.*, 1994) because only a small fraction of wild common bean populations was domesticated (Gepts *et al.*, 1986). The narrow genetic base of cultivars is attributed to the limited use of exotic germplasm (Miklas, 2000) and conservative breeding strategies employed by breeders (Singh, 1992). In order to broaden the genetic base and maximize gain from selection, it is essential to accumulate favorable alleles from the cultivated crops, wild populations and alien species (Miklas, 2000).

Recombination and selection methods depend mainly upon the genetic distance among parents, breeding objectives and available resources. Maintenance and availability of germplasm as a source of genetic variation is especially important to fulfill the increasing needs of breeders. Development of new varieties should not be the cause of overlooking the need for maintaining genetic resources. Problem of the loss of valuable germplasms in the last few decades has been widely observed due to the extensive cultivation of modern varieties and abundance of traditional agriculture in small units (Singh, 1992, 2001).

The objectives of this study were to evaluate genetic diversity of common bean germplasm present in Iran in order to obtain a baseline of information for the preservation, utilization and broadening the genetic base of this important food species. We were also interested in (1) comparing the diversity between two extraction methods of seed soluble proteins by ge-

netic parameters estimation and (2) identifying the contribution of storage proteins in differentiating the two weight groups.

MATERIAL AND METHODS

Seventy common bean genotypes randomly selected from genotypes collection exist in National Bean Research Station of Khomeyn, Iran. The genotypes under study were evaluated for several agronomic characters such as number of days to flowering, number of days to maturity, plant height, pod number per plant, grain number per plant and 100 grain weight in National Bean Research Station of Khomeyn, Iran. Seed length, width and thickness were also measured for three grains of each genotype. Factor analysis was also carried out to determine factors that explain most of the variation for each agronomic character.

Furthermore, protein patterns were studied by SDS-PAGE. The method of Krochko and Bewley (2000) was used for the extraction of soluble seed storage proteins in salt. In this research, both low salt (0.2 M NaCl) and high salt (1 M NaCl) solutions were used. After seed coat separation, seeds were ground and the resulting flour was filtered by a sieve (40 mesh). Forty mg of floured seed was poured in a micro tube. Then two extraction solutions were added in each micro tube and two soluble protein samples in low salt and high salt were prepared. Polyacrylamide gels and buffers were prepared by Hames and Richwood method (Hames and Richwood, 1990). The Laemmli method was used for protein electrophoresis (Laemmli, 1970). Electrophoresis was performed using vertical gels (10%) with 20 µl loading (Table 1). After staining, protein bands were evaluated qualitatively. Each band was named according to its relative mobility (RM). A zero-one coding was used for the presence or absence of proteins in a special location. Then G_{ST} statistic was calculated and tested by χ^2 method (Workman and Niswander, 1970).

Table 1

Consumed materials for the preparation of storage protein samples and loading of each sample in common bean

Sample type	Loaded samples in wells [µl]	2–mercaptoethanol [µl]	Reload buffer [µl]	Extracted soluble protein [µl]
S_1	20	7	2.5	15
S_2	20	14	5.0	30

S_1 = Soluble proteins in low salt, S_2 = Soluble proteins in high salt.

Statistical analyses were performed by SPSS, STATISTICA and POPGENE software.

RESULTS

Diversity for evaluated traits

Several variability statistics for agronomic and seed morphological characters are shown in Table 2. Phenotypic coefficient of variation for agronomic and seed morphological characteristics ranged from 10 (seed weight) to 62 (seed number per plant). Large coefficient of variation has been reported for yield seed, plant height, number of seeds per plant and pod length in common bean (Stoilova *et al.*, 2005). Furthermore, large range for days to maturity, seed characters, seed number per pod, seed number per plant and seed yield has been shown in this species (Santalla *et al.*, 2004).

Table 2

Some descriptive statistics for agronomic and seed morphological traits in the studied common bean genotypes

Characteristics	N	Range	Minimum	Maximum	Mean	Standard Error	Variance	PCV [%]
Seed thickness [cm]	70	0.4	0.42	0.82	0.59	0.009	0.006	15
Seed length [cm]	70	0.67	1.00	1.67	1.29	0.019	0.026	12
Seed width [cm]	70	0.37	0.61	0.98	0.80	0.010	0.007	10
Days to flowering	66	26	36	62	46.8	0.645	27.49	11
Days to maturity	66	60	67	127	97.3	1.445	137.8	12
Plant height [cm]	56	83	18	101	57.1	3.039	517.2	48
Pod number per plant	43	51	6	57	16.0	1.371	80.83	56
Seed number per plant	43	215	22	237	59.1	5.627	1361.7	62
Seed weight [g]	66	51	23	74	38.6	1.241	101.80	26

PCV= Phenotype Coefficient of Variation

Correlation among characters under study

The phenotypic correlations among various characters are presented in Table 3. Seed thickness, seed length and seed width were positively correlated. Seed thickness showed negative correlation with days to maturity. Among the developmental characters, days to maturity was significantly and positively correlated with plant height. Days to flowering had significant positive correlation with both pod number per plant and seed number per plant, however, the correlations were not high. Among the yield compo-

nents, pod number per plant and seed number per plant had significant negative correlation with seed weight. In another study, a negative relationship between number of pods per plant and seed weight was also reported (Duran *et al.*, 2005). This negative correlation should be taken into account in breeding programs in selecting for both seed weight and seed number simultaneously.

Table 3

Phenotypic correlation coefficients among various characters in common bean genotypes

Characters	ST	SL	SW	DtF	DtM	PH	PNpP	SNpP
SL	0.40**							
SW	0.74**	0.52**						
StF	0.11	0.20	0.06					
DtM	-0.31*	-0.12	-0.17	0.04				
PH	-0.05	-0.23	-0.18	0.17	0.41**			
PNpP	0.18	0.11	0.26	0.36*	0.30*	-0.13		
SNpP	0.17	0.16	0.29	0.29*	0.26	-0.10	0.93**	
SWgt	-0.18	0.04	0.00	-0.13	-0.01	0.22	-0.41**	-0.43**

*, ** Significant at 5% and 1% probability levels, respectively.
SL — Seed length
SW — Seed width
DtF — Days to flowering
DtM — Days to maturity
PH — Plant height
PNpP — Pod number per plant
SNpP — Seed number per plant
SWgt —Seed weight
ST — Seed tickness

Factor analysis

Factor analysis transformed nine variables into three factors (Table 4). These factors explained 72% of the total variation. Seed weight, seed number per plant and pod number per plant were the most important traits that characterized factor 1. This factor was, therefore, named as “yield components”. The sign for seed weight was opposite to pod number per plant and grain number per plant in this factor. Seed length, seed width and seed thickness had large coefficients in the second factor which was named “seed morphology”. These traits had similar signs in this factor indicating their positive interrelationships. Other researchers have also reported positive relationships among these traits (Corte *et al.*, 2010). Plant height, number of days to maturity and number of days to flowering were the most important characters contributing to the third factor that was named “phenology”. These characters were related positively which means that late maturing plants had larger value for day to flowering. Genotypes were distributed it

two dimensional plots based on their factor scores (Fig. 1). The factor scores of the "yield components", "seed morphology" and "phenology" ranged from -2.19 to 3.88, from -1.97 to 2.20 and from -2.11 to 1.95, respectively.

Table 4

Factor analysis based on principal component analysis of agronomic and seed morphological traits in studied common bean genotypes

Characteristics	Component			Communality
	Factor 1	Factor 2	Factor 3	
Seed thickness	0.114	0.868	-0.060	0.771
Seed length	0.038	0.772	0.007	0.598
Seed width	0.154	0.903	-0.031	0.839
Days to flowering	0.371	0.164	0.647	0.582
Days to maturity	0.151	-0.099	0.819	0.703
Plant height	-0.307	-0.111	0.830	0.796
Pod number per plant	0.903	0.111	0.201	0.868
Seed number per plant	0.890	0.139	0.176	0.842
Seed weight	-0.719	-0.067	0.186	0.556
Variance [%]	33.13	23.04	16.16	
Cumulative variance [%]	33.13	56.17	72.83	

Factor 1 = Yield components; Factor 2 = Seed morphology; Factor 3 = Phenology

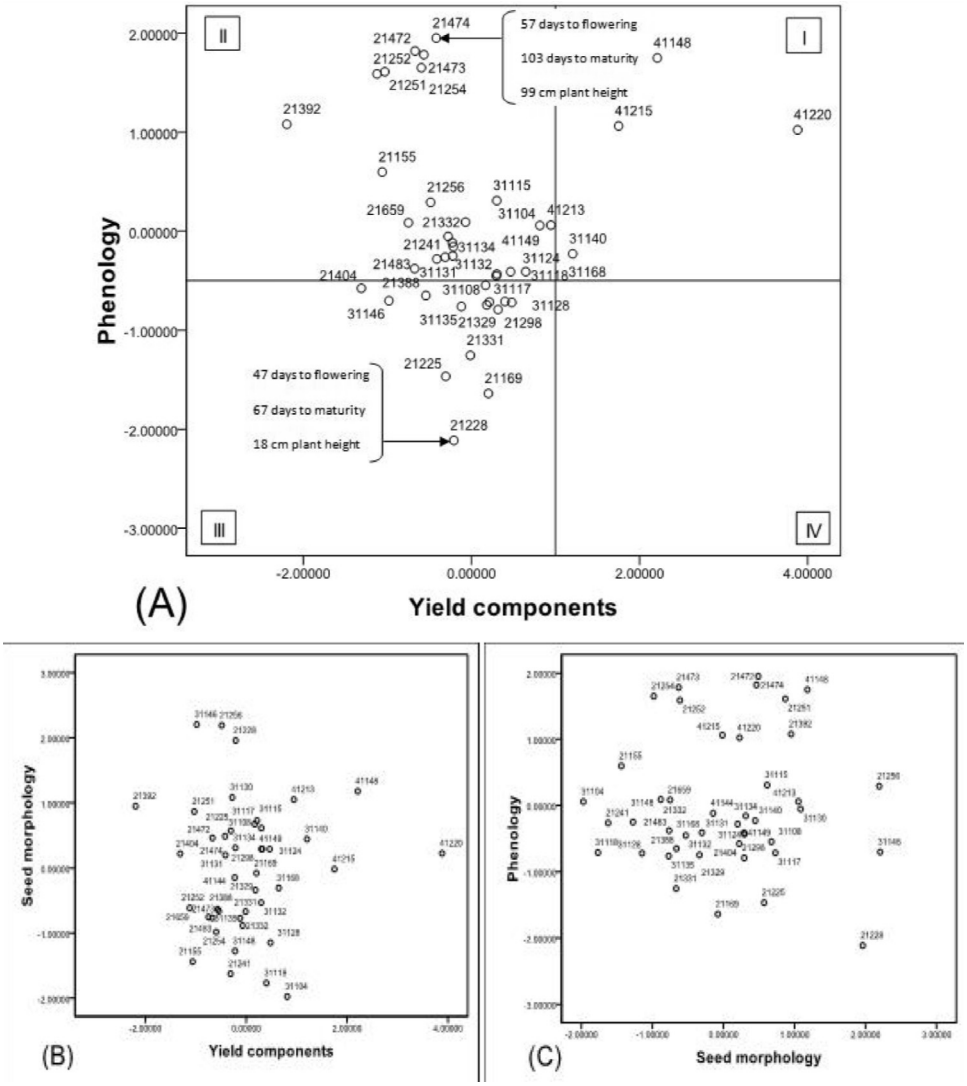

Fig. 1. Features of studied common bean genotypes based on their factor scores

Comparison of genetic variation between two extraction methods

Electropherogram of several common bean genotypes in terms of soluble proteins based on the method of extraction are shown in Figure 2. Nei's diversity coefficient (H_e =0.43) and effective number of alleles (A_e=1.69) for the extraction method of soluble proteins in low salt were almost similar to the extraction method in high salt (H_e=0.43 and A_e=1.75) (Table 5). However, the number of polymorphic loci for the extraction method in low salt was much higher than the other method

(17 and 6, respectively). It seems that a lot of soluble proteins in high salt were removed that caused the drastic reduction in polymorphism.

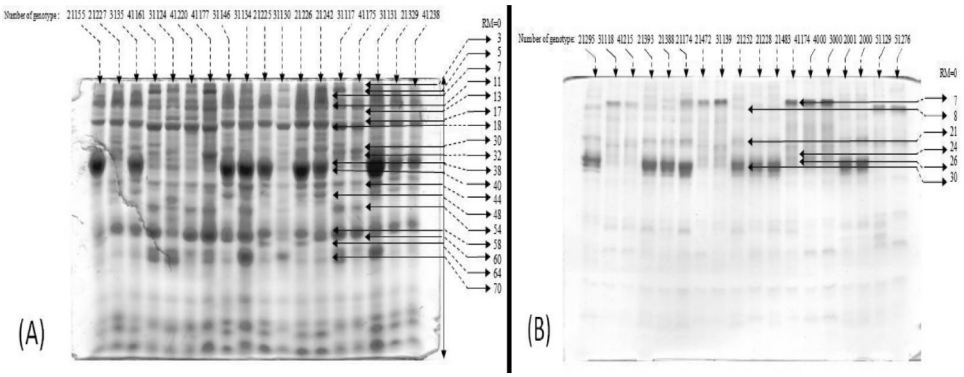

Fig. 2. Gel samples of several common bean genotypes for the extraction method of soluble proteins in low salt (A) and high salt (B)

Table 5

The genetic diversity statistics for the common bean genotypes under study for two extraction methods of soluble proteins in salt

Sample Size	Statistic					
	S_1			S_2		
	RM	A_e	H_e	RM	A_e	H_e
70	3	1.9085	0.4760	7	1.6336	0.3879
70	5	1.9780	0.4944	8	1.7792	0.4380
70	7	1.9580	0.4893	21	1.9431	0.4854
70	11	1.8876	0.4702	24	1.7555	0.4304
70	13	1.1771	0.1504	26	1.7314	0.4224
70	17	1.7792	0.4380	30	1.6827	0.4057
70	18	1.6336	0.3879			
70	30	1.7314	0.4224			
70	32	1.7314	0.4224			
70	38	1.6827	0.4057			
70	40	1.6827	0.4057			
70	48	1.6092	0.3786			
70	54	1.6336	0.3879			
70	58	1.5366	0.3492			
70	60	1.7068	0.4141			
70	64	1.4892	0.3285			
70	70	1.6827	0.4057			
Mean		1.69	0.40		1.75	0.43
N				6		

S_1 = Soluble proteins in low salt (0.2 M NaCl); S_2 = Soluble proteins in high salt (1M NaCl); RM = Relative Mobility; *A*e = Effective number of alleles; *H*e = Nei's diversity coefficient. N = Number of polymorphic loci.

Identifying the prevalence of two seed weight groups

There are two diversity centers for common bean based on seed weight (Evans, 1980). Genotypes from Andean region generally are large seeded (>40 g 100-seed weight) while genotypes from the Mesoamerican region are small (<25 g 100-seed weight) or medium seeded (25–40 g 100-seed weight). Therefore, protein bands under investigation were studied for the two weight groups. The results for G_{ST} statistics (Table 6) showed that electrophoresis bands of 30, 32, 38 and 40 (Fig. 2A) explained more differences in these two weight groups than other bands. Also, studying the relationship between these electrophoresis bands and agronomic and seed morphological characters by t-test showed that genotypes having bands with the RM of 30 and 32 had lower 100 seed weight and higher seed and pod number per plant while genotypes having bands with the RM of 38 and 40 had higher 100 seed weight and lower seed and pod number per plant (Table 7).

Table 6

The statistics of genetic diversity between two weight groups for bands from extraction method of soluble proteins in low salt

RM	Genetic statistic[1]		
	H_T	H_S	G_{ST}
3	0.4812	0.4326	0.1010
5	0.4938	0.4835	0.0209
7	0.4997	0.4233	0.1529
11	0.4604	0.4299	0.0664
13	0.1495	0.1483	0.0078
17	0.4708	0.3805	0.1919
18	0.3916	0.3268	0.1656
30	0.4374	0.3261	0.2546
32	0.4374	0.3261	0.2546
38	0.4533	0.3336	0.2641
40	0.4533	0.3336	0.2641
48	0.4077	0.3519	0.1370
54	0.3916	0.3268	0.1656
58	0.3434	0.3429	0.0015
60	0.3719	0.3717	0.0007
64	0.3229	0.3229	0.0000
70	0.4060	0.3670	0.0959
Mean	0.4101	0.3545	0.1355

[1] H_T = Total diversity; H_S = Population diversity; G_{ST} = Inter-population differentiation for all loci

Table 7

Results of t-test for several agronomic characters in common bean genotypes based on electrophoresis bands

RM	Absent or present band		Pod number per	Seed number per	Seed weight
30	Mean	0	14.26*	46.22*	44.18**
		1	20.07	72.57	33.41
32	Mean	0	14.26*	46.22*	44.18**
		1	20.07	72.57	33.41
38	Mean	0	20.07	72.57	33.41
		1	14.26*	46.22*	44.18**
40	Mean	0	20.07	72.57	33.41
		1	14.26*	46.22*	44.18**

* and **Significant at the 5% and 1% levels of probability, respectively.

DISCUSSION

There was a proper dispersion for seed morphology among the genotypes under study. Thus, it seems that there is a potential for the improvement of seed morphological characters in the breeding programs of common bean in Iran. Genotypes were distributed in almost two groups based on phenology. Most genotypes were located in the group having lower or medium scores. Apparently, the lower values for this factor are due to the climatic condition of common bean growing regions in Iran because the late maturing cultivars are not suitable for these regions.

One application of evaluation for diversity is to choose genotypes from two ends of the phenotypic distribution. For example, the genotypes 21474 and 21228 in Fig. 1A were located at the opposite end of the distribution for phenology. The genotype 21474 has high factor score for phenology with 57 days to flowering, 103 days to maturity and 99 cm plant height while the genotype 21228 had low scores for this factor with 47 days to flowering, 67 days to maturity and 18 cm to plant height. Crossing of the genotypes in the opposite locations in the distribution allows the breeders to increase the probability of heterosis and transgressive segregation. Significant heterosis has also been found for number of days to flowering (Barelli *et al.*, 2000; Mitranov, 1983), plant height (Gonçalves-Vidigal *et al.*, 2008), number of pods per plant, number of seeds per plant, seed weight (Barelli *et al.*, 2000; Gonçalves-Vidigal *et al.*, 2008; Nienhuis and Singh, 1988), seed thickness, seed length and seed width (Corte *et al.*, 2010) in beans. There-

fore, the factors recognized in this study can be used for choosing the parents to improve traits simultaneously.

Most of genotypes can be recognized by their soluble seed storage proteins in low salt. Considerate to stability and codominance heritability of seed storage proteins (Brown *et al.*, 1981a; 1981b), this extraction method can be used to seed purity certification and hybrids identification as complementary method for common bean.

Researchers reported that the two diversity centers of common bean are significantly different in some characters such as seed size (Evans, 1980) and phaseolin seed proteins (Gepts *et al.*, 1986; Pereira *et al.*, 2009). Results showed that about 54% of the genotypes had bands 38 and 40 and higher 100 seed weight. It seems that the contribution of the two diversity centers were almost similar in the studied common bean genotypes.

CONCLUSION

- Common bean has a potential diversity for some traits in this study. This variability could be exploited in breeding programs. While, other traits need to increase diversity by using of exotic germplasm and breeding strategies.
- Genotypes can be selected based on factor scores. Also, graphical information by biplot analysis such as factor analysis can help breeders to accumulate favorable alleles and broaden the genetic base and maximize gain from selection.
- Soluble proteins in low salt were more useful for measuring the genetic diversity present within common beans species than other extraction method.
- Some protein bands in this research may be useful to select indirectly for seed and pod number per plant.

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