

Genetic diversity analysis of faba bean (*Vicia faba* L.) germplasms using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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ABSTRACT. To investigate genetic diversity and relationships of 101 faba bean (*Vicia faba* L.), landraces and varieties from different provinces of China and abroad were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). A total of 2625 unambiguous and stable bands from 101 germplasms were detected, and 36 different bands were classified according to the electrophoretic mobility patterns of the proteins as determined by the SDS-PAGE analysis, of which 16 were polymorphic. Besides the common bands, the protein bands of 92, 75, 62, 40, 34, 17, and 13 kDa presented the highest frequencies of 92.08, 90.10, 99.01, 95.05, 95.05, 98.02, and 95.05%, respectively. The other 29 polymorphic protein bands showed higher polymorphism with 16.09 polymorphic bands in average. The genetic similarity of the 101 genotypes tested varied from 0.6111 to 0.9722, with an average of 0.7122. Cluster analysis divided the 101 genotypes into six major clusters, which was consistent with

the systematic classification of faba bean done in previous studies. The overall results indicated that SDS-PAGE was a useful tool for genetic diversity analysis and laid a solid foundation for future faba bean breeding.

Key words: Faba bean; Protein; SDS-PAGE; Genetic similarity; Cluster analysis

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important cultivated legume crops in the whole world. It is a legume, which is a primary food source for humans, and is popular due to its high seed protein content (Ye et al., 2003). Additionally, faba bean plays an important role in crop rotation, nitrogen fixation (Kaur et al., 2012), disease and weed control (Murray et al., 1988; Schmidtke and Rauber, 2000). Faba bean ($2n = 2x = 12$) is an autodiploid plant with fewer chromosome numbers than other *Vicia* species (Raina and Ogihara, 1995), but map-based cloning and marker assistant selection in faba bean have been enormously challenging because of the huge genome size (13,000 Mb) (Bennett et al., 1982; Johnston et al., 1999).

To date, a total of 38,360 faba bean germplasm from different countries have been conserved at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria (Duc et al., 2010). China is the biggest country with respect to growing area and production of faba bean, especially in the northwest and southwest areas. Various surveys have been conducted to describe the handling and genetic variation of faba bean collections.

Over the past few years, faba bean breeders have succeeded in improving yield, disease and pest resistance, as well as quality and agronomic performance, by using excellent germplasm resources. Genetic diversity is a key factor for crop improvement. However, due to the frequent utilization of a few elite germplasm lines, genetic diversity in faba bean is often decreased. Previous studies have made remarkable progress in analyzing genetic diversity of faba bean, especially with various DNA molecular markers. Random-amplified polymorphic DNA markers were first used to test relationships between three faba bean inbred lines obtained from Europe and the Mediterranean region (Link et al., 1995). Molecular variation among winter (Zong et al., 2009) and spring (Zong et al., 2010) faba bean ecotypes have been examined using amplified fragments length polymorphism markers. Inter-simple sequence repeat markers were used to analyze the genetic diversity and relationship of global faba bean germplasm (Terzopoulos and Bebeli, 2008; Wang et al., 2012).

The degree of genetic diversity can reflect the level of genetic progress in future breeding. Therefore, evaluation of faba bean genetic diversity will be helpful for breeding programs. Previous studies have indicated that the electrophoretic pattern of seed proteins can be utilized as a reliable tool to clarify specific phylogenetic relationships among species (Potokina et al., 2003). However, only few reports about protein genetic diversity of faba bean have been published; Eckert and Barrett (1993) studied clonal reproduction and patterns of genotypic diversity. Therefore, the study of genetic diversity of faba bean with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) is important. To the best of our knowledge, this is the first application of SDS-PAGE analysis for the assessment of genetic diversity among faba bean collections.

In order to measure the genetic diversity of faba bean with SDS-PAGE, we compared the genetic relationship of faba bean germplasm, and we have provided a basis for understanding the genetic diversity of faba bean. Moreover, we have also put forward suggestions for the further improvement of faba bean.

MATERIAL AND METHODS

Plant materials

A total of 101 faba bean accessions, including 74 varieties from different provinces of China and 27 accessions from abroad ([Table S1](#)) were selected from the China National GenBank, located at the Institute of Crop Science (ICS), Chinese Academy of Agricultural Sciences (CAAS), Beijing. All the representative samples of faba bean were grown on the experimental farm of Qinghai Agriculture and Forestry Academy. In this study, a representative collection of 74 elite faba bean accessions from the major faba bean-producing regions of China, and 27 germplasms from the rest of the world, were analyzed using SDS-PAGE analysis. The two main varieties in China, the spring and winter faba bean, were selected for this study to compare the different ecotypes of faba bean germplasm.

Sample preparation and electrophoresis

Total protein was extracted from bulk-crushed seeds as described by Chen (1995). Dry powder (0.05 g) was soaked in 0.4 mL water for at least 24 h, and then the mixture was placed in an Eppendorf centrifuge for 30 min at 8000 g. The supernatant was preserved at 4°C for sample preparation. Each sample was suspended in a medium containing 2% SDS (w/v), 5% 2-mercaptoethanol (w/v), 0.001% pyronin (w/v), 10% glycerol (v/v), and 1 M Tris-HCl, pH 6.8. The samples were left for 3 h at room temperature and shaken every 15 min. Then, the mixture was placed in a boiling water bath for 3-5 min and cooled at 4°C.

SDS-PAGE was performed on 4% slab gels in a discontinuous buffer system as described by Laemmli (1970). A constant electric current of 30 mA was used to run two gels for 10 h.

Statistical analysis

SDS-PAGE analysis was performed in duplicate, and only those bands obtained clearly twice were scored either 1 (present), or 0 (absent). The average diversity index among accessions and geographical groups was measured according to Shannon-Weaver. Data generated from SDS-PAGE analysis were analyzed with Jaccard similarity coefficient (Jaccard, 1908).

The cluster analysis of different geographical groups was carried out using unweighted pair-group method with arithmetic average (UPGMA), employing SAHN (sequential, agglomerative, hierarchical, and nested clustering) from the *NTSYSpc* (version 2.10) (Rohlf, 2005). The dendrogram was drawn by MEGA3.1.

RESULTS AND DISCUSSION

The SDS-PAGE analysis showed 2625 unambiguous and stable bands from 101 germplasms (Table 1). Each variety produced different numbers of bands; the numbers

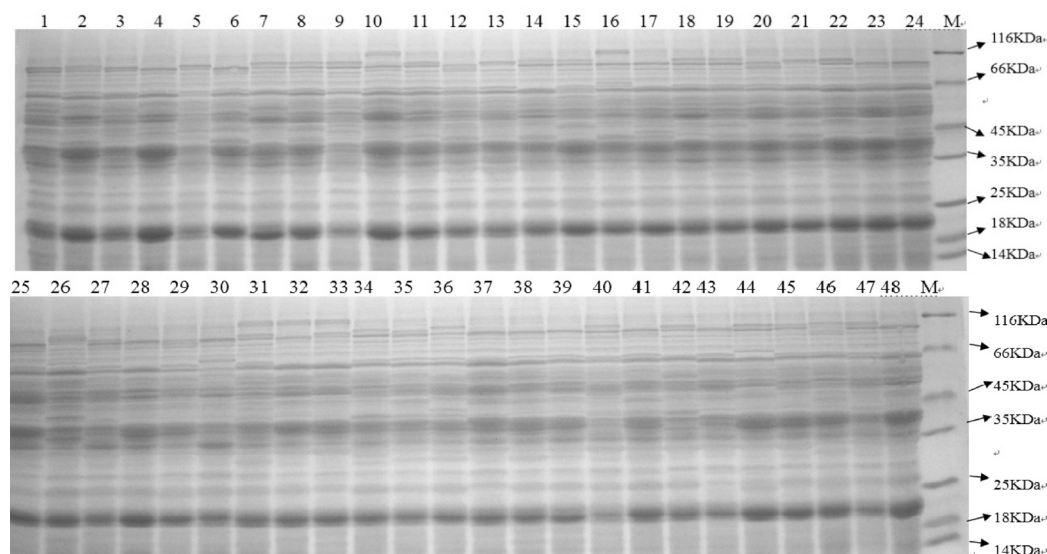
of protein bands ranged from 21 to 31 (Table 2). Based on the electrophoretic mobility of proteins, 36 different bands were present (Figure 1), of which 16 were polymorphic, with an average ratio of 44.69% polymorphic fragments per variety. Percentage of polymorphic bands ranged from 58.33% to a minimum of 30.56% with an average of 93%.

Table 1. Numbers of protein bands in 101 faba bean genotypes.

No.	Total bands	Polymorphic bands	Polymorphism percentage (%)	No.	Total bands	Polymorphic bands	Polymorphism percentage (%)
1	29	19	52.78	52	27	17	47.22
2	26	16	44.44	53	24	14	38.89
3	24	14	38.89	54	26	16	44.44
4	24	14	38.89	55	23	23	63.89
5	25	15	41.67	56	21	11	30.56
6	25	15	41.67	57	28	18	50.00
7	26	16	44.44	58	23	13	36.11
8	26	16	44.44	59	25	15	41.67
9	29	19	52.78	60	24	14	38.89
10	23	13	36.11	61	26	16	44.44
11	26	16	44.44	62	29	19	52.78
12	22	12	33.33	63	24	14	38.89
13	28	18	50.00	64	28	18	50.00
14	28	18	50.00	65	27	17	47.22
15	27	17	47.22	66	24	14	38.89
16	26	16	44.44	67	25	15	41.67
17	22	12	33.33	68	27	17	47.22
18	25	15	41.67	69	24	14	38.89
19	27	17	47.22	70	26	16	44.44
20	26	16	44.44	71	25	15	41.67
21	27	17	47.22	72	28	18	50.00
22	28	18	50.00	73	25	15	41.67
23	25	15	41.67	74	23	13	36.11
24	27	17	47.22	75	24	14	38.89
25	27	17	47.22	76	25	15	41.67
26	28	18	50.00	77	25	15	41.67
27	26	16	44.44	78	27	17	47.22
28	26	16	44.44	79	26	16	44.44
29	25	15	41.67	80	23	13	36.11
30	25	15	41.67	81	27	17	47.22
31	24	14	38.89	82	24	14	38.89
32	28	18	50.00	83	25	15	41.67
33	25	15	41.67	84	29	19	52.78
34	26	16	44.44	85	27	17	47.22
35	25	15	41.67	86	25	15	41.67
36	26	16	44.44	87	27	17	47.22
37	29	19	52.78	88	27	17	47.22
38	26	16	44.44	89	26	16	44.44
39	28	18	50.00	90	22	12	33.33
40	27	17	47.22	91	27	17	47.22
41	25	15	41.67	92	27	17	47.22
42	27	17	47.22	93	27	17	47.22
43	28	18	50.00	94	26	16	44.44
44	27	17	47.22	95	26	16	44.44
45	28	18	50.00	96	26	16	44.44
46	28	18	50.00	97	27	17	47.22
47	28	18	50.00	98	28	18	50.00
48	25	15	41.67	99	29	19	52.78
49	27	17	47.22	100	24	14	38.89
50	25	15	41.67	101	31	21	58.33
51	26	16	44.44				

Table 2. Distribution frequency of protein bands in 101 faba bean genotypes.

Total bands	Accession No.	Frequency (%)
31	1	0.99
29	6	5.94
28	14	13.86
27	21	20.79
26	20	19.80
25	19	18.81
24	11	10.89
23	5	4.95
22	3	2.97
21	1	0.99

**Figure 1.** Representative SDS-PAGE amplification pattern generated in part of 101 faba bean samples. Lanes 1 to 48 = partial faba bean germplasms; lane M = 100-kDa marker.

SDS-PAGE analysis indicated that 10 protein molecular masses, viz. 68, 42, 35, 29, 26, 22, 21, 20, 16, and 14 kDa were common bands for all the varieties tested, and 92, 75, 62, 40, 34, 17, and 13 kDa were detected in some varieties with high frequency. The genetic similarities of different germplasms were calculated with the genetic similarity method. Genetic similarity index of different geographical groups of germplasm ranged from 0.6111 to 0.9722 with an average of 0.7122. The collections from Northwest of China showed the highest genetic diversity, while the faba bean germplasm from central China showed the lowest level of diversity. The similarity matrix correlation analysis indicated a low level of genetic diversity among varieties, viz. *HUAN3*, *324*, *BEIYUAN*, *XIAOHUOUDOU*, *CHENDOU*, and *ZAOAN*. The *NEW1*, *SPANNIA299*, and *ENGLAND38* had similar genetic diversity parameters, which indicated that those groups had higher diversity.

Clustering analysis of faba beans belonging to different Chinese provinces and accessions obtained from other countries was carried out by POPGEN1.32 (Yeh and Boyle, 1997) based on the UPGMA method, and the dendrogram was drawn by the MEGA3.1

software (Tamura et al., 2007). The results showed that 101 faba bean accessions were divided into six major groups (Figure 2).

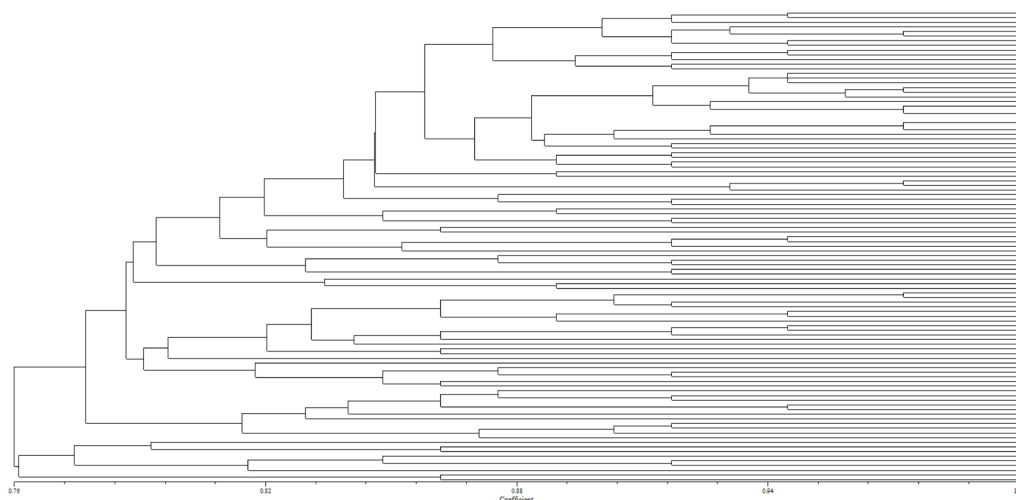


Figure 2. UPGMA dendrogram of 101 faba bean germplasms from different provinces in China and rest of the world.

The first group (group I) included 77 faba bean accessions, with a percentage of 76.23% among the 101 varieties tested, and the genetic similarity index of 77 different germplasm ranged from 0.6389 to 0.9722. Group I showed the highest polymorphism, which included 36 bands, and contained three subgroups. The second group (group II) comprised five faba bean accessions with a percentage of 4.95%. The diversity and genetic similarity index of group II were 3.6323, and 0.7778 to 0.8889, respectively. Two collections, viz. *WUSHAN* and *1638* in group II, showed a high level of genetic diversity, which indicated that they had a closer genetic relationship. This group generated 32 different bands, of which 18 bands were common, with a percentage of 56.25%. SDS-PAGE analysis showed that all faba bean genotypes in group II produced the specific band of 46 kDa, which can be used as a diagnostic characteristic. The third group (group III) consisted of one faba bean germplasm *0908* from Gansu Province in Northwest of China. Germplasm *0908* generated 25 types of bands, and produced the expected bands of 116 and 66 kDa. The fourth group (group IV) had three faba bean collections with a percentage of 3.96%. The diversity and genetic similarity index of group were 3.1826, and 0.7778 to 0.8611, respectively. Thirty-two bands were observed in group IV, and 22 of them were common bands. Compared with other groups, group IV had four specific bands of 66, 60, 55, 50, and 45 kDa. The fifth group contained 6 germplasms of the faba bean collections tested. The diversity and genetic similarity index of group V were 3.0753, and 0.8056 to 0.9167, respectively. Group V included 31 different bands, and 19 of these were common bands. The 6 germplasms in group V produced the band of 87 kDa, but lacked the bands of 81 and 46 kDa. The remaining 9 faba bean collections belonged to the sixth group. The genetic similarity index of group VI was 0.8333 to 0.9167. These 9 varieties

did not contain the bands of 116 and 81 kDa. The UPGMA clustering analysis indicated that there was a high level of protein diversity among different faba bean collections.

Group analysis of the genotypes according to their geographic origin showed that the landraces from Qinghai Province grouped together, other faba bean germplasms from different provinces of China formed another group, while the remaining faba bean collections from the rest of the world grouped together.

Based on SDS-PAGE analysis of 101 faba bean germplasms, genetic diversity between the three geographic groups was calculated (Table 3). Genotypes from Qinghai Province showed the highest genetic index among the three groups. The average genetic diversity of 101 faba bean germplasms was 5.1884, and the genetic diversity index between different groups was 0.0121, while the genetic diversity index in the group was 0.9879. These results indicated that the diversity of faba bean protein depended on the same group rather than different groups.

Table 3. Genetic index of three geographical groups.

Geographical group	Genetic index
Genotypes from Qinghai	5.3097
Genotypes from China	5.0703
Genotypes from abroad	4.9967
Average genetic diversity index	5.1255
Total average genetic diversity index	5.1884
Genetic diversity index within group	0.9879
Genetic diversity index between group	0.0121

The assessment of the genetic variation within SDS-PAGE is important for the effective conservation and utilization of genetic resources in breeding programs, and it could be dramatically enhanced by using molecular genotyping tools. Genetic variability in faba bean has been previously studied using different DNA molecular markers (Link et al., 1995; Terzopoulos and Bebeli, 2008; Zong et al., 2009, 2010; Wang et al., 2012). However, this is the first time that SDS-PAGE analysis has been utilized for estimating genetic diversity in faba bean. In this study, the genetic diversity of 101 accessions of different faba bean ecotypes from regions in China and the rest of the world was analyzed by SDS-PAGE.

In the current study, we identified that SDS-PAGE was a powerful technique for the assessment of genetic variability because it has shown a high degree of reproducibility and discriminatory power, as well as a high polymorphism rate in many previous genetic studies with molecular markers. In this study, SDS-PAGE analysis produced 2625 unambiguous and stable bands from 101 germplasms, and 36 different bands were classified based on electrophoretic mobility of proteins, of which 16 were polymorphic, with an average ratio of 44.69% polymorphic fragments per variety. Each variety produced different numbers of bands, the numbers of protein bands ranged from 21 to 31. All SDS-PAGE generated 100% polymorphic fragments, so the number of fragments and the polymorphism percentages in this study were higher than those obtained in other genetic diversity studies of faba bean using different molecular markers. The variation of polymorphism rate reflects the extent of genetic divergence among and within the populations and/or genotypes studied, and SDS-PAGE combinations used. These findings demonstrate the usefulness of SDS-PAGE analysis in detecting genetic variability in various plant species.

The genetic similarity of the genotypes tested varied from 0.6111 to 0.9722, with an average of 0.7122. The genetic diversity index within group was much higher than that between groups, which indicated that the main variation of protein occurred within populations. Cluster analysis divided the accessions into six major clusters, which was consistent with previous genetic diversity studies of faba beans.

In conclusion, our SDS-PAGE analysis results suggest that the 101 faba bean collections utilized, are genetically diverse, which is consistent with previous studies. The clustering results of the protein genetic diversity of faba bean were largely dependent on growth habit, geographical origin, and ecological distribution. However, a broader investigation of morphological and agronomical characteristics is required to confirm the distinct differentiation of gene pools between China and the rest of the world.

Conflicts of interest

The authors declare no conflict of interest.

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[Supplementary material](#)

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