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ORIGINAL PAPER

Molecular variation among Chinese and global winter faba bean germplasm

**Xuxiao Zong · Xiuju Liu · Jianping Guan ·
Shumin Wang · Qingchang Liu · Jeffrey G. Paull ·
Robert Redden**

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Abstract A sample of winter faba bean germplasm from China was compared with germplasm from outside China, using AFLP analyses. Both sets of germplasm were obtained from the National Genebank of China, Institute of Crop Sciences (ICS), Chinese Academy of Agricultural Sciences, Beijing, China. A sample of 39 winter type accessions from outside of China and 204 Chinese landraces and varieties (201 winter types and 3 spring types) were characterized with 10 AFLP primers. These detected 266 polymorphic bands. The Chinese germplasm was clearly separated from the rest of the world in principal component analysis and clustering analysis, with the spring types from China showing the greatest separation. Yunnan germplasm, both landraces and commercial varieties, showed the greatest separation among the germplasm of Chinese winter faba bean provinces. The landraces/varieties from Anhui, Zhejiang, Sichuan, Jianxi, Guizhou and Fujian provinces clustered in a central group.

Introduction

China is the largest faba bean (*Vicia faba* L.*)* producer in the world with an average grain production (2000–2005) of 2,067,200 metric tonnes (m.t.) on 1,151,800 ha, and 13,236 ha of green faba bean (FAO 2006). In China, large-seeded grain is used as human food both domestically and for export, medium size grain is used for both food and feed, and small-seeded faba mainly for feed as well as export to the Middle East (Redden et al. 2007). Faba is also consumed as a vegetable crop, cooked as shelled beans in north China, but with the seed coat removed in south China. There is a preference for green seed coat types with green cotyledons (Redden et al. 2007). China supplied 43% of the world faba bean production followed by Ethiopia (425,106 m.t.), Egypt (408,078 m.t.), France (297,645 m.t.) and Australia (252,000 m.t.) (FAO 2006). However Chinese faba bean germplasm is poorly represented in international collections with only 496 from China out of 5,927 accessions in the germplasm collection of the International Center for Agricultural Research in the Dry Areas (ICARDA) (K. Street, personal communication 2008). Faba bean is produced in 21 provinces in China, with 14 (Yunnan, Sichuan, Hubei, Jiangsu, Hunan, Zhejiang, Guizhou, Guangdong, Jiangxi, Anhui, Shanghai, Sha'anxi, Guangxi and Fujian) located in winter growing areas (Ye et al. 2003). Winter faba bean

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X. Zong · X. Liu · J. Guan · S. Wang
Institute of Crop Sciences/The National Key Facility for Crop Gene Resources and Genetic Improvement,
Chinese Academy of Agricultural Sciences,
100081 Beijing, China

X. Zong · Q. Liu
Department of Plant Genetics and Breeding,
China Agricultural University, 100094 Beijing, China

J. G. Paull
School of Agriculture, Food and Wine,
University of Adelaide, Adelaide, SA, Australia

R. Redden (✉)
The Department of Primary Industries,
Australian Temperate Field Crops Collection,
Grains Innovation Park, Private Bag 260,
Horsham, VIC 3401, Australia
e-mail: Bob.redden@dpi.vic.gov.au

comprised 85.5% of the total area and 78.2% of the total production of faba bean in China (Ye et al. 2003). Yunnan province with 340,000 ha cropped to faba bean has the largest production in China (Bao et al. 2006a).

Faba bean is a partially allogamous crop, consequently there is a high level of heterozygosity within varieties and landraces are generally highly heterogeneous (Link et al. 1994a). Population breeding methods can be applied for the improvement of landraces and open-pollinated varieties, both as a food grain (dry, fresh frozen or canned) and for feed (Duc et al. 2009), while synthetic varieties of faba beans are also developed (Link et al. 1994b). Faba bean was one of the earliest domesticated crops with the origin believed to be in the near or Middle East (Cubero 1974), and well preserved seed remains from north-west Syria date to the tenth millennium BP (Tanno and Willcox 2006). The timing of the introduction of faba bean to China is uncertain. There are ancient references to the introduction of faba bean (*V. faba* var. *major*) to the north part of China from the Middle East 2,100 years ago through the Silk Road (Lang and Ying 1997). Faba bean grain fossils found in Wuxing county of Zhejiang province indicated that faba bean has been grown in southern China for more than 4,000–5,000 years (Ye et al. 2003). A Yunnan historical document suggests that ancient landraces originated in the Lijiang area and wild faba beans appeared in the Binchuan area of Yunnan province (Ye et al. 2003).

In China, small to medium seed types of faba bean are traditionally produced in winter growing areas and medium to large seed types in spring sowing areas. Traditionally, medium (*equina*) to large (*major*) seed types of dry faba bean are used for human food, while small (*minor*) seed types are used for feed, for which respective 100 seed weights are 60.1–110, >110, and <60 g (Cubero and Nadal 2005). In addition, faba bean straw provides fodder for cows and pigs. Medium to large seed types with green seed coat (and preferably with a green cotyledon when the seed is mature) are consumed as vegetables while the pods are still green (Ye et al. 2003).

Gene pools of winter faba bean in China tended to be isolated in mountainous areas with very poor roads, until the advent of modern plant breeding and transportation systems in recent years. Faba bean genetic resources introduced from other countries (Table 1) are not well adapted to winter growing faba bean areas in China (Ye et al. 2003).

Chinese winter faba bean germplasm had not been compared previously with the genetic diversity in the rest of the world in a systematic way until now. Molecular diversity analyses were done by Link et al. (1995) using RAPDs analyses with inbred derivatives (F6–F11) from 28 European and Mediterranean faba beans of diverse seed sizes, and by Zeid et al. (2003) with AFLP analyses using eight selected primer combinations on inbred lines (F2–F10), from 79 elite varieties of European, North African and Asian origins. This study compared genetic diversity of winter faba bean germplasm among provinces within China and between China and the rest of the world. Since too few SSR primer pairs were available for faba bean (25 from chromosome 1 only) (Pozarkova et al. 2002), AFLP methodology was used.

Materials and methods

The 243 faba bean germplasm were sourced from National Genebank of China, Institute of Crop Sciences (ICS), Chinese Academy of Agricultural Sciences, Beijing, China. In addition, 14 varieties were sourced from provincial breeding programs. The accessions, representative of globally diverse winter faba bean genetic resources, comprised: 187 Chinese landraces and 14 commercial varieties from 11 provinces, 39 accessions from Asia, Africa and Europe, and three Chinese spring type accessions from Qinghai and Ningxia as a reference (Table 1).

Most landraces were of the intermediate *equina* type, but the 46 *minor* types included all 10 accessions from Sha’anxi.

For each accession, 10 randomly selected plants were sampled to provide pooled DNA samples for AFLP analyses. CTAB method (Dellaporta et al. 1983; Doyle and Doyle 1990), with modification (Liu et al. 2007), was used for DNA extraction. An optimized AFLP system of silver staining on 6% w/v denaturated polyacrylamide gels (Zong et al. 2003), with modification (Liu et al. 2007), was applied for genetic diversity analysis of faba bean germplasm in this study. The AFLP primer pairs were screened from all the possible three-nucleotide sequences of *Eco*RI and *Mse*I primer combinations, using six diverse faba bean DNA samples. The six diverse faba bean landraces from six provinces of China in winter sowing areas, were H0000105 (Qingpu area of Shanghai), H0001248 (Jiaxing city of Zhejiang), H0001335 (Feidong county of Anhui), H0001385 (Fuqing county of Fujian), H0001501 (Yichang county of Hubei) and H0001629 (Xindu county of Sichuan). Ten polymorphic primer pairs were selected (Table 2) to analyze 243 accessions for genetic diversity (The sequences of the selected primers are available on request from the authors), of which six (Table 2) were used by Zeid et al. (2003). One common check (H0001501) and ladder DNA were used in each gel plate to clearly identify each visible silver staining band.

Principal components analysis (PCA) and 3-dimension PCA graphs based on simple matching coefficient matrix converted from AFLP data, were conducted and drawn by NTSYSpc 2.20 (Rohlf 2006). Nei’s gene diversity (Nei 1973), Nei78 genetic distances (Nei 1978) among groups of genetic resources, Shannon’s information index (Lewontin 1972)

Table 1 Set of 243 winter sown faba bean accessions sampled for the study, with passport and seed data for Chinese accessions

Origin	Type	No. of accessions	Origin by province/country, and classification by seed size[a]	Latitude/longitude range of the province	Elevation range, meters
Winter sowing area in China	Local landraces	187	Zhejiang (A = 5, B = 48, C = 2);	27°12′–31°30′/118°–123°	0–1,000
			Sichuan (A = 1, B = 34);	26°03′–34°19′/97°21′–110°12′	0–3,099
			Anhui (A = 6, B = 16);	29°41′–34°38′/114°54′–119°37′	0–1,841
			Yunnan (A = 1, B = 8, C = 5);	21°8′–29°15′/97°31′–106°12′	0–6,740
			Guizhou (A = 7, B = 7, C = 2);	24°37′–29°13′/103°36′–109°35′	0–2,900
			Hubei (A = 9, B = 7);	29°25′–33°20′/108°21′–116°07′	0–3,105
			Jiangxi (A = 6, B = 6);	24°29′–30°4′/113°34′–118°28′	0–1,474
			Sha’anxi[b] (A = 8, B = 2);	21°42′–39°45′/105°29′–110°15′	0–3,767
			Fujian (A = 2, B = 2);	23°30′–28°22′/115°50′–120°40′	0–2,158
			Shanghai (B = 2);	30°40′–31°51′/120°51′–121°54′	0–100
			Guangxi (A = 1)	20°54′–26°20′/104°29′–112°54′	0–2,142
Winter sowing area in China	Commercial varieties	14	Sichuan (B = 3);	26°03′–34°19′/97°21′–110°12′	0–3,099
			Yunnan (B = 4, C = 1);	21°8′–29°15′/97°31′–106°12′	0–6,740
			Fujian (B = 6)	23°30′–28°22′/115°50′–120°40′	0–2,158
Spring sowing area in China	Local landraces	3	Qinghai (B = 2);	31°39′–39°19′/89°35′–103°04′	1,500–6,621
			Ningxia (B = 1)	36°14′–39°23′/104°17′–107°39′	1,000–3,556
Asia (except China)	Accessions	16	Afghanistan (A = 2, B = 1);		
			Syria (C = 2);		
			Turkey (C = 3);		
			Iraq (C = 3);		
			Iran (A = 1, B = 1);		
			Lebanon (B = 1, C = 1);		
			Japan (C = 1)		
Africa	Accessions	14	Morocco (B = 1, C = 1);		
			Egypt (B = 2);		
			Tunisia (A = 2, B = 2);		
			Sudan (A = 3, B = 1);		
			Ethiopia (A = 2)		
Europe	Accessions	9	Hungary (C = 1);		
			Former Soviet Union (B = 2);		
			Poland (B = 1);		
			Spain (B = 2, C = 1);		
			Netherlands (C = 2)		

[a] Seed size: *A* Number of accessions below 60 g in 100 seed weight, *B* Number of accessions between 60.1 and 110 g in 100 seed weight, *C* Number of accessions above 110 g in 100 seed weight

[b] Faba bean accessions from southern counties of Sha’anxi province, adjacent to Sichuan and Hubei

and UPGMA cluster analysis were carried out by Popgene V1.32 (Yeh et al. 1999). A dendrogram using Nei78 genetic distances was drawn by MEGA4 (Tamura et al. 2007). Diversity of accessions was measured using Nei’s gene diversity and Shannon’s information index.

Results

AFLP methodology was successfully employed in this study for the detection of molecular variation among Chinese and global winter faba bean germplasm. There were 266 polymorphic bands detected from a total of 323 unambiguous bands (Table 2). A significant level of variation in the overall level of diversity (Shannon’s information index) on primer pairs was detected. Primer pairs E-ACC/M-CAG, E-AGC/M-CTA and E-AGG/M-CTC, showed high diversity, while E-ATT/M-CAA and E-ATT/M-TGG showed lowest diversity, although all bands from primer pair E-ATT/M-CAA were polymorphic (Table 2). The results showed great difference in efficiency among AFLP primer pairs for faba bean genetic diversity analysis. The data successfully discriminated among the regional origins of the land races and accessions used in the study.

PCA and dendrogram analysis of faba bean accessions

Based on the simple matching coefficient matrix converted from the AFLP data matrix of the 240 winter type and three spring type accessions, both 2- and 3-dimension principal coordinate analysis (PCA) graphs were created using NTSYSpc 2.2d (Rohlf 2006), which represented 17.6%

Table 2 The AFLP primer pairs and their amplified polymorphic products of winter faba bean accessions

Primer pair no.	Primer pairs	Molecular range of bands (bp)	Total bands	Polymorphic bands	Polymorphic bands (%)	Shannon's information index
1[a]	E-AAC/M-CAC	70–530	22	17	77.3	1.605
2[a]	E-ACC/M-CAC	80–500	35	27	77.1	2.228
3[a]	E-ACC/M-CAG	75–400	36	34	94.4	4.790
4[a]	E-ACG/M-CTT	85–520	37	34	91.9	2.896
5[a]	E-AGC/M-CTA	75–520	37	33	89.2	4.028
6[a]	E-AGG/M-CTC	85–450	39	35	89.7	4.220
7	E-ATT/M-CAA	82–520	17	17	100.0	0.676
8	E-GTT/M-CTT	95–530	40	27	67.5	3.205
9	E-TAA/M-TCC	78–530	37	29	78.4	3.201
10	E-ATT/M-TGG	110–540	23	13	56.5	0.525
Total			323	266		
Mean			32.3	26.6	82.4	2.737

[a] AFLP primer pairs applied in the study by Zeid et al. (2003)

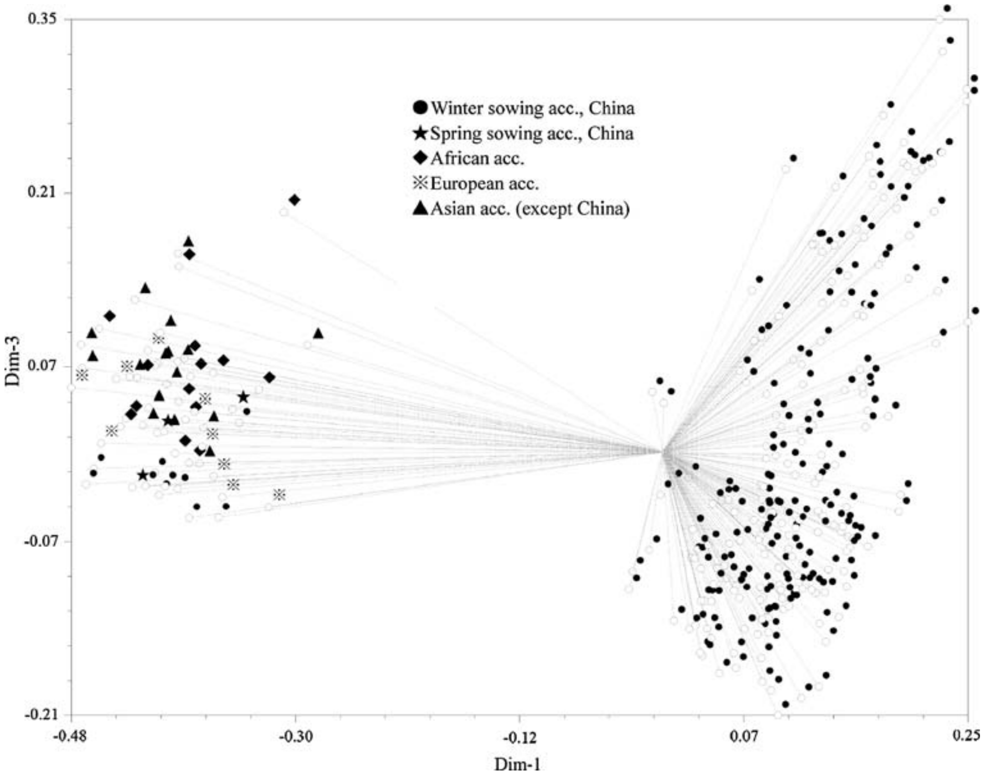

Fig. 1 Two dimension (dimension 1 and dimension 3) PCA graph for 240 winter type and 3 spring type faba bean accessions from different ecological areas, based on simple matching coefficient matrix converted from AFLP marker data

(7.94% for Dim-1, 5.65% for Dim-2 and 4.01% for Dim-3) of the total diversity information (Figs. 1, 2). The PCA graphs clearly showed a large separation of Chinese winter faba bean from European, African and Asian landraces, and also the three Chinese spring faba bean landraces.

Genetic distances calculated by Popgen 1.32 (Yeh et al. 1999) and the related dendrogram created by MEGA4 (Tamura et al. 2007) utilized 100% of the diversity information. The dendrogram showed levels of genetic relationships among the geographic groups (Fig. 3), with the closest relationship between Asian and African landraces, which were both closely related to European landraces. The most distantly related accessions were the three spring types from China followed by the Chinese winter accessions (Fig. 3). There was a general agreement in the diversity analyses between the PCA and the dendrogram, despite the relatively low proportion of total data captured in the PCA graph.

The overall results discriminate genetic diversity between Chinese winter faba bean and the winter germplasm from the rest of the world. The diversity index of Asia (except China) was higher than for Europe and Africa (Table 3).

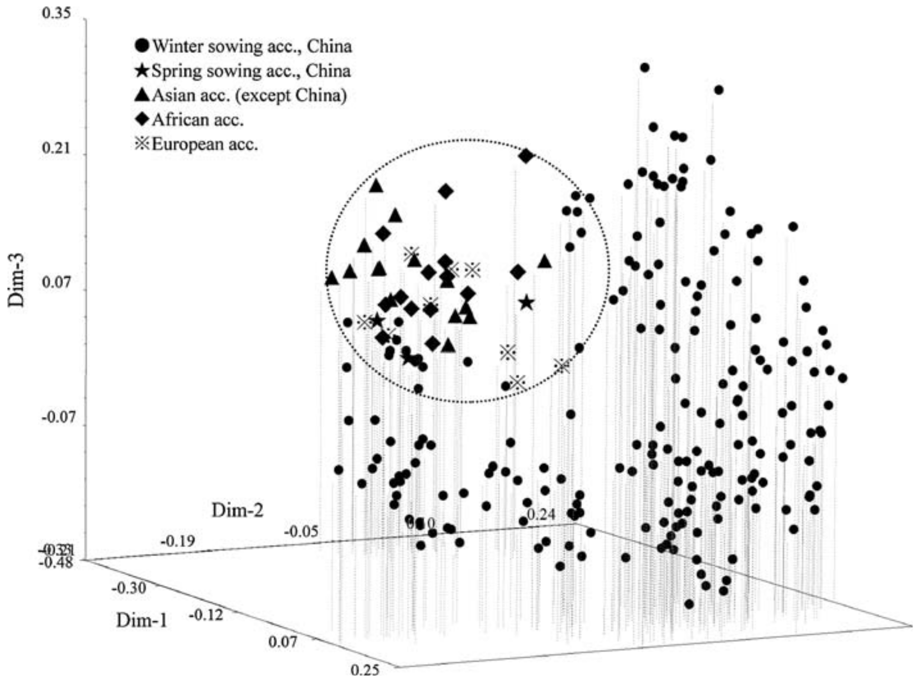

Fig. 2 Three dimension PCA graph for 240 winter type and 3 spring type faba bean accessions from different ecological areas, based on simple matching coefficient matrix converted from AFLP marker data

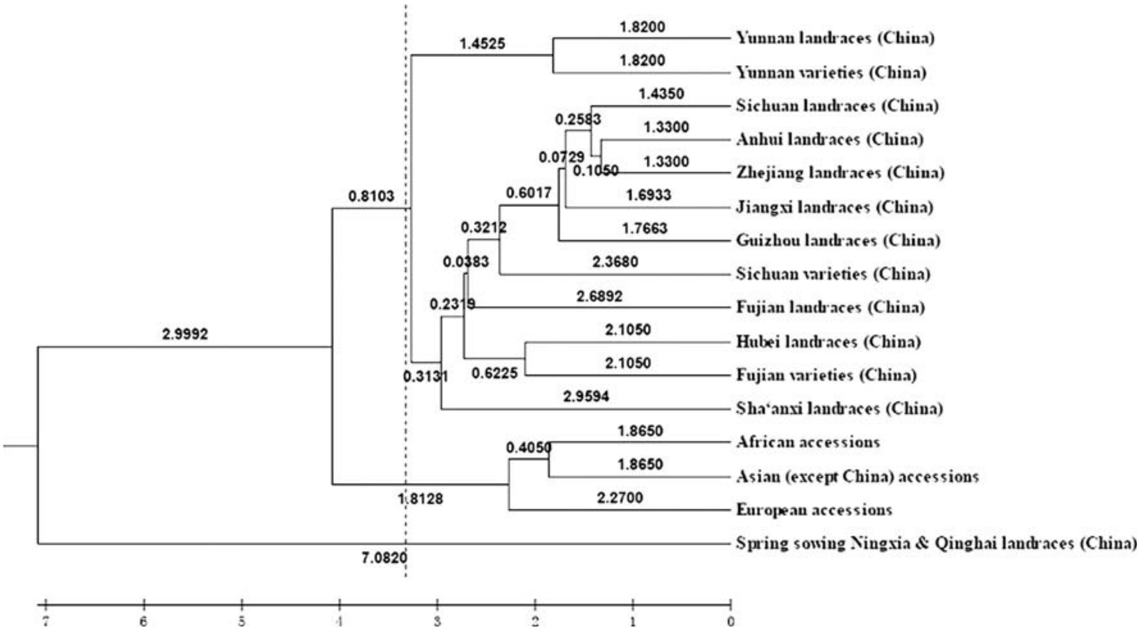

Fig. 3 Dendrogram of different ecological regions based on Nei78 genetic distance converted from AFLP marker data showing genetic relationships among groups of winter and spring types (5% confidence intervals for the node at *dashed line*)

Segmentation of diversity among Chinese provinces and the rest world

Accessions from Yunnan were the most distinct of the Chinese winter sowing germplasm (Figs. 3, 4). The dendrogram illustrated that both landraces and varieties from Yunnan have the greatest genetic distance from the other sources of Chinese winter faba bean. Yunnan varieties, developed from crosses of Yunnan landraces with introduced varieties and selected for adaptation to the Yunnan environment, clustered together with the Yunnan landraces, but had lower genetic diversity as per diversity indices (Table 3; Fig. 3). The winter type landraces from Sha'anxi were from southern counties near winter growing areas, and were the second distinguished group after Yunnan. Varieties from Sichuan had lower diversity than Sichuan land

Table 3 Polymorphism of AFLP amplified products of faba bean from different winter sowing origins and from two spring sown provinces Ningxia and Qinghai

Origin	Type of accessions	Accessions	Polymorphic bands	Nei's gene diversity	Shannon's information index
Yunnan	Landraces	14	119	0.1419	0.2078
Yunnan	Varieties	5	57	0.0679	0.0997
Sichuan	Landraces	35	158	0.1623	0.2452
Sichuan	Varieties	3	36	0.0433	0.0639
Guizhou	Landraces	16	137	0.1559	0.2297
Zhejiang	Landraces	55	142	0.1385	0.2112
Hubei	Landraces	16	104	0.1200	0.1774
Jiangxi	Landraces	12	78	0.0946	0.1382
Anhui	Landraces	22	99	0.1042	0.1562
Fujian	Landraces	4	107	0.1368	0.1969
Fujian	Varieties	6	36	0.0445	0.0649
Sha'anxi	Landraces	10	92	0.1105	0.1608
Ningxia, Qinghai	Landraces	3	88	0.1073	0.1577
Africa	Accessions	14	113	0.1329	0.1955
Europe	Accessions	9	110	0.1378	0.2000
Asia (except China)	Accessions	16	151	0.1724	0.2535

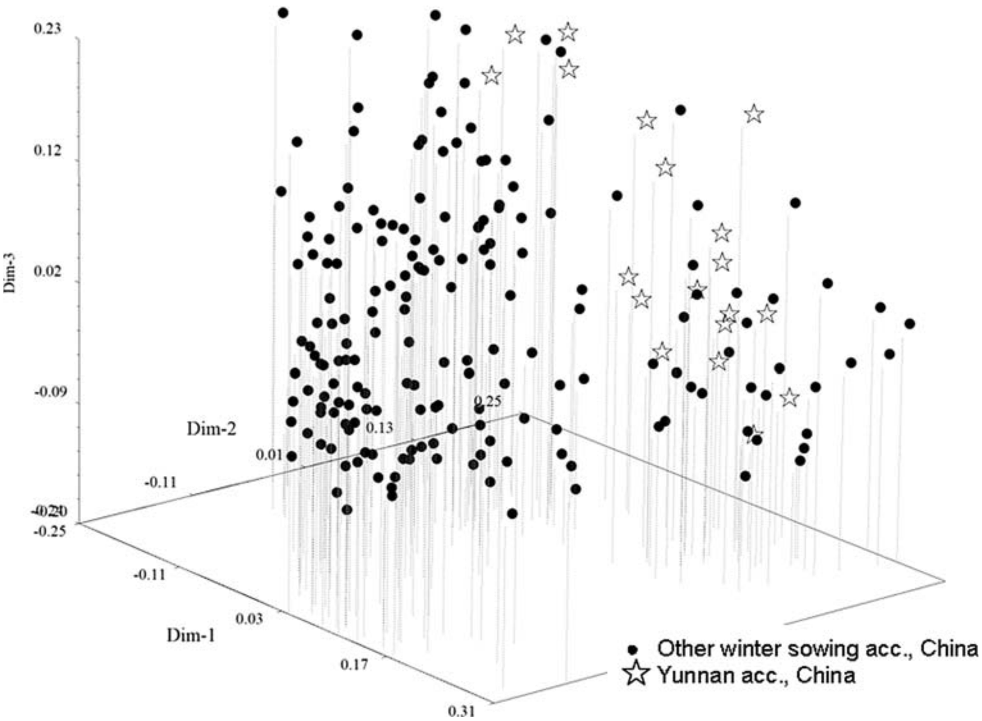

Fig. 4 Three dimension PCA graph for Yunnan local faba bean individuals and other Chinese local accessions based on simple matching coefficient matrix converted from AFLP marker data

races, however these varieties were also released in adjacent provinces Hubei and Guizhou (Bao, personal communication 2007). The provinces of Anhui, Zhejiang, Sichuan, Jiangxi, Guizhou, Fujian and Hubei, have broadly similar ecologies, and their landraces and varieties are clustered together (Fig. 3).

The provinces of Sichuan, Guizhou, Zhejiang and Yunnan had the greatest diversity (both Nei's gene diversity and Shannon's information index) within China (Table 3), and faba bean is a major crop in these provinces. The levels of Nei's gene diversity ranged from 0.1623 to 0.0946 and Shannon's information index ranged from 0.2452 to 0.1382. Although there were great differences of accession numbers among the 13 tested Chinese groups, no significant ($P > 0.01$) correlations were observed between the accession number and the Nei's gene diversity ($r = 0.5383$, $r_{0.01,12} = 0.661$), and Shannon's information index ($r = 0.5773$, $r_{0.01,12} = 0.661$), this means they and were not associated with the numbers of landraces analyzed per province. There was sufficient diversity to allow relationships to be compared among provinces.

Breeding programs within provinces were seen to narrow genetic diversity in comparison with local landraces, as demonstrated in all three cases in Yunnan, Sichuan and Fujian where the diversity (both Nei's gene diversity and Shannon's information index) of landraces was always much greater than for the varieties from these respective provinces.

Discussion

Winter faba bean germplasm from China appears to be in a different gene pool from the rest of the world, based on the sample of world diversity in this study. Within China, the distinctiveness of the winter faba bean from Yunnan was also reported by Bao et al. (2006b). Although only 17.6% of the diversity information was contained in the PCA graph, a useful preliminary outline of gene pool relationships is provided.

The dendrogram provided a more complete account of diversity among faba bean gene pools. Both the PCA graph and the dendrogram point to the uniqueness of the winter faba bean gene pool in China, with a further separation within China of the Yunnan gene pool. Winter faba bean is generally cultivated at higher elevations (400–2,700 m) in Yunnan (Bao et al. 2006a) than for other provinces except the southern counties of Sha'anxi (around 1,000 m), from which the local landraces clustered the next most diverse from the other winter faba bean provinces. Landraces from Sha'anxi were among the least diverse, with very small seed type in this relatively small production area. The genetic separation between Chinese winter and spring landraces requires more investigation, due to the low number of spring types included.

Winter faba bean landraces from Yunnan were recently collected in an Australian Centre for International Agricultural Research collaborative project with China (Bao 2007, unpublished). This germplasm was exchanged for elite lines from breeding programs in Australia. This exchange of new allelic diversity between the Chinese and non-Chinese winter faba bean gene pools provides potential benefits from reciprocal introgression into their respective breeding programs. Further phenotypic assessment is needed to optimize these new opportunities.

The results are suggestive of relative reproductive isolation of the Chinese winter faba bean gene pool from the rest of the world over a very long period, with different selection pressures leading to emergence of separate gene pools. In comparison the African and Asian gene pools are relatively similar. There is some parallel with the distinctiveness of Chinese pea germplasm from the rest of the world (Zong et al. 2008), probably with similar reproductive isolation. However for pea, the Chinese gene pools had greater diversity than for the rest of the world. The Chinese winter faba bean germplasm is distinct but it is not uniform with a major separation between the Yunnan and Sha'anxi gene pools, and of both from that of other provinces.

Molecular techniques have previously been applied to the study of genetic diversity of faba bean with significant variation identified between gene pools, however Chinese germplasm was poorly represented. Link et al. (1995) analyzed 28 large-seeded faba bean lines from European breeding programs by using RAPD methodology, and identified differences between large-seeded European types, small-seeded European types and Mediterranean faba bean types. The present study also identified separation between Mediterranean faba bean types (herein classified as Asia and Africa) and European types. Zeid et al. (2003), in an AFLP analysis of 79 elite faba bean cultivars with Asian, European (Northern and Southern) and North African origin, found only the Asian lines to be distinct as a group (Jaccard's similarity coefficient and PCA analysis).

In the current study, five out of the six primers in common with Zeid et al. (2003) showed reduced polymorphism, especially E-AAC/M-CAC and ACC/M-CAC, but E-ACG/M-CTT was more polymorphic, and all had a greatly reduced number of bands (or fragments) especially E-AAC/M-CAC, E-ACG/M-CTT and E-AGC/M-CTA. This further illustrates likely evolutionary differences between the Chinese and the European, Asian and African genepools studied by Zeid et al. (2003), as detected with the primers initially selected by the latter.

The present study extends the scope of previous studies on genetic diversity in winter faba bean, and demonstrates the uniqueness of the Chinese winter faba bean gene pool, with geographic/climatic separations of sub-gene pools within China particularly for landraces from Yunnan and Sha'anxi.

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