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Full Length Research Paper

Center of genetic diversity and dissemination pathway in adzuki bean deduced from seed protein electrophoresis

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Genetic diversity center plays important roles in collection of germplasm resources. To better understand the genetic diversity of adzuki bean (*Vigna angularis* L.), seed protein of 198 local strains collected from throughout China and Japan were analyzed by SDS-polyacrylamide gel electrophoresis. Four protein types were identified and the frequency of each protein type strain showed a clear geographical cline. The pattern of geographical distribution of the protein types reflected the region of genetic diversity, and the dissemination pathway in adzuki bean was proposed. The region of genetic diversity center in seed protein was southwestern China. Adzuki bean might have spread via middle and east of China to northern China and Japan from southwestern China, as only one seed protein type was detected in Japanese strains in this study. These results provide fundamental and important clues for genetic diversity preservation and exploitation of adzuki bean germplasm resources.

Key words: Genetic diversity, dissemination pathway, adzuki bean, *Vigna angularis*, seed protein electrophoresis.

INTRODUCTION

Adzuki bean (*Vigna angularis* (L.) (Willd.) Ohwi and Ohashi) is an important pulse crop cultivated traditionally in China, Korea, and Japan. Thus, it is also called “east Asia crop” (Hu, 1981, 1984). In recent years, it has spread to Africa, America and Australia. Since it has a very short maturity span, adzuki bean is grown under various cropping systems, hence contributing to the improvement of soil condition. It also provides an excellent source of easily digestible protein, and is used as a traditional medicine in these regions (Duan, 1989).

Adzuki bean originated in China (Vavilov, 1926) and this view has been supported by other authors inferred by morphology and wild species (Anishetty and Moss, 1988; Duke, 1981; Hoshikawa, 1987; Tasaki, 1963). In the past decade, effort has been made to elucidate the genetic diversity and classification of adzuki bean germplasm resources. Yee et al. (1999) believe that China is the genetic diversity center of adzuki bean revealed by RAPD and AFLP markers. Wang et al. (2002a, b, c) and Wang and Zhang (2002d) endeavor to evaluate the genetic diversity of adzuki bean and wild relatives using multiple technologies such as morphology, isozyme, RAPD and AFLP. They found that adzuki bean is mainly distributed in the region from northeastern China to southwestern China. The genetic diversity center is located in the southwestern China, as well as the middle and upstream of Yangtze River. When broader materials containing germplasm from Nepal-Bhutan and Himalaya regions are used for diversity research purpose implemented AFLP (Zong et al., 2003a, b), the results reveal that wild species resources from China and germplasm comprised of cultivated and wild strains from Himalaya area have the highest genetic diversity, whereas cultivated accessions from other regions as well as Japanese wild species lack diversity. These findings are also supported by the findings revealed using SSR markers (Xu et al., 2008). However, the center of genetic diversity of biochemical characters, which is considered

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Table 1. Germplasm of adzuki bean landraces used in this study.

Region	No. of samples	Identifier in National Gene Bank of China
Northern China	110	B0650 B0651 B0653 B0656 B0671 B0683 B0684 B0687 B0688 B0691 B0697 B0698 B0701 B0702 B0706 B0726 B0727 B0728 B0876 B0877 B0879 B0825 B0830 B0831 B0857 B0932 B0933 B0934 B0984 B0985 B1051 B1052 B1055 B1080 B1081 B2071 B2072 B2073 B2096 B2097 B0343 B0344 B0345 B00439 B0440 B0640 B0641 B0645 B0646 B1552 B1553 B1554 B1555 B1556 B1821 B2485 B2486 B2487 B2585 B2586 B2592 B2593 B2594 B2601 B2602 B2642 B2643 B2646 B2647 B2652 B0052 B0053 B0054 B0057 B0058 B0090 B0091 B1763 B1779 B1782 B0100 B0101 B0104 B0203 B0204 B0293 B0294 B0295 B0296 B0297 B2705 B2706 B2850 B2851 B2863 B2864 B2880 B2929 B2930 B2932 B1097 B1098 B1120 B1121 B1129 B1283 B1284 B1285 B1112 B1122
Middle and east China	43	B2663 B2664 B2667 B2668 B2669 B2696 B2697 B2698 B2703 B2704 B2137 B2138 B2139 B2169 B2170 B2174 B2175 B2178 B2179 B2184 B1426 B1427 B1428 B1474 B1475 B1476 B1477 B1478 B2210 B2249 B2254 B2257 B2258 B2277 B2281 B2282 B2286 B2287 B2288 B2289 B1802 B1803 B1804
Southwestern China	30	B1481 B1482 B1485 B1486 B1487 B1541 B1544 B1545 B1546 B1547 B1484 B1496 B2290 B2291 B2292 B2293 B2294 B2295 B2296 B2297 B2300 B2301 B2320 B2311 B2325 B2327 B2339 B2380 B2381 B2382
Japan	15	B1662 B3658 B3659 B3660 B3661 B3662 B3663 B3664 B3665 B2666 B3679 B3678 B3674 B3675 B3673
Total	198	

to be closely related to the origin of adzuki bean, has not been investigated. Among biochemical markers, the usefulness of the seed protein electrophoresis method was recognized, and this method has been used to establish the phylogenetic relationships of wild and cultivated forms and to identify the multiple centers of domestication and dissemination pathways in *Phaseolus vulgaris* (Gepts et al., 1986, 1988; Gepts and Bliss, 1988) and *Vigna radiata* (Tomooka et al., 1992). As for *Vigna angularis*, however, very few studies have been conducted using seed protein electrophoresis (Egawa et al., 1988; Hu and Wang, 1991). Moreover, these authors examined only interspecific variations of seed protein electrophoregrams and revealed the phylogenetic relationships among *Vigna* species including *V. angularis*. The present study was therefore conducted to investigate the intraspecific variations of seed protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and to identify the center of genetic diversity in seed protein and dissemination pathway in adzuki bean.

MATERIALS AND METHODS

Strains

A total of 198 local adzuki bean strains were used. They were supplied by the National Gene Bank of China as summarized in Table 1. Of the 198 strains used, 15 were collected from Japan, 110 from northern of China, 43 from middle and east China, and 30 from southwestern China. Since adzuki bean is cultivated and consumed in a local area, a given strain of adzuki bean collected from that area can be regarded as an endemic race that has been grown there for a long time. Adzuki bean strains used in the present study are therefore considered as ideal materials for studies on genetic diversity and geographical distribution.

Preparation of protein samples

Total seed protein was extracted from 10 mg of seed meal with 0.5 ml of 0.05 M Tris-HCl buffer (pH 8.0) containing 0.2% SDS and 20 μl of 2-mercaptoethanol. Samples were heated in boiling water for 5 min and then cooled at room temperature. Centrifugation was then executed at 6,000 rpm for 5 min. Thereafter, 30 μl supernatant of each sample was placed on the gel for electrophoresis.

Electrophoresis

The protein was analyzed by the slab SDS-PAGE system using 10% (w/v) polyacrylamide gel. The electrophoresis was conducted at 40 mA for the first 30 min and at 60 mA for a further 4 h. The molecular weight standards used were: phosphatase B (94,000), bovine serum albumin (67,000), myoalbumin (43,000), carbonic anhydrase (30,000), tobacco mosaic virus capsid protein (17,500). All gel were stained with

Table 2. Distribution frequency of adzuki bean seed protein electrophoregrams.

Region	No. of samples	No. (percentage) of seed protein electrophoregrams			
		Type 1 (%)	Type 2 (%)	Type 3 (%)	Type 4 (%)
Northern China	110	105 (95.5%)	5 (4.5%)	0	0
Middle and east China	43	34 (79.1%)	1 (2.3%)	8 (18.6%)	0
Southwestern China	30	21 (70%)	2 (6.7%)	1 (3.3%)	6 (20.0%)
Japan	15	15 (100%)	0	0	0
Total	198	175 (88.4%)	8 (4.0%)	9 (4.5%)	6 (3.0%)

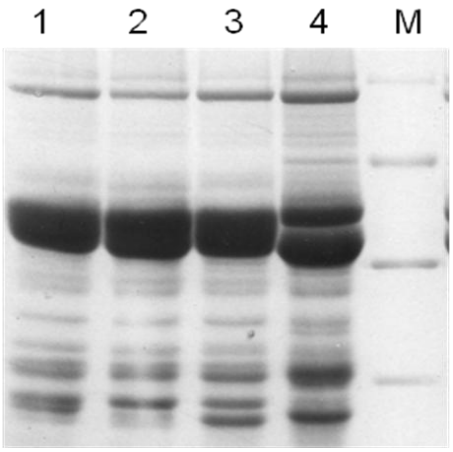

Figure 1. Adzuki bean seed protein electrophoregram types. 1, type 1; 2, type 2; 3, type 3; 4, type 4; M, molecular weight marker.

Coomassie Brilliant blue and de-stained by diffusion in 5% CH_3COOH-20% CH_3OH-water. The analysis of the banding patterns was performed with at least two electrophoregrams for each strain to confirm the consistency of the banding pattern.

RESULTS AND DISCUSSION

Types of electrophoregrams

Variation of the banding pattern was observed. 18 bands were recognized and molecular weight were estimated as 95, 88, 75, 67, 61.5, 57.5, 44, 41.5, 40, 38.5, 33.5, 31.5, 28.5, 27, 26kD, 23.5, 22.5, and 21.5 kD. The minor bands, which were lightly stained, were not included in the present analysis. Four different types of total protein electrophoregrams were recognized (Figure 1). They were designated as protein type 1, type 2, type 3, and type 4. The type 1 is deficient of four subunits (estimated molecular weight of 67, 57.5, 31.5, and 21.5 kD). Compared with type 1, type 2 is deficient of one subunit (22.5 kD), type 3 is deficient of one subunit (22.5 kD) but increased by one subunit (21.5 kD). Compared with the other three types, type 4 has three specific bands (67, 57.5, 31.5 kD) but is deficient of three bands (44, 23.5, 21.5 kD).

Geographical distribution of protein types

Of the 198 strains examined, 175 (88.4%) strains contained protein type 1, 8 (4.0%) protein type 2, 9 (4.5%) protein type 3, and 6 (3.0%) protein type 4 (Table 2). As shown in Table 2, the geographical distribution of the four protein type strains differed greatly. Protein type 1 strains, the most common, were distributed throughout China and Japan. Protein type 2 strains showed a wide geographical distribution covering all regions of China, whereas they were undetectable in strains from Japan. The distribution of the protein type 3 strains was restricted to the middle and east of China and southwestern China. Protein type 4 was detected in only six strains from southwestern China.

Based on the frequency distribution of the strains with different protein types, the region with the highest diversity was assigned to southwestern China which contained all of the four protein types (Table 2). In the middle and east of China, three kinds of protein type strains were detected and the diversity seemed to be lower than that in southwestern China. In the northern China, only protein types 1 and 2 were detected. In Japan, only one kind of protein type strain was detected, indicating they have the lowest protein diversity in this area. Considering the small number of strains from Japan examined in the present study (fifteen strains), it may be necessary to conduct further analyses using a large number of strains from that area.

Center of protein type diversity and dissemination pathway

The geographical cline of various protein type strains reflects the center of protein type diversity and possible dissemination pathway in adzuki bean. The center of protein type diversity appeared to be located in southwestern China rather than other areas. Adzuki bean probably spread from southwestern China via the middle and east of China to northern China. From China, type 1

spread to Japan.

Hoshikawa (1987) claims that northeastern China is the center of domestication. Central China has been suggested as a possible center of diversity with Japan serving as a secondary center (Duke, 1981). Anishetty and Moss (1988) have placed high priority on the future collection of adzuki bean germplasm on the Korea peninsula and in China, suggesting that these areas are either centers of origin or diversity. Tasaki (1963) used varietal differences in the degree of plant branching to suggest that adzuki migrated from central or southern China to southern Japan and then eventually up the Japanese archipelago. Although strains cultivated in Korea were not included in this study, results also reflected that southwestern China is the center of diversity. The center of genetic diversity as indicated by the seed protein electrophoresis, however, was considered to be the southwestern China rather than other areas. In recent years, DNA molecular markers have been employed in adzuki bean genetic diversity research. A number of genetic diversity results of adzuki bean are referred to China, especially southwestern China (Yee et al., 1999; Wang et al., 2002a, b, c; Wang and Zhang, 2002d; Xu et al., 2008). Our results are consistent with these conclusions made by using DNA markers. According to SSR marker data, China landraces and wild types of adzuki bean showed abundant genetic diversity, especially in South China (Wang et al., 2009a, b). Gong et al. (2008) also found highly genetic diversity among China landraces revealed by SSR markers. It should be noted that germplasm from Himalaya region including Nepal-Bhutan has the highest genetic diversity detected by AFLP markers (Zong et al., 2003a, b) and SSR markers (Zhao et al., 2011). However, our results were not in conflict with this conclusion because southwestern China is very close to Himalaya area and the genetic diversity center may be broadened to Himalaya regions containing Nepal-Bhutan. Nevertheless, it is worth examining the center of genetic diversity in adzuki bean by using other biochemical markers and DNA markers. A large number of local strains from broader regions such as Japan, Korea, Vietnam and other countries in the Indian subcontinent should be analyzed to obtain more precise information.

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