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Center of genetic diversity and dissemination pathways in mung bean deduced from seed protein electrophoresis

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Summary. Seed protein of 581 local strains of mung bean, *Vigna radiata* (L.) Wilczek, collected from throughout Asia, were analyzed by SDS-polyacrylamide gel electrophoresis. Eight protein types were recognized based on the combination of four albumin bands and three globulin bands. The frequency of each protein type strain showed a clear geographical cline. The pattern of geographical distribution of the protein types reflected the regions of genetic diversity, and two dissemination pathways in mung bean were proposed. The region of genetic diversity in seed protein was western Asia (Afghanistan-Iran-Iraq area). Mung bean may have spread mainly to the east by two routes from India, where the domestication of mung bean is believed to have occurred. One route led to Southeast Asia; strains consisting of a few protein types with prominent protein type 1 were disseminated from India to the Southeast Asian countreis. Thus, the strain composition in Southeast Asia was very simple, with the strains being similar to one another. Another dissemination pathway may have been the route known as the Silk Road. Since protein type 7 and 8 strains could not be found throughout Southeast Asia, it is assumed that these strains spread from western Asia or India to China and Taiwan via the Silk Road, and not by the route from Southeast Asia.

Key words: Genetic diversity – Dissemination pathways – Mung bean – *Vigna radiata* – Seed protein electrophoresis

Introduction

Mung bean (*Vigna radiata* (L.) Wilczek) is an important pulse crop cultivated traditionally by small landholders throughout tropical, subtropical, and temperate Asia. Since it has a very short maturity span (55–70 days) mung bean is grown under various cropping systems, hence contributing to the increase of the small landholders' income and to the improvement of soil conditions (Fernandez and Shanmugasundaram 1988). It also provides an excellent source of easily digestible protein in these regions (AVRDC 1975).

Vavilov (1926) hypothesized that mung bean originated in India. His theory has been supported by other authors based on the morphological diversity (Singh et al. 1974), existence of wild and weedy types (Chandel 1984; Paroda and Thomas 1988), and archeological remains (Jain and Mehra 1980) of mung bean in India. However, the center of genetic diversity of biochemical characters, which is considered to be closely related to the origin of mung 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; Gepts and Bliss 1988; Gepts et al. 1988). As for *Vigna radiata*, however, very few studies have been conducted using seed protein electrophoresis (Egawa et al. 1988; Thakare et al. 1988). Moreover, these authors examined only interspecific variations of seed protein electrophoregrams and revealed the phylogenetic relationships among *Vigna* species including *Vigna radiata*. The present study was therefore conducted to investigate the intraspecific variations of seed protein by SDS-polyacrylamide gel electrophoresis (PAGE), and to identify the center of genetic diversity in seed protein and dissemination pathways in mung bean.

Materials and methods

Strains

A total of 581 local mung bean strains was used. They were supplied by the Asian Vegetable Research and Development Center (AVRDC, Taiwan), Kyoto University (Japan), and the National Institute of Agrobiological Resources (NIAR, Japan). Of the 581 strains used, 6 were collected from Japan, 46 from Korea, 11 from China, 21 from Taiwan, 82 from The Philippines, 22 from Indonesia, 9 from Sri Lanka, 11 from Vietnam, 40 from Thailand, 7 from Burma, 246 from India, 17 from Pakistan, 34 from Afghanistan, 19 from Iran and Iraq, and 10 from Turkey, as summarized in Table 1. Since mung bean is cultivated and consumed in a local area, a given strain of mung bean collected from that area can be regarded as an endemic race that has been grown there for a long time. Moreover, the growth habit of the strains from each region used in the present study showed a clear geographical cline (Tomooka et al. 1991), suggesting that these materials are true landraces adapted to each local environment. Mung bean strains used in the present study are therefore considered as ideal materials for studies on genetic diversity and geographical distribution.

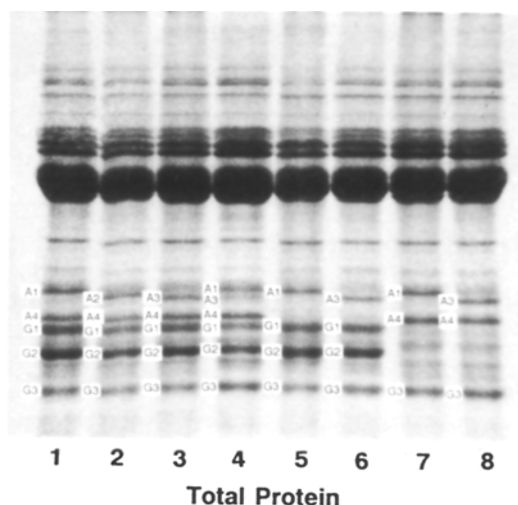

Fig. 1. Eight phenotypes of total protein electrophoregrams in mung bean

Preparation of protein samples

Total seed protein was extracted from 10 mg of seed meal with 1 ml of 0.05 *M* TRIS-HCl buffer (pH 8.0) containing 0.2% SDS and 5 *M* urea; 20 µl of 2-mercaptoethanol was then added to the extract. Thereafter, 15 µl of the crude extract was directly placed on the gel for electrophoresis. The globulin and the albumin fractions were prepared by the following procedures. Extracted protein from 100 mg seed meal with 2.5 ml of 0.02 *M* TRIS-HCl buffer (pH 8.0) was centrifuged at 3,000 rpm for 5 min, and 0.1 ml of 1 *M* CH_3COONa (pH 4.0) was added to the supernatant. The precipitate, referred to as globulin, was collected by centrifugation (3,000 rpm, 5 min). A fourfold volume of prechilled acetone was added to the clear supernatant, which contained albumin protein, and was kept at $-20\,^{\circ}C$ for 1 h. The precipitate, referred to as albumin, was collected by centrifugation (3,000 rpm, 5 min). The globulin and the albumin pellets were resuspended by dissolution into 5 ml and 1 ml of 0.05 *M* TRIS-HCl buffer (pH 8.0) containing 0.2% SDS and 5 *M* urea together with 100 µl and 20 µl of 2-mercaptoethanol, respectively. Thereafter, 15 µl of each extract solution was placed on the gel for electrophoresis.

Electrophoresis

The protein was analyzed by the slab SDS-PAGE system of Laemmli (1970) using 13.5% (w/v) polyacrylamide gel. The electrophoresis was conducted at 100 V for the first 30 min and at 150 V for a further 3 h. The molecular weight standards used were: Cytochrome c monomer (12,400), Cytochrome c dimer (24,800), Cytochrome c trimer (37,200), Cytochrome c tetramer (49,600), Cytochrome c hexamer (74,400). All gels were stained with Coomassie Brilliant blue and destained 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

Variation of the banding pattern was observed in the molecular weight range of 24,000 to 37,000, in which four albumin bands and three globulin bands were recognized. These were designated as A1 (estimated molecular weight of 37,000), A2 (36,300), A3 (34,400), and A4 (32,600) for the albumin bands and as G1 (31,400), G2 (28,200), and G3 (24,000) for the globulin bands. The minor bands, which were lightly stained, were not included in the present analysis. Six different banding patterns and two different banding patterns were observed for the albumin bands and globulin bands, respectively. Based on the combination of albumin and globulin banding patterns, eight different types of total protein electrophoregrams were recognized (Fig. 1). They were designated as protein type 1 (containing A1, A4, G1, G2, and G3 bands), protein type 2 (A2, A4, G1, G2, and G3 bands), protein type 3 (A3, A4, G1, G2, and G3 bands), protein type 4 (A1, A3, A4, G1, G2, and G3 bands), protein type 5 (A1, G1, G2, and G3 bands) protein type 6 (A3, G1, G2, and G3), protein type 7 (A1, A4, and G3), and protein type 8 (A3, A4, and G3).

Of the 581 strains examined, 372 strains contained protein type 1, 39 protein type 2, 107 protein type 3, 18 protein type 4, 2 protein type 5, 1 protein type 6, 24 protein type 7, and 18 contained protein type 8 (Table 1). As shown in Table 1, the geographical distribution of the eight protein type strains differed greatly. Protein type 1 strains, the most common, were widely distributed throughout Asia. Protein type 2 strains, in contrast, were distributed only in Iran and Iraq, Afghanistan, India, Thailand, and Indonesia with a low frequency. Protein type 3 strains, the second most frequent type, showed a wide geographical distribution covering Turkey, Iran and Iraq, Afghanistan, Pakistan, India, Burma, Sri Lanka, Indonesia, The Philippines, Taiwan, China, Korea, and Japan. The distribution of the protein type 4 strains was restricted to Afghanistan, Pakistan, India, Burma,

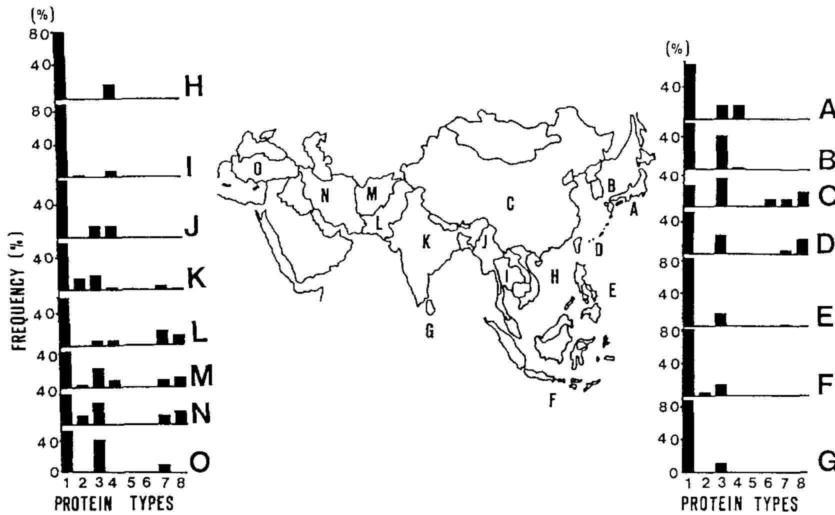

Fig. 2. Frequency of mung bean strains with each protein type in each region. A: Japan, B: Korea, C: China, D: Taiwan, E: Philippines, F: Indonesia, G: Sri Lanka, H: Vietnam, I: Thailand, J: Burma, K: India, L: Pakistan, M: Afghanistan, N: Iran and Iraq, O: Turkey

Table 1. Origin, protein types, and number of mung bean strains examined

Origin	No. of strains examined	Protein types							
		1	2	3	4	5	6	7	8
Japan	6	4	–	1	1	–	–	–	–
Korea	46	26	–	19	1	–	–	–	–
China	11	3	–	4	–	–	1	1	2
Taiwan	21	11	–	5	–	–	–	1	4
Philippines	82	69	–	12	–	–	–	1	–
Indonesia	22	18	1	3	–	–	–	–	–
Sri Lanka	9	8	–	1	–	–	–	–	–
Vietnam	11	9	–	–	2	–	–	–	–
Thailand	40	36	1	–	3	–	–	–	–
Burma	7	5	–	1	1	–	–	–	–
India	246	146	34	43	6	2	–	12	3
Pakistan	17	10	–	1	1	–	–	3	2
Afghanistan	34	15	1	8	3	–	–	3	4
Iran & Iraq	19	7	2	5	–	–	–	2	3
Turkey	10	5	–	4	–	–	–	1	–
Total	581	372	39	107	18	2	1	24	18

Thailand, Vietnam, Korea, and Japan with a low frequency. Protein type 5 was detected in only two strains from India. Protein type 6 occurred in only one strain from China. The geographical distribution of the strains with protein types 7 and 8 was quite similar. Protein type 7 strains were distributed in Turkey, Iran and Iraq, Afghanistan, Pakistan, India, The Philippines, Taiwan, and China. Protein type 8 strains were found in Iran and Iraq, Afghanistan, Pakistan, India, Taiwan, and China. These strains (protein types 7 and 8) could not be found in Southeast Asia, except for one strain from The Philippines.

Discussion

Geographical distribution of protein types

The frequency of the protein type strains in each region is shown in Fig. 2. Based on the frequency distribution of the strains with different protein types, the region with the highest diversity was assigned to western Aisa (the Afghanistan-Iran-Iraq area) (Fig. 2, Graphs M and N). Various protein types were distributed most evenly in this area. Protein type 1 strains, the most predominant type, accounted for approx. 40% of the total strains examined, and more than five kinds of protein type strains were found in this region.

In Turkey, which is located west of the Afghanistan-Iran-Iraq area, only three kinds of protein type strains were detected and the diversity seemed to be lower (Fig. 2, Graph 0). Considering the small number of accessions from Turkey (ten strains), however, it is necessary to examine the protein type diversity using a large number of local strains from this country. Frequency of protein type 1 strain began to increase gradually eastward from the Afghanistan-Iran-Iraq area. In Pakistan and India, which showed a similar pattern of strain composition, the predominant protein type 1 strains accounted for approx. 60% of the total strains examined (Fig. 2, Graphs K and L). Thus, the diversity of the protein types in these regions was considered to be lower than that in the Afghanistan-Iran-Iraq area. In Burma, the frequency of the protein type 1 strains further increased to 70% and only three kinds of protein type strains were found (Fig. 2, Graph J). Therefore, it was suggested that the diversity of the protein types in Burma was lower than that in India and Pakistan. However, Vavilov (1926) included Burma among the centers of diversity of mung bean. Considering the small number of strains from Bur-

ma examined in the present study (seven strains), it may be necessary to conduct further analyses using a larger number of strains from that area.

In the Southeast Asian countries (The Philippines, Indonesia, Vietnam, Thailand, and Burma), the frequency distribution of the protein types was very similar, i.e., the predominant protein type 1 strains accounted for 70–90% of the total and only two or three kinds of protein type strains were distributed (Fig. 2, Graphs E, F, H, I, and J). Protein type 5, 6, 7, and 8 strains were not detected throughout the Southeast Asian countries, with only one exceptional strain (protein type 7) found in The Philippines. In other words, the diversity in seed protein in the Southeast Asian countries was low and the composition of the strains was simple, with strains being similar to one another. However, some difference in the strain composition could be recognized between continental and insular Southeast Asia. In continental Southeast Asia (Thailand and Vietnam), the protein type 4 strains were detected at a frequency of ca. 10% in addition to the predominant protein type 1 strains (Fig. 2, Graphs H and I). However, in insular Southeast Asia (Indonesia and The Philippines), protein type 3 strains were detected at a frequency of ca. 10% in addition to the predominant protein type 1 strains (Fig. 2, Graphs E and F). In this sense, Sri Lanka's strain composition was closer to that of insular Southeast Asia than to that of continental Southeast Asia (Fig. 2, Graph G).

In Taiwan protein type 7 and 8 strains were distributed, and the diversity of the protein types was considered to be higher than that in the Southeast Asian countries (Fig. 2, Graph D). This tendency could also be recognized in the strains from China (Fig. 2, Graph C), despite the small number of strains examined (11 strains). Furthermore, the pattern of frequency distribution of the protein types was similar to that of western Asia, indicating the strong relationship between mung bean in China and Taiwan and that in western Asia.

In Korea, protein type 7 and 8 strains could not be found and the strain composition was rather simple (Fig. 2, Graph B). However, protein type 1 and 3 strains were distributed more evenly compared to the predominance of the protein type 1 strains observed in Southeast Asia. Strain composition in Japan seemed to be similar to that in Korea, i.e., protein type 1 strains predominated and protein type 3 and 4 strains were also distributed in addition to protein type 1 strains (Fig. 2, Graph A).

Center of protein type diversity and dissemination pathways

The geographical cline of various protein type strains reflected the center of protein type diversity and possible dissemination pathways in mung bean. The center of protein type diversity appeared to be located in western Asia (the Afghanistan-Iran-Iraq area) rather than in India. Mung bean probably spread to the east by two main routes. One route led to Southeast Asia from India. Strains consisting mainly of protein type 1 with few other protein types were disseminated from India to Southeast Asia. Thus, the strain composition in the Southeast Asian countries was found to be very simple and similar. Another dissemination pathway may have been the "Silk Road" from western Asia or India to Taiwan via China. Strains including protein types 1, 3, 7, and 8 spread to Taiwan by this route. Complete absence of strains with protein types 7 and 8 in Southeast Asia strongly suggested that the protein type 7 and 8 strains found in Taiwan did not originate from Southeast Asia but from China. From China, strains other than protein types 7 and 8 spread to Korea and Japan.

India had been considered to be the region with the greatest genetic diversity in mung bean (Vavilov 1926; Singh et al. 1974; Zeven and de Wet 1982; Tomooka et al. 1991). The center of genetic diversity as indicated by the seed protein electrophoresis, however, was considered to be the Afghanistan-Iran-Iraq area rather than India. Thus, it is worth examining the center of genetic diversity in mung bean by using other biochemical markers. A large number of local strains especially from Turkey, Iran, Iraq, Afghanistan, Burma, and China should be analyzed to obtain more precise information.

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