ISSN 0388-9394

Technical Bulletin

of

the Tropical Agriculture Research Center

No. 28 1991

GENETIC DIVERSITY AND LANDRACE DIFFERENTIATION OF MUNGBEAN, *VIGNA RADIATA* (L.) WILCZEK, AND EVALUATION OF ITS WILD RELATIVES (THE SUBGENUS *CERATOTROPIS*) AS BREEDING MATERIALS

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CONTENTS

Chapter 1. General background

Importance of mungbean in Asia

Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop cultivated traditionally by small landholders throughout tropical, subtropical and temperate Asia. Since mungbean has a very short maturity span (55-70 days), it is grown under various cropping systems, hence contributing to the increase of the small landholders' income and to the improvement of the soil conditions (Fernandez and Shanmugasundaram 1988). This popular and ancient crop is an excellent source of protein and is characterized by a high digestibility (AVRDC 1975). Mungbean has been used in a variety of foods depending on the region. It is principally prepared as dhal (split bean soup) in the Indian subcontinent (Thirumaran and Seralathan 1988), while it is mainly consumed as bean sprouts, noodle, vermicelli, bean jam, and sweets in Southeast and East Asia (Chen et al. 1988, Quyen 1988).

Vavilov (1926) considered that mungbean originated in India based on the wide morphological variations observed in India. Vavilov's theory has been supported widely in view of the morphological diversity of landraces (Singh et al. 1974), existence of wild and weedy types (Chandel 1984, Paroda and Thomas 1988) and based on archaeological remains (Jain and Mehra 1980) of mungbean in India. However, the pattern of geographical distribution of variations in the growth and morphological characters has been poorly documented (Smartt 1976, Duke 1981). Furthermore, the genetic variations in the biochemical characters have not been well studied for mungbean. Polymorphism of isozymes or subunit composition of seed proteins are considered to be neutral to the selection by human beings and regarded as important characters for phylogenetic analysis. Thus, a broad area of genetic variation still remains open to studies for elucidating phylogenetical aspects as the basis of improvement of this crop plant.

Mungbean accounts for about 12% of the total pulse production (consisting of mungbean, black gram, azuki bean, rice bean, horse gram, chick pea, pigeon pea, dry broad beans, dry peas, lentils, cowpea, khesari bean and vetch) in South and Southeast Asia. Annual mungbean production in Asia is estimated at 1.9 million t harvested from 3.8 million ha, and India produces nearly 70% of this total amount. The other major mungbean-producing countries in the regions are Thailand (0.3 million t), Burma (0.2 million t) and Indonesia (0.1 million t). Mungbean production in these regions increased at a growth rate of 4.5%, against a growth rate of only 0.5% for the total pulse production from 1976 to 1986 (Singh 1988). The highest growth rate for mungbean production was recorded in Sri Lanka (38.1%), followed by India (15.8%), the Philippines (9.6%), Thailand (8.5%), Pakistan (6.5%) and Korea (5.3%). Bangladesh has shown a negative trend in production (−5.2%). The utilization of mungbean has been increasing in most of the Asian countries despite a general decline in the consumption of total pulses (Babu and Hallam 1988). This fact indicates that mungbean is increasingly used as a substitute for other pulse crops.

Among the Asian countries producing mungbean, Thailand is the only country that exports its surplus to Japan, Taiwan, Malaysia, and Hong Kong. In 1982, mungbean exports accounted for 10% of the total value of food crops exported. The total mungbean exports volume has shown a steady increase in Thailand (Babu and

Hallam 1988). At present, Thailand is the leading mungbean exporter which ships overseas about 60% of the domestic mungbean production (Chainuvati et al. 1988). Thailand has also increased its production sixfold over the past 20 years so that it now ranks as the second major producer in the world (Lawn and Ahn 1985).

As discussed above, mungbean is considered to be one of the most important food legumes in Asia. However, its productivity is still low (about 500 kg/ha on the average) under the low input cultural practices of the small farmers in the regions. In view of the importance of this legume as a short duration crop in the cropping systems in Asia, it is essential to increase the yield by improving the varieties. To achieve the varietal improvement effectively, using a wide range of genetic sources, and collecting information on the genetic variations of the breeding materials are prerequisites.

The subgenus *Ceratotropis* as a wide genetic source for mungbean breeding

Mungbean belongs to the subgenus *Ceratotropis*, an extremely homogeneous and highly specialized taxonomic group in the genus *Vigna*, which originated in Asia (Maréchal et al. 1978). It is considered that *Ceratotropis* species form the primary gene pool for the breeding of *Ceratotropis* cultigens (Ahn and Hartmann 1978, Chen et al. 1983, Miyazaki et al. 1984, Egawa et al. 1990a, Siriwardahane et al. 1991). Species belonging to the subgenus *Ceratotropis* can in fact provide gene sources not only for mungbean breeding but also for the breeding of other *Ceratotropis* cultigens.

It is well known that black gram (*V. mungo*) exhibits a complete resistance to the azuki bean weevil (*Callosobruchus chinensis*), although mungbean experiences serious damage from this weevil (Sawa and Tan 1976, Fujii and Miyazaki 1987). Black gram has been used as a source of bruchid resistance for the mungbean breeding program at the Asian Vegetable Research and Development Center (AVRDC) (AVRDC 1982). Moreover, black gram is utilized as a gene source for the improvement of methionine content in seed storage protein of mungbean (AVRDC 1987). Rice bean (*V. umbellata*) showed a high level of bruchid resistance and tolerance to AMV (azuki bean mosaic virus) (Sawa and Tan 1976, Duke 1981). The possibility to incorporate useful genes from rice bean to azuki bean was examined (Sawa et al. 1984). *V. glabrescens*, a minor cultivated crop in the subgenus *Ceratotropis*, exhibits immunity against the bean fly, and is used in the mungbean improvement program at AVRDC (Fernandez and Shanmugasundaram 1988). It is anticipated that other wild species of the subgenus *Ceratotropis* may also be useful as genetic sources for the breeding of *Ceratotropis* cultigens. To date, however, very few wild species of the subgenus *Ceratotropis* have been collected and evaluated. Considering the genetic erosion occurring rapidly in the world, it is urgently needed to collect and evaluate wild species within the subgenus *Ceratotropis*.

Against this background, the geographical distribution and genetic variations in mungbean were analyzed in the present study. To elucidate the pattern of geographical distribution of the genetic variations, growth types (based on the number of days to flowering, stem length, and number of lateral branches), seed characters (seed weight, seed length, seed width, and seed shape) and protein types (subunit composition of seed proteins) of mungbean were investigated. Secondly, explorations were conducted to collect wild *Ceratotropis* species on the Nansei Archipelago, Japan and in Northern Thailand. Description and evaluation of the wild *Ceratotropis* species collected during the explorations were then performed at Chainat Field Crops

Research Center. Thirdly, attempts were made to develop a bruchid-resistant mungbean cultivar using wild mungbean germplasm (*V. radiata* var. *sublobata*) in Thailand. A mungbean line resistant to *C. chinensis* and *C. maculatus* and showing good agronomic characters was successfully developed after three consecutive back-crossings.

Chapter 2. Genetic variations and geographical distribution of mungbean in Asia

2-1. Geographical distribution of growth types in mungbean

Introduction

The center of genetic diversity in mungbean is considered to be located in India and Burma (Vavilov 1935, Zeven and de Wet 1982). This assumption is based on the great variability of growth characteristics observed in local varieties of mungbean in India and Burma. However, quantitative data relating to the range of variations and geographical distribution of growth characteristics of local mungbean strains collected from wide geographical areas in Asia are not available. Moreover, the evaluation of genetic diversity within germplasm collections is important for plant breeders who seek sources of genes for particular traits. As for soybean (Nagata 1959) and azuki bean (Tasaki 1963), which also originated in Asia, several plant types or ecotypes were classified and their geographical distribution was discussed. This basic information has been used for collecting and evaluating the breeding materials. However, very little information on this aspect is available for mungbean. This situation hinders the effective promotion of the varietal improvement of mungbean. It is thus important to investigate the range of variations and the geographical distribution of growth characters in mungbean local strains.

Quantitative characters such as number of days from sowing to flowering, stem length, and number of lateral branches are often influenced largely by the environmental conditions as well as by the agricultural practices. The present study was carried out at Chainat Field Crops Research Center (Chainat FCRC) in Thailand. Under the experimental conditions in Chainat, some strains may sometimes show characteristics different from those exhibited in the original regions. Nevertheless, comparative studies on the cultivation of the strains collected from various regions under the same conditions at the same time should reveal the relative variations and characteristics of the strains from each region.

Materials and methods

Four hundred ninety seven strains were used. All the strains used in this experiment were local varieties of each region. 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 497 strains used, 32 were collected from Korea, seven from China, four from Hong Kong, 17 from Taiwan, 69 from the Philippines, one from North Borneo, eight from Vietnam, 28 from Thailand, 18 from Indonesia, six from Burma, four from Sri Lanka, 234 from India, 10 from Pakistan, one from West Pakistan, 28 from Afghanistan, 12 from Iran, two from Iraq, eight from Turkey, three from Madagascar, two from Ethiopia, one from Kenya, and two from Nigeria, as summarized in Table 1. They were sown in the field of the Chainat FCRC on August 27 (Late rainy season).

The center is situated at 15° 10′ N and 100° 15′ E with an elevation of 16m above sea

Table 1. Variations in growth characters of local mungbean strains.

Origin	No. of Strains	Days to flowering Avg.[1]±SE[2] (Range)	Stem length Avg.±SE (Range)	No. of branches Avg.±SE (Range)
Korea	32	35±0.9(24~49)	58± 2.7(21~ 80)	1.9±0.2(0~ 4)
China	7	37±3.0(31~52)	54± 4.0(42~ 72)	1.4±0.4(0~ 3)
Hong Kong	4	38±2.3(35~45)	74± 5.6(59~ 85)	2.3±0.3(2~ 3)
Taiwan	17	36±0.7(31~41)	69± 3.1(51~ 85)	1.5±0.3(0~ 4)
The Philippines	69	37±0.6(30~55)	70± 1.6(40~ 95)	2.3±0.2(0~ 8)
North Borneo	1	31	65	1.0
Vietnam	8	43±2.8(34~58)	67± 3.4(53~ 83)	2.2±0.3(0~ 4)
Thailand	28	41±1.4(32~65)	75± 1.7(54~ 92)	3.4±0.3(1~ 7)
Indonesia	18	51±2.2(35~66)	82± 3.5(44~111)	5.6±0.7(1~12)
Burma	6	45±4.5(32~58)	66± 7.2(33~ 80)	4.5±0.9(0~ 6)
Sri Lanka	4	45±5.0(33~56)	87±11.6(53~105)	4.5±0.3(0~ 4)
India	234	38±0.4(24~62)	66± 1.2(23~119)	2.5±0.1(0~ 9)
Pakistan	10	40±2.1(35~52)	73± 6.0(51~106)	3.0±0.5(0~ 6)
West Pakistan	1	39	66	4.0
Afghanistan	28	36±0.7(31~46)	50± 2.5(29~ 75)	2.4±0.3(0~ 8)
Iran	12	36±1.5(30~47)	51± 3.8(37~ 76)	3.3±0.6(1~ 7)
Iraq	2	36±2.0(34~38)	59±30.5(28~ 89)	3.5±0.5(3~ 4)
Turkey	8	36±2.7(31~54)	62± 5.2(43~ 91)	3.3±0.7(0~ 6)
Madagascar	3	33±0.3(32~33)	60± 9.7(46~ 78)	3.0±0 (3~ 3)
Ethiopia	2	34±1.5(32~35)	60± 5.1(54~ 65)	2.0±0 (2~ 2)
Kenya	1	41	81	0
Nigeria	2	35±0 (35~35)	65±6.5(58~ 71)	2.5±0.5(2~ 3)
Total	497	38±0.3(24~66)	66± 0.7(21~119)	2.6±0.1(0~12)

1) : Average 2) : Standard Error

level. The average annual rainfall is 1100mm. Soil in the center consists of heavy clay from old river basin. Soil pH is 6.0-7.2 and organic matter content ranges from 0.7 to 2.1%. Total phosphorus content is around 412-843 ppm and potassium content is high enough so that its deficiency has not been recorded. The plants in the field were grown on ridges 60cm wide and 1m apart. On each ridge, two rows were made 50cm apart. Twenty plants for each strain were grown on a ridge 1m long which had two rows and ten hills. Two plants per hill were grown on each row with a 20cm distance. Irrigation, insecticide application, and hand weeding were performed as needed.

As in mungbean, it is generally difficult to determine the date of maturity due to the lack of synchrony in the pod maturity, the number of days from sowing to 50% flowering (the day when 50% of the plants in each strain exhibited flowering) was used as an indicator of the earliness of the strain. Two plants in the same hill were treated as a single plant for the determination of 50% flowering. Stem length and number of lateral branches longer than 10cm were recorded for one plant which showed a moderate growth in each strain at harvest. Harvesting was performed 30 days after the occurrence of 50% flowering.

Growth type classification was conducted basically by the demarcation line method used in the Revised Standard of Examination for Plant Type of Soybean (Watanabe et al. 1974). The number of days from sowing to flowering was also taken into account for classifying the growth types in the present study. The demarcation lines for the number of days from sowing to flowering, the stem length and the number of lateral branches were selected so as to divide all the strains into two groups consisting of nearly the same number of strains.

Results

Remarkable variations were observed in the number of days from sowing to flowering, the stem length, and the number of lateral branches (Table 1). The number of days to flowering, the stem length, and the number of lateral branches ranged from 24 days to 66 days, from 21cm to 119cm, and from 0 to 12, respectively. According to the analysis of variance, the number of days to flowering ($F_{21/475}=6.5$), the stem length ($F_{21/475}=4.3$), and the number of lateral branches ($F_{21/475}=5.3$) were significantly different at the 1% level depending on the origin of the strains.

The frequency distribution of the number of days from sowing to flowering is shown in Fig. 1-a. Based on the pattern of distribution of the days to flowering, the strains were divided into two groups with 35 days selected as the demarcation line, namely 249 strains belonged to the early maturity group and 248 strains to the late maturity group. The frequency distribution of the stem length is shown in Fig. 1-b. Based on this frequency distribution, the strains were divided into two groups by a demarcation line corresponding to the stem length of 65cm. Two hundred and thirty strains belonged to the short plant group (stem length 65cm or lower) and 267 strains belonged to the tall plant group (stem length above 65cm) based on this criterion. The frequency distribution of the number of lateral branches is shown in Fig. 1-c. The strains which had 0, 1, and 2 lateral branches were assigned to the low-branching group and the strains which had more than 2 lateral branches were assigned to the high-branching group. Based on this criterion, 258 strains belonged to the low-branching group and 239 strains belonged to the high-branching group.

Combining these three characters, i.e., number of days from sowing to flowering, stem length and number of lateral branches, eight growth types were defined as shown in Table 2. Growth type 1 was characterized by short plants with a low-branching habit and early maturity : 100 strains belonged to type 1. Type 2 was characterized by short plants with a high-branching habit and early maturity : 46 strains belonged to type 2. Type 3 was characterized by tall plants with a low-branching habit and early maturity : 67 strains belonged to type 3. Type 4 was characterized by tall plants with a high-branching habit and early maturity : 36 strains belonged to type 4. Type 5 was characterized by short plants with a low-branching habit and late maturity : 40 strains belonged to type 5. Type 6 was characterized by short plants with a high-branching habit and late maturity : 44 strains belonged to type 6. Type 7 was characterized by tall plants with a low-branching habit and late maturity : 51 strains belonged to type 7. Type 8 was characterized by tall plants with a high-branching habit and late maturity : 113 strains belonged to type 8.

A clear geographical cline was recognized for the distribution of each growth type (Fig. 2). This figure deals only with the countries which had more than ten strains as entries for this experiment. As for Indonesia (Graph D), about 80% of the strains examined belonged to the growth type 8. In other words, tall, high-branching and late

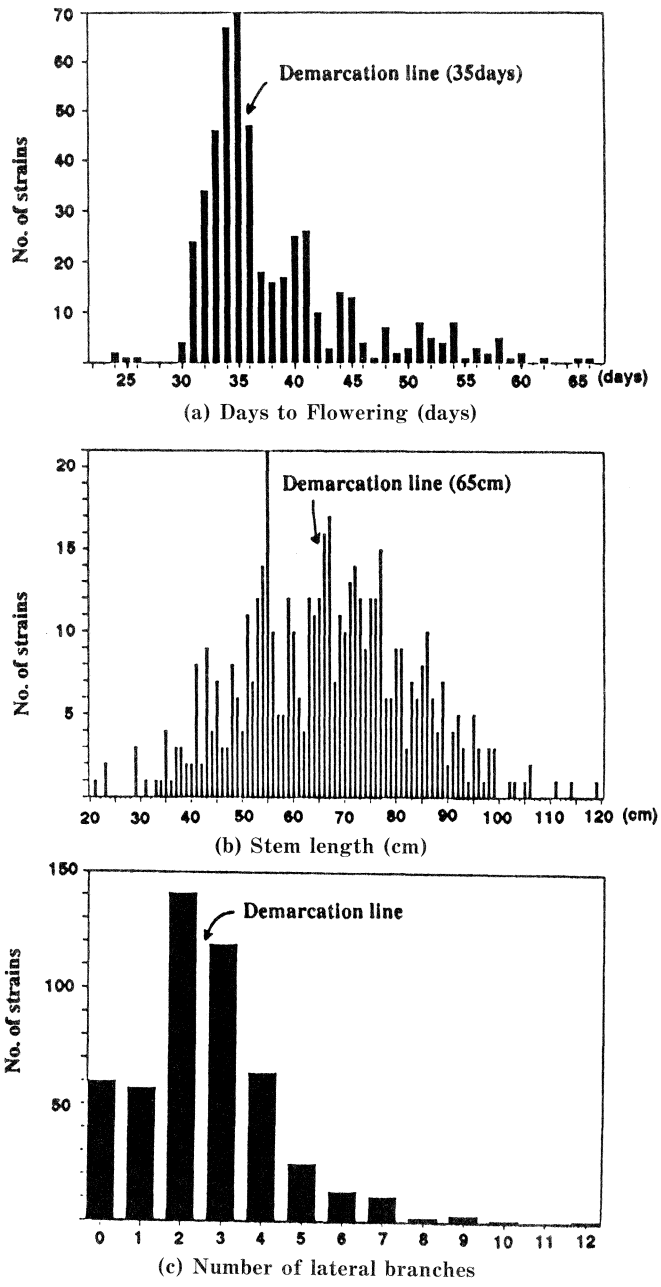

Fig. 1 Frequency distribution of (a) : the number of days to flowering, (b) : the stem length, and (c) : the number of lateral branches in mungbean, and the demarcation lines.

Table 2. Characteristics and number of strains in each growth type

Growth type	Characteristics			No. of strains
	Maturity	Stem length	Branching	
Type 1	Early[1)]	Short[3)]	Low[5)]	100
Type 2	Early	Short	High	46
Type 3	Early	Tall[4)]	Low[6)]	67
Type 4	Early	Tall	High	36
Type 5	Late[2)]	Short	Low	40
Type 6	Late	Short	High	44
Type 7	Late	Tall	Low	51
Type 8	Late	Tall	High	113

1) Days to flowering less than 36 days.
2) Days to flowering more than 35 days.
3) Stem length 65 cm or lower.
4) Stem length higher than 65 cm.
5) Number of lateral branches 0, 1, or 2.
6) Number of lateral branches more than 2.

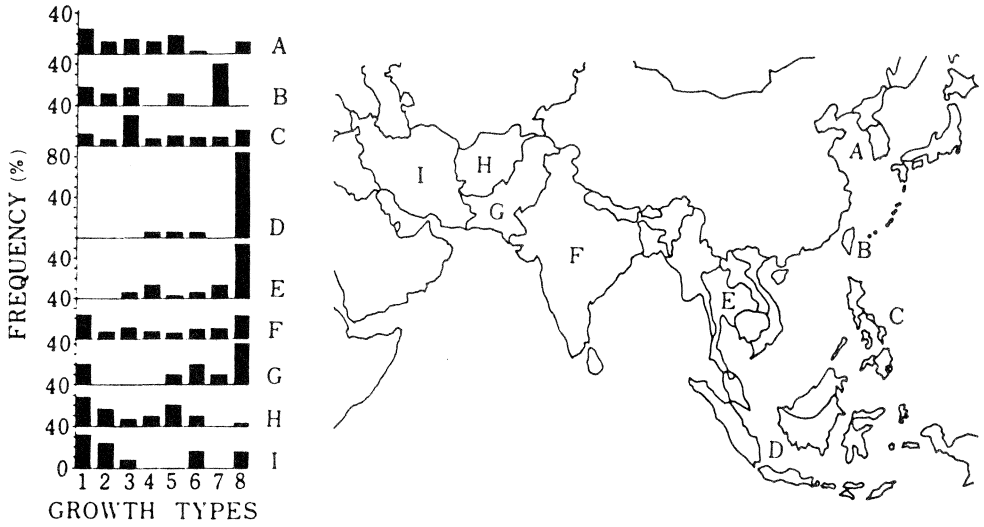

Fig. 2 Georaphical distribution of growth types in mungbean.

A : Korea, B : Taiwan, C : The Philippines, D : Indonesia, E : Thailand, F : India, G : Pakistan, H : Afghanistan, I : Iran

maturity strains were predominant in Indonesia. The same tendency could be seen for the strains from Thailand (Graph E), Pakistan (Graph G), Burma, Sri Lanka and Vietnam (Table 1). In contrast, the tall but low-branching types (Growth types 3 and 7) were dominant in Taiwan (Graph B) and in the Philippines (Graph C). The strains from Korea (Graph A), Afghanistan (Graph H) and Iran (Graph I) showed a similar pattern in the frequency of growth types. Short, low-branching and early maturity types (Growth type 1) prevailed in these regions. The same tendency could be seen for the strains from China and Turkey (Table 1). The strains from India (Graph F) showed the most even distribution of each growth type.

Discussion

The growth type of a local strain is considered to result from the adaptation of a plant to various climatic conditions and/or agricultural practices in a region. Among the climatic factors, day length and temperature mainly affect the growth type. The present study was carried out at Chainat which is located at a rather low latitude (15° N). The day length during the growing period in this experiment was decreasing from 13 hours to 12 hours and prevailing temperatures ranged from 27℃ to 33℃.

Mungbean is considered to be a short day plant (MacKenzie et al. 1975). The strains from higher latitudes are usually cultivated under longer day length and lower temperature regimes than under the current experimental conditions. Thus the strains from higher latitudes fulfilled the short-day requirement earlier than in the original region and a higher temperature accelerated flowering and ripening. As a result, their vegetative growth was restricted as expressed by the short stem length and a few lateral branches. These factors may explain why the strains from Korea, Afghanistan, and Iran which are located at higher latitudes consisted of short plants with a short maturity span and low-branching habit in this study.

Indonesian strains showed the latest flowering, indicating that the critical photoperiod of the Indonesian strains was the shortest among the entries. It is obvious that the shortest critical day length of the strains is due to the location of Indonesia around the equator. Therefore, the natural day length in Indonesia is about 13 hours all the year round. Late flowering habit was also observed in the strains from Thailand, which may be related to the cropping system in Thailand where mungbean is grown mainly in the late rainy season after corn. Since the day length during the late rainy season in Thailand is decreasing from 13 hours to 12 hours, most of the strains from Thailand have had the oppotunity to become adapted to the short day length. As a result, the strains from Indonesia and Thailand were characterized by an exceptionally high occurrence of growth type 8, i.e., tall, high-branching and late maturity type. It is interesting to point out that soybean (*Glycine max*) strains from Indonesia and Thailand showed a similar growth habit (called Indo-Chinese ecotype, Nagata 1959) to that of mungbean strains from the same region in this study.

The strains from Taiwan and the Philippines were characterized by a tall but low-branhing growth habit (Growth types 3 and 7, Fig. 2). However, many strains from the Philippines showed a shorter maturity span (Growth type 3) compared to that of the predominant strains from Taiwan (Growth type 7).

A wide range of variations in the growth characteristics was observed in the strains from India. India is considered to be the region of domestication of mungbean (Mollison 1901, Paroda and Thomas 1988), which may account for the fact that the largest diversity of growth types occurred in India. This fact indicates that the

genetic diversity of the other traits is also large in India. Therefore, it was suggested that the source of desirable genes such as disease and insect resistance could most probably be found in the strains from India.

The pattern of the latitudinal cline observed for the growth characteristics of the local mungbean strains in the present study is very similar to that of azuki bean (a close relative of mungbean) observed in Japan, i.e., the low-branching erect type was distributed in the northern part while the high-branching, late and prostrate type was predominant in the southern part of Japan (Tasaki 1963). Vavilov (1926) examined the geographical distribution of several cultivated crops, and recognized a general pattern of latitudinal distribution in the growth characteristics. He reported that the earlier strains characterized by shorter plants with a low-branching habit were distributed in the North, and the later strains characterized by taller plants with a high-branching habit were distributed in the South. Vavilov's concept on the pattern of latitudinal distribution of plant growth habit basically applied also to the case of mungbean.

2-2. Geographical distribution of seed characters in mungbean

Introduction

Mungbean is also called green gram based on the most frequent seed color of mungbean. However, the seed color in mungbean exhibits a wide range of variations from yellow, greenish yellow, light green, shiny green, dark green, dull green, black, brown, and mottled with black (Paroda and Thomas, 1988). Seed size also shows wide variations, i.e. seed length ranges from 3.5 to 6.2mm, seed width from 2.6 to 4.2mm, and 100-seed weight from 1.7 to 8.3g (Miyazaki, 1982). However, the pattern of geographical distribution in the variations of the seed color and size in mungbean has not been clarified.

In the present study, the geographical distribution of the seed color, seed weight, seed length, seed width, and seed shape (ratio of seed length/seed width) was analyzed. Then, a hypothesis to explain the pattern of geographical distribution was discussed in terms of ecological adaptation and ethnological preference in each region.

Materials and methods

Strains

A total of 651 local strains of mungbean 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 651 strains used, 48 were collected from Korea, 12 from China, 24 from Taiwan, 86 from the Philippines, 23 from Indonesia, 14 from Vietnam, 42 from Thailand, 266 from India, 62 from Pakistan, 38 from Afghanistan, 23 from Iran & Iraq, and 13 from Turkey as summarized in Table 3. Distribution of the growth types and protein types (the subunit composition of total seed protein) of the mungbean strains used in the present study showed a clear geographical cline (Tomooka et al. 1991b, 1992), suggesting that these materials are true landraces adapted to each local environment.

Analysis of seed characters

The strains were classified into five color categories based on visual observation (Fig. 3). Gr (shiny green) type included different shades of green with glossy or shiny seed luster. DG (dull green) type was represented also by a green seed color but with a dull seed luster. Yl (yellow) type consisted of yellow seed color with both glossy and dull seed luster. Bl (black) type showed variations from slightly black mottled to heavily black mottled green seed. This type exhibited a glossy seed luster. Br (brown) type was characterized by a brown seed color with dull seed luster. If more than two seed colors were present in a strain or an accession, they were listed separately.

Basically, the seed weight was determined using 100 seeds for each strain, but sometimes a smaller number of seeds were used depending on the availability of seeds. Seed length and seed width were measured with a dial caliper for five moderate-sized seeds per each strain. The ratio of seed length to seed width (length/width) was used as an indicator of the seed shape.

Results

Variations in seed weight, length, width and shape (length/width) of the mungbean strains are indicated based on the origin in Table 3. Seed weight (100-seed weight) ranged from 2.0 to 8.7g with an average of 4.4g. Seed length and width varied from 3.1 to 6.3mm (average=4.4mm) and from 2.3 to 4.5mm (average=3.4mm), respectively. Seed shape (length/width) ranged from 1.01 to 1.50 with an average of 1.29. To visualize the pattern of geographical distribution in the seed characters, the frequency distribution of the seed weight, seed shape and seed color was plotted for each origin (Fig. 4).

Table 3. Variations in seed weight, length, width, and length/width ratio of local mungbean strains

Origin	No. of strains used	100-seed weight (g)		Seed length (mm)		Seed width (mm)		Length/Width ratio	
		Avg.±S.E.	Range	Avg.±S.E.	Range	Avg.±S.E.	Range	Avg.±S.E.	Range
Korea	48	4.8±0.2	2.5-7.0	4.6±0.1	3.9-5.7	3.5±0.1	3.0-4.4	1.30±0.01	1.13-1.42
China	12	5.4±0.3	4.4-6.7	4.6±0.3	3.1-5.2	3.6±0.2	2.6-3.8	1.28±0.03	1.18-1.37
Taiwan	24	4.9±0.1	4.2-6.0	4.6±0.1	4.0-5.2	3.7±0.1	3.3-4.1	1.25±0.01	1.15-1.41
Philippines	86	6.1±0.2	3.1-8.6	5.0±0.1	3.8-6.3	3.8±0.0	3.1-4.5	1.31±0.01	1.16-1.49
Indonesia	23	4.9±0.3	3.0-7.4	4.7±0.1	4.0-5.4	3.6±0.1	3.0-4.3	1.31±0.02	1.17-1.44
Vietnam	14	5.7±0.2	4.5-6.5	5.0±0.0	4.9-5.2	3.9±0.0	3.6-4.1	1.28±0.01	1.23-1.33
Thailand	42	6.1±0.3	3.2-8.7	5.0±0.1	4.2-5.5	3.8±0.1	3.2-4.5	1.30±0.02	1.15-1.50
India	266	3.7±0.1	2.0-7.9	4.1±0.0	3.3-5.3	3.3±0.0	2.6-4.4	1.27±0.01	1.01-1.45
Pakistan	62	3.4±0.2	2.5-4.7	4.0±0.1	3.5-5.0	3.1±0.1	2.9-3.8	1.30±0.02	1.18-1.38
Afghanistan	38	3.5±0.1	2.4-4.8	4.1±0.1	3.5-5.1	3.2±0.1	2.8-4.0	1.28±0.01	1.18-1.42
Iran & Iraq	23	3.7±0.3	2.1-5.5	4.2±0.1	3.5-5.0	3.3±0.1	2.7-3.9	1.27±0.02	1.14-1.33
Turkey	13	3.7±0.3	2.6-4.9	4.4±0.1	3.7-4.8	3.2±0.2	2.3-3.6	1.29±0.02	1.17-1.35
Total	651	4.4±0.1	2.0-8.7	4.4±0.0	3.1-6.3	3.4±0.0	2.3-4.5	1.29±0.01	1.01-1.50

Avg.=Average, S.E.=Standard error.

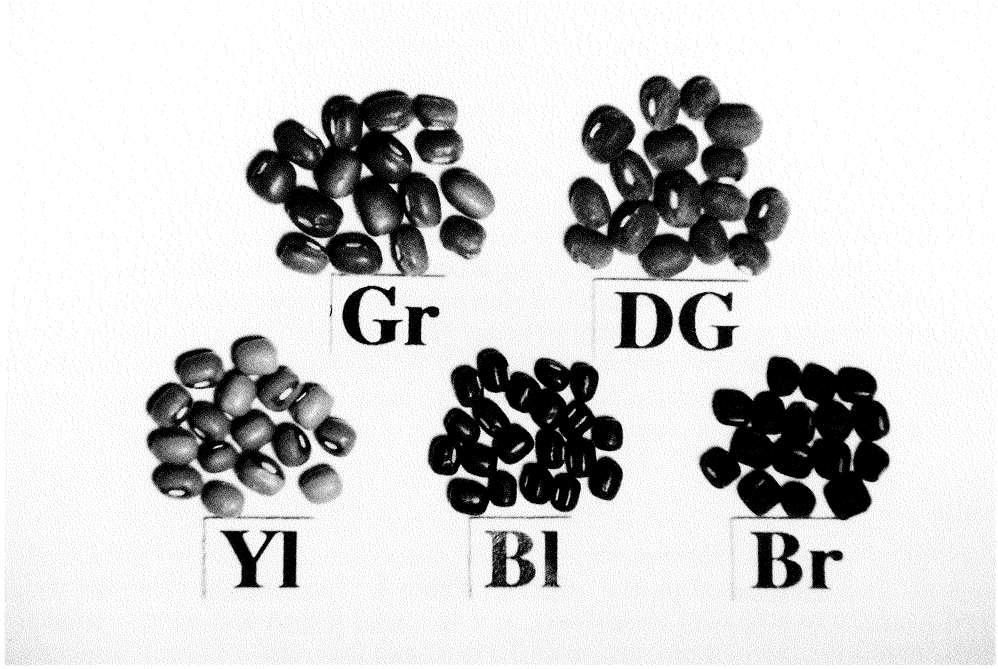

Fig. 3 Five types of seed color in mungbean.
Gr : shiny green, DG : dull green, Yl : yellow, Bl : black mottled, Br : brown

Seed color

Strains with a shiny green seed coat (Gr), the predominant type, accounted for 49% of the total strains, followed by the dull green type (DG, 33%), brown type (Br, 9%), black type (Bl, 5%) and yellow type (Yl, 4%). Strains with a green seed coat (Gr and DG) occurred througout Asia (Fig. 4). Among the green-seeded strains, the shiny green type (Gr) strains predominated in the Philippines, Vietnam, Thailand, India, Pakistan, Afghanistan, and the Iran & Iraq area, while dull green (DG) type strains predominated in Korea, China, Taiwan, Indonesia, and Turkey. Strains with a yellow seed coat (Yl) occurred in Korea, Taiwan, the Philippines, Indonesia, Thailand, and India in a low frequency. Strains with a black mottled seed coat (Bl) were detected only in India, Pakistan, and Afghanistan. Strains with a brown seed coat (Br) were mainly distributed in India and westward (Pakistan, Afghanistan, Iran & Iraq, Turkey), with only one exceptional Br-type strain found in Indonesia.

Seed weight

A clear geographical cline was recognized for the pattern of frequency distribution of the seed weight (Fig. 4). In the Indian subcontinent (India, Pakistan) and West Asia (Afghanistan, Iran & Iraq, Turkey), small-seeded mungbean strains (100-seed weight $\leq$ 4g) predominated (Fig. 4), and the average 100-seed weight in these regions ranged from 3.4 to 3.7g (Table 3).

In the Southeast Asian countries (the Philippines, Indonesia, Vietnam, Thailand), the strains showed a wide range of seed weight. In contrast with the strains in the

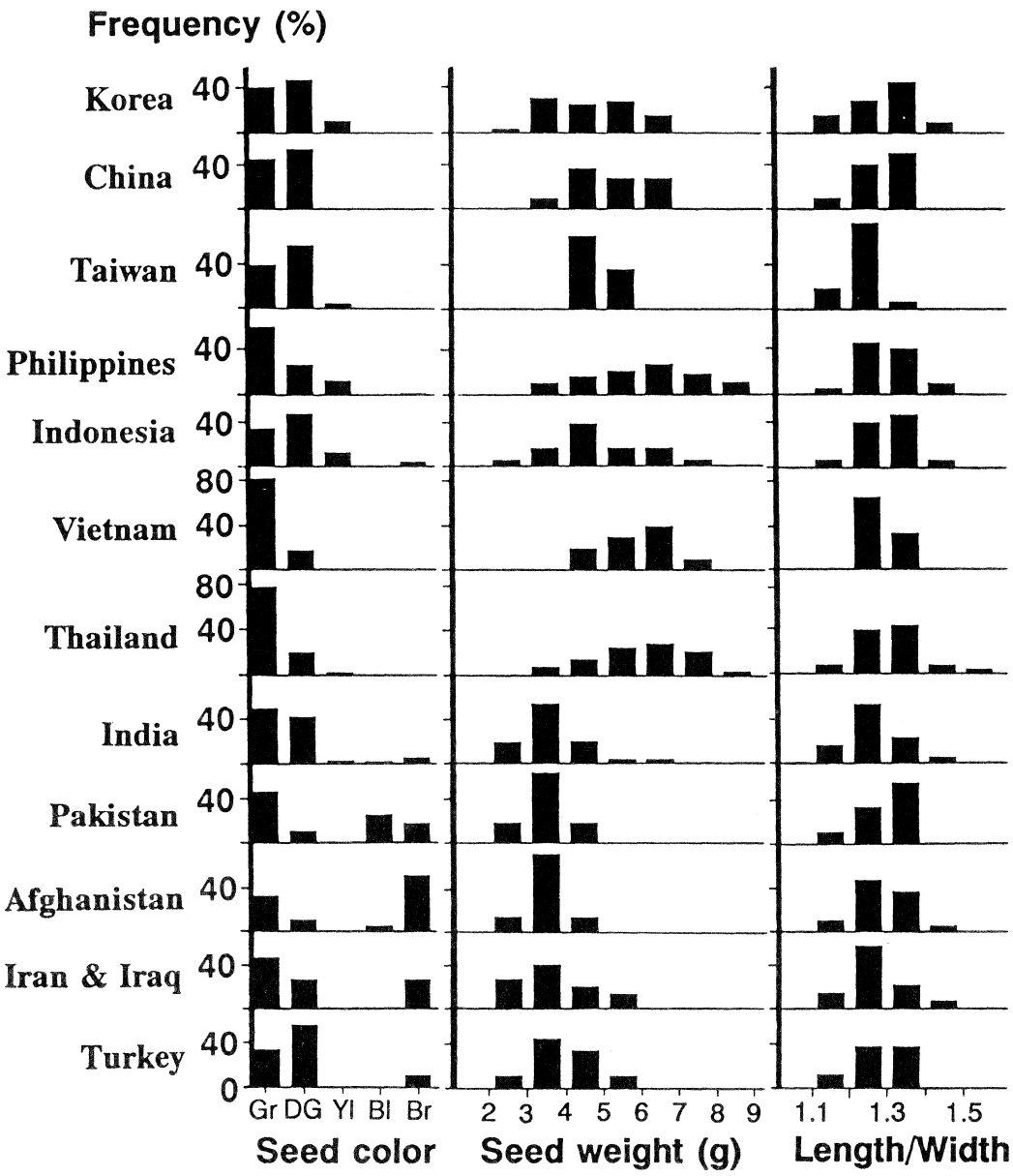

Fig. 4 Geographical distribution of seed characters in mungbean.

Indian subcontinent and West Asia, small-seeded strains (100-seed weight $\leq$ 4g) were not conspicuous, while extremely large-seeded strains (100-seed weight $>$ 7g) were detected throughout Southeast Asia (Fig. 4). Average 100-seed weight in these four countries ranged from 4.9 to 6.1g (Table 3).

In the East Asian countries (Korea, China, Taiwan), average 100-seed weight ranged

from 4.8 to 5.4g, which was an intermediate range between that of India and westward (3.4 to 3.7g) and that of Southeast Asia (4.9 to 6.1g) (Table 3). Neither extremely small-seeded strains (100-seed weight $\leq$ 3g) which were consistently distributed in India and westward, nor extremely large-seeded strains (100-seed weight $>$ 7g) which were found commonly in Southeast Asia, were detected in the East Asian countries (Fig. 4).

Seed size and shape

Geographical distribution of seed size (seed length and seed width) showed a similar pattern to that of seed weight (Table 3). Small-seeded strains predominated in India and westward (average seed length : 4.0-4.4mm, seed width : 3.1-3.3mm) and large-seeded strains were mainly distributed in Southeast Asia (seed length : 4.7-5.0mm, seed width : 3.6-3.9mm), while medium-seeded strains prevailed in East Asia (seed length : 4.6mm, seed width : 3.5-3.7mm).

The ratio of seed length to seed width (length/width, used as an indicator of seed shape) ranged from 1.01 to 1.50 (Table 3). However, 80% of strains fell into the range between 1.2 and 1.4. For the seed shape, it was thus considered that the variation was too small to consider a geographical cline by using the length/width ratio.

Discussion

Three mungbean-growing regions classified by the seed characters

Based on the pattern of geographical distribution in the seed characters, the mungbean-growing areas in Asia were classified into three regions with the following characteristics of mungbean strains.

(1) Indian subcontinent and West Asia : mungbean strains characterized by small seeds with brown and black mottles.
(2) Southeast Asia : predominance of mungbean strains with seeds with a shiny green color, and wide variations in seed weight.
(3) East Asia : medium-sized seeds with a relatively high occurrence of dull green color.

Since seeds are the main part that is utilized in mungbean, the characters of seeds are considered to result not only from the adaptation of the plant to the local environment but also from selection based on preference. The preference is considered to be closely related to the culture (e.g. racial traits, way of cooking and consumption, etc.) of the region. A hypothesis will be put forward to explain the above-mentioned pattern of geographical distribution from the environmental and cultural point of view.

Indian subcontinent and West Asia

The variable and unstable environmental conditions in India and westward may partly explain the reason why small-seeded mungbean strains with various seed colors were predominantly distributed in this area. Generally speaking, precipitation in the mungbean-producing region in West Asia (represented by desert and steppe climate) and in India (savanna climate) is scarce compared to that in Southeast Asia (savanna and tropical rain forest climate) and in East Asia (temperate rainy and temperate summer rain climate). In addition to the lower amount of precipitation, annual rainfall variability in West Asia (30-40%) and India (15-25%) is higher than that in Southeast and East Asia ($\leq$ 20%) (Riehl 1954). Singh et al. (1988) reported that

mungbean has been traditionally grown on marginal and submarginal land subjected to moisture and fertility stress in India. Mungbean is usually grown in mixed cropping or intercropped with other rainy season crops under dry land conditions where rainfall is highly erratic. Mixed cropping is also popular in Pakistan where mungbean is usually cultivated together with other food legumes (black gram, soy bean, field bean) and/or cereals (amaranths, maize, sorghum, pearl millet) (IBPGR mission 1990). The same conditions also apply to West Asia.

Mixed cropping is an insurance against total crop failure in the region especially where the environment is highly variable and unstable (Beets 1982). Under such conditions, genotype variability in a single crop is also high. In a variable environment, it may be desirable to plant a mixture of different genotypes. Extensive selection (e.g. to large-seeded and shiny green type) frequently leads to great uniformity of genotypes. Genetic variablity in such a type is very low, and the variety is therefore suitable only for a narrow range of environments. Against this background, a small-seeded strain with various colors can be considered as a population derived from non-extensive selection, and therefore, there may be a wide genotype variablility within a strain. Actually, 37 out of 43 accessions of mungbean collected in Pakistan showed multiple seed colors within an accession (IBPGR mission 1990). Vavilov (1926) proposed the concept of general geographical regularities in the distribution of cultivated plants. He pointed out that the cultivated plants in West Asia (e.g. wheat, barley, oats, peas, chick peas, grass peas, lentils, broad beans) showed primitive forms characterized by small seeds and small flowers. The difficulty in distinguishing the small-seeded cultivars from their wild forms was considered to be a characteristic in this region. Farmers may consciously conserve small-seeded primitive strains to maintain the genotype diversity under the unstable and unpredictable environments.

It was suggested that small-seeded strains with various seed colors in India and westward may result from the lack of extensive selection, and thus may harbour wide genetic variations. This hypothesis is supported by the results that the largest diversity in growth types of mungbean was found in India (Tomooka et al. 1991b), and that in seed protein types in West Asia (Tomooka et al. 1992). Introgressive hybridization between the cultivated and wild ancestral form of mungbean (*V. radiata* var. *sublobata*) may play an important role in the widening and maintenance of the genetic variations in this area. Paroda and Thomas (1988) reported that intermediate weedy forms of mungbean which were considered to have been derived from occasional crossing among primitive cultivars and wild forms were distributed in the disturbed habitats in India. They suggested that these forms may have undergone further natural and artificial selection so as to reach the present stage of domestication.

In addition to the ecological factors discussed above, the preference in the region also accounts for the fact that small-seeded strains with various seed colors were distibuted in the Indian subcontinent. The preference is closely related to the traditional practice of processing and consumption. Generally speaking, legumes are so hard that they must be cooked longer compared to cereals. To facilitate cooking (softening), legumes (including mungbean) are usually milled to remove the outer husk and made into split dehusked legume called "dhal" in the Indian subcontinent (Thirumaran and Seralathan, 1988). After being boiled with curry (salt and spices), legumes are consumed under the same name as "dhal", which is the most common and major dish in the Indian subcontinent (Singh et al. 1988). Rahman and Miah (1988) pointed out that since mungbean is mostly split and used in "dhal" soup, large-sized seed is not a consumer factor in Bangladesh, and preference is for well-filled, small-seeded types.

The preference for the small-seeded types is common in other countries in the Indian subcontinent where the seeds are prepared in the same way. Since the seed coat is usually removed before cooking "dhal" soup, the seed color is not a consumer factor, either.

Southeast Asia

In Southeast and East Asia, mungbean is consumed mainly as bean sprouts, mungbean noodle, vermicelli, soup (seasoned with sugar) and various kinds of sweets. Among them, bean sprouts are most popular throughout Southeast and East Asia.

As a reflection of the long history of consumption, various preferences for the quality of bean sprouts have developed in each region. In Malaysia, good quality sprouts should be short, stout, with hypocotyls large in diameter and with short roots (Siti and Mahmud 1988). Generally, the seed size is considered to be positively correlated with the hypocotyl diameter and large seeds are preferred by the people in the sprouting industry in Malaysia. In Thailand, the presence of large seeds (at least 5.5g/100 seeds) is a prerequisite for the release of a cultivar (Chainuvati et al. 1988). Preference for not only large but also shiny green seeds was reported from the Philippines (Lantican and Navarro 1988), Vietnam (Quyen 1988) and Thailand (Srinives and Yang 1988). Selection pressure resulting from this preference may account for the occurrence of large-seeded mungbean strains and the high frequency of a shiny green seed color in Southeast Asia (the Philippines, Vietnam and Thailand) (Fig. 4).

Interestingly, small-seeded mungbean strains were also detected in Southeast Asia (Fig. 4). Bamrungpol (1957) reported that three types of mungbean cultivars, i.e., (1) a small-seeded type (2) a golden gram (yellow seed color) (3) a mungbean cultivar with shiny seed coat, were grown in Thailand during the 1950s. The presence of small-seeded mungbean strains can be partly ascribed to the following fact. Two types of local strains, i.e., "Dâu Mõ" and "Dâu Móc", are popular in Vietnam (Quyen 1988). The "Dâu Mõ" (glossy bean) variety shows dark green, round, and large seeds with a shiny luster. "Dâu Móc" (dull bean) is a small-seeded variety which is elliptical at both ends, rough with a dull color, easy to cook, rich in starch, aromatic and has a good taste. This fact suggests that small-seeded mungbean shows some advantageous traits and is utilized not for bean sprouts but for soup, bean jam, or sweet.

East Asia

Preference for medium to small seeds with dull seed luster may explain the absence of extremely large-seeded strains (>7g/100 seeds) and the relatively high occurrence of dull green seeds in East Asia. In the East Asian countries (Taiwan, China, Korea), mungbean strains with a very large seed size (>7g/100 seeds) were not observed (Fig. 4). Preference for the quality of bean sprouts and seed size in East Asia is slightly different from that in Southeast Asia. In Taiwan, rootless or short-rooted mungbean sprouts about 9cm in length and 3-4mm in diameter with white color and crispy texture are preferred, and these characteristics along with a proper seed size are major seed properties desired for bean sprout production (Chen et al. 1988). It is considered that large seeds result in low yield. It was also reported that small seeds were preferred to produce bean sprouts and farmers appreciated seeds with a dull luster in Taiwan (Chen 1988). In Korea, the local bean sprout growers preferred small-seeded cultivars with dull seed luster, since these strains are considered to produce more sprouts, and thus two AVRDC high-yielding cultivars (100 seed weight

of 4.8g and 5.5g, respectively) were not appreciated by the sprout growers (Park and Hong 1988).

Yellow-seeded strains were distributed in Southeast and East Asia in a low frequency. In Thailand, such strains were designated as "tua tohn" (golden legume) and used for making dessert. They fetched the highest price in the 1950s, but are almost extinct presently (Srinives and Yang 1988). In the Philippines, some regions prefer seeds with a glossy yellow color, and breeding for yellow mungbean is also included in the government program (Lantican and Navarro 1988).

2-3. Geographical distribution of protein types in mungbean

Introduction

Biochemical characters such as isozymes are considered to be neutral to the selection by human beings and thus are regarded as important characters for the identification of the center of genetic diversity (Nakagahra 1978). Among the biochemical markers, the usefulness of the seed protein electrophoresis method was recognized and the 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 and Bliss 1988, Gepts et al. 1986, Gepts et al. 1988).

As for mungbean, however, very few studies have been conducted using seed protein electrophoresis (Egawa et al. 1988b, Thakare et al. 1988). Moreover, these authors examined only interspecific variations of seed protein electrophoregrams and revealed the phylogenetic relationships among *Vigna* species including mungbean (*Vigna radiata*). The present study was therefore conducted to investigate the intraspecific variations of seed protein by SDS-polyacylamide gel electrophoresis (PAGE) and to identify the center of genetic diversity in seed protein and dissemination pathways in mungbean.

Materials and methods

Strains

Five hundred eighty one local mungbean strains were 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, six were collected from Japan, 46 from Korea, 11 from China, 21 from Taiwan, 82 from the Philippines, 22 from Indonesia, nine from Sri Lanka, 11 from Vietnam, 40 from Thailand, seven from Burma, 246 from India, 17 from Pakistan, 34 from Afghanistan, 19 from Iran and Iraq and 10 from Turkey, as summarized in Table 4. Since mungbean is cultivated and consumed in a local area, a given strain of mungbean collected from a local area can be regarded as an endemic race that has been grown in that area for a long time.

Preparation of protein samples

Total seed protein was extracted from 10mg of seed meal with 1ml of 0.05M Tris-HCl buffer (pH8.0) containing 0.2% SDS and 5M urea. Then 20μl of 2-mercaptoethanol was added to the extract. Thereafter 15μl of the crude extract was

Table 4. Origin, protein types, and number of mungbean 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

directly placed on the gel for electrophoresis. The globulin and the albumin fractions were prepared by the following procedures. Extracted protein from 100mg seed meal with 2.5ml of 0.02M Tris-HCl buffer (pH8.0) was centrifuged at 3000rpm for 5 minutes and 0.1ml of 1M CH_3COONa (pH4.0) was added to the supernatant. The precipitate, referred to as globulin, was collected by centrifugation (3000rpm, 5min.). A fourfold volume of prechilled acetone was added to the clear supernatant, which contained albumin protein, and was kept at −20℃ for 1 hour. The precipitate, referred to as albumin, was collected by centrifugation (3000rpm, 5min.). The globulin and the albumin pellets were resuspended by dissolution into 5ml and 1ml of 0.05M Tris-HCl buffer (pH8.0) containing 0.2% SDS and 5M urea together with 100μl and 20μl of 2-mercaptoethanol, respectively. Then 15μl 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 100V for the first 30 minutes and 150V for another 3 hours. 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 the 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 and 37,000, in which four albumin bands and three globulin bands were recognized. They 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 (Fig. 5). 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. 5). 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).

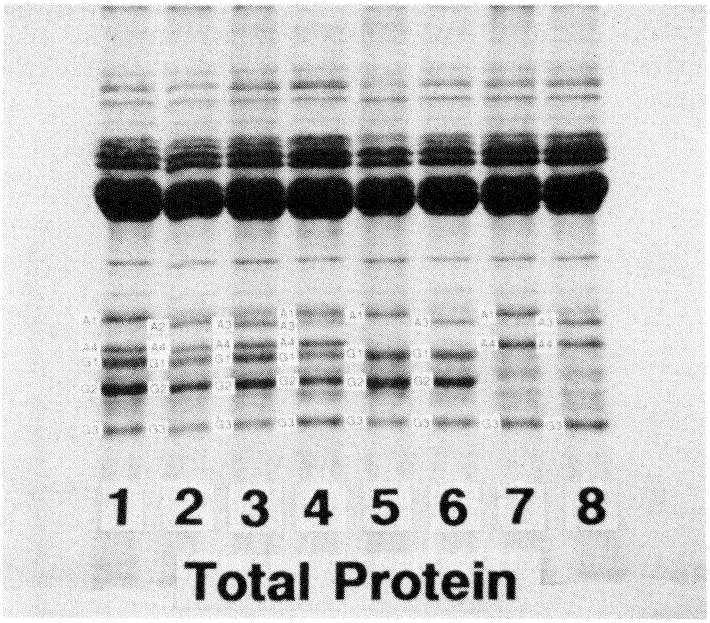

Fig. 5 Eight phenotypes of total protein electrophoregrams in mungbean.

Of the 581 strains examined, 372 strains contained protein type 1, 39 protein type 2, 107 protein type 3, 18 protein type 4, two protein type 5, one protein type 6, 24 protein type 7, and 18 contained protein type 8 (Table 4). As shown in Table 4, the geographical distribution of the eight protein type strains differed greatly. Protein type 1 strains, the most common type, were widely distributed throughout Asia. Protein type 2 strains, in contrast, were distributed only in Iran and Iraq, Afghanistan, India, Thailand, Indonesia, and the Philippines 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, Taiwan, China, Korea, and Japan. The distribution of the protein type 4 strains was restricted to Afghanistan, Pakistan, India, Burma, Thailand, 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 distibution of the strains with protein types 7 and 8 was quite similar to each other. 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

Frequency of the protein type strains in each region is shown in Fig. 6. Based on the frequency distribution of the strains with different protein types, the region with the largest diversity was assigned to West Asia (the Afghanistan-Iran-Iraq area, Fig. 6, Graphs M and N). Various protein types were distributed most evenly in this area. Protein type 1 strains, the most predominant type, accounted for about 40% of the total number of strains examined and more than 5 kinds of protein type strains were found in this region.

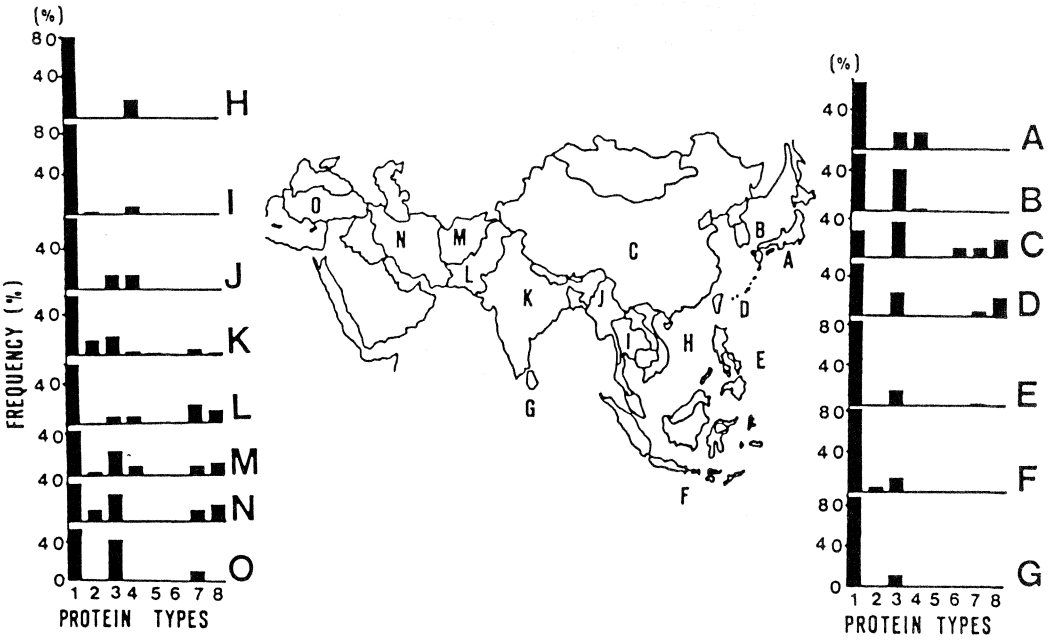

Fig. 6 Frequency of mungbean strains with each protein type in each region.

A : Japan, B : Korea, C : China, D : Taiwan, E : The Philippines, F : Indonesia, G : Sri Lanka, H : Vietnam, I : Thailand, J : Burma, K : India, L : Pakistan, M : Afghanistan, N : Iran and Iraq, O : Turkey

In Turkey, which is located west of the Afghanistan-Iran-Iraq area, only 3 kinds of protein type strains were detected and the diversity seemed to be lower (Fig. 6, Graph O). Coonsidering the small number of accessions from Turkey (10 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 about 60% of the total number of strains examined (Fig. 6, 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 isncreased to 70% and only 3 kinds of protein type strains were found (Fig. 6, 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 (1935) included Burma into the centers of diversity of mungbean. Considering the small number of strains from Burma that was examined in the present study (7 strains), it may be necessary to conduct further analyses using a larger number of strains from Burma.

In the Southeast Asian countries (the Philippines, Indonesia, Vietnam, Thailand and Burma), the frequency distribution of the protein types was very similar to each other, i.e., the predominant protein type 1 strains accounted for 70% to 90% and only 2 or 3 kinds of protein type strains were distributed (Fig. 6, Graphs E, F, H, I and J). Protein type 5, 6, 7 and 8 strain 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 and similar to each other. 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 in a frequency of about 10% besides the predominant protein type 1 strains (Fig. 6, Graphs H and I), whereas in Insular Southeast Asia (Indonesia and the Philippines), protein type 3 strains were detected in a frequency of about 10% besides predominant protein type 1 strains (Fig. 6, 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. 6, 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. 6, Graph D). This tendency could also be recognized in the strains from China (Fig. 6, Graph C) in spite of the small number of strains examined (11 strains). Furthermore, the pattern of frequency distribution of the protein types was similar to that of West Asia, indicating the strong relationship between mungbean in China and Taiwan and that in West Asia.

In Korea, protein type 7 and 8 strains could not be found and the strain composition was rather simple (Fig. 6, 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 besides protein type 1 strains (Fig. 6, Graph A).

Center of protein type diversity and dissemination pathways

Geographical cline of various protein type strains reflected the center of protein type diversity and possible dissemination pathways in mungbean. The center of protein type diversity appeared to be located in West Asia (the Afghanistan-Iran-Iraq area) rather than in India. Mungbean probably spread to the east by two main routes. One route led to Southeast Asia from India. Strains mainly consisting 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 West 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 suggests that the the protein type 7 and 8 strains found in Taiwan do 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 mungbean (Vavilov 1935, Singh et al. 1974, Zeven and de Wet 1982, Tomooka et al. 1991b). 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 mungbean by using other biochemical markers. A large number of local strains especially from Turkey, Iran, Iraq, Afghanistan, Burma and China should be analyzed for more precise information.

Chapter 3. Collection and Evaluation of wild relatives (subgenus *Ceratotropis*) of mungbean

3-1. Taxonomic position of the subgenus *Ceratotropis* (A literature review)

Introduction

The subgenus *Ceratotropis* of the genus *Vigna* includes five food legumes, i.e., mungbean (*V. radiata*), black gram (*V. mungo*), rice bean (*V. umbellata*), azuki bean (*V. angularis*), and moth bean (*V. aconitifolia*). Area of traditional cultivation, synonyms and natural distribution of wild forms of these five cultivated pulses are

Table 5. Five pulses in the subgenus *Ceratotropis* (after Tateishi and Ohashi 1990)

Cultivated form	Wild form
Vigna aconitifolia 1) *Phaseolus aconitifolius* 2) Moth bean 3) Pakistan and India	*Vigna aconitifolia* (Indistinguishable from cultivated form) 4) Arabia, Pakistan, India and W. China
Vina angularis var. *angularis* 1) *Phaseolus angularis* 2) Azuki bean 3) China, Korea and Japan	var. *nipponensis* 4) Himalaya, N. Burma, China, Taiwan and Japan
Vigna mungo var. *mungo* 1) *Phaseolus mungo* 2) Black gram, Black matpe, Urd 3) Pakistan, India and Burma	var. *silvestris* 4) India and Burma
Vigna radiata var. *radiata* 1) *Phaseolus radiatus, Phaseolus aureus* 2) Mungbean, Green gram 3) Tropics and subtropics in the old world	var. *sublobata* 1) *Phaseolus sublobatus* 4) E. and W. tropical Africa, India, Burma, Thailand, Indo-China, W. China, Taiwan, Malaysia, New Guinea and Australia
Vigna umbellata 1) *Phaseolus calcaratus, Phaseolus ricciardianus* 2) Rice bean 3) Subtropics and warm temperates in the old world	var. *gracilis* 4) E. India, Thailand, Indo-China and China

1) Synonyms ; 2) English names ; 3) Area of traditional cultivation ; 4) Natural distribution.

summarized in Table 5. Moth bean shows a high level of tolerance to drought and is grown mainly in dry areas in India and Pakistan. Azuki bean is principally cultivated in Japan, Korea, and North China. Black gram is one of the most important crops in India for producing "dhal", but it is not popular in other parts of Asia. Mungbean is the most common pulse among these five *Ceratotropis* cultigens. It is grown throughout Asia. Rice bean is commonly cultivated in Asia and Pacific on a small scale.

As discussed in Chapter 1, it is considered that species belonging to the subgenus *Ceratotropis* form the primary gene pool for the *Ceratotropis* cultigens (Ahn and Hartmann 1978, Chen et al. 1983, Miyazaki et al. 1984, Egawa et al. 1990a, Siriwardahane et al. 1991 Tomooka et al. 1991c). However, very few samples of wild germplasm of the subgenus *Ceratotropis* have been collected so far.

Considering the genetic erosion of wild species occurring rapidly due to the promotion of agriculture, deforestation and urban development, it is urgent to collect and evaluate the wild species within the subgenus *Ceratotropis*. For this purpose, it is essential to recognize the taxonomic characteristics of the subgenus *Ceratotropis*. Therefore, the key characters and the taxonomic position of the subgenus *Ceratotropis* were reviewed in the present section.

Subtribe level classification

Subgenus *Ceratotropis* belongs to the subtribe Phaseoliinae, tribe Phaseoleae, subfamily Fabaceae of the family Leguminosae. Up to this point, about 500 species in 33 genera are known in the subtribe Phaseoliinae. This subtribe is considered to have reached a high evolutionary level among the leguminous plants, because the flower parts show elaborate morphological characteristics (Ohashi 1979). Characters by which this subtribe can be distinguished from the other leguminous plants are as follows.

(1) Two callosities or one callosity are attached to the inner surface of the standard.
(2) Two keels are often united together at the upper and lower hem portions.
(3) Style is bearded on the inner side.

Genus level classification

Subgenus *Ceratotropis* belongs to the genus *Vigna* of the subtribe Phaseoliinae. The genus *Vigna*, together with the closely related genus *Phaseolus*, forms a very complicated taxonomic group, so called *Phaseolus-Vigna* complex. The taxonomical treatment of mungbean, black gram and their close relatives (which are now classified as the subgenus *Ceratotropis* in the genus *Vigna*) has also been confusing for this reason. History of taxonomical treatment of this group is summarized in Table 6.

Mungbean, black gram and their close relatives had been classified into the genus *Phaseolus* since the publication of De Candolle (De Candolle 1825). In 1953, Ohwi proposed a new genus *Azukia* for this group. Maekawk (1955) divided this group into two genera, i.e., *Azukia* and *Rudua*. Verdcourt (1970) proposed a very restricted concept of *Phaseolus*, limiting it exclusively to those Americal species with a tightly coiled style and pollen grains lacking coarse reticulation, hence, promoting significantly the concept of *Vigna* containing *Ceratotropis* as a subgenus. According to his proposal, mungbean, black gram and their close relatives were transferred to the genus *Vigna*. This proposal was supported by many taxonomists. Maréchal et al.

(1978) followed Verdcourt and presented a monograph on the *Phaseolus-Vigna* complex. They considered two evolutionary tendencies in Old World *Vigna* which can be summarized as follows.

(1) Specialization of the floral morphology which led to the homogeneous subgenus *Ceratotropis* in Asia.
(2) Simplification of the floral morphology which led to the species-rich subgenus *Vigna* in Africa.

Table 6. History of taxonomical treatment of the subgenus *Ceratotropis* (after Tateishi and Ohashi 1990)

References	Genus	*Ceratotropis*	Genus
De Candolle (1825) Bentham (1837, 1865) Baker (1876)	*Phaseolus*	(Strophostyles)	*Vigna*
Piper (1914, 1926)	*Phaseolus*	(Ceratotropis)	*Vigna*
Ohwi (1953)	*Phaseolus*	*Azukia*	*Vigna*
Maekawa (1955)	*Phaseolus*	*Azukia* & *Rudua*	*Vigna*
Verdcourt (1970) Maréchal et al. (1978)	*Phaseolus*	(*Ceratotropis*)	*Vigna*

Names in parenthesis refer to the subgenus or section of the genus in the same shade.

According to the monograph presented by Maréchal et al. (1978), useful discriminant characters between the genus *Vigna* (esp. subgenus *Ceratotropis*) and the genus *Phaseolus* are as follows (Table 7 and Fig. 7).

(1) Stipule : In the genus *Phaseolus*, the stipule is attached to the stem by its basal part (Fig. 7 : S-b 0) and does not spread underneath the attachment point. By contrast, in the genus *Vigna* especially in the subgenus *Ceratotropis*, the stipule is attached to the stem by its central part (Fig. 7 : S-b 3).
(2) Knob : Knobs are reduced from the inflorescence-branch in the genus *Vigna* (Fig. 7 : kb+), but knobs are not seen in the genus *Phaseolus* (Fig. 7 : kb-).
(3) Stigma : In the subgenus *Phaseolus*, stigma is positioned to the end of the style (Fig. 7 : stigma terminal, Bk-0). In the genus *Vigna*, the tip of the style sometimes elongates to some extent to form a style beak, so that the stigma is situated on a somewhat lateral part of the style (Fig. 7 : stigma lateral, Bk-1 or Bk-2). This feature is especially obvious in the subgenus *Ceratotropis* (Bk-2).

Seven subgenera of the genus *Vigna*

Maréchal et al. (1978) have shown that the genus *Vigna* can be subdivided into seven subgenera, i.e., *Vigna, Haydonia, Plectotropis, Ceratotropis, Lasiocarpa, Sigmoidotropis* and *Macroryncha* (Fig. 8).

The large subgenus *Sigmoidotropis* of the genus *Vigna* includes five different

Table 7. Comparison of *Phaseolus, Vigna, Plectotropis* and *Ceratotropis* (revised from Ohashi 1979)

	Genus *Phaseolus*	Genus *Vigna*	Subgenus *Plectotropis*	Subgenus *Ceratotropis*
Disstibution	America	Africa, Asia, America	Africa, Asia	Asia
No. of species	50	120	7	16
Stipule[1]	0	0, 1, 2, 3	2, 3	3
Knob[2]	−	+	+	+
Keel[3]	4	1, 2, 3, 4	3	3
Style[4]	6	1, 2, 3, 4, 5	1	1
Style-beak[5]	0	0, 1, 2	1	2
Keel pocket[6]	a	a	s	s, l
Stigma[7]	t	t, l	l	l
Standard[8]	+	+, −	−	−

1 : Stipule base as illustrated in Fig. 7.
2 : Knob reduced from inflorescence-branch, + : present, − : absent (see Fig. 7).
3 : Keel : Shape of keel petals (see Fig. 7).
4 : Style : Shape of style (see Fig. 7).
5 : Style-beak : Shape of style tip (see Fig. 7).
6 : Keel pocket : absent (a), short (s), long (l). (see Fig.7)
7 : Stigma : Position of stigma : stigma terminal (t), stigma lateral (l).
8 : Standard asymmetry : + : asymmetry, − : symmetry (see Fig. 7).

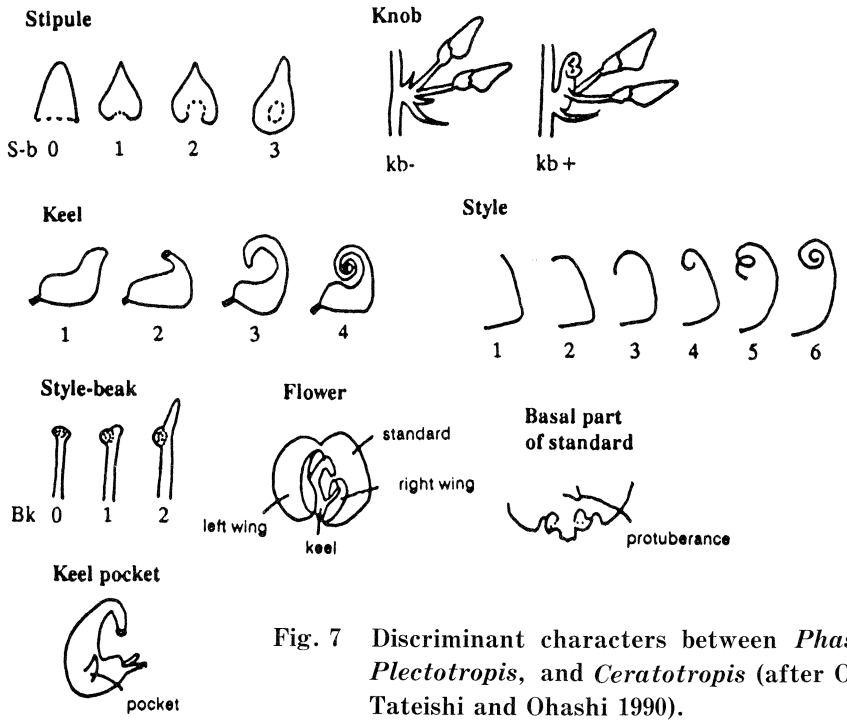

Fig. 7 Discriminant characters between *Phaseolus*, *Vigna*, *Plectotropis*, and *Ceratotropis* (after Ohashi 1979 and Tateishi and Ohashi 1990).

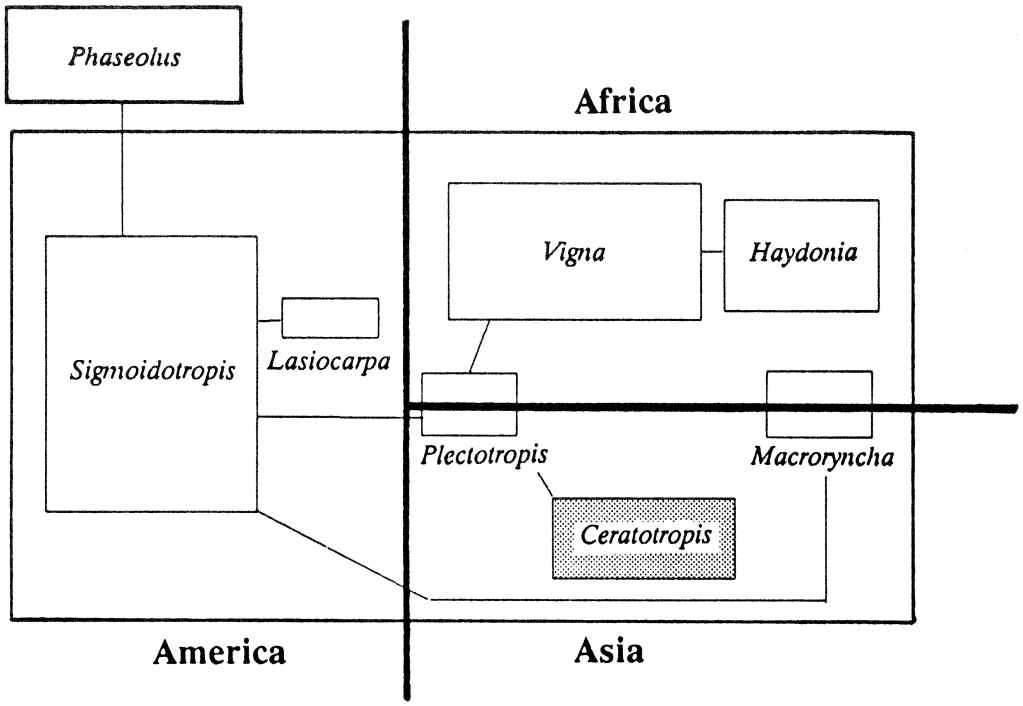

Fig. 8 Phylogenetic relationship and geographical distribution of seven subgenera in the genus *Vigna* and *Phaseolus* (after Maréchal et al. 1978).

Lines between each group indicate a close relationship
Size of the box represents the number of species belonging to the group

sections : *Sigmoidotropis, Caracallae, Pedunculares, Condylostylis* and *Leptospron*. All these sections have, at different degrees, accumulated only few of the typical *Vigna* characteristics, and the subgenus is thus considered as a rather primitive group. The geographical distribution of this group is confined to the American continent. Still confined to the neotropical regions, the subgenus *Lasiocarpa* shows most of the *Vigna* characteristics.

In the old world, the subgenus *Plectotropis* shows an intermediate position between the African and Asian *Vigna*. The Asian *Vigna*, the subgenus *Ceratotropis* is a very homogeneous group showing a high degree of specialization in floral morphology. The very rich African subgenus *Vigna* characterized by bilateral floral symmetry is divided into six sections : *Vigna, Comosae, Macrodontae, Reticulatae, Liebrechtsia* and *Catiang*. More or less closely related to the last, the subgenus *Haydonia* appears as a relatively recent evolutionary trend expressed by the loss of some typical *Vigna* characteristics, in particular pollen characters, and acquisition of some new ones.

Relationship between *Macroryncha* subgenus and typical *Vigna* appears more remote, and its maintenance in the genus *Vigna* is temporarily justified for reasons of convenience. Above-mentioned phylogenetic relationships and geographical distribution of seven subgenera in the genus *Vigna* together with the genus *Phaseolus* are schematically represented in Fig. 8.

Morphological characteristics of the subgenus *Ceratotropis*

The *Ceratotropis* subgenus is a homogeneous and specialized group of Asian origin. All the typical *Vigna* characters are represented with a high degree of expression. The flowers always show various shades of yellow color, but are never purple, violet, blue or white as is often found in other groups of *Vigna* (Baudoin and Maréchal 1988). The morphological characteristics so far known are summarized as follows (Tateishi and Ohashi 1990).

(1) peltate stipule (Fig. 7 : S-b 3)
(2) standard with a protuberance near the center of the inner surface of the lamina (Fig. 7 : Basal part of standard).
(3) keel petals incurved to the left in the upper part (Fig. 7 : Flower).
(4) horn-like appendage on the left keel petal (Fig. 7 : Keel pocket).
(5) style extending beyond the stigma as a beak (Fig. 7 : Style-beak, Bk-2).
(6) pollen grains with a coarsely reticulate sculpture.

"Mungbean group" and "Azuki group"

Maekawa (1955) suggested that *Ceratotropis* should be divided into two separate genera (*Azukia* and *Rudua*) on the basis of differences in seedling characters (Fig. 9). Species within the genus *Rudua* show epigeal germination (cotyledon appears on the ground after germination, having a primary leaf with very short petiole), while species belonging to the genus *Azukia* show hypogeal germination (cotyledon remains under-

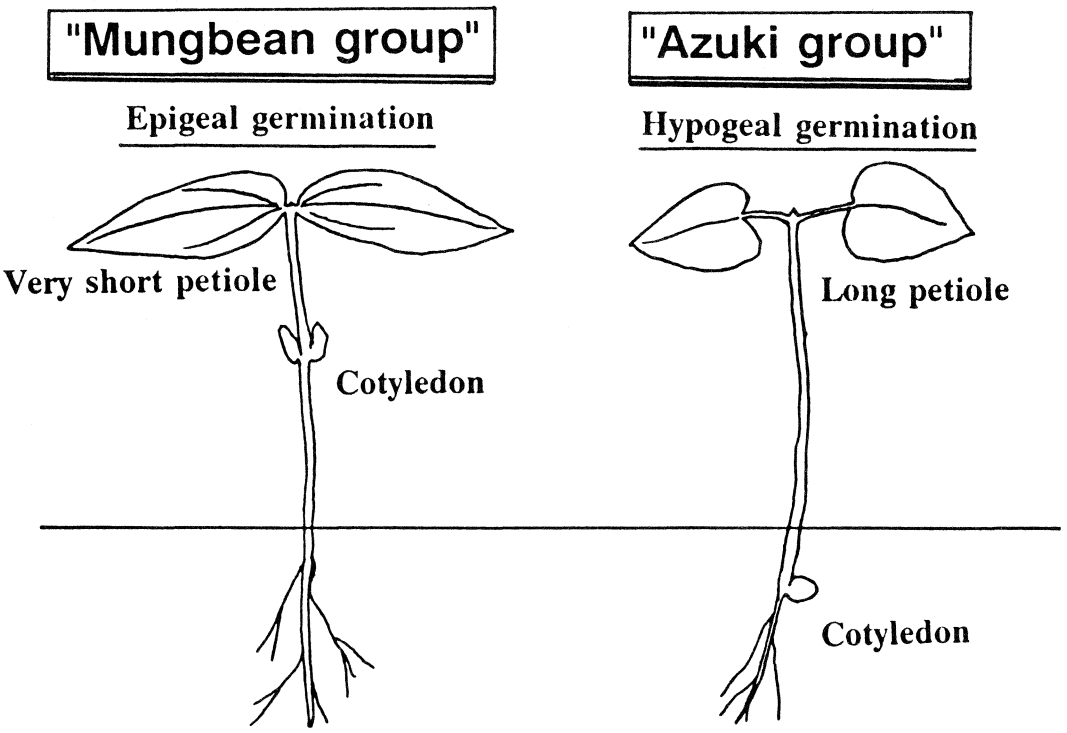

Fig. 9 Characteristics of seedling of "Mungbean group" and "Azuki group".

ground after germination, having a primary leaf with a long petiole ; hypocotyl does not elongate). Although this concept has not been supported by further studies, it is convenient to use it for the present study. The genus *Rudua* includes mungbean, blackgram and moth bean, while the genus *Azukia* contains azuki bean and rice bean as cultivated species. Therefore, the genus *Rudua* and genus *Azukia* are hereafter called "Mungbean group" and "Azuki group", respectively for reasons of convenience (Fig. 9). The species of the "Mungbean group" are distributed mainly in the Indian Subcontinent, while the species of the "Azuki group" are found principally in East Asia.

3-2. Collection and evaluation of wild *Ceratotropis* species on the Nansei Archipelago, Japan

Introduction

The wild *Ceratotropis* species which occur in Japan consist of *V. angularis* var. *nipponensis, V. nakashimae, V. riukiuensis,* and *V. reflexo-pilosa* (Ohwi 1953, Hatusima 1971). Most of these wild species are diploid species (2n=22) except for *V. reflexo-pilosa* which is a tetraploid species (2n=44). *V. angularis* var. *nipponensis*, which is considered to be an ancestor of azuki bean (*V. angularis* var . *angularis*), is distributed in Japan, Korea, Taiwan, North China, and the Himalayas. *V. nakashimae* occurs in the northern part of Kyushu island, which is located in the southern part of Japan. It is also found in Korea and North China. *V. riukiuensis* is restricted to the Yaeyama Islands, which is located in the southernmost part of Okinawa prefecture in Japan, and to Taiwan. *V. reflexo-pilosa* is found in the areas extending from Amami-Oshima Island (southern part of Kagoshima prefecture, Kyushu) through the islands of the Nansei Archipelago, South China, Southeast Asia, and Oceania (Tateishi 1984). Among these areas, the Nansei Archipelago, rich in indigenous flora but under the threat of urbanization, has not been explored systematically for the *Ceratotropis* species to identify useful gene sources for crop breeding. In the present study, an exploration of wild *Ceratotropis* was conducted on these islands.

Methods

Exploration

A total of six islands, i.e., Ishigaki, Iriomote, Yonaguni, Okinoerabu, Tokunoshima, and Tanegashima Islands, on the Nansei Archipelago was explored from 15 to 27 May, 1989 (Table 8). The Nansei Archipelago extends from the southern tip of Kyushu to Taiwan, covering a distance of about 1,200 kilometers (Fig. 10). The exploration was conducted along roadsides for the collection of the wild species belonging to the subgenus *Ceratotropis*.

The flowers of the subgenus *Ceratotropis* species showed various shades of yellow color, but are never purple, violet or white as is often found in other groups of *Vigna* (Baudoin and Maréchal 1988). Therefore, the plants with trifoliate leaves or yellow flowers were examined for their leaf and flower morphology, and classified based on the key characters proposed by Tateishi (1984). Seeds were collected wherever possible. All the accessions were planted in pots at Chainat FCRC, Thailand, and further examined for the morphological characteristics under more uniform condi-

tions.

Basically, the seed weight was determined using 100 seeds for each accession, but sometimes a smaller number of seeds was used depending on the availability of seeds. Seed length and seed thickness were measured for ten seeds with typical size per each accession. Number of seeds per pod and pod length were recorded for five pods per each accession.

Table 8. Itinerary of the exploration on the Nansei Archipelago, Japan

Date	Itinerary	Notes
15 May	Osaka = = Okinawa Island = = Ishigaki Island	
16 May	Ishigaki Island	One population of immature *V. riukiuensis* individuals was found on Ishigaki Island.
17 May	Ishigaki Island------**Iriomote Island**	Four Accessions of *V. riukiuensis* and one accession of *V. reflexo-pilosa* were collected on Iriomote Island.
18 May	Iriomote Island------Ishigaki Island = = **Yonaguni Island**	Three accessions of *V. riukiuensis* were collected on Yonaguni Island.
19 May	**Yonaguni Island**	Five accessions of *V. riukiuensis*, two accessions of *V. reflexo-pilosa* were collected.
20 May	Yonaguni Island = = Ishigaki Island	
21 May	Ishigaki Island = = Okinawa Island	
22 May	Okinawa Island------Okinoerabu Island	
23 May	**Okinoerabu Island** ------ Tokunoshima Island	One accession of *V. reflexo-pilosa* was collected on Okinoerabu Island. Two populations of immature *V. reflexo-pilosa* individuals were found on Okinoerabu Island.
24 May	**Tokunoshima Island**------	One population of immature *V. reflexo-pilosa* individuals was found.
25 May	------Kagoshima = = Tanegashima Island	
26 May	**Tanegashima Island**	
27 May	Tanegashima Island = = Osaka	

Note : = = Airplane, ------ Ship
The exploration was conducted on the islands indicated by the bold letter.

Fig. 10 Location of the Nansei Archipelago, Japan.

Tests for bruchid resistance

Two species of bruchid beetles, *Callosobruchus chinensis* and *C. maculatus* were collected at the mungbean seed storage house of Chainat FCRC. The insects were mass-reared in a petri dish (9cm in diameter) using cultivated mungbean seeds under laboratory conditions.

For the resistance test, ten seeds were placed in a petri dish (9cm in diameter) with two replications. The test seeds were infested with two pairs of freshly emerged bruchid adults and kept in an incubator which was maintained at 27℃ and 70% relative humidity. The adults were allowed to mate and lay eggs on the seeds for 2 days. The parental insects were then removed from the petri dish. Five days after infestation (DAI), when the eggs became distinctly visible, the number of eggs laid on seeds was counted. The petri dish was then placed in an incubator until the onset of adult emergence. When adults started emerging, the number of adults which emerged was counted daily until 50 DAI. The counting was discontinued thereafter to avoid double count of adults of the second generation. The counted adults were removed from the petri dish each day.

Results

Exploration

Twelve accessions of *V. riukiuensis* and four accessions of *V. reflexo-pilosa* were collected during the exploration (Table 9). Among the twelve accessions of *V. riukiuensis*, four were collected on Iriomote Island, and eight on Yonaguni Island. For *V. reflexo-pilosa*, one accession was collected on Iriomote Island, two on Yonaguni Island, and one on Okinoerabu Island.

Table 9. Location of sites for collection and some characteristics of *V. riukiuensis* and *V. reflexo-pilosa* collected on the Nansei Archipelago, Japan

Species	Accession name	Collection site*	100-seed weight (g)	Seed length (mm)	Seed thickness (mm)	Seeds/pod	Pod length (cm)
V. riukiuensis	I-1	Yonara, Iriomote Is.	1.2	2.4	1.5	5.6	3.6
	I-2	Yonara, Iriomote Is.	1.1	2.8	2.0	5.2	3.3
	I-3	Sonai, Iriomote Is.	1.2	2.9	2.2	6.6	3.4
	I-5	Near Yonara bridge, Iriomote Is.	1.1	2.7	1.9	7.4	4.0
	Y-1	1km W of Sonai, Yonaguni Is.	1.0	2.9	2.1	4.8	3.2
	Y-2	4km W of Sonai, Yonaguni Is.	1.4	3.0	2.4	4.8	3.1
	Y-3	16km W of Sonai, Yonaguni Is.	1.0	3.1	2.0	5.6	3.7
	Y-4-1	2km NW of Kubura, Yonaguni Is.	1.1	2.9	2.1	7.0	3.6
	Y-4-2	2km NW of Kubura, Yonaguni Is.	1.3	3.0	2.3	7.4	3.9
	Y-8-1	Azumazaki, Yonaguni Is.	1.0	3.1	2.1	6.8	4.0
	Y-8-2	Azumazaki, Yonaguni Is.	1.1	2.8	2.1	4.6	3.0
	Y-9	Sanninudai, Yonaguni Is.	1.2	2.9	2.3	-	-
Subtotal	12 accessions	Average	1.1	2.9	2.1	6.0	3.5
V. reflexo-pilosa	I-4	Near Yonara bridge, Iriomote	1.6	3.3	2.6	-	-
	Y-5	5km W of Kubura, Yonaguni Is.	1.7	3.2	2.4	5.2	3.9
	Y-7	3km N of Higawa, Yonaguni Is.	1.6	3.2	2.3	6.6	4.0
	E-1	1km E of Sumiyoshi, Okinoerabu Is.	1.4	3.1	2.4	-	-
Subtotal	4 accessions	Average	1.6	3.2	2.4	5.9	4.0
Total	16 accessions						

Collection site* : Is.=Island, E=east, NW=northwest, W=west, N=north

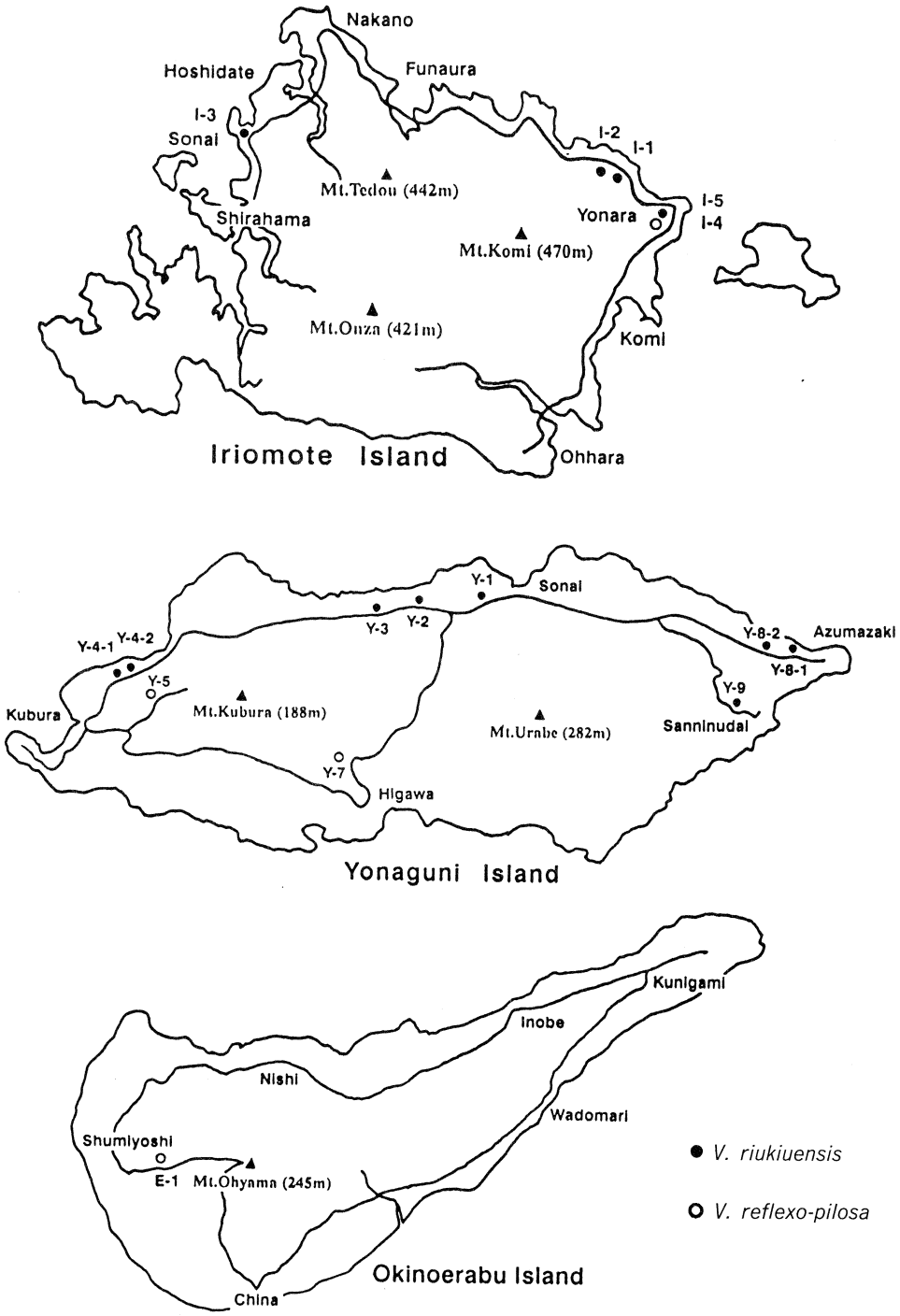

Fig. 11 Collection locality of *V. riukiuensis* and *V. reflexo-pilosa*.

One population of *V. riukiuensis* (near Kabira on Ishigaki Island) and four populations of *V. reflexo-pilosa* (two populations near the top of Mt. Ohyama on Okinoerabu Island, and two populations in the eastern part of Tokunoshima Island) were also found during the exploration (Table 8). However, since the plants did not reach the mature stage, no seeds could be collected at these sites.

On Iriomote Island, four accessions of *V. riukiuensis* and one accession of *V. reflexo-pilosa* were collected (Fig. 11). Among them, three accessions of *V. riukiuensis* (I-1, I-2, I-5, Fig. 11) and one accession of *V. reflexo-pilosa* (I-4) were collected in the area near Yonara. Especially, in the area near the Yonara bridge, a large number of plants of *V. riukiuensis* and *V. reflexo-pilosa* occurred widely at the edge of pastures along the roadside and were intermingled. In the Sonai area, *V. riukiuensis* individuals were found sporadically along the path to the small hill, which was used for reaching upland fields. The plants were twining on grasses. One accession was collected at this site (I-3).

On Yonaguni Island, eight accessions of *V. riukiuensis* and two accessions of *V. reflexo-pilosa* were collected (Fig. 11). In open and sunny areas along roadsides on the northern seacoast to the west of Sonai, three accessions of *V. riukiuensis* were collected (Y-1, Y-2, Y-3). A large population of *V. riukiuensis* was found in the area located 2km northeast from Kubura. Two accessions were collected there, one with black-colored mature pods (Y-4-1) and another with straw-colored mature pods (Y-4-2). In the eastern part of Yonaguni Island, several populations of *V. riukiuensis* were observed in the areas near Azumazaki and Sanninudai. Three accessions were collected there (Y-8-1, Y-8-2, Y-9). *V. reflexo-pilosa* was found and collected along roadsides in the areas located 5km east from Kubura (Y-5), and 3km north from Higawa (Y-7).

On Okinoerabu Island, a population of *V. reflexo-pilosa* was found at the site located 1km east from Sumiyoshi (E-1). The plants were still young, but a few mature pods could be collected at this site.

Morphological characters

Characteristics of seed and pod of *V. riukiuensis* and *V. reflexo-pilosa* are indicated in Table 9. Seed weight (100-seed weight), seed length, and thickness of *V. riukiuensis* ranged from 1.0g to 1.4g (average=1.1g), from 2.4mm to 3.1mm (average=2.9mm), and from 1.5mm to 2.4mm (average=2.1mm), respectively, while those of *V. reflexo-pilosa* ranged from 1.4g to 1.7g (average=1.6g), from 3.1mm to 3.3mm (average=3.2mm), and from 2.3mm to 2.6mm (average=2.4mm), respectively. Average number of seeds per pod was 6.0 in *V. riukiuensis*, while that in *V. reflexo-pilosa* was 5.9. Pod length of *V. riukiuensis* was shorter (average=3.5cm) than that of *V. reflexo-pilosa* (average=4.0cm). The morphological characters of the seeds of *V. riukiuensis* and *V. reflexo-pilosa* are shown in Fig. 12.

Bruchid resistance

Against the infestation by *C. chinensis*, the accessions of *V. reflexo-pilosa* exhibited significantly higher levels of resistance (Emergence (%)=15.1%, Damaged seeds (%)=18.3% on the average) compared with those of *V. riukiuensis* (Emergence (%)=45.4%, Damaged seeds (%)=61.4%) (Table 10). On the contary, the accessions of *V. riukiuensis* showed significantly higher levels of resistance to *C. maculatus* (Emergence (%)=13.4%, Damaged seeds (%)=25.5% on the average) compared with those of *V. reflexo-pilosa* (Emergence (%)=53.7%, Damaged seeds (%)=95.0%) (Table 11).

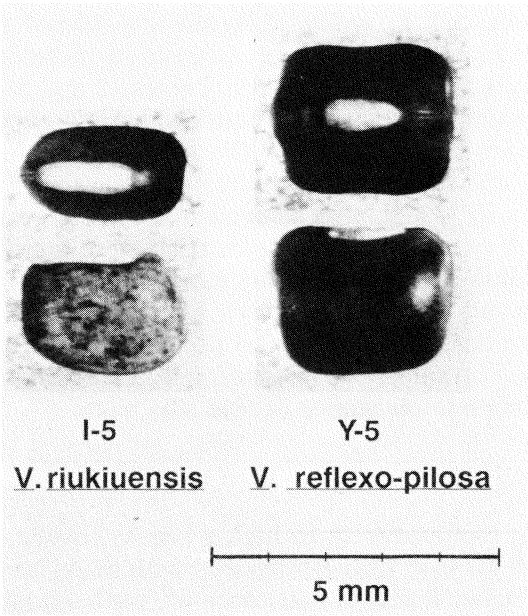

Fig. 12 Seed morphology of *V. riukiuensis* and *V. reflexo-pilosa*.

Table 10. Levels of resistance of *V. riukiuensis* and *V. reflexo-pilosa* to *C. chinensis*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. riukiuensis	I-1	1.2	13.5ab*	46.5a	55.0a	33.4ab
	I-2	1.1	11.5a	57.5a	65.0a	30.3ab
	I-3	1.2	21.5b	43.5a	85.0a	29.1a
	I-5	1.1	11.5a	39.0a	65.0a	35.7b
	Y-1	1.0	14.0ab	61.0a	75.0a	29.2a
	Y-2	1.4	16.0ab	79.0a	85.0a	29.7a
	Y-3	1.0	15.0ab	30.0a	55.0a	31.3ab
	Y-4-1	1.1	17.5ab	34.0a	60.0a	30.5ab
	Y-4-2	1.3	13.5ab	25.0a	30.0a	29.8ab
	Y-8-1	1.0	12.0a	60.0a	70.0a	28.9ab
	Y-8-2	1.1	17.0ab	23.5a	30.0a	33.1ab
	Average	1.1	14.8a**	45.4b	61.4b	31.1a
V. reflexo-pilosa	I-4	1.6	12.0a	25.0a	20.0a	30.2a
	Y-5	1.7	14.0a	10.5a	15.0a	32.0a
	Y-7	1.6	15.0a	10.0a	20.0a	29.3a
	Average	1.6	13.7a	15.1a	18.3a	30.5a

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate.
*, ** : Mean separation was performed by least significant difference at 99% level.

Since *V. riukiuensis* and *V. reflexo-pilosa* are considered to be closely related to azuki bean and rice bean, the levels of resistance of these cultivated legumes to *C. chinensis* and *C. maculatus* are indicated in Tables 12 and 13. The accessions (local varieties) of azuki bean were highly susceptible to both *C. chinensis* (Emergence (%)=56.1%, Damaged seeds (%)=100.0% on the average, Table 12) and *C. maculatus* (Emergence (%)=69.4%, Damaged seeds (%)=79.0%, Table 13). Among the seven accessions of rice bean tested, five showed a complete resistance to *C. chinensis* (Table 12). Although two accessions of rice bean were found to be susceptible to *C. chinensis*, only one adult could successfully emerge from each of these two accessions (Emergence (%)=0.5 and 1.0%, respectively). Furthermore, the emergence was significantly delayed in rice bean (Developmental period=42.5 days) compared with that in azuki bean (29.4 days). Against the infestation by *C. maculatus*, all the accessions of rice bean showed complete resistance (Table 13).

Discussion

Rice bean has been reported to show higher levels of resistance to *C. chinensis* and to AMV (Azuki bean Mosaic Virus) compared with azuki bean (Sawa and Tan 1976, Sawa et al. 1984). Higher levels of resistance of rice bean against *C. chinensis* were

Table 11. Levels of resistance of *V. riukiuensis* and *V. reflexo-pilosa* to *C. maculatus*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. riukiuensis	I-1	1.2	31.0a*	8.0a	25.0a	39.3a
	I-2	1.1	18.5a	5.5a	10.0a	38.0a
	I-3	1.2	33.0a	8.0a	30.0a	37.7a
	I-5	1.1	15.5a	19.5ab	30.0a	37.4a
	Y-1	1.0	19.0a	16.5ab	35.0ab	32.8a
	Y-2	1.4	19.0a	47.5b	75.0b	33.7a
	Y-3	1.0	20.5a	7.0a	10.0a	39.5a
	Y-4-1	1.1	16.0a	3.5a	5.0a	43.0a
	Y-4-2	1.3	23.5a	4.0a	5.0a	31.0a
	Y-8-1	1.0	21.5a	17.5ab	40.0ab	31.8a
	Y-8-2	1.1	19.0a	10.5a	15.0a	38.0a
	Average	1.1	21.5a**	13.4a	25.5a	36.5a
V. reflexo-pilosa	I-4	1.6	19.0a	47.5a	90.0a	31.2a
	Y-5	1.7	16.5a	58.0a	100.0a	30.3a
	Y-7	1.6	18.0a	55.5a	95.0a	31.2a
	Average	1.6	17.8a	53.7b	95.0b	30.9a

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

*,** : Mean separation was performed by least significant difference at 99% level.

confirmed, and complete immunity of rice bean against *C. maculatus* was revealed in the present study. Hybrids between azuki bean and rice bean are very difficult to produce (Rashid et al. 1988), and therefore attempts to incorporate useful genes of rice bean into azuki bean have not been successful. Using the materials collected in this exploration, it was revealed that *V. riukiuensis* was cross-compatible with both azuki bean (*V. angularis*) and rice bean (*V. umbellata*) when crossed as a pollen parent (Siriwardhane et al. 1991). Therefore, it was suggested that *V. riukiuensis* could act as a bridge species between azuki bean and rice bean. It may be possible to incorporate useful genes from rice bean to azuki bean through *V. riukiuensis*. Since *V. riukiuensis* showed higher levels of resistance to *C. maculatus* (Emergence (%)=13.4%, Damaged seeds (%)=25.5%, Table 11) compared to azuki bean (Emergence (%)=69.4%, Damaged seeds (%)=79.0%, Table 13), *V. riukiuensis* itself will also be useful for improving the level of resistance of azuki bean against *C. maculatus*.

Table 12. Levels of resistance of azuki bean (*V. angularis*) and rice bean (*V. umbellata*) to *C. chinensis*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. angularis	Kyoto dainagon	22.4	37.5b*	66.0abc	100.0a	28.7a
	Omuta	18.5	32.0b	55.0abc	100.0a	28.7a
	101	7.8	43.5bc	49.5abc	100.0a	28.5a
	102	6.8	34.0b	34.5a	100.0a	29.7a
	104	7.7	31.5ab	48.0abc	100.0a	30.3a
	105	8.6	30.5ab	54.5abc	100.0a	29.2a
	108	9.8	14.0a	78.5c	100.0a	30.9a
	118	10.6	61.0c	46.5ab	100.0a	29.2a
	119	11.1	39.0b	55.0abc	100.0a	29.4a
	126	9.8	28.0ab	73.0bc	100.0a	29.4a
	Average	11.3	35.1a**	56.1b	100.0b	29.4a
V. umbellata	Chainat	9.3	51.5a	0.0a	0.0a	
	070001	4.7	57.0a	0.0a	0.0a	
	220001	8.5	67.5a	0.0a	0.0a	
	220002	7.3	66.0a	0.5a	5.0a	42.0
	220003	6.4	56.0a	1.0a	5.0a	43.0
	220004	7.5	48.0a	0.0a	0.0a	
	220005	6.5	68.0a	0.0a	0.0a	
	Average	7.2	59.1b	0.2a	1.4a	42.5b

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

*,** : Mean separation was performed by least significant difference at 99% level.

Table 13. Levels of resistance of azuki bean (*V. angularis*) and rice bean (*V. umbellata*) to *C. maculatus*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
V. angularis	Kyoto dainagon	22.4	24.5abc*	69.0ab	45.0a	36.0a
	Omuta	18.5	32.5abcd	100.0b	100.0a	35.2a
	101	7.8	20.0a	46.0ab	50.0a	39.6a
	102	6.8	43.0bcd	54.5ab	80.0a	38.3a
	104	7.7	35.0abcd	46.5ab	80.0a	36.2a
	105	8.6	23.0ab	26.5a	70.0a	36.9a
	108	9.8	31.5abcd	94.0b	65.0a	34.6a
	118	10.6	15.5a	91.5b	100.0a	32.5a
	119	11.1	45.5cd	70.5ab	100.0a	35.4a
	126	9.8	47.5d	95.5b	100.0a	33.2a
	Average	11.3	31.8a**	69.4b	79.0b	35.8
V. umbellata	Chainat	9.3	53.5a	0.0a	0.0a	-
	070001	4.7	58.0a	0.0a	0.0a	-
	220001	8.5	58.5a	0.0a	0.0a	-
	220002	7.3	41.0a	0.0a	0.0a	-
	220003	6.4	54.5a	0.0a	0.0a	-
	220004	7.5	45.5a	0.0a	0.0a	-
	220005	6.5	43.5a	0.0a	0.0a	-
	Average	7.2	50.6b	0.0a	0.0a	-

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

*,** : Mean separation was performed by least significant difference at 99% level.

It was clarified that *V. reflexo-pilosa* was cross-compatible with *V. glabrescens* (Egawa et al. 1990b). These two species readily produced fertile hybrids when cross-pollinated with each other. Judging from the morphological similarities of seeds and primary leaves, the same ploidy level (2n=44, 4x), and high level of cross-compatibility, *V. reflexo-pilosa* seemed to be closely related to *V. glabrescens*. *V. glabrescens* exhibits pest and disease resistance, and has been used in the mungbean improvement program at AVRDC in Taiwan (Fernandez and Shanmugasundaram 1988). However, only one accession (V 1160) of *V. glabrescens* is currently available. Considering the close relationship between *V. glabrescens* and *V. reflexo-pilosa*, it is anticipated that *V. reflexo-pilosa* also harbours good genes for pest and disease resistance for the breeding of *Ceratotropis* cultigens.

V. reflexo-pilosa has been known to occur only in Taiwan and the Ryukyus (Okinawa prefecture, the southernmost islands in Japan). According to Tateishi (1984), however, many specimens from South China (Hainan), Thailand, the Philippines,

Indonesia (Sumatra, Java, Timor), New Guinea, Australia, New Caledonia, New Hebrides and Fiji, which have been identified as *V. mungo*, *V. radiata* var. *sublobata*, or *Phaseolus calcaratus* (= *V. umbellata*) may correspond to *V. reflexo-pilosa*. If so, a still wider range of variations can be expected to occur in *V. reflexo-pilosa*.

3-3. Collection and evaluation of wild *Ceratotropis* species in Northern Thailand

Introduction

The subgenus *Ceratotropis* is considered to have originated in Asia and is called Asian *Vigna*. As discussed in Chapter 3-1, the subgenus *Ceratotropis* can be subdivided into two groups based on the seedling characters, i.e., (1) "Mungbean group" showing an epigeal germination and (2) "Azuki group" showing a hypogeal germination (Fig. 9). Since the natural distribution of the "Mungbean group" and "Azuki group" overlaps in Southeast Asia, Thailand is considered to be a good location for collecting wild species of the subgenus *Ceratotropis* (Tomooka et al. 1991a). Against this background, and exploration for the collection of wild *Ceratotropis* species was conducted in Northern Thailand.

Methods

Exploration in Northern Thailand was carried out along roadsides for the collection of wild species of the subgenus *Ceratotropis*. The exploration was conducted from 19 to 25 November 1989, covering Chainat, Nakhon Sawan, Phisanulok, Uttaradit,

Table 14. Itinerary of the exploration in Northern Thailand

Date	Itinerary	Notes
19 Nov.	Chainat------Nakhon Sawan------Phisanulok	One accession of Species A and four accessions of Species B were collected.
20 Nov.	Phisanulok------Uttaradit------Phrae------Nan	Two accessions of Species B were collected.
21 Nov.	Nan------Phayao------Chiang Rai	Five accessions of Species B were collected.
22 Nov.	Chiang Rai------Chiang Mai	One accession of Species C and one accession of Species D were collected.
23 Nov.	Around Chiang Mai	
24 Nov.	Chiang Mai------Lumphun------Lampang------Tak	
25 Nov.	Tak------Kamphaeng Phet------Nakhon Sawan------Chainat	Two accessions of Species B were collected.

Phrae, Nan, Phayao, Chiang Rai, Chiang Mai, Lamphun, Lampang, Tak, and Kamphaeng Phet provinces (Table 14).

Seeds were collected wherever available. All the accessions collected were planted in pots on 27 November 1989 at Chainat Field Crops Research Center (Chainat FCRC), and the morphological characteristics were observed.

Basically, the seed weight was determined using 100 seeds for each accession, but sometimes a smaller number of seeds was used depending on the availability of seeds. Seed length and seed thickness were measured for ten seeds with typical size per each accession. Number of seeds per pod and pod length were recorded for five pods per each accession.

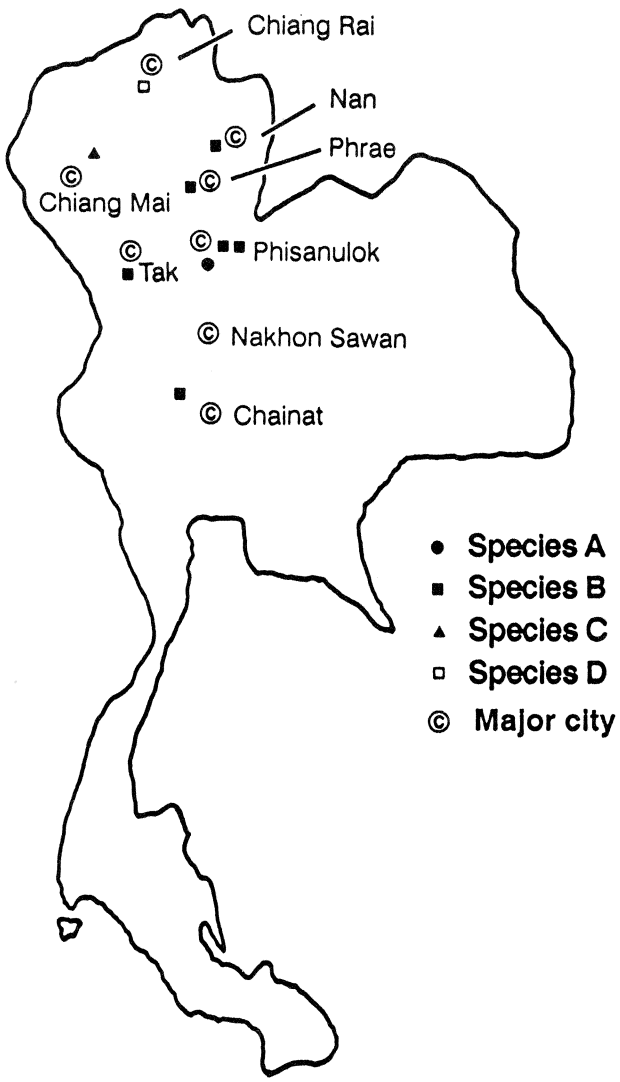

Fig. 13 Location of sites for collection locality of four wild *Ceratotropis* species in Northern Thailand.

Results and Discussion

Exploration

A total of sixteen accessions consisting of four wild species of *Ceratotropis* was collected at nine different sites during the exploration (Fig. 13, Table 15). Details of the collection sites are given in Table 16. Four species collected during the exploration were tentatively designated as Species A, Species B, Species C, and Species D. One accession of Species A was collected on the roadside near paddy fields at Amphur Ban Rakam in Phisanulok Province. A total of 13 accessions of Species B was collected at six different sites, i.e. Phisanulok (two sites), Phrae, Nan, Tak and Chainat Provinces. A population of Species B was also found at Amphur Samoeng in Chiang Mai province. However, since the plants did not reach the mature stage, no seeds could be collected at this site. One accession of Species C was collected along the road between Chiang Rai city and Chiang Mai city. The plants were found at shady sites near a mountain stream. One accession of Species D was found on sunny ground, located at about 6km south on the road from Chiang Rai city. Judging from the germination characteristics, Species A belonged to the "Mungbean group", while the other three species (Species B, Species C and Species D) belonged to the "Azuki group".

Table 15. Number of accessions and germination characters of wild *Ceratotropis* species collected in Northern Thailand

Species	Provinces	No. of Accessions	Germination Type	Remarks
Species A	Phisanulok	1	Epigeal	close to *V. radiata* and *V. mungo*
Species B	Phisanulok	4	Hypogeal	*V. umbellata* var. *gracilis*
	Phrae	2	Hypogeal	
	Nan	5	Hypogeal	
	Tak	1	Hypogeal	
	Chainat	1	Hypogeal	
Species C	Chiang Mai	1	Hypogeal	close to *V. minima*
Species D	Chiang Rai	1	Hypogeal	close to *V. reflexo-pilosa*, but diploid
		16		

Analysis of the species collected

Species A

The morphology of the primary leaf of Species A resembled that of *V. radiata* and *V. mungo*. The leaflet was sometimes lobate which was a characteristic of *V. radiata* var. *sublobata*, a wild ancestral form of *V. radiata*. However, the hilum of the seed (Fig. 14) was longer than that of *V. radiata* var. *sublobata* collected in India. Seed weight (100-seed weight) was 1.4g (Table 16), which was in the range of that in *V. radiata* var. *sublobata* (1.0-1.7g, Tomooka et al. 1991c). Seed color was dark brown with dull seed luster. Species A showed a short (3.4cm), hairy, erect pod. According

Table 16. Location of sites for collection and some characteristics of wild *Ceratotropis* species collected in Northern Thailand

Species	Collection site*	Accession name	100-seed weight (g)	Seed length (mm)	Seed thickness (mm)	Seed color	Seeds /pod	Pod length (cm)	Pod color
A	C. Phisanulok, A. Ban Rakam	(1)	1.4	3.6	2.6	black, dull	8.8	3.4	dark brown
B	C. Phisanulok, 48km E of A. Wang Tong	(2)-1	2.7	4.5	2.2	gray, dense black mottles	8.6	5.6	dark gray
		(2)-2	2.3	4.5	2.2	yellow, glossy	9.2	6.3	dark gray
	C. Phisanulok, 26km E of A. Wang Tong	(3)-1	1.9	4.4	2.1	gray, dense black mottles	10.0	5.5	light gray
		(3)-2	1.8	4.2	2.0	gray, dense black mottles			
	C. Phrae, Ban Nam Ram, 6km S of A. Denchai	(5)-1	2.2	4.2	2.2	yellow, glossy	10.8	5.5	dark gray
		(5)-2	2.3	5.0	2.3	gray, light black mottles	10.8	6.3	light gray
	C. Nan, Fai Gae, 6km W of Nan city	(6)-1-1	2.3	4.7	2.2	black, glossy	8.4	5.1	light gray
		(6)-1-2	2.4	4.6	2.3	gray, dense black mottles	8.8	4.8	dark gray
		(6)-2	2.2	4.3	2.1	gray, dense black mottles	7.6	4.8	dark gray
		(6)-3-1	1.8	4.7	2.0	black, glossy	-	-	gray
		(6)-3-2	2.0	4.5	2.1	gray, dense black mottles	6.8	3.9	gray
	C. Tak, 13km S of Tak city	(11)	2.1	4.6	2.2	gray, dense black mottles	8.6	6.0	light gray
	C. Chainat, Don Ken Luang, 5km W of A. Wat Sing	(10)	1.8	4.5	2.0	gray or dark red, dense black mottles	8.4	5.9	light gray
C	C. Chiang Mai, Wieng Doi, 15km S of Mae Kajan	(9)	0.9	2.8	1.7	gray, dense black mottles	16.0	5.9	dark gray
D	C. Chiang Rai, Tambon Sansai, 6km S of Chiang Rai city	(8)	0.7	2.3	1.6	gray, dense black mottles	11.2	3.6	gray

* : C.=Changwat (in Thai)=Province

: A.=Amphur (in Thai)=Administrative divisions in the Province

: E=east, S=south, W=west

to Maréchal et al. (1978), var. *sublobata* is widely distributed from the coastal regions of East Africa and Madagascar throughout tropical Asia, Southeast Asia, Indonesia and northern Australia. They also described another wild variety of *V. radiata* named *V. radiata* var. *setulosa*. Its distribution was more limited to the eastern part of tropical Asia, from India to Indonesia and southern China. In var. *setulosa*, the nearly orbicular-shaped stipule is wider and the stems are covered with dense hair compared to var. *sublobata*. These traits were consistent with the observation of Species A grown in Chaimnat FCRC. However, comparison of the morphology between Species A and the specimen of var. *setulosa* has not been conducted. Therefore, Species A needs to be studied taxonomically in more detail.

Species B

Species B showed very similar morphological traits to those of cultivated rice bean

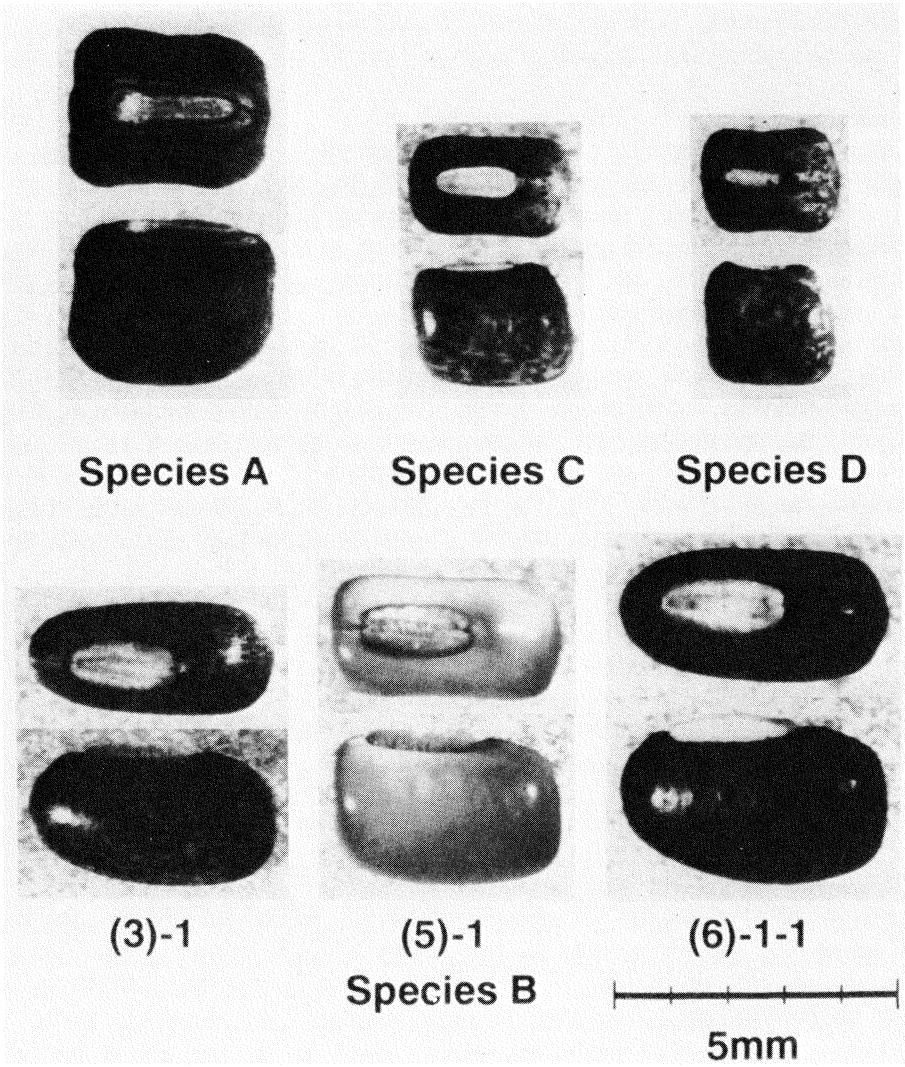

Fig. 14 Seed morphology of four wild *Ceratotropis* species.

(*V. umbellata*), i.e. lanceolate primary leaf with long petiole, multi-flowered inflorescence, drooping pods, cylinder-shaped smooth seeds and protruding hilum. However, the plants were smaller and lanker in Species B compared with *V. umbellata*. Seed weight (100-seed weight) of Species B ranged from 1.8 to 2.7g (Table 16), while that of cultivated *V. umbellata* collected in Chiang Rai province was 11.4g. These characters indicate that Species B is most probably a wild form of cultivated rice bean, *V. umbellata* var. *gracilis*. Seed color of Species B showed a range of variations from yellow, gray mottled with black, and black. The seeds showed a glossy luster. Seed color of cultivated *V. umbellata* also showed nearly the same range of variations. However, reddish brown seed testa type, which was sporadically cultivated in Northern Thailand, was not found in Species B. The distribution of *V. umbellata* var. *gracilis* is centered in the Indo-Chinese peninsula, covering most of Southeast Asia and southern China (Maréchal et al. 1978). Maréchal et al. (1978) also described *V. minima* and *V. dalzelliana*, which resemble *V. umbellata* var. *gracilis*. The discriminant character between var. *gracilis* and these two species is the presence of drooping pods in var. *gracilis* while the presence of flattened pods in *V. minima* and *V. dalzelliana*.

Species C

Tha plant type of Species C was similar to that of Species B. However, the seed size of Species C was smaller than that of Species B (Fig. 14). The seed weight (100-seed weight) of Species C was 0.9g, while that of Species B ranged from 1.8 to 2.7g (Table 16). Moreover, the hilum did not protrude as much as in Species B (Fig. 14). Maréchal et al. (1978) reported that the plant type of *V. minima* and *V. dalzelliana* was very similar to that of *V. umbellata* var. *gracilis*.

On the other hand, the morphology of the seed of Species C was quite similar to that of the seed of *V. riukiuensis* collected in Okinawa prefecture, Japan (Fig. 12). Tateishi (1984) suggested that *V. riukiuensis* should be reassigned and designated as *V. minima* var. *minor*. He also described *V. minima* var. *minima*, and reported that the number of ovules (seeds) of *V. minima* var. *minor* ranged from 7 to 9, while that of *V. minima* var. *minima* ranged from 9 to 12. On the contrary, the number of seeds of Species C was 16 (Table 16). Nevertheless, judging from the morphology of the seed, Species C was assigned to *V. minima* in the present paper.

Species D

Species D had a very lank twining stem, and its short-cylindrical seed was smaller than that of the other wild species collected during this exploration (Fig. 14, Table 16). The hilum of the seed did not protrude. The morphology of the seed of Species D was very similar to that of *V. glabrescens* (supplied from AVRDC, V1160) and of *V. reflexo-pilosa* (collected on Yonaguni, Iriomote and Okinoerabu Islands, Japan Fig. 12), but the seed size was far smaller in Species D (100-seed weight=0.7g) compared to *V. glabresces* (100-seed weight=4.1g) and *V. reflexo-pilosa* (100-seed weight=from 1.4 to 1.7g).

V. glabrescens is a tetraploid species (2n=44), and therefore, it shows a vigorous growth and has a great potential for high yield of good quality fodder. In fact, *V. glabrescens* was cultivated under the name of "Lentille de Créole" in Mauritius, and as a forage crop in Haringhata, West Bengal (Baudoin and Maréchal 1988). It also exhibits pest and disease resistance, and is used in the mungbean improvement program at AVRDC in Taiwan (Fernandez and Shanmugasundaram 1988). *V. reflexo-pilosa* is a wild tetraploid species closely related to *V. glabrescens*.

Based on the seed morphology, Species D was first designated as *V. reflexo-pilosa*. However, the observation of the chromosome number revealed that Species D was diploid (2n=22), which precludes its designation as *V. reflexo-pilosa*. Egawa et al. (1988) analyzed the genome constitution of tetraploid *V. glabrescens* and concluded that either *V. angularis*, *V. umbellata* or the species which have a homologous genome, donated one of the two genomes of *V. glabrescens*. Other donor species for the two genomes of *V. glabrescens* have not been identified yet. It was considered that Species D is one of the most probable candidates as genome donor to *V. glabrescens*, because of the similarity of the morphology. For the identification of Species D, further taxonomic studies are required.

Bruchid resistance

Levels of resistance of four wild subgenus *Ceratotropis* species collected in the exploration against *C. chinensis* and *C. maculatus* are indicated in Table 17 and 18, respectively. Accessions of Species A and Species C were found to be susceptible to both *C. chinensis* and *C. maculatus*. However, the larval developmental period on Species C (38.7 days for *C. chinensis* and 41.5 days for *C. maculatus*) was significantly longer compared with that on Species A (27.4 days for *C. chinensis* and 28.6 days for *C. maculatus*.)

Against *C. chinensis*, seven accessions of Species B showed a complete immunity among the eight accessions tested. Although one accession of Species B was infested by *C. chinensis*, the percentage of adult emergence was very low (3.0%, only one adult could emerge, Table 17). On the other hand, all the eight accessions of Species B

Table 17. Levels of resistance of wild *Ceratotropis* species to *C. chinensis*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
Species A	(1)	1.4	23.0a*	26.0a	60.0b	27.4a
Species B	(2)-1	2.7	16.0ab**	0.0a	0.0a	-
	(3)-1	1.9	17.0ab	3.0a	5.0a	35
	(5)-1	2.2	15.5ab	0.0a	0.0a	-
	(5)-2	2.3	21.5b	0.0a	0.0a	-
	(6)-1-1	2.3	21.0b	0.0a	0.0a	-
	(6)-1-2	2.4	12.0a	0.0a	0.0a	-
	(6)-2	2.2	18.5ab	0.0a	0.0a	-
	(6)-3-2	2.0	10.5a	0.0a	0.0a	-
	Average	2.3	16.5a	0.4a	0.6a	-
Species C	(9)	0.9	12.0a	34.5b	45.0b	38.7b
Species D	(8)	0.7	14.0a	0.0a	0.0a	-

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

*,** : Mean separation was performed by least significant difference at 99% level.

Table 18. Levels of resistance of wild *Ceratotropis* species to *C. maculatus*.

Legume species	Accession name	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
Species A	(1)	1.4	26.5a*	28.0a	70.0b	28.6a
Species B	(2)-1	2.7	23.5ab**	0.0a	0.0a	-
	(3)-1	1.9	22.0a	0.0a	0.0a	-
	(5)-1	2.2	25.5ab	0.0a	0.0a	-
	(5)-2	2.3	37.0b	0.0a	0.0a	-
	(6)-1-1	2.3	21.5a	0.0a	0.0a	-
	(6)-1-2	2.4	24.0ab	0.0a	0.0a	-
	(6)-2	2.2	20.5a	0.0a	0.0a	-
	(6)-3-2	2.0	14.0a	0.0a	0.0a	-
	Average	2.3	23.5a	0.0a	0.0a	-
Species C	(9)	0.9	7.5a	19.5a	15.0a	41.5b
Species D	(8)	0.7	17.0a	0.0a	0.0a	-

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

*,** : Mean separation was performed by least significant difference at 99% level.

showed a complete resistance to *C. maculatus*. Species D showed a complete resistance against the infestation by both *C. chinensis* and *C. maculatus*. Since Species B and Species D belong to the "Azuki group", these results strongly suggest that Species B and Species D are promising gene sources for breeding resistant azuki bean against *C. chinensis* and *C. maculatus*.

Chapter 4. Development of bruchid-resistant mungbean line using wild mungbean germplasm in Thailand

Introduction

In Thailand, two species of bruchid beetles (*Callosobruchus chinensis*, azuki bean weevil, and *C. maculatus*, cowpea weevil) were reported to cause serious damage to mungbean seeds during storage (Visarathanonth and Promsatit 1990). The initial infestation starts in the field, where the adult beetles lay eggs on green pods and the larvae bore into the pod and feed concealed within developing seeds (Southgate 1979). When such seeds are harvested and stored, the larvae continue to feed, emerge as adults, and cause secondary infestation which at times results in total destruction within a period of 3 to 4 months (Banto and Sanchez 1972).

Fujii and Miyazaki (1987) showed that one accession (TC 1966) of *V. radiata* var. *sublobata*, a wild ancestral form of mungbean, exhibited a complete resistance against *C. chinensis* in Japan. The resistance of TC 1966 was controlled by a single dominant gene (proposed gene symbol *R*, Kitamura et al. 1988). Moreover, Fujii et al. (1989) showed that TC 1966 was resistant to four species of bruchids, i.e. *C. chinensis*, *C. maculatus*, *C. phaseoli* and *Zabrotes subfasciatus* in Japan. On the other hand, Credland (1990) demonstrated that several biotypes differing in their capacity to utilize unusual hosts existed in *C. maculatus*.

Since TC 1966 is cross compatible with cultivated mungbean (Miyazaki et al. 1984), it was considered that a mungbean cultivar with good agronomic characters that would be resistant to *C. chinensis* and *C. maculatus* could be developed under the exvironmental conditions of Thailand.

In the present study, the levels of resistance of TC 1966 to *C. chinensis* and *C. maculatus* occurring at Chainat FCRC in Thailand were examined. Then the breeding procedure was described briefly, and the agronomic characters of the bruchid-resistant BC_3F_2 population were compared with those of recommended cultivars.

Materials and Methods

Mungbean cultivars and wild mungbean accessions

'Chainat 60' ('CN 60'), 'Kamphaeng Saen 1' ('KPS1') and 'Kamphaeng Saen 2' ('KPS 2') are mungbean cultivars officially released by the Government of Thailand. 'CN 60', which was used as a maternal parent in the breeding program, is the earliest mungbean cultivar (maturing in 55-60 days) bred by Chainat FCRC in 1987. Three accessions (TC 1965, TC 1966 and TC 2207) of wild mungbean (*V. radiata* var. *sublobata*) were supplied by the Asian Vegetable Research and Development Center (AVRDC).

Tests for bruchid resistance

Methods for the test of levels of resistance against *C. chinensis* and *C. maculatus* were described in Chapter 2-2. For screening resistant seeds in the course of the breeding procedure, two to five pairs of bruchid adults (*C. chinensis*) were released on the seeds in the petri dish depending on the number of test seeds. The petri dishes were kept in the incubator (27℃, 70% relative humidity). After incubation for 50 days, the number of susceptible and resistant seeds was counted. Seeds showing exit holes

for adult emergence as well as those easily crushed by the finger were designated as susceptible, and seeds showing no damage after more than 50 days were designated as resistant.

Breeding procedure

The breeding procedure to incorporate bruchid resistance from TC 1966 to 'CN 60', is illustrated in Fig. 15. 'CN 60' was used as a female parent and was crossed with TC 1966. Recurrent backcrossing using 'CN 60' as a male parent was started at the F_2 generation to improve the agronomic characters of the hybrids. After the F_2 generation, resistant seeds were selected using *C. chinensis* and planted for backcrossing.

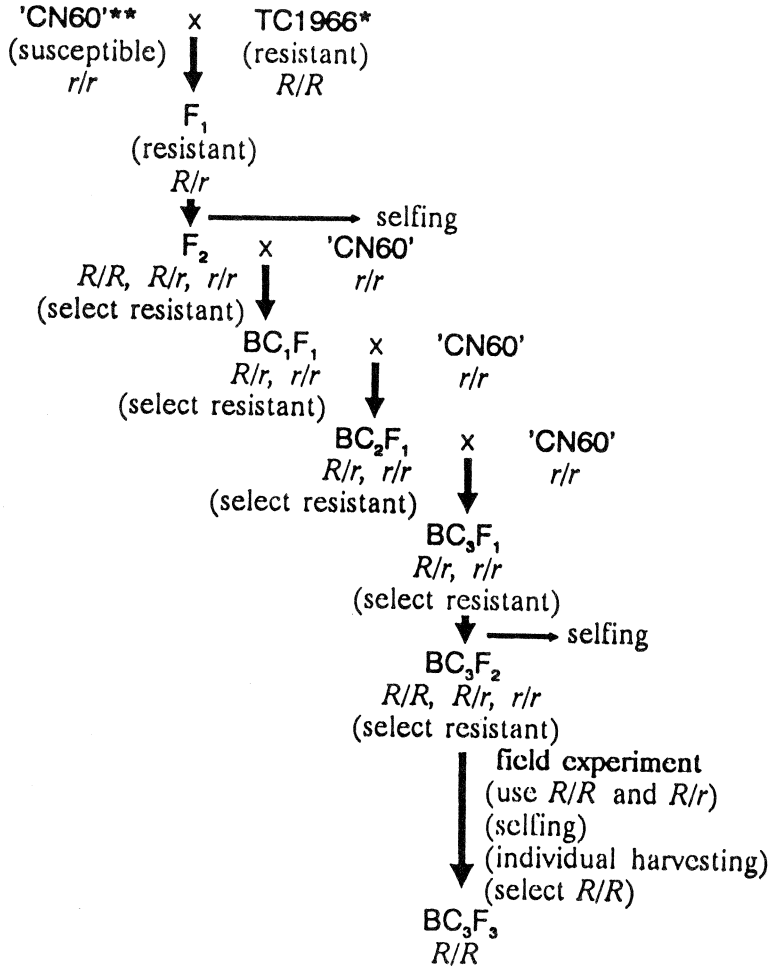

Fig. 15 Breeding procedure to imcorporate bruchid resistance from TC1966* to 'CN60'.**

* TC1966 is a bruchid-resistant accession of wild mungbean (*V. radiata* var. *sublobata*)

** 'CN60' is a recommended mungbean cultivar (*V. radiata*) in Thailand

BC_3F_1 plants were selfed to produce BC_3F_2 seeds. Resistant phenotypes (*R*/*R* and *R*/*r*) of the BC_3F_2 seeds were selected, and were used for the field experiment. The BC_3F_2 plants were harvested individually. Resistant BC_3F_3 line (*R*/*R*) was selected by checking the bruchid resistance of the BC_3F_3 seeds of each BC_3F_2 plant separately.

Field experiment for agronomic characters

The bruchid-resistant BC_3F_2 population and the check cultivars ('CN 60' and 'KPS1') were planted in the field of the Chainat FCRC on April 2, 1990 (early rainy season). The center is located in the central plain of Thailand (15°10'N and 100°15'E) at an elevation of 16m above sea level. Soil of the field consists of heavy clay from old river basin. Soil pH is 6.0–7.2 and organic matter content ranges from 0.7 to 2.07%.

The plants were grown on ridges 60cm wide, 5m long and 1m apart. Two replicates (ridges) for the BC_3F_2 population and four replicates (ridges) for each check cultivar were arranged at random (completely randomized design). On each ridge, there were two rows 50cm apart. One plant per hill was grown on a row at a 20cm distance. Irrigation, insecticide application, and hand weeding were performed as needed. The number of days from sowing to 50% flowering (the day when 50% of the plants in each ridge opened the first flower) was recorded for each ridge. Seed weight, number of pods per plant, seed yield per plant, and stem length were recorded for 10 plants of each replicate (ridge) which showed moderate growth. Harvesting was performed on the day when the color of about 80% of the pods in each ridge changed.

Results

Tests for bruchid resistance

Levels of resistance of cultivated and wild mungbean to *C. chinensis* and *C. maculatus* occurring at Chainat FCRC are indicated in Table 19. Among the six strains examined, only TC 1966 showed a complete resistance to both, *C. chinensis* and *C. maculatus*. Although the number of eggs laid on the seed surface of TC 1966 was equivalent to that in the other wild accessions, no adults emerged and no seeds were damaged in the case of TC 1966. Two other wild accessions and three recommended cultivars were susceptible at various levels to both *C. chinensis* and *C. maculatus*.

Segregation of resistance in F_1 and F_2 seeds from crosses between 'CN 60' and TC 1966 was investigated using *C. chinensis* (Table 20). All the ten F_1 seeds tested were found to be resistant. Genetic segregation of the F_2 seeds from each F_1 plant gave a close fit to a 3 resistant : 1 susceptible ratio.

Breeding procedure

'CN 60' could be easily crossed with TC 1966 and no abnormality was recognized in the F_1 and later generations. Since 'CN 60' was used as a female parent (Fig. 15), the seed coat color (a maternal trait) of the F_1 seeds was the same (green) as that of 'CN 60'. The seed coat color of all the F_2 seeds became brown (seed coat color of TC 1966), indicating that brown was dominant to green. The seed coat color segregated to green and brown in the BC_1F_1 seed generation. Resistant seeds with a green seed coat were selected in the segregating generation (Fig. 16). Although bruchids laid many eggs on the surface of the resistant seeds, larvae that hatched did not reach the adult stage.

Changes in mean seed weight induced by the recurrent backcrossing are shown in Fig. 17. Seed weight (100 seed weight) of TC 1966 was 1.7g and that of 'CN 60' was

7.1g. Hence, 100 seed weight was about 3.5g in the F_2 generation. The seed weight increased gradually by the recurrent backcrossing and attained the minimum standard for recommended cultivars (5.5g/100 seeds) in the BC_3F_1 generation (ca. 6.6g/100 seeds).

Table 19. Levels of resistance of cultivated ('CN60', 'KPS1' and 'KPS2') and wild (TC1965, TC1966 and TC2207) mungbean varieties to *C. chinensis* and *C. maculatus*

Bruchid species	Legume line	100 seed weight (g)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental period (days)
C. chinensis	'CN60'	7.1	38.0a*	80.3a	100.0a	23.4a
	'KPS1'	7.3	22.0ab	86.4a	95.0a	24.0a
	'KPS2'	7.3	29.0ab	89.7a	100.0a	24.2a
	TC1965	1.0	12.0b	66.7a	89.5a	26,7a
	TC1966	1.7	15.5b	0.0b	0.0b	-
	TC2207	1.4	15.0b	70.0a	90.0a	25.9a
C. maculatus	'CN60'	7.1	50.0a	60.0ab	100.0a	26.7a
	'KPS1'	7.3	30.0abc	78.1a	100.0a	27.4ab
	'KPS2'	7.3	35.0ab	75.7a	100.0a	27.3ab
	TC1965	1.0	19.0bc	39.5b	85.0a	31.6b
	TC1966	1.7	9.0c	0.0c	0.0b	-
	TC2207	1.4	15.5bc	45.2b	70.0a	28.8ab

Average of two replicates, 10 seeds infested with two pairs of freshly emerged bruchid adults per replicate

* : Mean separation for each bruchid was performed by least significant difference at 99% level.

Table 20. Segregation for bruchid (*C. chinensis*) resistance in the F_1 and F_2 seeds from the crosses 'CN60'* x TC1966**

Generation	No. of seeds tested			χ^2 (3 : 1)	Probability
	total	resistant	susceptible		
F_1	10	10	0	-	-
F_2	106	79	27	0.0126	0.91
	98	71	27	0.3401	0.56
	106	77	29	0.3145	0.57
	102	76	26	0.0131	0.91
Total (F_2)	412	303	109	0.4660	0.49

* 'CN60' is a recommended cultivar of mungbean (*V. radiata*) in Thailand.

** TC1966 is a bruchid-resistant accession of wild mungbean (*V. radiata* var. *sublobata*).

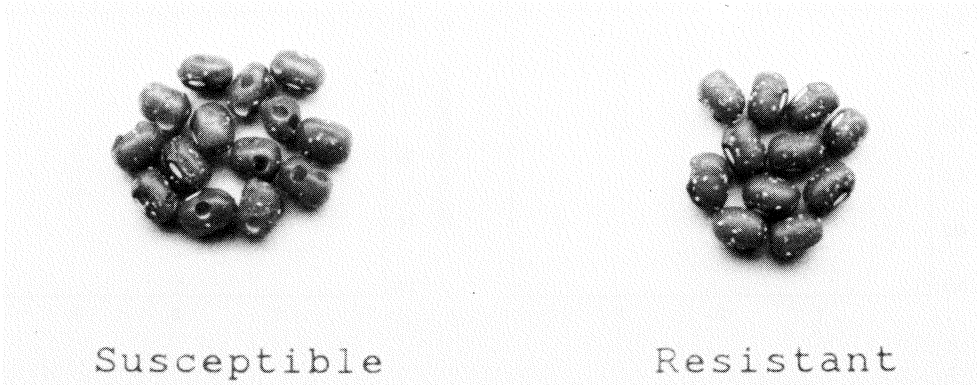

Fig. 16 Resistant and susceptible seeds in the segregating generation (BC_2F_1) of 'CN60' x TC1966.

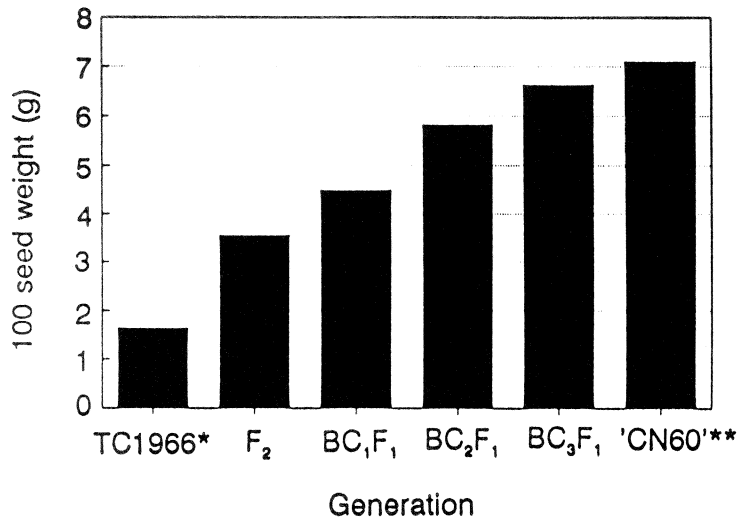

Fig. 17 Changes in mean seed weight of breeding line derived by recurrent back-crossing from the cross 'CN60' x TC1966.

Agronomic characters of the resistant BC_3F_2 population

Seed weight (100 seed weight), number of pods per plant, seed yield per plant, and stem length of the brucid-resistant BC_3F_2 population and check cultivars ('CN 60' and 'KPS1') are shown in Fig. 18 a-d. Seed weight (100 seed weight) of the resistant BC_3F_2 population was significantly lower (ca. 6.3g) than that of 'CN 60' (ca. 7.1g) and 'KPS1' (ca. 7.3g), but was large enough to satisfy the standard (5.5g) requested by the Thai Government (Fig. 18a). Since the resistant BC_3F_2 population contained a larger number of pods per plant than the check cultivars (Fig. 18b), seed yield per plant was

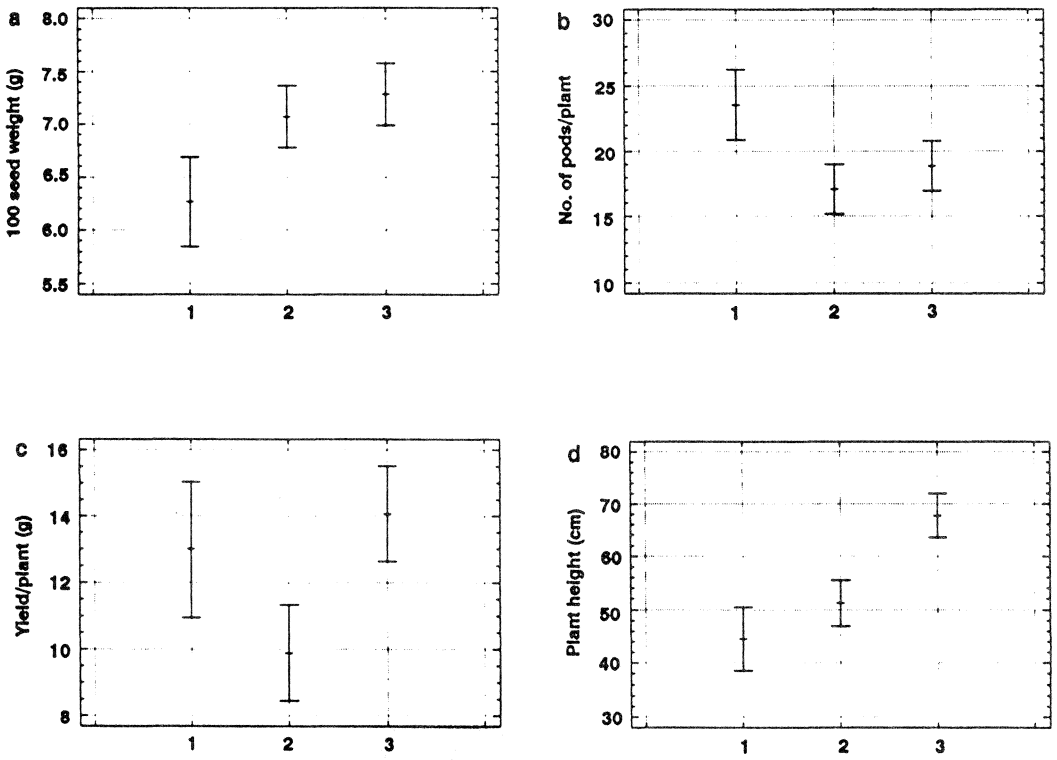

Fig. 18 (a) Seed weight (100 seed weight), (b) Number of pods per plant, (c) Seed yield per plant, and (d) Stem length of (1) the bruchid-resistant population (BC_3F_2 generation of 'CN60' x TC1966) and check cultivars (2) 'CN60' and (3) 'KPS1'.

Average of two replicates for the resistant BC_3F_2 population and four replicates for 'CN60' and 'KPS1'

Statiscal analysis by ANOVA, multiple range test with least significant difference at 95% level

comparable to that of 'CN 60' and 'KPS1' (Fig. 18c). Stems of the resistant BC_3F_2 population were shorter than those of 'KPS1' and comparable to those of 'CN 60' (Fig. 18d). Average number of days to 50% flowering of the resistant BC_3F_2 population was 30 days, as in the case of 'CN 60', and 5 days earlier than in 'KPS1'. All the three entries matured (with change in the color of 80% of the pods) 30 days after 50% flowering.

Discussion

It was revealed that the resistance of TC 1966 was also effective against the *C. chinensis* and *C. maculatus* species distributed in Thailand (Table 19). Genetic analysis indicated that the resistance was controlled by a single dominant gene (Table 20). Kitamura et al. (1990) suggested that a water-soluble substance with a high

molecular weight, and characterized by heat- and protease- stability was responsible for the resistance of TC 1966. This substance is considered to be the same as or a similar compound to that found in the seeds of black gram (*V. mungo*), which is also resistant to *C. chinensis* (Kitamura and Ishimoto, personal communication). Black gram is a close relative to mungbean and is a very popular crop in India. It has been consumed by Indians mainly as dhal soup. Recently, this legume has been imported and consumed as bean sprouts in Japan. Therefore, if the substance contained in TC 1966 is the same as that in black gram, it will not be harmful to human beings. The substance contained in TC 1966 which is responsible for the bruchid resistance should be further investigated in terms of toxicity to animals and human beings.

Since no reproductive barriers were recognized between 'CN 60' and TC 1966, it was possible to produce hybrids and to improve the agronomic characters of the hybrids by recurrent backcrossing (Fig. 15, Fig. 17). Based on the field experiment conducted in the early rainy season in Chainat FCRC, it was concluded that the agronomic characters of the resistant BC_3F_2 population reached the levels of the recommended cultivars. In the resistant BC_3F_2 population, the plant was short with many pods and early maturity habit. The agronomic characters of the breeding line should be examined also in other seasons and locations in Thailand.

AVRDC attempted to develop a line which would be resistant to *C. chinensis* by screening more than 500 mungbean and black gram accessions (Talekar and Lin 1981). However, they could not identify any mungbean accession showing a significantly higher level of resistance. On the other hand, TC 1966 which showed a complete resistance to both *C. chinensis* and *C. maculatus* was detected among only three wild mungbean accessions (Fujii and Miyazaki 1987). Furthermore, these three wild mungbean accessions showed three different types of seed protein profiles by means of SDS-PAGE method, while only eight protein types could be detected among 590 local mungbean varieties (Tomooka et al. 1991). Thus, it is considered that wild mungbean (*V. radiata* var. *sublobata*) harbours a larger genetic variation than cultivated mungbean (*V. radiata*).

V. radiata var. *sublobata*, which is distributed over a very wide area stretching from East Africa through Asia to North Australia, shows a wide genetic variation (Maréchal et al. 1978, Lawn et al. 1988). Moreover, no genetic barriers were recognized between *V. radiata* and *V. radiata* var. *sublobata* (Miyazaki et al. 1984, Kitamura et al. 1988), which was confirmed in the present study. Thus, *V. radiata* var. *sublobata* can be considered as a very useful genetic source for mungbean breeding. However, few accessions of *V. radiata* var. *sublobata* are currently collected and preserved in the world's genebanks. Therefore, it is important to collect and preserve a larger number of different accessions of *V. radiata* var. *sublobata* occurring in wide geographical regions.

Chapter 5. General Discussion

Genetic diversity of mungbean and its wild relatives was studied to identify germplasm that could be used for the breeding of mungbean and other crops of subgenus *Ceratotropis*. Landraces of mungbean throughout Asia as well as wild *Ceratotropis* species collected from Northern Thailand and the southwestern islands of Japan were examined for their taxonomic, phylogenetic, as well as agronomic traits. The wild relatives were evaluated for their usefulness as gene sources in cross breeding programs of mungbean in terms of favorable agronomic characters and cross-compatibility. Attempts were also made to develop a bruchid-resistant mungbean line using the resistance gene harboured by a wild relative species.

Pattern of landrace differentiation in mungbean

Evaluation of genetic diversity within germplasm collections is important for plant breeders who seek sources of genes for particular traits. Variations and geographical distribution of growth types, seed characters, and seed protein types of local strains of mungbean, *Vigna radiata,* collected throughout Asia, were investigated to determine the center of genetic diversity, dissemination pathways, and the pattern of landrace differentiation.

Based on the number of days to 50% flowering, stem length, and number of lateral branches, eight growth types were identified (Table 2). A clear latitudinal cline was recognized in the growth type distribution (Fig. 2). The strains consisting of tall plants with a high-branching and late maturity habit (Growth type 8) were predominantly cultivated in areas at low latitudes (Indonesia and Thailand). At the intermediate latitudes (Taiwan and the Philippines), the predominant types consisted also of tall plants but with a low-branching habit (Growth types 3 and 7). At high latitudes (Korea, Afghanistan, and Iran), the predominant type changed to short plants with early maturity and a low-branching habit (Growth type 1). The strains from India showed the largest diversity in terms of growth type variations. Various mungbean strains showing a wide range of growth habits were grown in India. These results were consistent with the theory on the general pattern of latitudinal distribution in the growth characteristics proposed by Vavilov (1926).

Based on the variations in the seed characters (seed color, seed weight, seed length, seed width, and seed shape=length/width), the following tendencies could be recognized (Fig. 4). Diversity of seed color was the largest in West Asia and the Indian subcontinent. Shiny green, dull green, brown and black mottled mungbean strains were found in these areas. In contrast to the wide variations in seed color, strains in these areas were characterized by small seeds with limited variations in seed size. On the contrary, strains in Southeast Asia showed wide variations in seed size, but the seed color was generally shiny green. In East Asia, medium-seeded strains with dull green seed color were predominantly distributed. The pattern of this geographical distribution in seed characters is considered to be closely related to the ecological adaptation of mungbean strains, racial preference, way of cooking and consumption in each region.

Based on the combination of four albumin bands and three globulin bands separated by SDS-polyacrylamide gel electrophoresis, eight protein types were recognized (Fig.

5). Frequency of each protein type also showed a clear geographical cline (Fig. 6). The regions of genetic diversity in seed protein were located in West Asia (the Afghanistan-Iran-Iraq area) rather than in India. The protein type composition of the Southeast Asian strains was very simple, with the strains being similar to one another. Protein type composition in East Asia was rather similar to that in West Asia, suggesting the strong relationship of the strains between West Asia and East Asia.

The center of genetic diversity (selection-neutral characters) of mungbean is located in West Asia. Mungbean may have spread mainly to the east by two routes from India, where the domestication of mungbean is considered to have occurred. One route led to Southeast Asia from India. Strains consisting of a few protein types with the predominance of protein type 1 were disseminated from India to the Southeast Asian countries. In Southeast Asia, the strains were subjected to a strong selection pressure for large seed size and shiny green seed color. Strains have also acquired late maturity (strong short day requirements) and high branching habits in Southeast Asia. Another dissemination pathway may have been 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 West Asia or India to East Asia (China and Taiwan) by the Silk Road, and not by the route from Southeast Asia. Based on a comparative morphological study, Vavilov (1931) concluded that mungbean and other major field crops cultivated in the Central Asian part (Xijiang Autonomous Region) of China were disseminated from West Asia. His conclusion supports the existence of the Silk Road pathway in the dissemination of mungbean. In East Asia, strains showing a dull green seed color with medium-sized seeds became the predominant type.

For a more detailed analysis of the center of genetic diversity, dissemination pathway, and the pattern of landrace differentiation, it is necessary to use a larger number of landraces, especially from West Asia, Burma, and China. Analysis of other selection-neutral characters, such as isozymes, should also be performed. Intraspecific hybrid sterility is considered to be one of the critical indicators of genetic differentiation. Intraspecific hybrid sterility has been extensively studied in relation to the phylogenetics of plant species (Stebbins 1950). Information on intraspecific hybrid sterility is also important in cross breeding programs through the utilization of a wide range of landraces and wild relatives of mungbean.

Landraces generally harbour wide genetic variations, especially in the traits which have been selected by the farmers. Landraces, therefore, have been considered to be an extremely important gene source for crop improvement since Vavilov's report (Vavilov 1935). Landraces are rapidly disappearing along with valuable information about special characteristics such as tolerance to drought, shade, insects and diseases, good taste, utilization pattern, usage in ritual or religious ceremonies, and traditional cultural practices. This kind of information which is useful not only for plant breeding but also for ethnology and cultural anthropology, as well as the landraces themselves should be collected and described before the landraces disappear from the region.

Incidentally, mungbean of the glossy green type had been cultivated and consumed in Japan covering an area of 200–220 ha in the 1950s. The area decreased to 131 ha in 1965, the crop was no longer cited in the agricultural statistics of 1966, and it totally disappeared in Japan around 1975.

Evaluation of wild *Ceratotropis* species

Wild species generally exhibit a wide range of genetic diversity in terms of agronomic characteristics involving pest and disease resistance, maturity span, environmental adaptation, resistance to lodging, root system and yield potential (Harlan 1976 Prescott-Allen and Prescott-Allen 1988). Wild relatives of cultivated plants are thus essential for crop improvement programs. Wild species are now being faced with the threat of extinction due to the recent trend of deforestation and urban development. For the breeding of mungbean and other Asian *Vigna* cultigens, the disappearance of wild species belonging to the subgenus *Ceratotropis* in the genus *Vigna* may lead to the erosion of the primary gene pool.

Against this background, the wild species in the subgenus *Ceratotropis* were collected in Thailand and the southwestern islands of Japan, and evaluated as a source of genes in the cross-breeding of crops of *Vigna* species. In the explorations described in Chapter 3, 16 accessions consisting of *V. riukiuensis* and *V. reflexo-pilosa* were collected on the Nansei Archipelago, Japan, and 16 accessions consisting of four wild *Ceratotropis* species were collected in Northern Thailand. *V. riukiuensis* showed a higher level of resistance to *C. maculatus*, while *V. reflexo-pilosa* exhibited a higher level of resistance to *C. chinensis* compared with that in azuki bean. It is also suggested that *V. riukiuensis* can act as a bridge species between azuki bean and rice bean. It may be possible to incorporate useful characters of rice bean, such as complete immunity to both *C. chinensis* and *C. maculatus*, into azuki bean. Among the four wild species collected in Northern Thailand, two species (designated as Species B and Species D) are considered to harbor resistance gene (s) against the infestation by *C. chinensis* and *C. maculatus*. Since Species B and Species D belong to the "Azuki group" in the subgenus *Ceratotropis*, these species may be used as gene sources in the breeding of azuki bean. Species B was considered to be a wild form of cultivated rice bean, *V. umbellata* var. *gracilis*. The morphology of Species D did not correspond to that of any of the species described before. Further taxonomic studies should be conducted to identify the taxon of this species.

The above results indicated that broader genetic sources belonging to *Ceratotropis* should be evaluated for their resistance to major diseases and insect pests in addition to *C. chinensis* and *C. maculatus*. Information regarding cross-compatibility between wild species and cultivated species is also necessary. Sixteen species were described in the subgenus *Ceratotropis* (Verdcourt 1970). Since the importance of the subgenus *Ceratotropis* as a gene pool for the *Ceratotropis* cultigens was confirmed in the present study, collection and evaluation should be further promoted. If the exploration area could be expanded to the other part of Asia in the future, more contribution to the collections of wild *Ceratotropis* species could be achieved.

Breeding of bruchid-resistant mungbean line

A mungbean (*V. radiata*) line which is resistant to two species of bruchid beetles (*C. chinensis* and *C. maculatus*) was successfully developed in Thailand using a wild mungbean variety (*V. radiata* var. *sublobata*). One accession (TC 1966) of wild mungbean was found to be resistant to *C. chinensis* and *C. maculatus* occurring at Chainat Field Crops Research Center in Thailand. The resistance was controlled by a single dominant gene. A breeding program for the development of a bruchid-resistant mungbean cultivar with good agronomic characters under the environmental

conditions of Thailand was initiated in 1987. 'Chainat 60' ('CN 60'), a recommended mungbean cultivar in Thailand, was crossed with TC 1966 to incorporate the resistance gene. Agronomic characters of the hybrids were improved by recurrent backcrossings using 'CN 60' as a pollen parent. Seed yield per plant, days to flowering and seed size of the bruchid-resistant bred line reached the level of 'CN 60' after three consecutive backcrossings (BC_3F_2 generation).

In addition to the bruchid resistance, many traits to be utilized in breeding, e.g. tolerance to yellow mosaic virus (Singh and Ahuja 1977), higher methionine content in the seed (Babu et al. 1988), higher photosynthetic efficiency, drought tolerance (Ignacimuthu and Babu 1987) and tolerance to salinity, alkaline calcareous soils and cool temperature (Lawn et al. 1988) have already been reported for *V. radiata* var. *sublodata*. This wild variety which is distributed over a very wide area stretching from East Africa through Asia to North Australia shows wide genetic variations (Maréchal et al. 1981, Lawn et al. 1988). Moreover, the present study confirmed the absence of genetic barriers between *V. radiata* and *V. radiata* var. *sublobata* (Miyazaki et al. 1984, Kitamura et al. 1988). Thus, *V. radiata* var. *sublobata* can be considered to be a very useful gene source for mungbean breeding. Unfortunately, few accessions of *V. radiata* var. *sublobata* have been collected and are currently preserved in the world's genebanks. In view of the rapid increase in the genetic erosion of wild species worldwide, it is urgently needed to collect and preserve a wider variety of germplasm of *V. radiata* var. *sublobata* occurring over a wide range of geographical regions.

Acknowledgements

The author is greatly indebted to Dr. Shoji Shigenaga, Professor at Kyoto University, for his kind supervision, continuous guidance, and fruitful discussion. His gratefulness is extended to Dr. Naoki Utsunomiya, Dr. Eiji Nawata, Faculty of Agriculture, Kyoto University, for their valuable suggestion.

The author also expresses his thanks to Dr. Yoshinobu Egawa of the National Institute of Agrobiological Resources (NIAR), for his fruitful advice throughout the course of the present study. Sincere thanks are also extended to Dr. Yoichi Tateishi, Tohoku University, for his valuable suggestion and identification of the plant specimens.

He is much indebted to Mr. Charaspon Thavarasook, Director, Mr. Chalermpol Lairungreang, Agronomist, Mr. Somyot Pichitporn, Breader, and Ms. Potjanee Nakeeraks, Breeder at Chainat Field Crops Research Center in Thailand, for their helpful advice and assistance. Special acknowledgement is due to Dr. J. Bartman of TARC, for her reading of the entire manuscript and her valuable suggestions. His deepest thanks are due to Mrs. Kazuko Tomooka for her warmhearted encouragement.

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