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Japanese Native *Vigna* Genetic Resources

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Abstract

This paper provides new information on the distribution of Japanese native *Vigna* species in the subgenus *Ceratotropis*. The azuki bean complex (*Vigna angularis*) in Japan consists of cultigen, wild, weed and complex population types. Population genetic studies of this complex based on AFLP and RAPD analyses have revealed clinal variation in Japan. Population analyses have also provided support for hypotheses related to the origin of weedy azuki bean. The complex population type displays greater genetic variation than other population types in the azuki bean complex. Thus complex populations are the logical focus for both *in situ* and *ex situ* conservation. The wild and weedy *Vigna* species of Japan are a potential source of useful traits for agriculture and agriculturally related industries. Currently *V. riukiuensis* (*V. minima* subsp. *minor* var. *minor*) is being used as a bridging species to enable gene transfer among sexually incompatible *Vigna* cultigens. The potential of the genus *Vigna* to become a genetic model to enhance the understanding and genetic improvement of the tropical grain legume cultigen group is discussed.

Discipline: Genetic resources
Additional Key words: *in situ* conservation, population genetics, RAPD, AFLP

Introduction

The genus *Vigna* consists of about 150 species that are divided into 7 subgenera[12)]. The genus *Vigna* consists of 11 cultigens of agricultural importance in different regions worldwide (Table 1). *Vigna* is closely related to the genus *Phaseolus*. Of the world legumes, a larger proportion of the human population probably relies for its nutrition on the *Phaseolus/Vigna* group compared with other legumes.

In Asia, the dominant group of *Vigna* species belongs to the subgenus *Ceratotropis* which has its center of species diversity in mainland South and Southeast Asia[18)]. In Japan, azuki bean (*V. angularis*) is the second most important legume. The Japanese people eat on average 1 kg of azuki beans a year but the cultural importance of this crop is far greater than this quantity suggests.

The objectives of this paper are to analyze the diversity of *Vigna* species in Japan, the distribution of native wild *Vigna* species in the subgenus *Ceratotropis*, present results of population genetic studies on the *Vigna angularis* complex and to discuss the evaluation and potential future use of Japanese native *Vigna* genetic resources.

Species diversity and distribution

Four wild species of the genus *Vigna* subgenus *Ceratotropis,* i. e. *V. angularis* var. *nipponensis, V. nakashimae, V. reflexo-pilosa* and *V. riukiuensis* grow in Japan (Fig. 1). Four other native wild *Vigna* species, i. e. *V. adenantha, V. luteola, V. marina* and *V. vexillata,* belonging to the subgenera *Vigna* and *Plectotropis,* occur in southern Japan. In addition, 4 species of *Vigna* are cultivated in Japan, i. e. azuki bean (*V. angularis*), mung bean (*V. radiata*), rice bean (*V. umbellata*) and cowpea (*V. unguiculata*). Of these cultigens, only *V. angularis* and *V. unguiculata* are widely grown in Japan. All these cultigens belong to the subgenus *Ceratotropis* except for *V. unguiculata* that belongs to the subgenus *Vigna*. Recently, a series of missions have been conducted

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Table 1. Cultivated species in the genus *Vigna*

Genus Subgenus Species	Common or local name	Origin
Vigna		
Subgenus *Ceratotropis*		
Vigna aconitifolia (Jacquin) Maréchal	Moth bean	South Asia
V. angularis (Willd.) Ohwi & Ohashi	Azuki bean	East Asia
V. mungo (L.) Hepper	Black gram	South Asia
V. radiata (L.) Wilczek	Mung bean	Asia
V. glabrescens Maréchal, Mascherpa & Stainer	Creole bean	Southeast Asia
V. trilobata (L.) Verdcourt	Jungli bean	South Asia
V. trinervia (Heyne ex Wight & Arnott) Tateishi	Tua pée (Thai)	South and Southeast Asia
V. umbellata (Thunb.) Ohwi & Ohashi	Rice bean	Southeast Asia
Subgenus *Vigna*		
V. unguiculata (L.) Walpers	Cowpea	East Africa
V. subterranean (L.) Verdc.	Bambara groundnut	West Africa
Subgenus *Plectotropis*		
V. vexillata (L.) A. Rich	Zombi bean	East Africa

throughout the Japanese archipelago to collect *Vigna* subgenus *Ceratotropis* genetic resources[9,22]. These missions, in addition to the information gathered from both Japanese and European herbaria, have clarified the distribution of native wild *Vigna* species in the subgenus *Ceratotropis* in Japan.

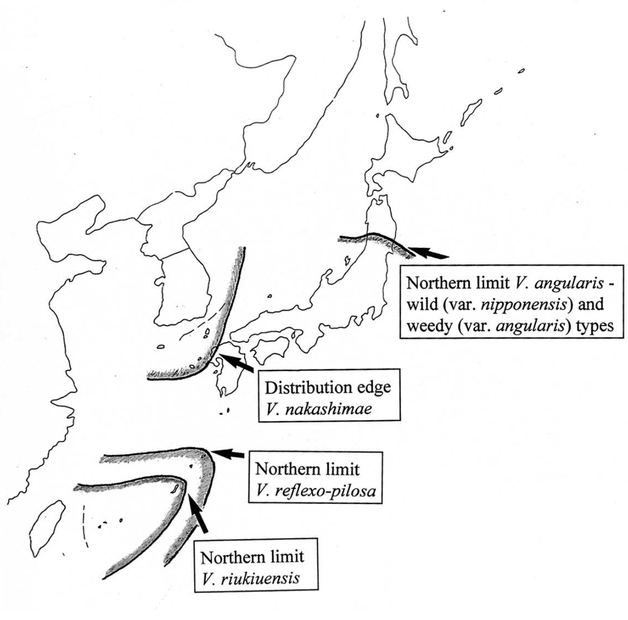

Fig. 1. Distribution limits of the wild *Vigna* subgenus *Ceratotropis* species in Japan

1) The Vigna angularis *complex*

The main production center for azuki bean in Japan is Hokkaido that accounts for 65% of the production area[13]. In other parts of Japan, azuki bean is generally grown on a small scale. In contrast to the cultigen, wild and weedy populations of azuki bean do not occur in Hokkaido. The northern limit of wild and weedy azuki bean is Akita and Iwate prefectures[22]. Wild and weedy populations can be distinguished in the field based on the plant habit, seed and pod size (Fig. 2). The greatest genetic diversity in Japan for the azuki bean complex can be found in central Japan surrounding the Kyoto-Osaka-Kobe industrial belt. For example, recently in Tottori and Okayama prefectures, wild, weedy, complex and wild and weedy mixed populations as well as a hybrid swarm population have been found[21]. Shikoku, Kyushu and northern Honshu do not show the same diversity or abundance of population types. The diversity of the population types in Japan reflects the dynamic evolutionary

Fig. 2. Dehisced pods and seeds of wild (bottom), weedy (middle) and cultivated (top) azuki bean

status of this crop complex.

2) Vigna nakashimae *(syn.* V. minima *subsp.* nakashimae*)*

Vigna nakashimae belongs to a group of closely related species that include *V. minima* and *V. riukiuensis*. Recent analysis of the *V. minima* complex has shown that *V. nakashimae* is genetically and geographically distinct from other taxa in this complex[26)]. This species is distributed in Northeast Asia. While this species is quite common in Korea, it is only occasionally found in Japan in Northwest Kyushu[26)].

3) Vigna reflexo-pilosa

The subgenus *Ceratotropis* consists of 2 closely related tetraploid species, *V. glabrescens* and *V. reflexo-pilosa*. *V. glabrescens* is occasionally cultivated in different parts of the world. This species does not occur in Japan but was presumably domesticated from the wild *V. reflexo-pilosa,* which can be observed in Japan.

V. reflexo-pilosa is widely distributed in East and Southeast Asia, Australia and the Pacific. In Japan, *V. reflexo-pilosa* shows a wider distribution in the Ryukyu islands than *V. riukiuensis*. This species has been found during recent collecting missions on the islands of Irabu, Iriomote, Ishigaki, Miyako, Okinawa, Tarama and Yonaguni. Based on herbarium specimens, it also grows on Amami Ohshima, Ikema, Kikai, Kume, Okinoerabu, Tokuna, Uke and Yoron islands[17)].

4) V. riukiuensis *(syn.* V. minima *subsp.* minima *var.* minor*)*

This perennial species has a restricted distribution in the southern Ryukyu islands and Taiwan. In Japan this species has recently been collected on Hateruma, Iriomote, Ishigaki, Miyako, and Yonaguni islands. It has also been reported from the main island of Okinawa and the small nearby islands of Ie, Kudaka, Aguni, Tonaki and Tokashiki.

Population genetics of the *Vigna angularis* complex

Vigna angularis shows the most extensive distribution of *Vigna* species in Japan and its wild form grows at a more northerly latitude than any other wild relative of the *Vigna*/*Phaseolus* group of cultigens[22)]. Since azuki bean is the second most important legume in Japan and little is known about the genetic diversity of its wild relatives, a series of studies have been initiated to analyze the genetic diversity of this species complex.

Isozyme and protein banding have revealed relatively little polymorphism in this species complex[19)]. Consequently the DNA marker methods, RAPD and AFLP, were applied to obtain estimates of population genetic parameters.

Materials and methods

For RAPD analysis, 29 populations representing wild (5), weedy (6), complex (6) and cultivar (12) populations were analyzed (Fig. 3A). For the wild, weedy and complex populations 54, 43 and 69 individuals were analyzed, respectively. Bulk samples from the cultigen were analyzed. For AFLP analysis, 41 populations representing wild (23), weedy (11), and cultivar (7) populations were analyzed as bulk samples (Fig. 3B). Individual plant samples from one each of wild, weedy and complex type populations from sites close to Tottori city were also analyzed by the AFLP method.

Details of the methods used can be found in the reports of Xu et al.[23, 24)].

Results and discussion

Intra-population genetic variation statistics for pop-

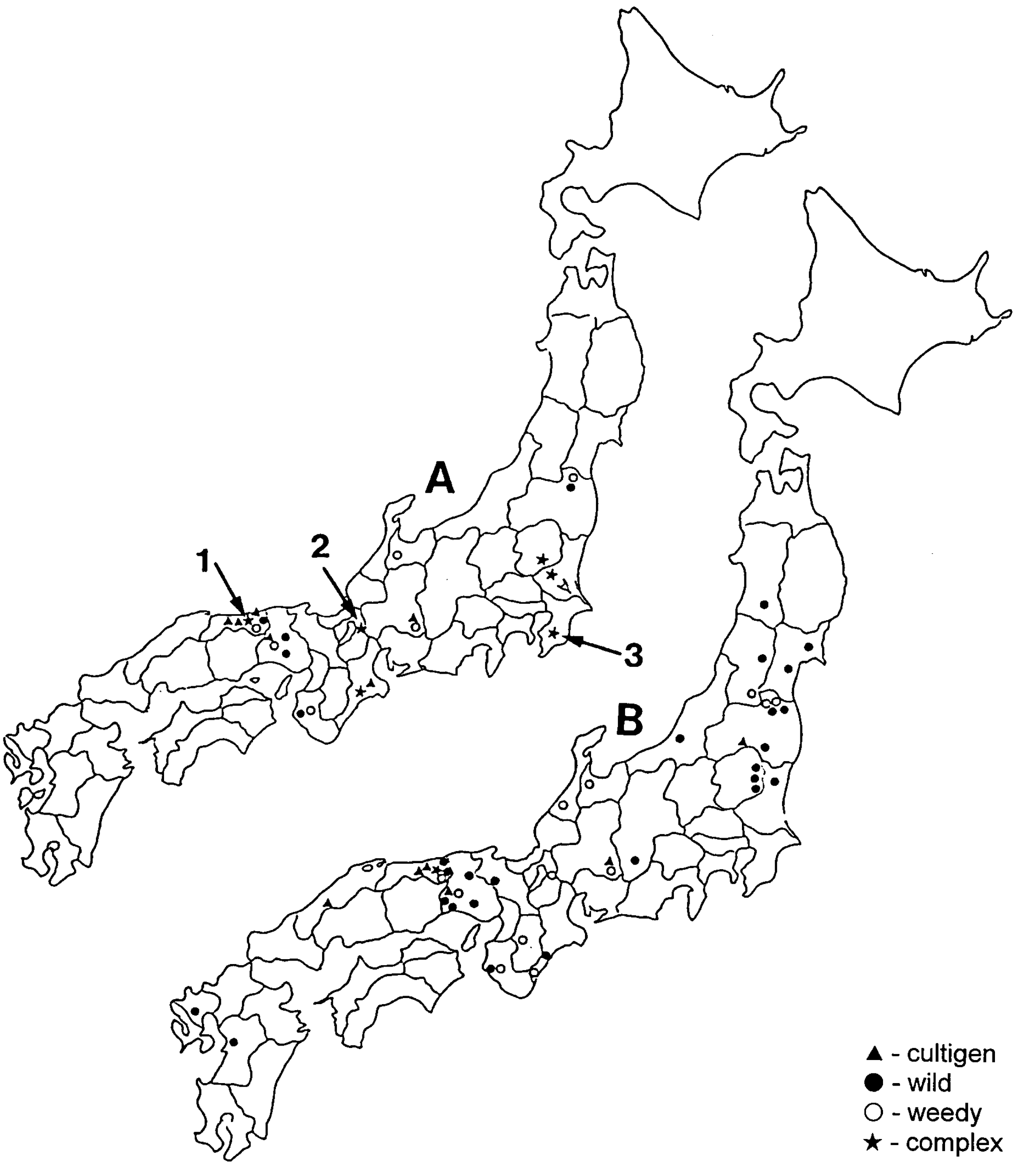

Fig. 3. Distribution of *Vigna angularis* complex samples used in (A) RAPD analysis (B) AFLP analysis
Numbered complex populations refer to those shown in Fig. 5. 1= ■ , 2= ● , 3= ✚ .

ulation types based on both RAPD and AFLP polymorphism are presented (Table 2). These statistics reveal that genetic diversity at the DNA level has decreased from the wild to weedy to cultivated populations. Since all the wild and weedy populations used in these studies originated from Japan, while varieties of the cultigen originated from Japan, China and, in the case of RAPD analysis, also from Nepal, it appears that a severe genetic bottleneck occurred during domestication. Such a phenomenon has been reported for other cultigens[15]. Our results suggest that at the DNA level, 2 or 3 fold more genetic variation can be found in the wild ancestor of azuki bean than in the cultigen. This fact suggests the potential genetic value of the wild genepool.

Inter-population type variation is similar using the two DNA methods. Both RAPD and AFLP analyses show that the genetic distances between population types, wild, weedy and the cultigen are similar. These results shed a new light on the origin of weedy azuki (discussed below).

1) Clinal variation

The samples of wild and weedy populations subjected to AFLP analysis originated from locations as far South and West as Saga and Kumamoto prefectures, Kyushu island, and as far North and East as Akita and Miyagi prefectures, Honshu island. Analysis of the results reveals that there is a clinal trend in variation

Table 2. Intra-population variation based on RAPD and AFLP polymorphism detected in *Vigna angularis* complex population types

Population types	RAPD polymorphism[1)]	AFLP polymorphism[1)]
Cultigen	0.079	0.353
Weedy	0.124	0.561
Wild	0.132	1.191
Complex	0.152	–

1): Based on Shannon's diversity index.

(Fig. 4). The Mantel test was applied to quantify the relationship between genetic variation measured by the AFLP similarity matrix and population location. The correlation between the AFLP similarity and longitude ($r = -0.231$, $P < 0.01$) and latitude ($r = -0.252$, $P < 0.01$) is highly significant but of low magnitude. These results suggest that wild and weedy populations are locally adapted and that comprehensive germplasm collection is necessary to capture the genetic variation of this species complex for *ex situ* conservation.

2) Weedy azuki bean

Currently weedy azuki bean is classified with the cultigen as *Vigna angularis* var. *angularis*. Three hypotheses have been proposed for the emergence of weedy races of cultigens: escape from cultivation, hybridization between wild and cultivated forms and a distinct evolutionary type with characteristics intermediate between the wild type and cultigen[4, 25)]. Both RAPD and AFLP analyses revealed that wild, weedy and cultivated azuki form distinct clusters (only AFLP analysis is shown in Fig. 4) and that the genetic distances between these population types are about equal (Table 3). RAPD analysis provides evidence that weedy azuki may generally have evolved from wild azuki bean since 2 polymorphic bands were found in the wild and weedy type populations but not in the cultigen. No common RAPD bands were shared only by the weedy type and cultigen. The 2 weedy populations associated with the wild azuki cluster shown in Fig. 4 may represent populations not yet clearly differentiated at the DNA level from wild azuki. Field observations suggest that weedy azuki beans may be a form adapted to specific, highly disturbed, ecological niches. In some locations, weedy azuki beans were found to grow in damp stream-side habitats, whereas wild azuki beans in the same locality were observed at a slightly higher elevation. Thus, the results from studies on genetic variation at the DNA level support the hypothesis that in some cases the weedy type of azuki directly evolved from the wild type.

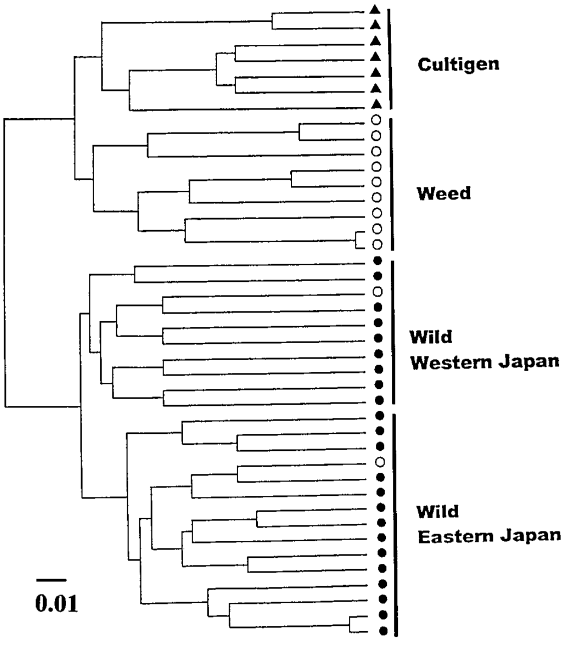

Fig. 4. UPGMA dendrogram representing the relationships between *Vigna angularis* population types based on AFLP data

The wild, weedy and cultigen populations are marked with ●, ○, ▲, respectively.

Hybridization between population types appears to occur and could explain the occasional observation of a hybrid swarm[21)]. Genetic analysis of such populations, using molecular techniques that can reveal co-dominant markers, in addition to pollination studies, may improve our understanding of the evolutionary dynamics of this species complex.

3) Complex population type

In addition to the cultigen, wild and weedy populations, there is another population type in the *Vigna angularis* complex. These populations are often large and consist of a wide range of plant types including wild and weedy plant types. Seed coat color of different individuals in these populations often varies and includes plants with black, tan or green seeds. Sometimes, but not always, cultivated azuki beans were found to grow at the same general location. Since these populations could not be readily classified, we designated them as complex

Table 3. Inter-population type variation based on RAPD and AFLP polymorphism detected in the *Vigna angularis* complex

Population type comparison	RAPD analysis	AFLP analysis
	Genetic distance[1)]	Genetic diversity[2)]
Cultigen vs. Weedy	0.348	0.505
Cultigen vs. Wild	0.452	0.561
Weedy vs. Wild	0.428	0.503

1): Based on Jaccard's dissimilarity index.
2): Shannon's diversity index.

populations. In other crop complexes, similar populations have been reported[2)].

RAPD analysis of complex populations revealed that these populations display more genetic variation than other population types (Table 2). Five polymorphic RAPD bands were found only in the complex populations. To better understand the association among individuals, principal coordinate analysis (PCA) was performed for individual plant RAPD data in 3 complex populations together with 12 cultivated accessions (Fig. 5). The cultivated accessions analyzed form a tight cluster except for one accession from Nepal. Individuals of the 3 complex populations are widely scattered but show some convergence towards the cultigen cluster.

The high level of genetic diversity in complex populations suggests that *in situ* conservation of the *V. angularis* complex, and by extension to other crop complexes, should focus on complex populations. Long-term monitoring of key populations revealed by RAPD and AFLP analysis is in progress.

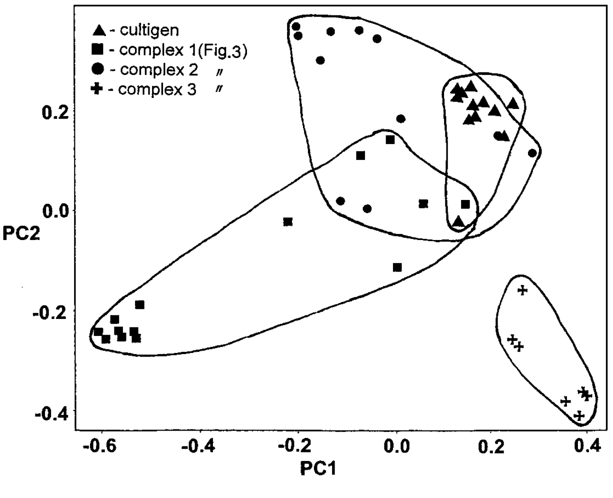

Fig. 5. Association among individuals from 3 complex populations (■ , ● , ✚) and 12 cultivated accessions (▲) of the *Vigna angularis* complex shown by principal coordinate analysis of RAPD data
The first and second principal coordinates represent 23.6 and 15.8% of the variation, respectively (Modified from Xu et al.[24)]).

Uses of Japanese native *Vigna* and prospects

Recent studies on the native species of *Vigna* in Japan are providing some basic information about their distribution and population genetics. To make further progress, research is currently being carried out to identify co-dominant molecular markers that may be used to improve the information on *Vigna* population dynamics. These markers should also enable an improved linkage map of *Vigna angularis* to be made[10)]. Since there are many cultivated *Vigna* species worldwide, basic knowledge from these studies is likely to have broad implications.

The wild and weedy relatives of *Vigna* in Japan have a potential value for agriculture. *V. marina* grows on sandy beaches and may be a source of salt tolerance. *Vigna riukiuensis* is being investigated as a source of heat tolerance for growing legumes in the tropics and subtropics[5)]. *V. riukiuensis* is also a useful bridging species between cross-incompatible species related to azuki bean[14,16)]. *Vigna nakashimae* is confined in its distribution to northeastern Asia and it is thus likely to be a source of cold tolerance early and late in the growing season.

One of the major constraints to legume production is the destruction of seeds by storage pests of which bean weevils are the most prevalent and serious. Recent evaluation of wild and cultivated *Vigna* genetic resources from Japan has revealed new sources of resistance (Table 4)[20)]. Resistance found in the variety Menaga of *V. umbellata* from Tsushima island, Nagasaki prefecture, is currently being investigated in detail since the resistance factor in this variety appears to be new[11)]. In addition, this source of resistance may be better than the currently available bean weevil resistance sources because it is found in a cultigen and is thus safe for human consumption. *V. riukiuensis* is being used as a bridging species to introduce this source of resistance to the bean weevils,

Table 4. Levels of resistance in Japanese *Vigna* species against *C. chinensis* and *C. maculatus*

Species	Accession	Origin	100 Seeds weight (g)	*C. chinensis*				*C. maculatus*			
				Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental duration ♀ (days)	Eggs/rep. (No.)	Emergence (%)	Damaged seeds (%)	Developmental duration ♀ (days)
V. angularis var. *angularis*	102	Japan	14.0	47.7	75.0	100.0	25.3	143.3	43.1	100.0	27.3
(Azuki bean)	Average		14.0	47.7	75.0	100.0	25.3	143.3	43.1	100.0	27.3
V. angularis var. *nipponensis*	96101204	Japan	2.7	79.5	17.6	100.0	25.4	50.0	20.9	93.3	26.8
(wild ancestor of azuki bean)	96101602	Japan	3.8	102.0	16.1	100.0	25.3	72.3	18.5	96.7	27.9
	96111108	Japan	2.8	119.7	10.5	100.0	25.8	53.3	22.6	96.7	27.2
	Average		3.1	100.4	14.7	100.0	25.5	58.6	20.7	95.6	27.3
V. nakashimae	Ukushima	Japan	2.0	23.0	43.9	90.0	27.9	51.3	14.0	63.3	27.2
(=*V. minima* subsp.*nakashimae*)	Average		2.0	23.0	43.9	90.0	27.9	51.3	14.0	63.3	27.2
V. riukiuensis	Yona4-1	Japan	1.1	28.0	24.5	56.7	30.4	30.7	5.7	16.7	43.0
(=*V. minima* var. *minor*)	Ri89018	Japan	1.3	15.0	5.7	10.0	31.0	31.3	2.0	6.7	29.0
	Average		1.2	21.5	15.1	33.3	30.5	31.0	3.8	11.7	36.0
V. reflexo-pilosa	Irio 4	Japan	1.5	21.0	6.9	13.3	32.0	37.0	22.1	83.3	27.4
var. *reflexo-pilosa*	Ref 1	Japan	1.8	28.0	26.5	63.3	28.7	42.3	24.5	80.0	26.3
(wild ancestor of var. *glabra*)	Average		1.6	24.5	16.7	38.3	30.4	39.7	23.3	81.7	26.9
V. umbellata (cultivated)	Bakaso	Japan	6.0	33.0	5.1	16.6	32.0	44.0	0.0	0.0	
(Rice bean)	Kagoshima	Japan	6.1	33.0	2.9	10.0	28.5	52.3	0.0	0.0	
	Menaga	Japan	6.2	30.0	0.0	0.0		45.0	0.0	0.0	
	Miyazaki	Japan	5.9	32.3	0.0	0.0		50.0	0.0	0.0	
	Average		6.1	31.2	2.0	6.7	30.3	47.8	0.0	0.0	0.0

Callosobruchus chinensis and *C. maculatus,* from cultivated rice bean (*V. umbellata*) to azuki bean.

Apart from the potential of wild and weedy *Vigna* species for agriculture, these species may have a potential to contribute to agriculturally related industries. Legumes are a particularly diverse source of secondary compounds, many of which are not found in other plant groups[7,8]. These secondary compounds may enable the development of new environmentally safe chemicals. For example, *Vigna* species, such as *V. angularis*, are known to show allelopathic activity and may be a source of allelochemicals[6]. Recently, *V. trinervia* has been found to be a cover crop under rubber trees in Thailand and seeds of this species in a local market command a price 10 times higher than that of rice (Tomooka, personal observation, 1999). Thai farmers also reported that *V. umbellata* showed strong allelopathic activity. A phytoalexin detected in the leaves of *V. unguiculata*, vignafuran, is fungitoxic and the flavonoid robinin, found in *Vigna* species, shows antibacterial activity[8].

The world major cultivated legumes can be classified into 2 main phylogenetically related groups[3]. The first group consists of the predominantly temperate, forage legumes that include genera such as *Trifolium* and *Vicia*. The second group includes the cultivated grain legumes that thrive in hot weather conditions and grow in tropical regions or temperate areas where hot summers prevail. This second group includes the genera *Vigna*, *Phaseolus*, *Glycine* and *Cajanus* that all have major cultivated species. The peanut (*Arachis hypogaea*) is not phylogenetically closely related to other cultivated legumes.

Within the tropical grain legume group, *Vigna* has the smallest genome that is only about 30% larger than the genome of rice which has one of the smallest genomes among major cultigens[1]. Among the *Vigna* species, a basic transformation system has been developed for *V. angularis* (Masao Ishimoto, 1999, personal communication), making *Vigna* species in general and *V. angularis* in particular, a useful model within the hot weather legume group for genetic and genome studies.

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