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日本における作物近縁野生種の保存：*Vigna*属

友岡　憲彦・ダンカン　ヴォーン・徐　如強・土井　考爾

農業生物資源研究所・遺伝資源第二部・集団動態研究室

Wild Relatives of Crops Conservation in Japan with a focus on *Vigna* spp.

Introduction.

Norihiko TOMOOKA, Duncan VAUGHAN, Ru Quiang Xu and Koji DOI

Crop Evolutionary Dynamics Laboratory, Department of Genetic Resources II, National Institute of Agrobiological Resources, Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan

Summary

The wild relatives of *Vigna* cultigens have been chosen as the first focus group for our studies on in-situ conservation and population dynamics. One reason is that this genus in Japan includes a crop complex within which geneflow between wild, "weedy" and cultivated components can occur. In this introduction to the following 4 exploration reports we summarise the 4 explorations undertaken in 1997 and a preliminary exploration made in 1996. In addition, we present the objectives of the collecting missions and related research.

KEY WORDS：*Vigna,* wild azuki bean, in-situ conservation, genetic resources

Overall introduction

In October 1996 a new laboratory was established in the National Institute of Agrobiological Resources called the Crop Evolutionary Dynamics Laboratory (集団動態研究室). Among the objectives of the laboratory was to focus on the topic of population dynamics and in-situ conservation. In autumn 1996 Tomooka (1997) undertook preliminary collecting of wild *Vigna* and *Glycine* in Honshu and Kyushu. Following that mission a plan was developed to undertake a nationwide survey for *Vigna* spp. for the following reasons:

1) *Vigna* is represented in Japan historically by 4 cultivated species (*Vigna angularis* var. *angularis, V. radiata, V. umbellata* and *V. unguiculata*) (Fig. 1). At present only *V. angularis* var. *angularis* and *V. unguiculata* are widely grown the other two species are either no longer grown or are grown in a very few localities. In addition four wild species of *Vigna* are found in Japan. Three species [*V. reflexo-pilosa, V. minima* var. *minor* (syn. *V. riukiuensis*), *V. minima* subsp. *nakashimae* (syn. *V. nakashimae*)] are restricted to southern Japan predominantly the Nansei islands, except *V. minima* subsp. *nakashimae,* which is reported only from western Kyushu (Fig.1). *Vigna angularis* var. *nipponensis,* the wild relative of azuki bean is widely distributed across Japan as far north as the Tohoku region (東北地方).

2) *Vigna angularis* sensu lacto is a species complex, the cultigen and its close wild relative are able to cross in natural habitats. Within this species complex is a distinctive type which has been called a weedy type of azuki.

3) Since *V. angularis* sensu lacto in Japan is a crop complex it is possible that azuki beans were domesticated in Japan.

4) The three species of *Vigna* which are found only in southern Japan, represent a group of island species of unclear taxonomy that are relatively rare. Thus these species are worthy of both conservation and studying to clarify their taxonomic status.

5) These island species can sometimes be in found in coastal areas and may have a range of physiological tolerances, such as salt tolerance, which may be of value in legume breeding.

6) No other economically important genus in Japan has this array of features which would enable it to become a model genus for in-situ conservation and populations dynamics studies.

1. Goals and objectives of the research.

General

To determine the inter and intra population genetic variation among important crop relatives in Japan. The overall objective of this research is to develop in-situ conservation models for crop relatives and determine evolutionary dynamics of these agriculturally important genetic resources by long term monitoring of a core set of populations.

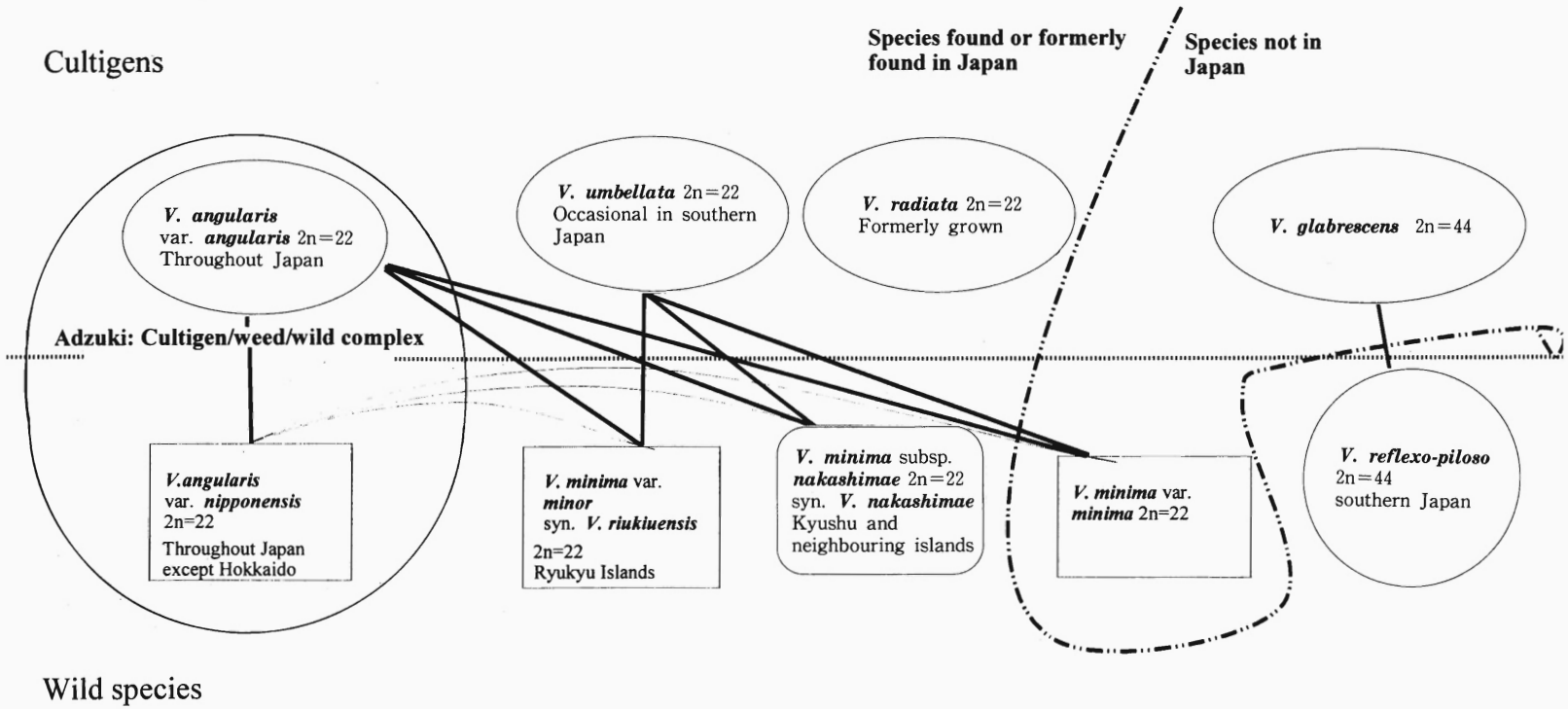

Fig.1 Japanese native species of *Vigna* and their close relatives.

Specific

a. With a focus on the *Vigna angularis* complex to sample as many populations as possible throughout its range of distribution in Japan. Since the habitat of *Glycine soja* is similar and in many places *Vigna* and *Glycine* grow together *Glycine* was also extensively collected. The populations sampled will be subjected to detailed analysis at the molecular level to determine which populations are most genetically heterogeneous and worthy of monitoring and long term in-situ conservation.

b. To conduct detailed population analysis where wild, weedy and cultivated *Vigna angularis* are sympatric to elucidate the origin of weedy azuki.

Literature review related to the *Vigna angularis* complex in Japan

The azuki bean as a crop of major importance has been studied extensively from an agricultural viewpoint. The topic has recently been reviewed by Lumpkin and McClary (1994). Regarding genetic resourdes of azuki bean several thousand accessions are held in worldwide collections, for example 2500 in the Tokachi azuki collection, Japan, 2500-3000 in the Institute of Crop Germplasm Resources, CAAS, China, 1212 in the Genebank of the Rural Development Administration, Korea (Lumpkin and McClary, 1994). Most of the germplasm collections consist of landrace varieties. Germplasm collections which consist of the complete species complex are few, one exception is the germplasm collection made by Yamaguchi (1992) between 1988-91 (see also Yamaguchi, 1989).

While there have been many investigations using different techniques to determine relationships between *V. angularis* and other species in the subgenus *Ceratotropis* [e. g. Egawa and Tomooka, 1994 (cross compatibility studies) ; Kaga et al., 1996 (RAPD analysis) ; Tateishi 1995 (morpho-taxonomic studies) ; Zink et al. 1994 (RFLP analysis)] there are very few scientific papers which have investigated the genetic resources of the *Vigna angularis* complex in Japan specifically. One paper which reports on the morphological and eco-geographic characteristics of this species complex was written by Yamaguchi (1992). A series of abstracts have been published in supplement issues of the journal Breeding Science (formerly the Japanese Journal of Breeding) on this complex. The main points made in previous publications are summarised as follows :

(a) Wild and cultivated azuki are easily distinguished at the juvenile stage. However, weedy azuki shows juvenile traits intermediate between wild and cultivated azuki. Variation in weedy types sometimes makes these forms difficult to identify. Appearance of weedy types in Japan suggests that cultivated azuki was derived from wild azuki. (Yamaguchi, 1990).

(c) SDS-PAGE banding showed little variation among samples of the azuki bean complex. However, one strain of wild azuki had a pair of extra bands (10/35kD) and two strains of cultivated lacked a pair of bands (30/27kD)(Yamaguchi and Kosuge, 1991). Polymorphism in wild azuki was also reported by Kuroda et al. (1997) based on SDS-PAGE banding at the both 60 and 70kD position.

(d) Weedy azuki can be easily distinguished from wild azuki based on characteristics of pods and size, habit, habitat, and flowering time. Domestication appears to have fixed many of the characteristics of cultivated azuki (Yamaguchi, 1993).

(e) Phylogenetic analysis using isozymes revealed that wild, weedy and cultivated azuki are very closely related based on 12 enzymes and 24 loci (Yasuda et al., 1994).

(f) Biomass studies suggest that weedy azuki is adapted to disturbed haditats and wild azuki is adapted to environments where it has to compete with other species (Yasuda and Yamaguchi, 1995).

(g) RFLP analysis revealed no polymorphism in the azuki bean complex (Takasawa and Yamaguchi, 1995).

(h) Based on SSCP (single strand conformation polymorphism) analysis of the ITS (Interstitial spacer) region wild and cultivated azuki showed very similar patterns. Two types of weedy azuki were found one type similar to wild azuki and one type similar to cultivate azuki. (Matsuomoto and Yamaguchi, 1997)

2. Methodology.

The trips aim was to obtain maximum information on populations collected, including, herbarium specimens, population sketch maps, for legumes collected, root nodule samples for *Rhizobium* analysis, in addition to, bulk population and individual plant seed samples. The collecting form used during the collecting missions is shown (Fig 2). Most of the data collected in the field is presented in the passport data tables as part of each collecting trip report.

NIAR 農林水産省農業生物資源研究所
遺伝資源第二部第四動態研究室長

CROP EVOLUTIONARY DYNAMICS LABORATORY
National Institute of Agrobiological Resources (NIAR)
Kannondai 2-1-2, Tsukuba, Ibaraki 305, Japan
Tel.81-298-38-7474 Fax.81-298-38-7408

Passport data				Date	d 7	m 11	y 97
Collectors	N. Kobayashi, R.Q.Xu, D.A. Vaughan			Collecting no.	97082		
				Plant no codes	1 — 17 + BULK		
Scientific name	Vo angularis var nipponensis		Local name				
Location							
GIS coordinate	33°53' 16.8" N		135° 10' 42.4" E				
Map reference							
Address	Land holder	J		E			
	Village	J		E	Iya		
	Nearest town	J 綾部市		E	Gobo CITY		
	Prefecture	J 和歌山県		E	WAKAYAMA		
Site							
Topography	Mountains	hills	plain	other specify			
Altitude	sea level						
Slope (degree)	Flat						
Land use	waste land						
Soil type(map)		Geology(map)					
Climate(map)							
Habitat							
Associated vegetation type		Forest	bushes	cultivated			
		grassland	other specify	waste land			
Associated plants specific		Dominant sp. Solidago Other spp.					
Shading(%)	heavy	medium	light	(open) (none)			
Degree of disturbance		(high)	med	low	none		
Population							
Size (m^2)	50m×10m						
State	vegetative	flowering	(mature)	past maturity			
Status	(wild)	weedy	cultivated	mixed			

NIAR 農林水産省農業生物資源研究所
遺伝資源第二部第四動態研究室
〒305 茨城県つくば市観音台 2-1-2
TEL 0298-38-7474
FAX 0298-38-7408

CROP EVOLUTIONARY DYNAMICS LABORATORY
National Institute of Agrobiological Resources (NIAR)
Kannondai 2-1-2, Tsukuba, Ibaraki 305, Japan
Tel.81-298-38-7474 Fax.81-298-38-7408

Introgression	yes(extent)		(no)	
Disease assessment	leaf		Pods/seed	
Pest assessment	leaf		Pod/seeds	
Plant characteristics				
Leaf pub.	High	med	low	none
Viable seeds/pod(10)				
Ovules/pod(10)				
Flower color	—			
Comments	Black seeded many small pods Pod color black or black/grey			
Special characteristics				
Collecting method	Individual + Bulk			
Observed var. In pop.				

Photo numbers	Herbarium spec.	Rhizobium collected		Collecting no.
Site ✓	(Yes) 4 sheets	(Yes)		Plant code no.
habitat ✓	no. sheets	No		
Plants ✓	No			1-17 + BULK

DRAW sketch map

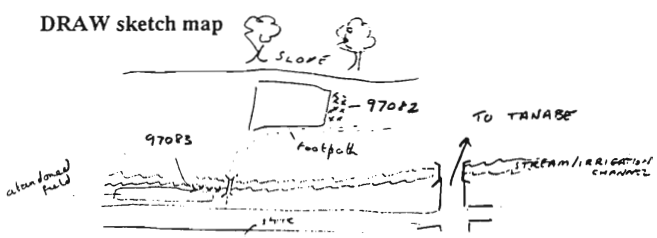

Fig.2 Passport data form used during the collection trips.

3. Trips undertaken in 1996 and 1997

Dates	Region	Team members
7,11-17th October	North-central Honshu[1)]	N. Tomooka
9-11 November	North-west Kyushu[1)]	N. Tomooka
6-11 October	Northern Honshu	N. Tomooka, M. Akiba, Elena Potokina
6,11,12 November	Kanto	N. Tomooka, S. Tsukamoto, H. Kuwahara
15-24 October	Central Honshu	N. Tomooka, S. Tsukamoto (entire trip), D. A. Vaughan, A. Konarev (15th to 20th)
3-12 November	Kinki	D. A. Vaughan, N. Kobayashi, R. Q. Xu

1) These trips are reported in Tomooka, 1997

4. Collections made

a. Summary of the collections made is presented (Table 1)

b. Maps routes (Fig. 3)

c. Collection sites (Fig. 4)

Table 1 A summary of the collecting trips collections made for wild relatives of crops with a focus on *Vigna* in 1996 and 1997.

Species	Kyushu[1)] 1996	Honshu[1)] 1996	North Honshu 1997	Central Honshu 1997	Kanto, Honshu 1997	Kinki, Honshu 1997	Total population number (individual number)
wild *Vigna*	5B[2)]	30B	5(54)	1B,7(75)	2(20)	15(97)	36B,29(246)
weed *Vigna*			2(9)	9(86)		3(34)	14(129)
Vigna complex				4(54)	3(35)	1(22)	8(111)
Cult *Vigna*				3B,1(3)		2B	5B,1(3)
Wild *Glycine*	7B	25B[2)]	3B,7(39)	7B,7(74)	5B	5B,5(54)	52B,19(167)
Cult. *Glycine*						3B	3B
Vicia spp.				3B			3B
Leersia spp.						1B	1B

1) Tomooka, 1997 ; 2) B=Bulk sample

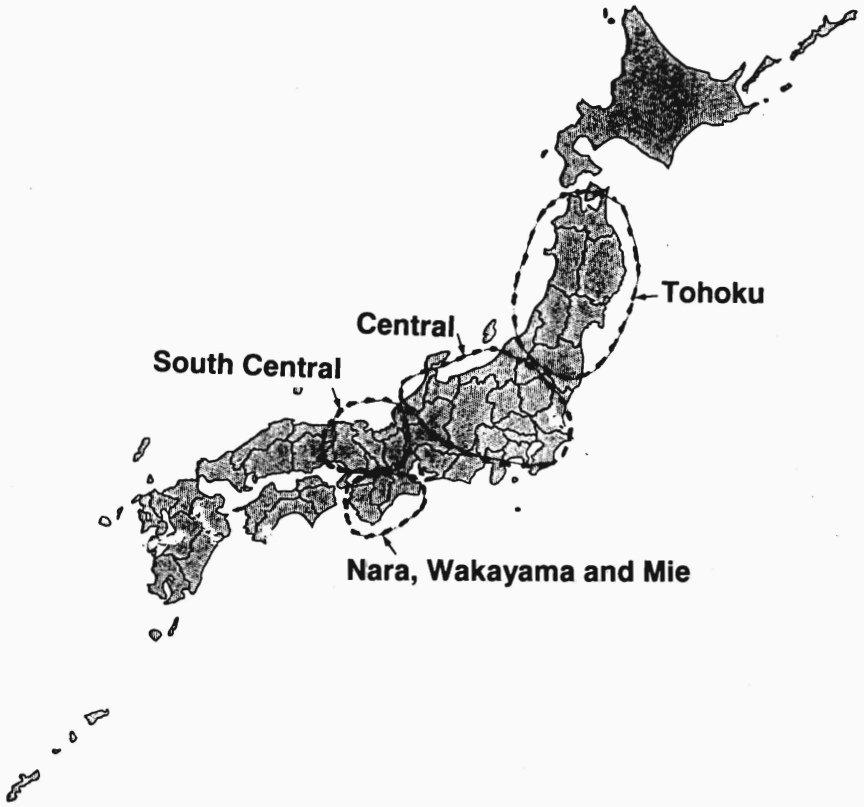

Fig.3 Regions which were used as the basis for quantitative data summary presented in Table 2.

5. General observations.

Ecological

Habitat

Habitats which appeared to represent "natural" habitats for both *Vigna angularis* var *nipponensis* and *Glycine soja* were river banks either adjacent to rivers or on sloping banks near by. Both spesies seemed to require open, herbaceous habitats characterised by *Solidago* and *Setaria*, but not *Pueraria*. *Vigna* appears to have two strategies to cope with disturbance, cut plants can regenerate from lower stem axils (observed at site 102 coll. no. 97095) and when a population is burnt new seedlings can grow (also observed at site no. 102 coll. no. 97095). Generally when *Vigna angularis* var. *nipponensis* and *Glycine soja* were growing together *G. soja* was the more abundant and appeared more vigorous. In most habitats neither *Vigna* nor *Glycine* were the dominant species. However they were frequently the most abundant climbing species.

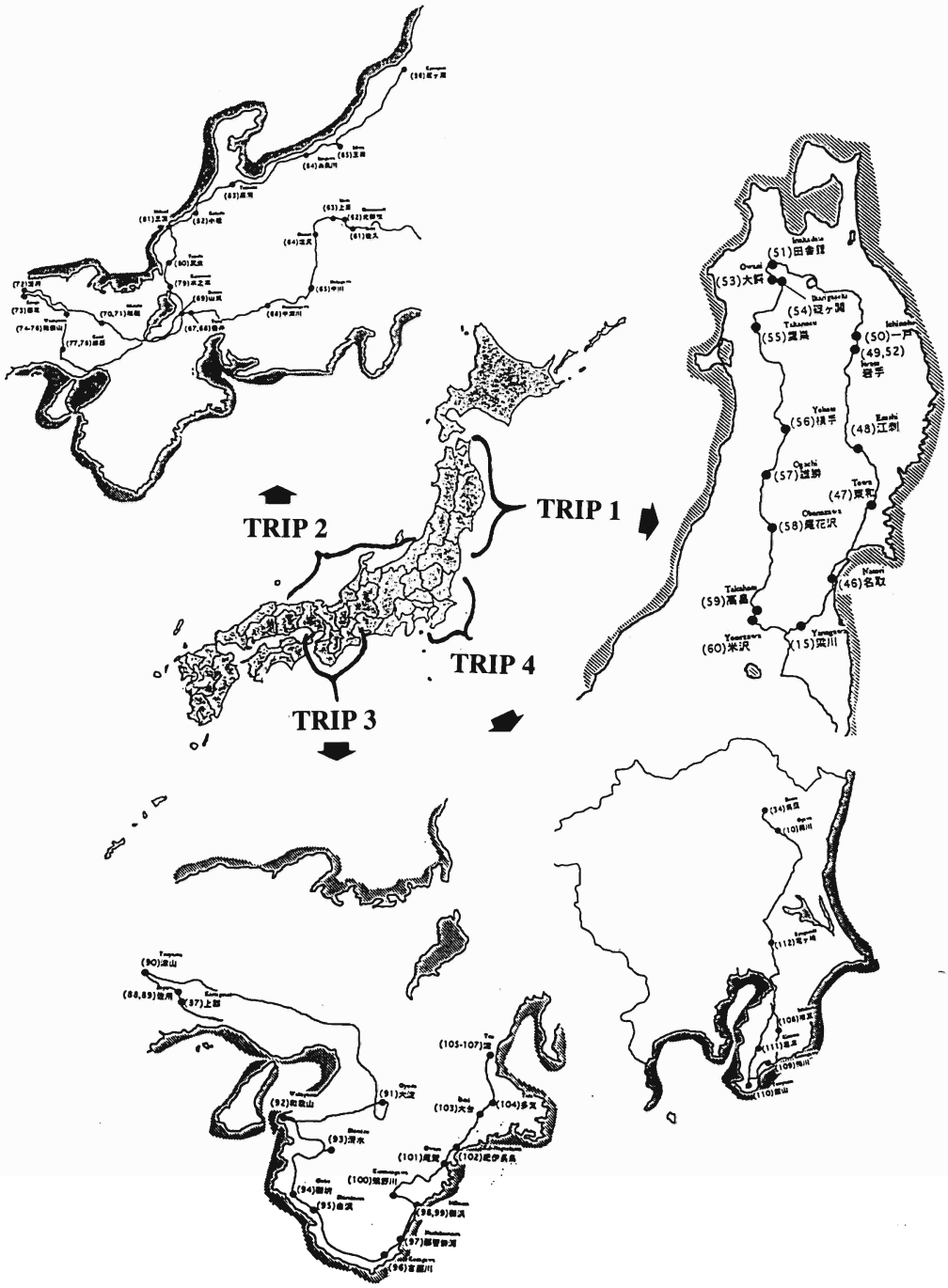

Fig.4 Collecting routes and sites in 1997 :
Trip 1. Tohoku (東北) trip report
Trip 2. Central Honshu (本州) trip report
Trip 3. Kinki (近畿) region trip report
Trip 4. Tochigi (栃木), Ibaraki (茨城) and Chiba (千葉) trip report

Habit

Despite growing in the same or similar habitats the plant architecture of wild *Vigna* and *Glycine* are very different. Some of the main differences are shown below.

Trait	*Vigna angularis* var. *nipponensis*	*Glycine soja*
Roots	Thick	Slender
Pods	Long	Short
Seeds/pod	3-11	2-3
Stem	Thick	Slender
Maturity	Late	Early

Comments on wild verses "weedy" azuki.

1) we have used the term weedy azuki for a type of azuki with a set of characteristics, such as larger seed size. However, generally weedy azuki was not found in field but generally near cultivated field a habitat similar to wild azuki. It is possible that this is not weedy but a wild ecotype. Yamaguchi and his co-workers use the terms weedy and semi-wild for this form (Yamaguchi, 1993 ; Yamaguchi and Kosuge, 1991 ; Yasuda and Yamaguchi 1995, 1996).

2) Population history. We observe a population at a moment in time. We do not know the history of the population during that year or during previous years.

3) Weedy *Vigna* was found in two places in low lying wet habitats beside small streams (97036 and 97083). Wild *Vigna* was not found during the trips in this type of habitat.

4) Field identification of wild/weedy plants was based primarily on habit, leaf size and maturity. The typical habit of wild and weedy azuki beans are shown (Fig 5 and 6 and below).

	Wild azuki	Weedy azuki
Maturity	Early	Late
Habit	Indeterminate, twining stems slender basal stem thick	Determinnate and indeterminate, thick stems
Leaf size	Small	Large

Quantitative characters (preliminary) (see Table 2)

A summary of quantitative data obtained from pods and seede collected in the field is presented (Table 2). The data summarises the record for regions shown in Fig. 3.

(A) *Vigna angularis* complex (Table 2a)

Clear differences are seen (Table 2a) between wild azuki (*V. angularis* var. *nipponensis*) and weedy azuki (*V. angularis* var. *angularis*). Pod length is longer and 100 seed weight is greater in weedy populations. However, the weedy populations for the characters shown, are much more similar to wild azuki than cultivated azuki. For example, 100 seed weight for cultivated azuki is generally about 10g. Regional differences cannot clearly be seen from the data presented, although pod length and 100 seed weight for Tohoku weedy azuki is greater than southern populations this may be a distortion since the sample number is only 10.

(B) *Glycine soja* (Table 2b)

Variation in pod length, seed number per pod and seed weight showed very little difference within and between regions. Generally pod length was about 2-3cm, pods have 3 seeds and 100 seed weight varies from 2.5-3g. Regional variation for the traits measure is not apparent.

Biochemical and molecular characters (preliminary)

Biochemical and molecular observations.

Variation at in total seed protein banding has been investigated in *Vigna angularis* var. *nipponensis* based on populations collected in 1996 from northern Honshu and Kyushu. Very little variation was observed (Fig.2) (Tomooka, 1997 ; Rao, 1997) although some other workers have reported genetic polymorphism in natural populations of wild azuki for seed protein banding (Kuroda et al., 1997). A survey of the 1996 collections for the four isozyme systems SDH, GOT, 6-PGDH and LAP did not reveal variation among different populations (Fig.3).

Using collections made in 1997 we have begun to look at variation at the DNA level. Preliminary screening of 11 populations has detected variation between populations for 45 primers out of 133 screened. 22 primers revealed clear intra population variation for one population which consisted of wild, weedy and cultivated *Vigna angularis* (Fig.9). Other DNA marker technologies, such as AFLPs and SSRs, which are more powerful at revealing polymorphism at the DNA level and provide different types of complementary genetic information, will be applied to the populations collected to obtain improved resolution of the inter and intra-population genetic diversity of this crop complex.

Table 2a Summary of data on 3 characteristics for field collected samples of wild, weedy and mixed wild and weedy populations of the *Vigna angularis* complex

Location	Pod length		Seeds/pod		100 seed weight[1]	
	Mean	Range	Mean	Range	Mean	Range
Vigna angularis var. *nipponensis* (wild)						
Tohoku	6.1 (59)[2]	4.6-7.3	8.28 (59)	3-11	2.51 (47)	1.85-4.15
Central	6.6 (53)	4.0-8.1	9.05 (53)	5-11.8	2.61 (41)	1.8-4.05
South Central	5.98 (87)	2.9-7.5	8.5 (87)	3-11.2	2.69 (62)	2.1-3.65
Nara, Wakayama and Mie	6.16 (107)	4.2-7.9	8.73 (107)	5-11	2.94 (84)	1.85-4.3
Vigna angularis var. *angularis* (weedy)						
Tohoku	7.84 (10)	3.3-9.7	9.25 (10)	5-11.5	4.65 (8)	2.4-7.05
North Central	7.17 (59)	3.9-9.5	8.58 (59)	5.2-11.8	4.0 (59)	3.3-5.45
South Central	6.49 (32)	5.2-8.0	8.46 (32)	5-10.6	3.11 (32)	1.75-6.4
Nara, Wakayama and Mie	6.51 (34)	2.9-9.2	7.6 (34)	4-11	3.62 (30)	2.7-5.3
Vigna angularis wild and weedy plants in mixed populations						
Tohoku	—	—	—	—	—	—
North Central	6.86 (60)	4.3-8.71	9.01 (60)	3.7-11.2	3.0 (48)	2.05-4.85
South Central	6.39 (46)	4.4-8.2	8.83 (46)	6.2-11.4	3.11 (34)	1.45-5.5
Nara, Wakayama and Mie	7.56 (22)	5.7-9.4	8.72 (22)	5-11	4.7 (14)	3.6-5.65

1) The seed weight of 20 seeds per plant or population in the case of bulk samples were weighed to obtain the value. In the case where less than 20 seeds were available this sample was not included in the calculation.

2) Numbers in parenthesis are the number of samples

Table 2b Summary of data on 3 characteristics of wild soydean (*Glycine soja*) based on field collected individual and population samples

Location	Pod length		Seeds/pod		100 seed weight[1)]	
	Mean	Range	Mean	Range	Mean	Range
Tohoku	2.72 (45)[2)]	2.5-3.1	3.02 (45)	2.6-3.4	2.52 (45)	2.05-3.65
Central	2.55 (46)	1.9-3.5	3.04 (46)	2.4-5.0	2.63 (40)	1.95-3.87
South Central	2.63 (11)	2.3-3.1	2.87 (11)	2.6-3.0	3.16 (11)	2.6-3.95
Nara, Wakayama and Mie	2.42 (66)	2.0-3.3	2.95 (66)	2.2-4.0	2.68 (35)	1.85-3.85

1) The seed weight of 20 seeds per plant or population in the case of bulk samples were weighed to obtain the value. In the case where less than 20 seeds were available this sample was not included in the calculation.

2) Numbers in parenthesis are the number of samples

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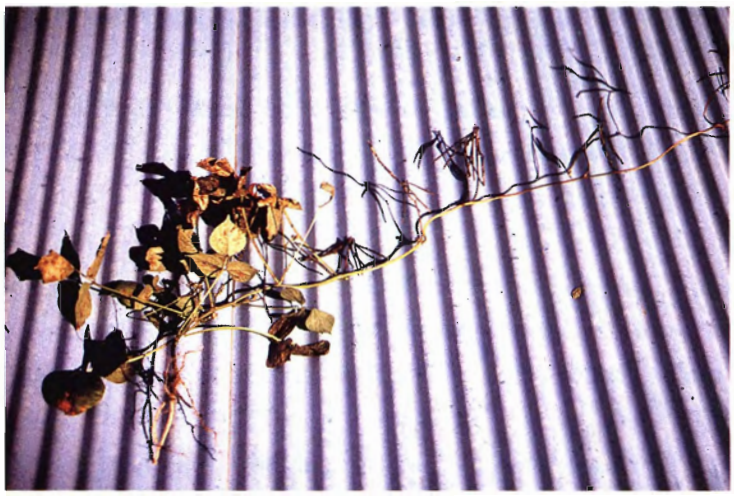

Fig.5 Habit of wild azuki (*Vigna angularis* var. *nipponensis*) collection number 97080, showing indeterminate habit.

Fig.6 Habit of weedy azuki (*Vigna angularis* var. *angularis*) collection nember 97071 growing in an abandoned field showing determinate habit.

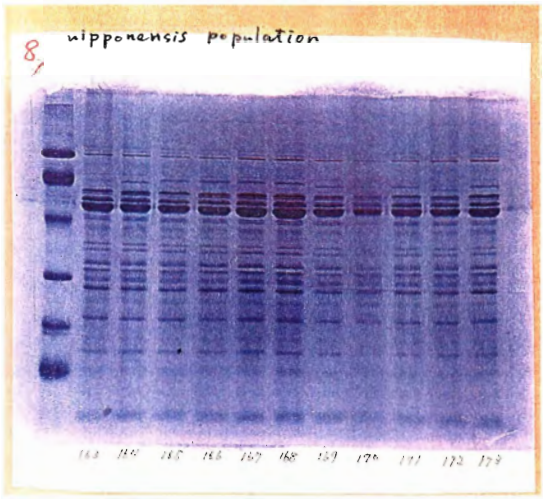

Fig.7 Total seed protein banding pattern using SDS PAGE method. Lane 1 protein molecular weight marker, 163 sample from Fukushima (福島),164-169 samples from Tochigi (栃木), 170 sample from Kumamoto (熊本), 171-173 samples from Saga (佐賀). 1996 collection. No variation was found in these samples.

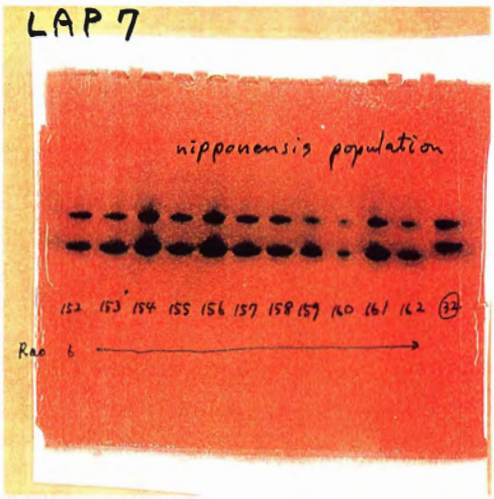

Fig.8 Analysis of *Vigna angularis* var *nipponensis* for variation in the isozyme LAP (Leucine amino peptidase) Samples 152-156 from Fukushima (福島), samples 157-161 from Niigata (新潟). 1996 collections. No variation was found in these samples.

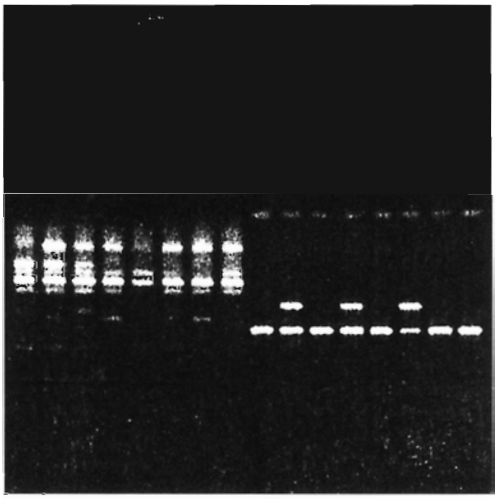

Fig.9 Banding pattern for two primers left (primer 41) right (primer 35). Polymorphism shown on the right using primaer 35 were samples from one population from Tottori (鳥取) (97047).