Glycine Genetic Resources

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A. The Soybean Phenomena

The very recent history of the ancient crop the soybean has been quite remarkable. It took several thousand years for soybean to reach a global production of about 27 million metric tons that was 1961. That production doubled in the following twelve years. By 2005 world soybean harvest was 214 million metric tons or about 8 times what it was 40 years earlier.

Soybean has become the dominant legume feeding the global population and the story of its history in the New World is illustrative of how rapidly agriculture can change. Between 1929 and 1939 soybean production increased by 970% due to a combination of factors but particularly the development of the soybean processing industry. The rise of soybean production in Brazil is equally remarkable and fast with Brazil's share of global soybean production rising from 1% in 1961 to 10% in 1973.

While the recent history of soybean has been dominated by events in the New World it is the Far East that is the home of soybean and its single close wild relative *G. soja*. The genus *Glycine* is split into two subgenera, subgenus *Glycine* and subgenus *Soja*. Subgenus *Glycine* consists of 26 perennial species mainly found in or near Australia. Subgenus *Soja* consists of the East Asian wild annual species *G. soja* and cultivated soybean, *G. max*.

In this paper we will briefly discuss the wild genetic resources of *Glycine* and their diversity and potential in crop improvement.

B. The Place of Soybean among other domesticated legumes

The genus *Glycine* consists of species with chromosome numbers of 2n=2x=40 or multiples thereof. One species, *G. tomentella* consists of a complex of cytotypes including various stable aneuploids with chromosome numbers of 38 or 78. The phylogenetically close relatives of *Glycine* such as *Vigna* and *Phaseolus* have chromosome numbers of 11 or rarely multiples thereof. *Glycine* is a model genus for studying ancient polyploidy (Doyle *et al*. 2004).

Glycine is phylogenetically closely related to a large number of important domesticated legumes including common bean (*Phaseolus* 2n=2x=22), cowpea and mungbean (*Vigna* 2n=2x=22), pigeonpea (*Cajanus* 2n=2x=22) and winged bean (*Phosocarpus* 2n=2x=18) (Fig. 1).

C. The Genepools of the Soybean

The genepools of soybean are shown (Fig. 2). It has proved extremely difficult to make successful hybrids using conventional techniques between the two subgenera, subgenus *Soja* and subgenus *Glycine*. To achieve gene transfer between these two subgenera radical techniques need to be used. To date only three of the 26 wild perennial species of *Glycine* have been hybridized with soybean, *G. argyrea* Tindale, *G. canescens* F.J. Hermann and *G. tomentella* Hayata.

D. The subgenus *Glycine*

In 1977 six perennial wild *Glycine* were recognized (Broué *et al*. 1977) but since that time there has been extensive exploration and biosystematic studies and currently 26 perennial *Glycine* species are recognized, many of then with highly restricted distribution in Australia. In Japan two perennial wild *Glycine* are found in highly restricted parts of Okinawa, *G. tabacina* (Labill.) Benth and *G. koidzumii* (Ohwi). *G. tabacina* is only known from a small islands of Ie (Okinawa) and Okinoerabu (Kagoshima) and *G. koidzumii* is only known from on the small islands of Miyako and Ishigaki (Okinawa).

Polyploidy

G. tabacina and *G. tomentella* have been studied in relation to their polyploidy origins. *G. tabacina* consists of two types of tetraploid that have different origins. One type which has adventitious roots (2n=80) (WAR) and another without (NAR). The former (WAR) is a segmental allopolyploid while the later (NAR) is a true allopolyploid (Singh *et al*. 2006). *G. tomentella* consists of 4 morphologically indistinguishable cytotypes 2n=38, 40, 78 and 80. Among these cytotypes there are three groups of aneutetraploids and three groups of tetraploids. Various studies suggest that these groups result from multiple independent allopolyploid events (Doyle *et al*., 2004; Singh *et al*. 2006).

Uses

The perennial wild *Glycine* harbor many potentially useful genes among them resistance to soybean rust (*Phackopsora pachyrhizi* Sydow) and soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) (Singh and Hymowitz, 1999). To date there is only one report of fertile backcross derived progenies between soybean and wild perennial soybean, *Glycine tomentella* (2n=78) (Singh *et al*., 1998). Introgression of SCN resistance was reported in lines derived from this cross (Riggs *et al*. 1988).

To fully exploit useful genes from perennial wild *Glycine* in soybean breeding new approaches are needed.

E. The subgenus *Soja*

Japan is a center of diversity for soybean and wild soybean (*Glycine soja* Sieb. & Zucc.). Over the past decade while collecting wild *Vigna* across Japan our laboratory also collected sympatric wild soybeans. The wild soybeans collected have become the basis for our studies of wild soybean diversity in Japan and beyond. In addition, our group has also undertaken large scale search for evidence of gene flow from cultivated to wild soybeans and subsequent monitoring of sites where this was found. Hundreds of hours have been spent searching, in the most likely areas of Japan, for clear evidence of soybean/wild soybean hybrids. These have been found mainly in Saga prefecture on the southern main island of Japan, Kyushu, where soybeans are widely grown and sympatric with wild soybeans. The record of the search for wild soybean/soybean hybrids can be found in the trip reports given in Appendix 1 authored by A. Kaga and Y. Kuroda. The rest of this paper will discuss the results of studies lead by Drs. Akito Kaga and Yosuke Kuroda.

(a) Wild soybean core collection (Kuroda *et al*. 2009)

SSR (microsatellite) markers were used to analyze a large collection of wild soybean accessions (1305) from across its area of distribution and Japanese cultivated soybeans. The results of the study revealed:

i. SSR allelic diversity could be clustered into 53 groups that largely corresponded with different geographic locations. This supports the view from other studies that wild soybeans are largely differentiated based on geography, suggesting long distance dispersal is uncommon.

ii. Average alleles per locus for wild soybeans (about 28 alleles/locus) were much higher than for Japanese cultivated soybean (about 5 alleles per locus)

iii. Allelic variation in Japanese wild and cultivated soybeans was similar supporting the view that soybean may have been separately domesticated in Japan.

iv. Russian germplasm showed a high level of allelic diversity that may reflect waves of introductions of wild soybean into Russia from China.

v. A high proportion of rare alleles were found in Korean wild germplasm and may reflect introductions from China and Japan and local evolution in isolated valley's in Korea.

This study provided information on which to select accessions for a core and mini core collection. The core collection consists of 192 accessions (14% of accessions) with 97% of the allelic diversity of the whole collection. The mini core collection consisted of 53% of accessions (5% of the whole collection) with 62.4% of the allelic diversity of the whole collection (Table 1).

(b) Population genetic structure of wild soybean across Japan (Kuroda *et al*. 2006)

The second study involved analysis of the population genetic structure of 77 wild soybean populations from across Japan based on 8 individuals each. The populations all showed low level of observed heterozygosity, low out-crossing rate (mean 3.4%) and strong genetic differentiation among populations. There was evidence of rare long distance dispersal over 10km and also populations within a radius of 100 kilometers showed a close genetic relationship to one another.

The genetic variation in wild soybeans was compared with Japanese cultivated soybeans and that revealed that the primary factor of genetic differentiation was the difference associated with domestication and the secondary factor was geographic differentiation.

Admixture analysis revealed that 6.8% of individuals appeared to show introgression from cultivated soybeans. These results indicated that population genetic structure of Japanese wild soybeans is (i) strongly affected by the founder effect due to seed dispersal and inbreeding strategy (ii) generally wild soybean is well differentiated from cultivated soybean, but (iii) introgression from cultivated soybean does occur.

(c) Fine scale genetic structure of wild soybean in selected parts of Japan (Kuroda *et al.* 2008)

To further understand the genetic structure of wild soybean 14 populations from different parts of Japan, northern, central and southern,were studied. 7 of these populations were adjacent to soybean fields

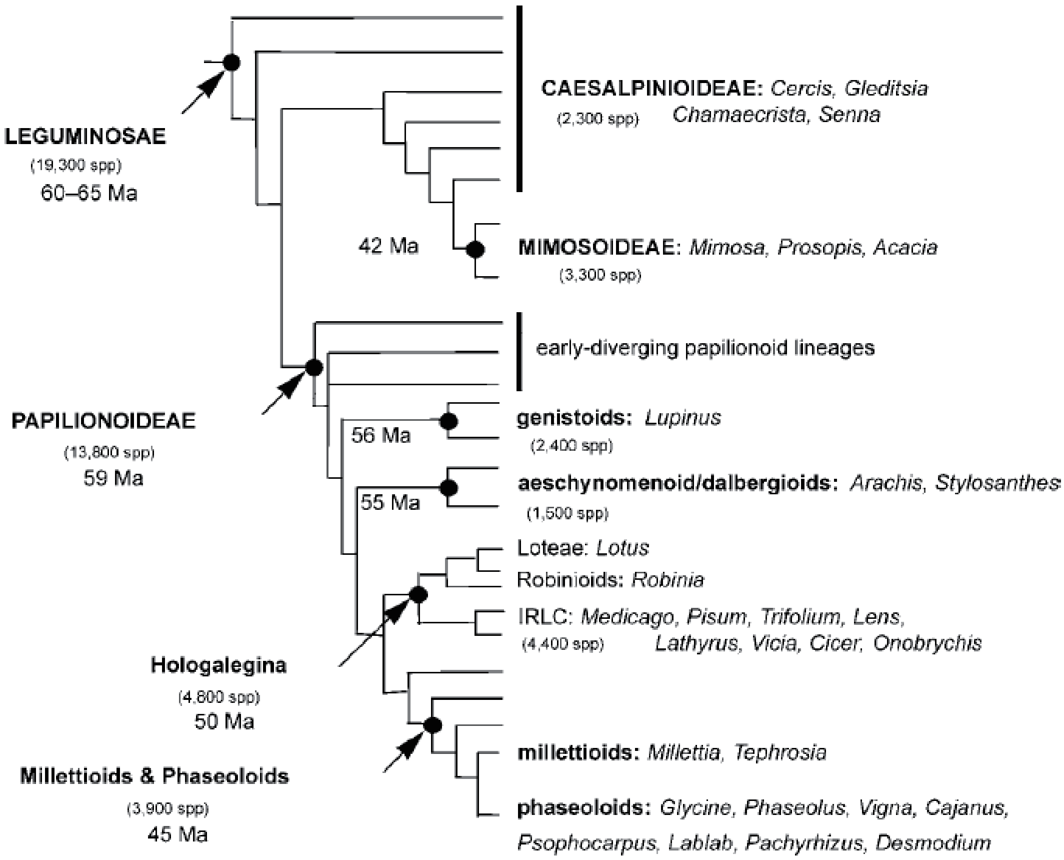

Fig. 1. The Phylogenetic position of *Glycine* (modified from Gepts *et al*.,2005)

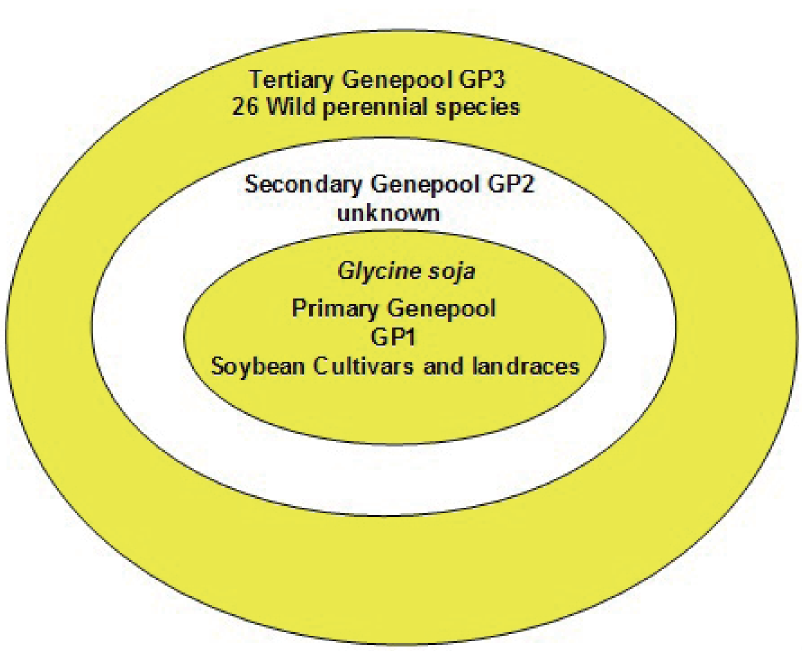

Fig. 2. The genepools of the soybean

Table 1. Composition of two core sets of germplasm (from Kuroda *et al*.2009)

Populations	*N*		*A*		allele cover(%) /total	allele cover(%) /region
Core53	53		353		62.4	
China		7		101		27.7
Russia		7		104		28.6
Korea		9		133		36.5
Japan		29		271		74.5
Max		1		20		5.5
Core192	192		549		97.0	
China		34		262		72.0
Russia		32		219		68.0
Korea		56		358		77.5
Japan		65		348		85.9
Max		5		44		40.4

N(number of samples), *A*(number of alleles)

and seven were more than 60m distant from soybean fields i.e. further than distance of pollen flow in soybeans. SRR analysis of individuals in these populations revealed no evidence of pollen from soybean to wild soybean. However, in populations of *G.soja* outcrossing rate ranged from 0-6.3% (average 2.2%) and the dispersal distance of pollen ranged from 5-25m (average 10.5m). In addition, 13 individuals of *G. soja* (7.7%) collected in four populations were assigned to neighboring populations (100-400m). The results suggest the occurrence of long-distance dispersal in *G. soja*.

Uses of G. soja

The actual use of *G. soja* in soybean breeding has been limited despite it having a much higher level of genetic variation than cultivated soybean and these being no barrier to hybrid formation. *G. soja* has been used a source of high protein and productivity (Hartwig 1973). Several small seeded cultivars of soybean have been developed for bean sprouts and natto using *G. soja* as non-recurrent parent (Carter *et al*. 1995; Fehr *et al*. 1990 a, b).

F. Concluding remarks

Despite the primary genepool of soybean being restricted to two species and secondary genepool being absent, cultivated soybean has become one of the world's most important crops. The large scale cultivation of soybean means it is vulnerable to pests and disease. Innovative breeding to tap the diverse sources of useful genes in the tertiary genepool of soybean are needed to strengthen the genetic basis of soybean.

References cited

Carter *et al*. 1995. Registration of 'Pearl' soybean. Crop Sci. 35:1713

Fehr W.R. *et al*. 1990a. Registration of 'SS201' soybean. Crop Sci. 30:1361

Fehr W.R. *et al*. 1990b. Registration of 'SS202' soybean. Crop Sci. 30:1361

Doyle, J.J., J. L. Doyle, J. T. Rauscher and A.D.H.Brown. 2004. Evolution of the perennial soybean polyploidy complex (*Glycine* subgenus *Glycine*): a study of contrasts. Biological Journal of the Linnean Society 82: 583-597

Paul Gepts*, William D. Beavis, E. Charles Brummer, Randy C. Shoemaker, H. Thomas Stalker, Norman F. Weeden, and Nevin D. Young 2005. Legumes as a Model Plant Family. Plant Physiology, Vol. 137, pp. 1228-1235.

Hartwig, E.E. 1973. Varietal development. In Soybeans: Improvement, production and Uses. American Society of Agronomy Publication 16. B.E. Caldwell (ed.) American Society of Agronomy, Madison, WI, pp. 187-210

Kuroda, Y., N. Tomooka, A. Kaga, S.M.S.W. Wanigadeva and D.A. Vaughan. 2009 Genetic diversity of wild soybean (*Glycine soja* Sieb. et Zucc.) and Japanese cultivated soybeans [*G. max* (L.) Merr.] based on microsatellite (SSR) analysis and the selection of a core collection. Genetic Resources and Crop Evolution (on-line)

Kuroda, Y., A. Kaga, N. Tomooka and D.A.Vaughan. 2006. Population structure of Japanese wild soybean (*Glycine soja*) based on microsatellite variation. Molecular Ecology 15:959-974

Kuroda, Y., A. Kaga, N. Tomooka and D.A.Vaughan. 2006. Gene flow and genetic structure of wild soybean (*Glycine soja*) in Japan. Crop Science 48:1071-1079

Riggs, R.D. et al. 1998. Possible transfer of resistance to Heterodera glycines from *Glycine* tomentella to *Glycine max*. J. Nematol. 30:547-552.

Shurtleff, W. and A. Aoyagi nd. A special report on history of soybean production and trade around the world. (online at http://www.soyinfocenter.com/HSS/history.php)

Singh, R.J., K.P. Kollipara and T. Hymowitz. 1998. Monosomic alien addition lines derived from *Glycine max* (L.) Merr., and *G. tomentella* Hayata: production, characterization and breeding behavior. Crop Sci. 38: 1483-1489.

Singh, R.J. and T. Hymowitz. 1999. Soybean genetic resources and crop improvement. Genome 42:605-616

Singh, R.J., R.L. Nelson and G. Chung. 2006. Soybean [*Glycine max* (L.) Merr.] in “Genetic Resources, chromosome engineering and crop improvement” R.J. Singh (editor)

Appendix 1. MAFF Domestic Exploration Trips for *Glycine* and *Vigna* species from 1997 to 2008.
Trip reports listed in References can be downloaded from NIAS genebank web page at
http://www.gene.affrc.go.jp/publications.php#plant_report (each report can be found in Annual report on Exploration and Introduction of Plant Genetic Resources, NIAS)
Most of the reports are written in Japanese with English Summary, Tables and Figures.

Year	Area	Species	No.	References
1997	Mie & Nara	*Glycine max*	26	Nakayama & Katsuta (S), 1998
		Vigna angularis var. *angularis*	10	
		Vigna umbellata	3	
1997	Yamagata	*Glycine max*	2	Kikuchi et al., 1998
		Glycine soja	94	
		Vigna angularis var. *nipponensis*	5	
1997	Shimane	*Vigna angularis* var. *nipponensis*	1	Nakanishi et al., 1998
1997	Fukushima, Miyagi,	*Glycine soja*	10	Tomooka et al., 1998
	Iwate, Aomori	*Vigna angularis* (weedy)	2	
		Vigna angularis var. *nipponensis*	5	
1997	Nagano	*Glycine soja*	16	Tomooka et al., 1998
	Gifu, Shiga,	*Vigna angularis* var. *angularis*	4	
	Kyoto,Tottori,	*Vigna angularis* (weedy)	8	
	Hyogo,Fukui,	*Vigna angularis* (wild - weedy complex)	4	
	Ishikawa,	*Vigna angularis* var. *nipponensis*	9	
	Toyama			
	Niigata			
1997	Hyogo,	*Glycine soja*	12	Tomooka et al., 1998
	Okayama	*Glycine max*	3	
	Nara,	*Vigna angularis* var. *angularis*	2	
	Wakayama,	*Vigna angularis* (weedy)	4	
	Mie	*Vigna angularis* (wild - weedy complex)	1	
		Vigna angularis var. *nipponensis*	13	
1997	Tochigi,	*Glycine soja*	5	Tomooka et al., 1998
	Ibaraki,	*Vigna angularis* (wild - weedy complex)	1	
	Chiba	*Vigna angularis* var. *nipponensis*	3	
1998	Wakaykama,	*Glycine max*	26	Nakayama et al., 1999
	Nara,Mie	*Vigna angularis* var. *angularis*	15	
		Vigna unguiculata	2	
1998	Yamanashi,	*Glycine soja*	3	Tomooka et al., 1999
	Tochigi	*Vigna angularis* var. *angularis*	4	
	Ibaraki	*Vigna angularis* (weedy)	4	
	Shiga	*Vigna angularis* var. *nipponensis*	6	
1998	Okayama,	*Glycine soja*	6	Tomooka et al., 1999
	Shimane,	*Vigna angularis* var. *angularis*	2	
	Yamaguchi,	*Vigna angularis* (weedy)	4	
	Fukuoka,	*Vigna angularis* var. *nipponensis*	10	
	Saga,	*Vigna nakashimae*	1	
	Nagasaki			
1998	Fukuoka,	*Glycine soja*	14	Vaughan et al., 1999
	Kumamoto,	*Vigna angularis* var. *angularis*	4	
	Oita,	*Vigna angularis* (weedy)	1	
	Kagoshima,	*Vigna angularis* var. *nipponensis*	10	
	Miyazaki	*Vigna unguiculata* (escaped)	4	
		Vigna unguiculata (cultivated)	3	
1998	Yamaguchi,	*Glycine soja*	9	Vaughan et al., 1999
	Hiroshima,	*Vigna angularis* var. *angularis*	1	
	Okayama,	*Vigna angularis* (weedy)	1	
	Aichi,	*Vigna angularis* var. *nipponensis*	3	
	Shizuoka	*Vigna umbellata*	1	
1998	Ehime, Kouchi	*Glycine soja*	14	Vaughan et al., 1999
		Vigna angularis var. *angularis*	1	
		Vigna angularis var. *nipponensis*	9	
		Vigna unguiculata	2	
1999	Ooita,	*Glycine max*	10	Nakayama & Miura, 2000
	Kumamoto,	*Vigna angularis* var. *angularis*	17	
	Miyazaki	*Vigna unguiculata*	1	
1999	Okinawa	*Vigna luteola*	3	Tomooka et al., 2000
	(Ishigaki,	*Vigna marina*	5	
	Iriomote)	*Vigna reflexo-pilosa*	8	
		Vigna riukiuensis	5	
1999	Tottori,	*Vigna angularis* (weedy)	1	Vaughan et al., 2000
	Okayama	*Vigna angularis* (wild - weedy complex)	5	
		Vigna angularis var. *nipponensis*	7	

Appendix 1.

1999	Okinawa (Miyako Yaeyama)	*Glycine max* *Glycine koidzumii* *Vigna luteola* *Vigna marina* *Vigna reflexo-pilosa* *Vigna riukiuensis* *Vigna unguiculata* (escaped)	1 5 2 17 10 27 5	Kaga et al., 2000
2000	Fukushima	*Glycine max* *Vigna angularis* var. *angularis*	26 14	Nagamine et al., 2001
2001	Niigata (Sado)	*Glycine max* *Vigna angularis* var. *angularis*	20 10	Fukuoka et al., 2002
2001	Niigata & Yamagata	*Glycine max* *Vigna angularis* var. *angularis*	35 12	Ishii et al., 2002
2001	Nagano	*Glycine soja* *Vigna angularis* (weedy) *Vigna angularis* var. *nipponensis*	13 2 10	Dharmadasa Weerasekera et al., 2002
2001	Okinawa (Aguni, Iheya, Tokashiki, Ie)	*Glycine tabacina* *Vigna luteola* *Vigna marina* *Vigna radiata* *Vigna reflexo-pilosa* *Vigna riukiuensis* *Vigna unguiculata*	1 1 8 1 3 4 4	Kalubowila et al., 2003
2002	Aichi, Gifu	*Glycine max* *Vigna angularis* var. *angularis*	12 5	Ebana & Ishii 2003
2002	Chiba	*Glycine max* *Glycine soja*	3 48	Hajika et al., 2003
2003	Ishikawa, Toyama	*Glycine max* *Glycine soja* *Vigna angularis* var. *angularis* *Vigna* sp.	9 2 5 4	Kojima & Kawase, 2004
2003	Akita, Iwate	*Glycine soja* *Vigna angularis* var. *angularis*	80 2	Kono et al., 2004
2004	Tokushima, Kouchi	*Glycine max* *Glycine soja* *Vigna angularis* var. *nipponensis*	1 35 3	Kikuchi et al., 2005
2004	Hiroshima, Akita	*Glycine max* *Glycine soja* *Vigna angularis* var. *angularis* *Vigna angularis* var. *nipponensis*	5 31 1 2	Kaga et al., 2005
2004	Akita, Ibaraki, Aichi, Hiroshima, Saga	*Glycine soja* *Glycine soja* (weedy) *Vigna angularis* (wild or weedy complex) *Vigna raidata* (weedy) *Vigna umbellata* (weedy) *Vigna unguiculata* (weedy)	86 19 12 1 1 1	Kuroda et al., 2005
2005	Akita, Ibaraki, Kouchi, Saga	*Glycine max* *Glycine soja* *Vigna angularis* var. *angularis* (escape) *Vigna angularis* var. *nipponensis* *Vigna radiata* var. *radiata* (escape) *Vigna umbellata* (escape) *Vigna unguiculata* (escape)	12 51 13 16 2 5 2	Kuroda et al., 2006
2006	Kouchi	*Glycine soja*	37	Saruta et al., 2007
2006	Akita, Hyogo, Saga	*Glycine max* *Glycine soja* *Vigna angularis* var. *nipponensis* *Vigna unguiculata* (escape)	3 44 26 1	Kuroda et al., 2007
2007	Shizuoka, Akita, Nagano, Fukushima, Chiba,Tochigi, Ibaraki	*Glycine soja* *Vigna angularis* var. *nipponensis*	53 7	Yamada et al., 2008
2007	Yamagata, Tottori, Hyogo, Kyoto, Saga, Fukuoka, Ooita	*Glycine max* *Glycine soja* *Vigna angularis* var. *angularis* *Vigna angularis* (weedy) *Vigna angularis* var. *nipponensis* *Vigna unguiculata*	2 28 7 7 15 1	Tomooka et al., 2008
2008	Shiga	*Glycine max*	9	Okuizumi et al, 2008

Appendix 2. MAFF Overseas Exploration Trips for *Glycine* and *Vigna* species from 1999 to 2008.
"Trip reports listed in References can be downloaded from NIAS genebank web page at
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Some reports are written in Japanese with English Summary, Tables and Figures.
Others are written in English with Japanese Summary."

Coll. Year	Country	Species	No.	References
1999	Greece	*Vigna unguiculata*	3	Hida et al., 2000
1999	Thailand	*Vigna exilis*	14	Tomooka et al., 2000
		Vigna grandiflora	1	
		Vigna hirtella	3	
		Vigna minima	9	
		Vigna trinervia	3	
		Vigna umbellata	31	
		Vigna unguiculata	1	
2000	Sri Lanka	*Vigna aridicola*	2	Tomooka et al., 2000
		Vigna stipulacea	2	
		Vigna trilobata	7	
		Vigna trinervia	4	
2000	Vietnam	*Glycine max*	30	Shimada et al., 2001
		Vigna angularis var. *angularis*	2	
		Vigna radiata	12	
		Vigna umbellata	5	
		Vigna unguiculata	16	
2001	Sri Lanka	*Vigna aridicola*	12	Tomooka et al., 2003
		Vigna dalzelliana	3	
		Vigna radiata var. *sublobata*	1	
		Vigna stipulacea	2	
		Vigna trilobata	15	
		Vigna trinervia	4	
2002	Myanmar	*Glycine max*	41	Takahashi et al., 2002
		Vigna mungo	5	
		Vigna radiata	5	
		Vigna umbellata	19	
		Vigna unguiculata	20	
2002	Myanmar	*Vigna mungo*	1	Nakagawa et al., 2002
2002	Myanmar	*Glycine max*	6	Tomooka et al., 2003
		Vigna angularis (weedy)	1	
		Vigna angularis var. *nipponensis*	7	
		Vigna hirtella	7	
		Vigna mungo	3	
		Vigna mungo var. *silvestris*	1	
		Vigna radiata	8	
		Vigna radiata var. *sublobata*	4	
		Vigna stipulacea	5	
		Vigna tenuicalulis	21	
		Vigna trilobata	1	
		Vigna trinervia	1	
		Vigna umbellata	10	
		Vigna umbellata (escape)	1	
		Vigna umbellata (wild)	1	
		Vigna unguiculata	8	
2003	Vietnam	*Glycine max*	32	Shimada et al., 2005
		Vigna angularis var. *angularis*	19	
2004	Myanmar	*Vigna stipulacea*	1	Uga et al., 2005
		Vigna tenuicalulis	1	
		Vigna sp.	3	
2004	Papua New Guinea	*Glycine tomentella*	3	Tomooka et al., 2005
		Vigna marina	1	
		Vigna radiata var. *sublobata*	14	
		Vigna reflexo-pilosa	5	
		Vigna unguiculata (wild)	1	
2004	Chinana (Yunan) & Laos	*Glycine max*	5	Tomooka et al, 2005
		Vigna angularis var. *angularis*	2	
		Vigna hirtella	10	
		Vigna minima	4	
		Vigna reflexo-pilosa	1	
		Vigna tenuicaulis	2	
		Vigna umbellata	20	
		Vigna unguiculata	4	

Appendix 2.

2005	Laos	*Vigna hirtella*	7	Tomooka et al., 2004
		Vigna minima	5	
		Vigna reflexo-pilosa	1	
		Vigna umbellata	5	
		Vigna unguiculata (escape)	1	
2005	Papua New Guinea	*Vigna luteola*	1	Vaughan et al., 2006
		Vigna marina	1	
		Vigna cf. *minima*	2	
		Vigna radiata var. *sublobata*	10	
		Vigna reflexo-pilosa	7	
2005	Myanmar	*Vigna angularis* var. *nipponensis*	2	Uga et al., 2006
		Vigna hirtella	1	
		Vigna unguiculata (wild)	1	
2005	East Timor	*Glycine max*	4	Tomooka et al., 2006
		Glycine sp.	1	
		Vigna radiata	2	
		Vigna radiata var. *sublobata*	1	
		Vigna reflexo-pilosa	1	
		Vigna trinervia	3	
		Vigna umbellata	19	
		Vigna umbellata (wild)	8	
		Vigna unguiculata	5	
2005	Laos	*Glycine max*	5	Tomooka et al., 2006
		Vigna angularis var. *nipponensis*	3	
		Vigna hirtella	6	
		Vigna mimina	3	
		Vigna mungo	1	
		Vigna radiata	5	
		Vigna reflexo-pilosa	1	
		Vigna tenuicalulis	4	
		Vigna trinervia	4	
		Vigna umbellata	10	
		Vigna umbellata (wild)	10	
		Vigna unguiculata	7	
2006	Papua New Guinea	*Vigna luteola*	4	Vaughan et al., 2007
		Vigna cf. *minima*	5	
		Vigna radiata var. *sublobata*	2	
		Vigna reflexo-pilosa	4	
		Vigna vexillata	3	
2006	Myanmar	*Glycine max*	1	Watanabe et al., 2007
		Vigna angularis var. *nipponensis*	2	
		Vigna umbellata	6	
		Vigna unguiculata	6	
2006	Laos	*Vigna minima*	9	Tomooka et al., 2007
		Vigna radiata	1	
		Vigna unguiculata	6	
2007	Bhutan	*Glycine max*	4	Tomooka et al., 2008
		Vigna angularis	3	
		Vigna angularis var. *nipponensis*	4	
		Vigna mungo	5	
		Vigna radiata	1	
		Vigna radiata var. *sublobata*	11	
		Vigna umbellata	2	
		Vigna unguiculata	3	
		Vigna vexillata	3	
2007	Tamil Nadu, India	*Vigna trilobata*	1	Fukui et al., 2008
2008	Tamil Nadu, India	*Vigna aconitifolia* (cult.)	2	Tomooka et al., 2008
		Vigna hainiana	1	
		Vigna mungo	1	
		Vigna mungo var. *silvestris*	1	
		Vigna radiata	1	
		Vigna radiata var. *sublobata*	1	
		Vigna stipulacea (cult.)	2	
		Vigna stipulacea	1	
		Vigna trilobata	1	
		Vigna unguiculata	3	
		Vigna vexillata	3	
		unidentified wild legume species	1	