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Morphological Traits of *Lotus japonicus* (Regal) Ecotypes Collected in Japan

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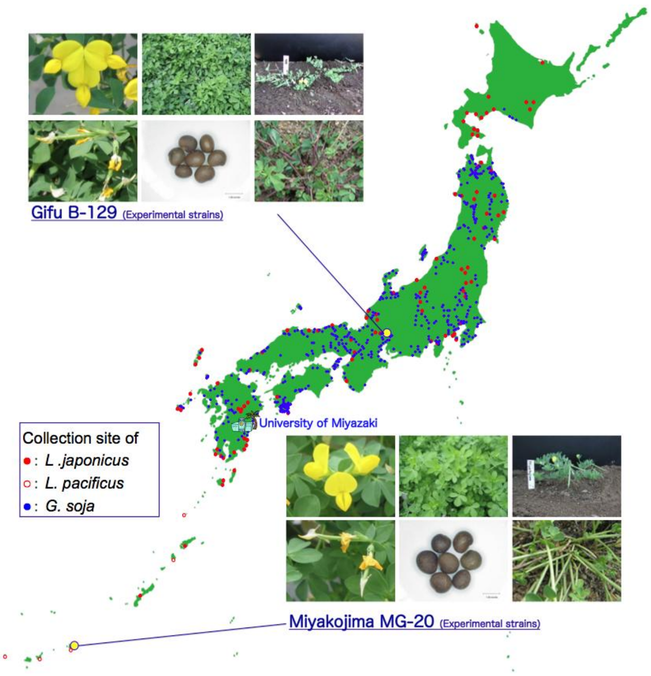
SYNOPSIS

Forty-seven wild accessions of *Lotus japonicus* Regal (Japanese trefoil) indigenous to Japan were investigated for nine morphological characters. Average temperature and annual precipitation were negatively correlated with stem color and seed weight. On the other hand, latitude was positively correlated with these traits. Consequently, accessions from sites at higher latitudes with low temperatures and precipitation tend to have dark red stems and heavy seeds. Cluster analysis based on nine morphological characters classified 47 wild accessions into six major groups. Cluster I included four accessions of tall and erect plants. These plants are phenotypically similar to commercial variety 'Empire'. Cluster II consisted of three accessions of creep plants with pale red stems. Cluster III contained 24 accessions that had average values for all morphological characters evaluated. Cluster IV included two accessions of erect plants with rounded leaflets and dark red stems. Cluster V included four accessions of small, creep plants with pale red stems. Cluster VI included seven accessions of small and erect plants, a phenotype that also applies to 'Gifu B-129', which is used as experimental strain worldwide. These data were deposited into LegumeBase, an online database (http://www.legumebase.brc.miyazaki-u.ac.jp/) supported by the National BioResource Project (NBRP) in Japan.



Keywords: *Lotus japonicus*, model legume, morphological traits, BioResource, LegumeBase

Introduction

The family *Fabaceae* (formerly *Leguminaceae*) is one of the morphologically diverse taxon that consists of over 20,000 species divided into 730 genera[1]. This agronomically and ecologically important group is responsible for much of the plant-associated biological nitrogen fixation, and contains major food-producing including soybean (*Glycine max* L), pea (*Pisum sativum*), azuki bean (*Vigna angularis*), and sources of traditional medicines, such as *Astragalus propinquus*. The genus *Lotus* consists of more than 200 species with the greatest diversity of species occurring in the Mediterranean[2]. *Lotus japonicus* Regal (Japanese trefoil) is distributed across East and Central Asia, including Japan, Korea, and China, extending west into Afghanistan[3].

L. japonicus was proposed as a legume research model two decades ago due to its small genome (470 Mb), generation time of 2 to 3 months, small plant size, large and abundant flowers, easy hand pollination, high levels of seed production, easy cultivation, regeneration from tissue culture and amenability to *Agrobacterium*-mediated transformation[4].

There are two important laboratory accessions of *L. japonicus*: Gifu B-129[5], and Miyakojima MG-20[6]. These two accessions have become global standards for legume research along with *Medicago truncatula*, and large number of mutants for legume-specific phenomena or related genes have been isolated[7]. While clearly of the same species, these two accessions have very different morphological traits[8]. Gifu B-129 is a late-flowering plant with errect growth pattern and anthocyanin accumulation in the stem. Miyakojima MG-20 is an early-flowering plant with creeping growth, lacking in stem anthocyanin. In addition, leaflets and petals of Miyakojima MG-20 are wider than those of Gifu B-129, stems and petioles are thicker, and seeds are darker and larger.

Recently, the Kazusa DNA Research Institute, Chiba, Japan, completed whole genome sequencing of Miyakojima MG-20[9]. *L. japonicus* is thus one of the most useful plants for *Rhizobium* nodulation and other aspects of legume biology. Many wild accessions of *L. japonicus* can be found growing in diverse natural habitats and have been collected for the conservation of a rich source of phenotypic and genetic variability.

Despite the importance of *Lotus* as a model plant, and its wide geographic distribution, little information is available about key morphological features, and how important genetic differences relate to its native habitats. Therefore it is very important that resources for investigating genotypic variability contributing to actual and potential traits of agronomic importance, such as seed yield, plant height, leaflet length and flowering day etc. are thus available for identification by mapping of quantitative trait loci (QTLs). In the present communication, we investigated nine morphological characteristics and establish an online database for Japanese accessions of *L. japonicus* in LegumeBase (http://www.legumebase.brc.miyazaki-u.ac.jp/) supported by the National BioResource Project (NBRP) in Japan.

Results

Collection sites of *L. japonicus* wild accessions

L. japonicus wild accessions used in this study were collected across several climatic zones from as far north as Tokoro, Hokkaido to Kaseda, Kagoshima, to the south. All collected materials were within the area of latitudes 44˚09'36'' to 31˚22'28'' N, and longitude 143˚56'10'' to 130°18'49'' E (Figure 1 and Table 1). Mean temperature of collection site varied from 5.3˚C; MG-92 (Tokoro, Hokkaido) to 17.9˚C; MG-1 (Tosashimizu, Kochi), MG-56 and MG-57 (Nichinan Miyazaki). Annual daylight of collection site varied from 1315.8 hr; MG-78 (Izumi Fukui) to 2202.3 hr; MG-1 (Tosashimizu, Kochi). Annual precipitation of collection site varied from 774.7 mm; MG-92 (Tokoro Hokkaido) to 3249.8 mm; MG-71 (Aso Kumamoto). Forty-seven accessions were obtained from subarctic to temperate zones and four distinct annual seasons. Most wild accessions were flowered from April to June in native areas.

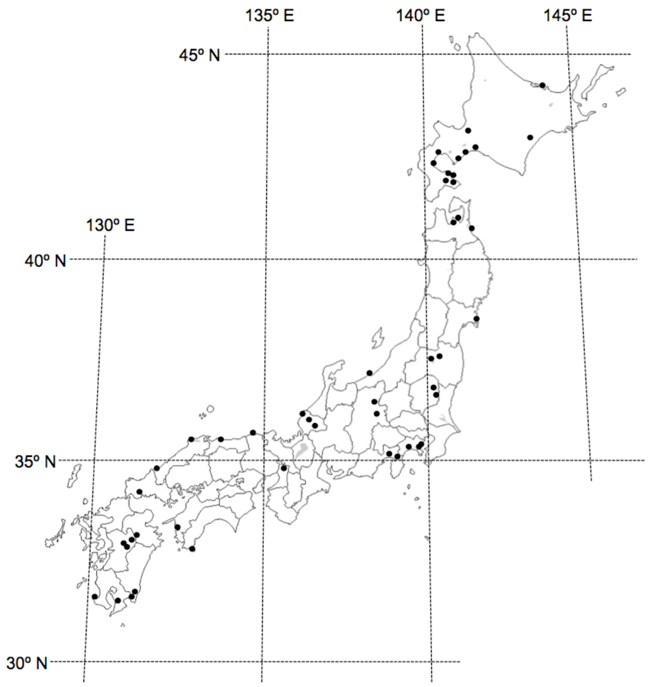


Figure 1. Collection sites of Japanese *L. japonicus* wild accessions.

Morphological traits

All wild accessions of *L. japonicus* were evaluated for nine morphological traits in the field (Figure 2). The evaluation methods are summarized in Table 2. Plant height varied from 2.8 (MG-17) to 19.5 cm (MG-75), and its distribution had a peak between 6.0 and 9.0 cm (Figure 2a). Plant length varied from 4.9 (MG-111) to 30.8 cm (MG-81), and its distribution had a peaked between 20.0 and 24.0 cm (Figure 2b). Plant type varied from 33.3 (MG-4) to 82.4% (MG-52), and its distribution had a peak between 60 and 70% (Figure 2c). Leaf length varied from 7.4 (MG-111) to 15.5 mm (MG-73), and its distribution had a peak between 10.0 and 12.0 mm (Figure 2d). Leaf width varied from 4.92 (MG-111) to 9.68 mm (MG-56), and its distribution had a peak between 7.00 and 8.00 mm (Figure 2e). Leaf shape varied from 51.3 (MG-73) to 78.9% (MG-16) and its distribution had a peak between 60.0 and 65.0% (Figure 2f). Stem thickness varied from 0.88 (MG-111) to 2.21 mm (MG-81), and its distribution had a peak between 1.50 and 1.75 mm (Figure 2g). Stem color varied from 1.00 (MG-111) to 7.17 (MG-34), and its distribution had a peak between 4.00 and 5.00 (Figure 2h). Mass of 1,000 seeds varied from 0.61 (MG-61) to 1.22 g (MG-91), and its distribution had a peak between 0.90 and 1.00 g (Figure 2i).

Correlation between morphological characters and collection sites

Correlation coefficients were determined pairwise for each of the measured traits (Table 3). A highly significant positive correlation was found between leaf width and leaf length and between stem thickness and plant length (r = 0.811 and r = 0.801, respectively). A similar result was found between plant height and plant length, between plant length and leaf width, and between leaf width and stem thickness (r = 0.746, r = 0.735 and r = 0.760, respectively).

Table 1. Wild accessions of *L. japonicus* and their passport data used in this study

Accession No.	Location				Meteorological data		
	Prefecture	City / Town	Latitude	Longitude	Mean temperature (ºC)†	Ann. daylight (hr)†	Ann. precipitation (mm)†
-1	Kochi	Tosashimizu	32°43'13"	133°01'03"	17.9	2202.3	2421.0
MG-4	Kagoshima	Kaseda	31°26'10"	130°18'49"	17.4	1532.7	2291.3
MG-5	Kanagawa	Ninomiya	35°18'08"	139°16'01"	15.1	1737.7	2024.2
MG-14	Kanagawa	Yokosuka	35°12'36"	139°36'20"	15.5	1920.6	1622.5
MG-16	Kanagawa	Miura	35°08'07"	139°40'35"	15.6	1867.7	1548.0
MG-17	Shizuoka	Mishima	35°07'59"	138°57'32"	15.6	1912.1	1864.1
MG-23	Aomori	Aomori	40°49'25"	140°45'43"	10.1	1675.6	1289.9
MG-34	Hokkaido	Toyokoro	42°36'51"	143°32'54"	5.7	1922.1	861.7
MG-35	Hokkaido	Noboribetsu	42°26'44"	141°09'38"	7.0	1511.2	1882.6
MG-36	Hokkaido	Oshamambe	42°24'34"	140°18'21"	7.3	1368.3	1275.9
MG-39	Aomori	Hiranai	40°56'39"	141°01'08"	10.1	1675.6	1289.9
MG-40	Aomori	Towada	40°35'13"	141°14'57"	9.4	1605.9	961.3
MG-44	Miyagi	Ishinomaki	38°23'12"	141°23'18"	11.4	1988.9	1064.5
MG-46	Fukushima	Shiokawa	37°35'15"	139°54'51"	11.4	1623.6	1133.8
MG-51	Tottori	Hojyo	35°30'37"	133°51'15"	14.4	1460.8	1754.4
MG-52	Shimane	Hikawa	35°23'03"	132°47'20"	14.4	1497.5	1695.0
MG-53	Shimane	Masuda	34°39'03"	131°49'35"	15.3	1602.3	1605.7
MG-56	Miyazaki	Nichinan	31°34'51"	131°24'58"	17.9	1960.2	2598.4
MG-57	Miyazaki	Nichinan	31°41'59"	131°28'56"	17.9	1960.2	2598.4
MG-61	Kagoshima	Kushira	31°22'28"	130°59'18"	17.2	1662.8	2458.6
MG-71	Kumamoto	Aso	32°56'45"	131°05'55"	9.6	1517.4	3249.8
MG-73	Oita	Kujyu	33°02'14"	131°16'01"	14.2	1802.2	1829.0
MG-75	Ehime	Uwa	33°20'19"	132°32'38"	14.6	1671.0	1943.1
MG-76	Yamaguchi	Yamaguchi	34°15'48"	131°35'28"	15.0	1907.6	1883.3
MG-77	Shizuoka	Fuji	35°07'09"	138°39'45"	15.6	1754.3	2098.3
MG-78	Fukui	Izumi	35°53'45"	136°43'23"	13.1	1315.8	2393.6
MG-79	Fukui	Mikuni	36°13'13"	136°08'29"	13.7	1529.2	2068.3
MG-81	Niigata	Omi	37°02'07"	137°51'00"	14.1	1442.6	2764.4
MG-83	Nagano	Sanada	36°32'38"	138°21'48"	6.3	1700.5	1220.9
MG-84	Fukushima	Nishi-Aizu	37°36'09"	139°39'01"	11.4	1623.6	1133.8
MG-86	Miyagi	Naruko	38°45'39"	140°45'35"	11.3	1576.2	1190.0
MG-88	Tochigi	Uji-ie	36°39'07"	139°58'38"	13.4	1938.0	1443.4
MG-90	Hokkaido	Sapporo	43°00'16"	141°25'57"	8.5	1774.8	1127.6
MG-91	Hokkaido	Ono	41°53'44"	140°38'21"	8.3	1420.9	1158.5
MG-92	Hokkaido	Tokoro	44°09'36"	143°56'10"	5.3	1604.9	774.7
MG-93	Hokkaido	Mori	42°02'01"	140°40'13"	-	-	-
MG-95	Hokkaido	Shiraoi	42°28'31"	141°13'13"	7.1	1581.0	1584.3
MG-96	Hokkaido	Tomakomai	42°36'10"	141°30'10"	7.5	1740.0	1227.7
MG-99	Hokkaido	Hakodate	41°45'25"	140°43'30"	8.8	1782.0	1160.3
MG-100	Hokkaido	Yakumo	42°13'44"	140°24'07"	7.8	1343.7	1292.0
MG-101	Hokkaido	Mori	42°00'11"	140°39'03"	-	-	-
MG-107	Osaka	Suita	34°49'04"	135°31'20"	16.5	1967.1	1306.1
MG-111	Kumamoto	Aso, Choyo	32°51'36"	131°00'03"	12.7	1576.5	2860.9
MG-113	Kumamoto	Aso, Takamori	32°52'47"	131°09'18"	13.0	1629.6	2482.0
MG-118	Fukui	Nyu,Asahi	35°58'29"	136°07'47"	14.3	1610.2	2257.9
MG-123	Nagano	Chino	35°59'44"	138°09'34"	10.8	2101.4	1307.0
MG-128	Tochigi	Hoki River	36°46'60"	140°07'41"	12.4	1715.3	1426.5

Meteorological data for each collection site were obtained from the Japan Meteorological Agency (http://www.data.kishou. go.jp/etrn/index.html).
†The average value calculated from the first year of observation to 2004.

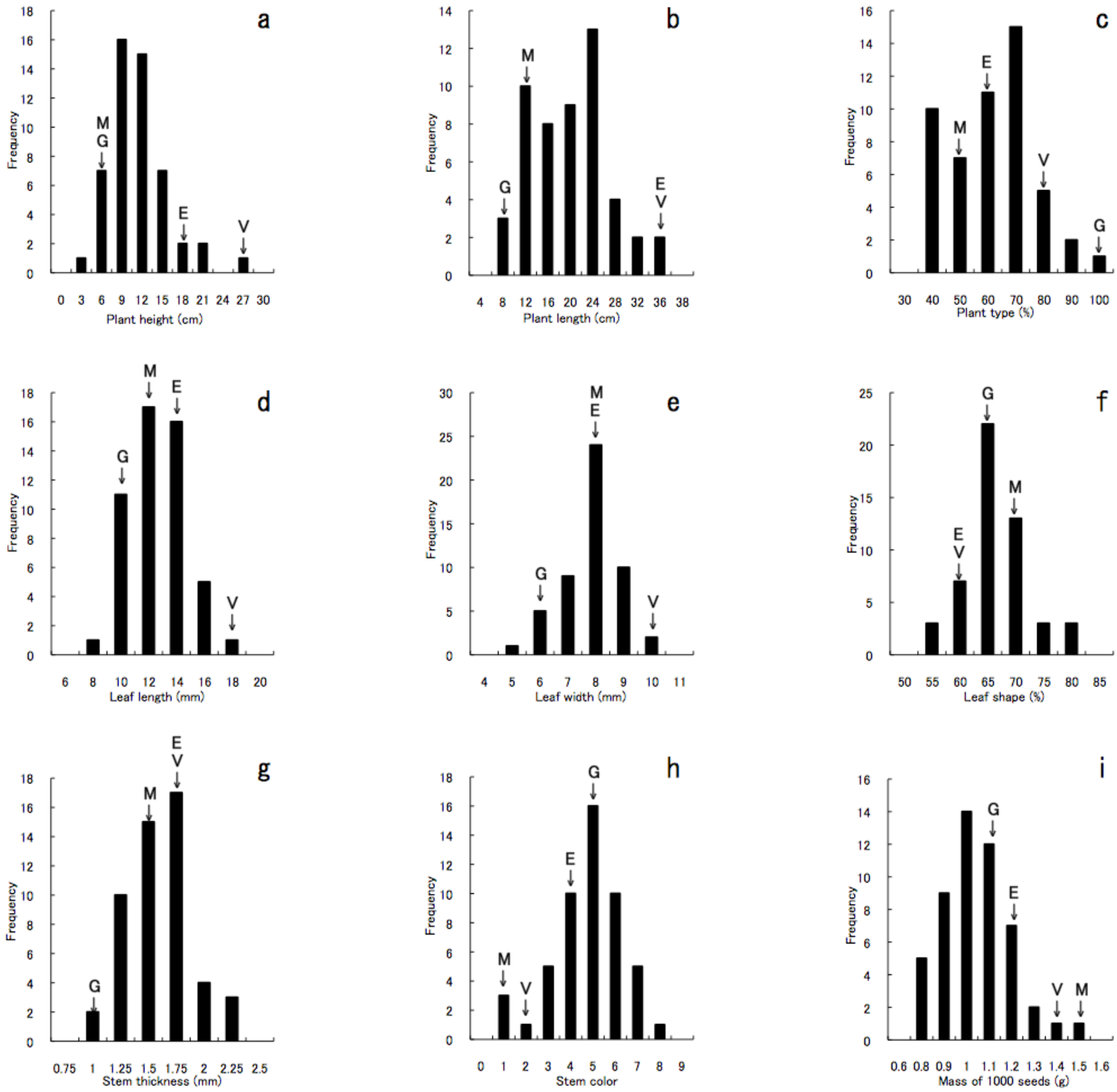


Figure 2. Frequency distribution of nine morphological characters of *L. japonicus* wild accessions. (a) Plant height, (b) Plant length, (c) Plant type, (d) Leaf length, (e) Leaf width (f) Leaf shape, (g) Stem thickness, (h) Stem color and (i) Mass of 1,000 seeds. Experimental lines and cultivar values are indicated by arrows (M: Miyakojima MG-20, G: Gifu B-129, E: Empire, V: Viking).

Table 2. Evaluation of the morphological characters of *L. japonicus* wild accessions

Morphological character	Number of tested samples	Methods	Rank or measurement unit	Remarks
Plant height	5 plants × 4 replicates	Measurement	cm	Plant height from the ground to the top of a plant
Plant length	5 plants × 4 replicates	Measurement	cm	Plant length from the ground to the top of a plant
Plant type	5 plants × 4 replicates	Calculation	%	Percentage of Plant height / Plant length
Stem thickness	5 plants × 4 replicates	Measurement	mm	Diameter of maximum thickness of the middle stem
Stem color	5 plants × 4 replicates	Observation	1:green 9:red	Observation at flowering stage
Leaflet length	5 plants × 4 replicates	Measurement	mm	Length of the middle leaflet of the largest leaf
Leaflet width	5 plants × 4 replicates	Measurement	mm	Width of the middle leaflet of the largest leaf
Leaflet shape	5 plants × 4 replicates	Calculation	%	Percentage of Leaflet width / Leaflet length
Mass of 1,000 seeds	250 mature seeds	Measurement	mg	Converted from weight of 250 original mature seeds

Table 3. Correlation between nine morphological characters of *L. japonicus* wild accessions

Morphological character	1	2	3	4	5	6	7	8	9
1: Plant height	1.000								
2: Plant length	0.746***	1.000							
3: Plant type	0.362*	-0.296*	1.000						
4: Leaf length	0.666***	0.676***	0.006	1.000					
5: Leaf width	0.672***	0.735***	-0.082	0.811***	1.000				
6: Leaf shape	-0.179	-0.117	-0.127	-0.551***	0.031	1.000			
7: Stem thickness	0.545***	0.801***	-0.317*	0.635***	0.760***	-0.009	1.000		
8: Stem color	0.221	0.228	-0.019	-0.034	0.074	0.120	0.043	1.000	
9: Mass of 1,000 seeds	0.011	0.086	-0.038	-0.287	-0.127	0.255	-0.097	0.410**	1.000

*, **, ***: significant at the 5, 1 and 0.1% levels, respectively.

Plant size, leaf size and stem thickness tend to related with plant growth but no correlation with stem color or seed weight. On the other hand stem color and seed weight were positively correlated with each other (r = 0.410).

Table 4 summarizes the results of correlating morphological characters with the meteorological data at plant collection sites. Mean temperature and annual precipitation was significantly and positively correlated with leaf length and negatively correlated with stem color and seed weight. Leaf length was negatively correlated with the latitude (r = -0.391) and positively correlated with both mean temperature and annual precipitation (r = 0.319 and r = 0.337, respectively). Stem color was positively correlated with latitude (r = 0.486) and negatively correlated with mean temperature and annual precipitation (r = -0.503 and r = -0.374, respectively). Mass of 1,000 seeds was positively correlated with latitude (r = 0.692) and negatively correlated with mean temperature and annual precipitation (r = -0.569 and r = -0.445, respectively). Daylight was not correlated with any morphological character. Stem color and mass of 1,000 seeds tended to decline with increasing temperature and was greater at higher latitudes (Table 4 and Figure 3).

Cluster analysis

Cluster analysis was used to group the 47 wild accessions into six well-defined clusters or clades with a dissimilarity value of 8.13 (Figure 4). All accessions were distinctly placed in this dendrogram and showed clustering into six clusters (I – VI) with a dissimilarity value of 8.13. Mean values of characters within each morphological cluster (Table 5). Cluster I consisted of four large and erect-type accessions and *Lotus corniculatus* ('Empire'). Cluster II includes three accessions that had a creeping habit and pale red stems. Cluster III consisted of 24 accessions that were of average value for all morphological characteristics. Cluster IV consists of two accessions that were erect plants with round-type leaflets and dark red stems. Four accessions grouped into Cluster V and were small, creep plants with pale red stems. Cluster VI consisting of seven accessions and 'Gifu B-129' that were small and erect plants. 'Viking', MG-78, MG-111, MG-61 and 'Miyakojima MG-20' were not included in any cluster. The clustering of accession was not correlated with the collected region.

Table 4. Correlation between nine morphological characters and meteorological data from the collection site of *L. japonicus* wild accessions

Morphological character	Latitude	Mean temperature	Annual precipitation	Annual daylight
Plant height	-0.077	0.024	0.137	-0.036
Plant length	-0.102	0.108	0.164	0.022
Plant type	0.045	-0.158	0.061	-0.216
Leaf length	-0.391**	0.319*	0.337*	0.039
Leaf width	-0.234	0.201	0.269	-0.137
Leaf shape	0.296	-0.227	-0.163	-0.229
Stem thickness	-0.188	0.126	0.251	-0.109
Stem color	0.486***	-0.503***	-0.374*	0.135
Mass of 1,000 seeds	0.692***	-0.569***	-0.445**	- 0.122

*, **, ***: significant at the 5, 1 and 0.1% levels, respectively.

Discussion

Use of genetic resources can involve a wide range of different, but often interrelated, actions including characterization and evaluation of material, use of wild accessions for biological and agricultural research and direct use (*e.g.* for restoration or production), as well as use in plant breeding programs. Therefore, characterization and evaluation of large, diverse collections is

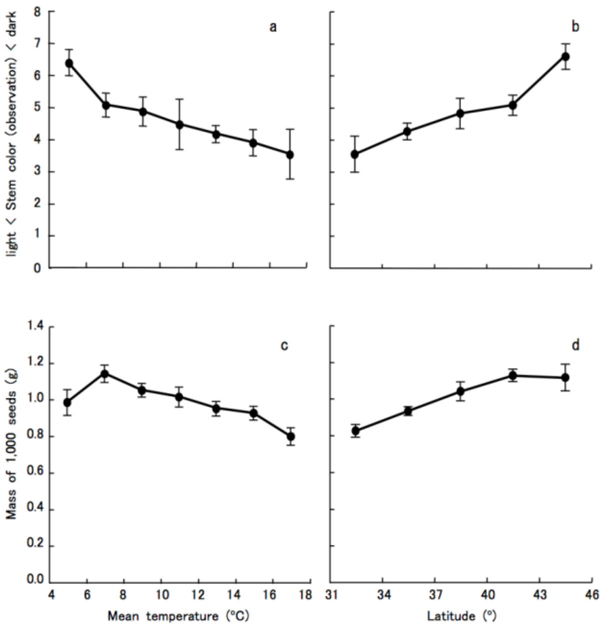


Figure 3. Relationships between stem color and mean temperature. Bar represent standard error around the mean. (a) stem color and latitude, (b) mass of 1,000 seeds and mean temperature (c) and mass of 1,000 seeds and latitude (d) of collection sites of *L. japonicus* wild accessions.

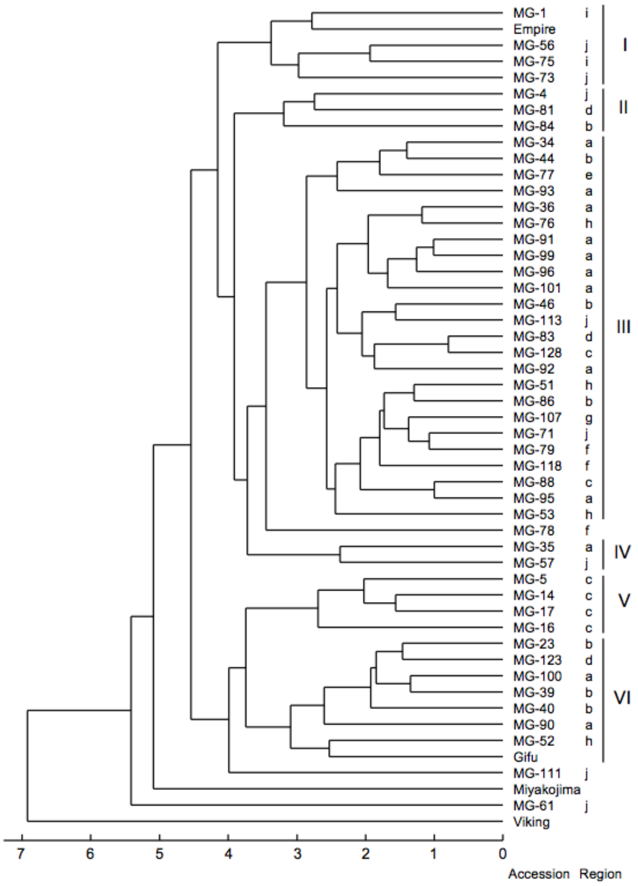


Figure 4. Phenogram of 47 *L. japonicus* wild accessions, 2 experimental lines and 2 cultivars generated from nine morphological characters using the UPGMA method. Scale on bottom indicates dissimilarity index. (a) Hokkaido, (b) Tohoku, (c) Kanto, (d) Koshin-etsu, (e) Tokai, (f) Hokuriku, (g) Kinki, (h) Chugoku, (i) Shikoku, (j) Kyusyu.

particularly valuable. A large number of accessions may have to be tested to find one or a few with the desired traits. In this report, stem color was positively correlated with latitude, and negatively correlated with mean temperature and annual precipitation (Figure 3). An earlier evaluation of the morphological characteristics of 66 wild accessions of *L. japonicus* wild accessions correlated leaf color, stem color, plant weight and harvest time with latitude[10]). The results of Suginobu and coworkers are very similar to the results of our study. The first quantitative trait locus (QTL) analysis of multiple agronomic traits in *L. japonicus* was performed with a population of recombinant inbred lines (RIL) derived from Miyakojima MG-20 × Gifu B-129[11], and indicated that stem color was the most informative QTL within the RILs variation. Genomic sequences near the QTL region include a candidate gene, which encodes a key enzyme in flavonoid biosynthesis[12,13]. On the other hand, mass of 1,000 seeds also correlated with the latitude and negatively correlated with average temperature in this study (Figure 3). However, Suginobu et al.[10] reported a correlation between seed yield and altitude. These results suggest that accessions from area with low temperatures (*i.e.* higher latitude or altitude) tend to have heavy but few seeds.

Miyakojima MG-20 has the highest level of polymorphism with AFLP markers relative to Gifu B-129 among 15 Japanese wild accessions[8]. In this study Miyakojima MG-20 differed from other wild accessions of *L. japonicus* by having green stems and heavy seeds (Figure 2). As a result, Miyakojima MG-20 was not included in any cluster based on morphological characteristics (Figure 4). These results suggest that there are remarkable differences between Miyakojima MG-20 and the wild accessions of *L. japonicus*, including Gifu B-129 genetically and morphologically.

A core collection can be defined as "consisting of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection. The core should include as much as possible of its genetic diversity"[14]. In this study, we collected not only pasportdata (latitude , longitude, mean temperature and /or annual precipitation of collection site) but also morphological data of Japanese ecotype of *L. japonicus*. These data will be useful information for developing a core collection of *L. japonicus* clones.

The Japanese government began the National BioResource Project (NBRP) in 2002 with the object of collecting, preserving and distributing biological materials that form the basis for life sciences research. Part of the NBRP mandate is to upgrade the responsiveness of culture collections, improve preservation technology, conduct genome analyses, and document significant biological phenomena, especially in relation to the other stated program goals. A key aspect of the NBRP is development of information center functions that provide information of collection locations, habitat, climatic data or genetic information that provide context for the biological resources (http://www.nbrp.jp/). At present, the NBRP is a consortium of twenty-seven core resource facilities, each of which curates a particular group of organisms, and by an information center[15]. As a part of this project, our group manages 'LegumeBase' (http://www.legumebase.brc.miyazaki-u.ac.jp/) for the legumes *Lotus japonicus* and *Glycine max*. LegumeBase is a collection of germplasm resources, including wild strains, experimental lines, recombinant inbred and mutant lines, but also includes DNA resources (BAC; Bacterial artificial chromosomes, TAC; Transformation-competent artificial chromosomes, cDNA and full-length cDNA clones). LegumeBase is accessible through a website that provides entry to the "*Lotus japonicus*" (http://www.shigen.nig.ac.jp/bean/lotusjaponicus/) and "*Glycine max / soja*" database (http://www.shigen.nig.ac.jp/bean/ glycinesoja/). Database users can identify accessions of interest by morphological, meteorological, passport or genetic data. Though relatively new,

Table 5. Mean values of characters within each morphological cluster

Cluster	Plant height (cm)	Plant length (cm)	Plant type (%)	Leaflet length (mm)	Leaflet width (mm)	Leaf shape (%)	Stem thickness (mm)	Stem color (observation)	Mass of 1,000 seeds (g)
I	16.92 ± 2.96^{a}	27.92 ± 3.59^{a}	61.14 ± 12.72^{ab}	14.80 ± 0.94^{a}	8.45 ± 0.76^{a}	57.19 ± 4.37^{d}	1.75 ± 0.23^{ab}	4.22 ± 0.92^{a}	0.92 ± 0.16^{a}
II	8.83 ± 1.44^{bc}	25.77 ± 4.42^{ab}	34.33 ± 1.17^{c}	11.80 ± 1.73^{bc}	8.15 ± 0.63^{a}	69.67 ± 5.92^{abc}	2.04 ± 0.16^{a}	3.50 ± 0.87^{a}	0.98 ± 0.17^{a}
III	10.37 ± 2.01^{b}	19.35 ± 3.84^{b}	54.38 ± 8.64^{abc}	11.99 ± 0.99^{b}	7.67 ± 0.49^{ab}	64.20 ± 3.20^{bc}	1.53 ± 0.18^{bc}	4.98 ± 1.11^{a}	1.01 ± 0.13^{a}
IV	12.55 ± 2.19^{ab}	15.95 ± 2.76^{bc}	78.55 ± 0.07^{a}	9.60 ± 0.57^{bc}	7.14 ± 0.06^{abc}	74.35 ± 3.99^{a}	1.43 ± 0.22^{bcd}	5.25 ± 1.06^{a}	0.85 ± 0.13^{a}
V	3.68 ± 0.62^{c}	9.65 ± 1.35^{c}	38.18 ± 3.53^{c}	9.48 ± 0.33^{c}	6.68 ± 0.59^{bc}	70.38 ± 6.07^{ab}	1.23 ± 0.09^{cd}	3.55 ± 1.07^{a}	0.89 ± 0.10^{a}
VI	7.54 ± 1.84^{bc}	11.48 ± 3.86^{c}	68.45 ± 13.72^{ab}	9.66 ± 0.79^{bc}	6.01 ± 0.45^{c}	62.27 ± 2.38^{cd}	1.10 ± 0.09^{d}	4.70 ± 1.45^{a}	1.06 ± 0.11^{a}

Values within a column followed by the same letter are not different at $P<0.05$ based on one-way ANOVA followed by Scheffe's test.

LegumeBase currently holds collated data for 108 *L. japonicus* and 1,169 *Glycine soja* accessions across Japan, a beginning that should provide researchers in many different fields a critical advantage for working on these important crop plants. In here we report nine morphological characters of wild accessions of *L. japonicus* and their correlation. The morphological data evaluated in this study have been opened in LegumeBase. These data could be useful information for utilize our resources.

Materials and Methods

Plant materials

A total of 51 strains, including 47 wild accessions of *L. japonicus*, two experimental lines (Gifu B-129 and Miyakojima MG-20) of *L. japonicus* and two commercial varieties of *L. corniculatus* ('Viking' and 'Empire') were used in this study. The collection sites of 47 wild accessions of *L. japonicus* are shown in Figure 1. The locations of all accessions were linked to latitude, longitude and meteorological data from the Geographical Survey Institute in Japan (http://watchizu.gsi.go.jp/) and the nearest observatory of the Japan Meteorological Agency (http://www.jma.go.jp/jma/ menu/report.html), respectively (Table 1). These strains except for 'Viking' and 'Empire' were obtained from "LegumeBase", an online database (http://www.legumebase.brc.miyazaki-u.ac.jp/) supported by the National BioResource Project in Japan.

Morphological traits

Nine morphological characters were evaluated at the Field Science Center, University of Miyazaki, Miyazaki, Japan (31˚49'48" N, 131˚24'32" E). Mean temperature, annual daylight and annual precipitations of Miyazaki were 17.4˚C, 1859.7 hr and 2898.3 mm respectively. A total of 51 wild accessions and varieties were sown into peat pots on 21 May 2004 and grown in a greenhouse. Seedlings were transplanted to the field in pots measuring 30 cm × 50 cm on 22 June 2004. Each plot was replicated four times with each plot containing five plants arranged according to a randomized block design. The nine morphological characters and methods for their investigation are summarized in Table 2. All investigation was performed in flowering stage.

Data analysis

Mean values were calculated for morphological characteristics except for flowering day and flowering degree from a total of 51 wild accessions and other varieties. The seeds of nine accessions did not germinate, and information about the collection site for one accession was not sufficient; therefore, these 10 accessions were not included in the study. Spearman rank correlation coefficient between trait and meteorological data were calculated by the Excel statistical software Ver. 1.07 (Social Survey Research Information, Tokyo, Japan). A phonogram of 51 accessions and varieties was constructed by using an unweighted pair-group method average (UPGMA) clustering based on a dissimilarity index calculated as Euclidean distance[16]. Cluster analyses were computed using the program STATISTICA Ver. 5.1 J (StatSoft Japan Inc., Tokyo, Japan). Comparisons of sets of density data from each cluster were by one-way analysis of variance (ANOVA) followed by Scheffe's test within the Excel statistical software.

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