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# A High-density Linkage Map of *Lotus japonicus* Based on AFLP and SSR Markers

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**Abstract**

**A collection of 94 $F_6$ individuals derived from crosses between *Lotus japonicus*, Gifu B-129 (G) and Miyakojima MG-20 (M) were used for mapping. By using the HEGS running system, 427 *Eco*RI/*Mse*I primer pairs were selected to generate a total of 2053 markers, consisting of 739 G-associated dominant markers, 674 M-associated dominant markers, 640 co-dominant markers, 95 SSR markers and 2 dCAPS markers. Excluding heavily distorted markers, 1588 were mapped to six chromosomes of the *L. japonicus* genome based on the 97 reference markers. This linkage map consisted of 1023 unique markers (excluding duplicated markers) and covered a total of 508.5 cM of the genome with an average chromosome length of 84.7 cM and interval distance of 0.50 cM. Fifteen quantitative traits loci for eight morphological traits were also mapped. This linkage map will provide a useful framework for physical map construction in *L. japonicus* in the near future.**

**Key words:** *Lotus japonicus*; AFLP; SSR; linkage map; HEGS (high efficiency genome scanning)

## 1. Introduction

Genetic mapping is a basic tool of eukaryotic genomic research. Molecular linkage maps provide information about the organization of the genome and can be used for genetic studies and breeding applications. A high-density genetic linkage map is essential to physical map construction and also a powerful tool for the location and map-based cloning of desired gene(s). PCR-based DNA markers make such a linkage map possible. Of these PCR-based markers, AFLP markers were demonstrated to be a powerful new class of markers making it feasible to develop linkage maps for plants with a large genome.[1] There are many linkage maps based on AFLP markers reported for various plants.[2–6]

*Lotus japonicus* is an autogamous diploid legume species.[7] As a model legume, it has many characteristics that make it a candidate for genomic research. These attributes are an autogamous diploid ($2n = 12$) and small genome (432∼494 Mb),[8–10] short life cycle and transformation ability,[6,11] making *L. japonicus* a model legume plant that can be used for molecular genetics and physiological studies. Genome synteny will help in marker preparation and gene cloning for other legume crops. To date, the cloning of various nodulation genes has been the subject of heated international competition. Therefore, the need for construction of a physical map covering the genome of *L. japonicus* is especially important. A primary genetic linkage map based on DAF (DNA Amplification Fingerprinting) markers with an $F_2$ population from a cross of *L. japonicus* accessions, Gifu B-129 and Funakura B-581 has

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been reported.[8] However, due to low polymorphism observed in this cross, the number of linkage groups did not cover all chromosomes of the genome. Crosses made with 'Miyakojima MG-20' showed the highest-level of polymorphisms relative to Gifu B-129 (>4%).[12] Although this recombination rate is still low when compared with other plants,[2–5] a fast and simple high efficiency genome scanning (HEGS)[10] AFLP protocol system can overcome this disadvantage. Although traditional AFLP protocols simultaneously assay for large numbers of polymorphic bands on a single gel, developing more than 2000 AFLP markers is time consuming and laborious. The HEGS system allows the development of AFLP markers in a short time.[10] The HEGS gel running apparatus is composed of a set of 24 cm × 26 cm glass plates. One hundred samples can be analyzed on a two-layer gel that is composed of 13% bis: arcylamide (19:1) separating gel and 5% stocking gel at 350 V for 4 h. As a result, 800 individuals can be analyzed on eight sets of plates per day by one person. By using the HEGS/AFLP system, some linkage maps have been developed for *L. japonicus*.[13,14] For example, Hayashi et al.[13] constructed a linkage map consisting of 287 markers (AFLP, SSR, dCAPS and other PCR-based markers) that spanned a total length of 487.3 cM and corresponded to six chromosomes in the *Lotus* genome using an $F_2$ population from a cross of 'Gifu B-129' and 'Miyakojima MG-20'. This study will use the map of Hayashi et al.[13] as reference to build a high-density linkage map in *L. japonicus*.

To construct a fine physical map, however, requires a linkage map of *L. japonicus* with sufficient high marker density (>1000 markers with less than 1 cM interval distance). This linkage map will also be used for gene cloning in future research. The resulting high-density linkage map created in this study will serve as a framework for building a genome physical map that will be suitable for map-based cloning in *L. japonicus* genetic research.

## 2. Materials and methods

### 2.1. Plant materials and DNA extraction

A cross of *L. japonicus* accessions 'Gifu B-129' and 'Miyakojima MG-20' was made at Kazusa DNA Institute, Japan. Gifu B-129 has a crawling habit with a red stem and Miyakojima MG-20 has erect habit with a green stem. A mapping population of 194 $F_6$ recombinant inbred lines (RILs) was obtained by single-seed descent method. A collection of 94 from the 194 RILs was randomly selected as a mapping population for convenient analysis of the HEGS/AFLP running system.

Genomic DNA was extracted from young leaves of the 94 individuals. In brief, 0.3 g of young leaves were collected in a 50 ml Falcon tube including five metal beads, immersed in liquid nitrogen and ground with a shaker (EYELA cute Mixer CM-100), 250 rpm, 30 s, twice. Immediately, the powder was incubated with 2 ml extraction buffer (10% CTAB: 10% SDS and sodium lanroylsarcosine = 1:2, preheated) at 65°C for 1 h with gentle shaking. About 2 ml of aqueous phase were transferred to a 6-tube strips and DNA was extracted automatically using KURABO NA-2000 (Japan) following the manufacturer's instructions. The DNA pellet was dried and dissolved in 50–100 μl 0.1 × TE (10 mM Tris–HCl and 0.1 mM EDTA, pH 8.0). The dissolved DNA samples were treated with RNase A (50 μg/ml) for 3 h at 37°C.

### 2.2. SSR analysis

Microsatellite (SSRs) and dCAPS analysis were performed based on the methods of Sato et al.[15] with the minor modifications. Genomic DNA (0.2 ng) was used in a total volume of 5 μl containing 0.0125 U Extaq polymerase (TaKaRa, Japan). The annealing temperature was set to 60°C. The sequence information of all SSR and dCAPS primers (written in TM prefix with serial number) can be found in Sato et al.[15] All the TM primers were synthesized from Invitrogen, Life Technologies, Japan.

### 2.3. AFLP analysis

The AFLP assays were performed as described by Vos et al.[1] with the following modifications. Genomic DNA samples (250 ng) were digested with 8 U of *Eco*RI and 5 U of *Mse*I (Biolabs Inc., New England) in a reaction volume of 25 μl with 1× *Not* I preservation buffer (10 mM Tris–HCl, pH 7.5, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, pH 8.0, 0.01% BSA, 0.15% Triton X-100, 50% glycerol), mixed briefly and incubated at 37°C for 3 h. Five microliters of the digestion solution were mixed with 1 μl *Eco*RI adapter (5 pmol/μl), 1 μl *Mse*I adapter (50 pmol/μl), 1 μl 10× ligation buffer and 28 U of T4 DNA ligase (TaKaRa) in a total of 10 μl of reaction volume and incubated at 37°C overnight. This digestion–ligation solution was diluted 10-fold in 0.1× TE buffer and used for pre-amplification. Pre-amplification was conducted in 25 μl reaction volume containing 2.5 μl diluted adaptor-ligated DNA, 1 μl *Eco*RI+A primer (5 ng/μl), 1 μl *Mse*I+C primer (30 ng/μl), 2 μl 2.5 mM dNTP each, 2.5 μl ExTaq buffer and 0.5 U ExTaq DNA polymerase (TaKaRa). The PCR profile was 94°C for 30 s, 56°C for 1 min, 72°C for 1 min and 20 cycles with a 10 min final extension at 72°C. The PCR products were diluted 100-fold in 0.1× TE buffer and stored at −20°C until further use. Selective amplification was performed in 5 μl

reaction volume containing 2.45 μl diluted pre-amplification products, 0.5 μl *Eco*RI +3 primer (7 ng/μl) and 1.125 μl *Mse*I+3 primer (7 ng/μl), 0.4 μl 2.5 mM dNTP each, 0.5 μl ExTaq buffer and 0.025 U ExTaq DNA polymerase (TaKaRa). The touchdown PCR profile was one cycle at 94°C for 30 s, 68°C for 30 s and 72°C for 60 s, 17 cycles with the annealing temperature reduced 0.7°C/cycle, and 23 cycles with an annealing temperature of 56°C and with final extension at 72°C for 10 min.

### 2.4. Gel running with HEGS running system

Five microliters of PCR samples were mixed with 1 μl loading buffer (0.25% xylene cyanol, 0.25% bromophenol blue, 1 mM EDTA, pH 8.0, 40% glycerol) and separated using the HEGS running system.[5] In brief, 6 μl of each sample per lane were loaded into polyacrylamide gels consisting of stacking gel (upper-side gel, 2.5 cm in depth, 5% bis-polyacrylamide contained 0.5 M Tris–HCl, pH 6.8) and running gel (lower-side gel, 13% bis-polyacrylamide contained 1.5 M Tris–HCl, pH 8.8). A total of 94 $F_6$ individuals and two parents and *ΦX174-Hae III* (50 ng/lane) in one gel was run in 1× Tris–glycine buffer (25 mM Tris–HCl, pH 8.3, 1.92 M glycine) at 100 V for 80 min followed 350 V for 4.5 h. The gels were stained in 1/10 000 volume Vistra Green dye solution (Amersham Pharmacia Biotech) for 10–20 min, washed in water for 5–10 min and scanned in FluorImager 575 (Molecular Dynamics, Amersham Pharmacia Biotech). We therefore employed the HEGS/AFLP analysis with 94 $F_6$ recombination lines from a cross of 'Gifu B-129' and 'Miyakojima MG-20' to develop a large number of markers. This approach attempts to construct a high-density linkage map containing ~2000 markers with an average of <1 cM interval distance between markers.

### 2.5. Data collection

Clear polymorphic bands were selected using the $F_2$ generation genome analysis software (Kazusa DNA Research Institute, Japan), and the bands were confirmed visually. This software also automatically assigned molecular weights to the fragments, distinguished the single polymorphic band from parents before manually setting paternal and maternal alleles, and generated reports of fragment presence/absence in 1/0 binary type. Only clearly visible markers were scored. For $F_6$ progeny, band presence associated with the Gifu B-129 allele was coded as A; band presence with Miyakojima MG-20 allele was coded as B, and those bands with both female and male parent were coded as H for heterozygote. Each AFLP marker was identified by a code referring to the primer combination, EM (*Eco*RI/*Mse*I) and character G (associated with Gifu B-129 allele), M (associated with Miyakojima MG-20 allele) or C (co-dominant marker), followed by the estimated size of the DNA fragment in nucleotides. The heterozygote (for the co-dominant markers) and missing data were coded as '-'.

Eight morphological traits, plant type (PT), stem pigment (SP), leaflet (LL), stipule (ST), petiole (PE), trichome (TR), seed color (SC) and seed size (SS), were scored as qualitative traits. We scored grade 1–5 for all traits. PT: 1—crawl stem and 5—stand stem; SP: 1—light color (green) stem and 5—dark color (red) stem; LF: 1—no LL and 5—long LL; ST: 1—no ST on the petal and 5—most STs; PE: 1—none and 5—longest; TR: 1—no TR and 5—most; SC: 1—lightest color and 5—dark (brown); SS: 1—smallest and 5—largest.

### 2.6. Linkage map construction

Before linkage analysis, chi-square tests ($\chi^2$) were performed on both SSR and AFLP markers for goodness of fit to the expected Mendelian 1:1 segregation ratio of each marker. Distorted loci that deviated significantly at $P < 0.01$ were excluded from map construction. Linkage analysis was performed with the Joinmap 3.0.[16] Initially, an LOD score of 14.0 was used to identify six linkage groups, corresponding to six chromosomes of *Lotus* genome based on the previously mapped SSR/dCAPS markers[13] (details can also be found from http://www.kazusa.or.jp/lotus/markerdb_index.html). The Kosambi mapping function[17] was used to convert recombination frequencies into map distances. Linkage maps were drawn using MapChart 2.1 software.[18]

A subset of markers spanned across the linkage map with even distance of 5 cM was selected and used for composite interval mapping.[19] Quantitative traits loci (QTL) analysis was carried out using WinQTL Cartographer 2.5.[20]

## 3. Results

### 3.1. AFLP markers generated in HEGS system

Ninety-four $F_6$ individuals and two parents were analyzed on a single gel (Fig. 1); 4096 *Eco*RI–*Mse*I primer combinations were first screened on the parental DNAs (data not shown). Of the primer pairs (Supplement 1) that produced the most polymorphic bands, 427 were selected for further AFLP analysis for 94 random individuals of $F_6$ population. Initially, a total of 2053 diagnostic AFLP markers, which included 739 Gifu B-129 dominant, 674 Miyakojima MG-20 dominant and 640 co-dominant markers, were scored (Fig. 2, Table 1). On average, 4.8 AFLP markers and 1.5 co-dominant markers were generated per primer combination, with a range of 2–8 visible markers (data not shown).

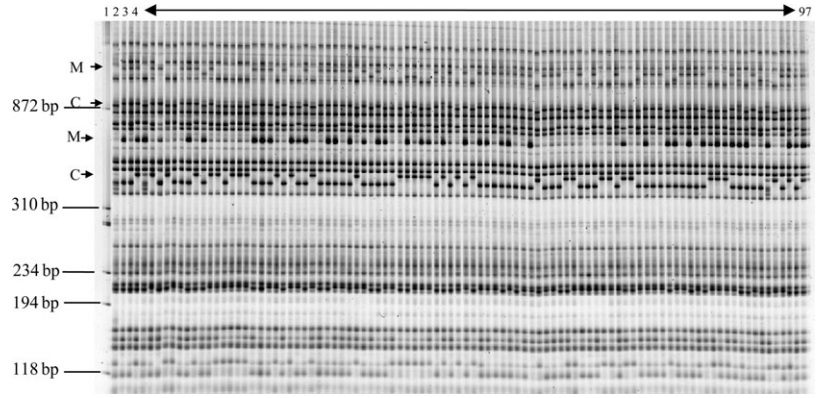



**Figure 1.** A sample of AFLP gel profile with a combination of E-ATG I/M-ACC primers for 94 $F_6$ individuals from a cross of *L. japonicus* accessions, 'Gifu B-129' and 'Miyakojima MG-20' with HEGS system. Lanes from left: lane 1, size marker *Φ*X174-*Hae* III; lane 2, maternal 'Gifu B-129' (associated allele was assigned as 'G', showed as arrow); lane 3, paternal 'Miyakojima MG-20' (associated allele was assigned as 'M', showed as arrow); lanes 4–97, $F_6$ individuals. Co-dominant allele was assigned as 'C', showed as arrow.

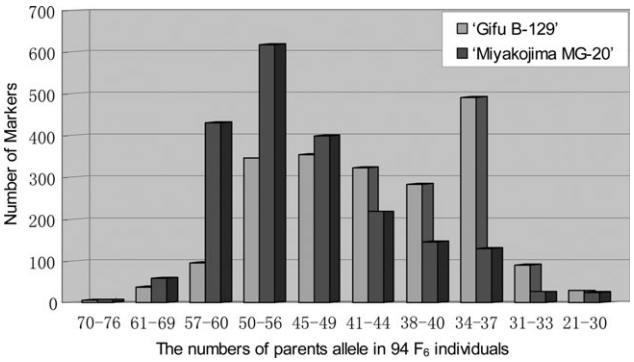



**Figure 2.** Distributions of 2053 AFLP alleles from 94 $F_6$ individuals derived from a cross of *L. japonicus*, 'Gifu B-129' and 'Miyakojima MG-20'.

The polymorphism ratio per primer pair combination agreed with that of Kawaguchi et al.[12] The electrophoresis profile (Fig. 1) scanned by the FluorImager 575 provided sufficient resolution to distinguish fragment mobility from 70 to 5000 bp, indicating a wide range of the amplification fragments with high resolution using the HEGS/AFLP-SSR running system (in ~24 cm long gel). A total of 95 SSR and 2 dCAPS markers that mapped on the previous linkage map[13] was also separated using HEGS running system.

An $F_6$ population is considered a RIL and presents an exception of 1:1 ratio for allele segregation among individuals, but a theoretical ratio of 3% of individuals should show as heterozygous. In this case, each segregating marker was tested with a $\chi^2$ test for goodness-of-fit to the expected 1:1 Mendelian segregating ratio. As a result, 131 of the 2053 markers (6.4%) were distributed to a skewed segregation with significance at $P \leq 0.01$, and 575 markers (28%) were distorted at $P \leq 0.05$. Most skewed segregating markers deviated to Miyakojima MG-20 alleles. In this study, the distributions of 82 of these 131 markers inclined to Miyakojima MG-20 and only 49 to Gifu B-129 (Table 1). Five SSR markers were distorted significantly at the 1% level, of which three were distorted to Gifu B-129 and two to Miyakojima MG-20. However, these five SSR markers were retained in the mapping analysis because their distribution frequencies were near to $P = 0.01$. The AFLP markers skewed at the 1% level were discarded and excluded in the data analysis. About 24% of the SSR markers were skewed at the 5% level and included in the map analysis (Table 1). As a result, a total of 1588 AFLP markers and 97 SSR including 2 dCAPS markers were mapped on the linkage map of *L. japonicus*.

**Table 1.** AFLP and SSR markers generated in the 94 $F_6$ individuals from a cross between *L. japonicus* accessions, 'Gifu B-129' (G) and 'Miyakojima MG-20' (M)

| | Total | Mapped markers | Distorted at $P \leq 0.05$ | Distorted at $P \leq 0.01$ |
|---|---|---|---|---|
| *Eco*RI/*Mse*I primer pairs | 427 | | | |
| G associated dominant markers | 739 | 504 | 194 | 44 |
| M associated dominant markers | 674 | 454 | 202 | 55 |
| Co-dominant markers | 640 | 533 | 179 | 32 |
| Total AFLP markers | 2053 | 1491 | 575 (28%) | 131 (6.4%) |
| SSR markers* | 97 | 97 | 23 (23.7%) | 5 (5.1%) |

The numbers in parentheses show the percentage of the markers stated to the total AFLP markers.
*Included two dCAPS markers.

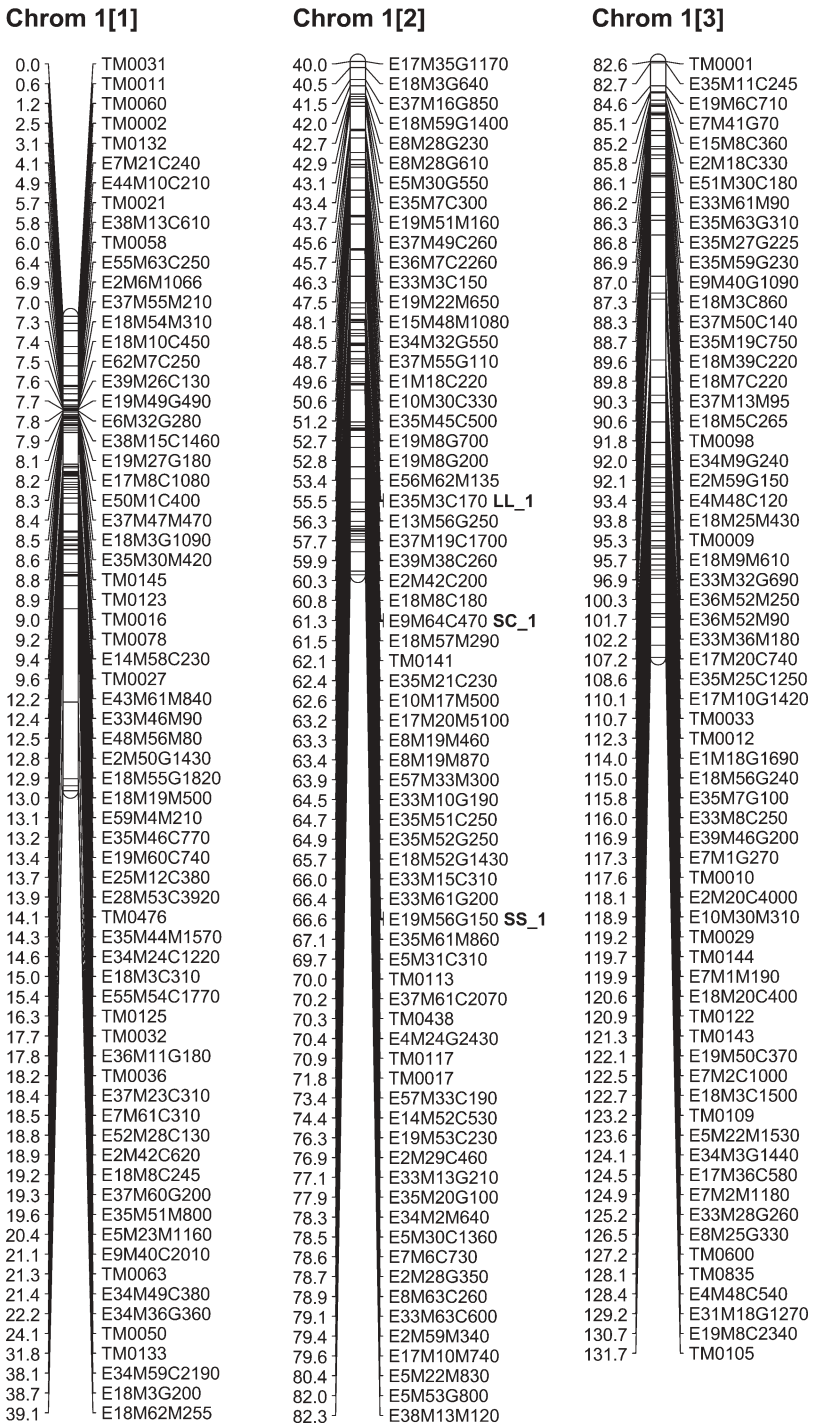



**Figure 3.** Linkage maps of chromosome 1–6 of *L japonicus* genome Marker names were assigned based on the combination of *Eco*RI and *Mse*I AFLP primers with generated marker size (in base pair). Letter G stands for maternal 'Gifu B-129' associated marker; M for paternal 'Miyakojima' associated marker and C for co-dominant markers. All SSR/dCAPS markers assigned as TM (Hayashi et al.[13] and visit at http://www.kazusa.or.jp/lotus/markerdb_index.html). Morphological QTL: SS stands for seed size; LL for leaflet; SC for seed color; ST for stipule; PE for petiole; TR for trichrome; PT for plant type and SP for stem pigment.

### *3.2. Construction of linkage map*

All AFLP markers and 95 SSR and 2 dCAPS markers were run on JoinMap® 4 to generate six groups at an LOD value of 14.0, with a maximum distance of 30 cM. Using the SSR markers on each group as references, these six groups were assigned to chromosome 1–6 of *L. japonicus*.[13] A high-density linkage map for *L. japonicus* was generated; 533 co-dominant AFLP markers and 97 SSR (including 2 dCAPS markers) were mapped on six chromosomes of

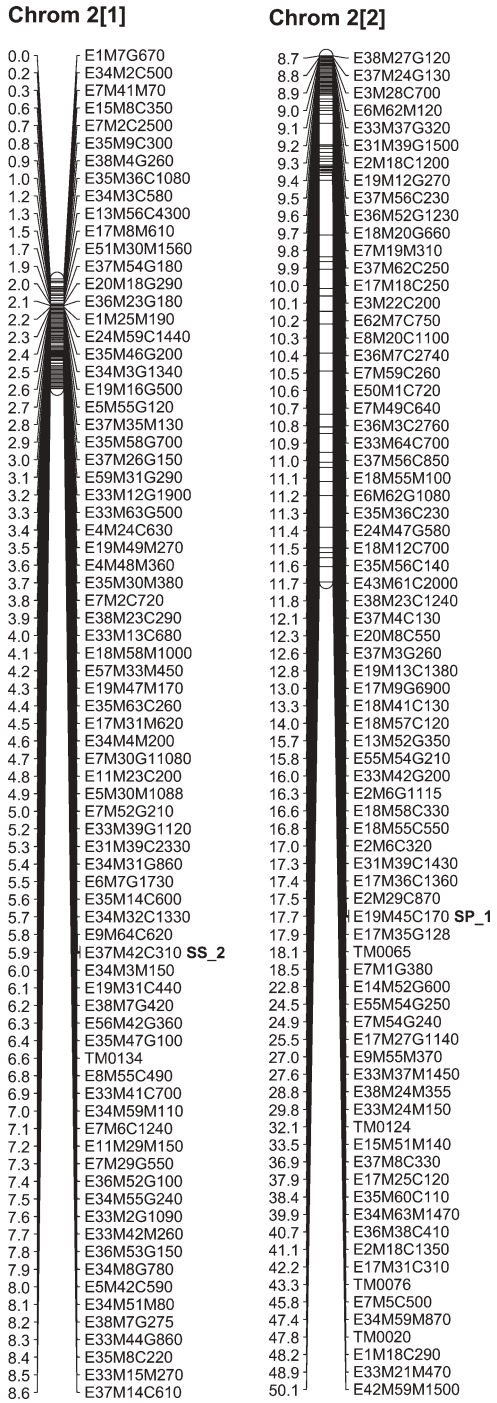



**Figure 3.** Continued

*L. japonicus*, with an additional 958 AFLP markers (504 Gifu associated and 454 Miyakojima associated markers, Table 2). A total of 1588 markers were mapped on the six chromosome maps (Supplement 2). This linkage map consisted of 1013 unique markers (excluded duplicated markers) and spanned a total length of 508.5 cM with an average of 0.50 cM between markers. The range of the length of each chromosome varies from 50.1 cM (chromosome 2) to 131.7 cM (chromosome 1), with a mean of 84.7 cM (Table 2, Fig. 3).

Eight morphological traits were recorded as QTL. After the map of each chromosome for each parent was constructed, several markers with almost an equal distance interval of $<10$ cM were used as frame markers to scan eight phenotypic markers within the linkage map of each chromosome. As a result, 14 QTLs were detected on six chromosomes for the eight morphological traits. There were six QTLs detected on chromosome 4, only one QTL on chromosomes 3, 5 and 6 (Table 2). SS showed five QTLs and was distributed to five chromosomes except for chromosome 5. LL and SC presented two QTLs. SP, PT, ST, PE and TR showed only one QTL, indicating control by a single gene (Table 2, Fig. 3).

The duplicated markers presented in six chromosomes, resulting in significant clustering in the whole genome, especially in the center region of each chromosome. To decrease the numbers of markers, all duplicated markers will be excluded on the each chromosome map (Fig. 3). So Fig. 3 presented only unique markers. All requirements about the details of mapped markers should be address to the corresponding authors.

### *3.3. Characterization of the linkage map*

Chromosome 1 had the largest number of markers (373) and longest genetic distance (131.7 cM). Chromosome 5 had the fewest number of markers (184) and chromosome 2 has shortest genetic distance (50.1 cM). The average interval distance between markers (excluded the duplicated markers) was 0.50 cM for all chromosomes. Chromosome 1 (0.64 cM) had the longest and chromosome 2 (0.33 cM) had the shortest average interval distance between markers. The other four chromosomes had similar average interval distances (Table 2). The distorted markers were mainly mapped on distal parts of chromosome 5 (marked with asterisk on chromosome 5 in Fig. 3). Chromosome 6 also showed some distorted markers, dispersed through the chromosome. The clustering of markers occurred on the center region of all six chromosomes (Fig. 3).

## 4. Discussion

### *4.1. Determination of the linkage map of each chromosome*

Before determining the chromosome of the genome, all AFLP and SSR markers were combined and analyzed with JoinMap® 3.0.[16] At the LOD of 14.0, six big groups were generated. On the basis of the distribution of SSR markers[13] within each group, we were able to locate six groups to six corresponding

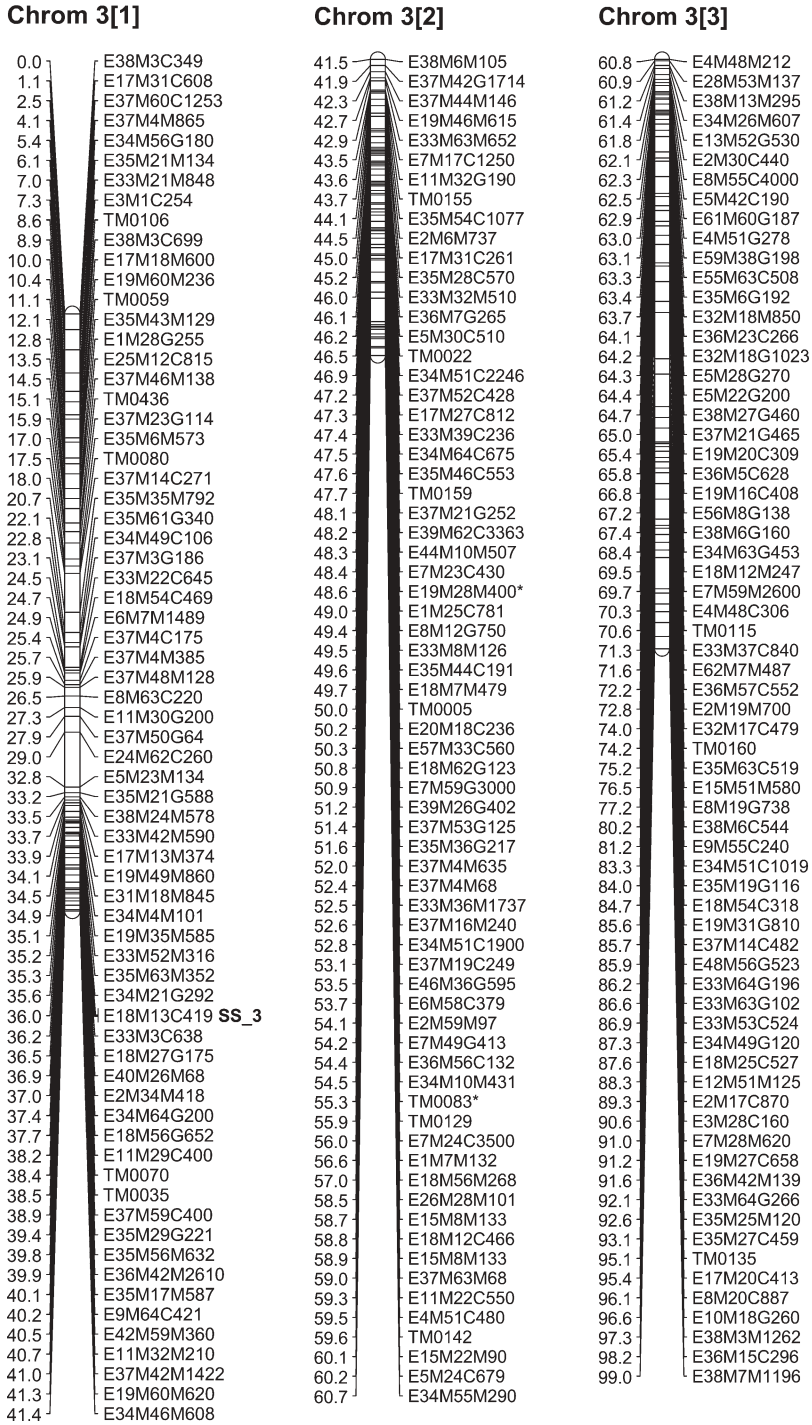
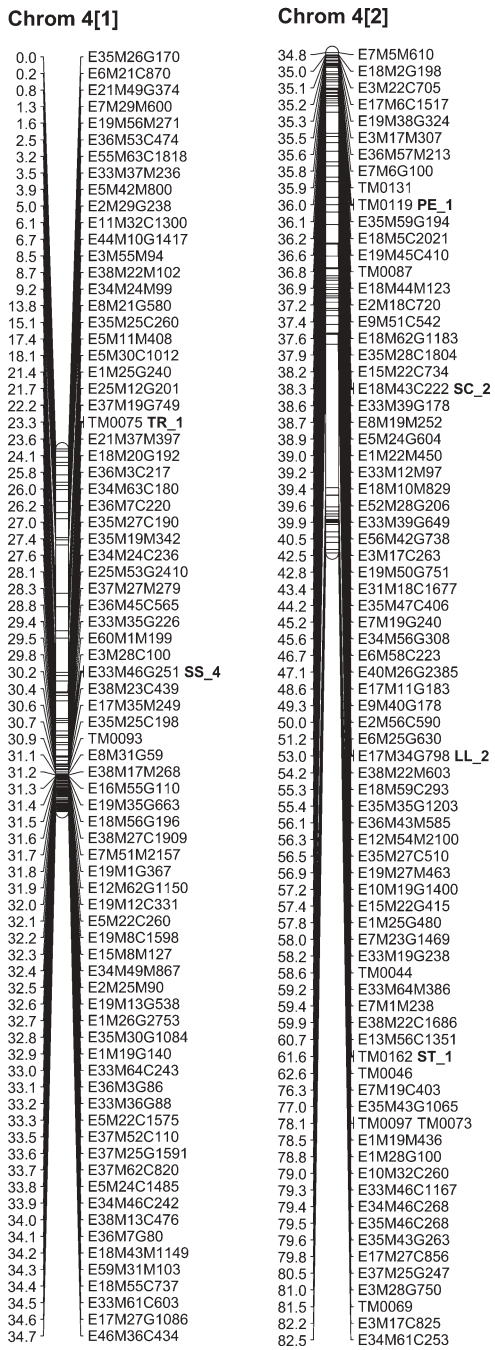



**Figure 3.** Continued

chromosomes of *L. japonicus*. After running the JoinMap® 3.0 program for each group, a linkage map on each chromosome was determined. The orders of these SSR markers on each chromosome were the same as those in the map of created by Hayashi et al.[13] except for some minor differences for some markers. Therefore, we confirmed the six groups correspond to the six chromosomes of *Lotus* genome.

### 4.2. *The length of linkage map*

This linkage map spans a total genetic distance of 508.5 cM in *L. japonicus* genome, slightly longer

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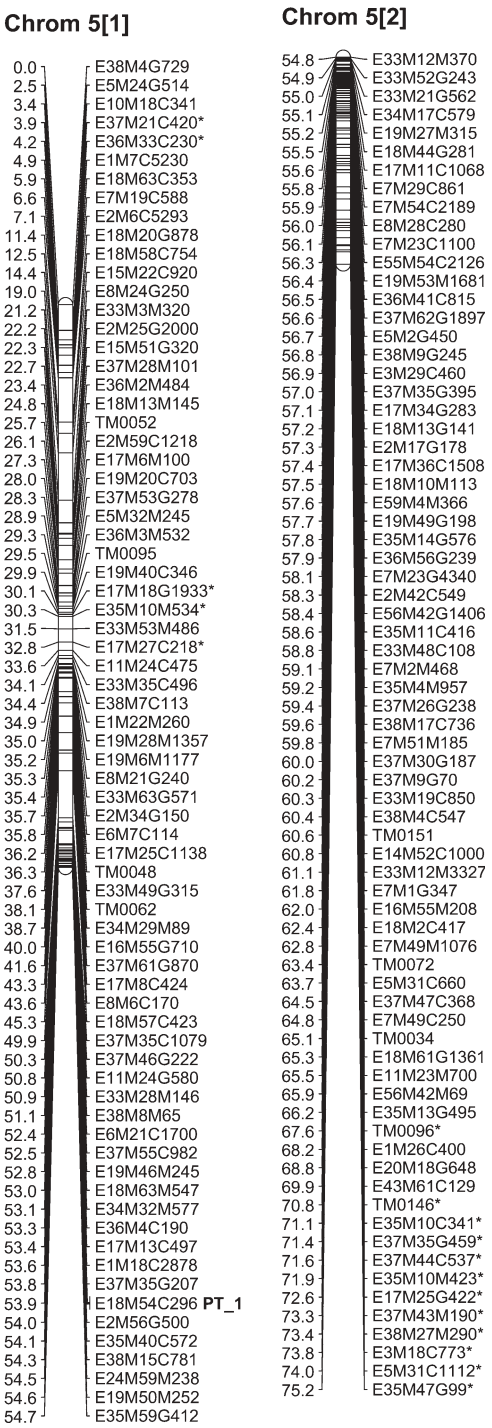


**Figure 3.** Continued

than those of previous maps.[13,14] This can be explained by the marker numbers dramatically increased in the present mapping population. The larger number of markers within one linkage group may enlarge the genetic intercrossing value between markers. Additionally, the small size of the $F_6$ population (94 individuals) compared with 127 $F_2$ individuals in Hayashi et al.[13] may not be enough for allele segregation and cause allele partial distribution.

The lengths of chromosome 3–6 were very close to the lengths of Hayashi's results.[13] However, they were nearly 30 cM longer for chromosome 1 and 30 cM shorter for chromosome 2 than the reference map.[13] Hayashi et al.[13] reported that there was a translocation region between the chromosome 1 and 2 in both parents' map. This translocation could have caused unequal crossover in the second generation and inherited to sixth generation. Fragment deletion may also occur during the translocation. In the

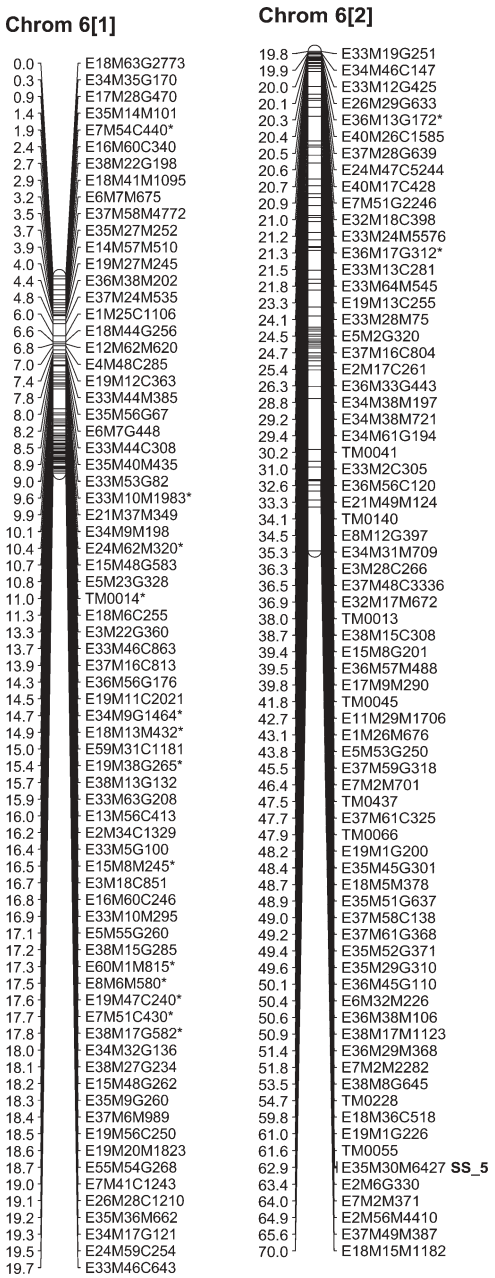


**Figure 3.** Continued

present map, chromosome 1 is 131.7 cM in length and chromosome 2 is only 50.1 cM in length. The difference of 30 cM is supposed to be the translocation fragment, compared with Hayashi et al.[13] chromosome 1 and 2 map. However, the map length of chromosome 1 and 2 are very close to that of Sandal et al.[14] map of *L. japonicus* from a cross of *L.filicaulis* × *L. japonicus*.

To further confirm the reliability of this linkage map, we developed a total of 300 AFLP co-dominant markers with the same primer-pair combinations and 97 SSR markers with 94 $F_9$ individuals derived from same $F_6$ individuals. With the same calculation, these 397 markers were located on six chromosomes and the order was generally the same within each chromosome, although there is a slight difference for some distances between markers. So this linkage map is reliable to use as a framework for physical map construction and map-based cloning in *L. japonicas*.

### 4.3. *Clustering of the markers*

The clustering of markers on each chromosome occurred significantly in this study. AFLP markers characteristically cluster in centromeric and/or telomeric regions in plant species with large genome.[21–24] Clustering of markers occurred mainly at heterochromatin-rich centromeric regions that ascribed to the great portions of repetitive sequences frequently present, and these repetitive sequences suppressed recombination between chromosomes.[21] Also, a high degree of clustering of markers in the AFLP map is much more pronounced than in the RFLP map.[25] There may be some very small variation, possibly, 1 bp deletion/insertion in repetitive sequences that can be detected by the AFLP technique, but not by RFLP technique. Thus, AFLP markers can be relatively easy to generate in highly repetitive regions near centromere.[22] In the present study, of the 1588 mapped markers, 575 duplicated markers will be excluded in the Fig. 3.

**Table 2.** The mapping characterization of six chromosomes of *L. japonicus* genome

| | SSR | AFLP markers | | | Total markers | Length (cM) | Unique markers* | Mean intervals | QTL |
|---|---|---|---|---|---|---|---|---|---|
| | | Co-dominant | G Dominant | M Dominant | | | | | |
| Chromosome 1 | 41 | 137 | 95 | 100 | 373 | 131.7 | 204 | 0.65 | 3 |
| Chromosome 2 | 7 | 102 | 83 | 67 | 259 | 50.1 | 154 | 0.33 | 2 |
| Chromosome 3 | 17 | 115 | 92 | 114 | 338 | 98.9 | 206 | 0.48 | 1 |
| Chromosome 4 | 12 | 71 | 100 | 58 | 241 | 82.5 | 157 | 0.53 | 6 |
| Chromosome 5 | 10 | 60 | 65 | 49 | 184 | 75.2 | 146 | 0.52 | 1 |
| Chromosome 6 | 10 | 48 | 69 | 66 | 193 | 70.1 | 146 | 0.48 | 1 |
| Total III | 97 | 533 | 504 | 454 | 1588 | 508.5 | 1013 | 0.50 | 14 |

*The duplicated markers were excluded.

Most significant clustering of markers located near the centromeric region.

Although the significant clusters presented, map gaps were found in whole genome, but much smaller than previous map. The biggest gap of 13.7 cM was found on chromosome 4. The gaps on other chromosomes ranged from 3.8 to 7.7 cM.

In this study, the rapid and efficient development of the linkage map with high resolution of *L. japonicus* was facilitated by the HEGS/AFLP system, by which a total of 1588 AFLP markers was mapped on the *L. japonicus* genome in 6 months. This map created a framework for anchoring EST, SSR and other sequence-based markers, and built the foundation for physical map construction in *L. japonicus* and gene cloning in other legume crops.

**Supplementary Data:** Supplementary data are available online at www.dnaresearch.oxfordjournals.org.

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