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ORIGINAL PAPER

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A genetic linkage map for azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]

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Abstract To make progress in genome analysis of azuki bean (*Vigna angularis*) a genetic linkage map was constructed from a backcross population of (*V. nepalensis x V. angularis) x V.angularis* consisting of 187 individuals. A total of 486 markers—205 simple sequence repeats (SSRs), 187 amplified fragment length polymorphisms (AFLPs) and 94 restriction fragment length polymorphisms (RFLPs) —were mapped onto 11 linkage groups corresponding to the haploid chromosome number of azuki bean. This map spans a total length of 832.1 cM with an average marker distance of 1.85 cM and is the most saturated map for a *Vigna* species to date. In addition, RFLP markers from other legumes facilitated finding several orthologous linkage groups based on previously published RFLP linkage maps. Most SSR primers that have been developed from SSR-enriched libraries detected a single locus. The SSR loci identified are distributed throughout the azuki bean genome. This moderately dense linkage map equipped with many SSR markers will be useful for mapping a range of useful traits such as those related to domestication and stress resistance. The mapping population will be used to develop advanced backcross lines for high resolution QTL mapping of these traits.

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Introduction

Azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] belongs to a group of legumes that includes mungbean [*V. radiata* (L.) Wilczek], known as the Asian *Vigna* (Kaga et al. 2005; Tomooka et al. 2002a). In addition to other Asian *Vigna*, azuki bean is closely related to cowpea [*V. unguiculata* (L.) Walp.] and species of the genus *Phaseolus*. Consequently, azuki bean genome map has the potential to be useful for a wide array of grain legumes.

Azuki bean is grown in East Asian and Himalayan countries. Its annual production in China and Japan has been estimated as 800,000 metric tons (Vaughan et al. 2005). Azuki bean is a diploid species with haploid chromosome number of 11 and small genome size estimated to be 539 Mbp (Parida et al. 1990). It has the best developed transformation systems among *Vigna* species (Yamada et al. 2001).

The relationships between cultivated azuki bean, its presumed wild progenitor (*V. angularis* var. *nipponensis*) and other Asian *Vigna* species have been well studied (Tomooka et al. 2002a; Zong et al. 2003). Based on morphological features and molecular analysis, the most closely related species to *V. angularis* is the Himalayan wild species *V. nepalensis* Tateishi & Maxted (Tateishi and Maxted 2002; Zong et al. 2003). Features of the inflorescence distinguish the two species. The two species occupy different habitats in the Himalayas with *V. nepalensis* growing at an altitude of about 1,000 m and wild azuki bean at higher elevations of about 1,700 m (Tomooka et al. 2002a). However, molecular analysis does not clearly distinguish *V. nepalensis* from Himalayan strains of *V. angularis* var. *nipponensis* (Zong et al. 2003), hence *V. nepalensis* can be considered to belong to the *V. angularis* complex (Tomooka et al.

2005). In addition, *V. nepalensis* and azuki bean are cross compatible, and hybrids between them are fully fertile. *V. nepalensis* exhibits resistance to the bruchid pests azuki bean weevil (*Callosobruchus chinensis* L.) and cowpea weevil (*C. maculatus* F.) (Tomooka et al. 2000).

Previously two different F_2 interspecific mapping populations have been developed for the construction of azuki bean linkage maps (Kaga et al. 1996, 2000). One map was between *V. angularis* and *V. nakashimae* (Ohwi) Ohwi & Ohashi and the other between *V. umbellata* (Thunb.) Ohwi & Ohashi and *V. angularis*. *V. angularis* is not as closely related to *V. nakashimae* and *V. umbellata* as it is to *V. nepalensis* based on AFLP analysis (Tomooka et al. 2002b). These two previous maps both resolved 14 linkage groups, three more than the haploid chromosome number of azuki bean. One of these populations involving a cross with *V. umbellata* had a high level of distortion (28.9%), and both populations were small with less than 90 individuals.

To date, azuki bean genome mapping has relied on using markers available for other legumes such as common bean (*Phaseolus vulgaris* L.), cowpea, mungbean and soybean [*Glycine max* (L.) Merr.]. However, recently simple sequence repeats (SSR) enriched libraries have been developed for azuki bean (Wang et al. 2004). These markers provide an opportunity to develop an enhanced azuki bean genome map. Development of an enhanced azuki bean genome map would enable it to be compared with those of related grain legumes and orthologous linkage groups to be determined.

The objectives of this study were:

(a) To construct a genome-wide saturated linkage map of azuki bean
(b) To determine the distribution across the genome of recently developed azuki bean SSR markers
(c) To compare the azuki bean linkage map with other grain legume linkage maps to seek orthologous linkage groups.

These objectives are pursued so that a range of useful traits can be analyzed in azuki bean including traits related to domestication and stress resistances.

Materials and methods

Plant materials

Cultivated azuki bean (*V. angularis* var. *angularis*, accession JP81481) was crossed to the wild relative (*V. nepalensis*, accession JP107881) at the National Institute of Agrobiological Sciences, Tsukuba, Japan. The *V. angularis* accession was a landrace from Tokushima prefecture, Japan, and the origin of the accession of *V. nepalensis* was a wild population collected in eastern Nepal. Both accessions came from the Genebank of the National Institute of Agrobiological Sciences, Tsukuba, Japan. In 2001, *V. angularis* grown from seeds of the original male parent was backcrossed to an F_1 plant (*V. nepalensis* x *V. angularis*) to obtain BC_1F_1 seeds in a vinyl greenhouse. The 187 BC_1F_1 individuals were grown in 20 cm diameter pots in a vinyl greenhouse from June to November in 2002 and used as the mapping population in this study.

DNA extraction

Total genomic DNA was extracted from three immature young leaves by the method of Draper and Scott (1988). DNA concentration was adjusted to 200, 50, 1 ng/μl for RFLP, AFLP and SSR analyses by comparing with known concentrations of standard λ DNA on 1.5% agarose gel, respectively.

RFLP analysis

Genomic DNA from parents was digested with six restriction enzymes (*Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Xba*I) under conditions recommended by the manufacturer (New England BioLabs, UK). The digested DNA was separated on 1% agarose gel and transferred onto Hybond N+ membrane (Amersham Pharmacia Biotech, UK) by alkaline solution (0.4 M NaOH, 1.5 M NaCl) and fixed by UV Cross linker at 60 mJ/cm^2 (Amersham Pharmacia Biotech, UK). Restriction fragment length polymorphism (RFLP) between parents was screened using mungbean, cowpea, common bean, and soybean probes. DNA from BC_1F_1 individuals was digested with enzymes that yielded clear polymorphisms between the parents and subjected to electrophoresis and blotted onto membranes. RFLP fragments between parents were scored as dominant/recessive in BC_1F_1 individuals on the basis of their presence or absence. Probe labeling, hybridization and detection were carried out by ECL direct nucleic acid labeling and detection systems according to the manufacturer's instructions (Amersham Pharmacia Biotech, UK).

AFLP analysis

AFLP was analyzed following the protocol of Vos et al. (1995). In brief, total genomic DNA from the parents and BC_1F_1 individuals was digested with *Eco*RI and *Mse*I (New England BioLabs, UK). Digested DNA fragments were ligated to *Eco*RI and *Mse*I adapters with T4 DNA ligase (Roche Molecular Biochemicals, Germany). The restriction ligation products were used as template DNA for the first PCR step (pre-amplification) with non-selective *Eco*RI and *Mse*I primers. The PCR products were diluted ten times with water and used as template DNA for the selective PCR step with selective *Eco*RI and *Mse*I primers. Forty-six primer combinations (40 *Eco*RI primers and 40 *Mse*I primers in

various combinations) with three selective nucleotides at the 3′-end were examined for the polymorphic survey between parents. To avoid redundant AFLP marker generation, most of the primer combinations were made from different *Eco*RI and *Mse*I primers. All the PCR products were amplified using GeneAmp 9700 (Applied Biosystems). Denatured second PCR products were run on 6% denaturing polyacrylamide gel (19:1) and stained according to the Silver Sequence DNA sequencing system (Promega, USA). Fragments showing AFLP between parents were scored as dominant/recessive in the BC_1F_1 individuals on the basis of the presence or absence of clear and unambiguous fragments. The segregating AFLP were named according to the primer combination name with estimated molecular weight of the fragment.

SSR analysis

Four hundred and one SSR primer pairs developed by Wang et al. (2004) were screened to detect polymorphism between the two parents. Ten microliter of PCR reaction mixture contained 1 ng of genomic DNA, 1U KOD-plus- DNA polymerase (TOYOBO, Japan), 1× PCR buffer for KOD-plus-, 0.2 mM dNTPs, 1 mM Mg_2SO_4 and 5 pmol of the forward and reverse primers. Five microliter of PCR products were run on precast gel Spreadex EL300, having the highest resolution in a narrow size range of 50–150 bp, using submerged gel electrophoresis system SEA2000 according to manufacturers instructions (Elchrom Scientific AG, Switzerland). One hundred and ninety-six primer pairs, showing polymorphism between the two parents, were selected for the mapping study (The list is available from the authors). The 5′-end of the reverse primer was labeled with one of the four following fluorescent dyes, 5-Fam, VIC, NED, and PET (Applied Biosystems, UK). Three or four differentially labeled primers were mixed into single PCR reaction mixtures and amplified at the same time. Fluorescent signal strength of each amplified fragment was adjusted to the same level by adding non-fluorescent labeled primer pairs instead of labeled primers and the multiplex sets were used to genotype BC_1F_1 individuals of the mapping population. All the PCR products were amplified using GeneAmp 9700 (Applied Biosystems) programmed as follows: 94°C for 2 min followed by 35 cycles of 94°C for 15 s, 45–65°C for 15 s, 68°C for 15 s. One microliter of PCR product was mixed with 13 µl of Hi-Di formamide containing 0.17 µl GeneScan 500 LIZ size standard (Applied Biosystems) and run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Using GENEMAPPER ver. 3.0 software (Applied Biosystems), alleles with four different colors in a multiplex PCR product were separated into respective loci and their sizes were determined.

Linkage map construction

JoinMap ver. 3.0 (Van Ooijen et al. 2001) was used to test the segregation ratio of the markers in the BC_1F_1 population and to construct a linkage map. The goodness-of-fit to a 1:1 segregation ratio of each marker was analyzed using the Chi-square test at 5, 1, and 0.1% significant levels. First, the pair-wise SSR loci that showed a recombination frequency smaller than the REC threshold of 0.4 and a LOD value larger than the LOD threshold 3.0 was used to create linkage groups and the recombination frequencies were converted into map distance (cM) using the Kosambi mapping function (Kosambi 1944). Then crossovers between SSR loci in a linkage group were verified manually and some doubtful data was reconfirmed by additional re-examination. After building the framework map, linkage analysis including AFLP and RFLP markers were repeated. AFLP and RFLP loci were verified at locations where the order of framework SSR loci was changed. The resultant linkage groups were named in descending order of the map length. Random marker distribution on the linkage map was tested using Poisson distribution as described by Young et al. (1999). An estimated genome length was calculated by adding 2 s (s = length of linkage group / the number of intervals) to the length of each linkage group to account for chromosome ends (Fishman et al. 2001).

Results

Polymorphism between *V. angularis* and *V. nepalensis* with different marker systems, SSR primer pairs, RFLP probes and AFLP primer combinations, were screened. Of 401 SSR primer pairs, 189 (47.1%) revealed clear polymorphism. Another seven primer pairs amplified multiple fragments in *V. nepalensis*. In total 196 primer pairs were used in linkage map development. These included 172 with prefix 'CEDG' and 18 primer pairs with prefix 'CEDC' that were designed to amplify dinucleotide repeat motif $(AG)_n$ and $(AC)_n$, respectively. Two primer pairs with 'CEDAAG' and one each of primer pairs with 'CEDAAT', 'CEDCAA', 'CEDGAG' and 'CEDGAT' were designed to amplify trinucleotide repeat motif $(AAG)_n$, $(AAT)_n$, $(CAA)_n$, $(GAG)_n$ and $(GAT)_n$, respectively. Of 187 RFLP probes screened, 13 (76.5%) out of 17 cowpea, 39 (70.9%) out of 55 mungbean, 38 (70.3%) out of 54 common bean, and 35 (57.4%) out of 61 soybean probes showed polymorphisms between the parents for at least one of the restriction enzymes. In total 74 low copy probes, from 7 cowpea, 28 mungbean, 24 common bean and 15 soybean, were used in linkage map development. Polymorphisms between parents and four BC_1F_1 individuals were screened with 46 AFLP selective primer combinations. Average number of fragments detected by each primer combination was

Table 1 Number of markers and average distance between markers in each linkage group in the azuki bean linkage map

Linkage group	Length (cM)	Average interval (cM)	No. of markers			
			Total	SSR	AFLP	RFLP
1	124.3	1.73	75	34	25	16
2	82.0	1.75	50	20	10	20
3	79.2	2.09	39	14	18	7
4	78.3	1.57	54	21	26	7
5	77.0	2.33	36	17	10	9
6	69.8	1.94	43	14	22	7
7	69.7	2.49	29	13	12	4
8	67.4	1.20	62	29	27	6
9	67.1	2.58	31	14	15	2
10	63.0	1.75	39	14	9	16
11	54.1	2.00	28	15	13	0
Total	832.1	1.85[a]	486	205	187	94

[a]Average of all linked markers

50.9 (range 14–82). Among a total of 2,575 bands, 234 (9.1%) were specific to *V. nepalensis* and most of them revealed segregation among four BC_1F_1 individuals in the preliminary experiment. Thirty-four AFLP selective primer combinations were used to develop the linkage map.

Construction of azuki bean linkage map

All 486 marker loci (205 SSR, 187 AFLP and 94 RFLP) detected by 196 SSR primer pairs, 74 RFLP probes and 34 AFLP selective primer combinations were assigned into 11 linkage groups, and there were no unlinked loci (Fig .1). The 11 linkage groups span 832.1 cM with an average marker distance of 1.85 cM (Table 1), and estimated genome length was 874.8 cM, and genome coverage was 95.1% over the genome. The length of the linkage groups ranged from 54.1 to 124.3 cM. Number of loci varied from 28 to 75 per linkage group, and marker density on linkage groups 4 and 8 were relatively higher than the other linkage groups (Table 1). Among 11 linkage groups, no RFLP locus was mapped to linkage group 11. SSR and AFLP loci were well distributed over all linkage groups whereas differences in marker density were observed when the number of markers at each 10 cM interval was counted. The marker density at 90–100 cM interval on linkage group 1, 30–50 cM interval on linkage group 3, 20–40 cM interval on linkage group 4, 50–60 cM interval on linkage group 5, 20–30 cM interval on linkage group 6, 40–60 cM interval on linkage group 8 was higher than other regions (Fig. 1). On linkage group 6, 26 markers were clustered at interval 20–30 cM. When the test for random marker distribution across the entire map was conducted, a significant deviation from expected Poisson distribution was observed for all markers ($\chi^2 = 232.0$; $df = 11$; $P < 0.0001$), AFLP markers ($\chi^2 = 114.2$; df = 5; $P < 0.0001$), SSR markers ($\chi^2 = 62.7$; df = 6; $P < 0.0001$) and RFLP markers ($\chi^2 = 53.8$; df = 4; $P < 0.0001$).

Table 2 Comparison of linkage groups in the present study to previously reported conserved groups between mungbean and common bean[a] and interspecific (rice bean *x* azuki bean) map[b] based on common RFLP markers

Azuki bean	Mung bean	Common bean	Common RFLP markers	Rice bean *x* Azuki bean	Common RFLP markers
1	1	C	Bng164, mc016, mgM228, mgM381, mgR002, sgA487, sgA509	10	mgR002, mgM186, sgA509
2	1	C	Bng123, mgM371, mgM456, sgA504	7	Mc003, mgM100, mgM371, mgM151
	8	K	Bng093, Bng099, mgM100, cgO008, cgO009		
	9	K	Bng110, Bng107, Bng134, mgM151		
3	6	F + H	Bng031, Bng041, mc003, mgR048	–	
4	2	F + H	Bng138, mgM241, sgA890	1	cgO109, mgM241, sgA890
5	7	G	Bng027, mgM208, mgM247	5	mgM208, sgA517
6	–	–		2	cgO091, mgR025, sgA064
7	–	–		–	
8	4	A	Bng081, Bng168, mgQ117	4	mgM316, mgQ117
9	–	–		–	
10	3	D	Bng119, mgM177, mgM307, mgM415, sgA106, sgA841, sgK070	3	mgM177, mgM415, cgO026, sgA106, sgA841
11	–	–		–	

More than two RFLP markers were not available between linkage groups
[a]Boutin et al. (1995)
[b]Kaga et al. (2000)

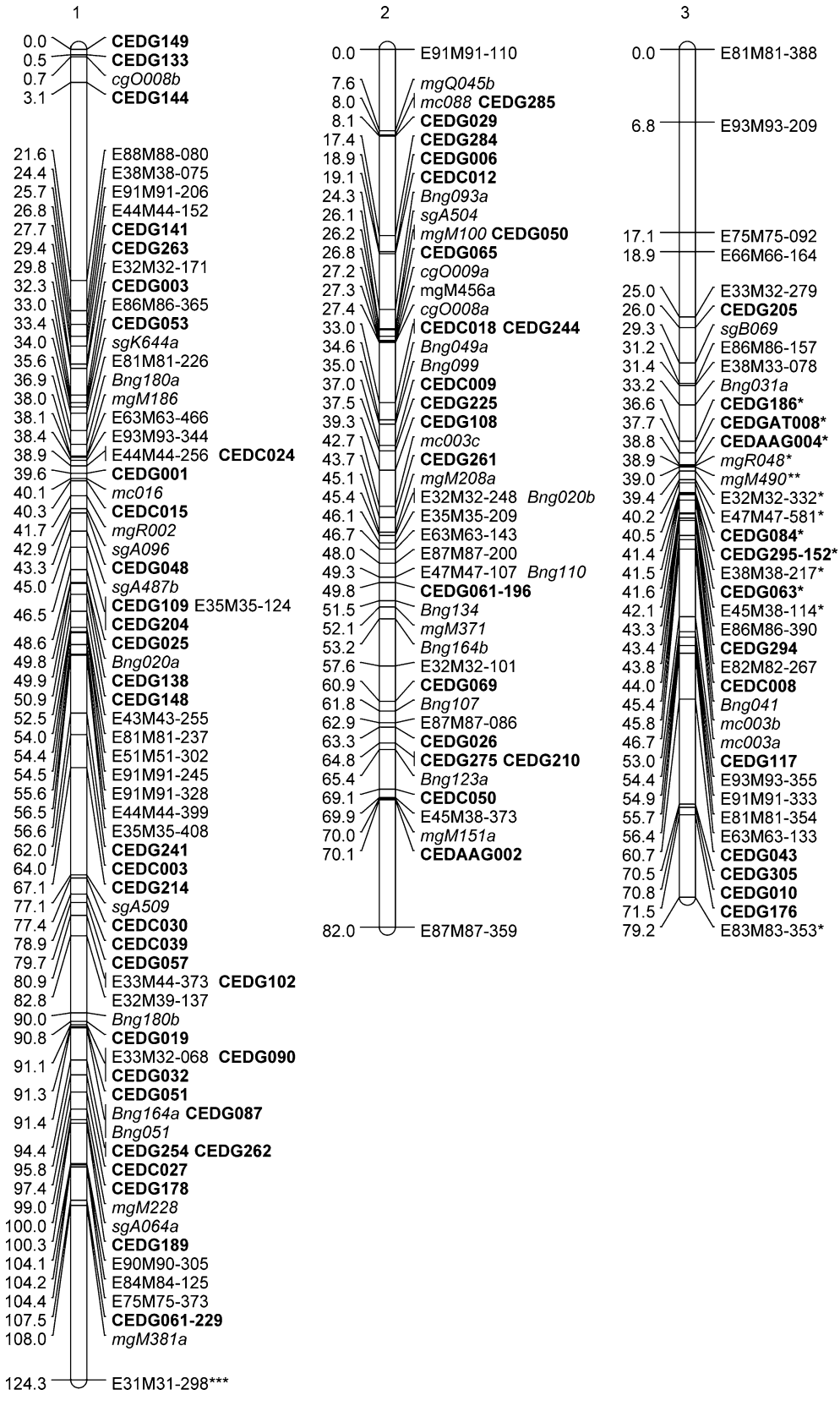
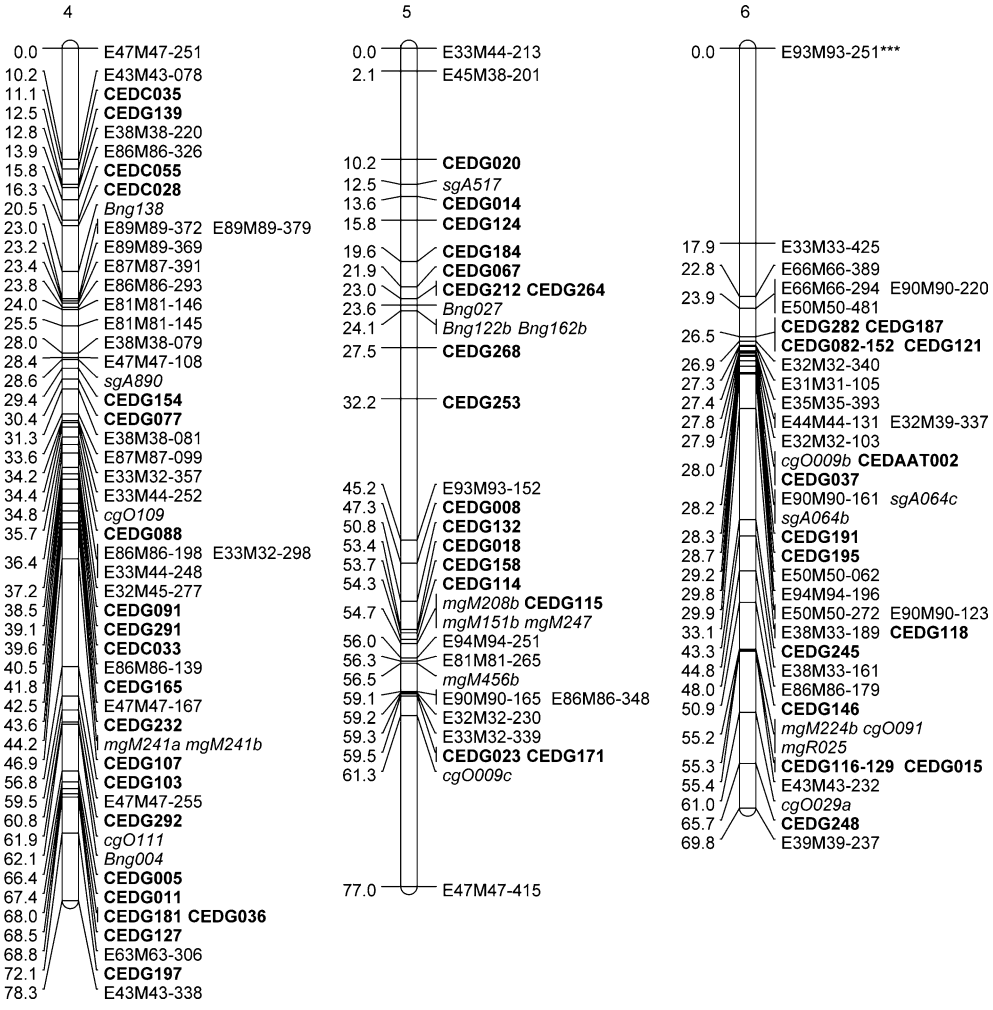


Fig. 1 A genetic linkage map of azuki bean based on SSR, RFLP and AFLP markers. This map was constructed from 187 BC_1F_1 individuals of (*V. nepalensis x V. angularis*) *x V. angularis*. Map distances and marker names are shown on the left and right sides of the linkage groups, respectively. *Bold* and *italicized* indicates SSR and RFLP markers, respectively. SSR and RFLP markers showing multiple loci are followed by numbers corresponding to the estimated size of the fragment (bp) and lower-case letters by signal strength of the fragment –a (strong), b (intermediate) and c (weak). Prefix mgM, mgQ, mgR, and mc indicate mungbean probes; cgO and cgP label cowpea probes; Bng identifies common bean probes; sgA, sgB, and sgK represent soybean probes. AFLP markers, prefix E..M.., were named according to the primer combination followed by the estimated size of the fragment. Markers showing significant deviation from the expected segregation ratios at 0.05, 0.01, and 0.001 levels are indicated with *, **, and ***, respectively

Multiple fragments were amplified by seven SSR primer pairs, CEDG061 (3 fragments), CEDG082 (2), CEDG-105 (2), CEDG116 (2), CEDG183 (2), CEDG290 (3) and CEDG295 (2) (Fig. 1). Multiple RFLP loci detected by a single probe were named with lower-case letters depending on the signal strength (Fig. 1) to enable

Fig. 1 (Contd.)



comparison with orthologous loci in other species in the future.

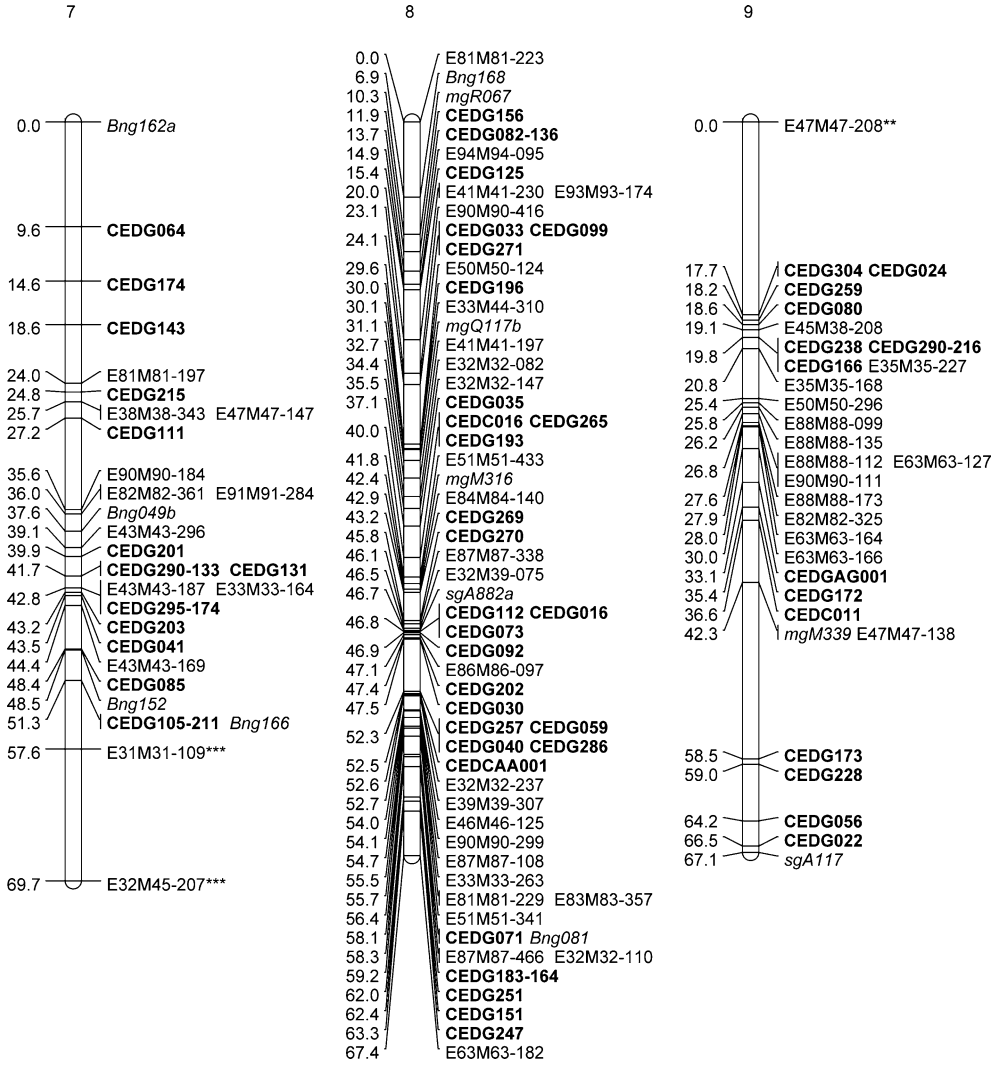
Among the 486 marker loci mapped, 19 loci (3.9%) revealed segregation distortion and were all skewed toward the heterozygote state except for E47M47-208 on linkage group 9, skewed toward homozygote state. Most of the loci showing severe distortion (significant at $P = 0.01$ and 0.001 levels) were AFLP loci located on the distal end of linkage group 1, 6, 7 and 9 (Fig. 1). A clustering of marker loci showing segregation distortion was found in the middle of linkage group 3.

Discussion

The genome map presented here is the most saturated for any of the *Vigna* maps to date. In addition, the linkage map consists of 11 linkage groups that correspond to the haploid chromosome number of *Vigna*. The observed coverage of the linkage map supported by no unlinked markers was about 95%. The linkage map length was greatly improved by re-examination of marker order and cross-over between linked markers, and the map information may facilitate accurate selection in advanced backcross progenies. Thus, this linkage map equipped with many SSR markers and a relatively large mapping population constitutes a comprehensive foundation for future genomic studies.

SSRs are an abundant resource in the genome and have a high level of allelic diversity. They are frequently used as genetic markers for plant genetic studies because of their co-dominant and multi-allelic nature (Powell et al. 1996). In order to discover SSRs comprehensively in azuki bean, Wang et al. (2003) developed SSR-enriched libraries and found more than five hundred clones containing SSR motifs. The 205 SSR loci detected by the 196 SSR primer pairs are distributed throughout the azuki bean genome. Although all these primer pairs were designed from non-redundant sequences, several SSR loci on each linkage group were clustered at the same position. Furthermore, marker loci were not evenly distributed across the genome contrary to our expectation of a random distribution across the genome. Since DNase I in the presence of manganese chloride has very low sequence specificity in its cutting site (Anderson 1981) and all SSR markers in the present study are developed from SSR enriched libraries for which DNase I was

Fig. 1 (Contd.)

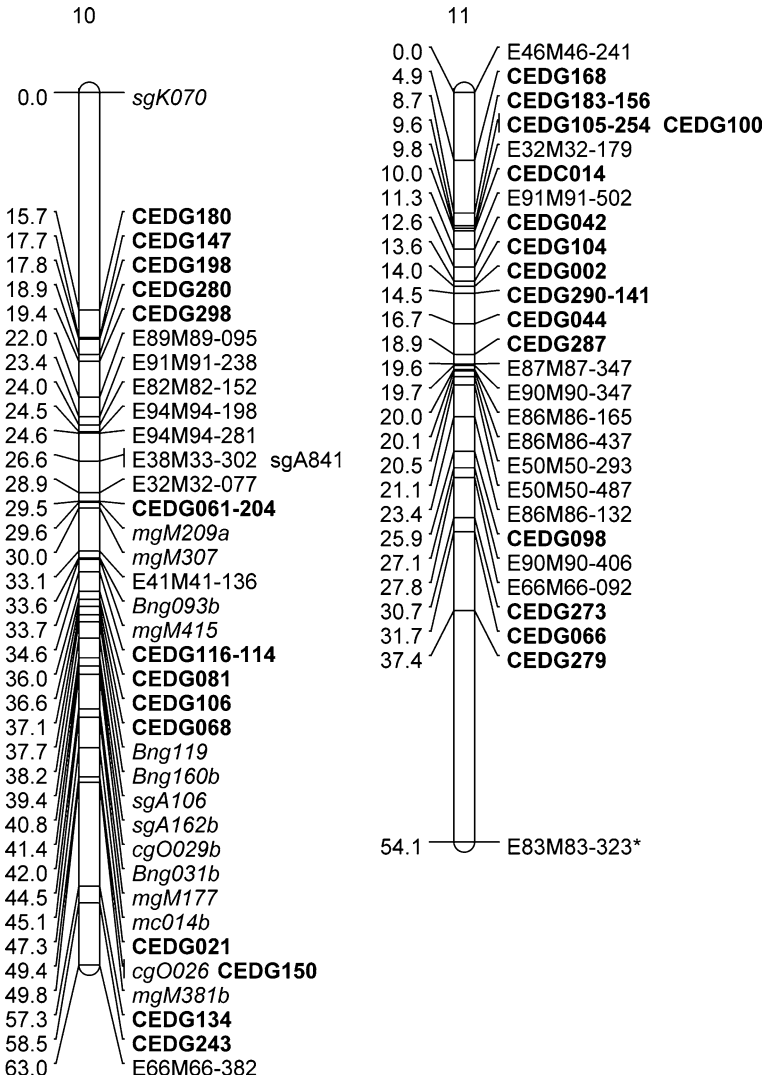


used in library construction, SSR markers are expected to have a random distribution in the azuki bean genome. One explanation for uneven marker distribution is the suppressed recombination in certain regions, most likely associated with the centromere (Areshchenkova and Ganal 2002; Keim et al. 1997; Young et al. 1999).

Although many markers are now available for azuki bean mapping, there remain gaps in the genome map of more than 15 cM between markers on linkage groups 1, 6, 9, 10 and 11. As indicated by the large number of recently published maps, AFLP markers are suitable for increasing markers on linkage maps because of their abundance and reproducibility. In the present study, we used the enzyme combination of *Eco*RI/*Mse*I to integrate AFLP markers, however, non-random distribution and clustering of AFLP markers were observed, as has been reported in other plant species. Young et al. (1999) proposed that the methylation sensitive enzyme *Pst*I would be more effective than *Eco*RI to develop AFLP markers randomly distributed on genome maps. In tomato, the SSR markers with the AT repeat motif from EST, the most prevalent in plant genomes but having some difficulties with primer design, are distributed more randomly than the other dinucleotide repeat motif (Areshchenkova and Ganal 2002). Therefore, to fill gaps in the azuki bean genome map and obtain a more saturated linkage map with markers evenly distributed, integration of these new markers must be considered.

Although the mapping population in the present study was constructed by interspecific hybridization, the proportion of markers showing segregation distortion (3.9%) was lower than that in the previous interspecific mapping population of *V. umbellata x V. angularis* (29.8%) (Kaga et al. 2000) and *V. angularis x V. nakashimae* (19.7%) (Kaga et al. 1996). Based on variation in the intergenic region of chloroplast DNA and ribosomal DNA ITS (Doi et al. 2002), AFLP (Tomooka et al. 2002b), *V. angularis* is more closely related to *V. nepalensis* than *V. nakashimae* and *V. umbellata*. The low level of segregation distortion may be correlated with the genetic similarity of parental species used. Several RFLP markers facilitated comparison of the distorted loci between previously reported maps and the present map. One RFLP marker 'mgR048' among a cluster of distorted loci on linkage groups 3 in

Fig. 1 (Contd.)



this study shows normal segregation in the populations of *V. umbellata x V. angularis* and *V. angularis x V. nakashimae*. However, 'mgM316' and 'mgQ117' on linkage group 8 and 'mgM415' and 'sgA841' on linkage group 10 show normal segregation in this study but segregation distortion in the population of *V. umbellata x V. angularis*. In addition, 'mgM241' on linkage group 4 shows normal segregation in this study but segregation distortion in the population of *V. angularis x V. nakashimae*. Different cross compatibilities and abnormalities in the progenies between *V. angularis* and *V. nepalensis*, *V. nakashimae* and *V. umbellata* may have a large effect on these differences. Crosses between *V. umbellata* and *V. angularis* require embryo rescue, and there is a high level of abnormality in segregating populations (Kaga et al. 2000).

RFLP markers from other legumes facilitated finding several orthologous linkage groups in other published RFLP linkage maps. Comparison of the mungbean and common bean linkage maps (Boutin et al. 1995) based on 40 markers, 7 out of 11 azuki linkage groups were found to be orthologous to mungbean and common bean conserved groups (Table 2). Azuki bean linkage group 1, the largest linkage group, was found to correspond to the largest mungbean linkage group 1. On the other hand, the second largest, azuki bean linkage group 2, was composed of three separate mungbean linkage groups, 1, 8 and 9 and two reported common bean linkage groups, C and K (Boutin et al. 1995). Since linkage groups 8 and 9 in mungbean correspond to a single linkage group in azuki bean and common bean this suggests that mungbean linkage groups 8 and 9, belong to a single linkage group. Although 114 RFLP markers were mapped to the interspecific map of *V. umbellata x V. angularis*, common RFLP markers, mostly from mungbean, were limited due to low polymorphism between parents in the present study. Based on several markers, seven out of 14 linkage groups in the interspecific map of *V. umbellata x V. angularis* are thought to be common to the seven linkage groups in the present study (Table 2). We also intended to compare the azuki bean linkage map with

that of soybean, based on RFLP markers, however, it was not possible due to limitation of low copy polymorphic probes incorporated into the azuki bean linkage map. The addition of SSR makers from *Phaseolus* species, both gene-based (Blair et al. 2003; Yu et al. 1999, 2000) and genomic SSR (Gaitán-Solís et al. 2002), will greatly facilitate comparison with the *Phaseolus* linkage map. Cross-genus amplification of soybean SSR markers are reported to be poor (13%) in *Vigna* (Peakall et al. 1998), however, more than 1,000 genomic SSR markers developed by Song et al. (2004) and Cregan et al. (1999) and more than 3,000 SSRs in unigenes from soybean identified by Tian et al. (2004) may become a large potential marker source for azuki bean. Most of the SSR markers (ca. ~70%) mapped here can be amplified in other *Vigna* species (Tomooka et al. 2005). Thus this moderately dense linkage map for azuki bean equipped with many SSR markers will provide a basic framework for constructing or improving linkage maps of related species.

This mapping population is now being used to analyze azuki bean domestication syndrome related traits. In addition, since the parents differ in response to the bruchid pests (*Callosobruchus* spp.), the population is also being analyzed for resistance to these pests.

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