Breeding Science 53 : 335-344 (2003)

Construction of a High-density AFLP and SSR Map Using Recombinant Inbred Lines of Cultivated × Weedy Soybean

Pilson Choi[1,4], Yoshiro Mano[1,5], Atsuko Ishikawa[1,2], Masashi Odashima[3,6], Taishi Umezawa[3,7], Tatsuhito Fujimura[3], Yoshihito Takahata[2] and Takao Komatsuda*[1]

[1] *National Institute of Agrobiological Sciences (NIAS)*, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2] *Faculty of Agriculture, Iwate University*, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan
[3] *Institute of Agricultural and Forest Engineering, University of Tsukuba*, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan
[4] *Present address*: *Eugentech, Korea Research Institute of Bioscience & Biotechnology*, 52 Oun-dong, Yusong, Taejon 305-333, Korea
[5] *Present address*: *National Institute of Livestock and Grassland Science*, 768 Senbonmatsu, Nishinasuno, Tochigi 329-2793, Japan
[6] *Present address*: *Iwate Agricultural Research Center*, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan
[7] *Present address*: *RIKEN Tsukuba Institute*, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

A genetic linkage map of soybean was constructed by using a cross between a cultivar 'Keburi' and a weedy form of soybean 'Masshokutou Kou 502'. The parents differed in their ability in somatic embryogenesis. The mapping population consisted of 117 recombinant inbred lines (F_{11}), and generated 30 linkage groups, which covered 2089 cM, including 515 amplified-fragment-length polymorphism (AFLP) markers and 85 simple sequence repeat (SSR) markers. For AFLP analysis, a simple technique using small polyacrylamide gels combined with silver staining was applied, which enabled a single person to construct a map in a short time at a reasonable cost. Comparison of this map with previously published maps revealed that 1) the level of polymorphism was the same as in a cultivar × cultivar cross; and 2) the cultivar × weed map was 13% shorter than the cultivar × wild map. The high-density map constructed in this study would contribute to the molecular mapping of quantitatively and qualitatively inherited characters from weedy forms of soybean.

Key Words: ***Glycine max*** **(L.) Merr., weedy soybean, molecular linkage map, amplified-fragment-length polymorphism (AFLP), simple sequence repeat (SSR), recombinant inbred line (RIL).**

Introduction

The genus *Glycine* consists of two subgenera, *Glycine* and *Soja*. The latter includes cultivated and wild forms, named respectively *G. max* and *G. soja*. Both species are annual and diploid with 2n = 40 (Hymowitz *et al.* 1998). A weedy form (*G. gracilis*) which is morphologically intermediate between the cultivated and wild forms also occurs in Northeast China (Skvortzow 1927). Crossing experiments show that the three forms in fact comprise a single biological species (Singh and Hymowitz 1989, Smartt 1990), and the weedy form is incorporated into *G. max* (Hymowitz *et al.* 1998). Nevertheless crossing cultivated and weedy forms provides a considerable polymorphism for both quantitatively and qualitatively inherited characters. More than ten years ago, one of us reported that some wild and weedy soybeans exhibited a higher competence for somatic embryogenesis and plant regeneration than did cultivated soybeans (Komatsuda and Ohyama 1988, Komatsuda and Ko 1990). This finding was of great interest for mapping quantitative trait loci (QTLs). For these purposes, in the present study we constructed recombinant inbred lines (RILs) and a molecular linkage map of cultivar × weedy combination.

Since molecular marker systems have been improved dramatically, the construction of genetic linkage maps has become possible in many plants. The first soybean genetic map was constructed with 57 classical markers (Palmer and Kilen 1987). Thereafter, high-density molecular maps have been gradually developed by using restriction-fragment-length polymorphism (RFLP) markers (Apuya *et al.* 1988, Keim *et al.* 1989, Shoemaker 1994), random amplified polymorphic DNA (RAPD) markers (Williams *et al.* 1990), simple sequence repeat (SSR) markers (Akkaya *et al.* 1992, Maughan *et al.* 1995), and amplified-fragment-length polymorphism (AFLP) markers (Maughan *et al.* 1996, Keim *et al.* 1997). The AFLP system was developed to provide a larger number of polymorphic bands per lane than in the other marker systems (Vos *et al.* 1995). Recently a simple AFLP technique using small polyacrylamide gels combined with silver staining has been developed (Mano *et al.* 2001), it enables a single person to construct an AFLP map in a short time at a reasonable cost.

To construct maps using hundreds of AFLP markers, 'known' markers, such as RFLP and SSR markers, are very useful as 'anchors'. SSR markers are ideal tools because they are highly abundant and evenly distributed in the genome, highly polymorphic, codominant, rapidly typed via

Communicated by K. Takeda
Received February 20, 2003. Accepted May 30, 2003.
*Corresponding author (e-mail: takao@affrc.go.jp)

PCR reactions, and highly accessible to other laboratories (Diwan and Cregan 1997, Morgante and Olivieri 1993). When the system was applied to soybean (Akkaya *et al.* 1992), a total of 606 SSR loci were mapped in three populations (Cregan *et al.* 1999). The integration of AFLP and SSR markers increases the information on AFLP maps, because the chromosomal location of each AFLP can be identified.

Because AFLP generates dominant markers, well-advanced RILs have major advantages over F_2 populations. They represent an important genetic resource for developing and exploiting linkage maps (Burr *et al.* 1988). RILs have been used to construct linkage maps for rice (Cho *et al.* 1998), barley (Mano *et al.* 2001, Qi *et al.* 1998), and soybean (Keim *et al.* 1997). RILs also have an advantage for the genetic analysis of quantitative traits, because they provide a sufficient amount of seeds for replicated field-testing and are permanent populations that can be propagated in a variety of environments by many investigators.

In this paper, we describe the construction of a high-density soybean AFLP and SSR map using RILs of the F_{11} generation developed from a cross between a cultivar and a weedy soybean. We compared the generated map with published cultivar × wild and cultivar × cultivar maps.

Materials and Methods

Plant materials

Keburi is a Japanese cultivar, and Masshokutou Kou 502 (synonymous with Mo-shi-dou Gong 502) is a weedy soybean originating from Manchuria (Fukuda 1933). The parents were chosen based on the difference in their ability in somatic embryogenesis (Komatsuda *et al.* 1991, Endo *et al.* 1998, Ito *et al.* 1999). Seeds were obtained from the Gene Bank of NIAS (formerly NIAR) and have been maintained in our laboratory since 1987. RILs (F_{11}) were generated by single-seed descent from a cross between Keburi as the female and Masshokutou Kou 502 as the male, with two or three generations per year in the field or in a greenhouse (Komatsuda, unpublished). A total of 117 RILs were used for genetic mapping.

DNA isolation

Total DNA was extracted from 2 g of leaf tissue from six-week-old seedlings following the procedure described by Dellaporta *et al.* (1985) with some modifications (Komatsuda *et al.* 1998). The DNA concentration was estimated by comparison with lambda DNAs stained with ethidium bromide in a 1.2% agarose gel.

AFLP analysis

The AFLP analysis essentially followed the methods described by Vos *et al.* (1995) and the instructions of Gibco BRL (Life Technologies, Inc., Rockville, MD, USA), with a modification for electrophoresis using a small slab gel (16 cm × 16 cm) consisting of 6% stacking gel and 7% separation gel with a discontinuous buffer system (Mano *et al.* 2001). Pre-selective and selective primers are listed in Table 1. The name of each AFLP band is derived from the primer combinations and the relative size of the amplified fragments. The fragment sizes ranged from 1 to 13 against DNA molecular weight marker VIII (Boehringer Mannheim, Tokyo, Japan). They were designated as follows: 1, > 1114 bp; 2, 1114–900 bp; 3, 900–692 bp; 4, 692–501 bp; 5, 501–489 bp; 6, 489–404 bp; 7, 404–320 bp; 8, 320–242 bp; 9, 242–190 bp; 10, 190–147 bp; 11, 147–124 bp; 12, 124–110 bp; 13, 110–67 bp. For example, "e13m29-10" indicates the polymorphic fragment in size range 10 (190–147 bp) that was amplified by the E-ATA and M-CTA primer combination.

Table 1. List of universal and selective primers

*Eco*RI universal primer		*Mse*I universal primer	
E-000	5′-GACTGCGTACCAATTC-3′	M-000	5′-GATGAGTCCTGAGTAA-3′
*Eco*RI-selective primers		*Mse*I-selective primers	
e01	E-000+AAA	m17	M-000+CAA
e02	E-000+AAC	m18	M-000+CAC
e03	E-000+AAG	m19	M-000+CAG
e04	E-000+AAT	m20	M-000+CAT
e05	E-000+ACA	m21	M-000+CCA
e06	E-000+ACC	m22	M-000+CCC
e07	E-000+ACG	m23	M-000+CCG
e08	E-000+ACT	m24	M-000+CCT
e09	E-000+AGA	m25	M-000+CGA
e10	E-000+AGC	m26	M-000+CGC
e11	E-000+AGG	m27	M-000+CGG
e12	E-000+AGT	m28	M-000+CGT
e13	E-000+ATA	m29	M-000+CTA
e14	E-000+ATC	m30	M-000+CTC
e15	E-000+ATG	m31	M-000+CTG
e16	E-000+ATT	m32	M-000+CTT

SSR analysis

We selected 150 SSR markers from the linkage map reported by Cregan *et al.* (1999). Reaction mixtures contained 30 ng of soybean genomic DNA, 0.15 μM forward and reverse primers, 0.15 mM dNTPs, 1 × PCR buffer (20 mM Tris · HCl [pH 8.0], 100 mM KCl, 1.5 mM $MgCl_2$, 0.5% Triton X-100; Nippon Gene, Tokyo, Japan), and 0.35 units of Taq DNA polymerase (Nippon Gene, Japan, or Promega, Madison, Wis., USA) in a total volume of 10 μL. The thermal cycler (MJ Research, Watertown, Mass., USA) was programmed as follows: 2 min at 95°C followed by 40 cycles of 25 s at 92°C, 25 s at 47°C or 40°C, and 25 s at 68°C. PCR products were separated by electrophoresis on 2% Agarose L03 (Takara Shuzou, Tokyo, Japan) or 3% MetaPhor agarose (FMC, Rockland, Maine, USA). For each primer set, the annealing temperature for PCR and the agarose gel for electrophoresis were determined from the preliminary test.

Seed storage protein

Because Keburi lacks the α′-subunit of β-conglycinin (Kitamura and Kaizuma 1981), we analyzed the phenotype of each RIL by SDS-PAGE according to the method described by Kitamura *et al.* (1984).

Map construction

We used the RI model of MAPMAKER ver. 3.0 (Lander *et al.* 1987). To choose "anchors" from the SSRs, we applied the "group" command at LOD > 3.0 and recombination fraction = 0.5 to the 86 SSRs. Using the resulting 59 SSRs as anchors, we tried to assign AFLP markers and the rest of SSR markers to 20 groups by using the "two-point" command at LOD > 3.0 and recombination fraction = 0.3. Unassigned markers were then grouped at LOD > 3.0 and recombination fraction = 0.5. A framework map was constructed by using the "three-point" and "order" commands at LOD > 3.0, and then at LOD > 2.0. The marker order was verified by use of the "ripple" command. Markers that could not be placed on the framework map at LOD > 2.0 were called "accessory" markers and were placed to the right of the nearest upper framework marker with their map distance (cM) from it marked (Fig. 1). For the linkage groups with few markers, the marker order and distance were calculated by using the "compare" and "map" commands. Kosambi's mapping function was used to calculate the map distances (Kosambi 1944).

Results

AFLP markers

The 16 selective primers for each of the *Eco*RI and *Mse*I restriction enzymes (Table 1), and the 256 possible primer combinations, were used to determine the levels of polymorphism in the parents (Keburi and Masshokutou Kou 502). On average, 35.5 AFLP bands were generated for each primer pair in Keburi or Masshokutou Kou 502 and 3.7 bands (11%) were polymorphic between the parents. For segregation analysis, we selected 91 primer combinations that showed more than five polymorphic bands per lane. Of 775 bands that were polymorphic in the parents, 532 clearly identified bands were scored as AFLP markers. The remaining 243 polymorphic bands were not included because of ambiguity in scoring, owing to a dense or overlapping banding pattern. The best two primer combinations were e04m31 and e12m26, which showed 42% and 44% polymorphic bands, respectively (data not shown).

The usefulness and applicability of these AFLP markers in the construction of a soybean linkage map were tested by the chi-squared method for goodness of fit to the expected 1 : 1 ratio. As a result, 56 out of 532 AFLP fragments showed a distorted segregation significant at the 5% level, and 27 at the 1% level (Fig. 1). Of the 532 AFLP markers, 280 (53%) were dominant for the Keburi allele and 252 (47%) for the Masshokutou Kou 502 allele.

SSR markers

Of 150 pairs of SSR primers preliminarily tested, 85 pairs generated single polymorphic bands identifying co-dominant alleles at each locus, and one pair of SSR primers generated double bands, of which one was polymorphic identifying co-dominant alleles (satt315.2) and the other was monomorphic (data not shown). Scoring of the SSRs was straightforward. Of a total of 86 SSRs, only three showed a distorted segregation significant at the 5% level, and none at the 1% level, showing good fit of the observed segregation to the expected 1 : 1 ratio. Heterozygosity was 0.08% overall, indicating that this was negligible in the linkage analysis.

Linkage maps

Because Cregan *et al.* (1999) established 20 linkage groups based primarily on RFLP and SSR markers, we tested some of the SSR markers in this study as possible anchors for grouping AFLPs. To choose reliable anchors, we applied the "group" command of MAPMAKER to the 86 SSRs (LOD > 3.0, recombination fraction = 0.5); 56 SSRs generated 17 groups, including at least two SSRs common with the consensus map of Cregan *et al.* (1999). We assumed that the 17 groups corresponded to all of the consensus maps of Cregan *et al.* (1999) except for D1a + Q, N and O. Including satt184, satt125 and satt094, respectively, as anchors for those exceptions, we used a total of 59 SSRs as anchors in our map construction.

Of 532 AFLP and 86 SSR markers, we assigned 462 AFLP and 82 SSR markers to the 20 consensus linkage groups. The remaining 70 AFLP and four SSR markers were then tested to construct additional groups. As a result, 53 AFLP and three SSR markers formed ten groups, which we named groups 21 to 30. The remaining 17 AFLP and one SSR markers were unlinked.

The linkage maps are shown in Fig. 1. The 20 maps from groups 1 to 20 probably corresponded to the consensus map of Cregan *et al.* (1999), because each map had at least two common SSRs and the orders of the SSRs were congruent.

The lengths of the consensus maps ranged from 19.1 cM (group 5 or consensus map C1) to 177.6 cM (group 9 or D2). The lengths of the remaining ten linkage maps ranged from 3.1 (group 30) to 43.6 cM (group 24). The total map includes 515 AFLPs and 85 SSRs and covers 2089 cM, with an average interval between markers of 3.7 cM.

The *cgy1* locus for the α′-subunit of β-conglycinin was mapped to the end of linkage group 25. Since this group did not correspond to the consensus map, the *cgy1* locus could not be assigned to a known linkage group.

Discussion

Ours is the first genetic linkage map constructed for cultivar × weedy soybean. We compared the level of polymorphism of this combination with a cultivar × cultivar combination. In this study 150 SSRs primer sets were used and 86 (57%) of them generated polymorphic DNAs. Soybean is strictly inbreeding, and Nei's (1987) gene diversity for this combination was estimated as 0.57. In the studies of Cregan *et al.* (1999), gene diversity within ten cultivars based on the common 150 primer sets can be estimated as 0.58. This result indicates that the cultivar × weedy combination has an equal level of potential for polymorphism as have cultivar × cultivar combinations. In their study, Cregan *et al.* (1999) separated SSR DNAs on sequencing gels, while we separated SSR DNAs on agarose gels. Therefore, we probably have underestimated the gene diversity in our study. In the AFLP analysis, the rate of polymorphic bands was 11%, which is almost the same as the 12% observed in a cultivar × cultivar cross (Keim *et al.* 1997). In their study, Keim *et al.* (1997) separated AFLPs on sequencing gels, while we separated AFLPs on small gels. This result indicates that a cultivar × weedy cross has an equal to or higher level of potential for polymorphism than a cultivar × cultivar cross.

A reduction of map distance has been recognized in interspecific crosses of plants, including cultivar × wild crosses of soybean (Griffin and Palmer 1987). Cregan *et al.* (1999) generated three linkage maps of two cultivar × cultivar crosses and one cultivar × wild cross, using mostly SSR, RFLP and RAPD markers. To study the recombinational suppression in soybean, we compared map distances between common SSRs for the cultivar × wild cross of Cregan *et al.* (1999) and our cultivar × weedy cross (Table 2). We calculated a total length of common SSR intervals of 1402 cM from the cultivar × wild map and 1216 cM from the cultivar weedy map, showing a 13% reduction (Table 2). Even though the reduction was not statistically significant ($t = 1.78$, $df = 19$, $P = 0.09$), this result seems to contradict the general finding that wider crosses lead to greater reduction in recombination values. In the study of Cregan *et al.* (1999) cultivar × wild and cultivar × cultivar crosses were compared,

Table 2. Comparison of map distances between two populations

Linkage group	Interval	Cultivar × wild[1] length (cM)	Cultivar × weedy[2] length (cM)
A1	Satt276–Satt385	64.8	59.6
A2	Satt207–Satt228	142.5	159.2
B1	Satt332–Satt359	18.8	23.0
B2	Satt168–Sct_064	55.1	22.3
C1	Sct_186–Satt194	7.5	19.1
C2	Satt291–Satt316	123.7	93.8
D1a+Q	Satt184–Satt179	59.5	69.6
D1b+W	Satt157–Satt271	91.9	104.8
D2	Satt135–Satt386	120.1	140.9
E	Satt212–Satt384	16.3	6.8
F	Satt149–Sat_120	93.8	51.8
G	Satt309–Sat_117	136.5	105.4
H	Satt253–Satt181	47.2	26.7
I	Satt367–Satt162	77.3	91.5
J	Satt414–Satt183	9.0	1.3
K	Satt178–Satt260	90.0	45.8
L	Satt182–Satt166	64.6	81.5
M	Satt245–Satt346	70.5	55.5
N	Satt125–Sat_091	51.8	33.3
O	Satt094–Satt331	61.2	43.7
Total[3]		1402.1	1235.6

[1] Cregan *et al.* (1999)

[2] This study.

[3] Since map distances between two common simple sequence repeats were compared, the length in this table is shorter than the full length in the linkage maps.

Fig. 1. An AFLP-SSR linkage map of soybean recombinant inbred lines (F_{11}) generated from a cross between a cultivar and a weedy form. RI model of MAPMAKER/3.0 was used. Twenty linkage groups (1 to 20) were assumed to correspond to consensus maps A1 to O of Cregan *et al.* (1999), because they contained at least two common SSRs and the order was congruent (except for group K). Of the ten additional linkage groups (21 to 30), groups 21, 22 and 23 were tentatively assigned to B2-2, J-2 and N-2, respectively, because each contained one SSR marker. Markers in boldface are SSR markers. Framework markers are placed immediately to the right of each linkage map. Accessory markers, which could not be assigned as framework markers, are shown in italics to the right of the nearest upper framework markers, together with their recombination distance (cM). The remaining markers, which were grouped but whose order could not be determined, are listed below the map. The following AFLP markers were excluded from the map because their inclusion considerably increased the map length: (529) e06m20-7-1, (531) e06m20-8-1, and (532) e06m20-8-2 on linkage group A2; (117) e04m29-7-1, (228) e04m32-7-2, and (347) e13m23-8 on linkage group C2; (148) e01m21-7 and (488) e02m17-8 on linkage group D1b+W; (316) e13m29-4-4 and (549) e10m20-8-2 on linkage group D2; (533) e06m20-8-3 and (535) e06m20-9-2 on linkage group F; (484) e03m31-8 on linkage group G; (123) e04m29-9 on linkage group L; and (595) e06m28-7 on linkage group O. These markers are also listed below the map. Group I was constructed with SSR markers only, because the 'order' command could not be run when AFLP markers were included. Distorted markers are indicated by * at the 5% level and ** at the 1% level of significance.

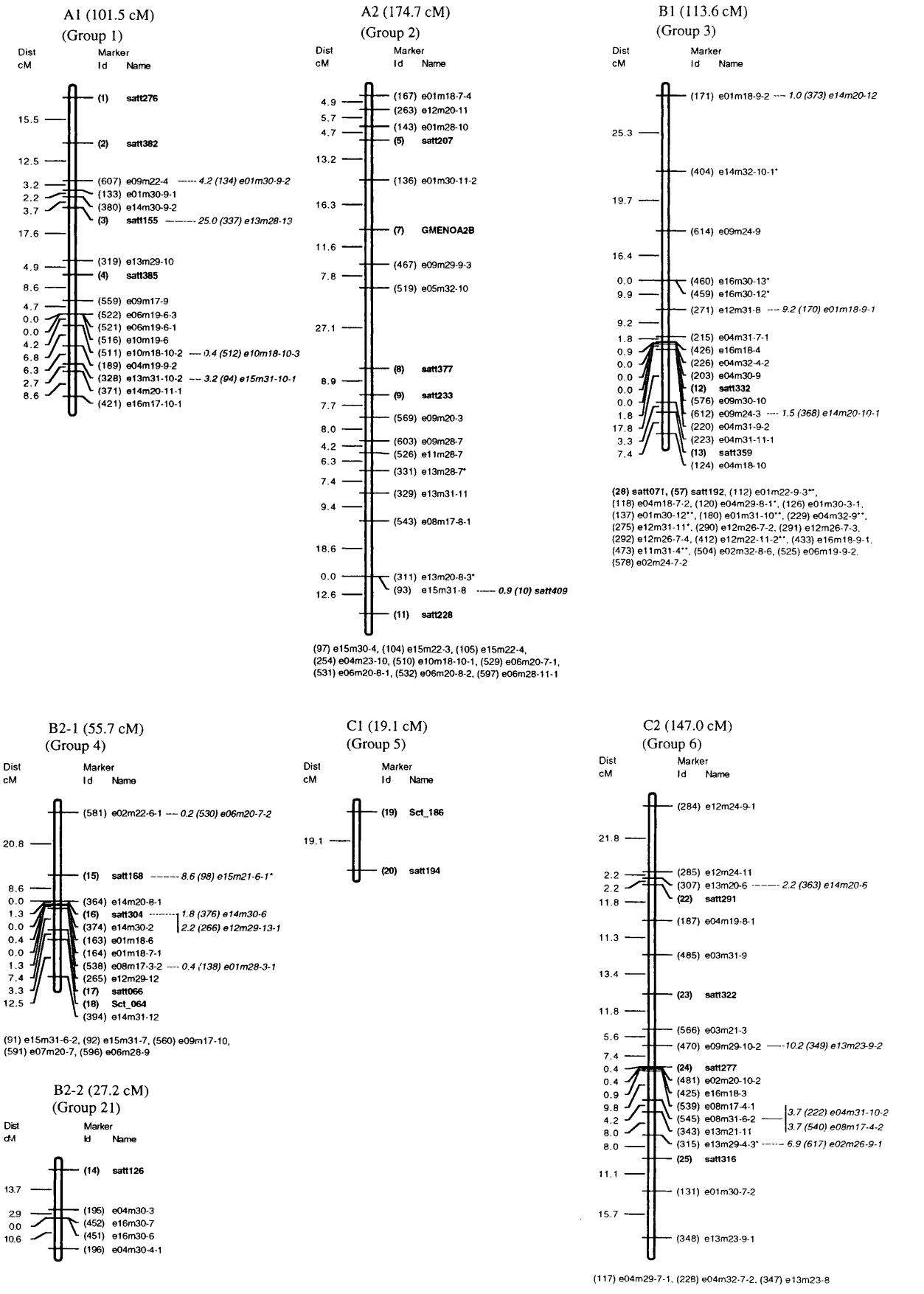

Fig. 1.

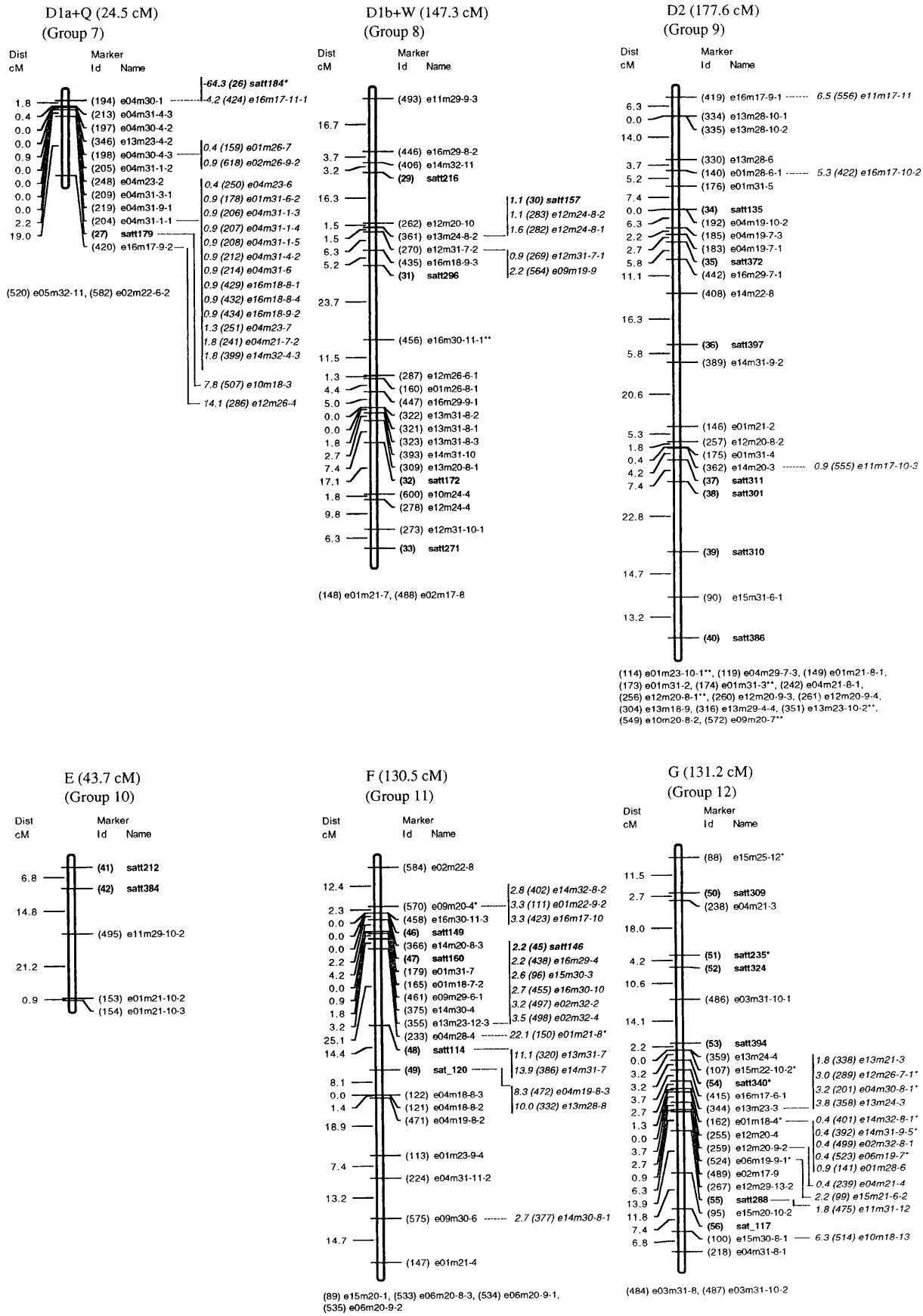

Fig. 1. (continued)

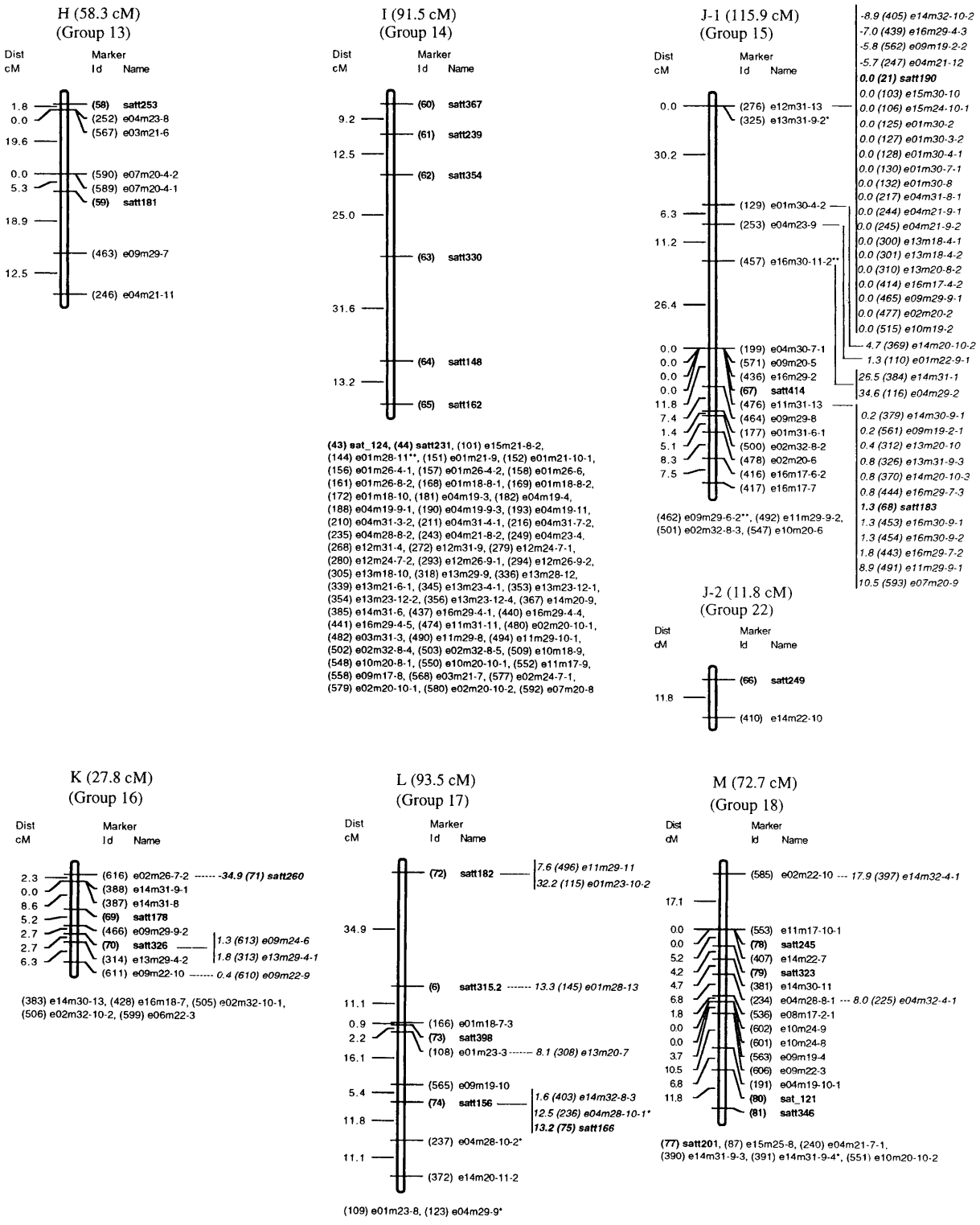

Fig. 1. (continued)

and the map length of the former was extended, particularly in linkage groups C2 and G. The SSR markers and other molecular markers were very rich in the regions where extension was considerable (Cregan *et al.* 1999). In these regions, therefore, the extension can be ascribed to the fact that the interspecific cross provides a higher degree of polymorphism, and the inclusion of a larger number of loci enabled us to detect more double crossing-over events.

Only one exceptional marker remarkably extended the map distance in the cultivar × weed map: satt315.2, a marker

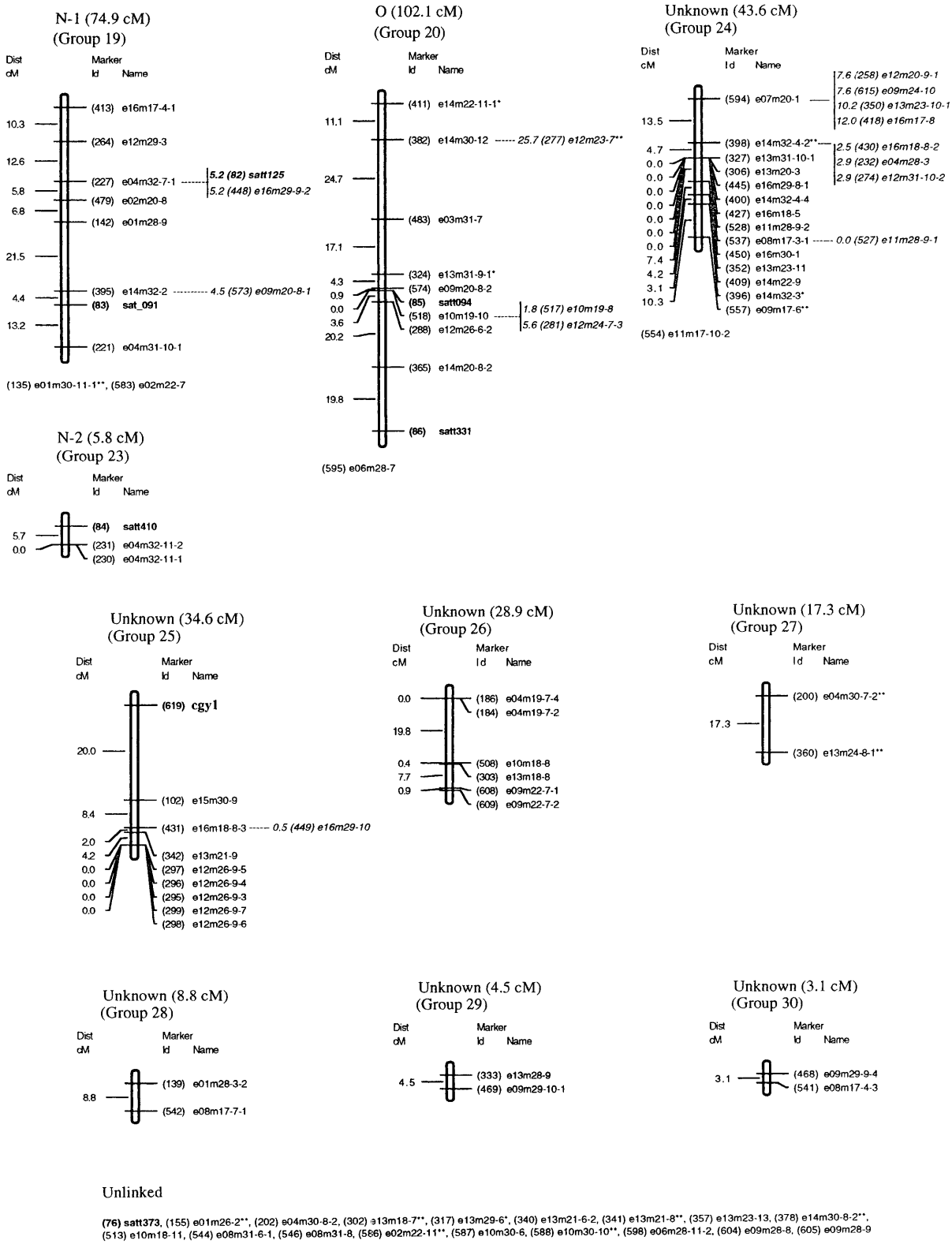

Fig. 1. (continued)

derived from satt315. The primer set generated two fragments in both Keburi and Masshokutou Kou 502, one monomorphic and the other polymorphic. The polymorphic fragment identified the satt315.2 locus in group L; therefore the monomorphic fragment seems to correspond to the satt315 locus mapped in group A2 of Cregan *et al.* (1999). In linkage group L, the map distance between the flanking markers, satt182 and satt398, was 46.9 cM (Fig. 1), excluding the

satt315.2 locus the distance became 23.7 cM, which agrees well with the 27.1 cM distance of the cultivar × wild cross of Cregan *et al.* (1999). It remains to be determined why the insertion of satt315.2 extended the distance; nevertheless, the interval in linkage group L showed a large difference also between cultivar × wild and cultivar × cultivar crosses in the study of Cregan *et al.* (1999), which indicates that the recombination rate within this region is highly variable depending on the cross combination.

In conclusion, we have constructed a genetic map of soybean containing 85 SSR loci that were selected as evenly distributed in the 20 consensus soybean linkage groups (Cregan *et al.* 1999) and 515 AFLP markers that were generated by a simple electrophoretic method. This study provides additional information, methodology, and materials to soybean geneticists and breeding scientists. The RILs generated in this study may be valuable for identifying novel gene loci that are monomorphic in cultivars. The mapping population is useful for detecting QTLs controlling agronomically important characters of soybeans.

Acknowledgements

We would like to thank Dr. P.B. Cregan for providing us with useful information about soybean SSR markers, and Dr. M. Ishimoto and Dr. A. Kaga for critically reviewing the manuscript. We are grateful to R. Yazawa, S. Hirashima, S. Yanagiya, and H. Tomiyama for their excellent assistance in the maintenance of plant materials. The scholarship (to P.C. and Y.M.) and the financial support (to T.K.) from the Science and Technology Agency of Japan are appreciated.

Literature Cited

Akkaya, M.S., A.A. Bhagwat and P.B. Cregan (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132: 1131-1139.

Apuya, N.R., B.L. Frazier, P. Keim, E.J. Roth and K.G. Lark (1988) Restriction fragment polymorphisms as genetic markers in soybean, *Glycine max* (L.) Merr.. Theor. Appl. Genet. 75: 889-901.

Burr, B., F.A. Burr, K.H. Thompson, M.C. Albertsen and C.W. Stuber (1988) Gene mapping with recombinant inbreds in maize. Genetics 118: 519-526.

Cho, Y.G., S.R. McCouch, M. Kuiper, M.R. Kang, J. Pot, J.T.M. Groenen and M.Y. Eun (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). Theor. Appl. Genet. 97: 370-380.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lohnes, J. Chung and J.E. Specht (1999) An integrated genetic linkage map of the soybean genome. Crop Sci. 39: 1464-1490.

Dellaporta, S.L., J. Wood and J.B. Hicks (1985) Maize DNA miniprep. *In* "Molecular Biology of Plants: A Laboratory Course Manual" Malmberg, R., J. Messing and I. Sussex (eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. p. 36-37.

Diwan, N. and P.B. Cregan (1997) Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor. Appl. Genet. 95: 723-733.

Endo, M., T. Komatsuda, Y. Takahata and N. Kaizuma (1998) Parent-offspring relationship in somatic embryogenesis from soybean immature embryo. Breed. Sci. 48 (Suppl. 2): 269.

Fukuda, Y. (1933) Cytogenetical studies on the wild and cultivated Manchurian soybeans (*Glycine* L.). Jpn. J. Bot. 6: 489-506.

Griffin, J. and R. Palmer (1987) Inheritance and linkage studies with five isozyme loci in soybean. Crop Sci. 27: 885-892.

Hymowitz, T., R.J. Singh and K.P. Kollipara (1998) The genomes of the *Glycine*. Plant Breeding Reviews 16: 289-311.

Ito, M., T. Komatsuda, Y. Takahata and N. Kaizuma (1999) Genotype × sucrose interactions for 2,4-D-induced somatic embryogenesis in soybean (*Glycine max* L.). Plant Biotechnology 16: 419-421.

Keim, P., R.C. Shoemaker and R.G. Palmer (1989) Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77: 786-792.

Keim, P., J.M. Schupp, S.E. Travis, K. Clayton, T. Zhu, L. Shi, A. Ferreira and D.M. Webb (1997) A high-density soybean genetic map based on AFLP markers. Crop Sci. 37: 537-543.

Kitamura, K. and N. Kaizuma (1981) Mutant strains with low level of 7S globulin in soybean (*Glycine max* Merr.) seed. Jpn. J. Breed. 31: 353-359.

Kitamura, K., C. Davies and N.C. Nielsen (1984) Inheritance of alleles for Cgy1 and Gy4 storage protein genes in soybean. Theor. Appl. Genet. 68: 253-257.

Komatsuda, T. and S. Ko (1990) Screening of soybean (*Glycine max* L. Merrill) genotypes for somatic embryo production from immature embryos. Jpn. J. Breed. 40: 249-251.

Komatsuda, T. and K. Ohyama (1988) Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean *Glycine max*. Theor. Appl. Genet. 75: 695-700.

Komatsuda, T., K. Kaneko and S. Oka (1991) Genotype × sucrose interactions for somatic embryogenesis in soybean. Crop Sci. 31: 333-337.

Komatsuda, T., I. Nakamura, F. Takaiwa and S. Oka (1998) Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. Genome 41: 680-685.

Kosambi, D.D. (1944) The estimation of map distance from recombination values. Ann. Eugen. 12: 172-175.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Mano, Y., S. Kawasaki, F. Takaiwa and T. Komatsuda (2001) Construction of a genetic map of barley (*Hordeum vulgare* L.) cross Azumamugi × Kanto Nakate Gold using a simple and efficient amplified fragment-length polymorphism system. Genome 44: 284-292.

Maughan, P.J., M.A. Saghai-Maroof and G.R. Buss (1995) Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. Genome 38: 715-723.

Maughan, P.J., M.A. Saghai-Maroof, G.R. Buss and G.M. Huestis (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor. Appl. Genet. 93: 392-401.

Morgante, M. and A.M. Olivieri (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J. 3: 175-182.

Nei, M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York. p. 176-207.

Palmer, R.G. and T.C. Kilen (1987) Qualitative genetics and cytogenetics. *In* "Soybean: Improvement, Production and Uses" Wilcox,

J.R. (ed.), 2nd edn. Agronomy 16: 135-199.

Qi, X., P. Stam and P. Lindhout (1998) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor. Appl. Genet. 96: 376-384.

Singh, R.J. and T. Hymowitz (1989) The genomic relationships among *Glycine soja* Sieb. and Zucc., *G. max* (L.) merr. and '*G. gracilis*' Skvortz. Plant Breed. 103: 171-173.

Shoemaker, R. (1994) RFLP map of soybean. *In* "DNA-Based Markers in Plants" Phillips, R. and I. Vasil (eds.) Kluwer Academic Publishers, Dordrecht, Netherlands. p. 299-309.

Skvortzow, B.V. (1927) The soybean—wild and cultivated in Eastern Asia. Manchurian Res. Soc. Publ. Ser. A, Nat. Hist. Sec. 22: 1-8.

Smartt, J. (1990) Grain Legumes—Evolution and Genetic Resources. Cambridge University Press, Cambridge. p. 246-257.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.