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ORIGINAL PAPER

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Demonstration of linkage and development of the first low-density genetic map of garlic, based on AFLP markers

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Abstract Garlic (*Allium sativum* L.) is a long-cultivated, clonally propagated diploid plant ($2n = 2x = 16$). With routine seed production now underway, we used populations (MP1 and MP2) generated by self-pollination of unrelated plants to generate two low-density genetic maps of garlic, consisting of amplified fragment length polymorphism (AFLP) and gene-specific markers. We did not observe any two plants with identical marker patterns in either population, indicating that they were the result of amphimixis rather than apomixis. This is an important finding, since several *Alliums* are facultative apomicts. A total of 360 markers segregated in MP1 (12.8 AFLP markers per primer combination) and 321 markers segregated in MP2 (13.9 per primer combination) to indicate a fairly high level of genetic heterozygosity in the garlic nuclear genome. Of these markers, 15.3% in MP1 and 24.3% in MP2 had segregation ratios distorted from the expected 3:1. Interestingly, 94.7% of those distorted segregations fit a 15:1 segregation ratio for duplicated loci, suggesting extensive levels of duplication in the garlic genome and supporting similar observations for onion. The genetic map for the MP1 family with 216 markers spanned 1,166 cM of the garlic genome (5.4 cM average), while 143 markers of MP2 spanned 862 cM (6.0 cM average). Gene-specific markers for alliinase, chitinase, sucrose 1-fructosyltransferase (*SST*-1), and chalcone synthase (*CHS*) were mapped, demonstrating the immediate utility of the garlic genetic map. These two garlic families had relatively few segregating AFLP markers in common, which supports their relatively distant relationship based on diversity analysis. Of those markers that were conserved, linkages were also conserved.

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Introduction

Garlic (*Allium sativum* L.) is an important crop with dramatically increasing world popularity, as indicated by a fourfold increase in production from 1986 to 1999 (http://www.fao.org/). The value of garlic as a crop has been recognized since ancient times, having been cultivated for more than 5,000 years and used as a food, spice, and medicine by many civilizations in Asia and the Mediterranean region (Mathew 1996; Woodward 1996) and now, worldwide.

Although it is an ancient crop, genetic and molecular studies in garlic are challenging due to its large genome size and, until recently, a strictly asexual life cycle for the cultivated crop. The DNA content of garlic is 32.7 pg per 2C nucleus, which is one of the largest genomes among the cultivated crops and only slightly smaller than that of the onion (33.5 pg/2C, Ranjekar et al. 1978). While no bona fide evidence of polyploidy in garlic has been presented ($2n = 2x = 16$), extensive intrachromsomal duplication has been suggested in *Alliums* (Jones and Rees 1968; King et al. 1998). The garlic genome has a low GC base composition of 36.9% and a high amount of repetitive DNA (Kirk et al. 1970).

Lack of flowering in most cultivated clones and seed sterility in those that do flower have also restricted the sexual breeding and genetic studies in garlic. Garlic had been described as an obligate apomict reproduced by only vegetative means (cloves or bulbils) until recently, when systems for seed production in garlic were

developed (reviewed by Etoh and Simon 2002; Simon and Jenderek 2003). Fertile garlic clones, able to set seed, have been discovered in the mountainous region of central Asia, the proposed center of garlic origin and diversity where wild forms occur yet today (Vavilov 1935; Etoh and Simon 2002; Simon and Jenderek 2003). The discovery of fertile garlic was somewhat unexpected, since floral abortion and sterility were widely observed among all cultivated garlic, and these floral dysfunctions were associated with chromosome aberrations in Asian garlic. Nevertheless, after several generations of selection, routine seed production has been achieved in garlic, and breeding programs have been initiated (Etoh and Simon 2002; Simon and Jenderek 2003).

With routine seed production underway, genetic analysis of this crop is now possible, but no garlic genetic map has been published. Genetic maps can serve to provide a better understanding of the genomic organization of garlic and provide a framework to locate genes of interest, with an eventual goal of marker-assisted breeding as is already possible with onion and leek, the other major *Allium* crops (King et al. 1998; van Heusden et al. 2000). Noting that King et al. (1998) reported the need for large amounts of pure DNA to evaluate restricted fragment length polymorphisms (RFLPs) in onion, a polymerase chain reaction (PCR)-based marker method requiring less DNA is a preferred marker system to use in genetic mapping of garlic (van Heusden et al. 2000). In this study, we primarily used amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995), since our previous genetic diversity study indicated that AFLP polymorphisms are numerous in garlic (Ipek et al. 2003), and they have been suitable markers to develop genetic maps in other *Alliums* and many plant species (e.g. Remington et al. 1999; Vuylsteke et al. 1999; van Heusden et al. 2000).

In this study, we used two families derived from self-pollinations to develop the first genetic map of garlic using AFLP and gene-specific markers and to provide a foundation to understand the garlic genome better.

Materials and methods

Plant materials, sampling, and DNA preparation

Two families, MP1 and MP2, each consisting of 53 plants, were provided by the Conagra Food Company (Gilroy, Calif., USA) for evaluation. MP1 and MP2 were developed by self-pollinating single plants from two different groups of the dendrogram developed by Ipek et al. (2003), so that MP1 and MP2 were relatively unrelated. Individual seedlings were grown to maturity, and leaves were collected. DNA was extracted from 100 µl of lyophilized and powdered leaf samples, using the method described by Fütterer et al. (1995). Concentration of DNA in Tris-EDTA buffer was measured using a TKO 100 Mini-Fluorometer (Hoefer, San Francisco, Calif., USA), and 100-µl aliquots with DNA concentrations at 60 ng/µl were prepared from each sample for molecular analysis.

AFLP procedure

The AFLP analysis was performed according to Vos et al. (1995), using *Mse*I primers with three selective nucleotides and *Eco*RI primers with three or four selective nucleotides as described by Ipek et al. (2003), with the AFLP Kit number I (Invitrogen, Carlsbad, Calif., USA) and AmpliTaq DNA polymerase (Applied Biosystems, Foster City, Calif., USA). Twenty-eight and 23 primer combinations for MP1 and MP2, respectively, of [^{33}P]-end-labeled *Eco*RI primers with four selective nucleotides and unlabeled *Mse*I primers with three selective nucleotides were used in the selective amplification reactions (Table 1). Starting from the same DNA extracts of individual plants, all AFLP reactions (digestions, ligations, preselective amplifications, and selective amplifications) were carried out in duplicate for each sample to confirm scores and minimize the experimental error in AFLP analysis. Only AFLP fragments that were identical in duplicated samples were scored as present, and inconsistencies between the duplicated samples were treated as missing data ($<0.67\%$).

Nomenclature for AFLP analysis and scoring of data

Each segregating AFLP fragment was identified and labeled with consecutive numbers, starting from the top of the autoradiogram. They were scored manually as dominant and recessive markers for presence (c) and absence (a), respectively. AFLP markers were named with the last three selective nucleotides of *Eco*RI primers and the last two selective nucleotides of *Mse*I primers, followed by a number reflecting relative position of AFLP fragment on the autoradiogram (e.g., TGAAG02).

Generation of gene-specific markers

Primer design

Using previously described garlic genes with sequences in GenBank (http://www.ncbi.nlm.nih.gov/), primer pairs were designed to amplify genomic sequences of alliinase (AF409953), chitinase (M94106), sucrose 1-fructosyltransferase (*SST*-1; AY098442), and *A. cepa* chalcone synthase (*CHS*; AF268382). Primers were chosen on the basis of their T_m (60°C) and location in the coding region of the gene.

PCR amplification

For all PCR reactions, the following reaction and thermal cycle conditions were used: each 25-µl PCR reaction contained 0.75 U of *Taq* DNA polymerase (Promega, Madison, Wis., USA), with reaction buffer at 1× concentration, 0.8 µM each primer, dNTPs at 200 M each,

Table 1 The number of segregating amplified fragment length polymorphism (*AFLP*) markers in each primer combination and their segregation ratios

Primer combination			AFLP markers		
*Eco*RI	*Mse*I	Total	3:1	15:1	1:1
MP1 family					
AAGA	CAA	11	8(3)[a]	1	2
	CAC	12	9(7)	2	1
	CAG	11	8(6)	2	1
	CCA	11	11(7)	–	–
	CCG	21	20(14)	1	–
	CCT	11	8(5)	3	–
	CGC	14	13(7)	1	–
	CGG	24	23(19)	1	–
	CGT	30	26(24)	3	1
	CTA	1	1(1)	–	–
AAGG	CAA	11	10(6)	1	–
	CAG	16	10(8)	6	–
	CCT	18	18(16)	–	–
	CTG	10	9(7)	1	–
ACGA	CAA	10	9(7)	1	–
	CAC	14	13(6)	1	–
	CAG	8	7(5)	1	–
	CAT	13	10(6)	2	1
	CCT	21	18(14)	3	–
	CTG	11	8(6)	3	–
ACGG	CAC	14	12(8)	2	–
	CAG	12	10(7)	2	–
	CTC	7	5(1)	2	–
	CATT	11	9(5)	2	–
AGCT	CAA	11	10(7)	1	–
	CAC	16	10(5)	6	–
ATGA	CAG	6	6(4)	–	–
	CAT	5	4(1)	1	–
Total		360	305(212)	49	6
MP2 family					
AAGA	CAC	15	12(7)	3	–
	CAG	16	14(8)	1	1
	CCA	7	7(5)	–	–
	CCG	18	12(7)	6	–
	CGG	23	17(9)	6	–
	CGT	23	20(10)	3	–
AAGG	CAA	12	6(1)	6	–
	CAG	9	4(3)	5	–
	CCG	16	12(10)	4	–
	CCT	14	8(3)	6	–
	CGC	9	8(7)	1	–
	CTG	12	11(6)	1	–
ACGA	CAC	20	15(8)	5	–
	CAG	11	9(3)	2	–
	CAT	10	9(6)	1	–
	CCT	23	18(13)	5	–
ACGG	CAT	7	4(2)	3	–
	CTC	6	4(3)	2	–
	CTG	17	17(11)	–	–
AGCT	CAG	14	7(3)	7	–
ATGA	CAT	17	14(8)	3	–
	CAC	9	7(3)	2	–
	CAG	13	8(5)	5	–
Total		321	243(141)	77	1

[a]Numbers within the *parentheses* are the number of markers mapped in either major or minor linkage groups

and 90 ng template DNA. The reaction was heated to 95°C for 2 min and exposed to 35 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min. PCR products were separated on 1% (w/v) agarose (Invitrogen) gels in 1× TAE buffer (40 mM Tris-acetate and 1 mM EDTA), stained with ethidium bromide (Sigma, St. Louis, Mo., USA), and photographed.

Cloning and sequencing of gene-specific DNA fragments

The PCR-amplified DNA fragments were excised from the agarose gel and purified using the QIAquick Gel Extraction kit (QIAGEN, Valencia, Calif., USA). These DNA fragments were cloned into the pGEM-T Easy

vector system (Promega), and vectors in the ligation mix were transformed into JM109 competent cells (Promega). Plasmids were extracted from bacterial cultures grown overnight using the Wizard Plus Miniprep DNA Purification system (Promega). Plasmids were then used for sequencing. *Taq* DNA polymerase (Applied Biosystems) cycle-sequencing reactions were performed according to the conditions recommended by Applied Biosystems, with reagents using a fluorescent-dye terminator, but using half-volume reactions. DNA was sequenced with a PE-Biosystem 377 XL automated DNA sequencing instrument at the Biotechnology Center in the University of Wisconsin, Madison. The sequences of each gene were aligned separately using the BioEdit program (Hall 1999).

Based on the sequence alignments of each gene, insertion/deletions (indels) were detected in the sequences of *SST*-1, alliinase, and chitinase, and oligonucleotide primer pairs flanking indels were designed to detect size polymorphisms (Table 2). For PCR amplification, each 10-µl reaction was composed of 200 µM each dNTP, 125 nM each primer, 2 mM $MgCl_2$, 90 ng DNA, 0.75 U *Taq* DNA polymerase (Promega), and 0.09 µl radionucleotide [α-P^{33}] dCTP (2,000 Ci/mmol, Amersham, Piscataway, N.J., USA) in 1× reaction buffer. The amplification included one cycle of 2 min at 94°C and 35 cycles of 94°C for 30 s, 58°C for 50 s, and 68°C for 1 min and 15 s. Polymorphisms were detected on 6% denaturing polyacrylamide gels containing 7.5 M urea in 1× Tris-Borate [(TBE) 45 mM Tris-Borate and 1 mM EDTA], 320 µl 10% ammonium persulfate (APS), and 30 µl TEMED. The PCR products were separated by electrophoresing at 25 W for 6 h. After the runs were completed, gels were cooled on ice, transferred onto 3-mm Whatman filter paper, and vacuum-dried using model SE 1160 dryer (Hoefer) at 80°C for 60 min. Dried gels were exposed to Kodak Biomax MR films (Kodak, Rochester, N.Y., USA) for 1–3 days for visualization.

There were no indels present in the sequence of *CHS*, so single-strand conformation polymorphism (SSCP) analysis (Beier 1993) was carried out to detect polymorphisms within the sequences of *CHS* in garlic, using a primer pair based on garlic *CHS* sequences (Table 2). The same reaction and thermal cycle conditions for SSCP analysis were used as described above for other genes, but PCR products were separated on MDE gels. After the addition of 1 µl reaction PCR product to 9 µl loading dye containing 10 mM NaOH, 0.25% bromophenol blue, and 0.25% xylene cyanol in 98% formamide, PCR-amplified DNA was denatured at 94°C for 3 min and then cooled and kept on ice. For the separation of reaction products, MDE gels containing 25 ml 2×MDE solution (Cambrex, East Rutherford, N.J., USA), 6 ml 10× TBE buffer, 40 µl TEMED, 400 µl 10% APS, and 69 ml H_2O were prepared. After running at 5 W for 14 h, vacuum-dried gels on 3-mm Whatman filter paper were also exposed to Kodak Biomax MR films for 1–3 days for visualization.

Linkage analysis

The segregation of each AFLP fragment was evaluated with a chi-square test, and all segregating AFLP fragments with 3:1 ratio ($\alpha = 0.05$) were included in linkage analysis. The computer package JoinMap, version 3.0 (van Ooijen and Voorrips 2001), was used to detect linkages among the markers, using the Kosambi mapping function. The calculation of linkage was done using all pairwise recombination estimates smaller than 0.45 and a LOD score larger than 3.0. Linkage groups of all markers were determined with a LOD score of 3.0.

Results

Generation of AFLP markers

Based on earlier AFLP studies (Ipek et al. 2003) in garlic, we added a fourth selective nucleotide to the *Eco*RI selective amplification primers (Table 1) A similar modification was also made in the analysis of onion (van Heusden et al. 2000) and leek (Smilde et al. 1999), where the number of nucleotides in *Mse*I selective amplification primers was increased from three to four.

Evaluation of 28 selective amplification primer combinations in family MP1 resulted in 360 segregating AFLP markers that were polymorphic (Table 1). On the average, 12.8 segregating AFLP markers per primer combination were scored, and polymorphisms ranged in number from one in primer combination EAAGA/MCTA to 30 in EAAGA/MCGT. Of 360 polymorphic AFLPs, 305 (84.7%) segregated according to the expected 3:1 ratio ($\alpha = 0.05$), while the remaining 55 AFLP markers (15.3%)

Table 2 Oligonucleotide sequences of gene-specific primers based on the genomic sequences of garlic

Gene	Primer set	Sequence (5′–3′)
Alliinase	As-Alliinase-1	CTCAACTCATCCATGGACTCGTCATCTCT
	As-Alliinase-2	GATCGTACGTTAGATCGATGTGTGC
SST-1	As-SST1-1	TGGACAATGATGAGTACATGTCAGTCGC
	As-SST1-2	CAGATAATTTTGATTACAGAGAATTTGCTGTCAACTT
Chitinase	As-Chtn-1	CAGCAACAGGCTATGCTGTAGC
	As-Chtn-2	GAATGAGTTTGCAGCTGCTATGAAGG
CHS	As-CHS-1	GTGAAGCGCTTCATGATGTACCA
	As-CHS-2	GGATGCGCTATCCAAAACACCT

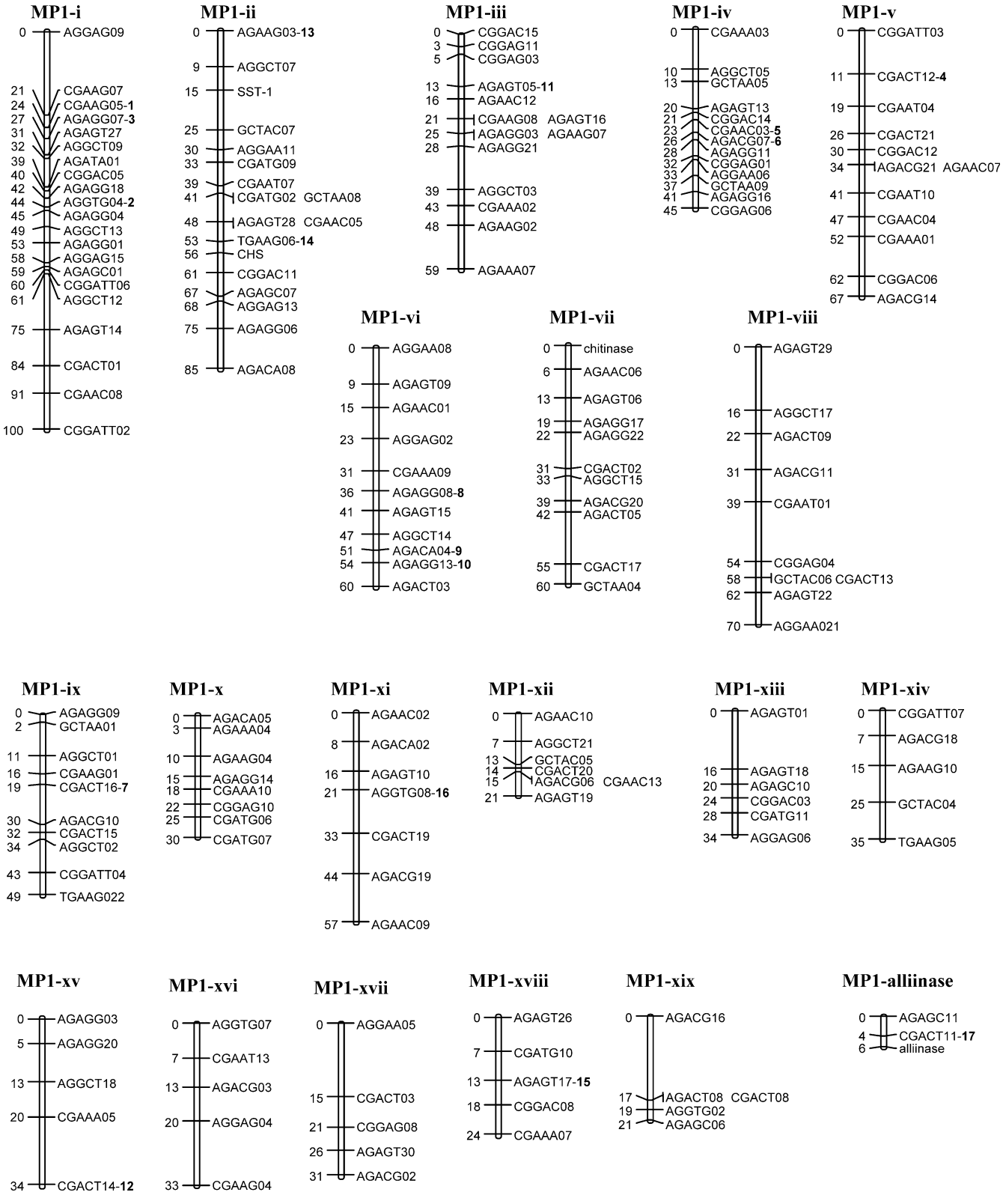

Fig. 1 Linkage groups in the genetic map of *Allium sativum*, based on the MP1 family. Map distances are in centiMorgans. *Numbers in boldface* are common markers segregating in both MP1 and MP2

had distorted segregation. Interestingly, among the markers with distorted segregation ratios, 49 (89%) markers fit a 15:1 ratio typical for duplicated genes. Using 23 selective amplification primer combinations in the MP2 family, 321 segregating AFLP markers were observed (Table 1). In this family, the average number of

segregating AFLP markers scored per primer combination was 13.9 and ranged from six in EACGG/MCTC, to 23 in EAAGA/MCGT. Like the MP1 family, the majority of segregating AFLP markers (243, or 75.7%) segregated according to the expected 3:1 ratio ($\alpha = 0.05$), while 78 AFLP markers (24.3%) had distorted segregation ratios. All but one of these 78 markers with distorted ratios (98.7%) fit a 15:1 ratio for duplicated loci (Table 1). Thus, combining the information of both garlic families, 681 polymorphic AFLP markers were observed, of which 548 (80.5%) segregated in a 3:1 ratio and 126 (18.5%) fit a 15:1 ratio. We did not attempt to map markers with distorted segregations. Apomictic reproduction mechanisms such as agamospermy and clonal reproduction generate progeny without recombination, so they are genetically identical. In this study, we found no two plants in either family with identical AFLP polymorphisms, thus indicating that garlic seed was the product of sexual reproduction and amphimixis.

Linkage analysis

In the analysis of markers fitting a 3:1 ratio among 53 plants of the MP1 population with a minimum LOD score of 3.0, a genetic map was composed of 212 AFLP and four gene-specific markers with 175 AFLPs and three gene-specific markers assigned to 19 major linkage groups with five to 21 markers (Fig. 1), while the remaining 38 markers were linked in 12 minor groups consisting of three or four markers (Table 3). This map spanned 1,166 cM of the garlic genome, with an average distance of 5.4 cM. To these linked markers, 93 AFLP markers (30.5%) remain unlinked in this population. In the MP2 family, 243 segregating AFLP markers and four gene-specific markers with 3:1 segregation ratios ($\alpha = 0.05$) were included for mapping (Table 1). With a minimum LOD score of 3.0, 141 AFLP and two gene-specific markers were placed in 13 major groups of five to 25 markers (Fig. 2) and 12 groups with three or four markers each. This map covered 862 cM of the garlic genome, with an average of 6.0 cM between loci (Table 3). A total of 102 segregating AFLP markers (41.9%) and two gene-specific markers were unlinked in this population.

Mapping gene-specific markers

In this mapping study, gene-specific markers for alliinase, chitinase, *SST*-1, and *CHS* were developed by using their previously identified sequences in garlic or other *Allium* species. After cloning the PCR-amplified gene products in garlic, multiple colonies carrying inserts were sequenced. Based on the alignment of these sequences, at least six alleles of alliinase, five alleles of chitinase, and three alleles of *SST*-1 were present in the garlic genome (data not presented). If all loci were heterozygous, these data suggest at least three copies of alliinase and chitinase and two copies of *SST*-1 in the garlic genome and further supports the hypothesis that duplications are common in the garlic genome. To avoid ambiguity in scoring, one dominant marker for each gene was selected and included in this mapping analysis, since the allelic constitution of each gene was not known. All four genes were mapped in MP1, while only the alliinase and *SST*-1 markers were mapped in MP2 (Figs. 1, 2). *CHS* and chitinase were each linked to only one AFLP marker in MP2 (data not presented). In order to map all copies of these genes present in the garlic genome, each allele should be identified by cloning and sequencing the DNA flanking each copy of these genes or introns to identify unique heterozygous sequences scorable with PCR, and heterozygous loci should be scored using segregation analysis in the subsequent progeny.

Table 3 Marker distribution among the linkage groups of garlic in both families

Linkage group	Marker number	Length (cM)	Average distance (cM)
MP1 family			
MP1-i	21	100	4.7
MP1-ii	18	85	4.7
MP1-iii	14	59	4.2
MP1-iv	13	45	3.4
MP1-v	12	67	5.5
MP1-vi	11	60	5.4
MP1-vii	11	60	5.4
MP1-viii	10	70	7.0
MP1-ix	10	49	4.9
MP1-x	8	30	3.7
MP1-xi	7	57	8.1
MP1-xii	7	21	3.0
MP1-xiii	6	34	5.6
MP1-xiv	5	35	7.0
MP1-xv	5	34	6.8
MP1-xvi	5	33	6.6
MP1-xvii	5	31	6.2
MP1-xviii	5	24	4.8
MP1-xix	5	21	4.2
Others	38	251	6.6
Total	216	1166	5.4
MP2 family			
MP2-i	25	89	3.5
MP2-ii	14	53	3.7
MP2-iii	11	59	5.3
MP2-iv	9	42	4.6
MP2-v	7	49	7.0
MP2-vi	5	54	10.8
MP2-vii	5	53	10.6
MP2-viii	5	42	8.4
MP2-ix	5	38	7.6
MP2-x	5	33	6.6
MP2-xi	5	27	5.4
MP2-xii	5	18	3.6
Others	42	305	7.2
Total	143	862	6.0

Discussion

The results of this study demonstrated that AFLPs are numerous and a powerful tool for mapping garlic. It was

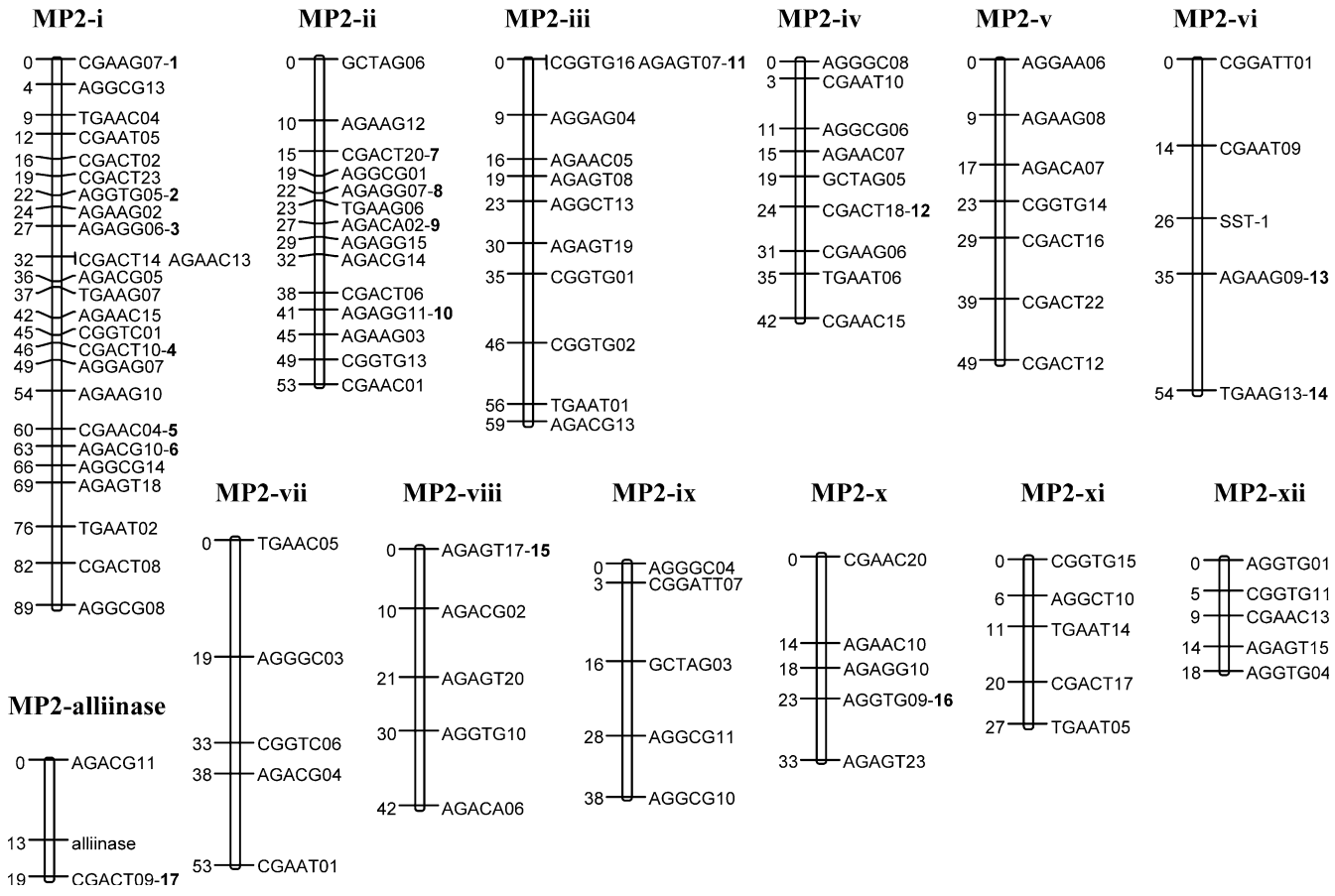

Fig. 2 Linkage groups in the genetic map of *A. sativum* based on the MP2 family. Map distances are in centiMorgans. *Numbers in boldface* are common markers segregating in both MP1 and MP2

not possible to develop linkage maps of garlic with classical genetic methods until the relatively recent success in producing true seed. With this, we can now generate segregating families, presuming seed is the product of amphimixis. In fact, no two plants had identical marker segregation patterns in the populations we evaluated, indicating that amphimixis accounts for the production of garlic seed. Pooler and Simon (1994) used isozyme markers to confirm the hybrid origin of garlic seedlings, but the current study is the first to evaluate numerous markers in segregating families and thus provide evidence that the garlic seed results entirely from amphimixis. This observation is not necessarily expected, since two other *Alliums*, *A. odorum* and *A. tuberosum*, are both facultative apomicts producing seed from a mixture of apomixis and amphimixis (Hakansson 1951; Gohil and Kaul 1981). Since facultative apomixis has been reported in these relatively closely related species in the same section of the genus *Allium*, our demonstration of a strictly amphimictic origin of garlic seed is important as we embark in garlic breeding and selection programs.

The number of segregating AFLP markers per primer combination in garlic (12.8 in MP1 and 13.9 in MP2) is comparable to those detected in mapping populations of leek (11, Smilde et al. 1999) and oat (12–15, Groh et al. 2001), but less than that for the interspecific progeny of *A. roylei* × *A. cepa* (21, van Heusden et al. 2000). The availability of a relatively high number of polymorphic AFLP markers reflects the heterozygous genome and outcrossing nature of garlic (Etoh and Simon 2002; Simon and Jenderek 2003).

The portion of markers segregating 15:1 in this study suggests that duplication may be common in the genome of garlic. It has been proposed that intrachromosomal duplications make up much of the *Allium* genome (Jones and Rees 1968; Ranjekar et al. 1978), and King et al. (1998) reported that about 21% of onion RFLP loci were duplicated. The 18.5% of markers we observed with 15:1 ratios in garlic closely approximated this value for onion, although the dominant genetic behavior of AFLP markers makes their designation as duplicated genes only speculative without proof that these ratios do, in fact reflect duplicated genes. To do this, more extensive genomic analysis will need to be done to identify and score two alleles at both loci. The relative abundance of markers with duplicate gene ratios observed in this study indicated that duplicated genes either must be on different linkage groups, or widely separated on the same chromosome if they are intrachromosomal, since closely linked duplications would approach single gene ratios.

Koul et al. (1979) reported that the average chiasmata per meiosis ranged from 24 to 42 in pollen mother cells of garlic. Therefore, we predict a total genetic distance of the garlic genome to range from 1,200 cM to 2,100 cM. For the MP1 population, the 1,166-cM map distance approaches the estimate derived from the cytological data, indicating that this map has fairly wide genome coverage. In the MP2 population, the lower total genetic distance of 862 cM reflects the lower number of segregating markers included in the mapping analysis.

In this study, 30.5% in MP1 and 41.9% in MP2 of segregating AFLP markers were not associated with any linkage groups. This portion of unlinked marker is high compared to the 17, 27, and 10% of AFLP markers in oat, an *A. roylei* × *A. cepa* interspecific hybrid, and melon, respectively (Wang et al. 1997; van Heusden et al. 2000; Groh et al. 2001). Detection of linkages among dominant markers with a reasonable statistical power depends on the linkage phases of markers and the size of the mapping population. If two dominant markers are at repulsion phase linkage, a population with about 200 plants is required to reliably detect linkages less than 10 cM (Liu 1998). Self-pollination of garlic plants typically produce 10 to 50 seeds per plant in garlic, so populations of 200 are difficult to attain. With relatively small population sizes of 53 plants in this study, the high percentage of markers not associated with any linkage group is not surprising. The high number of linkage groups we found may also be the consequence of our relatively small population sizes used and increasing the number of markers for mapping may not combine linkage groups (Kesseli et al. 1994; Keim et al. 1997; Wang et al. 1997).

The AFLP markers have been used to join genetic maps of plants such as potato and oat (Rouppe van der Voort et al. 1997; Groh et al. 2001). In order to compare the two genetic maps of garlic in this study, we identified common AFLP markers from the same primer combinations and segregating in both families, based on their positions on polyacrylamide gels. The relatively low number of common segregating markers that we identified between MP1 and MP2 likely reflects the distant genetic relationship between the plants from which these families were developed. However, a few common AFLP markers were identified and those linked in one family were always linked in the other family. For example, CGAAG05-1 and CGAAG07-1, AGAGG07-3 and AGAGG06-3, and AGGTG04-2 and AGGTG05-2 shared the same positions on polyacrylamide gels in MP1 and MP2, respectively, and −1, −2, and −3 were all in linkage group i of their respective maps (i.e., MP1-i and MP2-i, Figs. 1, 2). The MP2-i linkage group included not only these three markers from MP1-i, but also two from MP1-iv, and one from MP1-v. Similarly, MP2-ii had three common markers from MP1-vi and one from MP1-ix. These results suggest that the lower number of linkage groups in MP2 is likely due to the combination of linkage groups in genetic map of MP1.

While we successfully generated a low-density genetic linkage maps and demonstrated an amphimictic origin of the families evaluated, we also revealed several indications that the large garlic genome is complex. Duplicate segregation ratios of AFLP markers and multiple copies of gene-specific sequences in garlic suggest that a portion of the large garlic genome is present in more than one copy. Furthermore, the high numbers of segregating AFLP markers per primer combination and level of sequence polymorphisms of garlic genes confirm a high level of diversity for this out-crossing plant species, as had been suggested by assessment of germplasm diversity (Ipek et al. 2003). In spite of garlic's genomic complexity, this initial mapping study demonstrated that genetic maps of garlic based on AFLPs can be used to localize genes of interest with agricultural importance.

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