Comparison of AFLPs, RAPD Markers, and Isozymes for Diversity Assessment of Garlic and Detection of Putative Duplicates in Germplasm Collections

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Abstract. Garlic (*Allium sativum* L.) is an asexually propagated crop that displays much morphological diversity. Studies which have assessed garlic diversity with isozymes and randomly amplified polymorphic DNA (RAPD) markers generally agreed with the morphological observations but sometimes failed to discriminate clones. To discriminate among closely related garlic clones in more detail, we introduced amplified fragment-length polymorphism (AFLPs) to evaluate the genetic diversity and phenetic relatedness of 45 garlic clones and three *A. longicuspis* clones and we compared AFLP results with RAPD markers and isozymes. Three AFLP primer combinations generated a total of 183 polymorphic fragments. Although similarities between the clusters were low (≥0.30), some clones within the clusters were very similar (>0.95) with AFLP analysis. Sixteen clones represented only six different banding patterns, within which they shared 100% polymorphic AFLPs and RAPD markers, and likely are duplicates. In agreement with the results of other investigators, *A. longicuspis* and *A. sativum* clones were clustered together with no clear separation, suggesting these species are not genetically or specifically distinct. The topology of AFLP, RAPD, and isozyme dendrograms were similar, but RAPD and isozyme dendrograms reflected less and much less polymorphism, respectively. Comparison of unweighted pair group method with arithmetic averaging (UPGMA) dendrograms of AFLP, RAPD, and isozyme cluster analyses using the Mantel test indicated a correlation of 0.96, 0.55, and 0.57 between AFLP and RAPD, AFLP and isozyme, and RAPD and isozyme, respectively. Polymorphic AFLPs are abundant in garlic and demonstrated genetic diversity among closely related clones which could not be differentiated with RAPD markers and isozymes. Therefore, AFLP is an additional tool for fingerprinting and detailed assessment of genetic relationships in garlic.

The genus *Allium* consists of at least 500 species (Vvedensky, 1944) and garlic (*Allium sativum* L.) is the species in this genus under cultivation for the longest time. Garlic has been cultivated for at least 5000 years (Hahn, 1996) presumably having originated in central Asia (Hong and Etoh, 1996; Vavilov, 1951). Garlic is an asexually propagated crop, and it displays great morphological diversity in bulb and leaf size, color and shape, scape presence and height, and flower color, fertility, and bulbil (topset) development in inflorescence (Pooler and Simon, 1993).

Until recently, characterization of garlic germplasm has been based primarily on morphological data (Jones and Mann, 1963). However, morphological characters may differ under varying environmental conditions. This can complicate characterization of garlic clones (Al-Zahim et al., 1997; Bradley et al., 1996). To eliminate some of these complications, isozyme and RAPD markers have been used to reveal genetic diversity in garlic germplasm (Al-Zahim et al., 1997; Bradley et al., 1996; Lallemand et al., 1997; Maass and Klaas, 1995; Pooler and Simon, 1993). Flower-related morphological traits were correlated with isozyme data, while bulb-associated traits and geographic origin were not (Pooler and Simon, 1993). Furthermore, isozyme and RAPD studies indicated that *A. longicuspis* Regl., the proposed ancestor of garlic, is not a separate species from *A. sativum* (Al-Zahim et al., 1997; Maass and Klaas, 1995; Pooler and Simon, 1993). *A.*

longicuspis clones were discriminated morphologically from *A. sativum* clones by generally having exerted and purple anthers, higher flowering rate and seed production, and smaller bulbils in inflorescence (Etoh and Simon, 2002). The species have the same karyotype and *A. sativum* and *A. longicuspis* clones have been crossed successfully (Etoh, 1984; Pooler and Simon, 1994).

Garlic has been propagated by vegetative means for centuries and the presence of many closely related or duplicated garlic clones is likely in germplasm collections. Large numbers of molecular markers are needed to discriminate closely related clones or to identify duplicates. One particularly useful tool to achieve this goal is amplified fragment-length polymorphism (AFLP), which could produce high multiplex informative markers in a single reaction and, therefore, could generate sufficient markers to assess genetic diversity among garlic clones.

AFLP is a PCR-based DNA fingerprinting technique (Vos et al., 1995) and like randomly amplified polymorphic DNA (RAPD), it generates primarily dominant markers. Neither AFLP nor RAPD require any probe or sequence information (Barker et al., 1999) and they can be applied to any species after some minor modifications. Isozymes, RAPD, and AFLP have been used extensively for determining genetic diversity and relatedness within and between plant species (Knerr et al., 1989; Sharma et al., 1996; Spooner et al., 1996; Tohme et al., 1996; Zhu et al., 1998). Garcia-Mas et al. (2000) compared AFLP, RAPD, and RFLP for measuring genetic diversity in melon and found that AFLP, RAPD, and restricted fragment length polymorphism (RFLP) markers were equally informative, although AFLP detected polymorphism with highest efficiency.

Table 1. Origins, names, flowering data, AFLP group numbers, and marker types used for 45 *A. sativum* and three *A. longicuspis* clones.

Clones	Species	Country of origin[z]	Marker type[y]	AFLP grouping	Flowering[x]
DDRGRU2	*A. sativum*	DE	A, R, I	I	F
KS/10	*A. sativum*	FR	A, R	I	F
M8151	*A. sativum*	US	A, R, I	I	F
LAU	*A. sativum*	US	A, R	I	F
M/V	*A. sativum*	US	A, R, I	I	F
S/O/A	*A. sativum*	US	A, R, I	I	F
PI497945	*A. sativum*	PL	A, R	I	F
PI497949	*A. sativum*	PL	A, R, I	I	F
PI497951	*A. sativum*	SY	A, R	II	IF
W6-1961	*A. sativum*	ES	A, R	II	IF
PI493112	*A. sativum*	PL	A, R, I	III	NF
PI493118	*A. sativum*	PL	A, R, I	III	NF
PI383817	*A. sativum*	YU	A, R, I	III	NF
PI383819	*A. sativum*	YU	A, R, I	III	NF
PI383824	*A. sativum*	YU	A, R	III	NF
PI383823	*A. sativum*	YU	A, R, I	III	NF
PI497943	*A. sativum*	PL	A, R, I	III	NF
RE/MF	*A. sativum*	US	A, R	III	NF
PI515971	*A. sativum*	US	A, R, I	IV	NF
PI515974	*A. sativum*	US	A, R, I	IV	NF
U074	*A. sativum*	UZ	A, R, I	V	F
DDR6811	*A. sativum*	DE	A, R, I	V	F
R54RE	*A. sativum*	PL	A, R, I	V	F
R83RE	*A. sativum*	PL	A, R, I	V	F
RE6820	*A. sativum*	DE	A, R, I	V	F
U037	*A. sativum*	UZ	A, R, I	V	F
PI493116	*A. sativum*	CZ	A, R, I	V	F
U073	*A. sativum*	UZ	A, R, I	V	F
DDR6024	*A. sativum*	DE	A. R, I	V	F
DDR7116	*A. sativum*	DE	A, R, I	V	F
K/RO	*A. sativum*	FR	A, R, I	VI	F
McCOL59190	*A. sativum*	US	A, R, I	VI	F
DDR6822	*A. sativum*	DE	A, R, I	VI	F
DDR6807	*A. sativum*	DE	A, R, I	VI	F
DDR6819	*A. sativum*	DE	A, R	VI	F
U094-4	*A. longicuspis*	UZ	A, R, I	VII	F
U079	*A. longicuspis*	UZ	A, R, I	VIII	F
PI383831	*A. sativum*	YU	A, R, I	VIII	F
JN/EG	*A. sativum*	US	A, R, I	VIII	F
DDR130	*A. sativum*	DE	A, R, I	IX	F
DDR7087	*A. sativum*	DE	A, R, I	IX	F
DDR7099	*A. sativum*	DE	A, R	IX	F
DDR7085	*A. sativum*	DE	A, R, I	IX	F
PURPLE	*A. sativum*	US	A, R, I	IX	F
DDR6801	*A. sativum*	DE	A, R, I	X	F
PI576914	*A. longicuspis*	UZ	A, R, I	X	F
DDR7040	*A. sativum*	DE	A, R, I	X	F
M/PIT	*A. sativum*	CZ	A, R, I	X	F

[z]CZ = Czech Republic, DE = Germany, ES = Spain, FR = France, PL = Poland, SY = Syria, UZ = Uzbekistan, US = United States of America, and YU = Yugoslavia.
[y]A = AFLP, R = RAPD, and I = isozyme.
[x]Flowering data of 5 years observation: F = flowering, NF = nonflowering, and IF = incomplete flowering.

In this study, we applied AFLPs, RAPD markers, and isozymes to diverse garlic clones and its proposed ancestor *A. longicuspis*, and compared all three systems for the assessment of genetic diversity in garlic.

Materials and Methods

PLANT MATERIALS AND SAMPLING. In total, 48 clones representing a diverse subset of those studied by Pooler and Simon (1993),

and including 45 clones of *A. sativum* and three clones from *A. longicuspis*, were investigated in this study (Table 1). Most of these clones were collected from sources in the United States, Eastern Europe, and the former Soviet Union. These clones had been field propagated in Madison, Wis., for at least five years and young healthy leaves were collected and lyophilized. Flowering for all clones was also evaluated visually over five years.

DNA PREPARATION. Garlic DNA was extracted from freeze-dried leaf tissue according to the CTAB method (Murray and Thompson, 1980). DNA concentration was measured by using a minifluorometer (TKO 100; Hoefer), and then adjusted to 30 ng·mL^{-1} concentration with TE buffer.

AFLP PROCEDURES. Two to four individual plants of each clone were evaluated with AFLP analysis except for five clones where only a single plant was available. AFLP protocols were followed according to the manufacturer's procedure with minor modification using the AFLP Kit number I (Invitrogen, Carlsbad, Calif.) except for the modification of primers with both 3-base and 4-base selective nucleotide extensions as described below. Half of the recommended reaction volumes were used, except for preselective amplification which was performed at one-fifth the recommended volume. About 180 ng of the total genomic DNA of garlic was double digested with *Eco*R I and *Mse* I according to the manufacturer reaction conditions (Invitrogen, Carlsbad, Calif.) for 6 h. After heat inactivation at 70 ℃ for 15 min, *Mse* I and *Eco*R I restriction-site derived adapters were ligated with the DNA fragments. 10× diluted ligation product was used for preselective amplification with the preselective amplification primers. Preselective amplification products were diluted 50× for selective amplification. Selective amplification reactions were performed with a ^{33}P-end-labeled *Eco*R I primer (either 3 or 4 base extension) and unlabelled *Mse* I primer (3 base extension). Selective amplification products were denatured at 90 ℃ for 3 min in an equal volume of formamide loading dye buffer and AFLP fragments were separated on a 6% (w/v) polyacrylamide gel by running 3 to 4 µL of denatured selective amplification products at 60 W for 2 to 3 h. Dried gels were exposed to Kodak Biomax MR film for 1 to 3 d before developing. Eleven primer combinations (*Eco*R I + AAGG/*Mse* I + CAT, *Eco*R I + AAGG/*Mse* I + CTG, *Eco*R I + AAGG/*Mse* I + CAC, *Eco*R I + AAGA/*Mse* I + CAT, *Eco*R I + AAGA/*Mse* I + CTG, *Eco*R I + AAGA/*Mse* I + CAC, *Eco*R I + AACG/*Mse* I + CTG, *Eco*R I + ACGG/*Mse* I + CTA, *Eco*R I + ACGA/*Mse* I + CAT, *Eco*R I + ACGG/*Mse* I + CTC, and *Eco*R I + ACGG/*Mse* I + CAT) were tested for AFLP analysis in garlic.

Table 2. RAPD primers and number of bands scored for each primer.

Operon primer[z]	Bands scored (no.)	Operon primers	Bands scored (no.)
OPA-01[z]	2	OPD-03[y]	6
OPAB-04[y]	9	OPE-11[y]	3
OPAB-18[y]	8	OPE-17[y]	4
OPB-16[z]	4	OPG-13[y]	2
OPC-07[y]	5	OPG-19[y]	3
OPC-09[y]	8	OPM-17[x]	2
OPC-12[y]	6	OPN-01[x]	2
OPC-13[y]	4	OPO-01[x]	6
OPD-01[z,y]	6		

[z]Reported to be polymorphic in garlic by Bradley et al. (1996).
[y]Reported to be polymorphic in garlic by Maass and Klass (1995).
[x]Reported to be polymorphic in garlic by Al-Zahim et al. (1997).

RAPD PROCEDURES. RAPD analysis was performed by using the same DNA samples used for AFLP analysis. For each clone which exhibited no intraclonal AFLP polymorphism, one plant was evaluated and duplicate reactions were done to confirm scoring and minimize experimental error. The RAPD protocol was carried out as described by Bradley et al. (1996) with minor modification. Each 25 µL PCR reaction mixture contained 60 ng genomic DNA, 1 µM primer, 0.2 mM each dNTP mix, 1.5 mM MgCl$_2$, 2.5 µL of 10× PCR buffer, and 1.25 unit of *Taq* DNA polymerase (Promega, Madison, Wis.). The DNA was denatured at 95 ℃ for 2 min and followed by 40 cycles of 45 s at 95 ℃, 10 s at 50 ℃, 15 s at 45 ℃, 20 s at 40 ℃, 1 min at 35 ℃, 1 min 45 s at 72 ℃, with a final extension step of 5 min at 72 ℃, and then reactions held at 4 ℃. Reaction mixtures were separated on 1.5 % (w/v) agarose gels in 1× TAE (40 mM Tris-acetate and 1 mM EDTA) and stained with ethidium bromide. In total, 20 decamer primers (Operon Technologies, Alameda, Calif.) were tested for RAPD analysis and 17 of them selected for further analysis (Table 2). Of these primers, 12 were chosen from the set of 15 primers previously used by Maass and Klaas (1995). Three of these were also previously used by Bradley et al. (1996), and three had been used by Al-Zahim et al. (1997).

ISOZYME PROCEDURES. Enzymes were extracted by grinding 200 mg of young, fresh leaf tissue of two to three plants of 39 clones in 1 mL ice-cold extraction buffer (Pooler and Simon, 1993) and stored frozen in aliquots at –80 ℃ until analysis. Eight isozyme systems (esterase, diaphorase (DIA), glucose-6-phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), aspartate aminotransferase (AAT), and phosphoglucomutase (PGM)) were tested. Resolving banding patterns and staining gels for esterase, DIA, SKDH, G6PDH, and PGM were carried out according to the method described by Pooler and Simon (1993) while AAT, MDH, and IDH banding pattern were resolved and their gels were stained according to the methods described by Maass and Klaas (1995).

DATA ANALYSIS. All polymorphic fragments were identified for AFLP and RAPD and only unambiguously polymorphic bands were scored as present (1) or absent (0). The allelic constitution of isozymes cannot be determined without genetic analysis, so isozymes were scored as dominant markers, like AFLP and RAPD. Similarity matrices, generated according to the coefficient of Jaccard (Sneath and Sokal, 1973) were used to perform cluster analyses using the unweighted pair group method with arithmetic averaging (UPGMA) (Sokal and Michener, 1958) with NTSYS-PC v. 1.80 program (Rohlf, 1993). Dendrograms indicating the estimated similarity among the garlic clones were constructed with the TREE program of NTSYS-PC. Cophenetic values were calculated from AFLP, RAPD and isozyme dendrograms and used to compare dendrograms by using the Mantel test of significance (Mantel, 1967).

Results and Discussion

AFLP ANALYSIS. Our preliminary AFLP evaluations yielded more than 250 bands making data analysis ambiguous (bands were very close to each other; data not presented). This kit was recommended by the manufacturer for plant species with the genome size of 5×10^8 to 6×10^9 bp and has three base selective nucleotide extensions at the 3' end of both *Eco*R I and *Mse* I selective amplification primers. Since garlic has a large genome of over 3×10^{10} bp (Ranjekar et al., 1978), *Eco*R I selective
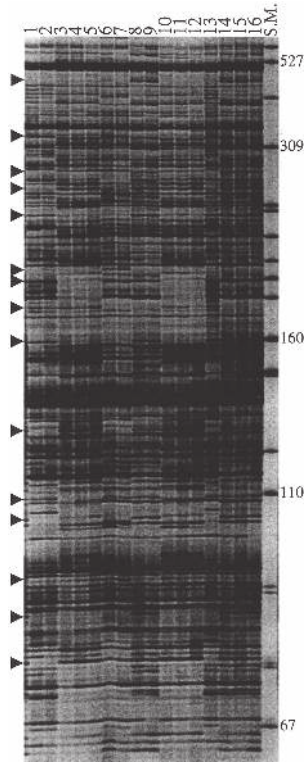
Fig. 1. Profiles of amplified fragment-length polymorphism (AFLP) fingerprinting patterns of garlic clones with the primer combination of *Eco*R-I + ACGG/*Mse*-I + CTC. SM = is the pBR322-*Msp*-I DNA molecular weight marker. Arrows point to representative bands scored as polymorphic AFLP fragments. Numbers on the top indicate clone number: 1–2 = LAU, 3–5 = DDR6801, 6–7 = PI515971, 8–9 = DDR6807, 10–12 = DDR7040, 13 = KS/10, and 14–16 = DDR6819.

amplification primers with four base nucleotide extensions were used to reduce the number of amplified bands to between 130 to 150, which could be scored unambiguously (Fig. 1). Among the eleven primer combinations tested, eight primer combinations revealed clustering of bands. This uneven distribution of bands made scoring difficult and these eight primer combinations were not used for further analysis. The remaining three primer combinations (*Eco*R I + ACGA/*Mse* I + CAT, *Eco*R I + ACGG/*Mse* I + CTC, and *Eco*R I + ACGG/*Mse* I + CAT) generated banding profiles with relatively uniform band intensity and distribution. Further analysis with these three primer combinations generated 64, 63 and 56 unambiguous polymorphic AFLP bands respectively for a total number of 183 polymorphic bands scored with a size range of 67 bp to 457 bp.

The Jaccard's similarity matrix used 183 AFLP fragments and demonstrated a similarity from 0.20 to 1.00 among the garlic clones analyzed (data not presented). Comparison of cophenetic values, calculated from the data of UPGMA cluster analysis, with Jaccard's similarity matrix demonstrated a correlation of 0.98, indicating that data in the similarity matrix were represented very well by the dendrogram. The UPGMA dendrogram revealed 10 groups by clustering clones with more than 60% similarity (Fig. 2). Except for groups III and V, clones in each group shared more than 80% of their AFLP markers.

RAPD ANALYSIS. For RAPD analysis, 20 decanucleotide primers (Operon Technologies Alameda, Calif.) reported to be polymorphic in previous studies for garlic (Al-Zahim et al., 1997; Bradley et al., 1996; Maass and Klaas, 1995) were tested. Since three primers generated inconsistent banding patterns or were monomorphic, they were not used for further analysis. The 17 consistent and polymorphic primers generated a total of 80 unambiguous polymorphic RAPD markers, ranging from 2 to 9 and averaging 4.7 bands per primer (Table 2). The UPGMA dendrogram constructed using Jaccard's similarity matrix of RAPD data was able to discriminate all groups identified by the AFLP dendrogram (Fig. 3A). All clones shared 100% of RAPD bands within each group and the similarity among groups was as low as 0.26 based on the dendrogram.

ISOZYME ANALYSIS. Eight isozyme systems were tested and eight consistent and polymorphic bands for G6PDH, EST, PGM, and DIA were scored. Previous studies also indicated that polymorphism in isozyme systems was similarly low (Maass and Klaas, 1995; Pooler and Simon, 1993). The dendrogram of the isozyme data placed garlic clones analyzed into six groups and within each group, clones shared 100% of their isozyme markers (Fig. 3B).

COMPARISION OF AFLP, RAPD, AND ISOZYME DENDROGRAMS. All clones having more than 70% similarity by AFLP data shared 100% of their RAPD bands. Above 50% similarity, groups were in the same relative locations in the AFLP and RAPD dendrograms, except DDR7116 which clustered with groups V and VI

Fig. 2. Dendrogram of 48 garlic clones constructed by unweighted pair group method with arithmetic averaging cluster analysis based on Jaccard's similarity coefficients of shared amplified fragment-length polymorphisms. Roman numerals indicate groups discriminated at 60% similarity.
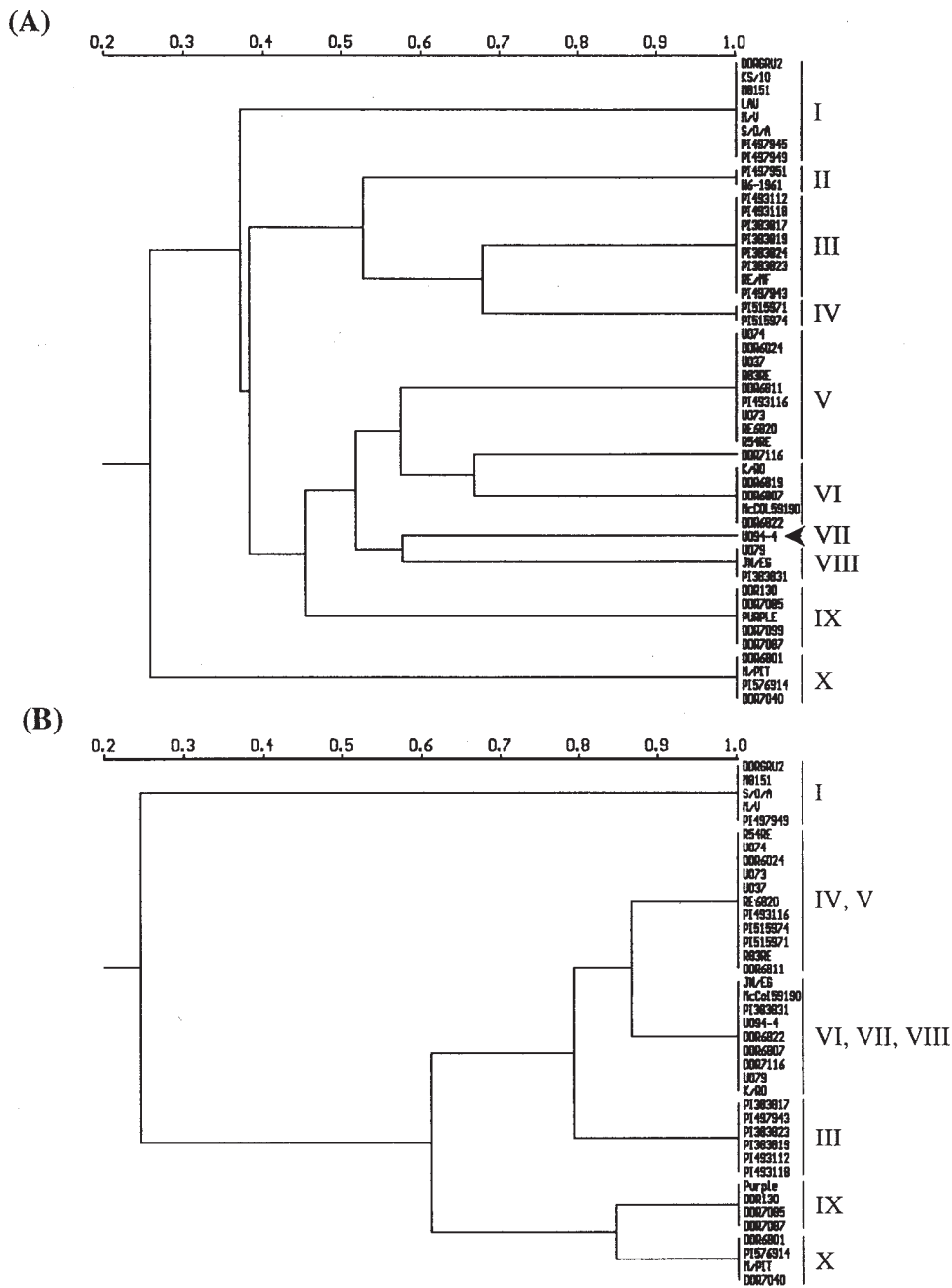
(A)

(B)
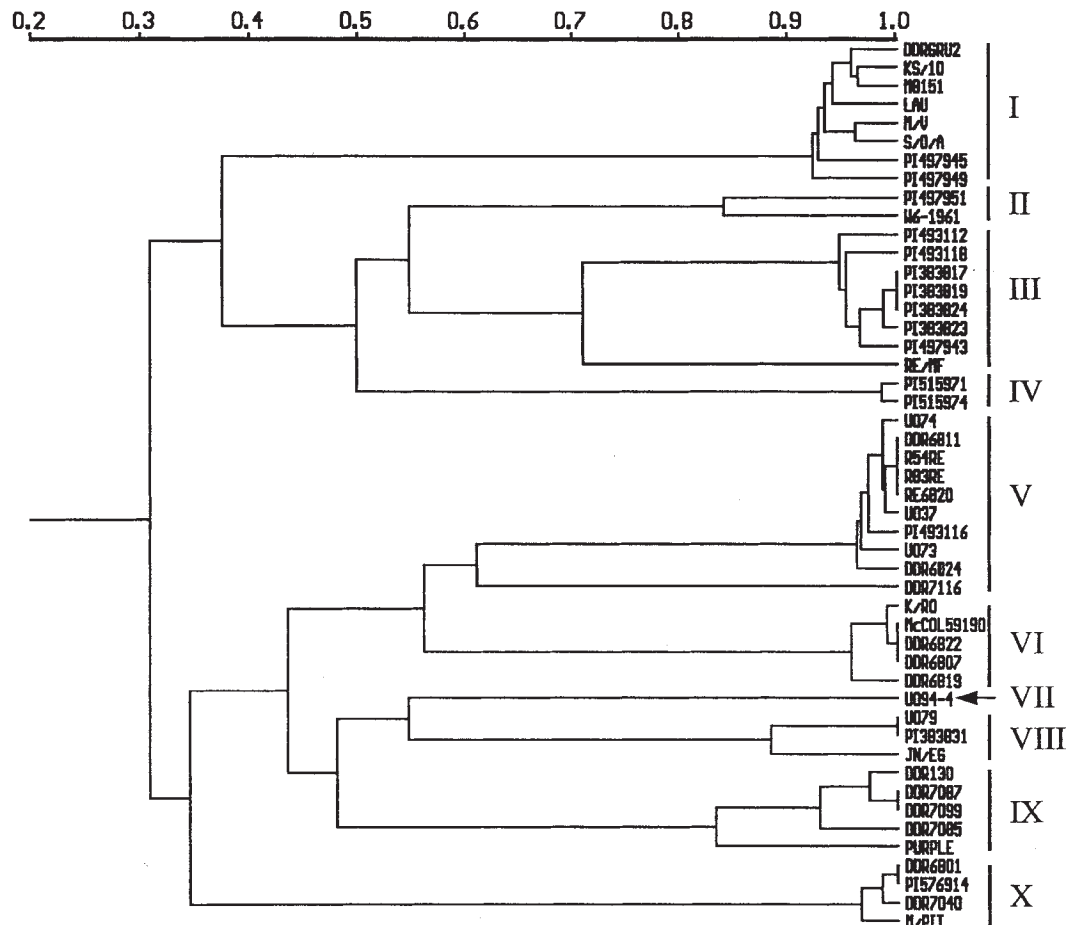
Fig. 3. Dendograms of unweighted pair group method with arithmetic averaging cluster analysis revealed by (**A**) randomly amplified polymorphic DNA markers and (**B**) isozymes. Roman numerals indicate groups from the amplified fragment-length polymorphism dendogram (Fig. 2).

in AFLP and RAPD analyses, respectively. Some differences in the association of AFLP and RAPD groupings were apparent for similarity values less than 50%. To provide a more objective comparison, matrices of cophenetic values, generated from AFLP and RAPD dendrograms, were compared using the Mantel test. Correlation between the dendrograms was 0.96, indicating high concordance between AFLP and RAPD for clustering of garlic clones. Good agreement between AFLP and RAPD analyses in characterization of genetic diversity was also reported in willow (Barker et al., 1999), melon (Garcia-Mas et al., 2000), rice (Virk et al., 2000), and pepper (Lefebvre et al., 2001). Virk et al. (2000) analyzed effectiveness of isozyme, ISSR, RAPD, and AFLP

using equal number of markers from each marker systems. They indicated that although each marker system generated similar clustering of 42 rice accessions, AFLPs and isozymes were most effective. In our study, randomly selected 80 AFLP markers were used to compare effectiveness of equal number of AFLP and RAPD on clustering of 48 garlic accessions. The dendrogram generated using 80 randomly selected AFLP markers demonstrated that general structure of dendrogram did not change but it discriminated 48 garlic clones less efficiently than using 183 AFLP markers (Fig. 4). This indicated that the use of more markers will better discriminate closely related accessions. For example, DDRGRU2, KS/10, and PI497949 shared more than 90% of the 183 AFLP markers but they happened to share all 80 randomly selected AFLP markers and consequently were not separable with this subset of bands. On the other hand, AFLP subsamples with 80 markers were more effective than the RAPD system with 80 polymorphisms for detecting genetic diversity in garlic. For instance, no RAPD polymorphism was detected among the accessions in group I, while 80 AFLP markers detected polymorphism among PI497945, M8151, and LAU.

The UPGMA cluster analysis of isozyme data resulted in much less resolution of clustering than AFLP or RAPD dendrograms. Cluster analysis of isozyme data was generally comparable to AFLP and RAPD, so that clones sharing more than 80% of the AFLPs and 70% of the RAPD markers were clustered together in the same group by isozyme markers. Thus the dendrogram pattern with isozymes was very similar to that with AFLPs and RAPD markers, but discrimination among clones in a cluster was minimal. One notable difference was that group I was widely separated from groups III and IV in the isozyme dendrogram while in the AFLP and RAPD dendrograms these three groups were more closely associated. Cluster analysis of isozyme data generated only six of the nine groups identified from AFLP and RAPD analyses. The reduction resulted from combining groups IV and V into one group and groups VI, VII, and VIII into another group. The Mantel test illustrated that the correlation between dendrograms of AFLP and isozymes was 0.55 for those taxa common to both studies. Similarly, correlation between matrices of cophenetic values of RAPD and isozyme was 0.57. Although correlations between isozyme and AFLP, and isozyme and RAPD were lower than the AFLP and RAPD correlation (0.96), both values were significant. We believe that the correlation between isozyme and AFLP, and isozyme and RAPD could be improved if there was more variation in isozymes, but based
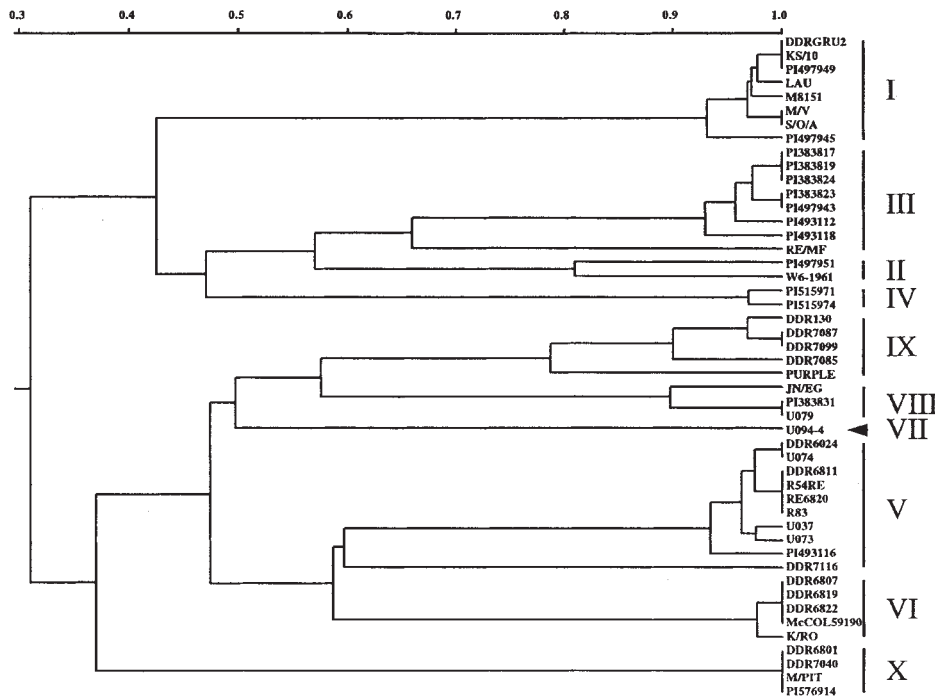
Fig. 4. Dendrogram of 48 garlic clones constructed by unweighted pair group method with arithmetic averaging cluster analysis based on Jaccard's similarity coefficients of randomly selected amplified fragment-length polymorphism (AFLP) markers. Roman numerals indicate groups from the AFLP dendogram (Fig. 2).

clusion. Fregene et al. (2000) used a similar approach in their analysis of African cassava germplasm using AFLP. They found that 10 African cassava landraces classified into three groups of five, three, and two accessions which shared 100% of their AFLP bands within each group. They also indicated that these duplicated landraces of African cassava are given different names by farmers since a variety can become widespread among the farms in Africa. Although our results suggest that PIs 383817, 383819, and 383824 might be the same genotypes, they had different names in their country of origin (http://www.ars-grin.gov/cgi-bin/npgs/). Perhaps, the same genotype was named independently by different growers and could be a popular clone of garlic in the country of origin. Bradley et al. (1996) analyzed 20 common Australian garlic cultivars with RAPD markers. They found that the cultivar 'California Early 2' shared 97% of its bands with 'Italian White 1'. They suggested that although these two cultivars were given different names, they might be identical genotypes. In addition, the AFLP analysis of our study found 36 of 48 clones to be grouped into nine clusters, where within each cluster a similarity of 0.95 or greater was noted based on the dendrogram (e.g., DDRGRU2, M8151, KS/10, M/V, and S/O/A in Fig. 2).

ASSOCIATION OF AFLP GROUPING WITH FLOWERING AND GEOGRAPHIC ORIGINS. Our results indicated that clusters were associated with flowering (Table 1). Groups II, III and IV included nonflowering (also called softneck or nonbolting) and incomplete flowering garlic types in which infrequent flowering occurs among the plants from the same clone and when flowering does occur, it results in a weak flower stalk. The garlic clones in all of the other groups were flowering (also called hardneck or bolting) types, where flowering was uniform and vigorous every year in Wisconsin. The same clones we classified as flowering were also classified as flowering in eastern Washington (GRIN garlic web site: http://www.ars-grin.gov/cgi-bin/npgs/). Flowering garlic types are classified as *A. sativum* var. *ophioscorodon* whereas nonflowering garlic types as var. *sativum* (Jones and Mann, 1963). Previous studies also demonstrated that there was some degree of relationship between morphological classification and molecular marker assessments of genetic relationship in garlic. Al-Zahim et al. (1997) were able to separate bolting type garlic clones from the nonbolting types with RAPD markers. They also indicated that cultivar 'Burgundy' with a 'weak flower stalk' clustered with nonbolting type garlic clones by RAPD analysis although it had been classified as a bolting type garlic. Pooler and Simon (1993) found that flower-related morphological characters were associated with isozyme data. Similarly, Bradley et al. (1996) reported that bolting and intermediate/nonbolting garlic forms could be separated from each other based on cluster analysis of RAPD markers.

Neither our results nor the results of Pooler and Simon (1993) noted an association between banding patterns and geographical origins (Table 1). In another study, Lallemand et al. (1997) found that the geographical origins of the some cultivars were associated with their isozyme patterns. Perhaps, the clones used in our

upon the large number of isozymes we evaluated with little variation observed, use of isozymes limits detailed fingerprinting of garlic. AFLPs and RAPD markers are very concordant markers to each other while isozymes are less but AFLP are much more informative than both RAPD and isozymes in garlic by generating the largest number of polymorphic markers (average 61 for AFLP, 4.7 for RAPD, and 2 for isozyme) per primer combination or assay.

DETECTION OF PUTATIVE DUPLICATE CLONES. Virk et al. (1995) suggested that two accessions could only be proven identical if their entire genomes were sequenced. Since this is not possible for garlic, a decision for accepting two accessions as genetically identical can only be made by using the most discriminating marker systems available. Virk et al. (1995) indicated that 86 RAPD markers would be enough to designate two accessions of rice as genetically identical with 99% confidence. They arbitrarily suggested that if two rice accessions share 100 RAPD markers without variation these accessions can be accepted as duplicate accessions.

The clonal nature of garlic provides an additional measure of comparison over seed-propagated species. Comparing fingerprints of the 48 clones in this study, sixteen clones in six different clusters shared 100% of 183 AFLPs within each group without any variation (Fig. 2). These included 1) PIs 383817, 383819, and 383824; 2) DDR6811, R54RE, R83RE, and RE6820; 3) McCOL59190, DDR6822, and DDR6807; 4) U079 and PI383831; 5) DDR7087 and DDR7099; and 6) DDR6801 and PI 576914. We point out that since our analysis included DNA samples from more than one plant when possible, the lack of both intraclonal and interclonal variation when two clones were compared reinforced our conclusion that they were duplicates. Morphological similarity among clones in a cluster further reinforced this con-

study have been passed between countries before their arrival in our collection and their true geographic origins were consequently lost. With this situation, the country of collection likely does not reflect the country of origin.

GROUPING OF *A. longicuspis* WITH GARLIC CLONES. The clones of *A. longicuspis* were clustered with other *A. sativum* clones although *A. longicuspis* is proposed to be a progenitor species of garlic. *A. longicuspis* clones UO94-4 and UO79 were clustered in groups VII and VIII, respectively and PI576914 was clustered in group X with flowering type garlic clones. Therefore, our AFLP data did not discriminate *A. longicuspis* from *A. sativum*, suggesting that these species are not genetically distinct. Morphologically, *A. longicuspis* clones evaluated closely resemble the clones of *A. sativum* var. *ophioscorodon* in the same group. Similar results were found with the isozyme study of Pooler and Simon (1993) and isozyme and RAPD studies of Maass and Klaas (1995). Al-Zahim et al. (1997) also found that *A. longicuspis* and *A. sativum* var. *ophioscorodon* were clustered together using RAPD markers.

Unlike seed propagated plants, a clonally propagated species such as garlic requires regeneration of each accession every year. This requirement increases the maintenance cost and also complicates maintenance. The results of AFLP analysis were in good agreement with RAPD and isozyme analysis and discriminated garlic accessions with higher efficiency. AFLP technology can help to identify duplication of accessions in germplasm resources of garlic to reduce the cost of maintenance. In conclusion, AFLP technology is a useful tool for analysis of genetic diversity of garlic along with morphological characters, isozymes, and RAPD markers. The very large number of polymorphic AFLP markers can provide a better approximation to true variation among clones and can help to detect duplication with higher confidence.

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