AFLP Assessment of Genetic Diversity of *Capsicum* Genetic Resources in Guatemala: Home Gardens as an Option for Conservation

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ABSTRACT

The genetic diversity of 74 chili pepper accessions (*Capsicum* spp.) was evaluated with amplified fragment length polymorphisms (AFLPs). Thirty-four accessions were collected from home gardens throughout the Department of Alta Verapaz, Guatemala. The remaining accessions were selected from the national ex situ germplasm collection representing the diversity of 12 other departments of Guatemala. Most of the accessions belong to *Capsicum annuum* L., both cultivated and semicultivated types. A few accessions of *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz & Pavon were also included. The analysis of banding patterns (68 polymorphic bands) were obtained with three AFLP primer combinations (+3/+4 and +4/+3 selective bases) and allowed the discrimination of all but two of the accessions examined. It also made it possible to conclude that the genetic diversity found in the home gardens of Alta Verapaz is representative of the total genetic diversity of *Capsicum* in Guatemala. This is the first time that molecular markers have been used to assess crop genetic diversity maintained in home gardens and to evaluate their importance for in situ conservation of genetic resources.

Ex situ and in situ are the two main mechanisms for the conservation of plant genetic resources (Frankel et al., 1995). In situ conservation has the advantage of allowing the process of evolution to continue (Hoyt, 1992). Jana (1999) recommends that the in situ conservation of crop genetic diversity maintained in traditional farming systems should be promoted and incorporated into agricultural development and land use planning.

Home gardens offer complex microenvironments in which farming families maintain populations of many useful plant species close to their dwellings (Hodgkin, 2002), where they can be closely observed and managed. They thus represent an important option for promoting in situ conservation of genetic resources, especially in the tropics where home gardens are significant reservoirs of genetic diversity (Knupffer, 2002). In Guatemala, home gardens contribute significantly to the sustainability of local production systems as a whole and represent an opportunity for conservation efforts in a region where deforestation continues to exacerbate genetic erosion (Leiva et al., 2002). However, thorough genetic diversity studies of the plant species found in these microenvironments are scarce, a situation that hinders the development of conservation strategies that explicitly include home gardens as in situ repositories of plant genetic resources.

A global project was undertaken to study plant genetic resources in home gardens in several countries—Guatemala, Cuba, Ghana, Nepal, Venezuela, and Vietnam—to evaluate their potential as a component for in situ conservation of agrobiodiversity (Eyzaguirre and Watson, 2002). In Guatemala, the project described the richness of plant species in home gardens in the northern (Department of Alta Verapaz) and the western (semiarid) parts of the country. Chili peppers (*Capsicum* spp.) were among the 500 species documented in Guatemalan home gardens. Inventories of species found in tropical home gardens in the Americas, Africa, and Asia reveal that chili pepper is a key home garden crop in all regions. Home gardens are thus a good site for the study of the global spread and distribution of genetic diversity in chili peppers from tropical America (Williams, 2003).

Four cultivated *Capsicum* species are found in Guatemala: *C. annuum,* scattered all over the country; *C. chinense*, mainly distributed in the department of Peten; *C. frutescens,* found in the northern parts; and *C. pubescens,* in the northern, central and western highlands of the country. In addition, *C. annuum* var. *annuum* and *C. annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill are found, as semicultivated varieties, in marginal areas throughout the country. Among these four species, several fruit types are identified with different local names, mostly on the basis of their shape, taste, pungency, or cultivation area (Azurdia et al., 1995).

The purpose of the work presented here was to compare by AFLPs the genetic variation of *Capsicum* found in home gardens of Guatemala with that conserved in the national ex situ collection and to determine the potential importance of home gardens for in situ conservation of plant genetic resources in the future.

Using molecular markers, allows a rapid and efficient assessment of genetic diversity (Hammer, 2003). The choice of AFLPs was based on the demonstrated success of using them to evaluate genetic diversity for studies in numerous species [e.g., soybean, *Glycine max* (L.) Merr., Maughan et al., 1996; *Phaseolus vulgaris* L., Tohme et al., 1996; *Arabidopsis,* Breyne et al., 1999; *Limonium,* Palacios and González-Candelas, 1999; *Azadirachta,* Singh et al., 1999; *Morus*, Sharma et al., 2000; and *Stylosanthes,* Sawkins et al., 2001).

MATERIALS AND METHODS

Plant Material

This work was performed on 40 *Capsicum* accessions from the national gene bank collection of the Universidad de San

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Abbreviations: AFLP, amplified fragment length polymorphism.

Carlos (Facultad de Agronomía) and 34 accessions collected from home gardens in the Department of Alta Verapaz, both in Guatemala (Table 1). Germplasm from the gene bank was selected on the basis of its representation of geographic origin and distribution (13 departments), its representation of the diversity of known Guatemalan fruit types, and the availability of seed. The selection of materials from home gardens was made to ensure the comparison of a set of contrasting environments both in terms of climate and indigenous culture (Fig. 1).

Seeds collected for the gene bank collection were harvested from as many plants with similar morphological traits as possible in a given place (farmer's field), and this group of seeds was identified as a single accession (Ayala, 1997). In the collections made from home gardens, each accession consisted of a mixture of seeds collected from whatever plants were available, normally only one or two (Azurdia et al., 2001b).

The total sample studied was composed of 24 semicultivated accessions [*C. annuum* var. *annuum* (Diente de Perro and Pico de Gallina) and *C. annuum* var. *glabriusculum* (Chiltepe and Chiltepe Grande)], three *C. pubescens* accessions, one accession of *C. frutescens*, one of *C. chinense*, and 45 accessions of cultivated *C. annuum*. The semicultivated accessions were almost evenly divided between the home gardens sample (13) and the gene bank collection (11).

The distribution of samples in terms of altitude, both in the home garden group and in the gene bank collection, ranged from 50 m (Department of Escuintla) to 1908 m (Department of Huehuetenango) above sea level.

DNA Extraction

Five individuals per accession were germinated in the greenhouse. Ten days after germination, a uniform amount of fresh leaf tissue (25 mm^2) was harvested from each individual. Leaf tissue harvested from all individuals of an accession was pooled for DNA extraction. Leaf tissue was crushed with 400 μL extraction buffer [200 m*M* Tris-HCl pH 7.5; 250 m*M* NaCl; 25 m*M* EDTA, 0.5% (w/v) SDS] in 1.5 mL tubes. After shaking, tubes were left at room temperature for 1 h and then spun down in a centrifuge at medium speed for 10 min. Supernatant was transferred to a clean tube, isopropanol was added (4:3 v/v) and the tube was gently inverted to help blending. The mixture was centrifuged at medium speed for 10 min at room temperature and the supernatant discarded. The DNA pellet was rinsed with ethanol 70% (v/v), centrifuged for 5 min more and resuspended in 100 μL TE. Each DNA extract was diluted to a final concentration of 25 ng/μL and stored at 4°C for future use.

Amplified Fragment Length Polymorphisms

Amplified fragment length polymorphisms were obtained with the Analysis System I and the AFLP Starter Primer kit of GIBCO-BRL (Life Technologies) with adapters and primers on the basis of the *Eco*RI and *Mse*I restriction enzymes sites. All steps in DNA restriction digestion, adapters' ligation, preselective and selective PCR amplifications were performed following the directions provided by the manufacturer.

Out of 40 combinations of +3/+3 selective bases, two were selected on the basis of the number of amplified fragments and the polymorphism detected in a set of four samples randomly taken. Given the high number of bands obtained per combination, an additional nucleotide was added to the selective primers to further restrict the last amplification and to increase the reliability of band scoring. A test was made with the 16 new combinations of selective primers and on the basis of the number of amplified fragments, the polymorphism observed in a set of four random samples and the clarity of bands, three primer combinations were finally chosen: *Eco*RI+AGC/*Mse*I+CAGC, *Eco*RI+AGG/*Mse*I+CAGT, and *Eco*RI+AGAG/*Mse*I+CAG. All PCR amplifications were performed in a PTC-100 programmable thermocycler (MJ Research, Inc., Watertown, MA).

Electrophoresis of AFLP products was performed in 6% (w/v) denaturing polyacrylamide gels containing 7 *M* urea and TBE 0.5× buffer. Six microliters of buffer [95% (v/v) formamide, 20 m*M* EDTA pH 8.0, 0.05% (w/v) bromophenol blue and xylene cyanol FF] were added to each PCR product and 5μL of each was loaded in the gel. Gels were left to migrate for approximately 2 h (110 W, 50°C), and immediately afterwards, stained with silver nitrate.

To check reproducibility of results and to ensure the comparison of similar bands in the two gels required for the analysis of each primer combination, a sample of control DNA was included in the gels. In addition, several accessions, randomly selected, were reprocessed and run in separate gels.

Data Analyses

Clear polymorphic bands were manually scored and a binary matrix produced (1 = presence, 0 = absence). The calculation of the heterogeneity or total genetic diversity was done according to Nei (1987). For this estimation, each band was considered as a diallelic locus, such that the band present is one allele and its absence is the alternative allele. Because AFLP bands detect dominant loci, Nei's coefficient may not be interpreted as the estimation of heterozygosis, but rather heterogeneity within groups. Then, total diversity ($H = Hs + Dst$) is the average locus heterogeneity (h_i) over the total number of evaluated loci ($i = 1, \ldots, n$); within group diversity (Hs) is the weighted average of diversity within each group and the diversity among groups (Dst) is the difference between H and Hs. The ratio Dst/H corresponds to the genetic differentiation coefficient (Gst), which measures the proportion of total variation explained by the difference among groups.

A multiple correspondence analysis (MCA) was performed with the binary matrix. The output of this test allows the representation of the distribution of the accessions in a multidimensional metric space in such a way that it reflects the relationships among samples based on their similarity in banding profiles. Its graphical representation shows the spatial location of the accessions, facilitating the visualization of their dispersion and also a possible structuring of the populations. For the present study, three dimensions were chosen. Calculations were done by the CORRESP procedure of the SAS statistical package v. 8.2 and clustering was performed by applying the average linkage method (UPGMA).

RESULTS

AFLP Polymorphism

The number of polymorphic bands obtained with each selective primer combination in the sample of 74 accessions was as follows: 18 bands were produced with *Eco*RI+AGC/*Mse*I+CAGC, 24 bands with *Eco*RI+AGG/*Mse*I+CAGT and 26 bands with *Eco*RI+AGAG/*Mse*I+CAG. The band size range of the polymorphisms scored was between approximately 330 and 80 bp. AFLP banding profiles were highly reproducible among replications of the same accession.

Only two (133 and 269) of the 74 accessions gave

Table 1. List of materials used in this study. Columns show the accession number as named at harvest time or assigned in the National ex situ collection, the cluster attribution as resulted from the multiple correspondence analysis, the botanical species and variety names, the local names when available, the municipality and the department where the accession was harvested, and the altitude. Accessions H1 to H37 correspond to materials collected in home gardens.

Accession	Cluster	Species	Local name	Municipality	Department	Altitude
H1	B	*C. annuum*	Chile S. Pedro	S. Juan Chamelco	A. Verapaz	1300
H3	C	*C. annuum*	Che-ik	S. Juan Chamelco	A. Verapaz	1300
H4	C	*C. annuum*	Bap-ik	S. Juan Chamelco	A. Verapaz	1300
H5	B	*C. annuum* var. *glabriusculum*	Chiltepe	Coban	A. Verapaz	500
H6	B	*C. annuum* var. *glabriusculum*	Chiltepe	Coban	A. Verapaz	1330
H7	C	*C. annuum*	Cuerudo	Chisec	A. Verapaz	250
H8	B	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	250
H9	C	*C. annuum*	Cahabonero	Chisec	A. Verapaz	250
H10	B	*C. annuum* var. *glabriusculum*	Chiltepe	Las Casas	A. Verapaz	300
H11	B	*C. annuum*	Bolonillo	Las Casas	A. Verapaz	300
H12	C	*C. annuum*	Tipo	Las Casas	A. Verapaz	300
H13	B	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	450
H14	B	*C. annuum*	Ik	Chisec	A. Verapaz	450
H15	C	*C. annuum*	Ik	Chisec	A. Verapaz	600
H16	B	*C. annuum*	Ik	Chisec	A. Verapaz	600
H17	B	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	400
H18	B	*C. annuum* var. *glabriusculum*	Chiltepe	Coban	A. Verapaz	300
H19	B	*C. annuum*	Cahabonero	Coban	A. Verapaz	300
H20	B	*C. annuum* var. *glabriusculum*	Chiltepe	Coban	A. Verapaz	300
H21	B	*C. annuum*	Chile Largo	Coban	A. Verapaz	300
H23	A	*C. annuum*	Cahabonero	Coban	A. Verapaz	300
H24	B	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	250
H25	A	*C. annuum*	Pico de Gallina	Chisec	A. Verapaz	250
H26	C	*C. annuum*	Cahabonero	Chisec	A. Verapaz	250
H27	D	*C. annuum*	Ik	Chisec	A. Verapaz	310
H28	D	*C. annuum*	Ik	Chisec	A. Verapaz	310
H29	B	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	310
H30	F	*C. annuum* var. *glabriusculum*	Chiltepe	Chisec	A. Verapaz	290
H32	B	*C. annuum*	Cahabonero	Chisec	A. Verapaz	290
H33	B	*C. annuum*	Cahabonero	Chisec	A. Verapaz	290
H34	C	*C. annuum*	Cahabonero	Las Casas	A. Verapaz	300
H35	A	*C. annuum*	Diente de Perro	Las Casas	A. Verapaz	300
H36	C	*C. annuum*	Chile	Las Casas	A. Verapaz	300
H37	E	*C. pubescens*	7 Caldos Rojo	S. Cristobal	A. Verapaz	1250
P8	B	*C. annuum* var. *glabriusculum*	Chiltepe	La Libertad	Peten	450
P9	C	*C. frutescens*	Tabasco	Poptun	Peten	450
P11	A	*C. annuum*	Chile Nance	La Libertad	Peten	80
P37	B	*C. annuum* var. *glabriusculum*	Chiltepe Grande	Sayaxche	Peten	150
P47	B	*C. annuum*	De Jardin	La Libertad	Peten	80
CH1	F	*C. annuum*	Chamborote	Chinautla	Guatemala	1200
CH2	D	*C. annuum*	Chamborote	Chinautla	Guatemala	1200
HU1	C	*C. annuum*	De Huerta	Huehuetenango	Huehuetenango	1908
HU2	A	*C. annuum*	De Huerta	Huehuetenango	Huehuetenango	1908
JOB	F	*C. chinense*	Habanero	Jobonpiche	Peten	150
51	B	*C. annuum* var. *glabriusculum*	Chiltepe	Bulbuxya	Suchitepequez	320
57	C	*C. annuum*	Chocolate	Samayac	Suchitepequez	600
58	C	*C. annuum*	Diente de Perro	Samayac	Suchitepequez	600
65	A	*C. annuum* var. *glabriusculum*	Chiltepe	N. Concepcion	Escuintla	50
66	A	*C. annuum*	Diente de Perro	N. Concepcion	Escuintla	50
68	A	*C. annuum*	Diente de Perro	N. Concepcion	Escuintla	50
85	C	*C. annuum* var. *glabriusculum*	Chiltepe	Jocopilas	Suchitepequez	550
89	C	*C. annuum*	Chile Nance	Chocola	Suchitepequez	550
119	B	*C. annuum*	Chile	S. Pedro Carcha	A. Verapaz	1360
120	D	*C. annuum*	Cahabonero	Lanquin	A. Verapaz	350
129	F	*C. annuum*	S. Pedro	Ixtapacapa	Suchitepequez	600
131	A	*C. annuum*	Chile Nance	Sto. Tomas	Suchitepequez	600
132	A	*C. annuum*	S. Pedro	S. Bernardino	Suchitepequez	600
133	A	*C. annuum*	S. Pedro	S. Antonio	Suchitepequez	600
162	E	*C. pubescens*	7 Caldos Rojo	Patzicia	Chimaltenango	1600
200	A	*C. annuum*	Chile Nance	Quezada	Jutiapa	800
201	C	*C. annuum*	Diente de perro	Quezada	Jutiapa	800
206	C	*C. annuum*	Chile Chocolate	Chiquimulilla	Sta. Rosa	260
224	B	*C. annuum*	Alargado	S. Pedro Pinula	Jalapa	1200
232	D	*C. annuum*	Chile Huerta	Santiago	Solola	1200
261	C	*C. annuum*	Chile	Cuilco	Huehuetenango	1300
269	A	*C. annuum*	Miracielo	Cuilco	Huehuetenango	1300
274	A	*C. annuum*	Chocolate	Cubulco	B. Verapaz	900
280	E	*C. pubescens*	7 Caldos Amarillo	Tamahu	A. Verapaz	1330
295	C	*C. annuum*	Chile Blanco	Panzos	A. Verapaz	900
302	B	*C. annuum* var. *glabriusculum*	Chiltepe	Uspantan	Quiche	1500
810	B	*C. annuum*	Cahabonero	Raxhuha	A. Verapaz	150
585	B	*C. annuum* var. *glabriusculum*	Chiltepe	Mataquescuintla	Jalapa	1500
765	C	*C. annuum*	Guaque			
820	D	*C. annuum*	Cahabonero	Coban	A. Verapaz	150

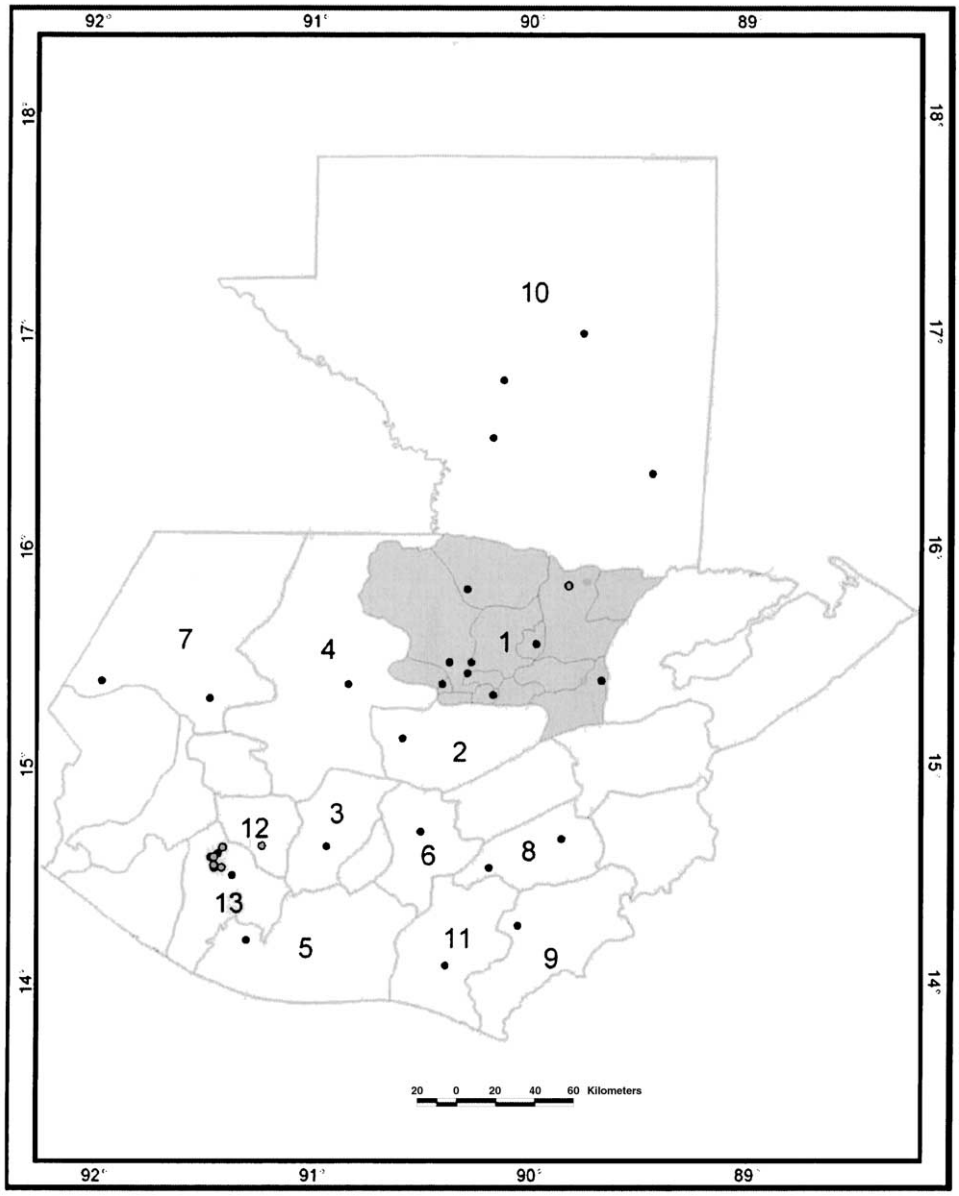

Fig. 1. Map of Guatemala indicating the collecting sites for home gardens and ex situ accessions. Departments: 1. Alta Verapaz (colored in gray), 2. Baja Verapaz, 3. Chimaltenango, 4. El Quiche, 5. Escuintla, 6. Guatemala, 7. Huehuetenango, 8. Jalapa, 9. Jutiapa, 10. Peten, 11. Santa Rosa, 12. Solola, 13. Suchitepequez.

similar AFLP genotypes for the 68 polymorphic bands scored. The other 72 accessions showed different band profiles, which means that the AFLP primer combinations selected had good discriminatory power, and that the sample under study was highly variable.

Genetic Diversity of Gene Bank Accessions versus Accessions Obtained from Home Gardens

The comparison of genetic diversity of AFLP bands obtained with the *Capsicum* accessions from home gardens and those from the national gene bank collection showed that the amount of diversity in the two samples was similar; that is, their divergence (*G*st) accounted for only 4% of the total diversity (Table 2). Because the gene bank collection also held six accessions from the department of Alta Verapaz, the source of the home garden samples, the analysis was redone after removing these accessions and similar results were obtained, which means that the contribution of these six samples to the total diversity of the *Capsicum* gene bank collection was not significant ($H = 0.280$, $H_S = 0.265$, $G_{ST} = 0.052$).

Considering the accessions from the home gardens and the gene bank as two different samples, four unique bands could be identified, three in the home gardens sample and only one in the gene bank sample.

The semicultivated accessions made up 38% of the home gardens sample and 27% of the gene bank sample. An analysis was performed comparing the diversity of these accessions with that of the cultivated types, and no significant differences were obtained (Table 2). In addition, the analysis of diversity performed between

Table 2. Diversity values as calculated for different types of samples (home gardens vs. ex situ collection and semicultivated vs. cultivated materials). *H*i is the diversity within each type of sample; *H*s is the average genetic diversity among samples; *G*st is the genetic differentiation coefficient; *n* is the number of accessions per sample; *H* = 0.280 (Total genetic diversity).

	n	*H*i	*H*s	*G*st
Origin				
Home gardens	**34**	**0.251**		
ex situ	**40**	**0.281**		
			0.268	**0.043**
Types				
Semicultivated	**24**	**0.248**		
Cultivated	**50**	**0.286**		
			0.274	**0.021**

the semicultivated accessions from home gardens and those from the gene bank revealed no significant differences (H = 0.248, Hs = 0.233, Gst = 0.060).

Likewise, an analysis was performed to evaluate the genetic diversity among the samples collected in different climatic or altitude conditions considering two groups, from 50 to 950 and from 951 to 1908 m above sea level. Some 15% of the home garden accessions and 32% of the gene bank accessions were from high altitude. The results again showed no significant difference in the genetic diversity of these two groups (H = 0.280, Hs = 0.280, Gst = 0.000). Once more, the comparison of the genetic diversity differences for the low altitude and high altitude accessions respectively, both in the home gardens and the gene bank samples, were detected to be nonsignificant (low altitude: H = 0.274, Hs = 0.258, Gst = 0.076; high altitude—without correction factor because n was small: H = 0.281, Hs = 0.269, Gst = 0.045).

The lack of significant differences in all genetic diversity comparisons explains the uniform genetic diversity values in the home gardens and the gene bank samples in spite of the fact that they differ in their composition, both in terms of type of materials and the collecting site altitude of the accessions.

Multiple Correspondence Analysis

The clustering procedure applied on the coordinates generated by the MCA distinguished a structure of six groups, whose presence explains 93% of the total variation in the sample (Fig. 2). The third dimension allowed the separation of the three accessions belonging to *C. pubescens* (group E) from the rest of the materials included in the study. The first and second dimensions differentiated the remaining 71 accessions of *C. annuum*, *C. frutescens*, and *C. chinense*.

Each of the six clusters was characterized by the presence or absence of certain AFLP bands (Table 3). For example, the *C. pubescens* group (E) was distinguished by the presence of two unique bands and the absence of nine bands that were present in all the other groups (data not shown). Group A lacked the presence of two bands and Group D lacked one band. Except for Cluster E, the presence of unique bands in a cluster was not a common feature of all the accessions. However, unique bands in Groups A and F represented an important percentage over the total number of bands (10 and 14%, respectively). Groups B, C, and D shared the highest number of bands, but their share of unique bands was the lowest (<3%).

Accessions from home gardens and the gene bank collection were scattered across the six groups formed by the MCA (Table 3). Group A was composed entirely of *C. annuum* accessions, including five semicultivated materials (C. *annuum* var. *glabriusculum* and C. *annuum* var. *anuuum*), and its accessions were distributed over seven of the 13 departments included in this study. Group B was also composed entirely of *C. annuum* accessions, mostly from home gardens (67%), and it contained 82% of the semicultivated variety *glabriusculum*, whose local name is "chiltepe". Group C contained *C. annuum,* with three semicultivated accessions, and the only *C. frutescens* accession. Group D included neither semicultivated materials nor any *Capsicum* species other than *annuum*. Group F contained three *C. annuum* accessions, one of them of the semicultivated types, and the only *C. chinense* accession.

The configuration of groups resulting from the MCA of the AFLP data matrix successfully discriminated the *C. pubescens* accessions. The single accessions of *C. frutescens* and *C. chinense* grouped with *C. annuum* in Groups C and F, respectively. Groups A, B, and D were composed exclusively of *C. annuum* accessions. It is noteworthy that Group B was composed mainly of the semicultivated variety *C. annuum* var. *glabriusculum.* Morphological characterization of the accessions in Group A could help to understand the reason why they significantly separated from B and D.

In terms of genetic diversity, Groups D and F brought together low numbers of accessions (6 and 4, respectively) yet were the most diverse. In contrast, Group A was the least diverse despite the fact that its accessions originated from seven different departments. The value of Gst, the genetic differentiation coefficient, indicated that the differences among the six distinct groups resulting from the MCA explained 45% of the total diversity of the sample (Table 4).

DISCUSSION

Genetic Diversity in Home Gardens versus that Conserved Ex Situ in the Gene Bank

The comparison of the samples collected from home gardens in Alta Verapaz with the accessions in the national gene bank collection shows that they differed only slightly in terms of their total genetic diversity (4%). This indicates that the diversity of peppers in the home gardens of the Department of Alta Verapaz is largely representative of the total diversity currently preserved in the national *Capsicum* germplasm collection, or at least of that portion of the collection representing the 13 departments included in the present study. Thus, a strategy of in situ conservation of *Capsicum* genetic resources in home gardens within Alta Verapaz, a relatively restricted area of the country, seems able to conserve nearly all the *Capsicum* diversity known in Guatemala, with the benefit that this conservation option allows

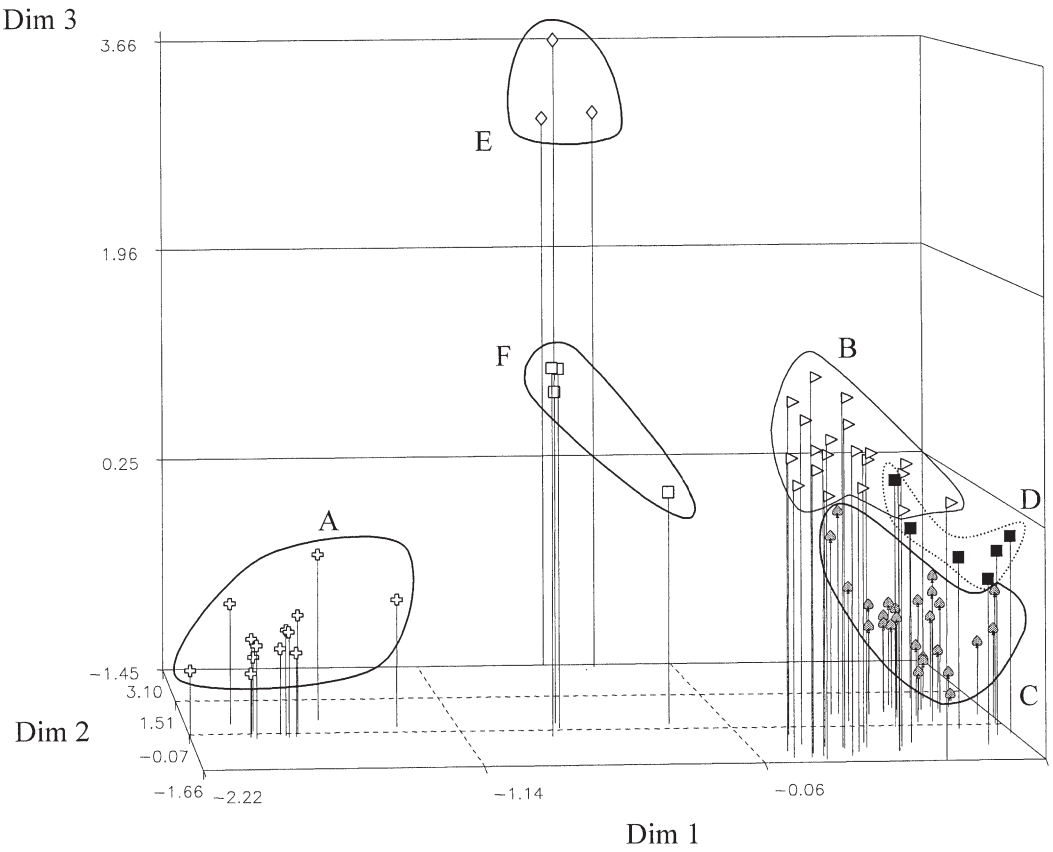

Fig. 2. Spatial distribution of the clusters of accessions, obtained with three principal axes of variation by multiple correspondence analysis.

evolutionary processes to continue and with the extra advantage that conservation goes along with continued use by local people.

It is important to note that, although the amount of genetic diversity in both samples was similar, our results showed that the polymorphism detected in the two sets of accessions, independently of their provenance, was different because we were able to discriminate all but two of the accessions in the samples. In addition, the accessions collected in home gardens showed a higher number of unique AFLP bands than those from the ex situ collection, which could indicate the fact that Alta Verapaz maintained a higher frequency of rare genetic variants. Alta Verapaz is the Department in Guatemala where consumption of pepper is highest, a fact likely because of the presence of the Ketchi and Pocomchi indigenous cultures which use pepper both as a basic component of their diet as well as for religious rituals. Furthermore, new *Capsicum* genetic types may have arrived from other regions. Lately, the northern part of Alta Verapaz has received a significant number of immigrants moving from other parts of the country, carrying seeds from their original locations.

Because the *Capsicum* accessions analyzed from the gene bank collection included material from 13 of the 22 departments of Guatemala, the question arises as to whether the breadth of the gene bank collection of *Capsicum* is sufficiently comprehensive or if further explorations are needed. Given its high diversity and the possibility of finding new pepper types there, Alta Verapaz may be considered a suitable region both for further collecting for ex situ conservation as well as for establishing in situ conservation priority areas, as already suggested recently for genetic resources of chayote and

Table 3. Cluster composition according to the origin of the accessions (home gardens vs. ex situ), number of polymorphic bands, and unique bands per cluster. *n* is the number of accessions per sample.

Cluster	*n*	Origin		Polymorphic bands	Unique bands
		Home Gardens	ex situ		
A	14	3	11	20	2
B	27	18	9	45	1
C	20	9	11	39	1
D	6	2	4	35	1
E	3	1	2	10	2
F	4	1	3	29	4

Table 4. Diversity values calculated per cluster as defined by the MCA. *Hi*: diversity within each cluster; *Hs*: average genetic diversity within cluster; *Gst*: genetic differentiation coefficient. *n* is the number of accessions per sample. $H = 0.280$, $Hs = 0.153$, $Gst = 0.450$.

Cluster	*n*	*Hi*
A	14	0.080
B	27	0.163
C	20	0.168
D	6	0.217
E	3	0.095
F	4	0.226

sapote (Azurdia et al., 2001a; Azurdia et al., 2002). In any case, in situ conservation must be always considered as a complementary approach to that of ex situ conservation. In this regard, increasing the ex situ collection with germplasm of *Capsicum* genotypes from Alta Verapaz would allow further characterization and evaluation of these materials. Moreover, seed kept in the ex situ collection would then be available for breeders and other users, and would help guarantee the conservation of these valuable genetic resources for the future in the event that they disappeared from home gardens.

Sample Genetic Structure

The configuration of the sample in six main clusters indicates that there is structure among the accessions analyzed. *Capsicum pubescens* separates significantly from the rest of the accessions, as expected according to results obtained previously with morphological characters (Pickersgill, 1971) and isozymes (Jensen et al., 1979). This species originated in South America, though its introduction in Mesoamerica seems to date from the pre-Hispanic times (Heiser, 1995). The clustering of *C. frutescens* together with *C. annuum* reflects the closeness of these two species (McLeod et al., 1979; Loaiza-Figueroa et al., 1989). On the other hand, *C. chinense* groups also with *C. annuum*, but it does so in a more differentiated cluster with the highest number of unique bands, which also may point to the fact that *C. chinense* has a South American origin (Prince et al., 1992, 1995).

In addition to the clusters that included accessions from species other than *C. annuum,* Groups A and B were particularly interesting because they mainly included the semicultivated accessions. These genetic resources grow either in home gardens or areas of cultivation in close proximity with other wild or semiwild peppers and are tolerated as weeds because of their importance for human consumption and with which introgression is likely to occur.

The combined clusters (C and F) of *C. annuum* with *C. frutescens*, *C. chinense* and the semicultivated types (*C. annuum* var. *annuum* and *C. annuum* var. *glabriusculum*) confirm the existence of gene flow among all these materials (Pickersgill, 1971; Prince et al., 1992). *Capsicum annuum*, *C. frutescens*, and *C. chinense* are in fact distinct morphologically, but can only be considered as different species from a taxonomic perspective and are not biologically distinct. Moreover, *C. annuum* var. *glabriusculum* is thought to be the taxon of origin of all cultivated *C. annuum* types (Azurdia et al., 1995).

Although more accessions per species would be needed to make firm conclusions with regard to their taxonomy, the presence of gene flow tends to even out the diversity among populations and could explain why, other than *C. pubescens,* the other species or varieties clustered together with *C. annuum*.

Molecular and Morphological Polymorphism

The three AFLP primer combinations used (modified with an extra selective base) produced a total of 68 polymorphic bands and 72 different banding patterns that allowed the distinction of all but two of the accessions involved (97%). This is an indication of the high discrimination power of the AFLP technology. Accessions 133 and 269, however, shared the same AFLP banding pattern, and this was so in spite of their morphological differences and the fact that they were collected in quite different regions. Accession 133, known as "San Pedro," was collected in San Antonio, Suchitepequez, a hot and humid environment. Accession 269, known as "Miracielo," is only found in Cuilco, Huehuetenango, with a temperate and dry climate. The extension of the experiment with one or more AFLP primer combinations would likely lead to the molecular differentiation of these materials.

On the other hand, the relative evenness in the AFLP genetic composition of *Capsicum* accessions collected in home gardens and those maintained in the ex situ collection may reflect the fact that most of them are landraces, which have not been involved in formal breeding programs, so that the relative levels of diversity among themselves and even with semicultivated materials are not great. Morphological differences among these accessions may be the result of just a few genes (Azurdia et al., 1995) and, consequently, are difficult to distinguish with the AFLP analysis of a few bands. To test this hypothesis, another type of molecular marker would need to be used, such as microsatellites, which are more powerful for infraspecific discrimination, but which because there were not publicly available sequences at the time of this research, could not be considered.

In the present work, the importance of home gardens as an agroecosystem to generate and conserve genetic diversity of *Capsicum* in situ has been proven. This is the first time that a molecular methodology was applied to assess and compare the genetic diversity maintained in two complementary conservation schemes, ex situ and in situ. Therefore, it can be taken as the basis for additional studies that include more species and more accessions per species to elaborate on the substantiation of the importance of home gardens as an attractive option for in situ conservation, especially in centers of origin and diversity of cultivated plants where both the biological and cultural richness are combined.

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