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Genetic diversity analysis of *Capsicum* spp germplasm bank accessions based on α/β-esterase polymorphism

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ABSTRACT. Genetic diversity and structure were analyzed in 10 accessions belonging to Banco Ativo de Germoplasma de *Capsicum* located at Federal University of Piauí in northwestern Brazil that receives pepper samples grown in community gardens in various regions and Brazilian states. Selections were made from seeds of *C. chinense* (4 accessions), *C. annuum* (5 accessions), and *C. baccatum* (1 accession). Samples consisting of leaves were collected from 4-10 plants of each accession (a total of 85 plants). Native polyacrylamide gel electrophoresis was used to identify α- and β-esterase polymorphisms. Polymorphism was clearly detected in 5 loci. Sixteen alleles were found at 5 α/β-esterase loci of the three *Capsicum* species. In the *C. chinense* samples, the highest H_O and H_E values were 0.3625 and 0.4395, respectively, whereas in *C. annuum* samples, H_O and H_E values were 0.2980 and 0.3310, respectively; the estimated H_O and H_E values in *C. chinense* samples were higher than those detected in *C. annuum* samples. A deficit of

homozygous individuals was found in *C. chinense* (F_{IS} = -0.6978) and *C. annuum* (F_{IS} = 0.7750). Genetic differentiation between *C. chinense* and *C. annuum* at these loci was high (F_{ST} = 0.1867) indicating that *C. chinense* and *C. annuum* are genetically structured species for α/β-esterase isozymes. The esterase analysis showed high genetic diversity among the *C. chinense* and *C. annuum* samples and very high genetic differentiation (F_{ST} = 0.6321) among the *C. chinense* and *C. annuum* samples and the *C. baccatum* accession.

Key words: Peppers; *Capsicum* spp; Esterase; Isozymes; Genetic diversity

INTRODUCTION

Peppers of the genus *Capsicum* are economically important in several countries. Fruits of *Capsicum* species are preferentially consumed fresh in the human diet and used in the preparation of sauces and dry seasonings (Kirschbaum-Titze al., 2002). Some species of the genus have medicinal and ornamental applications as well (Pickersgill, 1991; Bosland and Votava, 2000). Polymorphism for plant growth, flower color, and fruit form and color is large in *Capsicum* species (see Mosconeet al., 2007). Particularly in the cultivated peppers, fruit traits are highly variable within species owing to human selection (Pickersgill, 1988).

The *Capsicum* genus has 31 related species, among which *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz & Pav. are domesticated (Pickersgill, 1997; Moscone et al., 2007). *C. annuum* has high nutritional content, especially vitamins, and is the most cultivated species, represented by sweet pepper ("pimenta doce"), bell pepper ("pimentão"), and pimiento ("pimentão vermelho"). *C. baccatum* is represented by the peppers "dedo-de-moça" and "chapéu-de-frade", which are the most cultivated varieties in Brazil (Reifschneider, 2000). Most varieties of *C. chinense* are traditionally cultivated in northwestern Brazil, in which they are known as "pimenta-de-cheiro" and "pimenta-de-bode". The *C. frutescens* species known as "malagueta" is a highly pungent cultivar enjoyed in southwestern Brazil. *C. pubescens* is widely cultivated from Mexico to Peru, where it is grown in highlands in small family plots. It is grown on very limited acreage throughout the rest of the world (Bosland and Votava, 2000).

C. annuum, *C. chinense*, and *C. frutescens* have been considered closely related taxa according to karyotype features; these species are included in the *C. annuum* complex, in which poorly developed crossing barriers and great similarities with respect to morphology, isozymes, and DNA sequence suggest that they could be conspecific (Pickersgill, 1988, 1991; Park et al., 2000; Walsh and Hoot, 2001). In Brazil, various pepper species and their cultivars have been maintained in the germplasm collections of research institutes and universities. These collections have originated from traditional cultivars collected in various states and regions of Brazil among which the same genotype might be cultivated under different names, or different genotypes might be identified by the same name. The Federal University of Piauí in northwestern Brazil has a seed bank of peppers of the *Capsicum* genus [Banco Ativo de Germoplasma de *Capsicum* (BAGC)] that receives pepper samples that are often grown in community gardens in various regions and Brazilian states, mainly those in the northwest. The

main aim of the BAGC, which is located at the Federal University of Piauí, is to preserve and maintain the genetic diversity of *Capsicum* pepper accessions.

The seed bank collections of *Capsicum* represent quantitatively only a small fraction of the global biodiversity, but they are undoubtedly important as stocks of genetic diversity, and they represent a source of alleles for the genetic improvement of crops. Most samples of peppers that are deposited in the seed bank are identified only with the place and date of collection and the popular name or scientific name; many samples bear no additional specifications to ensure that an organization had approved proposals for the cultivation and breeding of the species. Therefore, the genetic characterization of the samples maintained in BAGC is important to confirm their genetic relationship. Genetic characterization of the samples allows the introduction of the accessions into an environment and guides the development of future cultural practices. The polymorphisms for morphological and agronomic traits of plant and fruit are extraordinarily important and frequently the main aim of selection programs (Monteiro et al., 2010). Therefore, molecular polymorphism is valuable information for the genetic characterization of *Capsicum* pepper accessions maintained at BAGC.

Protein electrophoresis studies (McLeod et al., 1983; Loaiza-Figueroa et al., 1989) in addition to DNA sequence analyses (Walsh and Hoot, 2001) and studies of restriction fragment length polymorphisms, polymerase chain reaction-amplified fragment length polymorphisms, randomly amplified polymorphic DNA, and polymorphic plastid DNA markers (Buso et al., 2001; Votava et al., 2005) have contributed to knowledge of the genetic diversity in the *Capsicum* genus and relationships among species. Polyacrylamide gel electrophoresis (PAGE) has been used to analyze α- and β-esterase genetic polymorphism in plants (Pereira et al., 2001; Carvalho et al., 2003; Orasmo et al., 2007; Frigo et al., 2009) and is also useful for analyses of the genetic diversity and structure of *Capsicum* accessions maintained at BAGC. Esterases are often found in multigene families (Robin et al., 1996), and a large number of esterase loci in plant tissues can be used simultaneously for the identification of genetic variation and polymorphism analyses. Esterase isozyme polymorphisms are co-dominant markers, which are useful for analyzing the genetic structure of plant populations. Thus, the goal of the current study was to use native PAGE to identify polymorphism in the α/β-esterase loci of the *Capsicum* pepper genus and analyze the genetic diversity and structure of *C. annuum*, *C. chinense*, and *C. baccatum* samples. Knowing the differences among these samples is important for the conservation of various genotypes as well as for carrying out crosses of these species in genetic breeding programs. Our hypothesis was that the polymorphisms of the α/β-esterase isozymes examined using a PAGE system represent adequate and promising markers with which to determine the differences, similarities, and identities of genotypes quickly.

MATERIAL AND METHODS

Capsicum samples

Seeds of 10 accessions were provided by Dr. Ângela C.A. Lopes (Federal University of Piauí, Brazil). The accessions belonged to BAGC. Selections were made from seeds of *C. chinense* (BAGC 06, BAGC 07, BAGC 23, and BAGC 24), *C. annuum* (BAGC 11, BAGC 36, BAGC 40, BAGC 59, and BAGC 67), and *C. baccatum* (BAGC 26; Table 1). The samples were collected in home gardens and community gardens and identified with the date and site

of collection and the scientific and common names. The seeds of 10 plants per accession were germinated in 128-cell trays using commercial substrates and maintained in an acclimatized chamber. After germination, seedlings (with 4-6 leaves) were transferred to pots containing soil and substrate (1:1) and maintained at a house garden (Department of Agronomy, State University of Maringá, Brazil). Figure 1 illustrates some *C. chinense*, *C. baccatum* var. *pendulum*, *C. annuum* var. *annuum*, and *C. annuum* var. *glabriusculum* plants used for electrophoresis analysis.

Table 1. Accessions belonging to Banco Ativo de Germoplasma de *Capsicum* (BAGC) for genetic diversity analysis.

Accession identifier	Common name	Species and variety	Site of collection (City / State)
BAGC 06	Murici	*C. chinense*	Teresina / PI
BAGC 07	Peito de moça	*C. chinense*	Piripiri / PI
BAGC 11	Peito de moça	*C. annuum* var. *glabriusculum*	Teresina / PI
BAGC 23	Olho de peixe	*C. chinense*	Teresina / PI
BAGC 24	Dedo de moça	*C. chinense*	Recife / PE
BAGC 26	Unknown	*C. baccatum* var. *pendulum*	Teresina / PI
BAGC 36	Pimenta de mesa	*C. annuum* var. *glabriusculum*	Teresina / PI
BAGC 40	Mexicana longa	*C. annuum* var. *annuum*	Teresina / PI
BAGC 59	Unknown	*C. annuum* var. *glabriusculum*	São Luis / MA
BAGC 67	Unknown	*C. annuum* var. *glabriusculum*	Água Branca / PI

PI = Piauí; PE = Pernambuco; MA = Maranhão.

Figure 1. Plants of *Capsicum chinense* (**a.** BAGC 23), *C. baccatum* var. *pendulum* (**b.** BAGC 26), *C. annuum* var. *annuum* (**c.** BAGC 40), and *C. annuum* var. *glabriusculum* (**d.** BAGC 36) used for electrophoresis analysis.

Electrophoresis

Samples consisting of leaves collected from 4-10 plants of each accession (a total of 85 plants) were evaluated using electrophoresis. Leaf fragments (7 mg) were individually homog-

enized with a glass rod in an Eppendorf microcentrifuge tube using a 70-mL extraction solution containing 1.0 M phosphate buffer, pH 7.0, with 5% polyvinylpyrrolidone-40 (Sigma), 1.0 mM ethylenediaminetetraacetic acid (Invitrogen), 0.5% β-mercaptoethanol, and 10% glycerol solution, and maintained in an ice chamber. After homogenization, the samples were centrifuged at 14,000 rpm (12,052 *g*) for 30 min at 4°C in a Jouan MR23*i*, and the supernatant was used for each sample.

Polyacrylamide gels (12%) were prepared using 0.375 M Tris-HCl, pH 8.8 (Pereira et al., 2001). A 6.2-mL volume of acrylamide/bis-acrylamide solution [30 g acrylamide and 0.8 g bis-acrylamide (Invitrogen) dissolved in 100 mL double-distilled water], 4.0 mL 1.5 M Tris-HCl, pH 8.0, 6.2 mL double-distilled water, 320 µL ammonium persulfate 2% (Sigma), and 16 µL tetramethylethylenediamine (Invitrogen) were used for the gel of separation. The stacking gel was prepared with 3.0 mL acrylamide/bis-acrylamide (5 g acrylamide and 0.25 g bis-acrylamide dissolved in 50 mL double-distilled water), 3.0 mL 0.24 M Tris-HCl, pH 6.8, 30 µL double-distilled water, 250 µL ammonium persulfate (2%), and 3 µL tetramethylethylenediamine. Electrophoresis was performed for 5 h at 4°C at a constant voltage of 200 V. The running buffer was 0.125 M Tris/0.0959 M glycine, pH 8.3.

The α/β-esterases were identified using staining techniques described by Pereira et al. (2001). Gels were soaked for 30 min in 50 mL 0.1 M sodium phosphate, pH 6.2, at room temperature. Esterase activity was visualized by placing the gels in a staining solution prepared with 50 mL sodium phosphate solution, 40 mg β-naphthyl acetate, 40 mg α-naphthyl acetate, 60 mg Fast Blue RR salt, and 5 mL N-propanol for 1 h. The staining solution was prepared using reagents, substrates, and dye (Sigma).

After being dried as described by Pereira et al. (2001), the polyacrylamide gels were kept at room temperature for 60 min in a mixture of 7.5% acetic acid and 10% glycerol embedded in 5% gelatin. They were then placed between two sheets of wet cellophane paper, stretched on an embroidering hoop and left to dry for 24 to 48 h.

Data analysis

Genetic variability in the *Capsicum* genus samples and in *C. annuum* and *C. chinense* was analyzed using the POPGENE 1.32 program (Yeh et al., 1999). The allele frequencies, percentage of polymorphic loci, chi-square test for deviation from Hardy-Weinberg equilibrium, observed and expected mean heterozygosity (H_O and H_E), mean number of alleles per locus, and effective number of alleles per polymorphic locus (N_E), were estimated for the esterase loci and 10 pepper accessions. The fixation index (F_{IS}) and Wright's (1965) *F*-statistic values (F_{IT} and F_{ST}) were also calculated for the esterase loci. The genetic identity (Nei, 1978) among *Capsicum* samples was calculated using the unweighted pair group method with arithmetic means.

RESULTS

Native PAGE analysis for esterase isozymes in the leaves of pepper plants from the *Capsicum* genus recorded using α-naphthyl acetate and β-naphthyl acetate indicated several α/β-esterases, but polymorphism was clearly detected in only 5 loci. The esterases produced from the *Est-4* locus were observed as strongly stained bands (Figure 2), and only 2 alleles were detected at the *Est-4* locus in the 85 pepper plants analyzed of *C. annuum* (5 accessions), *C. chinense* (4 accessions), and *C. baccatum* (1 accession) species. Three alleles each were

detected at the *Est-2*, *Est-3*, and *Est-5* loci, and 5 alleles for α/β-esterases were detected at the *Est-1* locus. A total of 16 alleles were found at the 5 α/β-esterase loci of the three *Capsicum* species. The less anodic α/β-esterases exhibited coincident migration in the electrophoresis gels, and the electrophoresis phenotypes could not be clearly determined. Only the polymorphisms at the *Est-1*, *Est-2*, *Est-3*, *Est-4*, and *Est-5* loci were analyzed, and allele frequencies were analyzed for these loci as well. Table 2 shows the number of alleles, N_E, and mean H_O and H_E in each of the accessions from *C. annuum*, *C. chinense*, and *C. baccatum*.

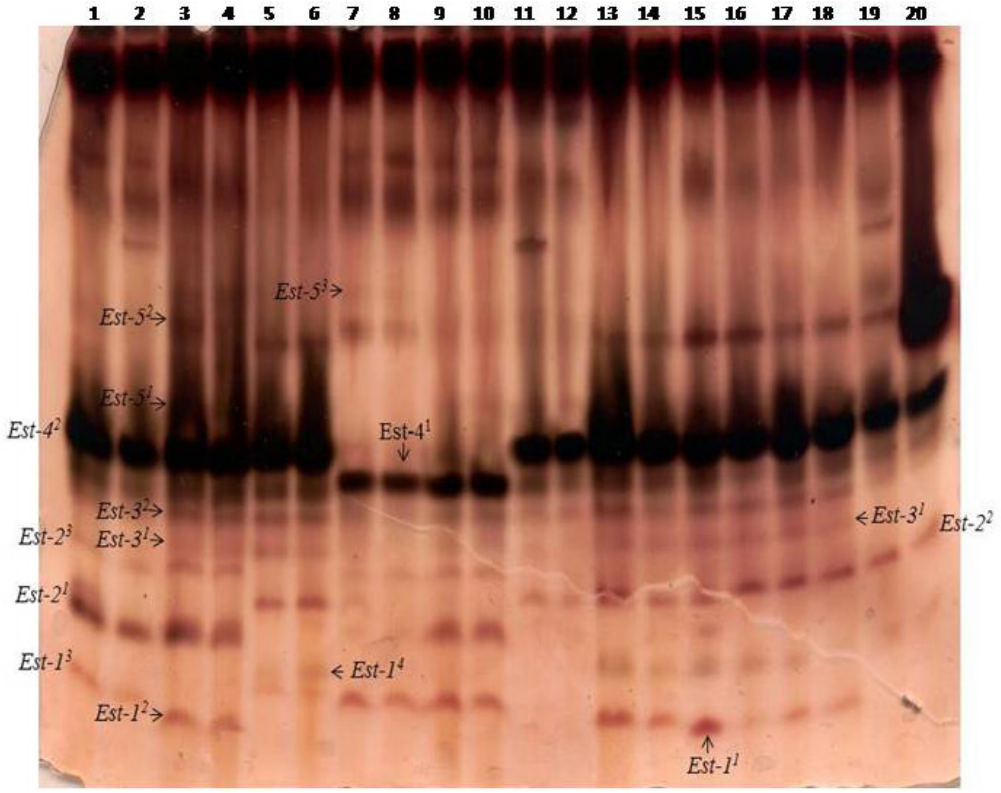

Figure 2. Polymorphism of α- and β-esterases detected in pepper plants from *Capsicum chinense* (BAGC 06: samples 1-2; BAGC 07: 3-4; BAGC 23: samples 7-8; BAGC 24: samples 9-10), *C. annuum* var. *glabriusculum* (BAGC 11: samples 5-6), *C. baccatum* var. *pendulum* (BAGC 26: samples 11-12), *C. annuum* var. *glabriusculum* (BAGC 36: samples 13-14; BAGC 59: samples 17-18; BAGC 67: samples 19-20), *C. annuum* var. *annuum* (BAGC 40: samples 15-16) accessions, produced from *Est-1*, *Est-2*, *Est-3*, *Est-4*, and *Est-5* loci; the different phenotypes are represented in lanes: *lane 1* = Est-$1^{3/3}$/Est-$2^{1/3}$/Est-$3^{2/2}$/Est-$4^{2/2}$/Est-$5^{1/2}$; *lane 2* = Est-$1^{3/3}$/Est-$2^{1/3}$/Est-$3^{2/2}$/Est-$4^{2/2}$/Est-$5^{1/1}$; *lane 3* = Est-$1^{2/2}$/Est-$2^{1/3}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{1/2}$; *lane 4* = Est-$1^{2/2}$/Est-$2^{1/3}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{1/2}$; *lane 5* = Est-$1^{3/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 6* = Est-$1^{3/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 7* = Est-$1^{3/3}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{1/1}$/Est-$5^{2/3}$; *lane 8* = Est-$1^{3/3}$/Est-$2^{1/3}$/Est-$3^{2/2}$/Est-$4^{1/1}$/Est-$5^{2/3}$; *lane 9* = Est-$1^{3/3}$/Est-$2^{1/3}$/Est-$3^{1/2}$/Est-$4^{1/1}$/Est-$5^{1/2}$; *lane 10* = Est-$1^{3/3}$/Est-$2^{1/3}$/Est-$3^{1/2}$/Est-$4^{1/1}$/Est-$5^{1/2}$; *lane 11* = Est-$1^{2/2}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{1/2}$; *lane 12* = Est-$1^{2/2}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{1/2}$; *lane 13* = Est-$1^{2/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 14* = Est-$1^{2/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 15* = Est-$1^{1/4}$/Est-$2^{1/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 16* = Est-$1^{2/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 17* = Est-$1^{2/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 18* = Est-$1^{2/4}$/Est-$2^{2/2}$/Est-$3^{1/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 19* = Est-$1^{4/4}$/Est-$2^{2/2}$/Est-$3^{2/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$; *lane 20* = Est-$1^{4/4}$/Est-$2^{2/2}$/Est-$3^{2/2}$/Est-$4^{2/2}$/Est-$5^{2/2}$.

Table 2. Number of alleles (N_A) per *Est-1*, *Est-2*, *Est-3*, and *Est-4* locus, effective number of alleles (N_E) per polymorphic locus, mean observed (H_O) and expected heterozygosities (H_E) in pepper plants from the 10 *Capsicum* accessions.

Accession	N	$N_{(pl)}$	N_A	N_E	H_O	H_E
BAGC 06 - *C. chinense*	10	3	1.6	1.58	0.5600	0.2960
BAGC 07 - *C. chinense*	10	2	1.4	1.18	0.1600	0.1280
BAGC 23 - *C. chinense*	6	4	1.8	1.39	0.3667	0.2361
BAGC 24 - *C. chinense*	6	2	1.4	1.38	0.3667	0.1972
Total (*C. chinense*)	32	5	2.4	1.82	0.3625	0.4395
BAGC 11 - *C. annuum* var. *glabriusculum*	10	1	1.2	1.20	0.2000	0.1000
BAGC 36 - *C. annuum* var. *glabriusculum*	10	2	1.4	1.25	0.2200	0.1470
BAGC 40 - *C. annuum* var. *annuum*	10	2	1.4	1.40	0.4000	0.2000
BAGC 59 - *C. annuum* var. *glabriusculum*	9	2	1.4	1.38	0.3556	0.1951
BAGC 67 - *C. annuum* var. *glabriusculum*	10	2	1.6	1.34	0.3200	0.2000
Total (*C. annuum*)	49	4	2.4	1.71	0.2980	0.3310
BAGC 26 - *C. baccatum pendulum*	10	2	1.2	1.20	0.2000	0.1000

N = number of plants analyzed; $N_{(pl)}$ = number of polymorphic loci.

The estimated H_O and H_E values in the *C. chinense* samples were higher than those detected in the *C. annuum* samples. In the *C. chinense* samples, the highest H_O and H_E values were 0.3625 and 0.4395, respectively, whereas in the *C. annuum* samples, H_O and H_E values were 0.2980 and 0.3310, respectively. The highest H_O (0.5600) and H_E (0.2960) values were recorded in the BAGC 06 sample of *C. chinense*. The lowest H_O value (0.16) was recorded in the BAGC 07 sample of *C. chinense*, and the lowest H_E value (0.10) was observed in the BAGC 11 sample of *C. annuum* var. *glabriusculum*.

Deviation from Hardy-Weinberg equilibrium was observed for the *Est-1*, *Est-2*, and *Est-3* loci in the *C. annuum* samples and for the *Est-1*, *Est-2*, *Est-3*, and *Est-4* loci in the *C. chinense* samples owing to an excess of heterozygous individuals (negative *F* values). The F_{IS} value was negative at all *Est-1*, *Est-2*, *Est-3*, *Est-4*, and *Est-5* loci in the *C. annuum* and *C. chinense* samples (Table 3). H_O was higher than H_E in all *C. annuum* and *C. chinense* samples (see Table 2), and therefore, a deficit of homozygous individuals occurred in all pepper accessions of the *Capsicum* genus analyzed in the present study, including the sole *C. baccatum* var. *pendulum* accession.

Table 3. Fixation coefficients (F_{IS}, F_{IT}, F_{ST}; Wright, 1965) in peppers of the *Capsicum chinense* and *C. annuum* accessions.

	F_{IS}	F_{IT}	F_{ST}
Capsicum chinense			
Est-1	-0.6259	-0.2522	0.2298
Est-2	-	1.0000	1.0000
Est-3	-0.7778	-0.6667	0.0625
Est-4	-0.0909	0.9165	0.9235
Est-5	-0.7872	-0.1468	0.3584
Total	-0.6978	0.1631	0.5070
Capsicum annuum			
Est-1	-0.8828	0.0101	0.4743
Est-2	-0.1765	0.8805	0.8984
Est-3	-0.8261	-0.5239	0.1655
Est-4	-	-	0.0000
Est-5	-0.1111	-0.0204	0.0816
Total	-0.7750	0.0978	0.4917

High genetic differentiation levels were detected in the samples of *C. chinense* (F_{ST} = 0.5070) and *C. annuum* (F_{ST} = 0.4917) accessions (see Table 3). The F_{ST} value detected in the *C. chinense* and *C. annuum* samples and *C. baccatum* accessions was 0.6321, indicating that 63.21% of the total variance in α/β-esterase allele frequencies in the three *Capsicum* species was due to genetic differences among samples. The genetic differentiation between *C. chinense* and *C. annuum* was high (F_{ST} = 0.1867), indicating that these species are genetically structured (F_{ST} > 0.15; Wright, 1978) for α/β-esterase isozymes.

In *C. chinense* samples, Nei's identity (*I*) values varied between 0.4202 (BAGC 07 and BAGC 23) and 1.0000 (BAGC 23 and BAGC 24; Table 4), showing that the BAGC 23 and BAGC 24 accessions are genetically identical for α/β-esterase polymorphism (allele frequencies) and indicating a broader genetic base for these samples. In *C. annuum* samples, *I* values varied between 0.5727 (BAGC 11 and BAGC 36) and 0.9297 (BAGC 59 and BAGC 67), showing that the BAGC59 and BAGC 67 samples had the highest level of genetic similarity and indicating a relatively lower genetic base in the *C. annuum* samples compared with that in the *C. chinense* samples. The *I* value between *C. chinense* and *C. annuum* was 0.7147.

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among accessions of *Capsicum chinense* (BAGC 06, BAGC 07, BAGC 23, BAGC 24), *C. annuum* (BAGC 11, BAGC 36, BAGC 40, BAGC 59, BAGC 67), and *C. baccatum* (BAGC 26).

Accession	BAGC 06	BAGC 07	BAGC 23	BAGC 24	BAGC 11	BAGC 36	BAGC 40	BAGC 59	BAGC 67	BAGC 26
BAGC 06	-	0.6325	0.7017	0.6723	0.6831	0.4317	0.4839	0.7759	0.6801	0.5566
BAGC 07	0.4580	-	0.4202	0.4414	0.4757	0.6823	0.8183	0.5040	0.4669	0.6569
BAGC 23	0.3543	0.8670	-	1.0017	0.5472	0.3000	0.3230	0.6549	0.5211	0.1419
BAGC 24	0.3970	0.8177	0.0017	-	0.5330	0.2922	0.3146	0.6217	0.5034	0.1579
BAGC 11	0.3812	0.7430	0.6030	0.6292	-	0.5727	0.6512	0.8265	0.9235	0.3343
BAGC 36	0.8400	0.3824	1.2040	1.2302	0.5573	-	0.8885	0.7552	0.7205	0.5384
BAGC 40	0.7260	0.2005	1.1301	1.1563	0.4289	0.1182	-	0.6412	0.7045	0.5328
BAGC 59	0.2538	0.6852	0.4232	0.4753	0.1906	0.2808	0.4444	-	0.9297	0.3542
BAGC 67	0.3855	0.7616	0.6518	0.6863	0.0796	0.3277	0.3502	0.0728	-	0.3552
BAGC 26	0.5860	0.4203	1.9529	1.8456	1.0958	0.6192	0.6296	1.0379	1.0351	-

Results from cluster analysis using the unweighted pair group method with arithmetic means separated 85 samples into 4 groups (Figure 3). The first group comprised the BAGC 06 (*C. chinense*) accession and BAGC 11, BAGC 59, and BAGC 67 accessions (*C. annuum* var. *glabriusculum*). The second group was composed of the samples BAGC 23 and BAGC 24 (*C. chinense*). The third group combined the BAGC 07 accession (*C. chinense*), BAGC 36 accession (*C. annuum* var. *glabriusculum*), and BAGC 40 accession (*C. annuum* var. *annuum*). The fourth group consisted of the BAGC 26 accession (*C. baccatum* var. *pendulum*). Among the 10 accessions of *Capsicum* species, the highest level of genetic differentiation was observed in the *C. baccatum* var. *pendulum* sample. *I* values ranged from 0.1419 (BAGC 26 and BAGC 23) to 1.000 (BAGC 23 and BAGC 24). The geographically distant accessions (BAGC 23 from Teresina, Piauí, and BAGC 24 from Recife, Pernambuco; 1.137 km apart; see Table 1) showed the highest genetic identity.

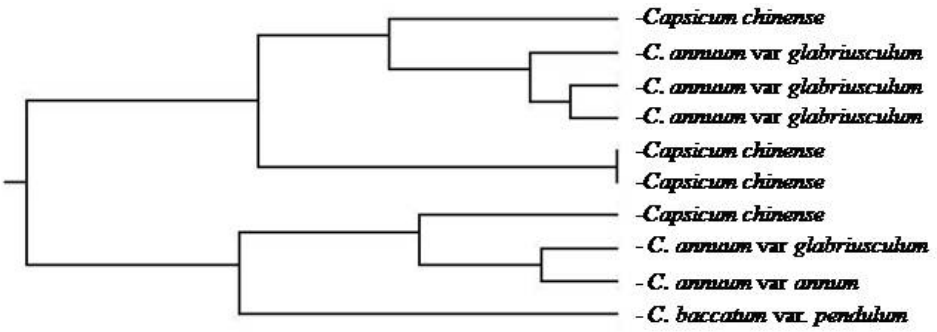

Figure 3. Dendrogram showing relationships of the 10 accessions including *Capsicum chinense*, *C. annuum*, and *C. baccatum*, based on UPGMA cluster analysis of the allele polymorphism at *Est-1*, *Est-2*, *Est-3*, *Est-4*, and *Est-5* loci, by the Jaccard similarity coefficient.

DISCUSSION

Our results confirmed a previous hypothesis that polymorphisms of the α/β-esterase isozymes examined using PAGE represent adequate and promising markers for analyzing the genetic differentiation of *Capsicum* samples maintained in germplasm banks such as the BAGC. Five polymorphic loci for the examined isoesterases were simultaneously evident in the leaves of peppers using only 1 enzymatic system. Because analyses using different enzymatic systems generally incur greater cost and time investments, α/β-esterase polymorphisms determined using PAGE are promising markers that may be useful in further studies to detect genetic diversity more quickly in other pepper accessions. Esterase isozymes have been described by Barrera et al. (2005) as the most polymorphic isozymes in *Capsicum* species (*C. chinense*, *C. frutescens*, *C. annuum*, and *C. baccatum*).

A high level of genetic divergence has been reported in various accessions of *Capsicum* species maintained at BAGC based on morphological and agronomic markers (color, form, measures of plant parts, flowers, and fruits, number of stems) (Monteiro et al., 2010). The results of α/β-esterase polymorphism investigation using PAGE in our study indicated that the genetic diversity of the *C. chinense*, *C. annuum*, and *C. baccatum* accessions is also characterized by high mean values for the proportion of polymorphic loci and the observed and expected proportion of heterozygous plants. The proportion of polymorphic loci and N_E were highest in the *C. chinense* samples, and the H_O and H_E proportions of heterozygous plants in this sample were also higher than those estimated in the *C. annuum* and *C. baccatum* samples. The highest genetic divergence based on morphological and agronomic traits was detected in the *C. chinense* accessions (Monteiro et al., 2010).

The negative F_{IS} values observed in the *C. chinense* and *C. annuum* samples indicating an excess of heterozygous plants contrasted with the expected moderate to high level of endogamy in *Capsicum* species. Self-compatibility has been described as the rule in the *Capsicum* genus (Onus and Pickersgill, 2004). However, the α/β-esterase phenotype patterns found in our study showed a deficit of homozygous plants or an excess of heterozygous plants. An excess of heterozygous plants ($H_O > H_E$) was found in the BAGC 06, BAGC 07, BAGC 23, and BAGC 24 samples of *C. chinense*; in the BAGC 11, BAGC 36, BAGC 40, BAGC

59, and BAGC 67 samples of *C. annuum*; and in the BAGC 26 accession of *C. baccatum*. Increased heterozygosity in the *C. chinense* and *C. annuum* accessions is important because it leads to an increase in the fitness of inbred individuals at loci for which heterozygous specimens have a relative advantage over homozygous specimens (Allendorf and Luikart, 2007). High heterozygosity indicates that a plant population likely includes a substantial amount of adaptive genetic variation, thus allowing it to escape the effects of a control agent. Therefore, polymorphism of α/β-esterases in the samples of seeds from the germplasm bank revealed that the accessions BAGC 06 of *C. chinense*, BAGC 40 and BAGC 67 of *C. annuum* var. *annuum*, and *C. annuum* var. *glabriusculum* are the samples most indicated for cultivation in various geographical regions (with differential climatic conditions, soil types, and pluvial precipitation). Seeds of BAGC 06, BAGC 40, and BAGC 67 samples showing high heterozygosity are also indicated for selection procedures.

No specific relationship of α/β-esterase phenotypes and morphological and agronomic traits was investigated in the present study; however, the selection pressure used for morphological and agronomic traits of plant and fruit in the *C. chinense* and *C. annuum* species might also select heterozygous phenotypes in α/β-esterase loci. Most esterase isozymes in plant tissues have been classified as carboxylesterases (Pereira et al., 2001; Carvalho et al., 2003; Orasmo et al., 2007). The carboxylesterase activity in plants has been correlated with differentiation processes (Gahan et al., 1983; Melati et al., 1996), and different physiological roles have recently been attributed to these compounds. Stuhlfelder et al. (2002) have proposed that carboxylesterases might have a role in plant signaling pathways. The possible substrates for carboxylesterases in plants include esters produced by plants to attract pollinators and deter herbivores (Pichersky and Gershenzon, 2002). The association of the roles of carboxylesterases in plant-pathogen interactions and programmed cell death suppression with the hypersensitive response during pathogen attack has been reviewed by Marshall et al. (2003). Thus, the various physiological roles attributed to carboxylesterases can be used to justify esterase loci as targets in artificial selection processes. In artificially selected plants of *Stevia rebaudiana*, specific heterozygous phenotypes at *Est-2* and *Est-4* loci have been observed in taller plants and plants with precocious flowering, respectively (Carvalho et al., 2011). These associations demonstrate that selection procedures for specific characteristics (e.g., height of plants and time of flowering) may lead to a fixation of alleles and α/β-esterase phenotype patterns in *Stevia* populations.

The differential allele frequencies and proportions of heterozygous loci found in the pepper accessions indicated that genetic divergence had occurred among the 4 *C. chinense* samples (F_{ST} = 0.5070) and among the 5 *C. annuum* samples (F_{ST} = 0.4917). According to Wright (1978), F_{ST} >0.25 indicate a very high interpopulational genetic divergence level. The observed establishment of isolation and structuring mechanisms in the *C. chinense* and *C. annuum* species can be justified by self-compatibility systems and as a consequence of frequent disturbances such as selection for traits such as multiple resistance genes and for the specific components of quality, such as degree of pungency, flavor, color, and pericarp thickness preferred by consumers; the selection pressure used in conventional *Capsicum* pepper management has resulted in highly structured populations within *C. chinense* and *C. annuum* species.

The α/β-esterase phenotype patterns found in *C. chinense* and *C. annuum* also revealed a high divergence level between these species (F_{ST} = 0.1867), unlike that expected according to previous reports in the literature. *C. annuum* and *C. chinense* (as well as *C.*

frutescens) have been described as closely related taxa. These 3 species have been included in the *C. annuum* complex, whereas poorly developed crossing barriers and great similarities with respect to morphology, karyotype features, isozymes, and DNA sequences suggest that they could be conspecific (Pickersgill, 1988, 1991; Park et al., 2000; Walsh and Hoot, 2001). Studies based on enzyme electrophoresis have indicated that this *C. annuum* complex forms an allozymically indistinguishable group of populations (McLeod et al., 1983; Barrera et al., 2005). However, in such studies, the data from zymograms have been entered as a matrix of presence/absence of isozymes for each enzyme system. The isozymes analyzed were treated as dominant markers, whereas the allele frequencies and homozygous and heterozygous plants were not detected. The isozymes were not useful for analyzing the genetic structure of the *Capsicum* plant populations. Conversely, the gene diversity estimates from 186 accessions of *Capsicum* from Mexico (76 alleles representing 20 genetic loci coding for 9 enzyme systems) suggested significant genetic differentiation, mainly among populations from different geographical regions (Loaiza-Figueroa et al., 1989). The BAGC accessions most geographically distant were not the most genetically divergent; however, significant genetic differentiation was observed among the *C. chinense* and *C. annuum* samples in our study, similar to that described by Loaiza-Figueroa et al. (1989) through isozyme analysis.

The level of genetic divergence in the *C. chinense* and *C. annuum* accessions revealed through *I* values (0.4202-1.0000 and 0.5727-0.9297, respectively) indicated a broader genetic base for these *Capsicum* species. In practical terms, this result represents an important finding for both species because the most divergent genotypes may be used to produce superior hybrids in breeding programs based on the assumption that the highest molecular dissimilarity in the genotypes may represent greater heterozygosity and consequently a greater probability of generating superior hybrids (Serafiniet al., 2001).

The *I* value between *C. chinense* and *C. annuum* was 0.7147. This low identity value may also be explained as a consequence of the different origins of the examined *C. chinense* and *C. annuum* samples. The cultivation of *C. annuum* has been postulated to have occurred first in Mexico, which is the major center of diversity for this species and in which archeological remains from approximately 7000 B.C. have been uncovered (Pickersgill, 1969). Support for this hypothesis has been found in cytogenetic data published by Pickersgill (1971). Patterns of genetic variation obtained through enzyme electrophoretic studies have also suggested a primary center of domestication in eastern Mexico and a second center in western Mexico (Loaiza-Figueroa et al., 1989). With respect to *C. chinense*, domestication reportedly began in the lowland Amazon basin, which is the area of greatest diversity (International Board for Plant Genetic Resources - IBPGR, 1983).

Our results enable us to suggest the use of α/β-esterase isozymes determined using PAGE for the analysis of the genetic structure and diversity among and within *Capsicum* species. The esterase analysis carried out in the current study showed that high genetic diversity exists within *C. chinense* and *C. annuum* samples and among 3 *Capsicum* species. Understanding the genetic structure of these pepper accessions is important and, in the near future, may lead to new management strategies for the culture and maintenance of various cultivars. In fact, knowledge of genetic diversity may be important for guiding management practices and for the development of more effective cultivation methods in these species. For example, future cultivation or control of pests in peppers of the *Capsicum* species showing a higher level of mean heterozygosity may require different types and concentrations of nutrients or

pesticides. Additionally, producing peppers from *C. chinense* and *C. annuum* accessions exhibiting high heterozygosity may be the goal of continuous medium- and long-term selection to generate inbred lines and for the future production of new cultivars in breeding programs. The α/β-esterase phenotype patterns obtained herein may be applied to the monitoring of genetic stability in selected populations for specific traits of interest.

REFERENCES

Allendorf FW and Luikart G (2007). Conservation and the Genetics of Populations. Edition Blackwell Publishing, Maden.

Barrera LQ, Garcia MC, Giraldo MC and Melgarejo LM (2005). Caracterizacíon por isoenzimas de accesiones de *Capsicum* pertenecientes a la colección Amazonica colombiana. *Rev. Colomb. Biotecnol.* 2: 59-65.

Bosland PW and Votava EJ (2000). Peppers: Vegetable and Species *Capsicum*. 12th edn. CABI Publishing, Oxon.

Buso GSC, Lourenço RT, Bianchetti LB, Lins TCL, et al. (2001). Espécies Silvestres do Gênero *Capsicum* Coletadas na Mata Atlântica Brasileira e sua Relação Genética com Espécies Cultivadas de Pimenta: Uma Primeira Abordagem Genética Utilizando Marcadores Moleculares. Embrapa Recursos Genéticos e Biotecnologia, Brasília.

Carvalho VM, Marques RM, Lapenta AS and Machado MFPS (2003). Functional classification of esterases from leaves of *Aspidosperma polyneuron* M. Arg. (Apocynaceae). *Genet. Mol. Biol.* 26: 195-198.

Carvalho VM, Marochio CA, Mangolin CA and Machado MFPS (2011). The use of esterase polymorphism for analysis of the genetic diversity and structure of stevia (*Stevia rebaudiana* Bert. Bertoni) populations. *Biochem. Syst. Ecol.* 39: 594-599.

Frigo MJ, Mangolin CA, Oliveira RS Jr and Machado MFPS (2009). Esterase polymorphism for analysis of genetic diversity and structure of wild poinsettia (*Euphorbia heterophylla*) populations. *Weed Sci.* 57: 54-60.

Gahan PB, Rana MA and Phillips R (1983). Activation of carboxylesterases in root cortical parenchymal cells of *Pisum sativum* during xylem induction *in vitro*. *Cell Biochem. Funct.* 1: 109-111.

International Board for Plant Genetic Resources (IBPGR) (1983). Genetic Resources of *Capsicum*, a Global Plan of Action. IBPGR Executive Secretariat, Rome.

Kirschbaum-Titze P, Hiepler C, Mueller-Seitz E and Petz M (2002). Pungency in paprika (*Capsicum annuum*). 1. Decrease of capsaicinoid content following cellular disruption. *J. Agric. Food Chem.* 50: 1260-1263.

Loaiza-Figueroa F, Ritland K, Labor de Cancino JA and Tanksley SD (1989). Patterns of genetic variation of the genus *Capsicum* (Solanaceae) in Mexico. *Plant Syst. Evol.* 165: 159-188.

Marshall SD, Putterill JJ, Plummer KM and Newcomb RD (2003). The carboxylesterase gene family from *Arabidopsis thaliana*. *J. Mol. Evol.* 57: 487-500.

McLeod MJ, Guttman SI, Eshbaugh WH and Rayle RE (1983). An electrophoretic study of evolution in *Capsicum* (Solanaceae). *Evolution* 37: 562-574.

Melati MR, Scialabba A and Bellani ML (1996). Carboxylestrase activity in the carrot seed and seedling. *Eur. J. Histochem.* 40: 159-166.

Monteiro ER, Bastos EM, Lopes ACA, Gomes RLF, et al. (2010). Diversidade genética entre acessos de espécies cultivadas de pimentas. *Cienc. Rural* 40: 288-283.

Moscone EA, Scaldaferro MA, Grabiele M, Cecchini NM, et al. (2007). The evolution of Chili Peppers (*Capsicum* - Solanaceae): a cytogenetic perspective. *Acta Hortic.* 745: 137-170.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Onus AN and Pickersgill B (2000). A study of selected isozymes in *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii* and two interespecific F_1 hybrids in *Capsicum* species. *Turk. J. Bot.* 24: 311-318.

Orasmo GR, Oliveira-Collet SA, Lapenta AS and de Fatima PSM (2007). Biochemical and genetic polymorphisms for carboxylesterase and acetylesterase in grape clones of *Vitis vinifera* L. (Vitaceae) cultivars. *Biochem. Genet.* 45: 663-670.

Park YK, Park KC, Park CH and Kim NS (2000). Chromosomal localization and sequence variation of 5S rRNA gene in five *Capsicum* species. *Mol. Cells* 10: 18-24.

Pereira AJ, Lapenta AS, Vidigal-Filho PS and Machado MF (2001). Differential esterase expression in leaves of *Manihot esculenta* Crantz infected with *Xanthomonas axonopodis* pv. *manihotis*. *Biochem. Genet.* 39: 289-296.

Pichersky E and Gershenzon J (2002). The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr. Opin. Plant Biol.* 5: 237-243.

Pickersgill B (1969). The Domestication of Chili Peppers. In: The Domestication and Exploitation of Plants and Animals

(Ucko PJ and Dimbleby GW, eds.). Duckworth, London, 443-450.
Pickersgill B (1971). Relationships between weedy and cultivated forms in some species of chili peppers (genus *Capsicum*). *Evolution* 25: 683-691.
Pickersgill B (1988). The genus *Capsicum*: a multidisciplinary approach to the taxonomy of cultivated and wild plants. *Biol. Zentralbl.* 107: 381-389.
Pickersgill B (1991). Cytogenetics and Evolution of *Capsicum* L. In: Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part B (Tsuchiya T and Gupta PK, eds.). Elsevier, Amsterdam, 139-160.
Pickersgill B (1997). Genetic resources and breeding of *Capsicum* spp. *Euphytica* 96: 129-133.
Reifschneider FJB (2000). *Capsicum*: Pimentas e Pimentões do Brasil. Embrapa Hortaliças, Brasília.
Robin C, Russell RJ, Medveczky KM and Oakeshott JG (1996). Duplication and divergence of the genes of the α-esterase cluster of *Drosophila melanogaster*. *J. Mol. Evol.* 43: 241-252.
Serafini LA, Fungaro MHP and Vieira MLC (2001). Marcadores Moleculares. In: Biotecnologia na Agricultura e na Agroindústria (Serafini LA, Barros MN and Azevedo JL, eds.). Livraria e Editora Agropecuária Ltda., Guaíba, 153-1999.
Stuhlfelder C, Lottspeich F and Mueller MJ (2002). Purification and partial amino acid sequences of an esterase from tomato. *Phytochemistry* 60: 233-240.
Votava EJ, Baral JB and Bosland PW (2005). Genetic diversity of Chile (*Capsicum annuum* var. *annuum* L.) landraces from northern New Mexico, Colorado, and Mexico. *Econ. Bot.* 59: 8-17.
Walsh BM and Hoot SB (2001). Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast *atpB-rbcL* spacer region and nuclear *waxy* introns. *Int. J. Plant Sci.* 162: 1409-1418.
Wright S (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395-420.
Wright S (1978). Evolution and the Genetics of Populations 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago.
Yeh FC, Yang R and Boyle T (1999). Popgene Version 1.32: Microsoft Windows-Based Freeware for Population Genetic Analysis. Centre for International Forestry Research, University of Alberta, Calgary.