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Genetic diversity among *Capsicum* accessions using RAPD markers

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ABSTRACT - *The genus* Capsicum *has 27 species, being five domesticated and 22 semi-domesticated and wild ones. For an enhanced use of the germplasm in breeding programs it is necessary to know the accessions in the collections, to identify them and evaluate the genetic diversity. Objectives of this research were to quantify the genetic diversity among 70* Capsicum *accessions, confirm their identification and verified possible duplicated accessions, using RAPD markers. Results showed that there is genetic diversity within and among species and were in agreement with botanical and morpho-agronomic classifications. The identification of 53 accessions was confirmed (16* C. annuum*, 7* C. frutescens*, 14* C. baccatum *and 16* C. chinense*) and among botanically non-identified accessions results suggested that six belong to* C. baccatum *and five to* C. chinens*e. The most distinct accession was UENF 1620, probably a wild species that is not represented in the collection yet.*

Key words: *Capsicum* spp., genetic diversity, multivariate techniques, RAPD markers.

INTRODUCTION

The genus *Capsicum* has a wide genetic diversity, composed by 27 species, being five domesticated and 22 semi-domesticated and wild ones (Reifschneider 2000). The taxonomy of the genus is confusing, and sometimes it is difficult to identify an accession using only subjective morpho-agronomic data.

Peppers are used worldwide as spices, condiments and vegetables. Furthermore, the genus has medical and ornamental uses. *Capsicum* is considered a self-pollinating crop (Allard 1971). However, it has an out-crossing rate and should be considered a facultative cross-pollinating plant (Tanksley 1984). Chromosome data recorded the universal presence of diploid complements in the genus and two basic chromosome numbers, x = 12 and x = 13, the latter restricted to a few wild species (Moscone et al. 1993).

Brazil is considered an origin and diversity center of many species of the *Capsicum* genus. However, little is known about its wild species, mostly found in Atlantic Forest areas and in the Amazon region (Embrapa 2002). Collections of pepper germplasm serve as repositories of useful genes. The understanding and preservation of the genetic diversity of *Capsicum* germplasm is therefore relevant for breeding purposes.

To use genetic resources adequately, it is necessary to understand how the genetic variation is distributed and which environmental and species characteristics influence this distribution (Vilela-Morales et al. 1997). These traits can be identified in gene pool conserved *in situ* and in *ex situ* germplasm collections through different techniques such as molecular markers. The molecular markers, such as RAPD (Randomly Amplified Polymorphic DNA), do not

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depend on the environmental conditions and are present in all plant parts (Rom et al. 1995). They can identify a great number of polymorphisms that allow the distinction among accessions and the identification of possible duplicates (Bastianel et al. 1998). Simplicity, cheapness and quick results are the main advantages of RAPD and make it efficient to analyze the genetic diversity in germplasm collections. The technique has been successfully used to distinguish accessions, to evaluate genetic diversity among them and to recognize duplications in germplasm collections (Waycott and Fort 1994, Virk et al. 1995, Daher et al. 2002, Teixeira-Cabral et al. 2002, Picoli et al. 2004, Palomino et al. 2005). In *Capsicum*, numerous studies using RAPD have identified genes of agronomic interest and helped construct linkage maps or studies on genetic diversity and phylogeny, among others (Prince et al. 1995, Vazquez et al. 1996, Livingstone et al. 1999, Rodriguez et al. 1999, Engle et al. 2001, Ilbi 2003).

Objectives of this research were to estimate the genetic diversity of 70 *Capsicum* accessions, to confirm their identifications and verify possible duplicates using RAPD markers.

MATERIAL AND METHODS

Seventy accessions of *Capsicum* were evaluated in this study (Table 1). Part of them had previously been botanically identified, characterized and evaluated by Sudré et al. (2005), which correspond to numbers 1 through 53, while the others (54 through 70) were not botanically identified yet.

Young leaves from 10 to 15 plants per accession were harvested for DNA extraction, according to the protocol of Doyle and Doyle (1987). The amplification reactions were performed according to Williams et al. (1990) and modified to a final volume of 25 µL. To detect molecular polymorphisms among and within accessions, eight out of 20 primers were tested and used for the amplification of polymorphic loci, which were: OPAW-02, OPR-06, OPAX-08, OPAW-15, OPV-05, OPR-19, OPAV-06, and OPAU-08 (Operon Technologies). The amplification products (bands) were separated in 1.2% agarose gel, stained with ethidium bromide and visualized under ultraviolet light using an Eagle Eye II image system.

The presence or absence of RAPD band was scored as "1" or "0", respectively. One hundred out of 112 bands were polymorphic. The data matrix of the RAPD scores was generated and dissimilarity coefficients were calculated using Jaccard's arithmetic complement index on software Genes. The dendrogram was constructed using UPGMA cluster algorithm from the Statistica software.

RESULTS AND DISCUSSION

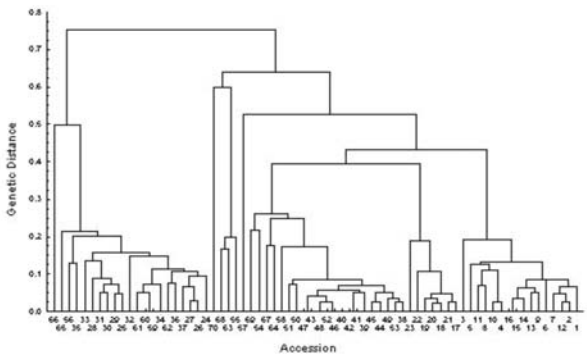

Figure 1. UPGMA dendrogram based on Jaccard's dissimilarity index of 70 *Capsicum* spp. Accessions 1 to 70 are coded in Table 1

Cluster I grouped the accessions from *C. baccatum* var. *pendulum* and var. *baccatum*. Morphologically different from the other domesticated species, *C. baccatum* has white flowers with diffuse spots at the base of the corolla, while the others do not have such spots (IBPGR 1983, IPGRI 1995). In relation to cross compatibility, *C. baccatum* only generated partially fertile hybrids when crossed with *C. praetermissum* (Pickersgill 1991, Zijlstra et al. 1991) and/or with *C. tovarii* (Tong and Bosland 1999). Six non-identified accessions (numbers 56, 59, 60, 61, 62 and 65) were also grouped in this cluster. Considering that these six accessions have flowers with yellow spots on a white corolla, an intrinsic trait to this species (IBPGR 1983, IPGRI 1995), we suggest that they belong to *C. baccatum* var. *pendulum*. The varieties *pendulum* and *baccatum* were not distinguished. The distinction might not have been possible because of the small number of polymorphic bands observed in the species, as shown in Table 2, or because of the molecular technique used. According to Ferreira and Grattapaglia (1998), RAPD markers amplify DNA random sequences, so when two species (or varieties, in this case) are very similar, with similar genotypes, the small differences in the genome may not be identified in sufficient quantity to individualize them at variety level. Morphologically, the main difference between

Table 1. *Capsicum* spp. accessions and their provenances

DNA sample number	Accession	Species	Provenance
01	UENF 1381	*C. annuum* var. *annuum*	PESAGRO/Itaguaí
02	UENF 1382	*C. annuum* var. *annuum*	PESAGRO/Itaguaí
03	UENF 1420	*C. annuum* var. *annuum*	AGROCERES
04	UENF 1421	*C. annuum* var. *annuum*	AGROCERES
05	UENF 1422	*C. annuum* var. *annuum*	TOPSEED
06	UENF 1423	*C. annuum* var. *annuum*	Aracaju, SE
07	UENF 1502	*C. annuum* var. *annuum*	México
08	UENF 1503	*C. annuum* var. *annuum*	México
09	UENF 1562	*C. annuum* var. *annuum*	Viçosa, MG
10	UENF 1565	*C. annuum* var. *annuum*	Viçosa, MG
11	UENF 1567	*C. annuum* var. *annuum*	Viçosa, MG
12	UENF 1569	*C. annuum* var. *annuum*	Viçosa, MG
13	UENF 1575	*C. annuum* var. *annuum*	Campos, RJ
14	UENF 1578	*C. annuum* var. *annuum*	México
15	UENF 1559	*C. annuum* var. *glabriusculum*	Cachoeiras de Macacu, RJ
16	UENF 1559	*C.annuum* var. *glabriusculum*	Cachoeiras de Macacu, RJ
17	UENF 1425	*C. frutescens*	Campos, RJ
18	UENF 1491	*C. frutescens*	RJ
19	UENF 1557	*C.frutescens*	Goiânia, GO
20	UENF 1560	*C.frutescens*	Cachoeiras de Macacu, RJ
21	UENF 1561	*C.frutescens*	Campos, RJ
22	UENF 1587	*C.frutescens*	Parintins, AM
23	UENF 1588	*C.frutescens*	Parintins, AM
24	UENF 1417	*C. baccatum* var. *pendulum*	UFLA/MG
25	UENF 1426	*C. baccatum* var. *pendulum*	Campos, RJ
26	UENF 1489	*C. baccatum* var. *pendulum*	RJ
27	UENF 1490	*C. baccatum* var. *pendulum*	RJ
28	UENF 1492	*C. baccatum* var. *pendulum*	RJ
29	UENF 1494	*C. baccatum* var. *pendulum*	RJ
30	UENF 1496	*C. baccatum* var. *pendulum*	RJ
31	UENF 1499	*C. baccatum* var. *pendulum*	RJ
32	UENF 1500	*C. baccatum* var. *pendulum*	RJ
33	UENF 1501	*C.baccatum* var. *pendulum*	RJ
34	UENF 1556	*C. baccatum* var. *pendulum*	Goiânia, GO
35	UENF 1573	*C. baccatum* var. *pendulum*	Duas Barras, RJ
36	UENF 1495	*C. baccatum* var. *baccatum*	RJ
37	UENF 1584	*C. baccatum* var. *baccatum*	Rio das Ostras, RJ
38	UENF 1418	*C. chinense*	UFLA/MG
39	UENF 1419	*C. chinense*	UFLA/MG
40	UENF 1424	*C. chinense*	Campos, RJ
41	UENF 1497	*C. chinense*	RJ
42	UENF 1498	*C. chinense*	RJ
43	UENF 1551	*C. chinense*	Goiânia, GO
44	UENF 1553	*C. chinense*	Goiânia, GO
45	UENF 1554	*C. chinense*	Goiânia, GO
46	UENF 1555	*C. chinense*	Goiânia, GO
47	UENF 1558	*C. chinense*	Campos, RJ
48	UENF 1570	*C. chinense*	PA
49	UENF 1571	*C. chinense*	Aracaju, SE
50	UENF 1572	*C. chinense*	Aracaju, SE
51	UENF 1577	*C. chinense*	Goiânia, GO
52	UENF 1585	*C. chinense*	Parintins, AM
53	UENF 1586	*C. chinense*	Parintins, AM
54	UENF 1605	*Capsicum* sp.	UFV, Viçosa, MG
55	UENF 1606	*Capsicum* sp.	UFV, Viçosa, MG
56	UENF 1607	*Capsicum* sp.	UFV, Viçosa, MG
57	UENF 1608	*Capsicum* sp.	UFV, Viçosa, MG
58	UENF 1609	*Capsicum* sp.	UFV, Viçosa, MG
59	UENF 1610	*Capsicum* sp.	UFV, Viçosa, MG
60	UENF 1611	*Capsicum* sp.	UFV, Viçosa, MG
61	UENF 1612	*Capsicum* sp.	UFV, Viçosa, MG
62	UENF 1613	*Capsicum* sp.	UFV, Viçosa, MG
63	UENF 1614	*Capsicum* sp.	UFV, Viçosa, MG
64	UENF 1615	*Capsicum* sp.	UFV, Viçosa, MG
65	UENF 1616	*Capsicum* sp.	UFV, Viçosa, MG
66	UENF 1617	*Capsicum* sp.	UFV, Viçosa, MG
67	UENF 1618	*Capsicum* sp.	UFV, Viçosa, MG
68	UENF 1619	*Capsicum* sp.	UFV, Viçosa, MG
69	UENF 1621	*Capsicum* sp.	UFV, Viçosa, MG
70	UENF 1620	*Capsicum* sp.	UFV, Viçosa, MG

two varieties is the corolla spot color, which is yellowish in the *pendulum* and greenish in the *baccatum*, and the number of flowers per node, one in *pendulum* and two or three in *baccatum* (IBPGR 1983). Although accession 66 was grouped in this cluster, it was not morphologically identified as *C. baccatum* species.

Cluster II grouped *C. annuum, C. frutescens* and *C. chinense* accessions and ten other non-identified accessions. Even though they were grouped together, they formed subgroups, corresponding to these three species. The accessions 69, 54, 67, 64, and 58 were closer to the *C. chinense* subgroup and probably belong to this species. According to Pickersgill (1991), *C. annuum*, *C. chinense* and *C. frutescens* may be grouped in a single complex, called *C. annuum* complex. In this complex, all accessions present white or white-greenish flowers and are cross compatible. The closest species are *C. chinense* and *C. frutescens*. This is confirmed in IPGRI (1995), where the main difference is described as an annular constriction observed on the *C. chinense* calyx.

Although accessions number 15 and 16 had been taken from the same genotype (UENF 1559), they were considered different because of the morphological variations in the flower in field observations. Accession number 15 had completely purple flowers and number 16 had white flowers with a thin purple contour. However, by molecular analysis, there were no differences between them. This might have been the case due to small degree of intraspecific polymorphism found (Table 2) or due to technical limitation. According to Williams et al. (1990), the heterozygous genotypes cannot be differentiated from the homozygote using RAPD; the presence of bands in the gel may correspond to these two different forms (homo and heterozygote).

Another subgroup was formed by three non-identified accessions (55, 63 and 68). It is possible that they belong to *C. praetermissum* species, based on flower morphology. Their flowers have green spots on a white corolla and a large purple board around the petals (IPGRI 1995). Although accession 70 was closer to this little group, it was the most divergent genotype in this analysis. It is possible that this accession does not belong to any species studied here and it is probably a wild *Capsicum* species. Its flowers are white with yellow anthers and very different from all others we evaluated.

Duplicates

According to cluster analysis, the accessions number 41 and 42, 43 and 47, 46, 52 and 40, and 59 and 60 were considered similar. Barring accessions 59 and 60, all the others were classified by Sudré et al. (2005) as genetically dissimilar, based on 15 qualitative and 11 quantitative traits. The qualitative characteristics are subjective and hard to score while quantitative ones are influenced by the environment. On these grounds, they cannot be confirmed as different accessions. Considering furthermore the molecular results established in this study, one could suppose that these accessions are duplicates of the same genotype. However, in a more in-depth molecular analysis, where polymorphism is not only evaluated among but also within species, the number of polymorphic bands drops (Table 2) compared to the initial 100 bands, which invalidates the duplicate hypothesis. So, it would be appropriate to obtain a greater number of interspecific marks or to use another methodology to come to conclusions about the question. We suppose that the case of accessions 59 and 60 is similar to the one previously described; more research is necessary to reach a conclusion about the similarity of these accessions.

Table 2. Interspecific molecular analysis of 53 previously botanically identified accessions of the UENF collection

Species	Nr. of Accessions	Total markers	Polymorphic markers
C. annuum	16	41	18
C. frutescens	7	36	16
C. baccatum	14	32	15
C. chinense	16	33	8

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Diversidade genética entre acessos de *Capsicum* por marcadores RAPD

RESUMO - *O gênero* Capsicum *é composto por 27 espécies, cinco domesticadas e 22 semi-domesticadas e silvestres. Para melhor utilização destas espécies em programas de melhoramento é preciso conhecer os acessos existentes nas coleções, identificando-os e também avaliando a diversidade genética entre eles. Os objetivos deste trabalho foram quantificar a diversidade genética entre 70 acessos de* Capsicum*, verificar a identificação dos mesmos e a presença de duplicatas, utilizando marcadores RAPD. Os resultados revelaram expressiva diversidade genética entre e dentro das espécies e foram concordantes com a classificação botânica e a caracterização morfo-agronômica. Foram confirmadas as identificações de 53 acessos (16* C. annuum*, 7* C. frutescens*, 14* C. baccatum *e 16* C. chinense*), e entre os não identificados botanicamente, os resultados sugeriram que 6 pertençam a espécie* C. baccatum *e 5 à espécie* C. chinense*. O acesso mais divergente foi o UENF 1620, provavelmente uma espécie silvestre ainda não representada na coleção.*

Palavras-chave: C*apsicum* spp., diversidade genética, análise multivariada, RAPD.

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