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Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill.)

ABSTRACT

In this study we used ISSR molecular markers to evaluate the genetic variability of 96 tomato accessions from *Banco de Germoplasma de Hortaliças* (BGH) of *Universidade Federal de Viçosa*, Minas Gerais, Brazil. Ten ISSR primers were individually amplified to allow the differentiation of the material. All ten primers generated 144 DNA bands, 53 being polymorphic, with an average of 14.4 per primer. The profiles generated by primer 840-$(GA)_8$YT contained the largest number of polymorphic bands (13 bands). The primer 855-$(AC)_8$YT detected the greatest differentiation of the accessions (12 accessions) while the primer HBH-884$(AG)_7$ did not detect any. The evaluation of the dendrogram obtained by UPGMA and Tocher' grouping method allowed the differentiation of all the accessions. Accession BGH-980 was located in a separate group, being the most divergent amongst the accessions tested by both methods. DNA profiles based on ISSR markers revealed the potential of the digital fingerprints in the diagnosis of all the accessions. From the results obtained in this study, ISSR markers have a high efficiency to differentiate the germplasm of wild species, besides origin studies.

Key words: Breeding, genetic distance, molecular diversity, germplasm bank.

Variabilidade genética em tomate (*Solanum lycopersicon* Mill.) por marcadores ISSR

RESUMO

Neste estudo foram utilizados marcadores moleculares ISSR para avaliar a variabilidade genética de 96 acessos de tomateiro pertencentes ao Banco de Germoplasma de Hortaliças (BGH) da Universidade Federal de Viçosa, Minas Gerais, Brasil. Dez *primers* ISSR foram amplificados individualmente para permitir a diferenciação do material. Os dez *primers* geraram 144 bandas de DNA, sendo 53 delas polimórficas, com média de 14.4 bandas por *primer*. O perfil gerado pelo *primer* 840-$(GA)_8$YT continha o maior número de bandas polimórficas (13 bandas). O *primer* 855-$(AC)_8$YT detectou a maior diferenciação dos acessos (12 acessos), enquanto o *primer* HBH-884$(AG)_7$ não diferenciou nenhum. A avaliação do dendrograma obtido pelos métodos de agrupamento UPGMA e Tocher permitiu diferenciar todos os acessos. O acesso BGH-980 foi localizado em um grupo separado, sendo o mais divergente entre os acessos testados por ambos os métodos. Os perfis de DNA com base em marcadores ISSR revelaram o potencial das impressões digitais no diagnóstico para todos os acessos. A partir dos resultados obtidos neste estudo, os marcadores ISSR têm uma alta eficiência para diferenciar o germoplasma de espécies silvestres, além de estudos de origem.

Palavras-chave: Melhoramento genético, distância genética, banco de germoplasma, diversidade molecular.

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INTRODUCTION

The highest diversity of both genera and species is found in the Neotropics. The largest genus in the family is *Solanum,* however members of the Solanaceae family occur worldwide. Species of *Solanum* have an estimated of 1500–2000 species ranging from tiny herbs to medium sized forest trees, with many different morphological forms (Nee et al., 2001).

The expansion of the use of genetic resources in *Solanum* species has been limited by a number of factors, including: the lack of a core collection, the lack of evaluation information, the difficulty in the introgression of traits from some wild species, most notably *S. peruvianum* and *S. chilense*, and a lack of reliable screening criteria for several traits of interest, such as insect resistance and fruit flavor (Nee et al., 2001). The increased erosion of plant genetic resources has resulted in a reduction of the genetic variability of cultivated species and their wild relatives. The genetic vulnerability can only be avoided with the genetic variability, which depends on the genetic resources (Silva et al., 2001). In 1996, the Food and Agriculture Organization (FAO) identified about 1300 germplasm banks preserving more than 5.5 million accessions, 78.000 of which were tomato accessions (Fraleigh, 2006).

With the rapid increase in the number of accessions in the germplasm banks, redundant genetic resources have become an obstacle to the effective maintenance and utilization of these collections. The characterization and evaluation of germplasm banks help bank curators use the genetic resources in breeding programs, find out and eliminate duplicate accessions, and discover the value of the collection. According to Karp (2002), the characterization of germplasm has been based mainly on morphological descriptors and, in less intensity, on agronomic traits. The advance in molecular techniques, initially with the use of isozymes and, subsequently, based on the variation of DNA sequence, has contributed in assessing genetic variability fast, precisely and efficiently. In Brazil, before the establishment of the International Plant Genetic Resources Institute (*IPGRI)* in the 70s, there was already some vegetable genetic resources collection aiming to maintain the variability existing. The *Banco de Germoplasma de Hortaliças* (BGH), which belongs to *Universidade Federal de Viçosa* (UFV), was established in 1960s and currently has 6.559 accessions with 25 families and 106 species. *Solanaceae* represents 44.21%, *Leguminosae* 16.83% and *Cucurbitaceae* 15.70% of the germplasm bank content. This germplasm is incorporated in breeding programs for new varieties and it remains as germplasm source of cultivated species (Silva et al., 2001).

The characterization of the current tomato accessions has not yet been completely concluded. The agronomic characterization of over than 350 tomato accessions of the BGH (*http://www.bgh.ufv.br*) has been accomplished together with the characterization of different resistance to *Tuta absoluta* (Oliveira et al., 2009; Moreira et al., 2005), potyvirus (*Zucchini yellow mosaic virus*, ZYMV and *Pepper yellow mosaic virus,* PepYMV) (Moura et al., 2005; Juhász et al., 2008), whitefly (*Bemisia tabaci* biotype B) (Fernandes et al., 2009), *Phytophthora infestans* (Abreu et al., 2008) and geminivirus (*Tomato yellow spot virus*, ToYSV) (Aguilera et al., 2008). The agronomic characterization and molecular information could provide a great support for breeding programs by including all these data in a joint analysis that takes in account all the variables that enhance the selection process of parents with high divergence.

In spite of the tomato genome be considered one of the most investigated among other plant species, with large number of molecular markers previously described (Tikunov et al., 2003; Kocheiva et al., 2002a; 2002b), it is still necessary to create new markers with high degree of polymorphism (Tikunov et al., 2003). ISSRs (*inter-simple sequence repeats*) or ISA *(Inter-SSR amplification*) molecular markers amplify regions between microsatellite loci (small repeated sequences of 2-5 pairs of bases). This class of markers does not require any prior knowledge about the sequences to be amplified and shows high polymorphism in the material, being very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology (Reddy et al., 2002). The ISSR is being used by several authors in the molecular characterization of many plant species, such as: tomato (Kamel et al., 2010), rice bean (Muthusamy, et al., 2008) and coffee (Masumbuko & Bryngelsson, 2006). When compared with the SSR (*simple sequence repeats*) markers (Goulão & Oliveira, 2001), AFLP (*amplified fragment length polymorphism*) (Saini et al., 2004), RFLP (r*estriction fragment length polymorphism*) (Kesari et al., 2010) and RAPD (*randomly amplified polymorphic DNA*) (Marotti et al., 2007) the ISSR markers were the most efficient to obtain a large number of polymorphic bands.

DNA molecular marker techniques exhibit great potential for determining the genetic diversity among tomato germplasm. In this study, 10 ISSR molecular markers were employed in the evaluation of the genetic diversity of 96 tomato accessions belonging to BGH-UFV germplasm bank and evaluate the efficiency of ISSR markers in the discrimination of accessions according to their geographical origin collection.

MATERIAL AND METHODS

Plant material and DNA extraction

Ninety-six accessions of *Solanum lycopersicon* belonging to BGH-UFV and two commercial varieties (Santa Clara and Debora Plus, Table 1) were used in the study. The study was conducted at the Laboratory of Plant Molecular Genetics (BIOMOL) of the Applied Agricultural Biotechnology Institute (BIOAGRO), UFV. The seeds were sown in polystyrene trays. After the germination, the seedlings were transplanted to pots of 1 liter volume containing previously sterilized substrate. Samples of young leaves (three plants per accessions) were collected and frozen at -80ºC. Genomic DNA was extracted from young leaves following the method of Doyle & Doyle (1990) using 3 individuals conducted in bulk. The DNA extracted of the three individuals was quantified and adjusted to a concentration of 10 ng/µl and mixed in equal quantities.

Table 1. Tomato accessions used in the current study.

Tabela 1. Acessos de tomate empregados neste estudo.

No	Code[a]	Origin[b]	No	Code	Origin
1	BGH 24	Minas Gerais, Brazil	50	BGH 994	São Paulo, Brazil
2	BGH 55	Bahia, Brazil	51	BGH 997	São Paulo, Brazil
3	BGH 83	Bahia, Brazil	52	BGH 1019	Minas Gerais, Brazil
4	BGH 121	Bahia, Brazil	53	BGH 1020	Minas Gerais, Brazil
5	BGH 160 Salada	Bahia, Brazil	54	BGH 1211	Minas Gerais, Brazil
6	BGH 160 Santa Cruz	Bahia, Brazil	55	BGH 1214	Mato Grosso, Brazil
7	BGH 161	Bahia, Brazil	56	BGH 1254	Goiás, Brazil
8	BGH 166	-	57	BGH 1282	Paraná, Brazil
9	BGH 168	Alagoas, Brazil	58	BGH 1485	Rio Grande do Sul, Brazil
10	BGH 181	-	59	BGH 1490	São Paulo, Brazil
11	BGH 184	Pernambuco, Brazil	60	BGH 1497	São Paulo, Brazil
12	BGH 185	Pernambuco, Brazil	61	BGH 1498	São Paulo, Brazil
13	BGH 186	Pernambuco, Brazil	62	BGH 1499	São Paulo, Brazil
14	BGH 216	Pernambuco, Brazil	63	BGH 1532	Minas Gerais, Brazil
15	BGH 218	Pernambuco, Brazil	64	BGH 1538	Goiás, Brasil
16	BGH 224	Bahia, Brazil	65	BGH 1706	São Paulo, Brasil
17	BGH 225	Bahia, Brazil	66	BGH 1708	-
18	BGH 227	Bahia, Brazil	67	BGH 1985	Indiana, USA
19	BGH 243	Pernambuco, Brazil	68	BGH 1987	Indiana, USA
20	BGH 279	-	69	BGH 1988	Indiana, USA
21	BGH 320	Goiás, Brazil	70	BGH 1989	Indiana, USA
22	BGH 322	-	71	BGH 1990	Indiana, USA
23	BGH 327	Goiás, Brazil	72	BGH 1991	Indiana, USA
24	BGH 349	Goiás, Brazil	73	BGH 1992	Indiana, USA
25	BGH 351	Goiás, Brazil	74	BGH 1993	Indiana, USA
26	BGH 378	Goiás, Brazil	75	BGH 2000	Indiana, USA
27	BGH 406 Salada	Goiás, Brazil	76	BGH 2119	Indiana, USA
28	BGH406 Santa Cruz	Goiás, Brazil	77	BGH 2202	Indiana, USA
29	BGH 468	-	78	BGH 2203	Indiana, USA
30	BGH 489	-	79	BGH 2205	Indiana, USA
31	BGH 603	Minas Gerais, Brasil	80	BGH 2208	Indiana, USA
32	BGH 606	-	81	BGH 2211	Indiana, USA
33	BGH 616	Minas Gerais, Brazil	82	BGH 2213	Indiana, USA
34	BGH 674	Minas Gerais, Brazil	83	BGH 2214	Indiana, USA
35	BGH 700	-	84	BGH 2216	Indiana, USA
36	BGH 773	Mato Grosso, Brazil	85	BGH 2219	Indiana, USA
37	BGH 850	Espírito Santo, Brazil	86	BGH 2223	Indiana, USA
38	BGH 970	São Paulo, Brazil	87	BGH 2229	Indiana, USA
39	BGH 975	São Paulo, Brazil	88	BGH 2234	Indiana, USA
40	BGH 978	São Paulo, Brazil	89	BGH 3472	North Carolina, USA
41	BGH 980	São Paulo, Brazil	90	BGH 4006	Minas Gerais, Brazil
42	BGH 981	São Paulo, Brazil	91	BGH 4035	-
43	BGH 985	São Paulo, Brazil	92	BGH 4053	Espírito Santo, Brazil
44	BGH 987	São Paulo, Brazil	93	BGH 4054	Espírito Santo, Brazil
45	BGH 989	São Paulo, Brazil	94	BGH 4055	Espírito Santo, Brazil
46	BGH 990	São Paulo, Brazil	95	BGH 4206	-
47	BGH 991	São Paulo, Brazil	96	BGH 4309	Minas Gerais, Brazil -
48	BGH 992	São Paulo, Brazil	97	* Hybrid Débora	Sakata Seed Sudamerica
49	BGH 993	São Paulo, Brazil	98	*Cultivar Santa Clara	Sakata Seed Sudamerica

[a] registration code at *Banco de Germoplasma da Universidade Federal de Viçosa* (BGH-UFV), [b] state and country of collection origin of accessions and (-) unknown origin, * standard varieties.

Amplification conditions and fragments separation

A total of 10 ISSR primers (Table 2) producing well-amplification and visible bands were selected later for determining the best temperature for annealing. The range of the temperatures tested was between 47 and 57ºC using thermal gradient model 'Robocycler Gradient 96' (Stratagene). For each primer, the annealing temperature was selected when it generated a clear pattern of the bands. All amplification reactions were performed in a final volume of 15 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.4 mM $MgCl_2$, 25 mM of each deoxynucleotide (dATP, dTTP, dGTP and dCTP), 0.5 mM of primer, 0.6 units of *Taq* DNA polymerase enzyme, 0.5% BSA (bovine serum albumin) and 20 ng of DNA. The amplification was performed in thermo-cycler model "GeneAmp PCR System 9600" (Applied Biosystem 9600). After an initial denaturation for 4 min at 94°, 35 cycles were performed each consisting of a denaturation step at 94° for 1 min, an annealing step at primer-dependent temperatures (Table 2) for 2 min and an extension step at 72° for 2 min. At the end, a final extension step (72° for 10 min) was included. The samples were held at 4° until further processing occurred. The final product was separated by electrophoresis and visualized in 1.2% (w/v) agarose gel (Invitrogen) at 1X TBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light in the photo-documentation system Eagle-Eye II (Stratagene). It was evaluated the most intense bands and the ones with high reproducibility.

Statistical analysis

The genetic dissimilarity was obtained based on Jaccard coefficient (Jaccard, 1901) using ISSR primers data. The interpretation of band patterns exhibited by gels was performed as (1) present and (0) absent of bands for each relative position. The absence of amplification product was used as a criterion for considering the polymorphic marker. With information of the bands analyzed for each primer, a matrix of binary data was built to calculate the Jaccard similarity coefficient ($S_{ii'}$). The dissimilarity matrix (d) among the accessions was obtained by $d = 1 - S_{ii'}$. The number of bands amplified by primer allowed calculating the percentage of polymorphism of each primer evaluated and the number of accessions differentiated by markers in each primer. The grouping of the accessions was performed by the Tocher sequential optimization (Vasconcelos et al., 2007) and the hierarchical UPGMA method (*Unweighted pair group method with arithmetic averages*) using the dissimilarity matrix obtained by the complement of Jaccard's coefficients. Analysis of bootstrap was conducted to evaluate the trust of the nodes of the cluster formed by the UPGMA method conducted with 500 permutations.

Discriminant analysis based on the non-parametric method *k nearest-neighbor* was used to determine the possible origin of accessions of unknown origin through binary data matrix obtained by the markers. The $k = 3$ value is assigned a priori and arbitrarily, according to Khattree & Naik (2000). The geographic origin was used as a criterion for a prior classifying which provided the allocation of 86 accessions in two geographical areas, Brazil and The United States (USA). The other 12 accessions (of unknown origin, Table 1) were assigned a posteriori. All tests were performed using the software GENES (Cruz, 2006).

RESULTS

The different sites of collection and origin of 96 tomato accessions used in this work are shown in Table 1. The sites of collection are distributed in 10 Brazilian states, representing

Table 2. ISSR primers, annealing temperature tested (ATT), annealing temperatures used (T_a), number of total amplified bands (NTB), number of polymorphic bands (NPB), percentage of polymorphism (P), size of amplified fragments in base-pairs (FS) and number of accessions identified per primer used to access genetic diversity of 96 tomato accessions

Tabela 2. Primers ISSR, temperatura de anelamento testada (ATT), temperatura de anelamento empregada (Ta), número total de bandas amplificadas (NTB), número de bandas polimórficas (NPB), porcentagem de polimorfismo (P), tamanho das bandas em pares de bases (FS) e número de acessos identificados por primers para acessar a diversidade genética de 96 acessos de tomate

Primer[a]	ATT(°C)	T_a(°C)	NTB	NPB	P(%)	FS(pb)	No of accessions identified per primer
810-$(GA)_8$T	47-54	51	22	11	50.00	300-2000	10
812-$(GA)_8$A	47-54	50	10	2	20.00	300-1100	2
835-$(AG)_8$YG	50-57	52	9	2	22.22	250-1000	1
840-$(GA)_8$YT	49-57	51	18	13	72.22	500-2000	10
841-$(GA)_8$YC	50-57	51	9	2	22.22	300-1000	1
855-$(AC)_8$YT	49-55	52	15	9	60.00	320-1200	12
884-HBH$(AG)_7$	48-55	51	15	4	26.67	250-1450	0
885-BHB$(GA)_7$	48-56	51	19	3	15.79	250-2000	1
888-BDB $(CA)_7$	48-56	51	14	5	35.71	300-1200	6
889-BDB $(AC)_7$	48-55	54	13	2	15.38	300-1200	1
Total	-	-	144	53	-	-	44
Mean		-	14.4	5.3	34.02	-	4.4

[a]: Y= (C or T); H= (A, C or T); B= (C, G or T); D= (A, G or T).

the Southeast (35 accessions), Central West (11 accessions), South (2 accessions) and Northeast regions (16 accessions), and two sites located in the states of Indiana and North Carolina, in The United States, with 21 and 1 accessions, respectively.

Ten ISSRs primers were used to characterize the genetic divergence of the 96 accessions of *S. lycopersicon.* In Table 2, it is shown the annealing temperatures of each primer, the number of amplified loci, the number of polymorphic loci, the polymorphism percentage obtained, the amplitude of the amplified fragments and number of accessions identified by each primer in the groups formed using the method of UPGMA. A total of 144 bands were amplified and 53 of them were polymorphic, representing 36.8% of the amplified loci, ranging between 250 to 2000 bp. The average of the total bands per primer studied was 14.4, ranging from 9 to 22 bands. For the polymorphic bands, the average was 5.3 bands per primer, representing 34.02 % of polymorphism (ranged between 2 to 13 bands). The data obtained from each individual primer allowed the differentiation of a total of 44 accessions with general mean of 4.4 accessions per primer, ranging between 0 to 12 accessions identified.

In general, a high number of bands per primer was detected, although a low number of polymorphic bands was found, except for the primers 840 (Table 2, Figure 1), 810 and 855 (Table 2), which showed a high polymorphism, representing 72, 50 and 60% of polymorphic bands, respectively. The identification of the largest number of accessions coincided with the primers that revealed the highest degree of polymorphism with emphasis on primer 855, which allowed the identification of the larger number of accessions (12). The primer 884 identified the lowest number of accessions (0), even though having a large number of bands (15) and 4 polymorphic bands (26.67%) (Table 2, Figure 1). The primers 812, 835, 841 and 889 produced the lowest number of polymorphic bands, with only 2 of the total polymorphic bands analyzed (Table 2). The percentage of polymorphic bands ranged between 15 to 72% of the total amplified bands with an average of 34.02%, and the size of the amplified fragments ranged between 250 to 2000 bp.

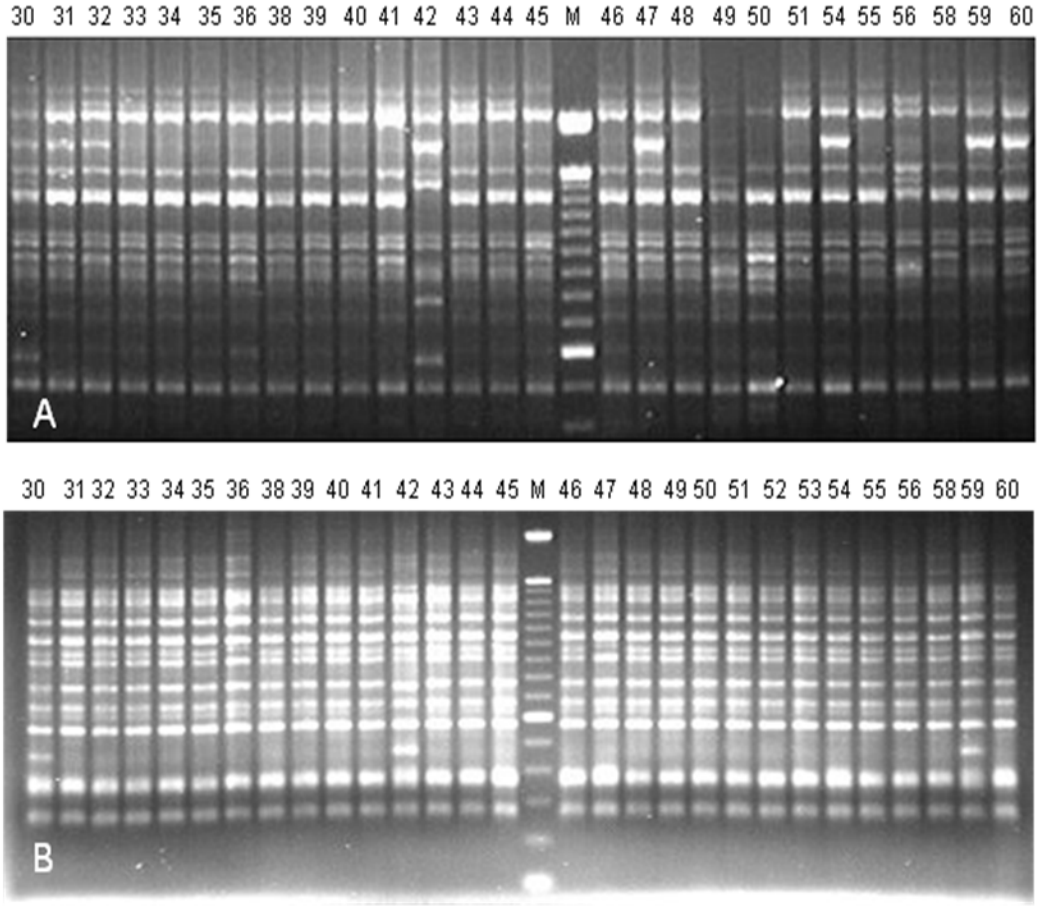

Figure 1. Profiles of DNA amplification of *S. lycopersicon.* accessions (agarose gel 1.2 %, stained with ethidium bromide). Profiles of the primers ISSR: 840-$(GA)_8$YT (A) and 884-HBH$(AG)_7$ (B). The identification of each accession is shown in Table 1. M is marker of 100 pb (Invitrogen)

Figura 1. Perfis da amplificação do DNA de acessos de S. lycopersicon. (gel de agarose 1,2 %, corados com brometo de etideo). Perfis dos primers ISSR: 840-$(GA)_8$YT (A) e 884-HBH$(AG)_7$ (B). A identificação de cada acesso é mostrada na Tabela 1. M é o marcador de 100 pb (Invitrogen)

Table 3. Evaluated characteristics in four tomato accessions: origin (Or), plant height (PH), nodes length (LN), fruit weight (FW), number of fruits (NF), total production (TP), total soluble solids (SS) fruit color (FC), fruit size (FZ), fruit shape (FS)

Tabela 3. Características avaliadas em quatro acessos de tomate: origem (Or), altura da planta (PH), comprimento dos entrenós (LN), peso do fruto (FW), número de frutos (NF), produção total (TP), sólidos solúveis totais (SS) cor do fruto (FC), tamanho dos frutos (FZ), tipo de fruto (FS)

Acession	Or	PH (cm)	LN (cm)	FW (g)	NF	TP (g/plt.)	SS (ºBrix)	FC[1]	FZ[2]	FS[3]
BGH-616	MG	30.47	58.52	67.94	9.01	4125	3.33	1	1	1
BGH-970	SP	31.08	73.4	78	7.32	2537	5.03	1	1	1
BGH-674	MG	29.83	53.88	79.44	7.28	3088	3.8	1	1	1
BGH-991	SP	32.33	63.4	85.5	6.95	2205	4.78	1	2	1

[1]: 1-red; [2]: 1-small, 2-medium; [3]: 1- very round. Data adjusted from the database of *Banco de Germoplasma de Hortaliças*- UFV is available at *http://www.bgh.ufv.br*

Table 4. Grouping of 96 tomato accessions and two commercial cultivars using the Tocher sequential method obtained from the dissimilarity matrix of Jaccard of 10 ISSR molecular markers

Tabela 4. Agrupamento de 96 acessos de tomate e duas cultivares comerciais empregando o método sequencial de Tocher obtido ao empregar a matriz de dissimilaridade de Jaccard de 10 marcadores moleculares ISSR

Group	Sub-group	$\alpha^{(1)}$	Accessions
I (0.1374)*	1	0.0726	BGH-616, BGH-970, BGH-351, BGH-674, BGH-991, BGH-700, BGH-987, BGH-St406, BGH-603, BGH-606, BGH-975, BGH-378, BGH-1211, BGH-985, BGH-227, BGH-320, BGH-186, BGH-83, BGH-243, BGH-279, BGH-S406, BGH-989, BGH-224, BGH-349, BGH-185, BGH-225, BGH-322, BGH-1990, BGH-981, BGH-S160, BGH-1020, BGH-St160, BGH-184, BGH-216, BGH-1989, BGH-1991, BGH-218, BGH-2234, BGH-978, BGH-327, BGH-168, BGH-121, BGH-850, BGH-993, BGH-994, BGH-1254, BGH-24, BGH-166, BGH-1019, BGH-2229, BGH-1214, BGH-990, BGH-468, BGH-4006, BGH-4054, BGH-1988, BGH-55, BGH-1485, BGH-1498, BGH-1532, BGH-992, BGH-4053, BGH-489
	2	0.0859	BGH-2205, BGH-2208, BGH-2214, BGH-2219, BGH-1708, BGH-2203, BGH-2202, BGH-1992, BGH-2000, BGH-2119, BGH-1985, BGH-1706, BGH-2223, BGH-1538, BGH-997, BGH-2213, BGH-4055, BGH-4309, Santa Clara, BGH-2211, BGH-2216, BGH-1499, BGH-4035, Débora
	3	0.1181	BGH-181, BGH-773, BGH-161, BGH-3472, BGH-1282, BGH-4206, BGH-1497
	4	0.127	BGH-1987, BGH-1993
	5	-	BGH-1490
II			BGH-980

(1) Maximum value of the measure of dissimilarity found in all the smaller distances involving each accession; * Value of á in the initial grouping.

Among the selected primers effectively employed in this work, the repetition of the sequence of the amino acids guanine and adenine (GA) was present in 5 of the 10 primers selected. Primers with the same amino acids sequence but different in the anchoring sequences produced very different amplification profiles, in the total number of bands amplified (TNB), number of polymorphic bands (NPB), percentage of polymorphism (P) and number of accessions identified (Table 2).

The discrimination of most accessions evaluated (UPGMA dendrogram) showed that the accession BGH-980 was the most divergent among the other accessions (Figure 2) with 100% of dissimilarity (0.1811). Regarding the 10 primers evaluated, the accession BGH-980 had bands only in primers 840 (2 bands) and 855 (1 band) which allowed a clear differentiation from the rest of the accessions tested. The values of genetic distance ranged from 0 to 0.25, with an average of 0.1205. The lowest values of genetic dissimilarity (0) were between the pairs of accessions: BGH-674 and BGH-991, BGH-616 and BGH-970 (Figure 2).

In Table 3, it is shown the values of 10 traits: 3 qualitative (fruit color [FC], fruit size [FZ] and fruit shape [FS]), 6 quantitative (plant height (PH), length of nodes (LN), fruit weight (FW), number of fruits (NF), total production (TP) and total soluble solids (SS)) and origin (Or) of the four accessions displayed on the website of BGH-UFV (available at http://www. bgh.ufv.br). These data show not only the genetic proximity, as observed in the UPGMA clustering and the sequential method of Tocher, but also the phenotypic characteristics, which are very similar, too.

The Tocher sequential method uses Jaccard's dissimilarity matrix and initially forms two groups, in which the accession

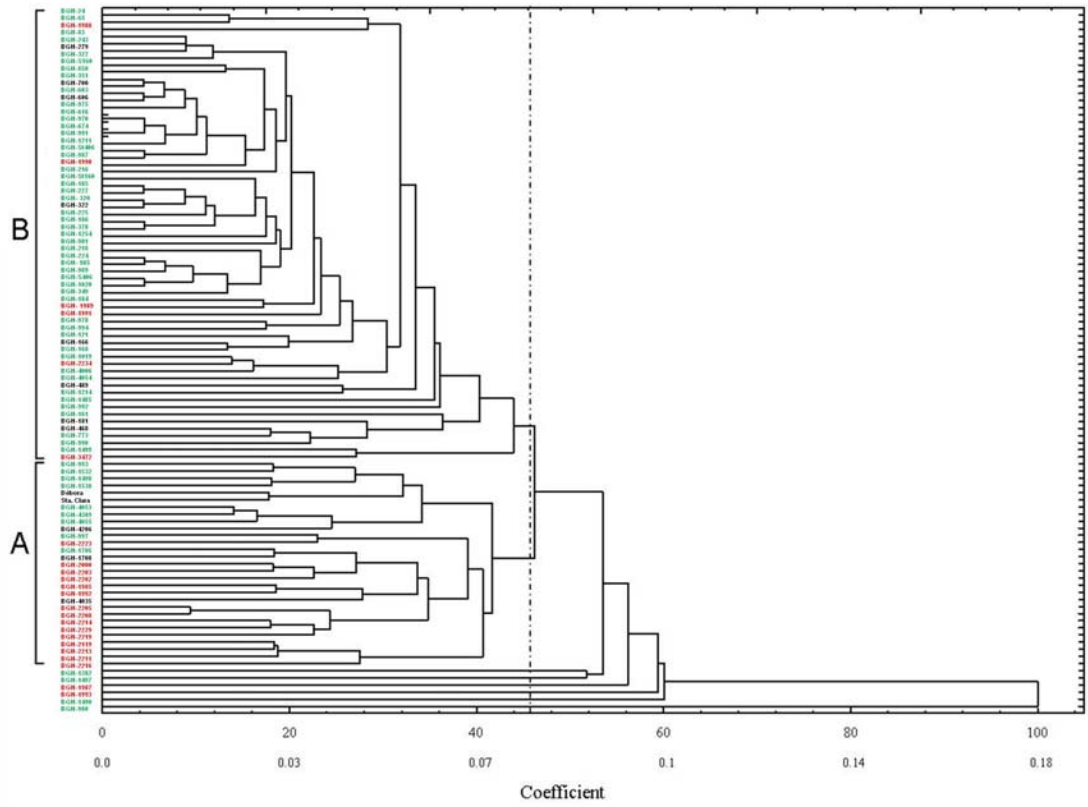

Figure 2. Dendrogram of 96 accessions plus two commercial cultivars of tomato (*S. lycopersicon* Mill.) of BGH-UFV. The scale is based on the Jaccard coefficient of dissimilarity considering the marks of 10 ISSR molecular marker. Group A with accessions from The United States and Group B from Brazil. The accessions from The United States are shown in red color, the ones from Brazil in green and the accessions with unknown origin, in black collor.

Figura 2. Dendrograma de 96 acessos mais dois cultivares comerciais de tomate (S. lycopersicon Mill.) do BGH UFV. A escala é baseada no coeficiente de dissimilaridade de Jaccard considerando as marcas geradas por 10 marcadores moleculares ISSR. Acessos do Grupo A dos Estados Unidos e do Grupo B do Brasil. Os acessos dos Estados Unidos são mostrados em cor vermelha, os do Brasil em verde e os acessos de origem desconhecida, em cor preta.

BGH-980 was located in a separated group whereas the other group was formed by the rest of the accessions (Table 4), confirming the result shown by UPGMA (Figure 2). Conducting a new grouping analysis without the accession BGH-980, the group I formed 5 sub-groups (Table 4) showing different levels of dissimilarity within the group. The cultivar 'Santa Clara' and the hybrid 'Débora' were used as standard varieties and were grouped together by both methods (UPGMA and Tocher) indicating the low level of dissimilarity between them, accessed by markers (0.03). This result was expected since the individuals of this group are commercial cultivars resulted from breeding process sharing the same genetic basis.

When compared with the accession BGH-980, the values of dissimilarity were 0.16 and 0.18 for 'Débora' and 'Santa Clara', respectively. The two standard varieties were always grouped together in groups formed by the most accessions of The United States, identified in the dendrogram (Figure 2) as Group A and in the Tocher grouping (Table 4) in group I and sub-group 2. Whereas the Group B of the UPGMA's dendogram (Figure 2) included accessions that share the same country origin according to data from the BGH passport. In the dendrogram (Figure 2), it was used 46% of dissimilarity to differentiate the groups from Brazil and The United States, where the highest differentiation of the groups was obtained.

The discriminant analysis using the average distance (distance of the individual in each population) and *k nearest neighbor* (similarity of the individual in relation to the k coming from different populations) was performed in the population defined previously by the passport data (Table 5).

The formation of the first population contained 62 accessions, collected from 5 states of Brazil, while the second population, formed by accessions collected from two states of The United States, included 23 accessions. The method of the k nearest neighbor allocated 75.81%, which corresponds to 47 of the 62 accessions originally from Brazil, in the Brazil's group, 82.61%, which corresponds to 19 of the 23 accessions originally from the USA, in the USA's group, and 22.35% of apparent error. The discrimination method using the mean distance allocated 88.71%, which corresponds to 55 of the 62 accessions originally from Brazil, in the Brazil's group, 69.57%, which corresponds to 16 of the 23 accessions originally from the USA, in the USA's group, and 16.47% of apparent error. From both methods the allocation of the accessions with unknown origin was the same, with two accessions (BGH-1708 and BGH-4035) in the USA population and the others in the Brazilian population.

Table 5. Discriminant analysis of two populations *of accessions of S. lycopersicon* from different origins based on the k nearest neighbor method (k = 3) and mean distance estimated from 10 ISSR markers. The detected values in bold refer to the percentage of allocation of the accessions in their respective groups of origin.

Tabela 5. Analises discriminantes de duas populações de acessos de S. lycopersicon de diferentes origens baseadas no método de k vizinho mais próximo (k = 3) e a distância média estimada por 10 marcadores ISSR. Os valores em negrito referem-se à percentagem de alocação dos acessos em seus respectivos grupos de origem

Group of origin	Nº of accessions[1]	% of allocation of accessions in the different groups			
		k nearest neighbor		Mean distance	
		Brazil	USA	Brazil	USA
Brazil	62	75.81	24.19	88.71	11.29
USA	23	17.39	82.61	30.43	69.57
		BGH-166		BGH-166	
		BGH-181		BGH-181	
		BGH-279		BGH-279	
		BGH-322	BGH-1708	BGH-322	BGH-1708
A_p	11	BGH-468	BGH-4035	BGH-468	BGH-4035
		BGH-489	(18.18)	BGH-489	(18.18)
		BGH-606		BGH-606	
		BGH-700		BGH-700	
		BGH-4206		BGH-4206	
		(81.82)		(81.82)	
T_{AA}[2]	60	36	71	25	
TEA (%) [3]	22.35	16.47			

[1]Total of accessions allocated previously in populations based on the origin, [2]Total of accessions allocated in each population, [3]Rate of apparent error.

DISCUSSION

ISSR molecular markers have been used successfully in germplasm bank characterization (Terzopoulos & Bebeli, 2008; Tikunov et al., 2003; Kocheiva et al., 2002b) especially in the assessment of the differences among species or varieties belonging to the same genus. In the current study, the ISSR markers were also useful in the characterization of *S. lycopersicon* accessions, amplifying a relatively large number of loci per primer, which were consistently generated in all genotypes. However, the level of polymorphism detected was low in comparison to other results obtained from other species (Isshiki et al, 2008). On the other hand, the degree of genetic divergence detected between *S. lycopersicon* accessions, using these molecular markers, was considered high in comparison to the level of polymorphism among accessions of other species of the genus (Terzopoulos & Bebeli, 2008; Kochieva et al., 2002b), however the variation detected was sufficient to distinguish the majority accessions in the current study.

Within the gender *Solanum*, members of different species have distinct phenotypic and genotypic characteristics and a high level of polymorphism can be expected among them, but a low level is expected among the members of the same species. However, the observed values are compatible with those obtained by Park et al. (2004), who assessed the genetic diversity of 74 cultivars of *S. lycopersicon*, and by AFLP. Kochieva et al. (2002a) who estimated the genetic polymorphism of the genus *Solanum* using RAPD. The analysis showed that the inter-specific polymorphism of the representatives of the genus was 98.8% while the intra-specific polymorphism of *S. lycopersicon* cultivars was 65.6%, with lower differences between populations and cultivars than between species. The polymorphism accessed by different molecular markers is low when comparing individuals or accessions of the same species of *Solanum*.

Among the 10 ISSR markers used, the amino acids sequence $(GA)_7$ and $(GA)_8$ produced the highest number of amplified bands (78 of 144 amplified bands) and polymorphism level (31 of 53 amplified bands), showing the presence of the combination of nucleotides in the tomato genome. Smulder et al. (1997) identified 80 polymorphic loci of microsatellite in *Solanum* genotypes containing sequences from one to five nucleotides, since the amino acid $(AT)_n$ was the most common found in 33 of the 80 loci identified. However, ISSR primers were used to study five species of the genus (*S. lycopersicon, S. pennellii, S. cheesmanii, S. humboldtii and S. hirsutum*), generating many polymorphic bands with primers $(CA)_n$, $(CA)_n$, $(GA)_n$ and $(AG)_n$ that expressed a great number of polymorphic bands (Tikunov et al., 2003). These results coincide with those obtained in this work, showing the high level of polymorphism of the amino acids sequence GA, though it is less common in representatives of the species *S. esculentum*, in comparison to the rest of the combinations.

The genetic variability of the accessions could be classified as high, since many genetic differences among them were obtained, as evidenced by the large number of groups formed

by the sequential method of Tocher and the differentiation of most accessions by the hierarchical method UPGMA. In this study, the results reflect the genetic divergence existing among the accessions and the importance of retaining these collections conserved at the BGH-UFV germplasm bank as a source of variability for breeding programs and scientific purposes. Few differences among representatives of *S. lycopersicon* species has been found (Tikunov et al., 2003; Kocheiva et al., 2002b).

The data obtained by ISSR markers were more efficient in the discrimination of the accessions collected in Brazil than in those from the USA by the discriminant analysis, also evidenced by the coherence of clusters obtained by UPGMA methods (Group A and B) and by the sequential method of Tocher, which allowed the allocation of 11 accessions in the same groups in the three methods used. These results could be considered as evidence of the importance of the origin in the genetic variability of the tomato. The commercial cultivars 'Débora' and 'Santa Clara' were more similar to the material collected in the USA than in Brazil. Terzopoulos & Bebeli (2008) detected a clear diversity of the commercial cultivars from the landraces by using 12 ISSR markers.

The results obtained by the discriminant analysis considering the different origins and different populations showed that the loci of ISSR markers were adequate to discriminate the accessions in both populations when considering the TEA value. The results showed that the discriminant analysis depended on the method of discrimination (*k nearest neighbor* or average distance). The results showed that there were some differences among the accessions collected in Brazil and in the USA with the same standard classification in most accessions.

Conclusions

In this study it was shown that the ISSR markers were effective in the estimation of the genetic diversity of the *Solanum* accessions characterized with different levels of polymorphism. The information obtained by the markers allowed the differentiation and grouping of the germplasm, enabling the discrimination into 2 groups. The application of ISSR molecular markers could be an efficient tool in the characterization of over than 800 accessions of the BGH-UFV collection.

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