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Molecular characterization of reference husk tomato (*Physalis ixocarpa* Brot. ex Horm.) varieties using ISSR markers

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Abstract

Husk tomato is an important crop that has been subjected to a wide range of domestication process all across Mexico; therefore, there is an extensive genetic variability available. Consequently, it is convenient to characterize molecularly commonly cultivated varieties that are used as reference to classify new accessions or to register new improved varieties. Accordingly, 12 husk tomato varieties belonging to eight different races were fingerprinted using ISSR (Inter Simple Sequence Repeat) markers. Twenty-two ISSR primers produced a total of 208 bands, and 172 of them were polymorphic (representing 83 % of polymorphism). DNA fingerprinting table was compiled. Six primers were absolutely required for analyzing tested cultivar cultivars. UBC-835, UBC-873, and UBC-823 primers showed high discernment power, and therefore they will be very useful in further on genetic studies of this crop. In addition, Jaccard's distances between pairs of races were used to create a dendrogram. The results obtained showed that ISSR molecular markers can be used for reliable identification of husk tomato varieties.

Keywords: *Physalis ixocarpa* Brot., germplasm characterization, molecular ISSR profiles.

Introduction

Husk tomato (*Physalis ixocarpa* Brot.) is a Mexican species with enormous genetic variability. More than 70 different species from *Physalis* genera have been reported, and at least eight different breeds of *Physalis ixocarpa* (Vargas-Ponce, O., Pérez-Alvares, L. F., Zamora-Tavares, P., & Rodríguez, A., 2011). This Solanaceous crop is cultivated in all Mexican states and fruit production is directed to both, national and international markets. It occupies the fifth place among horticulture crops, and it is planted on 43 500 ha. About 700 thousand tons are produced annually; with average yield of 14 $t \cdot ha^{-1}$, however, it has a potential of 40 $t \cdot ha^{-1}$ (FAO, 2014).

Fresh fruits are round, watery, with seeds enclosed in a calyx; they come in a variety of colors from green and yellow to purple. They are a bit acid, slightly sweet, earthy, with a hint of citrus, and are the main ingredient to elaborate traditional Mexican dishes such as green sauces, as ingredients in various stews, soups, preserves, beverages, pies, jams, and other Mexican recipes. Calyx's infusion has antibacterial activity against respiratory infections caused by *Staphylococcus*. The juice of the fruit is used as eyewash and to control gastrointestinal problems and respiratory disease (Sharma, N., Bano, A., Dhaliwal, H. S., & Sharma, V., 2015). It has potential as an anticancer drug since the ixocarpalactone A compound isolated from the juice has shown antiproliferative and apoptotic activity against colon cancer cells (Choi et al., 2006).

Husk tomato fruits have been found to be a good safe source of antioxidants. Fruits contain approximately 93.3 % water,

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but they also contain raw fiber, digestible carbohydrates, carotene, flavonoids, vitamins such as A, E, K, niacin and ascorbic acid, and minerals as calcium, iron, and phosphorus (USDA, 2015).

Mexico possesses a wide genetic diversity of husk tomato, which has been classified into eight breeds: 'Silvestre', 'Milpero', 'Arandas', 'Tamazula', 'Manzano', 'Rendidora', 'Salamanca', and 'Puebla', distributed all across the country from eight to 3 350 m of altitude. The most important breeds due to commercial use are 'Rendidora', 'Puebla', 'Tamazula', and 'Salamanca'; however, there are no improved varieties belonging to the last race (Santiaguillo et al., 2012).

Husk tomato is an obligated autogamous crop due to gametophytic self-incompatibility(Escobar-Guzmán, R. E., Hernández-Godínez, F., Martínez de la V., O., & Ochoa-Alejo, N., 2009) (Escobar-Guzmán et al., 2009), controlled by two genes with multiple alleles; therefore, its populations is are heterogeneous, heterozygous, highly variable, and thus difficult to classify. In addition, the presence of intermediate populations at different stages of evolution (wild, tolerated, fomented, domesticated, and cultivated) (Ponce-Valerio et al., 2011), also makes classification difficult. Then, the International Union for the Protection of New Varieties of Plants (UPOV, 2007) has proposed the use of 12 reference varieties for morphological characterization of husk tomato materials. Currently, it is not required for variety holders to submit correspondent DNA fingerprinting for registration, it becomes convenient to have the DNA fingerprints of reference varieties as an extra parameter to compare molecular profiles of new developed husk tomato varieties. ISSR (Inter Simple Sequence Repeat) markers have proved to be a suitable tool to obtain husk tomato fingerprints (Vargas-Ponce et al., 2011).

Materials and methods

Plant material. A total of 12 husk tomato varieties belonging to seven different breeds were used in this study (Table 1). Seedliing production was performed under lab conditions. One hundred seeds from each accession were sown in Petri dishes and kept in germination chambers at 28 °C for 5 d.

DNA extraction. The protocol of De la Cruz, M., Ramírez, F., y Hernández, H. (1997); was used; 0.3 g of fresh leaf tissue was powder grounded with liquid nitrogen and then transferred to 1.5 mL plastic tubes preheated at 65 °C using a themoblock, containing 700 µL of extraction buffer (Tris-HCl 100 mM, EDTA-Na_2 50 mM, NaCl 500 mM, 2-mercaptoetanol 10 mM, SDS 1.3 %, pH 8.0). Tubes were agitated for homogenization and reheated at 65 °C for 10 min. A total of 200 µL of 5 M potassium acetate were added to each tube and ice cooled for 30 to 60 min and then centrifuged for 20 min at 12,000 × g. Supernatant was transferred to new plastic tubes containing 600 µL of precooled isopropanol andkept at -20 °C for 60 min to precipitate DNA. Afterwards, tubes were centrifuged for 5 min at 10 000 × g and decanted; DNA's pill was dissolved with 700 µL of solution and left at 4 °C overnight. Afterwards, 4 µL of RNase was added to each tube and incubated at 37 °C for 1 h. DNA was reprecipitated using 75 µL of 3 M sodium acetate and 500 µL of cooled isopropanol for 2 h. Then, tubes were centrifuged for 5 min at 10 000 xg, supernatant was disposed and the DNA's pill was washed with 70 % ethanol. Tubes were again centrifuged for 5 min at 8 000 × g, the supernatant was discarded and the DNA's pill was dried at room temperature. Finally, it was dissolved in 50 µL of TE and then storage in a fridge at 4 °C. Six replicates per accession were performed, yielding a total of 72 extractions.

DNA quantification and quality evaluation

DNA concentration for the different samples was determinate using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, USA) and dilutions were made in order to get a uniform concentration of 10 ng·µL^{-1}. Later the 10 replicates per accession were mixed to have one sample per genotype. DNA quality was quantified by running an 8 % agarose gel using 100 mL of TAE 1X buffer (Tris base 40 mM, pH 7.8; sodium acetate 20 mM and EDTA 2 mM, pH 8.0). Over a parafilm tape, 2 µL of load buffer (0.2 % blue bromophenol, 0.2 % xylenecianol, 25 % glycerol, 5 mM EDTA, 50 mM Tris-HCl, pH 8.0) were mixed with 10 µL from each sample, while at both ends 3 µL of 1 Kb molecular ladder was loaded. Electrophoresis was performed at 90 volts for 1.5 h. Afterwards, the gel was removed and dyed with ethidium bromide solution (0.6 µg·µL in TAE 1 X) for 20 min. Subsequently, the gel dried and was placed inside a DigiDoc-It ® Imaging System UV transilluminator to be photographed using a Kodak EDAS 290 camera.

ISSR molecular profiles

A total of 66 Sigma® primers were originally tested to evaluate their ability of producing clear and reproducible amplification products. From them 22 primers were finally selected. The PCR protocol was as follows: 2.5 µL of DNA (10 ng µL^{-1}) were placed in 1.5 mL plastic tubes containing 22.5 µL of reaction mixture integrated by PCR buffer (2.5 µL 10X), Taq DNA polymerase (0.3 µL, Invitrogen®), DNTP's (10 µL 500 µM), $MgCl_2$ (3 µL 50 mM), primer (1.5 µL 10 ng µL^{1}) and water (5.2 µL) to complete a total volume of 25 µL. Tubes were incubated in a Techne TC-412 thermocycler using the following program: one pre-denaturation cycle of 20 min at 93 °C, 40 denaturation cycles (1 min at 93 °C each), alignment (1 min at different temperatures for each primer), extension (1 min at 72 °C), and a final extension cycle at 72 °C. The amplification

Table 1. Reference varieties for varietal description of husk tomato (*Physalis ixocarpa* Brot.) established by UPOV (2007).

Breed	Variety	Breed	Variety
Milpero	Milpero Tetela	Arandas	Morado R
Manzano	Manzano Tepetlixpa	Puebla	Puebla SM3
	Yema de Huevo		Tecozautla 04
Tamazula	Tamazula SM3	Rendidora	Rendidora
Salamanca	Salamanca		CHF1 Chapingo
	Potrero	Hybrid (Rendidora x Puebla)	Diamante

products were separated by electrophoresis in agarose gels at 2 % at 200 volts for 2 h, then stained with ethidium bromide for 20 min, reveled inside the UV transilluminator and photographed using the Kodak EDAS 290 camera.

Statistical analysis

Genetic similarities and differences between pairs of accessions were computed from band patterns allocating the 0 value to the absence and a value of 1 to the presence of each band. Amplification products were recorded for each primer, and a consecutive number was assigned according to their migration distance in the agarose gel. The registration of ISSR patterns obtained from each of the 22 selects primers for the 12 accessions created the basic data matrix (BDM).

To evaluate the discrimination power of tested primers a descriptive analysis of amplified products was obtained where quantitative variables such as number of amplified bands, proportion of mono and polymorphic loci, and polymorphic information content per primer were quantified using InfoGen® software (ver. 2011, Universidad de Córdoba, Argentina). Because genetic distance can be used to compare similarity among different breeds, several groups were constructed under the criteria of Ward's minimum variance using Jaccard's distance (1 – s) to construct a dendrogram. The cut distance was estimated based on the cubic clustering criterion (r) and Hotelling's T^2 pseudostatistic (Johnson, 2000), using PROC CLUSTER and PROC TREE of SAS® Ver 9.0.

Results and discussion

ISSR analysis

The descriptive analysis of amplified products (Table 2) shows the 5'-3' sequence, and the annealing temperature for the 22 tested primers. Only 36 out of a total of 208 amplified bands were monomorphic, which represents 83 % of polymorphism. The number of bands per primers ranged from 5 to 16. All primers produced different fingerprinting profiles for each variety, confirming the ISSR value in husk tomato variety discrimination. These results are in good concordance to those achieved by Vargas-Ponce *et al.* (2011), who by the use of six primers in 8 species of *Physalis* genera obtained 101 bands that allowed them to obtain DNA profiles to unmistakably distinguish among species.

Even when PI01 and ISSR05 primers showed the highest percentage of amplification through all 12 accessions (30.4 and 26.7 %, respectively), it was primer UBC835 that showed the highest polymorphic information content (PIC=0.27). In addition, it exhibited a low probability for two individuals to share the same allele by chance (PSSA), and therefore a high discrimination power among varieties. This performance was also observed for UBC873 and UBC823 primers; therefore, they were the most recommendable to be used in further studies of molecular characterization of husk tomato to accurately distinguish among varieties. Low PSSA values were also observed for ISSR01, ISSR02 and ISSR04 primers; so, it could be recommendable the use of at least these six primers to obtain reliable DNA profiles for this crop (Table 3).

Genetic relationships among breeds. Genetic distances between pairs of breeds were estimated using Jaccard's similarity coefficient transformed into genetic distance as 1-S. Afterwards, a clustering analysis was conducted using Ward's minimum variance criteria. To ensure the obtained clustering was robust, 1 000 cycles of resampling by bootstrapping were performed and results were represented in a hierarchical dendrogram (Figure 1). The dendrogram cutting value (r = 0.60) was estimated using the cubic clustering criterion and Hotelling's T^2 pseudostatistic. The cutting point divided breeds into four groups. The most genetically distant breeds were 'Puebla' and 'Tamazula' with a distance value of 0.86; meanwhile, the more related breeds where 'Rendidora' and 'Manzano' (d = 0.44).

Results seem to reflect the importance of growth habit to separate the evaluated breeds. These results agreed to those found previously for other crops such as beans Gill L., H. R., Rosales C., R., Hernández D., S., & Mayek P., N., 2014) and pepper (Tilahun, S., Paramaguru, P., & Hanna,

Table 2. Primers, 5'-3' sequence, annealing temperature (AT), number of polymorphic bands (PB), monomorphic bands (MB), total bands (TB), proportion of polymorphic loci (PPL), polymorphic information content (PIC), standard error (SE), percentage of amplification (PA), and probability for two individuals to share the same allele by chance (PSSA) of ISSR fingerprints from husk tomato reference varieties.

Name	(5' - 3') Sequence	AT	PB	MB	TB	PPL(95)	PIC	SE	PA	PSSA
ISSR01	$(CA)_8$AAGG	62	15	1	16	0.94	0.19	0.01	13.19	4.2E-16
ISSR02	$(CA)_8$AAGCT	62	16	0	16	1.00	0.18	0.01	12.08	8.7E-16
ISSR03	$(GA)_8$CTC	58	8	0	8	1.00	0.18	0.01	12.50	3.6E-13
ISSR04	$(AG)_8$CTC	58	4	4	8	0.50	0.18	0.01	12.04	3.5E-16
ISSR05	$(AG)_8$CTA	56	7	3	10	0.70	0.25	0.01	26.67	2.2E-12
ISSR06	$(AG)_8$CTG	58	11	2	13	0.85	0.22	0.01	19.79	8.9E-12
ISSR07	$(AG)_8$CTG	58	7	0	7	1.00	0.20	0.01	14.68	3.1E-13
ISSR08	$(AC)_8$CTT	56	8	1	9	0.89	0.20	0.01	14.47	2.2E-15
ISSR10	$(GA)_8$T	50	7	2	9	0.78	0.19	0.01	13.41	2.5E-13
UBC811	$(GA)_8$C	52	7	3	10	0.70	0.20	0.01	17.59	4.5E-10
UBC822	$(TC)_8$A	50	10	0	10	1.00	0.19	0.01	12.88	3.9E-15
UBC823	$(TC)_8$C	52	3	1	4	0.75	0.20	0.01	13.73	9.3E-16
UBC835	$(AG)_8$CTC	54	8	3	11	0.73	0.27	0.01	24.17	4.0E-16
UBC836	$(AG)_8$CTA	52	6	2	8	0.75	0.21	0.01	15.97	3.0E-14
UBC841	$(GA)_8$CTC	58	13	0	13	1.00	0.21	0.01	15.48	3.7E-15
UBC844	$(CT)_8$AC	56	8	0	8	1.00	0.18	0.01	12.15	1.3E-13
UBC848	$(CA)_8$AGG	56	1	4	5	0.20	0.21	0.01	15.53	1.5E-14
UBC873	$(GACA)_4$	48	8	0	8	1.00	0.22	0.01	17.67	8.9E-16
PI01	$(CA)_6$AGCT	48	5	2	7	0.71	0.24	0.02	30.36	1.2E-10
PI02	$(CA)_6$AGG	48	3	4	7	0.43	0.26	0.02	23.91	2.0E-14
PI03	AGCT$(GACA)_3$	48	6	4	10	0.60	0.23	0.02	20.83	2.0E-12
PI04	$(CT)_8$AGC	58	11	0	11	1.00	0.16	0.01	10.26	1.5E-15
Total			172	36	208	0.83			16.40	1.5E-295

B. J. R., 2013), in which clustering by morphological characterizations was strongly influenced by growth habit. Thus, Group I was integrated by 'Rendidora' and 'Manzano' breeds, both of semi-erected growth habit, Group II by 'Puebla' and 'Salamanca', Group III by 'Tamazula' of erected habit and Group IV by 'Arandas' and ''Milpero' with prostrated growth habit.

Breeds classified into Group I also have in common that both produce large fruits, with calyxes strongly adhered to them covering fruits completely. In addition, fruits have intermediate firmness, with intermediate number of seeds per fruit of medium size. Their fruits reach commercial maturity quickly and have short a shelf life.

On the contrary, Group II breeds have in common the production of fruits with high firmness, with intermediate to late physiological maturity and the production of a large number of seeds per fruit. Group IV breeds produce small round fruits with light peduncle cavity and calyxes medially adhered to them. Fruits reach physiological maturity in an intermediate time compared to other breeds but they maintain good commercial standards through longer storage periods.

Group III was integrated exclusively by 'Tamazula'. Even though the variety evaluated belonging to this particular race ('Tamazula 03') it also has erected growth habit, however, it was not included in Group I, probably because the fruit and seed characteristics were more similar to those of Group IV, since this variety produces medium size purple fruits containing small seeds in an intermediate number per fruit, with calyxes completely closed around the fruit, strongly pigmented and with intermediate adherence to it. This variety has late flowering but an intermediate period to reach commercial maturity and a long storage life.

Table 3. Size of the alleles (bp) carried by twelve husk tomato reference varieties.

Primer	PS†	P	DM	ML	MR	MZ	RN	CH	YH	SL	TC	TA
PI01	1772	2656	2656	2656	2656	2656	2656	2656	2656	2656	1719	1719
	1099	1719	1678	2189	1678	2243	1678	1678	2298	2243	1061	1113
	913	1287	1086	1719	896	1719	896	1113	1678	1719	874	896
	738	1086	896	1287		1086		1086	1086	1086		738
	458	896		1086		896			896	896		
				896								
PI02	1359	1683	1644	1644	1683	1890	1847	1847	1644	1606	1606	1644
	1029	1334	1334	1334	1365	1683	1644	1644	1365	1303	1303	1303
	843	1215	1187	1215	1187	1334	1334	1334	986	1187	986	986
	710	1009	986	1009	1009	1215	1009	1187	837	986	818	799
	597	818	818	837	818	1009	837	1009	695	781	679	679
	537	728	695	712	712	799	695	818		712		
	452					695		712				
	338											
PI03	1099	1665	1665	2123	1588	2123	1626	2061	1588	1626	1588	2123
	866	1410	1061	1965	1036	1874	1410	1588	1410	1345	965	1626
	673	1061	661	1626	661	1588	1061	1410	1036	818	661	1377
	597	661		1410		1223	818	1087	818	677		1036
	509			780		988	677	661	677			661
				661		661						
PI04	1099	1898	2123	2844	1658	3461	3664	2477	2053	2102	2102	1586
	729	705	1814	2600	1385	2659	2719	1662	1513		1742	1254
	516		1550	2076	1057	2221	2172	1197	1284		1411	969
			1324	1585	945	2030	1855	925	1040		1090	682
			705	1385	674	1515	1295	651	903		803	145
				690		1266	789	484			138	
ISSR01	978	1105	1185	1924	1080	1880	1880	1837	1754	1754	1837	1795
	400	877	1105	1675	800	1637	1675	1675	1212	1185	1637	1299
	340	782	940	1563	680	1105	1425	1185	1055	1055	1330	1158
		540	838	1080	553	857	1158	1055	665	877	1158	1055
		429	729	940	391	620	1055	838	340	592	1055	857
			566	782		391	819	492		528	696	696
			481				680	382		391	566	553
			400				391				373	382
ISSR02		1392	707	1425	1757	3817	1799	2349	1493	2897	1493	1717
		1155	615	1210	1425	1458	1564	1493	1298	1929	1155	1564
		1028	510	1102	1077	1182	1052	1128	1182	1528	958	1210
		833		1004	560	1077	853	691	1052	1238	833	
		560		833	465	741	724	573	936	1128	724	
				587		587	587		777	853	630	
									547			

†P=Potrero, PS=Puebla SM3, RN=Rendidora, S=Salamanca, DM=Diamante, MZ=Manzano Tepetlixpa, MR=Morado R,YH=Yema de Huevo, CH=CHF1 Chapingo, ML=Milpero Tetela, TC=Tecozautla 04, TA=Tamazula SM3.

Table 3. Size of the alleles (bp) carried by twelve husk tomato reference varieties. (*cont.*)

Primer	PS†	P	DM	ML	MR	MZ	RN	CH	YH	SL	TC	TA
ISSR03	1144	1212	2177	1212	1212	1304	2124	1438	1510	1212	1369	1336
	889	904	1473	950	927	1183	1547	1183	1242	997	1127	1154
	700	569	1183	744	841	950	1212	904	973	861	820	950
	613		841	555	744	820	973	542	781	709	569	762
	523		709		569	555	861	457	659	555		569
	452		569		480	424	692		529	468		468
	376		457				542		468			
							468					
ISSR04	1359	1791	1881	1835	1438	1927	1975	1975	1975	1975	2073	2120
	1114	1404	1404	1438	1155	1510	1474	1548	1548	1586	1586	1625
	963	1183	1155	1155	862	1183	1213	1243	1273	1273	1305	1337
	821	974	998	998	727	905	883	1073	1073	950	927	974
	700	841	821	862	628	745	763	905	883	821	801	628
	530	763	745	542	556	542	556	782	745	583	598	
		529	542					569	598			
ISSR05	1183	2498	2498	2498	2498	2498	2498	2498	2498	2591	2591	2498
	950	1472	1472	1437	1508	1437	1472	1508	1472	1508	1472	2128
	628	1099	974	974	1183	998	998	1272	998	1303	1154	1472
		974	782	821	998	821	821	998	821	1154	974	1154
		763	644	644	801	660	660	821	660	998	821	998
		644		517	644		505	660		660	644	821
								517				660
ISSR06	1893	2268	2351	2351	2268	2351	2351	2268	1697	2187	2268	2351
	1594	1697	1697	1697	1697	1697	1637	1637	1270	1637	1697	1637
	1433	1270	1270	1270	1317	1225	1366	1317	793	1270	1270	1225
	1029	1022	1099	1022	986	1022	765	1022	474	765	1099	950
	889	917	884	765	765	765	483	765	433	513	853	686
	655	765	738	507	513	487	445	507	375	491	765	478
	490	491	507	462	466	445	383	449		400	507	400
	429	445	474	412		387		408			478	
	366	404	437								404	
	324		367									
ISSR07	1460	1745	685	1745	1000	1706	1745	1706	1397	1397	1397	1366
	1000	1460	469	1493	801	1460	1397	1428	957	1000	670	1093
	783	1069	402	1222		1169	1118	1143	429	685	449	502
	685	819		1000		1000	525	978		548		
	525			801		701		685		491		
				685		480		491				
ISSR08	2128	1624	1624	1941	1709	2042	1666	1666	1709	1753	1753	1753
	816	1504	1504	1624	1504	1624	976	927	1001	1624	1583	1001
	572	859	859	881	1053	904	881	756	617	1027	904	529
	432	491	516	491	859	737	543	543	516	881	529	
					586	529				601		
					478					516		

†P=Potrero, PS=Puebla SM3, RN=Rendidora, S=Salamanca, DM=Diamante, MZ=Manzano Tepetlixpa, MR=Morado R,YH=Yema de Huevo, CH=CHF1 Chapingo, ML=Milpero Tetela, TC=Tecozautla 04, TA=Tamazula SM3.

Table 3. Size of the alleles (bp) carried by twelve husk tomato reference varieties. (*cont.*)

Primer	PS†	P	DM	ML	MR	MZ	RN	CH	YH	SL	TC	TA
ISSR10	938	1319	1350	1382	1382	1350	1382	1382	1382	1382	1414	1783
	770	1022	1022	1175	1046	1046	1230	1230	1096	1096	1121	1481
	597	849	869	1022	889	910	910	910	932	932	932	976
	512	510	522	910	586	774	739	534	369	756	573	792
	350	352	344	722	498	600	643	360		614		614
				534	360	360	522			369		395
							360					
UBC811	1143	1486	1451	1451	1966	1557	1451	1418	1071	1418	1451	1966
	938	1071	1096	1096	1261	1096	1096	1096	809	1176	1022	1670
	792	848	809	809	1022	828	809	809	484	1022	809	1451
	737	507	507	507	809	641	507	484		809	484	1122
	549				507	495				495		809
	519											626
	370											484
												318
UBC822	1095	1702	1956	2002	1742	2114	2192	2192	2192		2192	2002
	826	1382	1742	1825	1148	739	1825	1867	1230		1588	1481
	589	1096	1096	1414	600	586	1447	1121	658		1230	1319
		830	600	910		476	1096	910	487		1096	1121
		586	433	600			889	658			954	830
		433					658				658	673
UBC823	1168	1771	1740	2629	1740	2671	3512	1618	1618	1837	1804	1804
	965	1504	1504	1771	1147	1589	1589	1211	1233	1589	1647	1647
	718	1147	1168	1618		1326	1190			1233	1233	1256
	549			1147		1168						
	457											
UBC835	784	3393	3131	3393	1862	3000	2868	2868	3000	2868	3000	2868
	577	1826	1780	1488	1826	1862	1922	1862	1910	1922	1691	1922
	482	1450	1488	805	708	1780	1780	1780	1735	1735	1450	1735
	337	826	963	708	577	1378	1488	1450	1450	1414	805	1414
	261	708	805	548	482	826	963	963	805	826	690	805
		495	708	482	328	708	826	784	708	726	470	690
		413	548	320		562	726	690	562	470	422	548
		320	482			482	577	548	470	320	312	470
			424			413	482	482	304			312
			320			320	422	422				
							320	320				
UBC836	778	1537	1537	15372	1571	1571	1503	1318	1318	1606	2254	1571
	682	1234	1289	1289	1289	1318	1347	1035	1035	1181	13475	1347
	490	1130	1105	1058	1155	1013	1058	697	712	1058	1155	1081
	361	1013	991	697	1013	682	697	459	469	697	1058	728
		667	682	459	682	449	459			469	697	512
		449	439		469						469	

†P=Potrero, PS=Puebla SM3, RN=Rendidora, S=Salamanca, DM=Diamante, MZ=Manzano Tepetlixpa, MR=Morado R,YH=Yema de Huevo, CH=CHF1 Chapingo, ML=Milpero Tetela, TC=Tecozautla 04, TA=Tamazula SM3.

Table 3. Size of the alleles (bp) carried by twelve husk tomato reference varieties. (*cont.*)

Primer	PS†	P	DM	ML	MR	MZ	RN	CH	YH	SL	TC	TA
UBC841	858	2441	2497	2497	2229	2441	2555	2441	877	939	2613	2179
	443	2082	2179	2179	1352	2179	2130	918	715	748	939	939
	345	898	1415	1383	1127	1352	1415	715	570	464	748	732
		699	898	898	898	1153	939	454		370	464	464
		454	668	668	699	918	715	361			370	370
		353	434	454	434	699	454	294				
			345	345	353	443	353					
						361						
UBC844	2323	1207	1234	1207	1291	2018	2120	1973	1129	1104	1056	1056
	1233	863	1032	1056	1104	1104	1320	1207	755	772	772	755
	1049		844	844	826	826	1080	1010			516	
	917			577		552	826	807				
	749						604	552				
	499											
UBC848	1392	1974	1320	1292	1264	1712	1349	1320	1379	1379	1440	1349
	1049	1320	1040	1018	1040	1349	1040	1063	1110	1110	1349	1110
	834	1040	802	820	8382	1063	838	838	856	856	1110	894
	729	802			704	820		768	768	785	856	785
						736					785	
UBC873	1615	2119	2222	2174	2119	2170	1751	2170	2069	2119	2276	
	1289	1630	2020	1973	2020	2069	1347	2069	1670	1710	2170	
	10157	1315	1630	1670	1670	1518	1036	1670	1347		1751	
	664	965	1284	1315	1347	1284	857	1284	1036		1012	
	559	797	1012	1012	1012	1036	675	1036	836		675	
		613	817	836	836	836		836	708			
			675	659	691	675		691				

†P=Potrero, PS=Puebla SM3, RN=Rendidora, S=Salamanca, DM=Diamante, MZ=Manzano Tepetlixpa, MR=Morado R,YH=Yema de Huevo, CH=CHF1 Chapingo, ML=Milpero Tetela, TC=Tecozautla 04, TA=Tamazula SM3.

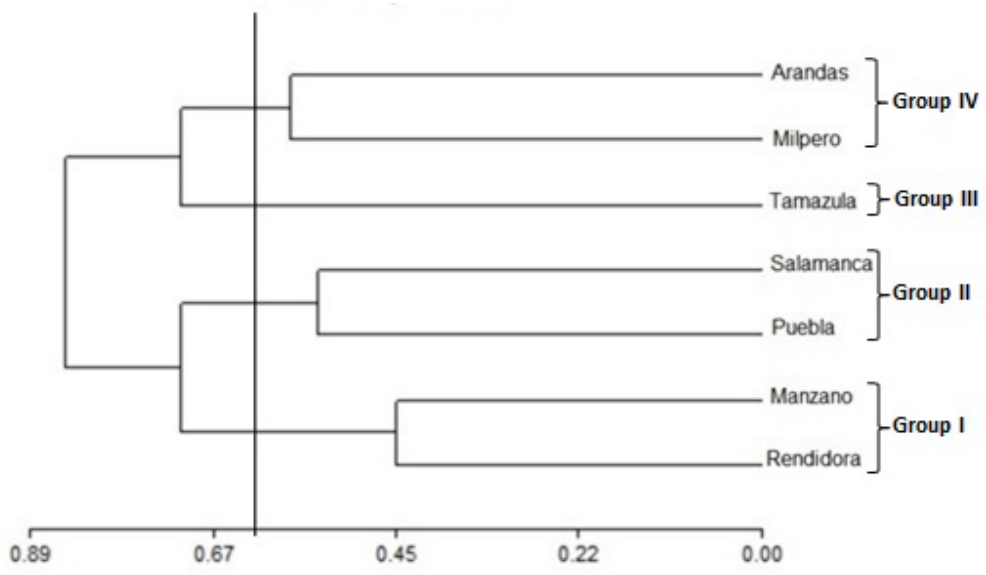

Figure 1. Dendrogram generated from Jaccard's distance (1 - S) for seven breeds of husk tomato.

Conclusions

The results of this study provide genomic DNA fingerprints of husk tomato reference varieties that complement morphological characterizations available for the registration of new varieties of this crop. ISSR markers showed genetic variability and are found to be powerful tools for estimating genetic similarities and diversity among and within husk tomato varieties. The genetic relationships presented among breeds are useful for future breeding programs through selection of genetically divergent parents.

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