Study of the genetic diversity of tomato (*Solanum* spp.) with ISSR markers[1]

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10.1590/0034-737X202067030005

ABSTRACT

In this study 55 tomato genotypes from different geographical regions were evaluated with ISSR markers (Inter Simple Sequence Repeats). The seven ISSR primers originated 63 amplified bands, of which 90.48% were polymorphic. The cluster analysis based on the Nei-Li similarity coefficient using the average genetic clustering method (UPGMA) revealed the conformation of five clusters at a level of similarity of 72%. The ISSR technique did not discriminate tomato genotypes according to the species or region of provenance. The structure analysis and the dendrogram did not reveal a genetic structure in the population evaluated. The genotypes of the species of *S. pimpinellifolium*, *S. l. cerasiforme*, *S. lycopersicum* and *S. peruvianum* were found consistently grouped, showing a close genetic relationship among them. A high genetic variation among the individuals within each of the groups formed was suggested by the AMOVA. The ISSR markers were effective in assessing the genetic diversity and structure of populations of tomato genotypes. The high genetic variability found in this study indicates the valuable genetic potential present in tomato germplasm, especially of wild species, which could be used for future breeding programs of the species.

Key words: wild tomato; germplasm; genetic variability; population structure.

INTRODUCTION

The tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) is a major vegetable crop in the world (Rothan *et al.*, 2019). Wild species related to tomato are native to Bolivia, Perú, Ecuador, Chile, Colombia, including the Galapagos Islands (Lucatti *et al.*, 2013). These species disperse indiverse habitats from the arid Pacific coast at sea level and up to 3,300 meters above sea level in the mountainous regions of the Andes (Nakazato & Housworth, 2011).

The genetic base of the cultivated tomato has been narrowing due to the continuous selection processes caused by domestication and genetic improvement. (Miller & Tanksley, 1990). The inclusion of wild species in breeding programs is necessary, since they are sources of variability and possess valuable genes that can be introduced to cultivated species (Bergougnoux, 2014). Knowledge of the genetic diversity of a germplasm is of utmost importance for plant breeding activities that seek the development of varieties with high quality, yield, resistant to biotic and abiotic factors, among other characteristics of economic importance (Herison *et al.*, 2018).

DNA markers allow a more complete evaluation of the genome of a certain species, are little affected by the environment, reveal polymorphism at the DNA level, hence they are a widely used technique in characterizing genetic diversity (Ansari *et al.*, 2016).

Intersimple sequence repeat markers (ISSR) are a variant of the microsatellite assay, and combine the advantages of other types of markers such as AFLP, RAPD and SSR (Henareh *et al.*, 2016). It is a PCR-based technique and

Submitted on May 29th, 2019 and accepted on May 11th, 2020.

[1] This work is part of the first author's Undergraduate Dissertation.

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uses primers generally 16 to 25 bp in length, which can be di-, tri-, tetra- or penta-nucleotides (Zietkiewicz *et al*., 1994). Despite being a dominant marker, it has been widely used for studies of genetic diversity, phylogeny, genomics and evolutionary biology (Muñoz *et al*., 2008), and also because of the sequences to be amplified it is not necessary to have a preliminary knowledge of them (Zietkiewicz *et al*., 1994), shows high polymorphism in the material, it is better reproducibility than RAPD as well as technical simplicity. ISSR can be used for fast preliminary characterization of breeding material as well as for source of locus-specific molecular markers. This study aimed to examine the genetic diversity of tomato (*Solanum* spp.) using ISSR markers.

MATERIAL AND METHODS

Vegetable material

Fifty-five (55) tomato genotypes were evaluated from the germplasm bank of the University of Caldas. Representatives of the wild species *S. pimpinelifolium, S. esculentum* var. cerasiforme and *S. peruvianum*, as well as introductions from Colombia and two commercial controls were included (Table 1). The genotypes were established in the production system of hydroponic crops, located in the Botanical Garden of the University of Caldas, at a height of 2,130 m altitude, with an average temperature of 19 °C and a relative humidity of 75%.

Extraction and quantification of DNA

DNA extraction was carried out in the laboratory of Biotechnology and Tissue Culture of the University of Caldas. From each tomato plant, young leaves were taken, from which the total DNA was extractedby means of the protocol of Dellaporta *et al*. (1983). The quality and quantity of DNA obtained was performed with 0.8% agarose gels, run in 0.5X TBE buffer for 45 minutes at 80 volts and stained with GelRed™. DNA concentrations were determined by analysis in the Spectrograph NanoDrop™ 2000 spectrophotometers.

HPLC-type water was used to dilute the quantified DNA and bring it to a volumeof 100 µl to 10 ng / µl. Seven ISSR type primers (Technologies Inc.) were used, whose sequence is shown in table 2.

The amplification reaction consisted of 10 ng of genomic DNA, 0.2 mM of dNTPs, 1.5 mM of MgCl2, 2.0 µm of primer, 1X buffer and 1U of Taq Polymerase Bioline® in a final volume of 25 µl. The amplification was carried out in a Biorad Gradient 96 Well DNA Engine Thermal Cycler. The PCR cycles were started with an initial denaturation at 95 °C for 5 minutes; denaturation at 95 °C for 30 seconds, annealing at 50 °C (primers AG, CA and ACA), 55 °C (primer CCA-CT) and 58 °C (primer GT-CGA) for 45 seconds, an extension at 72 °C for 2 minutes, 37 cycles from the denaturation to extension and finally an extension at 72 °C for 7 minutes. In each amplification assay, a negative or blank control without DNA was included to check the absence of contamination.

The amplified products were separated by horizontal electrophoresis in 1.2% (w/v) agarose gels, run for 2 hours at 80 volts in 0.5X TBE buffer, using a DNA sequencin System, FB-SEQ- 3545. A weight marker of 100 bp DNA Ladder (Bioline®) was included to determine the size of the amplicons. The gels were stained with GelRed™ and visualized in a UV transilluminator for subsequent analysis in digitalized images.

Data analysis

A binary matrix of absence (zero) and presence (one) was constructed. The estimates of similarity were calculated with the method of Nei & Li (1979). A cluster analysis was made with the average linkage algorithm (UPGMA), generating the corresponding dendrogram using the statistical package NTSYS-pc version 2.0. The coefficient of cofenetic correlation was calculated to measure the distortion between the original distance matrix and the resulting dendrogram by means of the COPH and MXCOMP program of the NTSYS-pc package.

For each primer, the content of polymorphic information (PIC) was calculated based on the formula of Botstein *et al*. (1980) as follows:

$$PIC = 1 - \sum_{i=1}^{j} P_i^2 - 2 \sum_{i=j\neq 1}^{j} \sum_{j=1}^{i=1} P_1^2 - P_j^2$$

Where P_i y P_j are the frequencies of the i^{th} y j^{th} alleles at the locus.

An analysis of molecular variance (AMOVA) was carried out using the GenAlex program version 6.2 (Peakall & Smouse, 2012) with 999 permutations, in order to determine the differences between and within the groups formed.

The average heterozygosity (He) and the percentage of polymorphic loci, were obtained through the statistical package TFPGA (Tools for Population Genetic Analyses, version 1.3, Miller, 1997). The unbiased statistical f was determined with a confidence interval of 95%.

The population structure was examined employing a Bayesian analysis based on a grouping algorithm, with the program Structure v2.3.1 (Pritchard *et al*., 2000). The software estimates the natural logarithm of the probability Ln P (D) that a given genotype belongs of a given population (K). A mixing model with 200,000 interactions was used after a burnin period of 100,000. The number of clusters (K-value) was 2 to 10. For each cluster number (K) ten runs were made. The ΔK value was calculated to detect the most probable number of clusters (Evanno *et al*., 2005).

Table 1: Tomato genotypes used for molecular evaluation with ISSR markers

Genotype	Species	Provenance
IAC 416	*T. cereja rosado*	Brazil
IAC 426	*T. cherry Juliet*	Brazil
IAC 421	*T. cereja Alemán Vermelho*	Brazil
IAC 1622	*T. cereja 12*	Brazil
IAC 1621	*T. cereja Alemán 12*	Brazil
IAC 445	*T. cereja Jundiai*	Brazil
IAC 1688	*T."Lili"cereja*	Brazil
IAC 1685	*T. cereja 11B*	Brazil
IAC 391	*T. red cherry*	Brazil
IAC 420	*T. cereja*	Brazil
IAC 424	*T. cherry*	Brazil
IAC 1624	*T. cereja*	Brazil
IAC 1686	*Tomate cereza*	Brazil
LA 2076	*Tomate cereza*	California
LA 0168	*Tomate cereza*	California
LA 2131	*Tomate cereza*	California
LA 1546	*Tomate cereza*	California
LA 2710	*Tomate cereza*	California
LA 1705	*Tomate cereza*	California
LA 1480	*Tomate cereza*	California
LA 1334	*Tomate cereza*	California
IAC402	*T. cereja Alemán*	Brazil
LA443	*Tomate cereza*	California
IAC1684	*Tomate cereja 11A*	Brazil
IAC460	*T.cereja Perita TG0154*	Brazil
IAC418	Tomate cereja Lago Unicamp	Brazil
LA460	*Tomate cereza*	California
LA1623	*Tomate cereja "Mini Pepe"*	Brazil
LA424	*Tomate cereza*	California
IAC443	*Tomate cereja Milao*	Brazil
IAC451	*Tomate cereja 14A*	Brazil
LA2640	*Tomate cereza*	California
IAC401	*Tomate cereja "Rubi"*	Brazil
COLY 002	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 003	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 004	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 007	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 013	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 011	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 016	*Solanum lycopersicum var. cerasiforme*	Colombia
LA 416	*Solanum lycopersicum var. cerasiforme*	California
LA 1621	*Solanum lycopersicum var. cerasiforme*	California
Sweet Million	*Solanum lycopersicum*	Comercial
COLY 009	*Solanum lycopersicum var. cerasiforme*	Colombia
COLY 006	*Solanum lycopersicum var. cerasiforme*	Colombia
LA0420	*Solanum lycopersicum var. cerasiforme*	California
IAC1645	*Solanum lycopersicum var. cerasiforme*	Brazil
IAC1687	*Solanum lycopersicum var. cerasiforme*	Brazil
LA 445	*S. peruvianum*	California
LA 412	*S. pimpinellifolium*	California
LA 1685	*S. pimpinellifolium*	California
LA 1688	*S. pimpinellifolium*	California
LA1626	*S. pimpinellifolium*	California
LA1686	*S. pimpinellifolium*	California
Santa Clara	*Solanum lycopersicum*	Commercial

*** IAC:** Introductions from the Agronomic Institute of Campinas, Campinas, Brazil. ** **LA:** Introductions from the Tomato Genetics Resources Center (TGRC), University of California-Davis.

RESULTS AND DISCUSSION

The analysis of the 55 tomato genotypes (*Solanum* spp.) with the seven ISSR primers originated a total of 63 amplified bands for all population, of which 90,48% were polymorphic. The band average revealed by each primer was 14.29, ranging from seven for primer CGA and 10 for primers AG and ACA (Table 3).

The size of the amplified products varied between 100 and 1400 base pairs. The content of polymorphic information (PIC) ranged between 0,23 (CA) and 0,31 (CGA), with an average of 0,27. According to the classification of Botstein *et al*. (1980), the PIC value obtained in our study, five ISSR primers were moderately informative, and therefore very useful for studies of genetic diversity in tomato. The highest values of PIC were observed with primers AG, ACA and CGA. Primers CA and GT were not very informative.

Kaushal *et al*. (2017) found PIC values between 0,0 to 0,54 with an average of 0,27 in the evaluation of 25 tomato genotypes with 20 microsatellite markers. Likewise, Marin-Montes *et al*. (2019), found an average PIC value of 0,63 when evaluating the genetic diversity of 26 accessions of native Mexican tomatoes with 18 SSR molecular markers.

The expected heterozygosity average (He) was 0,37, which reveals a high value of genetic diversity in the evaluated tomato genotypes (Table 3). Primer CCA was the one that obtained the highest value of He corresponding to 0,41, followed by primers CGA (0,39), AG (0,39) and CA (0,38). The genetic diversity found is possibly due to the inclusion of wild genotypes of the species *S. peruvianum*, *S. pimpinellifolium* and *S. l.* var. *cerasiforme*, which present a great variation in terms of their morphological, physiological, and reproductive characters, among others. According to Peralta & Spooner (2005), the wide range of ecological conditions to which wild tomato species have adapted have contributed to their genetic diversity.

Table 2: Sequence of the ISSR primers used to determine genetic diversity in tomato (*Solanum* spp.)

Primer	Sequence (5' to 3')	Molecular weight (MW) g/mol
CCA	$DDB(CCA)_5$	5338,4
CGA	$DHB(CGA)_5$	5523,0
GT	$VHV(GT)_5G$	5620,5
AG	$HBH(AG)_7A$	5660,1
CT	$DYD(CT)_7C$	5310,9
CA	$DBDA(CA)_7$	5410,5
ACA	$BDB(ACA)_5$	5451,0

The following designations are used for the degenerated sites: H (A or T or C); B (G or T or C); V (G or A or C) and D (G or A or T).

Cultivated tomato is a highly autogamous species and exhibits a wide range of morphological diversity, whether in the size of the fruits, colors, shapes, quality, among other characteristics, but compared to other species related to the genus, it presents a low diversity, this due to intensive selection through domestication and breeding of improved varieties (Miller &Tanksley, 1990). However, Rick (1958) reported that depending on the region of origin different degrees of diversity are presented in tomato, being the Andean region where the highest levels of diversity occur, extending this phenomenon to species like *S. l. cerasiforme* which are mainly self-compatible and autogamous. Likewise, Ranc *et al*. (2008) reported that in different geographic regions the wild species of *S. pimpinellifolium*, which are mostly self-pollinated, have exhibited different levels of allogamy.

Ceballos-Aguirre *et al*. (2017), reported similar results, they found great genetic diversity (He = 0,6946) when evaluating 30 cherry tomato introductions with 36 microsatellite markers.

The average polymorphism percentage obtained was 90,63%, where primers CT, AG, CGA and CCA showed 100% polymorphism, while primer CA generated the lowest value of 66.67%. Hernández-Ibánez *et al*. (2017), using 29 ISSR molecular markers in elite tomato lines, found a polymorphism of 77.9%. Gonias *et al*. (2019) revealed a high level of genetic diversity (He= 0,73) and a PIC value of 0.74, in the assessment of tomato genotypes with nine SSR. Similarly, Kandel *et al*. (2019), found great genetic diversity when they evaluated tomato genotypes with SNPs. High values of polymorphisms have also been found with RAPD markers (Herison *et al*., 2018).

These results contrasted with those of Aguilera *et al*. (2011) who, when using ISSR molecular markers to evaluate the genetic variability of 96 tomato accessions, found a lower level of polymorphism corresponding to 34%, with an average of 5,3 polymorphic bands per primer.

The fixation index (Fst) showed a value of 0,09. According to Wright (1978), this value indicates a moderate genetic differentiation. This is possibly due to the genetic flow between the genotypes evaluated. According to Levy *et al*. (1978), in works carried out with *Solanum lycopersicum* they mention that although the tomato is a self-pollinated species, cross-pollinated percentages ranging from 2 to 4% under normal growing conditions have been observed, but could increase in certain genotypes or in conditions of high temperature that tend to pronounce the exsertion of the style.

In contrast, Ceballos-Aguirre *et al*. (2017) obtained a higher Fst value of 0,3474 showing a great genetic differentiation in the cherry tomato introductions evaluated. Marin-Montes *et al*. (2019), obtained inbreeding and fixation coefficients close to 1. This indicated the

presence of inbreeding and wide differences within of the identified tomato groups. Similar results were found by Henareh *et al.* (2016), in their study of diversity in tomato with 14 ISSR markers.

Clustering analysis

The UPGMA average genetic clustering analysis grouped the genotypes into five groups at a level of similarity of 72%, with a value of r = 0,98, showing an excellent correlation between the dendrogram and the values of the initial distances (similarity matrix) (Figure 1).

Group I is the one that covers the largest number of genotypes evaluated (33), which corresponds to 60% of the total population; in group II, 10 genotypes were grouped, representing 22%. Both groups III and IV were represented by two genotypes (3.64%), and in group V gathered eight genotypes, representing 14,54%. As can be seen in the dendrogram, the ISSR technique did not discriminate tomato genotypes according to the species or region of origin. However, the genotypes collected in Colombia, identified as Coly, tended to unite in a single group with a coefficient of similarity of 0,78. These results

Table 3: Statistics of genetic diversity (Nei, 1983) and estimated PIC from the ISSR data evaluated in tomato genotypes

Primer	No. Bands	% loci poli 95%	Estimated He	Fst	PIC
CT	9	100	0,36	0,11	0,25
GT	9	77,78	0,29	0,07	0,24
AG	10	100	0,39	0,11	0,29
ACA	10	90,00	0,35	0,09	0,29
CA	9	66.67	0,38	0,09	0,23
CGA	7	100	0,39	0,03	0,31
CCA	9	100	0,41	0,11	0,26
Average		90,63	0,37	0,09	0,27

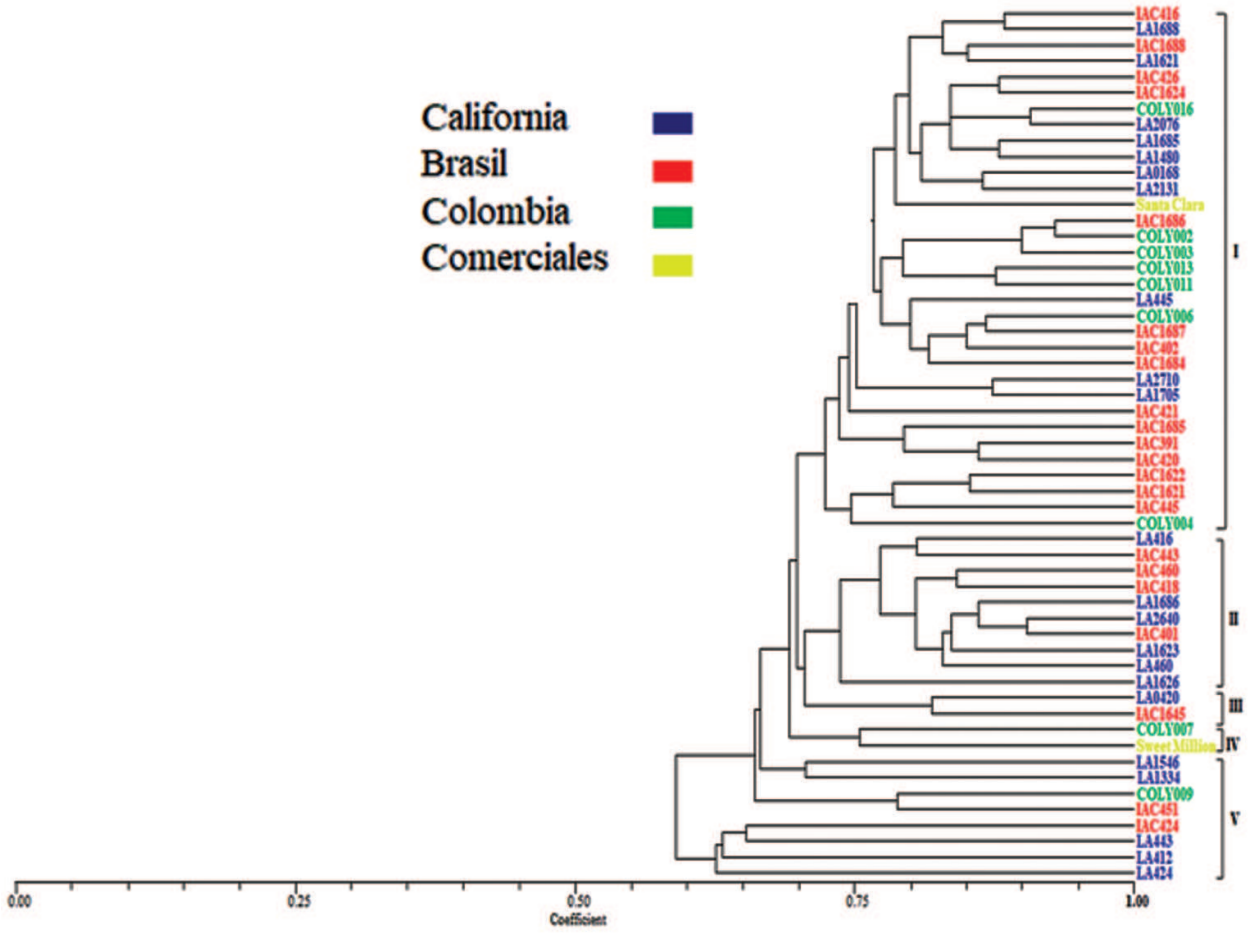

Figure 1: Dendrogram of 55 genotypes of *Solanum* spp. based on the Nei Li similarity coefficient and the UPGMA algorithm.

agree with those of Ceballos-Aguirre *et al*. (2017), where the microsatellite markers used did not group the tomato introductions according to the geographical area where they were collected. Although genotypes with the same geographic origin tend to group together, introductions from different regions were also closely related, regardless of their geographical origin, so they were grouped in the same cluster. This suggests the high degree of genetic diversity of the genotypes evaluated, consistent with the value of He found.

According to the Molecular Variance Analysis (AMOVA) based on the PhiPT values, it was found that the differentiation between the groups formed was significant ($P < 0.001$) and explained 5% of the total genetic variance, whereas the component contributed 95% due to the differences of the individuals within each group (Table 4).

Population structure analysis

The results obtained by the structure analysis corresponded well with the groups obtained in the cluster analysis, where the 55 tomato genotypes were grouped into four genetic groups, taking into account the ΔK value (Evanno *et al*., 2005). The groups obtained on average a membership coefficient (Q value) of 36% (Figure 2). It can be observed that most of the genotypes were grouped in groups 1 (color red) and 3 (color blue), with a total of 10 individuals respectively; meanwhile, eight individuals were grouped in group 2 (color green) and seven individuals in group 4 (color yellow). The rest of genotypes constituted the subgroup mix, which corresponds to 20 genotypes. The probability of membership of an individual in each group was considered greater than 70%.

The structure analysis did not reveal a genetic structure in the population evaluated, which is evidenced by the distribution of genotypes of different species along the groups formed, that is, genotypes of the species of *S. pimpinellifolium*, *S. l. cerasiforme*, *S. lycopersicum* and *S. peruvianum* consistently grouped, showing a close genetic relationship among them. The above is consistent with the Fst value found of 0,09, which indicates a moderate genetic structure. Likewise, the accessions did not show grouping patterns according to the geographical area of origin. This is possibly due to some degree of genetic flow between the tomato genotypes evaluated, due to interspecific crossings, processes of introgression of alleles from wild species to cultivated ones, the type of reproduction characteristic of each species, anthropogenic events, among others.

Table 4: Analysis of molecular variance AMOVA for the groups formed of *Solanum* spp. using ISSR markers

Source	Degrees of freedom	Sum of squares	Mean square	Components of variance	% of variance
Among groups	3	49,541	16,514	0,502	5%
Within groups	51	536,313	10,516	10,516	95%
Total	54	585,855		11,018	100%

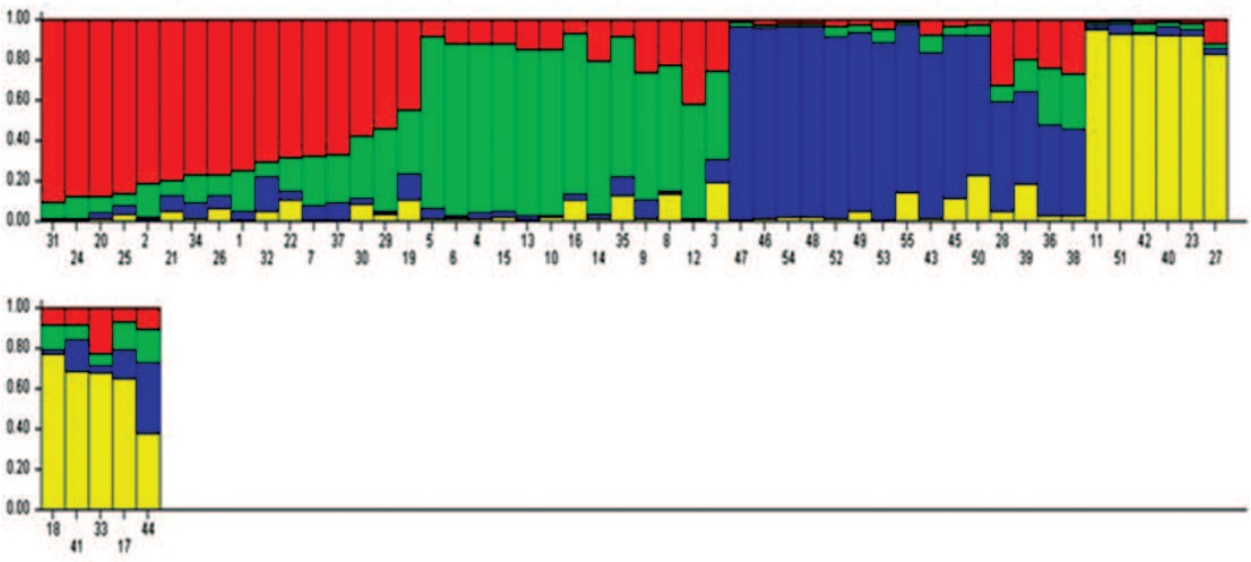

Figure 2: Graphical representation of the results of the analysis of the genetic structure of four tomato populations (*Solanum* spp.) Using seven ISSR microsatellite markers. Calculated with software structure v. 2.3.4.

S. lycopersicum var. *cerasiforme* presents both plants with flowers of inserted and exserted stigma and *S. pimpinellifolium* has always exserted stigmas, which could favor interspecific crossing and allogamy between the two species (Morales-Palacio *et al*., 2016).

Cherry tomato accessions have been observed to show great genetic diversity and an interesting feature is that they have an intermediate fruit size between *S. pimpinellifolium* and cultivated tomato, hence botanists have stated that cherry tomato accessions are plants wild or constitute a possible genetic combination between wild and cultivated germplasm (Peralta & Spooner, 2007).

According to Ranc *et al*. (2008), domesticated and wild tomatoes have developed as a complex of species through an intense hybridization process, which has been demonstrated through molecular analysis of the genetic structure of a large number of not only wild accessions of *S. pimpinellifolium* and cherry tomato but also from cultivated accessions. For years, the *S. lycopersicum* L. var. cerasiforme expression has been employed in a wide variety of forms featuring cherry-sized fruit that included escaped (feral) *Solanum lycopersicum* L. var. lycopersicum worldwide and hybrid offspring between *Solanum lycopersicum* L. var. lycopersicum and wild species (Ranc *et al.*, 2008). However, recent studies such as those carried out by Blanca *et al.* (2015) have shown that the species *S. lycopersicum* L. var. cerasiforme is considered an authentic taxonomic group.

CONCLUSIONS

In this study, ISSR molecular markers were found to be a useful tool for examining the genetic diversityand also to establish the relationship of tomato genotypes from different geographical areas. However, for a more complete and detailed assessment of genetic diversity, a combination of different molecular markers is indispensable.The high genetic diversity found could be exploited for future breeding programs of the species, since the inclusion of parental genotypes with a wide genetic variability to obtain hybrids is of great relevance for plant breeders, since they will contribute a supply of allelic variants that can generate new favorable combinations for the characteristics of economic importance in tomato.

Knowledge of the distribution of genetic variation within and between populations, as well as population structure, is of utmost importance for germplasm management, crop improvement and genetic mapping. This information could be useful for a better understanding of the phylogenetic relationships of the cultivated tomato and its related wild relatives, elucidate the mechanisms of dispersion and domestication, among others; also for the formulation of strategies of conservation and rational use of the phytogenetic resources of *Solanum* spp.

REFERENCES

Aguilera JG, Pessoni LA, Rodrigues GB, Elsayed AY, Silva DJH & Barros EG (2011) Variabilidad genética por marcadores ISSR en tomate (*Solanum lycopersicon* Mill.). Revista Brasileira de Ciências Agrárias, 6:243-252.

Ansari A, Sikarwar P, Lade S, Yadav H & Ranade SH (2016) Genetic diversity clusters in germplasm of Cluster Bean (*Cyamopsis tetragonoloba* L., Taub), an important food and an industrial legume crop. Journal of Agricultural Science and Technology, 18:1407-1418.

Dellaporta S, Wood J & Hicks J (1983) A plant DNA minipreparation: version II. Plant Molecular Biology Reporter, 1:19-21.

Bergougnoux V (2014) The history of tomato: from domestication to biopharming. Biotechnology Advances, 32:170-189.

Blanca J, Montero-Pau J, Sauvage C, Bauchet G, Illa E, Díez MJ, Francis D, Causse M, van der Knaap E & Cañizares J (2015) Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. BMC Genomics, 16:257.

Botstein D, White RL, Skolnick M & Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American journal of human genetics, 32:314-331.

Ceballos-Aguirre N, López W, Orozco-Cárdenas M, Morillo CY & Vallejo-Cabrera FA (2017) Use of microsatellites for evaluation of genetic diversity in cherry tomato. Bragantia, 76:220-228.

Evanno G, RegnautS & Goudet J (2005) Detecting the number of clusters of individuals using the software structure: A simulation study. Molecular Ecology, 14:2611-2620.

Gonias ED, Ganopoulos I, Mellidou I, Bibi AC, Kalivas A, Mylona PV, Osanthanunkul M, Tsaftaris A, Madesis P & Doulis AG (2019) Exploring genetic diversity of tomato (*Solanum lycopersicum* L.) germplasm of genebank collection employing SSR and SCAR markers. Genetic Resources and Crop Evolution, 66:1295-1309.

Henareh M, Dursun A, Abdollahi-Mandoulakani B & Haliloðlu K (2016) Assessment of genetic diversity in tomato landraces using ISSR markers. Genetika, 48:25-35.

Hernández-Ibáñez L, Sahagún-Castellanos J, Rodríguez-Pérez JE & Peña-Ortega MG (2017) Prediction of fruit yield and firmness of tomato hybrids with BLUP and RR-BLUP using ISSR molecular markers. Revista Chapingo Serie Horticultura, 23:21-33.

Herison C, Sutjahjo SH, Sulastrini I, Rustikawati R & Marwiyah S (2018) Genetic diversity analysis in 27 tomato accessions using morphological and molecular markers. Agrivita - Journal of Agricultural Science, 40:36-44.

Kandel DR, Bedre RH, Mandadi KK, Crosby K & Avila CA (2019) Genetic Diversity and Population Structure of Tomato (*Solanum lycopersicum*) Germplasm Developed by Texas A&M Breeding Programs. American Journal of Plant Sciences, 10:1154-1180.

Kaushal A, Singh A & Singh AJ (2017) Genetic diversity in tomato (*Solanum lycopersicum* L) genotypes revealed by simple sequence repeats (SSR) markers. Journal of Applied and Natural Science, 9:966- 973.

Levy A, Rabinowitch HD & Kedar N (1978) Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica, 27:211-218.

Lucatti AF, Van Heusden AW, De Vos RCH, Visser RGF & Vosman B (2013) Differences in insect resistance between tomato species endemic to the Galapagos Islands. BMC Evolutionary Biology, 13:175.

Marin-Montes IM, Lobato-Ortiz R, Carrillo-Castañeda G, Rodríguez-Pérez JE, García-Zavala JJ & Velasco-García AM (2019) Riqueza alélica de poblaciones nativas de jitomate (*Solanum lycopersicum* L.) para el mejoramiento genético. Agrociencia, 53:355-370.

Miller MP (1997) Tools for population genetic analysis (TFPGA), 1.3: A windows program for the analysis of allozyme and molecular population genetic data. CD-ROM. (Compute software distributed by the author).

Miller JC & Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theoretical and Applied Genetics, 80:437-448.

Morales-Palacio MN, Morales-Astudillo ÁR, Artiles-Valor A, Milián-García Y & Espinosa-López G (2016) Caracterización fenotípica y genética de cuatro especies silvestres del género *Solanum*, sección *Lycopersicon*. Cultivos Tropicales, 37:109-119.

Muñoz JE, Morillo CA & Morillo CY (2008) Microsatélites amplificados al azar (RAM´s) en estudios de diversidad genética vegetal. Acta Agronomy, 57:219-226.

Nakazato T & Housworth EA (2011) Spatial genetics of wild tomato species reveals roles of the Andean geography on demographic history. American Journal of Botany, 98:88-98.

Nei M (1983) Estimation of genetic distance and phylogenetic trees from DNA analysis. Proceedings of 5th World Congress on Genetics Applied to Livestock Production, 21:405-412.

Nei M & Li WH (1979) Mathematical model for studying genetic variation in terms of restricción endonucleasa. Proceedings of the National Academy of Sciences, 76:5269-5273.

Peakall R & Smouse P (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28:2537-2539.

Peralta IE & Spooner DM (2005) Morphological characterization and relationships of wild tomatoes (Solanum L. Section Lycopersicon) Monographs in Systematic Botany from the Missouri Botanical Garden, 104:227-257.

Peralta IE & Spooner DM (2007) History, origin and early cultivation of tomato (Solanaceae). In: Razdan MK & Mattoo AK (Eds.) Genetic improvement of Solanaceous crops. Beltsville, Science Publisher. p.01-24.

Pritchard JK, Stephens M & Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155:945-959.

Ranc N, Muños S, Santoni S & Causse M (2008) A clarified position for *Solanum lycopersicum* var. cerasiforme in the evolutionary history of tomatoes (*Solanaceae*). BMC Plant Biology, 8:130.

Rick CM (1958) The role of natural hybridization in the derivation of cultivated tomatoes of western South America. Economic Botany, 12:346-367.

Rothan C, Diouf I & Causse M (2019) Trait Discovery and Editing in Tomato. Plant Journal, 97:73-90.

Wright S (1978) Evolution and the genetics of populations: Volume 4: Variability within and among natural populations. Illinois, University of Chicago Press. 590p.

Zietkiewicz E, Rafalski A & Labuda D (1994) Genome fingerprinting by simple sequence repeats (SSR)- anchored polymerase chain reaction amplication. Genomics, 20:176-183.