Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):933-939

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Agronomic and molecular characterization of four genotypes of *Solanum betaceum*. Cav tolerant to *Colletotrichum acutatum* from the region of Patate in Ecuador

Ibeth Bolagay[1], Darwin Rueda Ortiz[1]*, Norman Soria Idrovo[1], Maritza Tulcan[1], Bangeppagari Manjunatha[1], Rajesh R. Kundapur[2], Sikandar I. Mulla[3] and Maddela Naga Raju[4]

[1]*Department of Life Sciences, Universidad de las Fuerzas Armadas-ESPE, Sangolquí, Quito, Ecuador, South America*
[2]*Department of Zoology, University of Pune, Pune, India*
[3]*Department of Biochemistry, Karnatak University, Dharwad, Karnataka, India*
[4]*Department of Environmental Engineering, Universidad Estatal Amazonica, Puyo, Ecuador, South America*

ABSTRACT

This research was performed simultaneously in the greenhouse and in the laboratory with the aim of agronomic and molecular characterization of four genotypes of Solanum betaceum Cav. tolerant to Colletotrichum acutatum of Patate area. Further to determine the phenotypic characteristics and genetic variability will allow segregating from productive to non productive plants. 40 agronomic descriptors were used to characterize four strains of the tree tomato from different locations like Leitillo, Los Andes and El Triunfo of Patate region. The result of the Ward hierarchical clustering revealed the existence of four genetic groups and among them plant with the code 138L2S1 was found to be a possible promising material with desirable characteristics: fruit size, earliness, production and tolerance to pests and diseases. In the laboratory phase or molecular characterization of four strains of tree tomato a molecular technique called Random Amplification of Polymorphic DNA (RAPD) was used, with the coefficient of Jaccard's similarity matrix for different genotypes was calculated and determined genetic variability through dendrogram analysis.

Keywords: Tree tomato, Descriptors, RAPD, Variability, Genotype.

INTRODUCTION

The tree tomato is a native fruit to the eastern part of the Andes of Peru, Ecuador and Colombia. This crop is traditional and ancient in Ecuador in areas such as Patate and Banos, and also well known and cultivated throughout the Ecuadorian highlands [1]. The fruits of this species are edible, slightly acidic flavor and rich in Vitamins (provitamin A, vitamin C and B_6) and minerals (iron and potassium) due to this its cultivation and consumption is very popular in the tropical and subtropical Andean region [2]. Its cultivation has been increased in the past 15 years for its good quality, quick production and low market price when compared to other fruits. The cultivation has spread to worldwide, it is because of free trade of export of Andean fruit mainly tree tomato to Europe and other parts of the world [3].

In the Ecuador tomato growing tree is mainly cultivated by small and medium producers who have increased in the last decade the harvested area of 820 ha in the eighties to 2200 ha in 1995, while the census of 2011 reported the increase of 4462 ha. The area has been increased gradually year by year. But on the contrary the yield was found to be decreased from 15.3 to 8.1 ton / ha. The main cause behind the low yield is due to pests and diseases [4]. Despite having an increased demand in the market, the cultivation has not managed adequately and this is due to the lack of elite planting materials. In most cases, the planting is carried out with genotypes selected by the producers, with

materials having a narrow genetic base and are heterogeneous [5]. In Ecuador the expansion of its production is limited by factors such as low quality of the fruit (heterogeneity, health problems) use of unsuitable varieties or replacement of local varieties of material from other origins. Even though it is a subsistence crop and is listed as marginalized species, it is not considered under conservation programs and enhancement of plant genetic resources [6].

The understanding of genetic variability within the genotypes of tree tomato would help in designing guidelines for future molecular studies or breeding programs or conservation of this species. It also evaluates the genetic erosion and allows getting a proper use of the species and its conservation [7]. Morphological characterization is intended to protect the genetic resources that are usually lost by in the crop mismanagement either by replacing varieties originating of a region by improving varieties or destruction of mountain vegetation [8].

The need for agronomic and molecular characterization of *S. betaceum* will establish the genetic variability among genotypes which is essential for its use, conservation and in turn identify promising traits that will be useful for fruit growers and helps in solving agricultural problems related to production, adaptability and resistance to different pests and diseases. So this study significantly contributes to the knowledge of conservation of genetic resources and breeding of tree tomato, which is important for the development of this marginalized crop with high potential for Andean countries, especially in Ecuador.

EXPERIMENTAL SECTION

The research was conducted in two phases: field phase and laboratory phase.

Field phase

For agronomic characterization a total of 15 accessions of *S. betaceum* of three locations of the Patate region (Leitillo, Los Andes and El Triunfo) and all are tolerant to *Colletotrichum acutatum* were selected. All tree tomato plants were grouped into four genotypes (Table 1) as mentioned by Lucero and Novoa [9].

In Andean fruit, greenhouse the tomato plants were planted in a bed containing sterile soil. The pot holes had a dimension of 0.20 x 0.25 x 0.25 cm with a distance of 1.20 m between plants and the bed had the following dimensions: 1 x 20 m long. The total area of the assay was 32 m² and a total net area of 20 m². Concurrently, the standard procedures like weeding, hoeing, watering and phytosanitary measures were also performed. The incidence of pests (mainly for whitefly) and diseases were also monitored. The data were recorded from the beginning of flowering until the first harvest and the agronomic features were recorded as described by Albornoz [8]. There were 40 different agronomic descriptors were considered in the present study. All the agronomic data are evaluated through the statistical methods. To set the variable of the agronomic characteristics of the tree tomato (*S. betaceum* Cav.) the coefficient of variation was used [10].

Laboratory phase

The Random amplified polymorphic DNA (RAPD) assay was carried out in the laboratory of Agricultural Biotechnology to all the 15 tree tomato DNA samples.

Isolation of genomic DNA from tree tomato

The total genomic DNA was extracted separately from tree tomato leaves using Sorbitol-cetyltrimethyl ammonium bromide (CTAB) method [11], with slight modifications and with column based Pure Link Plant Total DNA Purification Kit, the extraction was performed as per the kit insert. The genomic DNA, thus isolated was separated by electrophoresis on 0.8% agarose gel in 1x TBE buffer at 120 V for 2 h. The gels were stained with ethidium bromide (0.5µg/ml) and visualized by a UV transilluminator. The 2kb DNA ladder (Invitrogen) was used as a marker. Subsequently, the gel was photographed.

Random amplified polymorphic DNA (RAPD) analysis

The RAPD performed in the present investigation was based on the method of the Wisconsin University, UW-Madison, with slight modifications. The primers belong to the series OPA, OPAC, OPAN, OPAM, OPR, OPW and OPAA (Operon Technologies Inc., USA) were used for amplification. The primers and their nucleotide sequences at 5'- 3' direction are given in Table 2**.** The recipe for Polymerase chain reaction (PCR) was presented in Table 3. The cycling program for DNA amplification consisted of Initial denaturation for 5 min at 94°C followed by 40 cycles of 30 s at 94°C for denaturation, 1 min at 45°C for primer annealing and 2 min at 72°C for extension. A final extension was at 72°C for 7 min. The amplified products, thus obtained were separated by electrophoresis on 0.8% agarose gel in 1x TBE buffer at 120 V for 1 h. The gels were stained with ethidium bromide (0.5µg/ml) and visualized by a UV

transilluminator. The 2kb DNA ladder (Invitrogen) was used as fragment size markers. Subsequently, the gel was photographed.

RESULTS

In the present study the characterization was performed for 15 *S. betaceum* plants which belong to four genotypes and also tolerant to *Colletotrichum acutatum*.

Agronomic characterization and cluster analysis

The agronomic characterization of the tree tomato through the coefficient of variation was presented in Table 4. There were ten discriminant variables which have a coefficient of variation more than 20% (Table 5) and the same were used to draw a dendrogram to establish groups or clusters. The evaluation of the variability of 15 accessions of the tree tomato through agronomic characterization identified 4 groups (Figure 1), which are similar to the report published by Albornoz [8]. For discriminating groups, they used multivariate analysis and in the present study, we have been using the coefficient of variation which allowed more precise variability of data characters, providing a comparison of the variability of the same characteristic in four genotypes or collection measured on the same characters. This is important because at the time this analysis was conducted it was established new differences in each of the descriptors, because the genotypes were studied under controlled conditions. Using the Euclidean distance similarity between 15 tree tomato plants that have been evaluated on a set of metric variables (quantitative), using SPSS statistical software was measured. In the matrix obtained Cluster analysis technique was applied using the UPGMA clustering method.

Table 1: Genotypes, code and place of selection of Tree Tomato (*Solanum betaceum* Cav.)

Genotypes	*Plants*	*Code*	*Place of Selection*
G1	4	60L1S1	Leitillo
G2	4	534L1S1	Leitillo
G3	4	138L2S1	Los Andes
G4	3	451L3S1	El Triunfo

Table 2: RAPD primers used in the characterization of Tree Tomato (*Solanum betaceum* Cav.)

Primer	Nucleotide sequence 5'-3'
OPAC – 09	AGAGCGTACC
OPAC – 19	AGTCCGCCTG
OPAN – 12	AACGGCGGTC
OPAM – 07*	AACCGCGGCA
OPAM – 15	GATGCGATGG
OPAM – 16	TGGCGGTTTG
OPR – 16	CTCTGCGCGT
OPS – 13	GTCGTTCCTG

** Primer used in the characterization of fifteen accessions of Tree Tomato (Solanum betaceum Cav.).*

Table 3: The receipe for Polymerase chain reaction (PCR)

Components	Concentration	1x (in µl)	15x (in µl)
H20	--	6.7	100.5
Buffer	10X	1.5	22.5
MgCl2	25mM	0.9	13.5
dNTP`s	10mM	1.2	18
Primer	0.2µM	2	30
Taqpolymerase	5U/µl	0.7	10.5
DNA	5ng/µl	2	--
TOTAL		15	

Identification of promising material

With the aim of characterizing four genotypes of the tomato (*Solanum betaceum* Cav.) and in order to identify the most promising materials all results of morphological and agronomic characteristics evaluated in the present study was summarized in Table 5. The overall analysis of morphological and agronomic descriptors allowed identifying the genotype 138, L2, S1 as possible promising material within four genotypes of the tree tomato of Patate region. For the selection of elite material traits like production, tolerance to viruses and fruit characteristics (demand in the international market) were taken into account. The morphometric and phenotypic parameters that were used in the present study are similar to those key features mentioned in the findings of Bioversity International [12]. The selected material produced fewer flowers and buds per inflorescence, but develops the greatest number of fruit per inflorescence, this material has an elliptical shape and fruit with a size of 6.9 to 7.12 cm with a light orange color for

the epidermis, the thickness of the pulp was 6.93 mm and the light orange colored slurry and finally this selection showed no presence of pests such as aphids, bug and boat but had a mean incidence of whitefly.

Table 4: The coefficient of variation calculated for all the forty agronomic characters observed in fifteen accessions of Tree Tomato (*Solanum betaceum* Cav.)

S. No.	Variables	C.V %
1	Shaft height (m)	10.56
2	Density of the cup	0
3	Habit cup	34.50*
4	Petiole shape	0
5	Petiole length	10.75
6	Blade shape	0
7	Longitudinal axis of the leaf blade	8.61
8	Transverse axis of the leaf blade	9.28
9	Color of the leaf blade	14.74
10	Rib blade	0
11	Rib sheet color	0
12	Sizacaliz (mm)	4.72
13	Color caliz	0
14	Size of the corolla	5.69
15	Corolla primary color	0
16	Corolla shape	0
17	Type of inflorescence	0
18	No. of flowers and buds per inflorescence	49.19*
19	Number of fruits set per inflorescence	42.66*
20	Fruit shape	36.40*
21	Shape the apical end of the fruit	23*
22	Longitudinal axis size	8.73
23	Transverse axis size	6.10
24	Primary color of the skin of the fruit	20.94*
25	Secondary color of the skin of the fruit	35.21*
26	Grain of the skin of the fruit	23.00*
27	Flesh color	47.88*
28	Pulp thickness	9.06
29	Thickness musilagocontainingseed	18.99
30	Color mucilagecontainingseed	0
31	Number of fruits per inflorescence	38.73*
32	Number of fruits fallen per inflorescence	17.65
33	Number of seeds per fruit	7.99
34	Size axis longitudinal seed (mm)	4.20
35	Size axis transverse seed (mm)	8.10
36	Seed color	0
37	Incidence of aphid	0
38	Incidence bug	0
39	Incidence of Boat	0
40	Incidence of whitefly	0

** Coefficient of variation >20 %*

Table 5: Agronomical character with coefficient of variation >20 % of Tree Tomato (*Solanum betaceum* Cav.) considered for the establishment of groups

Variables	Genotypes (Groups)			
	60L1S1	**534L1S1**	**138L2S1**	**451L3S1**
Habit cup	2 nor-2corv	3nor-1conv	Normal	Normal
Number of flowers and buds per inflorescence	Jun-34	Jun-35	May-31	Jun-39
Number of fruits set per inflorescence	4	6	7	4
Fruit shape	Elliptical	Elliptical	Elliptical	Ovoid
Shape the apical end of the fruit	Punton	Punton	Punton	Round
Primary color of the skin of the fruit	Light purple	Light purple	Dark purple	Dark
Secondary color of the skin of the fruit	Light orange	Light orange	Light orange	Dark
Grain of the skin of the fruit	Slightlyveined	Slightlyveined	Slightlyveined	Not
Flesh color	Light orange	Light orange	Light orange	Light
Number of fruits per inflorescence	4	4	6	5

Molecular Characterization

DNA extraction

A reliable genetic analysis of plants is very much dependent on pure and high yield of DNA, out of two DNA extraction methods followed in the present study only DNA isolated from Pure Link Plant Total DNA purification kit gave relatively pure DNA compared to SORBITOL-CTAB method. Upon gel electrophoresis the DNA isolated from Pure Link Plant Total DNA Purification Kit gave impurity free genomic DNA bands (Figure 2) where as the

genomic DNA isolated from Sorbitol-CTAB method contained salts and impurities (Figure 3). So the kit method was more efficient in extracting DNA of good quality and suitable for genetic analysis experiments. So, the genomic DNA isolated from Sorbitol-CTAB method was excluded assuming that the impurities may interfere with RAPD analysis. The DNA isolated from kit method was further quantified using Qubit Fluorometer 2.0 and the same was subjected for RAPD analysis.

Figure 1. Dendrogram of fifteen accessions of Tree Tomato from forty agronomic descriptors and establishment of four clusters

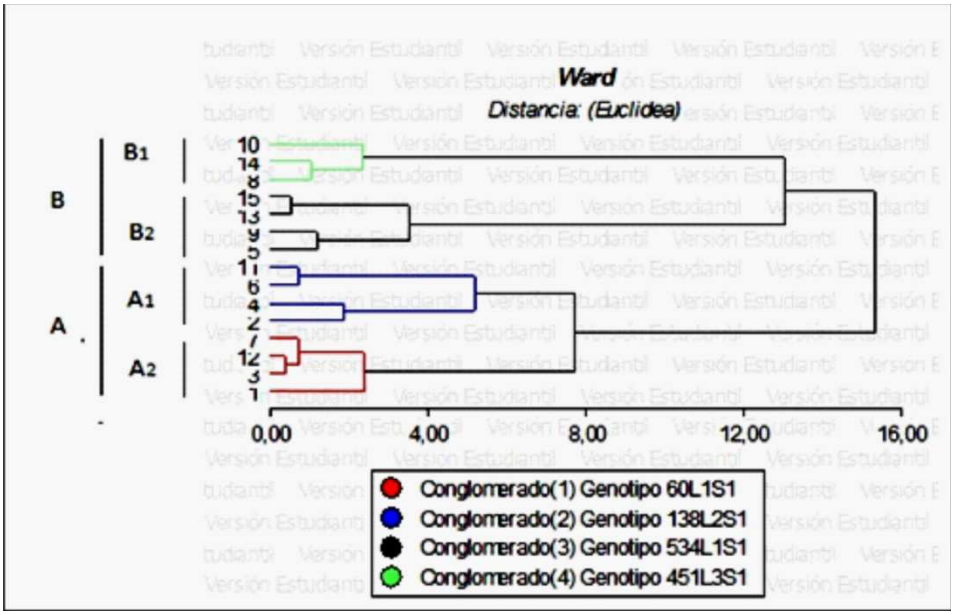
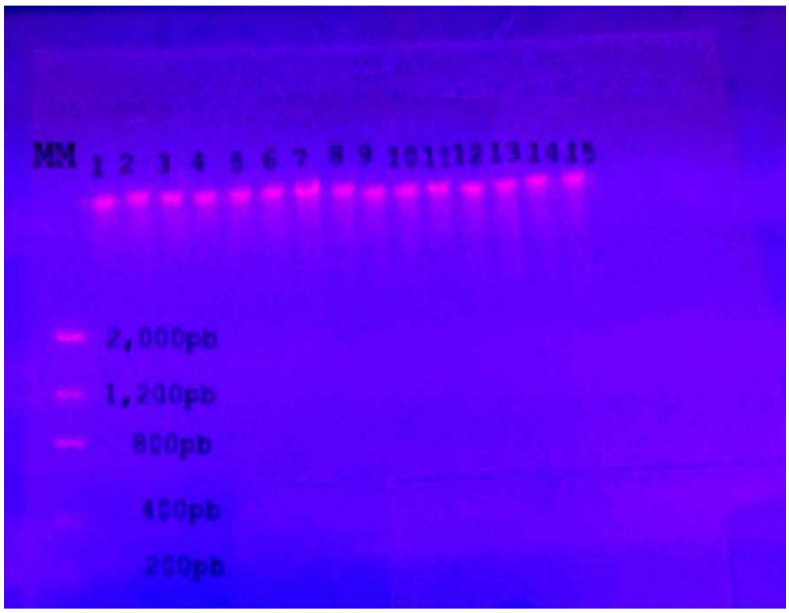


Figure 2. The total genomic DNA isolated from *Solanum betaceum* Cav. using link pure plant total DNA purification kit



RAPD and Cluster analysis

There were eight primers used for RAPD analysis. In the initial screening of primers it was observed that only OPAM - 07 gave polymorphic bands and the same was further used to amplify DNA from 15 accessions (Figure 4). Only intense bands that showed a consistent amplification were analyzed, while doubtful or blurred bands were not considered for analysis.

The Jaccard's coefficient was used to calculate the genetic similarity among 15 accessions of the tree tomato for this work the SPSS statistical program was used. The bands were scored as discrete variables, using 1 to indicate presence and 0 to indicate the absence. UPGMA cluster analysis was performed to develop a dendrogram (Figure 5). The dendrogram analysis divided the 15 accessions of the tree tomato into 4 main branches or groups.

Figure 3. The total genomic DNA isolated from *Solanum betaceum* Cav. using Sorbitol-CTAB method

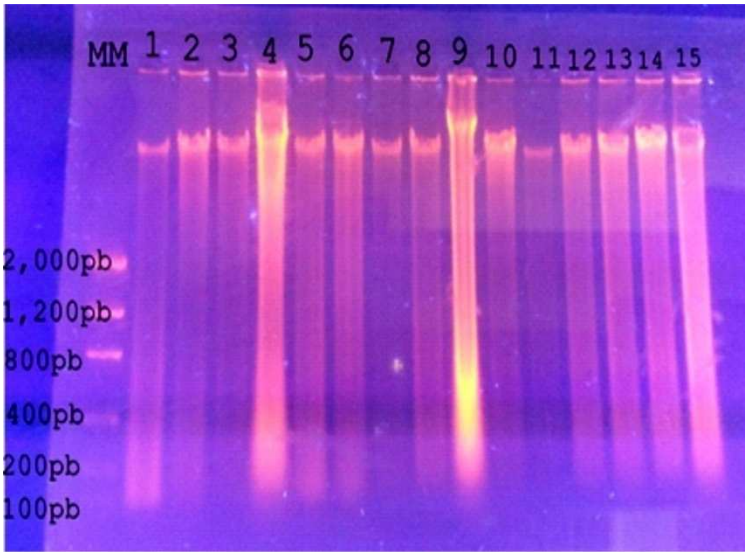


Figure 4. RAPD banding pattern obtained from 15 Tree Tomato (Solanum betaceum Cav.) plants of Patate area
Lane MM: Molecular weight marker. Each lane contains genomic DNA amplified through OPAM-07 Primer

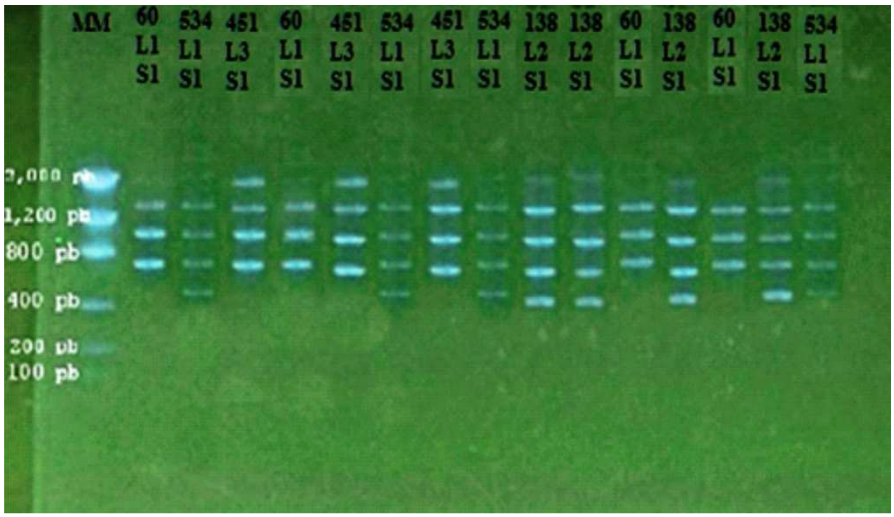
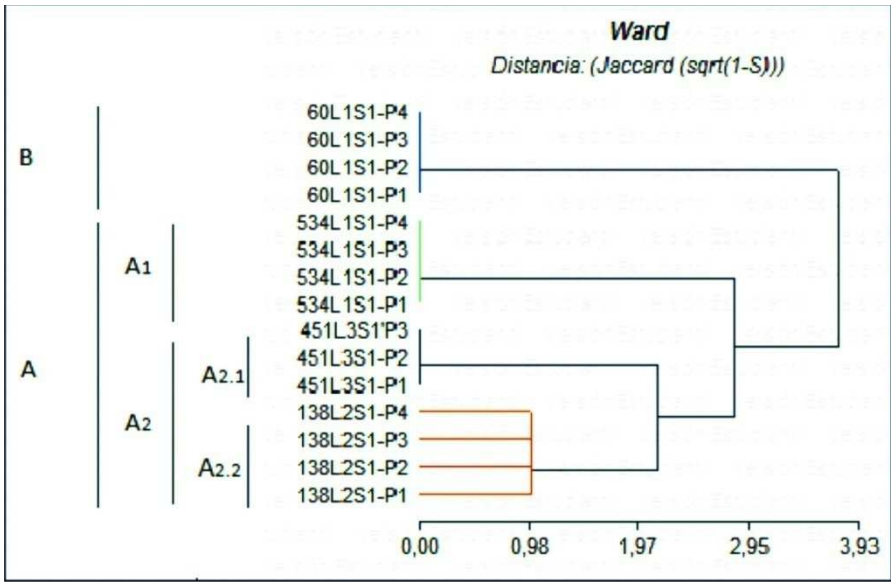


Figure 5. Dendrogram generated based on molecular data from fifteen accessions of Tree Tomato using Jaccard's coefficient and UPGMA clustering method



DISCUSSION

From the agronomic and molecular characterization study carried on 15 accessions of tree tomato it was found that the genotype 138L2S1 was the promising materials with the features of high production and tolerance to pests and diseases. The forty morphological and agronomic variables, ten are with greater discriminatory power: drink habit, number of flowers and buds per inflorescence, number of sets per inflorescence, fruit shape, form the apical end of the fruit, colorful fruits epidermal primary fruit, secondary color of the skin of the fruit, grain of the skin of the fruit flesh color and number of fruits per inflorescence.

The morphological descriptors used in the present study were useful to know phenotypic characteristics of interest in each of the studied materials and by grouping the Euclidean distance and Ward resulted four groups, formed by plants of the same genotype these are 60L1S1, 534L1S1, 138L2S1 and 451L3S1. The RAPD markers allowed analyze different ranges according to minimal migration banding pattern, determined that this variability was low and that the genotypes of the tree tomato studied are highly related to each other. The similarity percentages revealed that groups established are highly similar to what is observed in the clusters of the genotypic and phenotypic dendrogram.

The similarity found in the genotypes studied by cluster analysis and the groups formed in the dendrogram indicates that the genetic variability is low, because the pattern of spread of tree tomato is clonal, by so when the molecular analysis found that plants under study are highly related. Penafiel [7], mentioned in previous results in tree tomato, reduced genetic variability could be explained by a relatively recent process of domestication of the species, and by a combination of geographical/ecological and cultural factors that encourage phenological genetic homogeneity between and within crops.

The use of RAPD molecular marker technique allowed us to obtain accurate data for the establishment of genetic variability within 4 genotypes of tree tomato. Throughout the process of agronomic and molecular characterization of the genotypes studied, in a period in which the climatic conditions were optimal for the favorable development of *Colletotrichum acutatum* (high temperature, high humidity and abundant rainfall) and no symptoms of anthracnose in any of the test plants was observed

Acknowledgements
The authors would like to gratefully acknowledge the Department of Life Sciences, Universidad de las Fuerzas Armadas-ESPE for providing all the necessary facilities throughout my research work. We would also like to acknowledge the contributions of colleagues in our respective institutes for helpful comments on the manuscript.

REFERENCES

[1] Solagro. Tree Tomato. The solution for Agro, **2006**.
[2] L Bohs. *Cyphomandra (Solanaceae).* New York: Flora Neotropica, **1994.**
[3] J Pilco. Evaluation two chemical bases NPK formulations for the growth and development of treetomato, **2009**.
[4] MAGAP. The Ministery of Agriculture, Livestock, Aquaculture and Fisheries: http://servicios.agricultura.gob.ec/sinagap/phocadownloadpap/spr/tomate_arbol/tomatea3.pdf*,* **2014.**
[5] FONTAGRO. **2010**. Manual handling: harvest and postharvest of tree tomato. Bogota. http://www.fontagro.org/sites/default/files/TomateArbol_final.pdf.
[6] P Acosta. Morfological and molecular characterizacion of tree tomato, *Solanun betaceum* Cav. (Solanaceae). PhD Thesis, Technical University of Madrid, Fachhochschuleagronomists engineers. Madrid, Spain, **2011**.
[7] N Penafiel. Evaluation of the genetic variability of the tree tomato (*Solanum betaceum* Cav.) in cultures of three provinces of Ecuador by microsatelites markers, **2007**.
[8] G Albornoz. The tree tomato in Ecuador. Quito: Central University of Ecuador, **1992.**
[9] N Lucero, D Novoa. Selection of genotypes of tree tamato (*Solanum betaceum* Cav. tolerant to anthracnose (*Colletotrichum acutatum*) and high productivity in three locations using mass selection area Patate, **2013**.
[10] T Franco, R Hidalgo. Statistical Analysis Data Morphological Characterization of Plant Genetic Resources. Cali: IPGRI, **2003**.
[11] C Mariac; P Trouslot; C Poteaux; G Bezancon; JF AnRenno, *In Biotechniques.*, **1999**, 28, 110-113.
[12] Acosta-Quezada, Riofrío-Cuenca, Martínez-Laborde, Prohens, **2013**, Descriptors for tree tomato (*Solanum betaceum* Cav.) and wild relatives. http://www.bioversityinternational.org/e-library/publications/detail/descriptors.-for-tree-tomato-emsolanum-betaceum-emcav-and-wild-relatives/.