

Morphological characterization of wild tomato (*Solanum* sp.) in farms of the Universidad Estatal del Sur de Manabí, Ecuador

Caracterización morfológica del tomate silvestre (*Solanum* sp.) en predios de la Universidad Estatal del Sur de Manabí, Ecuador

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Abstract: In order to morphologically characterize the wild tomato populations found on the grounds of the Universidad Estatal del Sur de Manabí, in Los Angeles, a survey and identification of 15 wild tomato populations was carried out. Six quantitative variables and 24 qualitative variables were evaluated. For quantitative variables, single-factor analysis of variance and comparison of means were performed. Pearson correlations were performed for the variables evaluated; and multivariate analysis of principal components and hierarchical clusters was carried out. Likewise, Chi-square analysis, Spearson correlations and multiple correspondence analysis were performed for qualitative variables. The results obtained showed two different populations or differentiated ecotypes of wild tomato, which due to the morphological characteristics of the plant and the fruit were identified as belonging to the species *Solanum pimpinellifolium*, vernacularly called "tomatillo". It is distributed in Los Angeles between 313 and 385 meters above sea level, and was located at coordinates 1°21'0.31" to 1°21'10.14" south latitude and 80°33'49.87" to 80°34'0.32" west longitude. This species is in danger of genetic erosion, due to construction, road opening and clearing of the natural sites where it lives.

Keywords: erosion, ecotype, multivariate, multivariate, species, *Solanum pimpinellifolium*

Resumen: Con el objetivo de caracterizar morfológicamente las poblaciones silvestres de tomate que se encuentran en los predios de la Universidad Estatal del Sur de Manabí, en los Angeles, se realizó un recorrido de reconocimiento e identificación de 15 poblaciones de tomate silvestre. Se evaluaron seis variables cuantitativas y 24 variables cualitativas. Para las variables cuantitativas se hicieron análisis de varianza unifactorial y comparación de medias. Se realizaron correlaciones de Pearson para las variables evaluadas; y se ejecutó análisis multivariante de componentes principales y conglomerados jerárquicos. Así mismo, para las variables cualitativas se realizaron análisis de Chi-cuadrada, correlaciones de Spearson y

análisis de correspondencia múltiple. Los resultados obtenidos mostraron dos poblaciones diferentes u ecotipos diferenciados de tomate silvestre, que por las las características morfológicas de la planta y el fruto se identificó que pertenece a la especie *Solanum pimpinellifolium*, denominado vernáculamente como “tomatillo”. La misma está distribuida en los Ángeles entre los 313 a 385 msnm, y se las ubicó en las coordenadas 1°21'0,31” a 1°21'10,14” de latitud Sur y 80°33'49,87” a 80°34'0,32” de longitud oeste. Esta especie está en peligro de erosión genética, por las construcciones, apertura de caminos y desbosque de los sitios naturales donde habita.

Palabras clave: Erosión, ecotipo, multivariante, especie, *Solanum pimpinellifolium*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable plants in the world. It originated in western South America and domestication is believed to have occurred in Central America. Because of its importance as a food, tomato has been bred for improved productivity, fruit quality, and resistance to biotic and abiotic stresses. There are 13 recognized wild tomato species in Ecuador that have a wide variety of phenotypes and can be crossed with cultivated tomato. These wild tomatoes are important for breeding, as sources of desirable traits and for evolutionary studies (Kimura and Sinha, 2008).

Genetic diversity is a broad term that includes all the variability present within a genetic baggage of different related species, as is the case of the different species of wild tomatoes distributed throughout the world. Wild tomatoes are found in a large number of habitats, and are located from sea level to altitudes greater than 3000 m a.s.l., from the arid Pacific coast to the humid highlands of the Andes on both slopes of the mountain range (Peralta & Spooner, 2006).

Wild tomato populations grow at different altitudes in these narrow valleys, are geographically isolated from each other, and are adapted to very particular soil and microclimate conditions. This diversity of habitats has contributed to the great diversity that can be found among wild tomatoes (Peralta & Spooner, 2006).

The production or collection of wild tomato and the works that document the gene pool of native tomatoes are insufficient; so it is necessary to document the quality, yield and agromorphological characteristics of wild tomato, native populations have a high potential for adaptation, plant development, yield and resistance to attack by some pests and diseases; In addition, the variation in size, shape, color and chemical composition, as well as in nutritional and nutraceutical

value, make it viable to be cultivated, marketed or used as a rootstock (Berrospe Ochoa *et al.*, 2015).

It is important to study the genetic diversity of wild species and their variability, since from a genetic point of view, wild species constitute a large reservoir of genes for resistance to multiple diseases, tolerance to drought, high and low temperatures, salinity, high content of antioxidants, vitamins and sugars among others, the genes of wild species can be used to improve and adapt cultivated tomatoes to changing environmental conditions and human needs (Torrico, 2011). However, there are still many sites where these studies have not been carried out, as is the case of the Los Angeles property of the Universidad Estatel del Sur de Manabí, where there are natural populations of wild tomatoes that grow and are in danger of extinction due to anthropic intervention.

Methodology

Location

This research was carried out at the Universidad Estatel Del Sur de Manabí (UNESUM), located 1 ½ km via Noboa, Los Angeles, Canton Jipijapa, Province of Manabí. It is located at 1°21'10.14" South latitude and 80°33'50.40" West longitude at an altitude that varies between 230 and 313 meters above sea level.

Factors under study

The research was monofactorial. The study factor was 15 wild tomato populations.

Treatment

The research was carried out in the Los Angeles farm at the Universidad Estatel del Sur de Manabí and the treatments were the identified wild tomato populations, whose characteristics were randomly selected and characterized by their quantitative and qualitative variables using the descriptors of IPGRI (1996).

Statistical analysis

A descriptive inferential experimental design was used. Quantitative variables were tested for normality and homogeneity. Once these

assumptions were met, a factor-specific analysis of variance was performed to test the hypotheses. Then the comparison mean was calculated using Tukey multiple testing at $P < 0.05$ probability. To complement the analysis, a Pearson correlation analysis was performed and finally, a multivariate principal component analysis was performed to define new quantitative variables and determine the contribution of the greatest variability among them. For qualitative variables, Chi-square tests were performed to analyze the perceived importance of qualitative variables, Spearson correlation analysis and multivariate multiple correspondence analysis (Gabriel *et al.*, 2021). All these analyses were performed with SPSS software (Pardo Ruiz, 2002).

Variables evaluated

Six quantitative variables were evaluated: petal length (LP), sepal length (LS), stamen length (LE), fruit weight (PF), fruit length (LF), fruit width (AF) (IPGRI, 1996), and 24 qualitative variables: Growth type (TCP), Plant size (TP), No. of leaves (NH), Leaf position (PH), Leaf type (TH), Stem pubescence density (DPT), Stem internode length (LET), Inflorescence type (TI), Corolla color (CC), Corolla type (TC), Flower sterility type (TEF), Style position (PE), Style shape (FE), Style pubescence (PE), Anther dehiscence (DA), External color of unripe fruit (CEFNM), Green back stripes (RVF), Intensity of green back (IGB), Fruit pubescence (PF), Predominant fruit shape (FPF), Fruit size (TF), Fruit homogeneity (HF), External color of ripe fruit (CEFM), Intensity of external color (ICE), which will be evaluated according to the manual of descriptors for tomato (*S. lycopersicum* L.) of the International Plant Genetic Resources Institute (IPGRI, 1996).

The geographic coordinates of the populations were determined with a GPS, considering the latitude, longitude and altitude of the study sites, with which a distribution map of the populations was generated.

Research management

This was an inferential descriptive research, whose purpose was to describe the morphological characteristics of 15 wild tomato populations. For this purpose, a total tour was made of the Los Angeles property at the Universidad Estatal del Sur de Manabí, and the concentrations of wild tomato populations were identified, which were geo-referenced and in each population three randomly selected plants were morphologically characterized, considering the descriptors of the IPGRI (1996). Likewise, a comparison of the plants of the populations found was made with reference literature such as that of Paralta and

Spooner (2000, 2007) and Zeballos *et al.* (1987), in order to identify to which species they belonged.

Identification of collection sites

For the determination of wild tomato populations, the Angels' farms were visited *in situ*, through observations, measurements and systematization of local information. Each site identified was geo-referenced with a GPS. Likewise, a route map was used to visit the Los Angeles farms, after identifying the sites where the morphological characteristics of the plants were evaluated, considering the qualitative and quantitative variables using the descriptors recommended by IPGRI (1996). Measuring instruments were used, such as a GPS for geo-referencing, tape measure and gram scale to measure the quantitative characteristics, and the IPGRI descriptors for the qualitative variables.

Results

The Shapiro-Wilk test ($P < 0.05$) (Table 1), showed that the data for the variables evaluated were not significant and the coefficients of variation were within the range allowed for this type of research (CV from 17% to 32%). This suggests that the data were normally distributed. Likewise, Levene's test showed that all the variables were not significant at $P < 0.05$ probability, with the exception of the stamen length variable (LDE), so the data of the evaluated variables showed homogeneity of variances. These results suggest the continuity of the analysis of variance.

Table 1. Analysis of normality and homogeneity of variances for quantitative data.

Variable	n	Media	D.E.	Var	CV	Asymmetry	Kurtosis	Shairo-Wilk	Levene's test
LDP	45	11,73	2,89	8,34	24,61	0,82	0,03	0.97ns	2,10ns
LDS	45	6,31	1,72	2,95	27,20	-0,23	-0,63	0.94ns	0.50ns
LDE	45	8,80	2,83	7,98	32,10	1,11	4,89	0,8**	7,60**
AFRU	32	35,88	7,51	56,44	20,94	0,25	-0,57	0.96ns	12,00ns
LFRU	32	13,13	3,53	12,44	26,87	0,26	-0,47	0.93ns	3.00ns
PFRU	32	7,13	3,26	10,63	17,43	0,15	-0,72	0.96ns	1.30ns

** : highly significant at $P < 0.01$, ns: not significant, LDP: petal length (mm), LDS: sepal length (mm), LDE: stamen length (mm), PFRU: fruit weight (g), LFRU: fruit length (mm), AFRU: fruit width (mm).

Analysis of quantitative morphological characters

The analysis of variance (ANOVA) between groups (populations) and within groups (study variables) showed significant differences at $P < 0.05$ probability for the variable petal length (PLL) and highly significant differences at $P < 0.01$ probability for the variable fruit weight (FRW) (Table 2). This would indicate that at least one of the wild tomato populations evaluated showed outstanding differences.

Table 2. Single-factor analysis of variance, for populations and response variables.

Variables		Sum of squares	gl	Root mean square	F	Sig.
LDP	Inter-group	182,13	14	13,010	2,113	,042
	Intra-group	184,67	30	6,156		
	Total	366,80	44			
LDS	Inter-group	56,31	14	4,022	1,645	,123
	Intra-group	73,33	30	2,444		
	Total	129,64	44			
LDE	Inter-group	80,53	14	5,752	,638	,812
	Intra-group	270,67	30	9,022		
	Total	351,20	44			
PFRU	Inter-group	240,33	11	21,848	4,901	,001
	Intra-group	89,17	20	4,458		
	Total	329,50	31			
LFRU	Inter-group	140,50	11	12,773	1,043	,449
	Intra-group	245,000	20	12,250		
	Total	385,500	31			
AFRU	Inter-group	839,667	11	76,333	1,678	,152
	Intra-group	909,833	20	45,492		
	Total	1749,500	31			

Variables in bold were significant at $P < 0.05$ and highly significant at $P < 0.01$ probability respectively. PDL: petal length (mm), SLD: sepal length (mm), SLD: stamen length (mm), PFRU: fruit weight (g), LFRU: fruit length (mm), AFRU: fruit width (mm).

The comparison of means by Tukey's multiple test ($P < 0.05$) showed significant differences in some variables compared between populations (Table 3). It should be clarified that only the comparisons that were significant were systematized, thus outstanding differences were observed for LPD between populations 2 and 11 and between 2 and 14.

Table 3. Multiple comparisons using Tukey's test at $P < 0.05$ probability.

Dependent variable	(I) Popul ation	(J) Populat ion	Difference of means (I-J)		Sig.
			Lower limit	Upper limit	
LDP	2	1	5,33	2,03	0,39
		3	7,00	2,03	0,08
		4	3,67	2,03	0,88
		5	5,67	2,03	0,30
		6	6,00	2,03	0,23
		7	5,33	2,03	0,39
		8	6,67	2,03	0,12
		9	8,00	2,03	0,03
		10	5,33	2,03	0,39
		11	7,67*	2,03	0,04
		12	6,33	2,03	0,17
		13	6,33	2,03	0,17
		14	9,00**	2,03	0,01
		15	6,67	2,03	0,12

* Mean difference is significant at the .05 level, 1-15: wild tomatillo species populations in Los Angeles, UNESUM, PDL: petal length (mm),

Principal Component Analysis (PCA)

Correlation analysis

Pearson's correlation analysis performed for the variables evaluated (Table 4), showed high and significant correlations ($P < 0.01$) for the variables LPD vs LDS ($r = 0.71$), PFRU vs LFRU ($r = 0.57$) and PFRU vs

AFRU ($r=0.59$).

Correlation analysis of quantitative variables.

	LDP	LDS	LDE	PFRU	LFRU	AFRU
LDP	1,00	0,71**	0,00	0,32	0,22	0,12
LDS		1,00	0,11	0,26	0,42	0,16
LDE			1,00	0,19	0,07	0,15
PFRU				1,00	0,57**	0,59**
LFRU					1,00	0,30
AFRU						1,00

** Correlation is significant at the 0.01 level (bilateral). PDL: petal length (mm), SLD: sepal length (mm), SLD: stamen length (mm), PFRU: fruit weight (g), LFRU: fruit length (mm), AFRU: fruit width (mm).

The PCA generated components, which represented the number of components and their eigenvalue on the abscissa and the percentage of variance on the ordinate. This showed the decrease of the first components in relation to the others and the most significant components were selected (Table 5). Significant components were considered to be those values prior to the inflection point (Figure 1). Two components whose eigenvalue was ≥ 1 and which expressed more than 100% of the total variance were retained.

Table 5. Total variance explained.

Component	Initial eigenvalues			Sums of the squared saturations of extraction		
	Total	% of variance	Accumulated	Total	% of variance	Accumulated
1	4,782	79,706	79,706	4,782	79,706	79,706
2	1,218	20,294	100,000	1,218	20,294	100,000
3	3,21E-016	5,35E-015	100,000	4,782	79,706	79,706
4	2,46E-018	4,10E-017	100,000			
5	-6,19E-017	-1,03E-015	100,000			
6	-8,32E-017	-1,39E-015	100,000			

Extraction method: Principal Component Analysis .

Figure 1. Sedimentation plot for 24 quantitative variables.

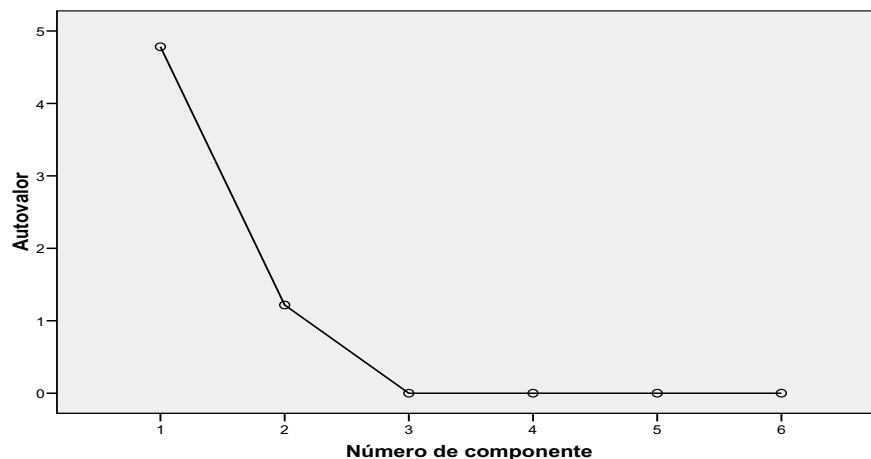


Table 6 showed that the first component contributed 79.71% of the total variance. The variables LFRU, LDP, LDE, LDS, PFRU contributed positively (Table 8). The second component contributed 20.29% of the total variance, in which the AFRU variable contributed positively.

Table 6. Matrix of extracted components(a).

Variables	Component	
	1	2
LFRU	1,000	-,005
LDP	,996	,095
LDE	,976	,218
LDS	,943	-,332
PFRU	,759	,651
AFRU	-,611	,792

PDL: petal length (mm), SLD: sepal length (mm), SLD: stamen length (mm), PFRU: fruit weight (g), LFRU: fruit length (mm), AFRU: fruit width (mm).

Therefore, two new variables were generated that explain more than 100% of the variance. The first component refers to the reproductive characteristics of tomatillo, the second component groups the morphology of the fruit (Table 6).

differences ($P < 0.01$), where the highest frequency was for intermediate LDE with 49%, short with 42% and long with 22% . Regarding style position there were highly significant differences ($P < 0.01$), where the highest frequency was for PDE very projected with 56%, slightly projected with 31%, and at the same level with 13%. In reference to CEFNM there were highly significant differences ($P < 0.01$), where 51% were greenish white, and 18% were light green. Regarding the TDF, there were significant differences, determining that 42% were of intermediate size and 20% were small. Finally, in reference to HDTF, highly significant differences were detected, where 64% showed intermediate uniformity.

Table 7. Contrast statistics by Chi-square test and frequencies.

Character	Nominal scale	Absolute frequency	Relative frequency	Chi-square
Plant size (TDP)	small	5	11,1	
	intermediate	23	51,1	
	grande	17	37,8	
Total		45	100,0	11,20**
Stem Pubescence Density (SPDD)	scarce	15	33,3	
	intermediate	20	44,4	
	Long	10	22,2	
Total		45	100,0	3,33ns
Stem internode length (SIL)	Cut	19	42,2	
	intermediate	22	48,9	
	Long	4	8,9	
Total		45	100,0	12,40**
Style Position (PDE)	same level as the worsted	6	13,3	
	slightly projected	14	31,1	
	very projected	25	55,6	
	Total	45	100,0	
External color of unripe fruit (CEFM)	blanco	23	51,1	
	verduzco			

	light green	8	17,8	
	dark green	1	2,2	
	Missing data	32	71,1	
	Total	13	28,9	
Total		45	100,0	23,69**
Intensity of greenback (green shoulders)	Slight	47	88,68	
	intermediate	6	11,32	
Total	strong	53	100,00	1.00ns
Fruit pubescence	scarce	12	26,7	
	intermediate	5	11,1	
	dense	32	71,1	
	Total	13	28,9	
Total		45	100,0	4.39ns
Fruit size (TDF)	very small	1	2,2	
	small	9	20,0	
	intermediate	19	42,2	
	grande	3	6,7	
	Total	32	71,1	
	System	13	28,9	
Total		45	100,0	24,50**
Homogeneity of fruit size	intermediate	29	64,4	
	much	3	6,7	
	Total	32	71,1	
	System	13	28,9	
Total		45	100,0	21.12**
External color of ripe fruit	orange	29	64,4	
	Red	3	6,7	
	Total	32	71,1	
	orange	13	28,9	
Total	Total	45	100,0	4.54ns
External color intensity	Little	9	20,0	
	intermediate	8	17,8	
	much	5	11,1	
	Total	22	48,9	
	System	23	51,1	
Total		45	100,0	1.18ns

Multiple Correspondence Analysis

This analysis was carried out for the qualitative variables, which allowed discriminating, based on Spearman's correlation (Table 8), which variables are highly associated, and then interpreting the distribution of these variables on a Cartesian axis with two dimensions. Table 8 shows that there were not many highly associated variables, the main ones being the following: the external color of the unripe fruit is highly significant and moderately associated with the homogeneity of fruit size ($r = 0.49$) and the external color of the fruit is moderately related to the intensity of the external color ($r = 0.51$).

The degree of association can be seen in the two dimensions described in (Table 9), which confirms what was observed in the Spearson correlations obtained. As shown in Table 9, some variables contribute to the first component (plant size, stem pubescence density, stem internode length, style position, external color of unripe fruit, greenback intensity, fruit pubescence, fruit size, fruit size homogeneity, external color of ripe fruit and external color intensity). The second component is contributed by stem pubescence density (Figure 4).

Discrimination measures

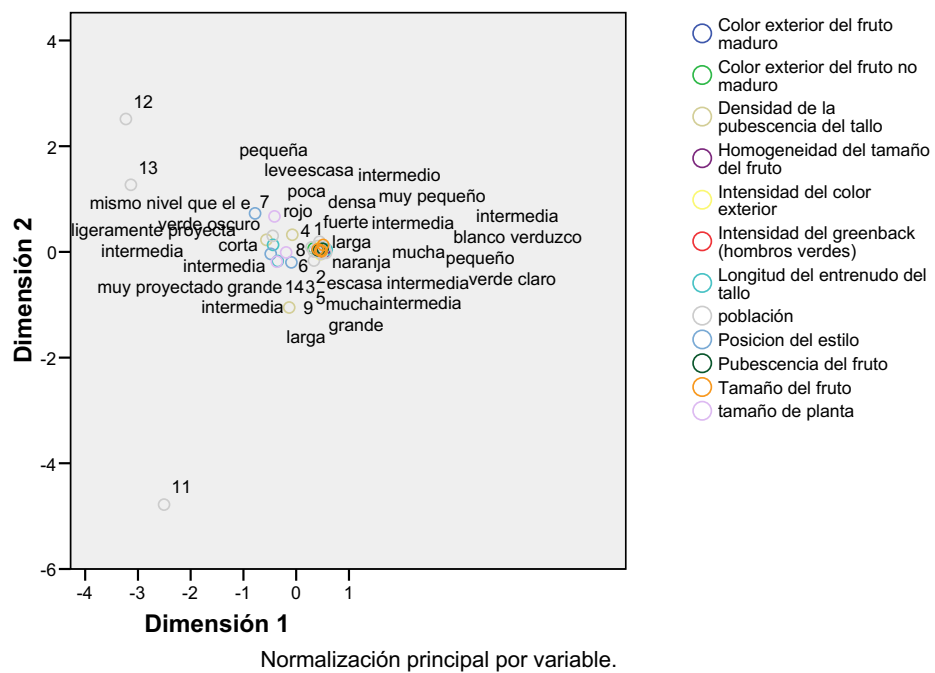
Variables	Variable weighting	Dimension		Media
		1	2	
Plant size	3		,068	,084
		101		
Stem pubescence density	3	,147	,304	,226
Length of stem internode	3	,171	,022	,096
Style position	2	,158	,093	,126
External color of unripe fruit	5	,137	,001	,069
Intensity of greenback (green shoulders)	3	,136	,001	,069
Fruit pubescence	3	,137	,001	,069
Fruit size	5	,137	,001	,069
Homogeneity of fruit size	3	,137	,001	,069
External color of ripe fruit	6	,103	,000	,052
Intensity of exterior color	3	,102	,001	,052
Total assets		33,918	32,373	33,145

a. The weights of the variables are incorporated in the statistics of

Total assets.

In Figure 4, it is observed that populations 11 and 12 show that they have differential characteristics to the rest of the populations. This is possibly due to differences in climate and soil.

Figure 3. Joint plot of category points of 15 populations of *Solanum pimpinellifolium*.



Geographical distribution of wild tomato populations

It was determined that the populations are distributed at different latitudes, longitudes and altitudes (Table 10) and a distribution map of the 15 populations was drawn up (Figure 3). It was determined that the wild species found apparently correspond to *Solanum pimpinellifolium*, based on the morphological characteristics of the plants and fruit. They are distributed in a range of 313 to 385 m asl, at a latitude between 1°21'0.31" to S 1°21'10.14" South latitude and W 80°33'49.87" to 80°34'0.32" West longitude (Figure 1).

Table 10. Altitude, latitude and longitude of the wild tomato populations in the farms of the Universidad Estatal del Sur de Manabí.

Population	Altitude (masl)	Latitude	Length
1	339	S 1°21'10,14"	W 80°33'50,40"
2	339	S 1°21'9,28"	W 80°33'50,22"
3	339	S 1°21'9,16"	W 80°33'50,73"
4	328	S 1°21'6,51"	W 80°33'49,97"
5	328	S 1°21'5,65"	W 80°33'50,18"
6	328	S 1°21'5,75"	W 80°33'49,87"
7	328	S 1°21'5,69"	W 80°33'49,88"
8	328	S 1°21'4,40"	W 80°33'50,93"
9	342	S 1°21'1,17"	W 80°33'54,76"
10	313	S 1°20'59,81"	w 80°33'51,78"
11	342	S 1°21'0,31"	W 80°33'56,14"
12	318	S 1°20'58,12"	W 80°33'57,00"
13	313	S 1°20'58,54"	W 80°33'52,25"
14	342	S 1°21'3,05"	W 80°33'59,98"
15	385	S 1°21'3,88"	W 80°34'0,32"

Figure 4. Distribution map of the 15 populations of *Solanum pimpinellifolium*.



We have determined that in Los Angeles on the grounds of the Universidad Estatal del Sur de Manabí there is a wild species of tomato, which we have studied carefully in order to determine to which species it belongs and if there is genetic variability of the same. In our study we found 15 populations distributed throughout Los Angeles, but which are in danger of genetic erosion, due to the constant predation of the natural sites where it grows, an aspect that no one has given it due importance, particularly because of how valuable it could be as germplasm to be used in genetic improvement programs of cultivated tomatoes (Flores Hernández *et al.*, 2017; Medina Litardo *et al.*, 2022) and in this regard Morales Palacios *et al.* (2016), mention that with the objective of evaluating morphological and genetic variation in the germplasm collection of the National University of Loja (UNL) in Ecuador, four wild species were selected: *Solanum pimpinellifolium*, *Solanum neorickii*, *Solanum habrochaites*, *Solanum lycopersicum* var. *Cerasiforme*. In which they detected phenotypic differences in the vegetative components and those related to the flower and fruit. These species were described by Peralta *et al.* (2006) and Peralta and Spooner (2007).

Acosta Quezada (2022) reported five wild species in Ecuador, two of green fruits (*S. neorickii* and *S. habrochaites*) and four of red fruits (*S. pimpinellifolium*, *S.77 alapaguense* and *S. cheesmaniae*), the latter two endemic to the Galapagos Islands. An interesting study was reported by Zeballos Bravo *et al.* (1987), who reported the presence of the wild species *Solanum pimpinellifolium* L., in the fields of Bolívar canton, Manabí province. This species is self-compatible and red-fruited (Peralta and Spooner, 2000; Van der Knaap *et al.*, 2014). This was observed in our study where we determined that the fruits of the species found are medium to small red fruits. In our study we found significant diversity in flower petal length and fruit weight; as well as plant size, stem pubescence density, stem internode length, style position, external color of unripe fruit, fruit size and fruit size homogeneity. This suggests that the species studied is *Solanum pimpinellifolium* (Natualista, 2023), but that it has diversified into new ecotypes that are distributed in two defined populations, an aspect that was described by Zuriaga *et al.* (2008) and Zevallos *et al.* (2017), who suggest that this species has a wide adaptive plasticity, despite having a high genetic homozygosity due to its high autogamy.

It should be noted that, contrary to what was believed, *S. pimpinellifolium* originated in Ecuador and spread to northern and

southern Peru, as suggested by Ya-Ping Lin *et al.* (2020), who consider that the populations of this species occupy different ecological niches and have the ability to adapt to climate changes, making it an ideal species for investigating the environmental isolation hypothesis.

Conclusions

The morphological characterization of wild tomato from 15 populations found in the farms of the Universidad Estatal del Sur de Manabí was carried out, for which six quantitative variables and 24 qualitative variables were considered, determining two different populations or ecotypes, which probably belong to *Solanum pimpinellifolium* due to the morphological characteristics of the plant and the fruit.

The wild tomato species "tomatillo" is distributed in a range of 313 to 385 masl, from 1°21'0.31" to 1°21'10.14" south latitude and 80°33'49.87" to 80°34'0.32" west longitude.

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