Morphological, Molecular and Pollen characterization of potatoes of local use in Venezuelan Andean communities to determine its genetic diversity

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Abstract

Potato (*Solanum tuberosun* L.) is one of the main agricultural products in the communities of Merida state in Venezuela; where the wild, native or unnamed species coexist with the commercial varieties and the promising clones, which are cultivated in different manners.

The introduction of foreign varieties, either native, commercial or improved, have led to the gradual replacement of local varieties, prompting the disappearance of our gene pool. Therefore, the characterization of local varieties is needed in order to determine their diversity and proper identification, which would lead to their preservation as an intangible heritage of Venezuelan communities and their contribution to their country's food security. For this reason, we present the morphological, molecular and pollinic characteristics of potato samples located in the "Dr. Eduardo Ortega Cartaya" Mucuchies Experimental Field. These samples came from different locations in the state of Mérida.

Our results showed that 29 samples share molecular, morphological and pollinic expressions that allow their identification and registration over time. The statistical analyzes helped us to discriminate and group the samples in each level, i.e. morphological, molecular and pollinic level, which allowed to identify varieties with high potential to ensure the preservation of wild, native and commercial local varieties.

Keywords: *Solanum*, local varieties, promising clones, SSR markers, microsatellites.

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Introduction

Potato (*Solanum tuberosum* L.) was domesticated approximately 8000 years before our era in the South American Andes, with the greatest variability of species in the southwest of Peru and northwestern Bolivia (Gabriel, 2010)

Currently, there are nine cultivated species of potatoes, and almost 200 wild species distributed throughout the American continent, from the south of the United States to the north of Chile (Ochoa, 2001). Potatoes are cultivated in more than 148 countries around the world and are one of the most versatile foods for both, small producers (own consumption) and the industry (main fast food component). Potatoes are without doubt, a fundamental pillar of food sustainability projects and one of the most precious treasures that America has given to humanity, especially to the poor people of Europe, Asia and Africa that have faced or are facing famine (Ortega et al., 2005; Graves, 2000).

In Venezuela, potato production is of great importance because of its cultivated area and volume of production. It is planted mainly in farms of small producers in the Andean states (Táchira, Mérida and Trujillo), which constituted approximately 83% of the country's potato production. In 2014, the harvested area was 25,757 ha (Merida: 11,494.05 ha, Tachira: 8,131.46 ha and Trujillo: 6,131.47 ha), and the production was 490,196 ton, with an average yield of 19,032 tons. ha-1 (FEDEAGRO, 2014).

In the Venezuelan Andean region, local production of potatoes is made by: wild, commercial, and native species. The so - called native potatoes are named by the peasants of the region. These potatoes are identified as ancient cultivars, and the assigned names are related with their form, color, period of growth or references to peasant's yearnings. Some potatoes, have been used for more than 50 years in the locality, but they do not have a specific name or denomination, i.e. the potatoes are called "No Name". Often these potatoes came from exchanges between farmers, which do not conserve their original names, only their value for high yields and tolerance. Lately, native potatoes along with the varieties of commercial potatoes and the so-called improved clones, has been gaining ground for their preference for the producer and the consumer (Monasterio, 2002; Romero and Monasterio, 2005 González, 2013).

The lack of knowledge on the effect of how local varieties are affected by the existence of wild species, native varieties and improved clones, constitutes a latent threat of loss of diversity and variability. This problem, is accentuated by genetic erosion, characterized by the reduction of cultivation areas, the reduction of agricultural activities and the lack of market opportunities (Ortega et al., 2005).

So far, studies of genetic diversity and inventories of locally produced potatoes maintained by Venezuelan Andean farmers have been restricted, in most cases, to the use of morphological markers. These markers are limited to the phenotype and although they have determined great genetic diversity (Osorio et al., 2011), they are affected by the environment, which constitutes a limitation for the formulation of conservation strategies, that tend to safeguard the genetic potential and the potential adaptability of a certain species (Soto, 2006). Therefore, it became necessary to corroborate the results of the phenotypic studies, with other techniques that would be independent of the environmental effect. The morphological, molecular and pollen characterization, was performed in order to determine in a more precise way the existing degree of diversity between the species tested.

Molecular markers are DNA sequences that can be used to analyze minimal genotypic variations (polymorphisms) without the intervention of environmental factors and have proved to be a powerful, reliable and reproducible tool for the unambiguous

identification of potato cultivars (*Solanum tuberosum* L.). These markers have contributed to greater genetic knowledge in many plant species, including potatoes, which efficiently select individuals with specific characteristics, even if they are in juvenile stages. Its use is widely supported by its dominant genetic base, simplicity, high degree of polymorphism and high reproducibility between laboratories (Norero et al., 2002, Izpizúa et al., 2003, Mathias et al., 2004).

The pollen characterization can be done by comparing the pollen morphology (shape and size) and the characterization of the exine structure. The structure of the pollen exine allows the formation of subgroups according to the ornamentation of the same, that is, the ornamentation of the exine and the pollen form is a notorious characteristic among the different species which allows its identification, being of great help when establishing taxonomical descriptors, since it can varies from one species to another, but remain constant within the same species, so it is a good feature to separate the different types of pollen (Saez, 1978; et al., 2005; Mondragón, 2006; Pirpeno, 2006).

Materials and Methods

Morphological, molecular and pollen characterization was carried out in 29 samples obtained from the collection of the Germplasm Bank located in the Mucuchies Experimental Field Station "Dr. Eduardo Ortega Cartaya" -INIA Mérida, located at 3100 masl. (08°45'86" N and 070°53'09" W). This station in located in La Toma sector, Mucuchies -Rangel municipality, Mérida state. Samples came from the rural communities of the Rangel, Pueblo Llano, Cardenal Quintero, Miranda, Libertador, Campo Elías, and Arzobispo Chacón, all belonging to Mérida state and the Jáuregui municipality of the Táchira state. The Granola variety was included as reference because it was the most planted in the Andean Paramo Merino.

Morphologic characterization

Forty varieties were planted in the Mucuchies Experimental Field "Dr. Eduardo Ortega Cartaya". The characterization was done in five plants per variety. Fifteen plants per variety were planted in two plots with 17 rows of 6 meters long, with 0.90m space between rows and 0.40m space between plants. Ferlitization was done in the sowing, using a granulated fertilizer of 12-12-17/2 applied in a dose of 1000 kg/ha, and before the hilling, using potassium sulphate and calcium nitrate in a dose of 250 kg/ha. The morphological evaluation of qualitative characteristics was done using the descriptors of the International Potato Center (CIP) (Huamán and Gómez, 1994). These characteristics were: primary color of the flower, shape of the tuber, primary color of the tuber, secondary color of the tuber, distribution of the secondary color of the tuber, primary color of the flesh of the tuber, depth of eyes.

Pollen characterization

Pollen characterization was done using the following taxonomic descriptors: number and characteristics of the colpos and pores[4] (number, absence or combination), shape, contour, and structure presents (ornamentation of the exine), in the polar and equatorial view of the grain of pollen, and pollen form (Sáenz, 1978). The processing of the samples was carried out in the Archaeobotanical Laboratory of the Gonzalo Rincón Gutiérrez Archaeological Museum of the University of Los Andes. No chemical treatment was applied, pollen was transported

[4] Pollen grains contain openings where the genetic material leaves to fertilize the ovule: colpos (elongated) and pores (circular).

for direct observation in the scanning microscope at the Structural and Chemical Analysis of Materials Laboratory (LAQUEM) -Faculty of Science, University Los Andes. Each of the samples was photographed for their further characterization.

Molecular characterization

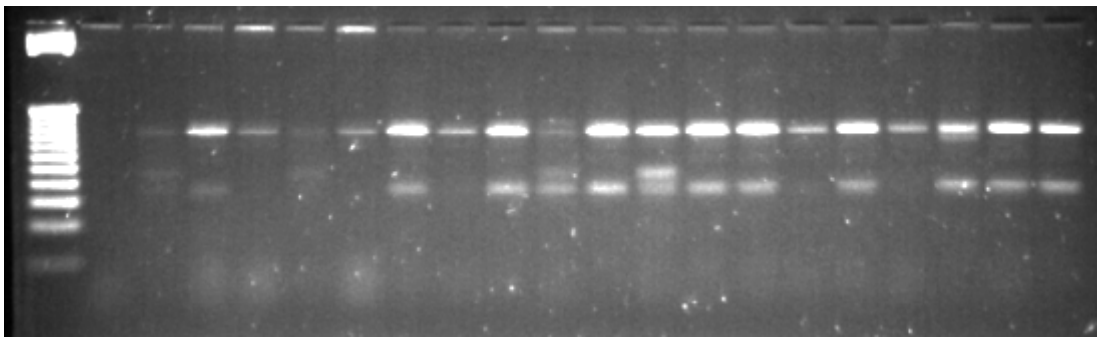
Tubers and foliar tissue from samples collected in the plots of the aforementioned municipalities as well as their in vitro replicas conserved were used. For the extraction of DNA, we used the methodology proposed by Zambrano et al. (2002), which allowed us to obtain high concentration, quality and purity of DNA, optimal for our amplification process. Microsatellites were used for the amplification following the methodology described by Osorio et al. (2011). Nine specific initiators developed for potato were used. These initiators were selected for their high polymorphic content index (PIC). For the separation of the amplified products, MS-8 high resolution agarose gels were used, in 3% (w/v) 1X TBE buffer and 0.5X TBE buffer for running. After amplification, genotyping by segregation was performed. In the genotyping of potato materials from the Venezuelan Andean region, the maximum number of bands with specific and particular molecular weights exhibited by the electrophoretic patterns that described the materials with the most polymorphic initiator was determined. Therefore, the code assigned to each material has as many digits as bands described by the electrophoretic pattern and its intensity. The genotyping code was composed of a maximum of five digits, where each digit was repeated depending on the intensity of bands observed.

Statistical analysis

Morphological and pollen data were analyzed in conjunction with the molecular data. A basic data matrix (MBD) was constructed and the analysis of components was made. The similarities of the materials were evaluated (molecular, morphological and pollen characteristics). The distances (0 to 1) were calculated according to the Gower algorithm (Gower similarity index). To visualize the dispersion of the samples with respect to the possible descriptive variables, it was complemented with Clustering and network analysis. The statistical analyzes and graphs were made with the SPSS version 21 programs (IBM Corporation, 4 Redmond, US). The clustering analysis of the main components was carried out with the program Past v3.06 (Natural History Museum, University of Oslo, Oslo, Norway). We obtained a dendrogram of relations with the UPGMA method (Unweighted Pair Method Method using Arithmetic Averages), done with the matrix of similarities (Gower similarity index) of molecular, pollen and morphological variables.

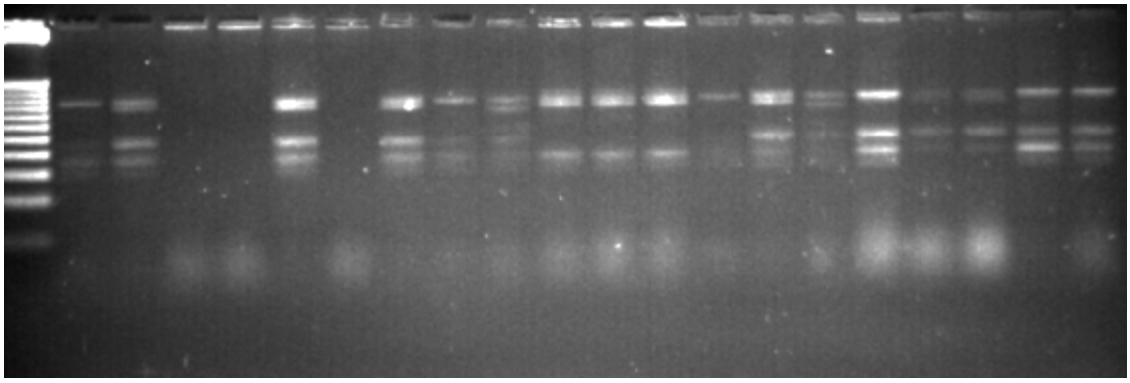
Results and Discussion

STM 0037 was the most polymorphic initiator of the nine used in the study, which produced the greatest discrimination and genotyping by segregation among the individuals evaluated (Figures 1 and 2). From the varieties evaluated, only 29 were considered for statistical analysis, which were those that possess -in addition to the molecular characterization -the morphological and pollen characterization. The varieties on lanes 1, 2, 17, 23, 24, 26, 36, 37, 38, 39 and 40, were excluded. These varieties correspond to: No Name Luís Quintero, No Name Ismael Sánchez, No Name, No Name Sector Nanjar, Papa Negra, Papa Negra Los Trigales, Papa Negra Jesús Santiago, Papa Negra Homero Santiago, Papa Negra Juan Santiago, Papa Negra José R. Santiago, Papa Negra Benjamín Quintero, and Papa Negra Timotes, respectively.



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure 1. Gel with varieties 1 to 20 all call No Name



M 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Figure 2. Gel with varieties 21 to 40 with Black and colour potatoes.

The clustering analysis the molecular characterization of 29 samples, formed 11 groups, which matched with the groups formed by the morphological and pollen characterization. The results showed that within these groups, four subgroups were formed with unique varieties such as Reinosa de Gavidia (Group III), the No Name from the communities of La Toma and La Laguna of the Rangel municipality (Groups VIII and X, respectively) and No Name from Pueblo Llano of the Pueblo Llano municipality (Group IX). In group I, a very particular discrimination was observed, as it is conformed by varieties that, although they come from very distant places, were grouped as materials with identical sequences for the portion of the genome explored with the primer STM 0037. These are the case of the coloured potatoes from El Molino and the red potatoes from Atilio González and Bernave Torres, both from the municipalities of Arzobispo Chacón and Rangel, respectively. In group II, the unification of the potatoes Arbolona negra, Bernave Torres, and Negra Liccia Romero, both from Gavidia. Ponce Almeri et al. (2013) indicate that the evaluation of the groups from the regional point of view, has a moderate tendency to group according to the geographical region. Groups IV, V and VI were the most homogeneous, with the majority of the Black potatoes being grouped in the first two, while in Group VI, 10 materials named No Name were grouped, seven of them from the Rangel municipality and three from the Cardenal Quintero municipality. A similar effect was observed in group VII with five materials of

potatoes, but for Papa Cucuba. The clustering analysis was done using the type of potato and the skin color of the tuber as characters for grouping, which agrees with the results obtained by Madroñero et al, (2013), in the characterization of promising genotypes of creole potatoes in Nariño. The use of microsatellite molecular markers was efficient for the analysis of the genetic diversity of accessions of potatoes (*Solanum spp*.), as we got as result the formation of population conformed by local groups of wild, native and improved varieties (Tenorio-Bautista et al, 2019).

The pollen analysis showed two fundamental characteristics which distinguish the pollen grains of species and varieties, i.e. the pollen corresponding to species have three colpos, with a circular rosette contour at the polar view and prolato at the equatorial view. The sample clustered at Group III, identified as Reinosa, correspond to the specie *Solanum phureja*.

Pollen grains with three or four colpos, belongs to wild potatoes pollen grains; which present variations in the contour level at polar and equatorial view. In our study, groups I, II, V, VI and VII presented three and four colpos in the pollen grains. These results were similar to the ones obtained at the molecular analysis. Groups IV, VIII, IX, X and XI, presented four colpos in the pollen grains with similar results to those obtained in the molecular analysis. Group IV samples correspond to the black potatoes varieties and Group XI to the Granola sample.

Our study also determined that exine structure can not be used as a descriptor to distinguish between species and varieties, since it maintained uniformly a granular ornamentation for the entire sample. The size and shape of the pollen grains, the shape of the openings and types of ornamentation are the most useful characters for the identification of the species. The variability of pollen in terms of morphology is very diverse and it seems to be correlated with the form of dispersion (Tovar et al., 2016). Other authors point out that the usefulness of pollen as a biomarker at the species level is limited (Collao-Alvarado et al., 2016).

The analysis of similarity (Figure 3) allowed grouping the samples into three large groups, with high degree of similarity ranging from 100% to 92% (0.00 -0.08) between the samples. The samples M11, M12, M13, M14, M15, and M7 have a similarity of 0.00 between them. The samples M11, M15 and M12, constitute a single individual, located in the group of the No Name potatoes. The samples M16, M17, and M20 presented a percentage of similarity of approximately 92% (0.08-0.09), both corresponding to the the black potatoes. The sample M28 presents a percentage of similarity in relation to the M3 sample. M4, M8, M27, M17, M18 and M26 samples had a similarity ranges from 60% to 46%. A similar percentage was found between sample M27 with M5, M8, M9, M16, M19, M20, M23, M24, and M25. Theses samples correspond to the No Name potatoes (the first three samples), the next three to the "black and coloured potatoes", and the last one to the potato called "Cucuba". Sample M29 presented a similarity distancing percentage of 6% and 58% (0.40-0.42). in relation to the samples M18, M21, M6 and M8.

M2 sample presented a percentage of similarity distancing from 60% to 54% (0.40-0.46) in relation to samples M19, M22, M23, M24, M8 and M29. These samples corresponded, the first four to the "black potatoes and coloured potato" and the last one is a potato called Granola. The combined analysis of the morphological, molecular and pollen characters of the 29 samples, gave as result four groups, with a coefficient of similarity between 0.00 to 0.40 (Figure 3). The first group is represented by sample M27, being the most divergent of the samples. The second group is conform by the samples M24, M23, M22 and M28, which maintain a similarity range between 0.20 and 0.10, and it is constituted by the coloured

potato samples and the so-called Peruvian potato. The third group is composed of the commercial variety Granola (M29) and the sample M10 (potato called “No name”). The fourth group comprised 22 of the total samples analysed, which represents 75.9% of the total, with a similarity range of 0.25 to 0.00. In this group, is represented the samples corresponding to the “No name" potatoes and the "black potatoes", in a first subgroup are the samples: M8, M3, M20, M16, M21, M26, M18 and M17.

The second subgroup, is integrated by the samples: M1, M19, M25, M9, M5, M15, M12, M11, M7, M14, M2, M6, M4 and M13. Samples M15, M12 and M11 present a similarity of 0.00 with a range of difference of M27 of 0.40, as can be visualized in the dendrogram. It was also observed that morphological markers had low discrimination of the analyzed individuals, which increases when molecular markers data. This result coincides with the one reported by Onamu et al. (2012) when they analyzed 15 potato genotypes using molecular markers.

	M01	M02	M03	M04	M05	M06	M07	M08	M09	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29
M01	0																												
M02	0,27	0																											
M03	0,34	0,19	0																										
M04	0,18	0,09	0,23	0																									
M05	0,15	0,29	0,22	0,23	0																								
M06	0,23	0,04	0,18	0,08	0,28	0																							
M07	0,19	0,23	0,15	0,17	0,09	0,19	0																						
M08	0,47	0,35	0,16	0,39	0,34	0,34	0,28	0																					
M09	0,23	0,24	0,17	0,25	0,20	0,23	0,14	0,24	0																				
M10	0,30	0,32	0,18	0,23	0,26	0,30	0,20	0,30	0,28	0																			
M11	0,20	0,22	0,14	0,16	0,08	0,20	0,01	0,27	0,13	0,18	0																		
M12	0,20	0,22	0,14	0,16	0,08	0,20	0,01	0,27	0,13	0,18	0,00	0																	
M13	0,24	0,13	0,17	0,07	0,17	0,11	0,10	0,33	0,22	0,19	0,09	0,09	0																
M14	0,18	0,19	0,17	0,13	0,10	0,18	0,04	0,29	0,15	0,16	0,03	0,03	0,07	0															
M15	0,20	0,22	0,14	0,16	0,08	0,20	0,01	0,27	0,13	0,18	0,00	0,00	0,09	0,03	0														
M16	0,42	0,38	0,24	0,38	0,29	0,37	0,23	0,21	0,27	0,33	0,22	0,22	0,31	0,24	0,22	0													
M17	0,35	0,33	0,14	0,28	0,23	0,32	0,16	0,14	0,25	0,18	0,15	0,15	0,21	0,18	0,15	0,18	0												
M18	0,33	0,26	0,22	0,25	0,30	0,30	0,24	0,22	0,33	0,26	0,23	0,23	0,28	0,25	0,23	0,25	0,08	0											
M19	0,21	0,40	0,25	0,30	0,19	0,38	0,23	0,25	0,21	0,26	0,21	0,21	0,27	0,24	0,21	0,24	0,14	0,21	0										
M20	0,40	0,37	0,18	0,36	0,28	0,36	0,21	0,14	0,25	0,27	0,20	0,20	0,29	0,23	0,20	0,07	0,11	0,19	0,22	0									
M21	0,35	0,31	0,22	0,30	0,22	0,35	0,16	0,27	0,25	0,23	0,15	0,15	0,24	0,17	0,15	0,19	0,15	0,18	0,22	0,17	0								
M22	0,27	0,46	0,27	0,37	0,25	0,45	0,26	0,31	0,22	0,28	0,25	0,25	0,34	0,27	0,25	0,27	0,25	0,33	0,19	0,23	0,30	0							
M23	0,32	0,46	0,32	0,37	0,32	0,42	0,23	0,36	0,22	0,32	0,24	0,24	0,33	0,27	0,24	0,23	0,30	0,37	0,26	0,24	0,23	0,12	0						
M24	0,23	0,40	0,31	0,36	0,23	0,41	0,22	0,35	0,21	0,32	0,23	0,23	0,33	0,26	0,23	0,29	0,29	0,32	0,18	0,28	0,17	0,15	0,13	0					
M25	0,37	0,25	0,24	0,19	0,24	0,24	0,18	0,38	0,27	0,28	0,17	0,17	0,13	0,19	0,17	0,27	0,26	0,33	0,32	0,30	0,26	0,30	0,29	0,38	0				
M26	0,30	0,27	0,23	0,26	0,28	0,31	0,22	0,28	0,30	0,24	0,20	0,20	0,30	0,23	0,20	0,25	0,16	0,09	0,28	0,18	0,09	0,31	0,32	0,26	0,31	0			
M27	0,39	0,35	0,39	0,34	0,44	0,36	0,35	0,50	0,49	0,32	0,37	0,37	0,38	0,34	0,37	0,47	0,38	0,31	0,50	0,40	0,35	0,53	0,52	0,46	0,50	0,25	0		
M28	0,25	0,47	0,40	0,43	0,28	0,48	0,32	0,49	0,35	0,39	0,33	0,33	0,39	0,33	0,33	0,34	0,41	0,44	0,27	0,38	0,36	0,18	0,23	0,21	0,39	0,42	0,54	0	
M29	0,39	0,46	0,32	0,37	0,40	0,43	0,32	0,31	0,39	0,18	0,33	0,33	0,34	0,30	0,33	0,45	0,33	0,40	0,38	0,38	0,41	0,36	0,38	0,37	0,46	0,42	0,38	0,42	0

Figure 3. Matrix of similarities of the materials evaluated according to their molecular, morphological and pollen characteristics. The distances (0 to 1) were calculated according to the Gower algorithm (*gower similarity index*).

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Conflicts of interest

The authors express that there are no conflicts of interest with the present investigation.

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