



Analysis of phenotypic traits for the differentiation of *Mussa* sp. clones and their association with environmental variables

Análisis de rasgos fenotípicos para la diferenciación de clones de *Mussa* sp. y su asociación con variables ambientales

 María Victoria Vega*,  Juana Cáceres,  Graciela María Castillo

Facultad de Recursos Naturales, Universidad Nacional de Formosa, Av. Dr. Luis Gutnisky 3200, Formosa, Argentina. CP 3600

ABSTRACT: In order to determine morphological characteristics of *Musa* sp' clones collected in Riacho He He, Laguna Blanca, Clorinda, and Laguna Naick-Neck, Formosa province, banana standard descriptor from IPGRI-INIBAP/CIRAD and Banana and Plantain Breeding International Network, were used. Nine quantitative descriptors related to the plant, to the leaves, and to the fruits were studied. Outstanding descriptors were observed in detail. Data was analyzed through Multivariate Variance Analyses, Components Principal Analysis and Regression Trees. The comparisons between provenances were highly significant and phenotypic characteristics were associated with one environmental variable. The Principal Component Analysis explained 87 % of the phenotypic variance in two extracted components. A genetic improvement program is very important to obtain new clones due to traits can be highly heritable.

Key words: phenotypic variability, plant breeding, environmental variables.

RESUMEN: Para determinar las características morfológicas de clones de *Musa* sp recolectados en Riacho He He, Laguna Blanca, Clorinda, y Laguna Naick-Neck, se utilizaron los descriptores estandarizados de banano del IPGRI - INIBAP/CIRAD y la Red Internacional para el Mejoramiento del Banano y el Plátano, considerándose 9 descriptores correspondientes a caracteres cuantitativos de la planta, hojas, pseudotallo, flores, frutos, etc. Se hizo énfasis en los descriptores altamente sobresalientes. Los datos fueron analizados estadísticamente mediante un Análisis de Varianza Multivariado, Análisis en Componentes Principales y Árboles de Regresión. Las comparaciones entre procedencias fueron altamente significativas para todos los caracteres analizados. Las características fenotípicas evaluadas estuvieron relacionadas con el ambiente, según el análisis basado en los árboles de regresión. El análisis en Componentes Principales explicó el 87 % de la varianza fenotípica existente en las dos primeras componentes. De tal manera, es muy importante el desarrollo de un programa de mejoramiento genético a través de descriptores morfológicos para obtener nuevos y mejores clones, ya que los rasgos analizados pueden ser altamente heredables.

Palabras clave: variabilidad fenotípica, fitomejoramiento, variables ambientales.

INTRODUCTION

Banana belongs to the *Musa* genus and is one of the most developed crops in the world. Most of the cultivars come from *Musa acuminata* (genome A) and *Musa balbisiana* (genome B), edible diploids that are crossed with their wild progenitors, giving rise to sterile progeny, forming diploid (AB), triploid (AAA, AAB), and tetraploid (ABBB and AAAB) genomes. Section *Eumusa* comprises the

Cavendish subgroup with eleven species, distinguishing triploid cultivars (1).

In Formosa province, Argentina, the banana-growing area is located in the Pilcomayo and Pilagás Departments, with 90 % of the farms in the locality of Laguna Naick-Neck (Pilcomayo Department). The behavior of plants is associated with the agroclimatic conditions, manifesting itself through the variability and magnitude of the growth processes.

*Author for correspondence: mavivega@yahoo.es

Received: 12/06/2023

Accepted: 08/08/2024

Conflict of interest: Authors declare that they have no conflict of interest.

Authors' contribution: **Conceptualization:** Graciela Castillo. **Research:** Juana Cáceres, María Victoria Vega. **Methodology:** María Victoria Vega, Juana Cáceres, Graciela Castillo. **Supervision:** Graciela Castillo. **Initial draft writing:** María Victoria Vega. **Final writing and editing:** María Victoria Vega, Graciela Castillo. **Data curation:** María Victoria Vega, Juana Cáceres.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial (BY-NC 4.0). <https://creativecommons.org/licenses/by-nc/4.0/>



One of the main problems of banana is low yields, due to problems caused by black *Sigatoka*, black weevil and virosis. As for local material, it is essential to characterize both morphological and agroecological zones suitable for cultivation, since the 1950s, material from different origins (presumably from Brazil and Paraguay) has been introduced, of which, in many cases, only their passport data are known.

An alternative that can improve commercial production in the medium and long term is the development of new cultivars through genetic improvement programs, based on the characterization of morphological descriptors (leaves, plant height and stem perimeter) and agronomic descriptors (number of fruits, net and gross weight of banana), which allow taxonomic differentiation of plants (3). These characters can be highly heritable, easily observable, expressing themselves in the same way in any environment (2), and once the plants are identified, it is essential to select the best ones that present a good productive aptitude or tolerance to certain diseases. Although, the efforts to achieve improvement in the last 50 years have determined that at higher productivity levels, high levels of genetic vulnerability are detected, the essential and fundamental is the genetic valorization of germplasm as a response to achieve stability and productivity of the methods to extend its genetic base (3).

The objective of this work was to evaluate the phenotypic diversity in clones of *Mussa* sp in four localities of Formosa province, Argentina, based on morphological characters.

MATERIALS AND METHODS

The sites sampled corresponded to banana growers' farms, located at parallels 25°17'10" South Latitude and 57°43'21" West Longitude, in the Pilcomayo Department, approximately 110-141 km from the capital city of Formosa Province, Argentina. Mother plants of the Sub - Group: *Cavendish*, Variety: Nanika, were sampled in four origins (Riacho He He, Laguna Blanca, Clorinda, and Laguna Naick-Neck), and in each of them two banana farms (Figure 1).

The selected mother plants had a history of performance under adverse climatic conditions (low temperatures -5 °C and extreme temperatures: 45 °C) and diseases (*yellow Sigatoka*), as well as their productivity potential. The plantation design was 2 x 3 m (2 m between plants and 3 m between rows) in some cases, and in others the design was 1 x 4 (1 m between plants and 4 m between rows), being geo-referenced with GPS (Garmin Etrex 10), and passport data were recorded (Figure. 1).

The zones were characterized by soils with natural drainage and the most representative was the loam soil with abundant organic matter, with average annual rainfall ranging from 60 to 61 mm and average temperatures of 27.9 °C during the study.

Morphological characterization was carried out based on morphological descriptors used by IPGRI (5) and the International Banana and Plantain Improvement Network. Quantitative characters evaluated were plant height (m) (measured from the base to the V-shaped insertion of the last emitted leaves), pseudostem diameter (cm) (measured

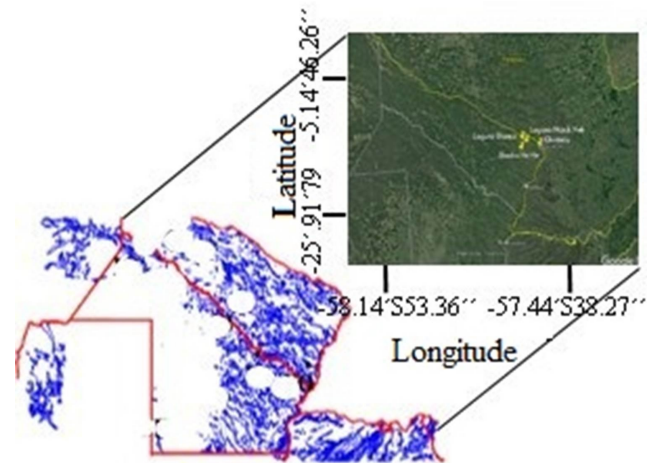


Figure 1. Location of the sampling sites

from the base to 1 m height), leaf length and width (cm). In addition, petiole length (cm), second hand central finger length (cm) measured with tape measure, while second hand fruit diameter (cm) was measured with a Vernier caliper (Mitutoyo 530-122, graduation 0.02 mm, measuring range up to 150 mm) were evaluated.

Simple random probability sampling was applied, where each of the phenotypes had a known probability of being selected. The sample size was $n = 40$ phenotypes per origin, evaluating 320 plants.

The variable system applied consisted of Independent Variable (X): provenances, Dependent Variables (Y):

1. pseudostem height (PH) (m)
2. pseudostem diameter (PD) (cm)
3. leaf length (LL) (cm)
4. leaf width (LW) (cm)
5. number of leaves (NL)
6. petiole length (LP) (cm)
7. number of hands
8. second hand central finger length (SHCFL2nd) (cm),
9. second hand finger diameter (SHFD2nd) (cm).

Variation between provenances was characterized through a multivariate analysis of variance (MANOVA) based on the linear model: "equation (I)".

$$Z_{ij} = \mu + P_i + e_{ij} \quad (I)$$

Where: Z_{ij} : trait observation for individual j of provenance i ; μ : overall mean; P_i : effect of provenance; e_{ij} : residual component.

The *Hotelling* test was applied for the treatment averages at a confidence level of 95 %.

To determine which descriptors showed the greatest variability, a Principal Component Analysis was used based on Spearman's correlation matrix. The genetic variation of phenotypic traits and environmental variables

was established using regression trees, to estimate the regression by a recursive partitioning of the sample in binary mode dividing the data set from the environmental variables. For each sampled site, mean annual precipitation and temperature were recorded. Data were obtained from meteorological stations located in Laguna Naick-Neck, Laguna Blanca and Riacho He He (Pilcomayo Department), Formosa Province, Argentina.

All data were analyzed, through the statistical program InfoStat version 2017 on Windows. Tests were performed with a significance level of 5 % (4).

Todos los datos se analizaron, a través del programa estadístico InfoStat versión 2017 sobre Windows. Las pruebas se efectuaron con un nivel de significación del 5 % (4).

RESULTS

The variance showed highly significant differences between provenances (Wilks $p < 0.0001$) for all the traits

evaluated, i.e. the phenotypes behaved differently in the different locations (Table 1). In addition, there were significant ($< 0.01^*$) and highly significant ($< 0.0001^{**}$) differences for the environmental variables of the different provenances analyzed. In the Hotelling test, it was observed that the means obtained between groups were significantly different between provenances. The provenances of Laguna Blanca and Riacho He He differed from each other, and from Laguna Naick-Neck and Clorinda (Table 2). Table 3 shows the descriptors evaluated that contributed to the characterization of the germplasm.

The Principal Component Analysis explained 87 % of the phenotypic variance in the first two extracted components. The first component contemplated the AP characteristic that contributed approximately 65 % of the observed variability. The second component was composed of the PD variable that contributed 87 % (Table 3). In component 1, the variables that contributed moderately to the characterization were PH, PD, LL and NH. For component 2, the variables that stood out were SHFD2th, and WL (Table 3).

Table 1. Analysis of Variance of the phenotypic traits evaluated (Wilks)

F.V.	Statistical	F	gL (num)	gL (den)	p
Sources		3.0	27	1143	< 0.0001**
MAR	0.4	54.6	9	391	< 0.0001**
MAT	0.9	2.3	9	391	< 0.01*

*significant ($p > 0.05$), ** highly significant ($p > 0.05$)
MAR= Mean Annual Rainfall, MAT= Mean Annual Temperature

Table 2. Multiple Comparisons Test: Hotelling Test

Source	PH	PD	LL	LW	NL	PL	NH	SHFD2 nd	SHCFL2 nd	
RH	3.0	0.7	2.0	0.8	9.4	0.3	16.8	0.03	0.18	A
LB	2.9	0.6	2.1	0.8	8.1	0.3	15.5	0.03	0.18	B
CI	2.8	0.6	2.0	0.7	8.4	0.3	15.3	0.03	0.17	C
LN	2.9	0.6	2.0	0.8	8.3	0.3	16.2	0.03	0.17	C

Means with a common letter are not significantly different ($p > 0.05$).

PH = pseudostem height, PD = pseudostem diameter, LL = leaf length, LW = leaf width, NL = number of leaves, PL = petiole length, NH = number of hands (taken in the middle hand of the bunch), SHFD2nd = second hand finger diameter, SHCFL2nd = second hand central finger length

Table 3. Análisis de Componentes Principales para variables cuantitativas analizadas

Component	C1	C2
Eigenvalues	5.87	1.97
Ratio	0.65	0.22
Prop. Acum	0.65	0.87
Autovectors		
PH	0.39	-0.20
PD	0.40	0.16
LL	0.38	0.14
LW	0.28	-0.50
NL	0.38	0.20
PL	0.17	0.45
NH	0.41	0.03
SHFD2 nd	-0.11	0.65
SHCFL2 nd	0.34	0.02

PH = pseudostem height, PD = pseudostem diameter, LL = leaf length, LW = leaf width, NL = number of leaves, PL = petiole length, NH = number of hands (taken in the middle hand of the bunch), SHFD2nd = finger diameter of second hand, SHCFL2nd = length of central finger of second hand

The regression trees (Figures 2 and 3) showed that all traits were significantly or highly significantly associated with at least one environmental variable. In both Figure 2 and Figure 3, the trees presented terminal nodes, which were determined by differences in mean annual precipitation and temperature. A MAT and MAR threshold of approximately 28.2 °C and 61.9 mm was shown to be linked to the traits LW, PH, PD, LL, and NH (Figure 2).

Sixty-four percent of the sampled individuals were assigned to the partition with MAT and MAR values below or equal to the threshold mentioned in the paragraph above. Both MAR and MAT presented low variances ranging from 0.01 to 0.2 for almost all phenotypic traits, with the exception of NH whose variance was higher 11.2 -12.9. The means ranged from 0.6 to 16.7 depending on the trait analyzed.

In Figure 3, three terminal nodes were observed for the traits PL, NL, SHCFL2th and SHFD2th with a MAT and MAR threshold of approximately 27.6 °C and 60.2 mm. Twenty-seven percent of the sampled individuals were assigned to the partition with MAT and MAR values below or equal to the threshold mentioned in the paragraph above. 2th

DISCUSSION

The studies carried out in the genus *Musa* show that morphological characterization, based on growth characters, made it possible to obtain groups of individuals with common phenotypic characteristics in a much greater degree of detail than that achieved by classical systematics (6-10).

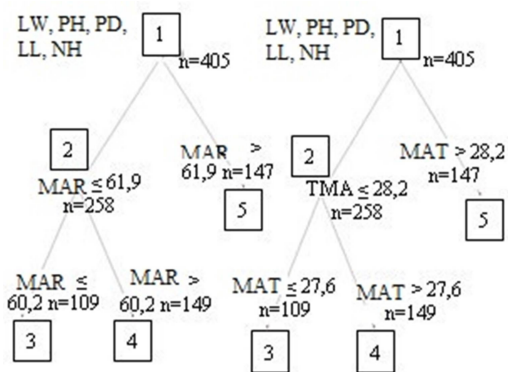
Statistical analysis of the collected phenotype samples determined the presence of heterogeneous material, with high variability among provenances. Other researchers have reported statistically similar data (8-14).

The height and diameter of the pseudostem formed the first component, constituting a source of phenotypic variability among clones with similar values reported in other studies (8). As for the trait NH, which also contributed greater variability in the first component, the values per bunch were lower than those obtained in the varieties Tafetan Rojo (dessert banana), Cachaco sin bellota, and Cachaco espermo (cooking bananas) (14).

Regression tree analysis showed that the variability of most of the quantitative traits was associated with the environment. In this sense, several authors (15-18) proved the existence of significant associations between morphological variables and some climatic factors, suggesting a control of rainfall on growth in *Faidherbia albida* and *Neltuma alba* (15). The observed dependence on mean annual precipitation and temperatures allowed grouping individuals whose parents developed in environments with different precipitation and temperatures.

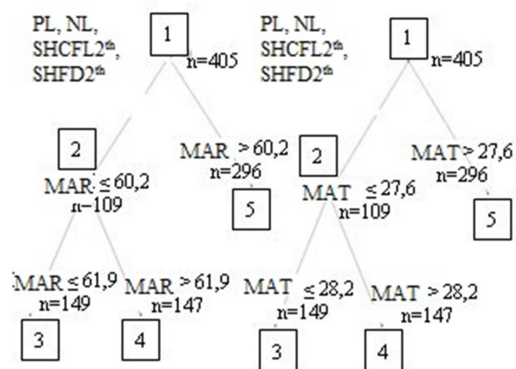
CONCLUSIONS

The evaluation and analysis of the morphological descriptors of the four *Musa* sp. provenances allowed a differentiation based on the phenotypic characteristics of the clones. For the morphological characterization, nine quantitative descriptors were analyzed, being the height and diameter of the pseudostem, leaf length, number of leaves,



MAR=Mean annual rainfall; MAT=Mean annual temperatures. PH=Pseudostem height, PD=Pseudostem diameter, LL=Leaf length, LW=Leaf width, NH=Number of hands.

Figure 2. Regression trees for the traits pseudostem height, pseudostem diameter, leaf length, leaf width, number of hands depending on rainfall and mean annual temperatures



MAR = Mean annual rainfall; MAT = Mean annual temperatures. PL= petiole length of, NL=number of leaves, SHCFL2nd = length of the central finger of the second hand), and SHFD2nd (diameter of the finger of the second hand).

Figure 3. Regression trees for the traits petiole length, number of leaves, length of the central finger of the second hand, and diameter of the finger of the second hand, depending on rainfall and mean annual temperatures

petiole length, and finger diameter of the second hand, the variables that most contributed to the differentiation of the clones. In addition, it was observed that the analysis based on regression trees determined a significant or highly significant association with at least one environmental variable.

BIBLIOGRAPHY

1. Ermini J, Tenaglia G, Pratta G. Molecular Diversity in Selected Banana clones (*Mussa* AAA Cavendish) Adapted to the Subtropical Environment of Formosa Province (Argentina). *American Journal of Plant Sciences*. 2018. <https://doi.org/10.4236/ajps.2018.912181>
2. Bustamante Intriago E. Selección de material genético de banano cv. Williams (*Musa* AAA) resistente a sigatoka negra (*Mycosphaerella fijiensis* Morelet), mutados con radiación gamma. Quevedo. 2019. UTEQ. p 63. <http://repositorio.uteq.edu.ec/handle/43000/3842>

3. Cedeño Vera, J., y Puerta Napa, A. Caracterización morfoagronómica de 51 selecciones élites de plátano CV. Barraganete (*Musa* AAB Simmonds) en el valle del Río Carrizal. Proyecto de investigación. Escuela de Ingeniería Agrícola. Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López. 2023.
4. Di Rienzo J, Casanoves F, Balzarini M, González L, Tablada M, Robledo C. InfoStat Software Estadístico 2017.
5. IPGRI. Descriptores para el Banano (*Musa* sp.). INIBAP, Montpellier. 1996. ISBN: 92- 9043-307-8.
6. Mera C. Caracterización agronómica y sanitaria de mutantes de banano cv. Williams en la Estación Experimental Tropical Pichilingue, Provincia de Los Ríos. Tesis de Grado. Guayaquil, Guayas, Ecuador: Universidad de Guayaquil. 2019. p 78. <http://repositorio.ug.edu.ec/handle/redug/39405>
7. Mozombite Tello M. Caracterización botánica y evaluación preliminar del rendimiento en tres ecotipos de *Musa paradisiaca* L. Tesis para optar el título profesional de Ingeniero Agrónomo. Facultad de Ciencias Agrarias. Universidad Nacional de San Martín- Tarapoto. 2019. p 54. <http://hdl.handle.net/11458/3601>
8. Navia M. Caracterización morfológica de cultivares recolectados de banano y plátano. Lima, Perú: PIA. 2019. <http://repositorio.uteq.edu.ec/handle/43000/6089>
9. Zambrano Saltos C. Caracterización morfológica de un banco de germoplasma de banano cv. Williams proveniente de mutagénesis física en la Estación Experimental Tropical Pichilingue. Proyecto de Investigación previo a la obtención del título de Ingeniero Agrónomo. Facultad de Ciencias Agrarias. Universidad Técnica Estatal de Quevedo. 2020. p 77. <http://repositorio.uteq.edu.ec/handle/43000/6089>
10. Gerónimo F, Ibarra Q, Navia M, Aguirre G. Caracterización morfológica de plátano (*Musa paradisiaca* L.) en la Provincia NorYungas de la Paz y Provincia Chapare de Cochabamba, Bolivia. *Agrocencias Amazonia*. 2013;1(2):35-41. <https://es.scribd.com/document/307843358/Caracterizacion-Morfologica-de-Platano-Musa-Paradisiaca-I-en-La-Provincia#download>
11. Buitrago Bitar, M., Enrique Valencia, A., Londoño Caicedo, J., Muñoz Flores, J., Villegas Estrada, B., & Santana Fonseca, G. Molecular and morphological characterization of *Musa* spp. (*Zingiberales: musaceae*) cultivars. *Bol. Cient. Mus. Hist. Nat. Univ. Caldas*. 2020; 24(1). ISSN 0123-3068. <https://doi.org/10.17151/bccm.2020.24.1.2>
12. Mera C. Caracterización agronómica y sanitaria de mutantes de banano cv. Williams en la Estación Experimental Tropical Pichilingue, Provincia de Los Ríos. Tesis de Grado. Guayaquil, Guayas, Ecuador: Universidad de Guayaquil; 2019. 78 p. <https://repositorio.uteq.edu.ec/server/api/core/bitstreams/cc8e8ff2-4881-4a52-ac70-df45316aa8ad/content>
13. Navia Montañó M. Caracterización morfológica de cultivares recolectados de banano y plátano. Lima, Perú: PIA ACC-UMSS08. 2019. <https://docplayer.es/140038862-Pia-acc-umss-o8-caracterizacion-morfologica-de-cultivares-recolectados.html>
14. Torres Cabrera D, García Águila L, Bermúdez Carabaloso I, Sarria Z, Hurtado Ribalta O, Delgado E, Pérez A, Fernández Martínez O. Respuesta morfo-agronómica y organoléptica de cinco cultivares de banano (*Musa* sp.) en condiciones de campo. *Biotecnología Vegetal*. 2020; 20 (1): 43 - 50. ISSN 2074-8647, RNPS: 2154. <https://revista.ibp.co.uk/index.php/BV/article/view/654/pdf>
15. Roser, L. *Genética del paisaje en poblaciones de *Prosopis alba* de la provincia de Santiago del Estero*. (info:eu-repo/semantics/doctoralThesis). Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. 2017. https://hdl.handle.net/20.500.12110/tesis_n5874_Roser
16. Fontana, L., Pérez, V. y Luna .C. Efecto del origen geográfico sobre la calidad morfológica de plantas de *Prosopis alba* (Fabaceae). 2018. *Rev. Biol. Trop*, 66; (2), 593-604. *Inst. J. Trop. Biol.* ISSN 0034-7744. <http://dx.doi.org/10.15517/rbt.v66i2.33383>
17. Fredrick, C., Muthuri, C., Ngamau, K. & Sinclair, F. (2015). Provenance variation in seed morphological characteristics. germination and early seedling growth of *Faidherbia albida*. *Journal of Horticulture and Forestry*, 7 (5), 127-140. <https://doi.org.10.5897/JHF2015.0392>
18. Vega, M.V. (2023). Instalación de un huerto experimental y evaluación fenotípica de *Neltuma alba* (Fabaceae) para la estimación ex situ de parámetros genéticos: heredabilidad y diferenciación entre procedencias. Tesis para optar el título de Doctor en Recursos Naturales. Facultad de Ciencias Agrarias. Universidad Nacional del Nordeste. Corrientes. Argentina.