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Original Paper

Morphological and phenological variability of Guaraní maize germplasm from Northeastern Argentina (NEA)

María Florencia Realini [1,2,6], *Lidia Poggio* [1,4], *Ana María Miante Alzogaray* [3] & *Graciela Esther González* [1,5]

Abstract

Morphological and phenological variability of Guaraní maize germplasm from Northeastern Argentina (NEA) are potential sources of genetic resources, constituting valuable repositories of alleles associated with tolerance and resistance to biotic and abiotic stresses. Here, we studied the morphological and phenological variability in maize populations of 14 Guaraní landraces from NEA and analysed the relationship among them. These landraces were characterised according to the endosperm consistency of grain as Popcorn (Pc), Floury (F) and Floury with corneal periphery (F-Pc). There were inter-population differences in phenological characters as time from germination to tassel emergence, from germination to spike emergence and from germination to anthesis (length of vegetative cycle -LVC). The morphological and phenological data here reported were used to carry out a cluster analysis, in which the F and Pc populations appeared as two separate groups and the F-Pc populations were included in the F group. Thus, the well-defined F (including F-Pc) and Pc genetic pools should be prioritised within the framework of breeding plans as well as *ex situ* and *in situ* conservation programs. For these purposes it is essential the morphological, phenological and genetic characterisation the maize Guaraní landraces.

Key words: cluster analysis, diversity conservation, genetic resources, Guaraní maize landraces.

Resumen

Las razas de maíz Guaraní, nativas del noreste de Argentina (NEA), son fuentes potenciales de recursos genéticos y constituyen depósitos valiosos de alelos asociados con tolerancias y resistencia a estreses bióticos y abióticos. En este trabajo, estudiamos la variabilidad morfológica y fenológica en poblaciones de 14 razas guaraníes de maíz del NEA y analizamos las relaciones entre ellas. Estas razas se caracterizaron según la consistencia del endosperma de sus granos como Popcorn (Pc), Floury (F) y Floury con periferia cornea (F-Pc). Se observaron diferencias interpoblacionales en caracteres fenológicos como el tiempo desde la germinación hasta la emergencia de la panoja, desde la germinación hasta la emergencia de la espiga y desde la germinación hasta la antesis (longitud del ciclo vegetativo-LVC). Los datos morfológicos y fenológicos reportados aquí se emplearon en un análisis de conglomerados, en el cual las poblaciones F y Pc formaron dos grupos separados, mientras que las poblaciones F-Pc se incluyeron en el grupo F. Por lo tanto, los pooles génicos bien definidos F (incluyendo F-Pc) y Pc deben priorizarse en el marco de planes de mejoramiento y programas de conservación *ex situ* e *in situ*. Para estos propósitos es esencial la caracterización morfológica, fenológica y genética de las razas nativas de maíz Guaraní.

Palabras clave: análisis de conglomerados, conservación de la diversidad, recursos genéticos, razas nativas de maíz Guaraní.

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[1] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Inst. Ecología, Genética y Evolución IEGEBA-CONICET, Depto. Ecología, Genética y Evolución, Lab. Citogenética y Evolución (LaCyE), Ciudad Autónoma de Buenos Aires, Argentina.

[2] Universidad de Buenos Aires, IFEVA-CONICET - Facultad de Agronomía, Depto. Recursos Naturales y Ambiente, Cátedra de Ecología, Ciudad Autónoma de Buenos Aires, Argentina. ORCID: <https://orcid.org/0000-0002-9527-1190>.

[3] Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Botánica Agrícola, Ciudad Autónoma de Buenos Aires, Argentina. ORCID: <https://orcid.org/0000-0003-4946-4597>.

[4] ORCID: <https://orcid.org/0000-0003-2223-2374>. [5] ORCID: <https://orcid.org/0000-0003-1071-7665>.

[6] Author for correspondence: floreal282@gmail.com

Introduction

Maize is a major cereal crop worldwide, adapted to different climatic conditions and farming practices. It is cultivated from Alberta in Canada to the province of Chubut in Argentina, and from sea level to 4,000 m.a.s.l (Samayoa *et al*. 2018). The first extensive study of maize morphological diversity in Latin America was led by Sergey Bukasov from the Soviet Institute of Applied Botany, as part of Nikolai Vavilov's efforts to investigate the diversity and utility of crops worldwide. The analysis of over 8,000 maize populations sampled globally revealed that at extreme latitudes (*e.g*., Argentinian Patagonia region) plants are short, have few leaves and a short vegetative cycle, whereas in tropical regions (*e.g*., as Yucatan and southern Colombia) plants are tall, have many leaves and show a long vegetative cycle (Kuleshov 1930, 1933).

Maize landraces are classified by the characteristics of the ear, grain, tassel, whole plant and their geographic distribution (Goodman & Brown 1988). Cámara Hernández *et al*. (2011) described morphologically and phenologically about 50 Argentine native maize landraces cultivated by small farmers for their own consumption and cultural practices and provided a guide to their identification. Two centres of maize diversity have been recognised in Argentina based on the geographical distribution of genetic factors: the Northwestern Argentina Region (NWA), which represents an expansion of the Peruvian Andes, and the Northeastern Argentina Region (NEA), also called Chaco-Mesopotamian plain (Horovitz 1935). So far, 28 landraces have been reported from NWA and 23 from NEA, 15 of which are cultivated by the Guaraní indigenous communities from the subtropical forests in Misiones Province (Cámara Hernández *et al*. 2011).

The Guaraní's landraces grow in an area of little topographic variation and relatively stable environmental conditions. Their agronomic traits (*e.g*., marked differences in length of the vegetative cycle, resistance to fungi and high soil humidity) make them a potentially important source of germplasm for genetic improvement (Melchiorre *et al*. 2006, 2020; Realini 2017; Realini *et al*. 2016). The Guaraní communities cultivated native landraces maintaining the use of long-standing agricultural practices in small parcels (Keller 2007). In the last decades, however, the use of commercial high-yielding varieties and the expansion of the agricultural frontier led to loss of native maize biodiversity in NEA (Bracco *et al*. 2012; Cámara Hernández *et al*. 2011). Genetic and karyotype studies of Guaraní maize revealed the existence of two gene pools, the Popcorn and the Floury landraces, which differ in endosperm consistency. The F-Pc landraces, endosperm floury with corneal periphery, has been included in the Floury gene pool (Bracco *et al*. 2016; Realini *et al*. 2016, 2018).

The study and conservation of Guaraní landraces is important because they are well adapted to the environmental conditions in NEA and meet the needs and traditions of local communities (Melchiorre *et al*. 2020).

The determination of clearly defined maize genetic groups is useful for implementing *ex situ* and *in situ* conservation and breeding programs. The objective of this study was to analyse the phenotypic diversity and the relationship among Guaraní maize landraces from NEA based on morphological and phenological traits.

Materials and Methods

Plant material

Twenty-six Guaraní maize populations belonging to 14 landraces were collected from Guaraní farmers in the Misiones Province, NEA (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>). Each population was assigned to a maize landrace according to the classification of Cámara Hernández *et al*. (2011). The term landrace is used to define a taxonomic entity delimited by a set of morphological traits, and the term population refer to a group of individuals that are managed by a single local farmer and share similar characteristics (*i.e.*, can be assigned to the same landrace). The specimens were deposited at the seed bank of the Laboratory of Genetic Resources N. I. Vavilov at the Facultad de Agronomía (FA), Universidad de Buenos Aires (UBA), and a voucher number was assigned for the Vavilov seed bank (VAV).

Methods

In up to 5 ear per population, the following traits were measured: number of grains rows and diameters of ear, cob, rachis and pith. Ten grains in three adjacent rows were removed from the middle of each ear to measure the length, thickness and weight of each grain, and then data were averaged. To classify the grain type as Floury (F),

Floury with corneal periphery (F-Pc) or Popcorn (Pc), the endosperm consistency was determined after making a longitudinal cut along the grain with a razor blade, according to Cámara Hernádez *et al*. (2011). These grains were germinated at 28 °C for 2 to 3 d in Petri dishes containing wet filter paper. Seedlings of about 20 cm high were manually transplanted to the experimental field of the Botanical Garden Lucien Hauman (FA, UBA), (34º35'S, 58º29'W and 25 m.a.s.l.). Ten to 15 grains per ear from 1 to 5 ears belonging to each population were planted in 15 rows of 20 plants each, with a spacing of 30 cm between plants and 75 cm between rows. The cross-pollinations were carried out between plants from the same population. For this purpose, the female inflorescences (spikes) of all plants and the male inflorescences (tassels) of selected plants were covered with waxed bags. The pollen grains from mature tassels were collected and dusted over the stigmas. Pollinated spikes were rebagged to prevent free pollination.

The following phenological traits were recorded for each plant: time (d) from germination to tassel emergence, to spike emergence and to anthesis, naming LVC-length of vegetative cycle as the time from germination to anthesis (Melchiorre *et al*. 2006). After harvest, ears were photographed, assigned voucher numbers and deposited at the N. I. Vavilov seed bank for *ex situ* conservation.

Statistical analysis

A UPGMA cluster analysis was performed using the mean values of morphological and phenological traits (time to spike emergence and LVC). Data were standardised and an Euclidean distance-based similarity matrix was built. Statistical analysis was done with the program Infostat, FCA, Universidad Nacional de Córdoba (Di Rienzo *et al*. 2012). Box-plot graph was obtained by GraphPad Prism 8.0.1 software (2018).

Results

The mean values of the morphological and phenological traits of the studied Guaraní maize populations are shown in Tables S2 and S3 (available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>), respectively. Entire ears from Pipoca Colorado landrace were not available, for this reason, is not shown in Table S2 (available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>). The analysis of endosperm consistency allowed to classify each population into the Floury (Fig. 1a-c), F-Pc (Fig. 1d-f) and Popcorn (Fig. 1g-i) types. The mean ear diameter ranged from 3.27 to 4.96 cm in Floury, 3.95 to 3.98 cm in F-Pc and 3.17 to 4.06 cm in Popcorn populations. The mean number of grain rows per ear ranged from 10 to 14 in Floury, 12.67 to 14 in Popcorn and 13.33 to 16 in F-Pc populations (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>). The mean weight of 10 grains ranged from 1.43 (VAV6827) to 1.90 g (VAV2011/09) in Popcorn populations and from 2.03 (VAV6819) to 3.48 g (VAV6847) in Floury populations, whereas F-Pc populations showed intermediate values, ranging from 2.38 (VAV6851) to 2.42 g (VAV6837) (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>).

The time to tassel emergence ranged from 87.10 to 118.58 d (VAV6818 and VAV6827, respectively), time to spike emergency ranged from 97.60 to 125.71 d (VAV6818 and VAV6843, respectively), and LVC ranged from 89.55 to 124.78 d (VAV6818 and VAV6827, respectively) (Tab. S3, available on supplementary material <https://doi.org/10.6084/m9.figshare.22151345.v1>). The Floury populations showed short to intermediate LVC, with values between 89.55 and 114.33 d (VAV6818 and VAV6847, respectively). The Popcorn populations showed long LVC values, ranging from 108 to 124.78 d (VAV2011/09 and VAV6827, respectively), the F-Pc populations had intermediate LVC values, from 104.86 to 112.75 d (VAV6837 and VAV6851, respectively) (Fig. 2).

The UPGMA cluster analysis based on Gower distances (cophenetic correlation = 0.8) revealed the presence of two groups (Fig. 3). One group included the Pc populations (VAV6827, VAV6826. VAV2011/09 and VAV6843) and the other comprised all the Floury and the two F-Pc populations (VAV6837 and VAV6851) (Fig. 3).

From each population, cross-pollinated ears were obtained from sister plants for the *ex situ* conservation purpose and some of them are show in Figure 4.

Discussion

The analysis of 26 maize populations belonging to 14 Guaraní landraces from NEA revealed an important phenotypic diversity in terms of ears and grains. In this work, landraces were characterised according to the endosperm consistency of grains. Most of the endosperm is

composed of starch granules at different degrees of compression. In the Floury landrace, starch granules are slightly compressed, giving grains a white colour and a floury consistency, while in the Popcorn landrace, with very compressed granules, grains have a glassy appearance and a hard consistency (Cámara Hernández & Arancibia de Cabeza 2007). The consistency of the grains and other morphological traits of the populations studied here allowed corroborating the racial classification provided by these authors.

The plants transplanted to the experimental field were used to estimate the phenological traits of the studied Guaraní maize populations. These showed differences in time from germination to tassel emergence, to spike emergence and to anthesis. In particular, a wide inter-population variability was observed for LVC, the time from germination to anthesis. The Pc populations had the longest LVC (108 - 124 d), F populations were precocious or had intermediate LVC (90 - 114 d), whereas F-Pc populations showed intermediate

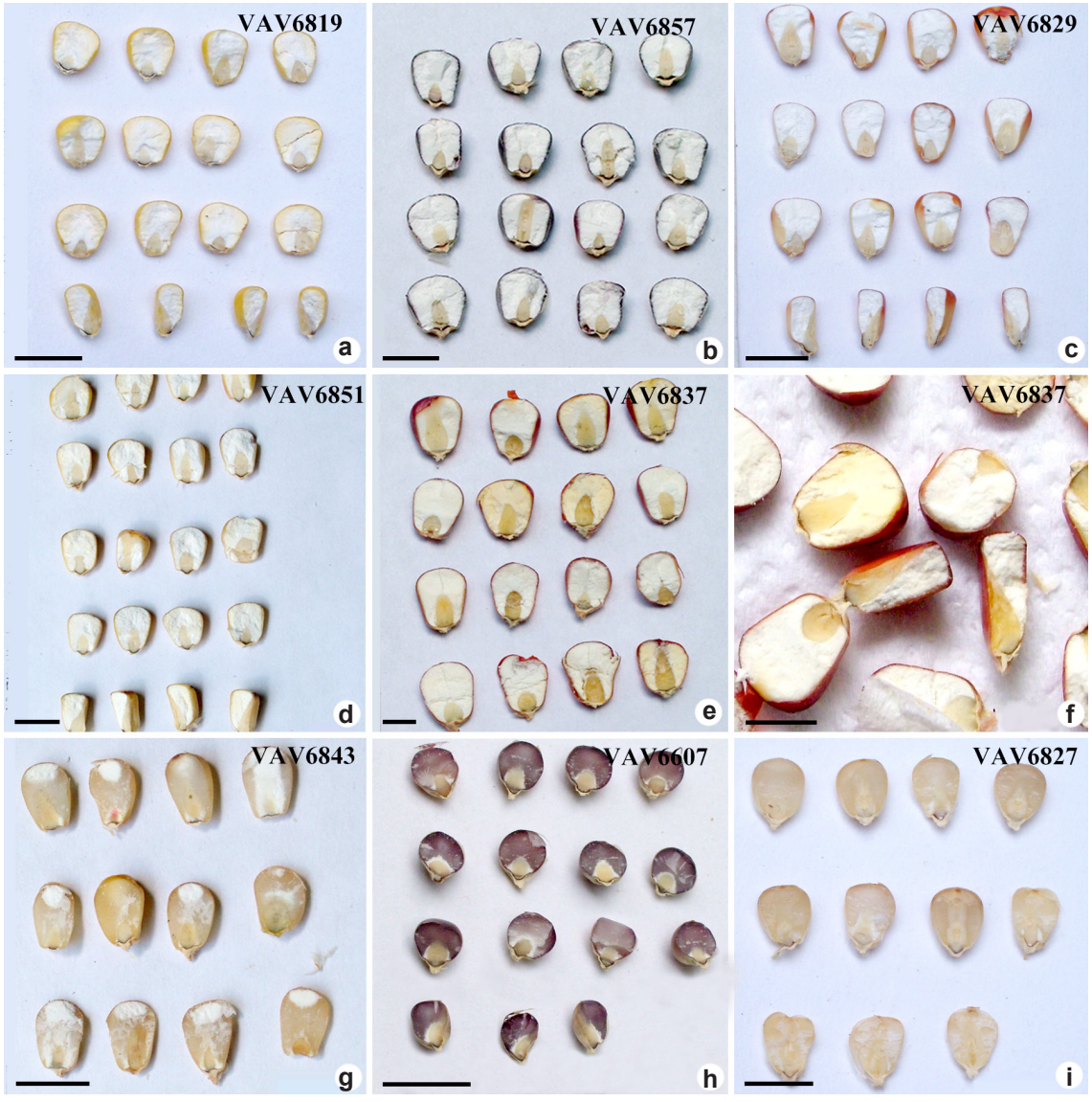

Figure 1 – a-i. Longitudinal-radial cuts of grains of Guaraní maize landraces – a-c. grains of Floury populations; d-f. grains of F-Pc populations (f. also shows longitudinal-tangential cuts); g-i. grains of Popcorn populations. Ref.: numbers correspond to voucher ears of the studied population. Bar = 1 cm.

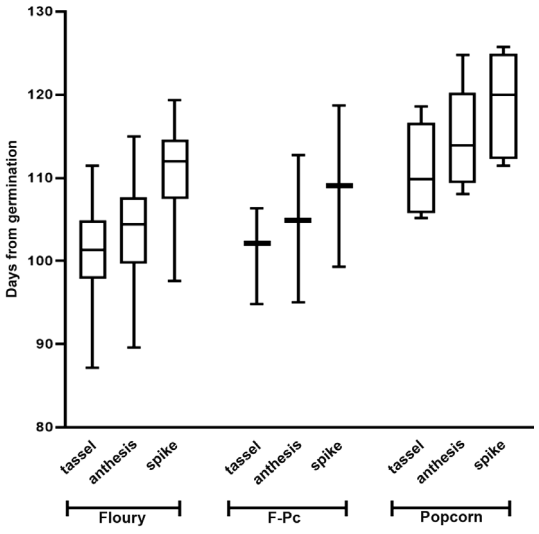

Figure 2 – Days from germination to emergence of tassel, to anthesis (LCV) and to spike emergence for the Floury, Popcorn and F-Pc populations. Ref.: Whiskers represent the range.

LVC (104 - 112 d). In general, these results are in agreement with those reported by Melchiorre *et al*. (2006) and Cámara Hernández *et al*. (2011) for Guaraní populations cultivated in Misiones province (26º58'S, 55º02'W and 166 m.a.s.l). The LVC values of Guaraní maize cultivated in the Buenos Aires province (34º35'S, 58º29'W and 25 m.a.s.l., present study) were higher than those recorded in the Misiones province. However, the relationship between the long and short LVC populations was maintained in both locations. The variation in LVC values between localities was possibly due to differences in edaphic, environmental, climatological and biological factors. The study of genotype-environment interactions is relevant when selecting material for maize breeding programs, as they can vary under different environmental conditions (Melchiorre *et al*. 2020). Therefore, the knowledge of phenological characters of the Guaraní maize landraces studied here provides useful information for planning breeding and *in situ* conservation strategies.

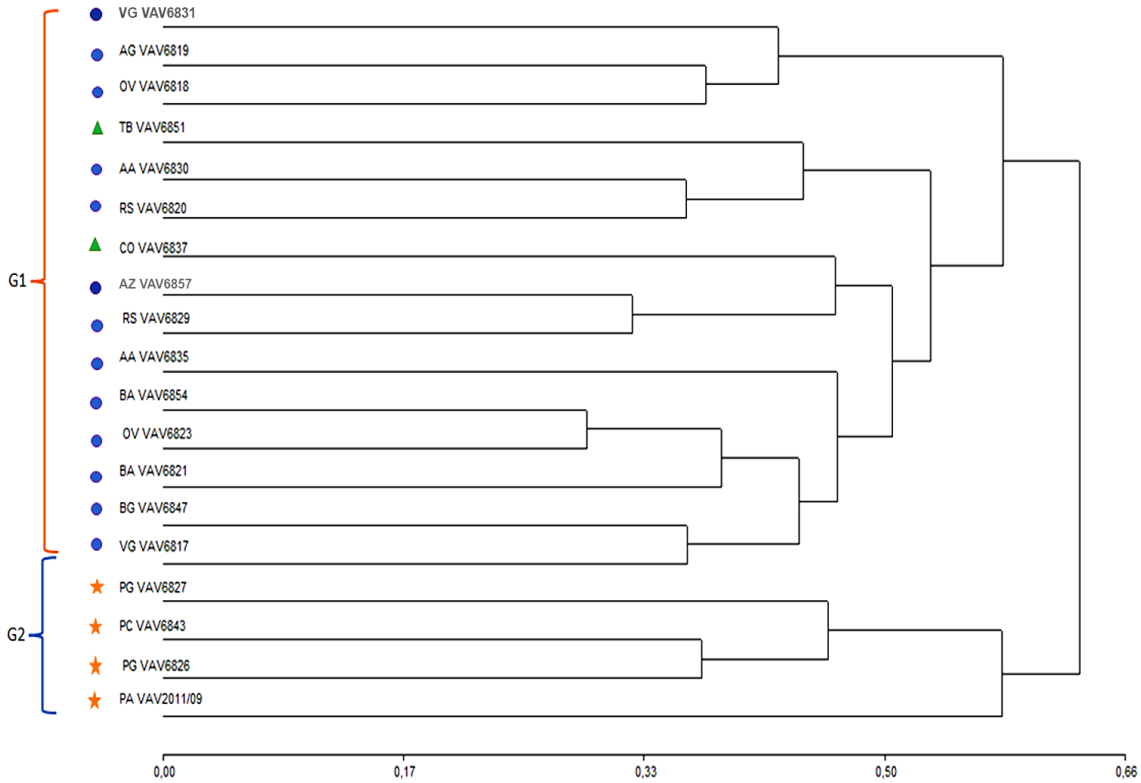

Figure 3 – UPGMA cluster analysis algorithm based on Gower distances [sqr (1-S)]. Stars = Popcorn populations; Circles = Floury populations; Triangles = F-Pc populations; PA = Pipoca Amarillo; PC = Pororó Chico; TB = Tupí Blanco; PG = Pororó Grande; BA = Blanco Ancho; AZ = Azul; RS = Rosado; VG = Variegado; AG = Amarillo Angosto; CO = Colorado; OV = Overo; BG = Blanco Angosto; AA = Amarillo Ancho. G1= Group 1; G2= Group 2.

Figure 4 – a-j. Harvested ears – a. Pororó Chico; b. Pororó Grande; c. Blanco Ancho; d. Amarillo Angosto; e. Colorado; f. Variegado; g. Overo; h. Amarillo Angosto; i. Blanco Angosto; j. Variegado.

Morphological and phenological traits were used as variables for the cluster analysis, which showed two distinct groups corresponding to the Floury and Popcorn landraces, with the F-Pc (VAV6837 and VAV6851) being included in the former group. This result is consistent with the clusters obtained by Melchiorre *et al*. (2006), who also included morphological and phenological traits in the analysis. It is interesting to note that these two groups of NEA maize landraces have also been identified using microsatellite markers (Bracco *et al*. 2012). Studies on cytogenetic characterisation showed that Popcorn populations had a higher percentage of heterochromatin and number and frequency of heterochromatic *knobs*, compared with Floury populations (Realini *et al*. 2018). Recently, Realini *et al*. (2021) found that heterochromatin percentage is positively correlated with the length of the vegetative period in maize populations from NEA. All these results demonstrate that the Guaraní maize from NEA presents two well-defined genetic pools, namely, the Floury (including F-Pc) and the Popcorn landraces. It is probably that selection made by Guaraní farmers for certain morphological and phenological characters, in relation to food and ritual practices, have selected indirectly the cytological characteristics related with the LVC.

Studies carried out so far indicate that future conservation programs should prioritise the protection of the Popcorn and the Floury genetic pools. The landraces belonging to these genetic pools, which are cultivated by smallholder farmers, are severely threatened by the expansion of the agricultural frontier and the introduction of commercial hybrid varieties. Therefore, part of this work was aimed at multiplying the material collected from the field and reintroducing it into the Guaraní communities in Misiones province, as a strategy for its *ex situ* conservation. Indeed, studies undertaken within the framework of *in situ* conservation programs need to be directed at improving the quality of life of local farmers through their economic growth.

The NEA maize landraces are potential sources of genetic resources, constituting useful repositories of alleles for tolerance and resistance to biotic and abiotic stresses. Thus, a comprehensive morphological, phenological and genetic characterisation is crucial for their inclusion in future breeding plans and agrobiodiversity conservation.

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