Agrociencia Uruguay 2022 | Volume 26 | Number NE2 | Article 1077
DOI: 10.31285/AGRO.26.1077
ISSN 2730-5066

25 years in 25 articles

# Classifying Uruguayan maize collection to develop a core collection

## Clasificación del germoplasma de maíz de Uruguay para establecer una colección núcleo

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**Article originally published in:**

Agrociencia (Uruguay). 1998;2(1):103-11.
doi: 10.31285/AGRO.02.1007

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### Abstract

Core Collections were suggested to improve germplasm utilization. A Core Collection is a subset of reduced size, chosen to represent the diversity of a collection with a minimum of redundancies. Because diversity is distributed between and within groups with different degrees of organization, accessions should be classified adequately into related groups before the selection of a Core Collection. In this research, different classification strategies for the Uruguayan Maize Collection were compared, and the best was used to select a Core Collection. The following classification strategies were compared following a multivariate approach using the available maize data base: i) racial classification, ii) geographic origin (south and north of the country), and iii) a combination of kernel type and geographic origin. The third option was considered the best classification criterion, since it takes into account two points which are closely related to the distribution of diversity: genotypic composition and geographical origin. The following five groups were identified in the collection: a) Pop, b) Floury, e) Dent, d) Southern Flint-semiflints, and e) Northern Flint-semiflints. A total of 90 accessions were selected to constitute the Uruguayan Maize Core Collection. Each group was represented in the Core in proportion to the logarithm of its size. The ranges of 17 variables in the Core were compared with those in the entire collection to verify the representativeness of the subset. On average, 91.2% of the ranges were retained in the Core, confirming its representativeness.

**Keywords**: maize, germplasm collection, core collection, germplasm utilization, classification

### Resumen

El desarrollo de Colecciones Núcleo ha sido propuesto como una alternativa para aumentar el uso del germoplasma conservado. Una Colección Núcleo es una muestra representativa de una colección de germoplasma, en la cual se mantiene la variabilidad genética de una colección con un mínimo de redundancia. Previo a la selección de una Colección Núcleo, es necesario definir una clasificación de los materiales ya que la variabilidad se distribuye entre y dentro de grupos con diferentes grados de organización. En este trabajo se comparan diferentes criterios de clasificación de la Colección de Maíz de Uruguay para designar una Colección Núcleo. En base al análisis multivariado de la base de datos existente, se compararon los siguientes criterios de clasificación: i) por raza, ii) por origen geográfico (sur y norte del país), iii) una combinación de tipo de grano y origen geográfico. El criterio iii) mostró ser el más adecuado, identificándose los siguientes cinco agrupamientos: a) Pop, b) Floury, c) Dentados, d) Flint-semiflint Sur, y e) Flint-semiflint Norte. Esta clasificación



Malosetti M, Abadie T. Classifying Uruguayan maize collection to develop a core collection. Agrociencia Uruguay [Internet]. 2022 [cited dd mmm yyyy];26(NE2):e1077. doi:10.31285/AGRO.26.1077

refleja dos factores importantes en la distribución de la variabilidad: la composición genotípica y el origen geográfico. En base a estos agrupamientos se seleccionaron las 90 accesiones que pasaron a componer la Colección Núcleo. El número de accesiones que se muestrearon de cada uno de los grupos fue determinado en forma proporcional al logaritmo del tamaño del grupo. Para verificar la representatividad de la Colección Núcleo se compararon los rangos de 17 variables en la muestra seleccionada con los de la colección base. En promedio, un 91.2% de los rangos de estas variables quedó incluido en la Colección Núcleo, confirmando su representatividad.

**Palabras clave**: maíz, colección de germoplasma, colección núcleo, utilización de germoplasma, clasificación

## INTRODUCTION

The Uruguayan Maize Collection consists of 852 recessions collected from farmers' fields during the year 1978, within the framework of a project of the IBPGR (Intemational Board for Plant Genetic Resources).

This collection has been highlighted for its improvement potential due to the variability found in characters of interest (De María and others, 1979; Ozer Ami and others, 1995). Interest in this collection has recently increased, mainly considering the suitability for forage and silage production of some groups of materials (Abadie and others, 1996). Despite this, the maize collection has not been much used for improvement.

Establishing a Core Collection represents an alternative to promote the evaluation of germplasm, support its use and improve the collection management (Frankel and Brown, 1984). A Core Collection is a representative sample of the collection that includes the genetic variability of a crop and its related species with a minimum of repetitions (Frankel and Brown, 1984; Brown, 1995). Accessions that are not included in the Core Collection become the Reserve Collection. The development of a Core Collection implies a change in the organization of the collection allowing to: a) identify those areas of the collection that require greater variability, b) rationalize the process of monitoring and regeneration of accessions, and e) facilitate the exchange of germplasm. On the other hand, the Core Collection reduces the efforts required for evaluation by facilitating the search for new characters of interest for improvement. Before the selection of a Core Collection, it is necessary to establish an adequate classification of the base collection, since the variability is not distributed randomly, but presents different degrees of organization (Brown, 1989a; Hintum, 1995). The geographical origin and genotypic composition are two basic factors in the distribution of variability, so information such as: a) origin of collection or improvement program, b) characterization data, and e) agronomic evaluation, are useful to define an appropriate classification (Brown, 1989a).

This study aimed to define a classification criterion for the Uruguayan Maize Collection and based on this, to designate a Maize Core Collection.

The establishment of this Core Collection, in addition to favoring the exploration of characters of interest potentially present in this collection, will allow the organization of other activities related to the collection such as the monitoring of germination, regeneration and exchange of germplasm.

## MATERIAL AND METHODS

### Classification

The patterns of variation in the collection and its association with different classification criteria were estimated using the database generated by De Maria and others (1979), published in the Catalog of Maize Genetic Resources of South America - Uruguay (Femández and others, 1983). Collections were evaluated during the agricultural year 1978/79 at the Experimental Station Dr. A. Backhaus of the Agronomy College. The evaluation was carried out on plots of a 6 meters furrow at a density of 47,620 plants per hectare (De María and others, 1979), in which 17 morphological and agronomic quantitative variables were recorded (Table 1). Eleven of these variables were selected to be included in a principal component analysis (Table 1). For this, the correlations between variables were analyzed as well as the associations of each of them with the first three principal components according to the criterion proposed by Hair and others (1995). The principal component analysis was carried out using the information of 845 accessions for which complete information is available. The accessions were plotted according to the first three principal components grouping them according to different classification criteria. The criteria analyzed were: a) race, b) region of origin (north and south of the country), and c) a combination of kernel type and region of origin,

a criterion that emerged from the analysis of the two previous criteria.

In addition to the graphical study, for the grouping criterion e), a discriminant analysis was performed including the information of the 17 quantitative variables (Table 1), determining the percentage of correctly classified cases, as recommended by Klecka (1980) and Crossa *and* others (1995).

**Table 1.** List of quantitative characteristics evaluated by De Maria *et. al.* (1979) in the Uruguayan Maize Collection. The table shows which components were included in the principal component analysis.

| Variable | Included in the PCA a | Variable | Included in the PCA • |
|---|---|---|---|
| plant height | YES | grain length | YES |
| cob height | YES | days to male flowering | YES |
| prolificacy | YES | days to female flowering | YES |
| tillering | NO | tipping percentage | NO |
| number of rows | YES | grain yield | NO |
| cob length | NO | forage yield | NO |
| cob diameter | NO | weight 100 grains | YES |
| grain thickness | YES | % grains on the cob | YES |
| grain width | YES | | |

PCA: Principal component analysis

### Selection of the Core Collection

The Core Collection was selected from the groupings defined in the previous stage. The relative weight of each of the groups in the Core Collection was determined according to the logarithmic strategy (Brown, 1989b). The following procedure was followed for the selection of accessions for each group: i) analysis of clusters with standardized data, using the Ward method, and the Euclidean Distance squared, according to the recommendation by Crossa and others (1995), ii) in the resulting dendogram, both subgroups and accessions representing the group were identified in the Core Collection (Relative Diversity Method proposed by Diwan and others, 1994), and iii) within each subgroup, a completely randomized accession was selected, as recommended by Ozer Ami (1997). As verification of the representativeness of the Core Collection, a comparison of the variable ranges in the Core Collection regarding the base collection was first performed, determining the percentages of the ranges kept in the Core Collection (Diwan and others, 1994).

This indicator would tend to 100% if the Core Collection includes the most common and the least frequent cases. Secondly, a graphical analysis was performed based on the principal components.

## RESULTS AND DISCUSSION

### Classification

The first three principal components accounted for 67.1% of the variation (Figure 1). Accessions plotted based on the first three principal components did not show a tendency to group according to race. Despite this, it was possible to observe four groups; the first three were composed of materials from the Pisingallo, Morotí, and White Dent races, respectively, while the rest of the races were grouped in the remaining one (Figure 1). It is noteworthy that the four groups observed coincide with the four types of maize cited by Brieger and others (1958), according to the kernel type:

a) Pop, b) Floury, c) Dent, and d) Flint. While showing that racial classification alone is not a clear criterion for classifying the collection, these results are suggesting the kernel type as an alternative criterion.

The criterion of geographical origin of the accessions, allowed distinguishing two groups, those coming from the south and the north of the country. The value of geographical origin as a criterion associated with the distribution of variability has been highlighted by different authors (Brown, 1989a; Spagnoletti Zeuli and Qualset, 1993; Hintum, 1995), and is confirmed by these results, demonstrating its effect even in a small geographical extension as it

is the Uruguayan territory. However, this criterion, although satisfactory, was not considered sufficient since it allows distinguishing only two groups (Figure 2).

Based on the results above, the kernel type was proposed as an alternative criterion, combining it with the geographical origin in the case of the largest group (Flint-semiflint).

**Figure 1.** Distribution of accessions according to the first three principal components by classifying the materials in: a) White Dent, b) Pisingallo, c) Morotí and d) other accessions. The variation percentage explained by the principal components are indicated on the axes.

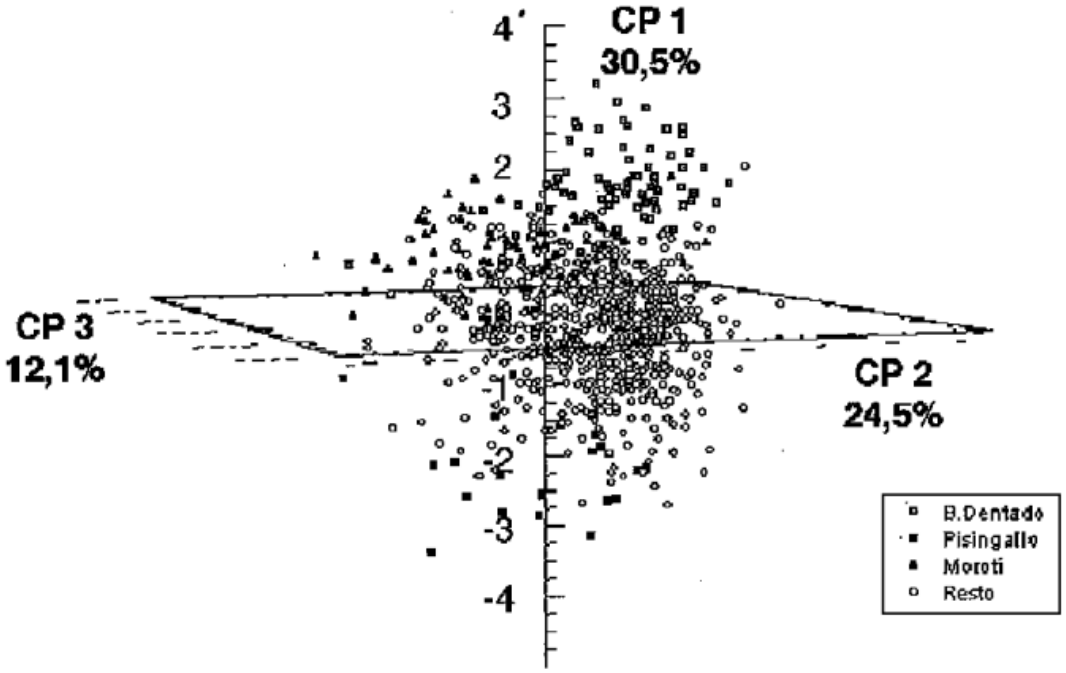
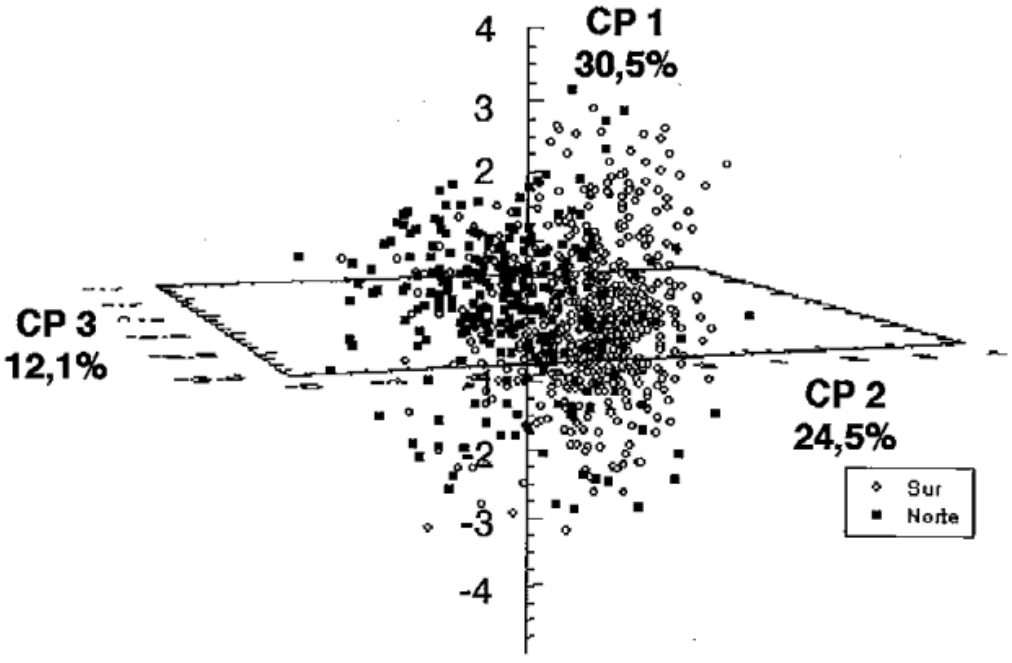



**Figure 2.** Distribution of accessions according to the first three principal components using the origin of collection as the only classification criterion: a) south of the country, and b) north of the country.



The following five groups of materials are thus distinguished: (a) Pop, (b) Floury, (c) Dent, (d) Southern Flint-Semiflint, and (e) Northern Flint-Semiflint, which are observed as separate groups based on the first three principal components (figure 3). On the other hand, this classification showed a high percentage of cases correctly classified according to the discriminant analysis (Table 2).

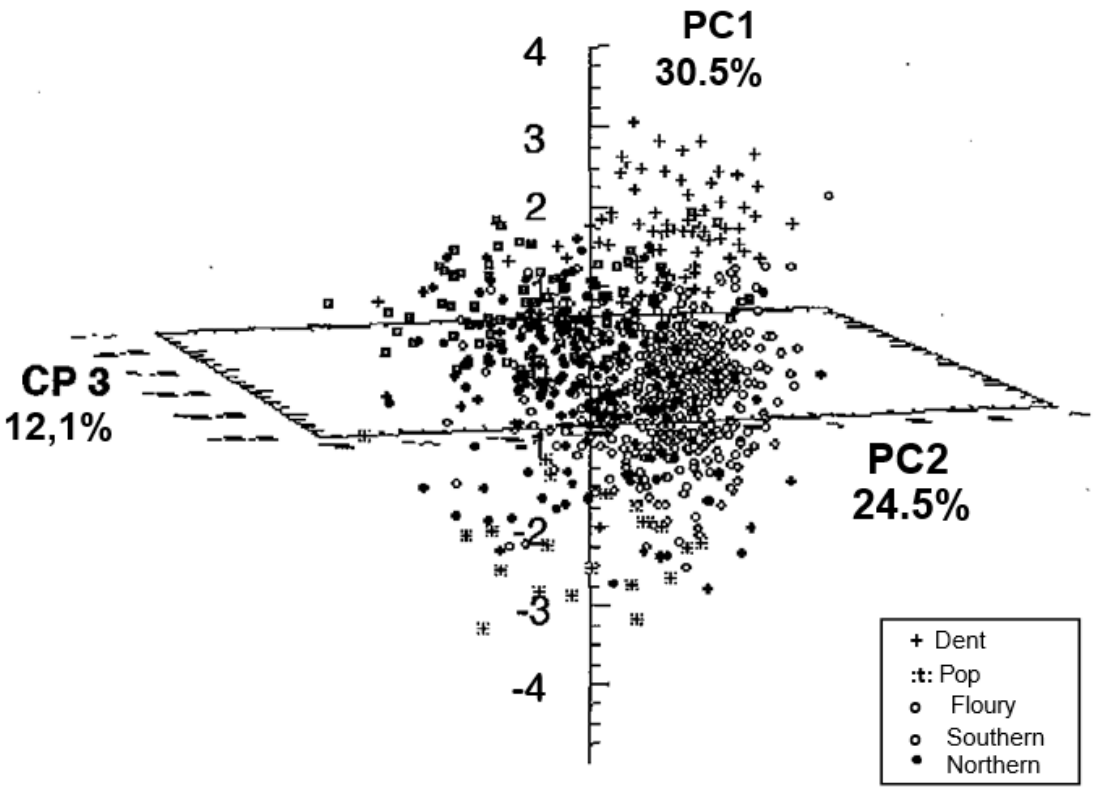
**Table 2.** Percentage of cases correctly classified according to the discriminant analysis.

| Group | % correct classification |
|---|---|
| Dent | 92.2 |
| Pop | 100.0 |
| Floury | 87.8 |
| Southern Flint-Semiflint | 91.8 |
| Northern Flint-Semiflint | 70.0 |

Brieger and others (1958) associated the kernel type with the different stages in maize domestication, mentioning the Pop type as the most primitive followed by the Flint, Floury and Dent. This is also reflected in the distribution of the groups in the graph, being the Pop group the most distant from the Dent, and the other two groups in an intermediate position between them (Figure 3).

Goodman and Bird (1977) point out that the germplasm of this region represents a complex, product of the entry of exotic germplasm and subsequent interbreeding with native germplasm. The proposed classification provides a basis for a better understanding of this complex. On the one hand, the materials of the *Pop* and *Floury,* groups are distinguished, which could be associated with germplasm of Guarani origin (Paterniani and Goodman, 1977). On the other hand, most of the collection corresponding to the groups *southern Flint-Semiflint* and *northern Flint-Semiflint,* includes widely disseminated germplasm and adapted to this region as the Cateto group. Precisely, Brieger and others (1958), and Paterniani and Goodman (1977), based on the considerable degree of ecological adaptation to specific conditions of this region, suggest this area as a possible origin of this germplasm, although Goodman (1976) also links its origin with Flint populations from the Caribbean. In these groups, apart from typical materials of the Cateto Sulino race (majority in the collection with approximately 450 accessions), populations with different degrees of introgression with exotic germplasm are included. Such is the case of the races Riograndense Dent and Riograndense Semident, which were identified by Brieger and others (1958) as Cateto-DentSynthetics.

**Figure 3.** Distribution of accessions according to the first three principal components, classified in five groups: a) Dent, b) Pop, c) Floury, d) Southern Flint-Semiflint, and e) Northern Flint-Semiflint.



These populations are the result of the combination of modern germplasm (dent type) with germplasm adapted to the region (Cateto Sulino), being the tendency towards the parent Cateto Sulino consequence of the increase by natural selection of the frequency of alleles of the best adapted parent (Brieger and others, 1958).

Discrimination found between southern and northern native populations of the country could reflect adaptation to different environmental conditions. Finally, the Dent group corresponds to modern germplasm, mainly introduced from the United States in this century, which determined a lower introgression with maize from the region.

The proposed classification contains a strong biological basis, since it is based on aspects of main relevance in the study of diversity such as genotypic composition and geographical origin, which makes a good starting point for the selection of a Core Collection (Brown, 1989a). On the other hand, the consistency observed with the research on the classification of creole populations in Brazil (Abadie and others, 1997), encourages similar classification in other countries of the region. This would also constitute a homogenizing factor of the different maize collections, promoting regional efforts for future research related to this germplasm.

**Selection of the Core Collection**

A total of 90 accessions were defined as the appropriate size of the Core Collection, representing 10.6% of the size of the base collection.

The relative weight of each of the five groups was determined according to the logarithmic strategy (Table 3). The 90 accessions that became part of the Maize Core Collection were designated according to the methodology described.

**Table 3.** Relative weight of the different groups in the Core Collection according to the logarithmic strategy (proportional to the logarithm of the group size).

| Group | Size | N° acc. to select |
|---|---|---|
| Dent | 90 | 17 |
| Pop | 23 | 12 |
| Floury | 90 | 17 |
| Southern Flint-Semiflint | 449 | 24 |
| Northern Flint-Semiflint | 193 | 20 |
| Total | 845 | 90 |

In the case of the Dent group, Ozer Ami (1997) used the same method to select the accessions corresponding to the Core Collection.

As can be seen in Table 4, the retained ranges of the variables varied between 60% and 100%, being less than 80% only for 2 of the 17 variables. The mean range retained was 91.2% (Table 4). Diwan and others (1994) found that the best strategies retained, on average, between 74% and 81% of the ranges for a total of 14 variables, when comparing different selection strategies of a Core Collection for annual species of the genus Medicago. Results of this study show a higher retention, which indicates that the designated Core Collection is effective in representing a good part of the variation of the Uruguayan Maize Collection.

Figure 4 shows that the constituent materials from the Core Collection satisfactorily cover the variation of the maize collection. Another aspect that should be highlighted is the difference in the distribution pattern of the points that represent the Core Collection and the rest of the collection. While the Core Collection shows a homogeneous pattern of distribution, the rest of the collection presents areas of greater concentration of points.

This responds to the existence of very similar materials and therefore to possible redundancies, which is satisfactorily overcome by the Core Collection. That is, in the Core Collection, not only the most common cases were represented, but also the less common, thus ensuring a lower level of redundancy between materials and a greater coverage of the variability present in the collection. The full list of materials designated as the Core Collection is presented in Table 5.

The Core Collection represents the logical starting point in the search for new characteristics, decreasing evaluation costs. On the other hand, it increases the possibilities of applying more expensive methodologies of germplasm exploration, such as molecular techniques. From the point of view of exchange, this can be favored by involving a smaller number of accessions, for which a greater amount of seed is available. Finally, the established Core Collection, together with the one designated for Brazil by Abadie and others (1997), may mean an incentive for similar efforts in other maize collections in the region.

**Table 4.** Percentage of the range retained in the Core Collection for each variable. The mean range retained in the Core Collection is indicated at the end of the box.

| | Range BC + | Range CC * | (CC/BC) x 100 (%) |
|---|---|---|---|
| Male flowering (days) | 37 | 33 | 89.2 |
| Female flowering (days) | 38 | 35 | 92.1 |
| plant height (cm) cob | 110 | 108 | 98.2 |
| height (cm) prolificacy | 118 | 1 18 | 100.0 |
| (cob/plant) tillering | 1.96 | 1.95 | 99.5 |
| (stems/plant) % tipping | 2.50 | 2.45 | 98.0 |
| Grain yield (kg/ha) | 85 | 53 | 62.4 |
| Forage yield (kgDM/ha) | 6439 | 5284 | 82.1 |
| cob length (cm) cob | 34200 | 26800 | 78.4 |
| diameter (cm) | 14 | 12 | 85.7 |
| n° of rows grain | 4 | 4 | 100.0 |
| thickness (mm) | 16 | 14 | 87.5 |
| grain length (mm) | 10 | 10 | 100.0 |
| grain width (mm) weight | 8 | 7 | 87.5 |
| 100 seeds (g) | 7 | 7 | 100.0 |
| % grains on the cob | 58 | 58 | 100.0 |
| **Mean range retained** | 56 | 50 | 89.3 |
| | | | 91.2 |

+ **BC**: Base Collection

* **CC**: Core Collection

**Figure 4.** Distribution of the accessions designated as the Core Collection (CC) and the Reserve Collection (RC), according to the first three principal components.

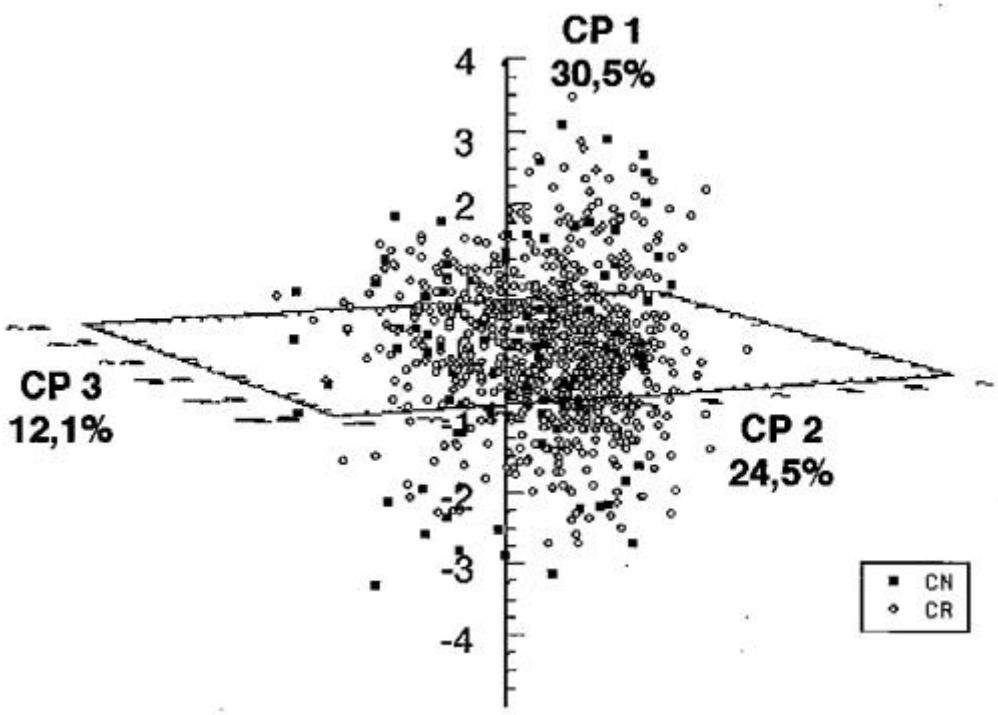


**Table 5.** List of accessions that are part of the Uruguayan Maize Collection. The accessions identification corresponds to the denomination in the Uruguayan Maize Catalog (Fernandez and others, 1983). The group to which each accession belongs is indicated next to it: Pop, Floury, Dent, Southern Flint-Semiflint (Southern F-SF), and Northern Flint-Semiflint (Northern F-SF).

| URZM | GROUP | URZM | GROUP | URZM | GROUP |
|---|---|---|---|---|---|
| 1194 | Pop | 1005 | Dent | 12033 | Southern F-SF |
| 1195 | Pop | 1007 | Dent | 12041 | Southern F-SF |
| 1196 | Pop | 5002 | Dent | 13048 | Southern F-SF |
| 1199 | Pop | 6003 | Dent | 13064 | Southern F-SF |
| 1200 | Pop | 11001 | Dent | 13068 | Southern F-SF |
| 1201 | Pop | 11002 | Dent | 13078 | Southern F-SF |
| 1204 | Pop | 11005 | Dent | 13112 | Southern F-SF |
| 8015 | Pop | 11011 | Dent | 16009 | Southern F-SF |
| 10013 | Pop | 11012 | Dent | 16013 | Southern F-SF |
| 11110 | Pop | 11013 | Dent | 16020 | Southern F-SF |
| 13126 | Pop | 11017 | Dent | 6030 | Northern F-SF |
| 18129 | Pop | 12003 | Dent | 6031 | Northern F-SF |
| 1017 | Floury | 13027 | Dent | 6056 | Northern F-SF |
| 5010 | Floury | 13028 | Dent | 6059 | Northern F-SF |
| 5011 | Floury | 18001 | Dent | 6060 | Northern F-SF |
| 6008 | Floury | 18002 | Dent | 6061 | Northern F-SF |
| 6009 | Floury | 1044 | Southern F-SF | 6092 | Northern F-SF |
| 8003 | Floury | 1063 | Southern F-SF | 9002 | Northern F-SF |
| 13032 | Floury | 1074 | Southern F-SF | 9018 | Northern F-SF |
| 13044 | Floury | 1123 | Southern F-SF | 10002 | Northern F-SF |
| 18010 | Floury | 1141 | Southern F-SF | 10004 | Northern F-SF |
| 18011 | Floury | 1152 | Southern F-SF | 18052 | Northern F-SF |
| 18020 | Floury | 1154 | Southern F-SF | 18054 | Northern F-SF |
| 18023 | Floury | 1173 | Southern F-SF | 18055 | Northern F-SF |
| 18033 | Floury | 2014 | Southern F-SF | 18084 | Northern F-SF |
| 18036 | Floury | 3005 | Southern F-SF | 18086 | Northern F-SF |
| 18037 | Floury | 11019 | Southern F-SF | 18101 | Northern F-SF |
| 18042 | Floury | 11030 | Southern F-SF | 18115 | Northern F-SF |
| 18043 | Floury | 11071 | Southern F-SF | 18120 | Northern F-SF |
| 1003 | Dent | 11101 | Southern F-SF | 18122 | Northern F-SF |

## ACKNOWLEDGMENTS

This study was funded by the Sectoral Commission for Scientific Research of the Universidad de la República (CSIC by its Spanish acronym), within the framework of the research initiation support scholarships program.

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