Investigation note

Evaluation of mestizos and use of multivariate techniques to identify outstanding corn lines

Francisco Javier Sánchez-Ramírez[1]
Ma. del Carmen Mendoza-Castillo[2§]
Carmen Gabriela Mendoza-Mendoza[2]

[1]Department of Plant Breeding-Autonomous Agrarian University Antonio Narro. Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México. (javier.sanchez@uaaan.edu.mx). [2]Postgraduate in Genetic Resources and Productivity-Montecillo *Campus*-Postgraduate College. Mexico-Texcoco Highway km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. CP. 56230. (mendoza.carmen@colpos.mx).

[§]Corresponding author: camen@colpos.mx.

Abstract

It is common for plant breeding programs based on hybridization schemes to start with hundreds of inbred lines; the foregoing makes discrimination essential to continue with desirable attributes. This work was carried out in 2017 at the Graduate College and the Superior Technological Institute of the West of the State of Hidalgo, to choose outstanding mestizos, which were formed with 34 lines (S_3-S_4) developed for the production of grain in conditions of limited or temporary irrigation, these were identified by integral phenotypic selection, principal component analysis and graphical dispersion analysis. High performance criteria and agronomic-morphological characteristics of interest for grain production and for its improvement were applied. The analysis of main components and the analysis of graphic dispersion contributed to a reliable characterization and easy graphic visualization of the phenotypic expression of the mestizos. The outstanding lines were those that gave rise to the mestizos identified as ^1, ^4, ^5, ^8, ^10, ^11, ^14, ^16, ^19 and ^27, which yielded between 8.5 and 6.2 t ha^{-1}. They also had high values of ear weight, diameter and length of ear, and grain weight; the mestizo ^27 also stood out for its prolificity.

Keywords: graphical dispersion analysis, High Valleys of Central Mexico, line x tester, principal component analysis.

Reception date: January 2020
Acceptance date: March 2020

Plant breeding with conventional methodologies (selection or hybridization) takes advantage of additive genetic effects or dominance and epistasis (Hallauer *et al*., 2010). In general, the selection is applied first, since it improves the genetic basis of the populations that will give rise to the inbred lines. Hybridization improvement programs commonly begin with a large number of inbred lines (Jugenheimer, 1981), which makes their discrimination indispensable in order to identify the best for their combinatorial aptitude and outstanding agronomic characteristics.

To determine the potential of a line, the behavior of its crosses is analyzed, but this is impractical when the number of possible crosses is very large. Given this problem, Jenkins (1929) proposed to cross lines with a common tester, a methodology called ‘mestizo test’ or ‘line x tester’, which is efficient for the early evaluation of lines since it allows to determine what their combinatorial aptitude is.

Although we regularly seek to increase the yield of grain, in the improvement process other variables are also evaluated to choose useful phenotypes, but it has been observed that some of these have limited variation, which makes it difficult to select the superior phenotypes based on these features. Iezzoni and Pritts (1991); Yan and Fregeau-Reid (2008); Maji and Shaibu (2012) suggest that for the optimal selection of phenotypes, principal component analysis and graphical dispersion analysis can be used as tools to identify the variables of greater weight, genotypes and the interrelation between them that best explain the variation studied.

Based on the above, the objective of this study was to discriminate lines of a genetic improvement program in maize and identify the outstanding ones, through the mestizo test, the principal component analysis and the graphic dispersion analysis, as well as the Agronomic-morphological expression of mestizos formed with 34 inbred lines (S_3-S_4) developed for grain production in limited or temporary irrigation.

Vegetal material

The 34 S_3-S_4 lines with which the mestizos were formed were obtained from 20 varieties of free pollination (VPL), which were derived by recurrent selection of native maize populations with conical cobs of the Central High Valleys of Mexico (> 2 200 meters above sea level) and transition areas of the Mezquital Valley (1 700 to 2 100 meters above sea level), in the state of Hidalgo. VPLs were characterized by being suitable for grain production under rainfed conditions, for having rusticity for agronomic management of low fertilization doses and limited cultivation work and with grain quality for the elaboration of tortillas.

Training and evaluation of mestizos

In the spring-summer 2016 cycle, 34 mestizos were trained at the Postgraduate College using a synthetic, broad-based synthetic program of the Postgraduate College’s corn program, called P6.

In 2017, mestizos were evaluated at the Superior Technological Institute of the West of the State of Hidalgo in Mixquiahuala, Hidalgo, located at 2 000 meters above sea level, with an annual average temperature of 18.4 °C and 480 mm of annual rainfall and at the Graduate College in

Montecillo, State of Mexico, located at 2,265 meters above sea level, with 16.4 °C annual average temperature and 546.7 mm of annual rainfall. The trials were established under the experimental design of randomized complete blocks with three repetitions. The experimental unit consisted of two furrows 5 m long and 0.8 m wide, 50 000 plants ha^{-1} were handled and as control the commercial hybrids San Jose (H_1), San Josecito (H_2) and the P6 tester were included.

Conduction of the essays

The crop management in both trials was based on the technical recommendations of each institution. In Montecillo it was fertilized with the dose 120-60-60 and in Mixquiahuala fertilizer was not applied because in that locality it is irrigated with sewage, with sufficient organic matter 'aguas negras', coming from Mexico City. The sowing was carried out on May 10 in Montecillo and on April 26 in Mixquiahuala.

Characteristics evaluated

The days to male (DFM) and female (DFF) flowering were determined, quantified from that 50% + 1 of the plants on the plot exhibited anthers or stigmas, respectively; floral asynchrony (AsinF) was the difference between DFM and DFF. The morphological variables and yield components were determined with the average value of five representative cobs obtained from each experimental unit and were: number of rows (Nhil) and number of grains per row (Ghil), ear length (Lmz), diameter of cob (Dmz) and diameter of olote (Dolo), in cm; average ear weight (Pmz) and grain weight (Pgr), in kg.

Grain yield per experimental unit (Rto_{UE}) was determined as: Rto_{UE}= ((PMz1 + PMz2) ∗ Id∗ ((100 -%Hum)/100))/0.86; where Pmz1 and Pmz2 are the weights, in kg, of cobs of primary and secondary order, respectively; the shelling index (Id), is the difference between the weight of the cob and its grain; %Hum, the humidity of the sample from the field, determined with the stove method, correction factor of 0.86 to define the grain yield with 14% humidity. With this the grain yield (Rto) in t ha^{-1} was estimated.

In the field, the number of primary cobs (Mz1) and secondary cobs (Mz2), plant (Apta) and cob (Amz) heights, in cm, and the position of the cob (Amz/Apta) were recorded; the weight of 100 grains (P100grn), was quantified in g, in three repetitions.

Data analysis

The data analysis through both locations was done as a series of experiments in space, a combined analysis of variance was performed, with the SAS Institute (2004), system for statistical analysis) as well as the comparison of means with the Tukey test with a significance level of 0.05. With the data averaged across the locations, a principal component analysis was performed using PRINCOMP (SAS Institute, 2004). In addition, with the matrix of approximate correlations between the variables and the first two main components (CP1 and CP2), a graphical dispersion analysis was performed in a 'biplot' (Gabriel, 1971) that was developed with NTSYS for PC (Rohlf, 2009).

Discrimination of lines and selection of outstanding lines

Based on the phenotypic expression of the mestizos, the analysis of main components and the analysis of graphic dispersion, the lines that showed poor performance were discriminated in comparison with the controls (P6, H1 and H2); that is, when in the graphic dispersion the mestizos were far from the latter and were not associated with the vectors of the evaluated characteristics. It was considered that the lines that formed the mestizos with superior characteristics were those of superior combinatorial aptitude.

Analysis of combined variance

It was found that in most of the variables evaluated there was phenotypic variation between genotypes ($p\leq 0.01$) and that the effect between locations was statistically differentiated ($p\leq 0.01$) but there was no statistical significance ($p\leq 0.01$) in their interaction (Gen x Loc; Table 1); that is to say, that the expression of the genotypes presented the same order in each locality. The differences between genotypes were associated with the variation between the mestizos, which was related to the genetic diversity of the germplasm, the effect per se of their parents and the specific combination of each line with the tester. In a similar study, Velazquez-Cardelas *et al*. (2018a) associate such differences with the contrasted expression between hybrids and mestizos.

Table 1. Mean squares and statistical significance of the F values in the combined variance analysis.

FV	Gl	Rto	Pmz1	Pmz	Pgr	Dmz	Lmz	DFM	DFF	AsinF	Apta
Genotypes	36	2.7^{*}	2.8^{*}	1.1^{*}	0.9^{*}	0.1^{**}	2.2^{*}	36.6^{**}	31.6^{**}	4.4^{*}	0.26^{*}
Rep (Loc)	4	3.3^{ns}	3.6^{ns}	1.1^{ns}	1^{ns}	0.21^{ns}	2.4^{ns}	24.8^{**}	15.7^{ns}	1.6^{ns}	1.1^{**}
Loc	1	242.4^{**}	160.2^{**}	62.5^{**}	40.4^{*}	4.75^{**}	91.1^{**}	7322.6^{**}	10434^{**}	274.8^{**}	456^{**}
Gen x Loc	36	1.77^{ns}	2.2^{ns}	0.6^{ns}	0.51^{**}	0.06	1.1^{ns}	10^{ns}	10.29^{ns}	4^{ns}	0.31^{*}
Error	144	1.51	1.73	0.56	0.46	0.05	0.95	6.6	8.03	2.53	0.15
CV (%)		21.8	22.4	13.5	14.1	4.6	6.3	3.1	3.3	40	5.79

FV	Gl	Amz	Amz/Apta	Nhil	Ghil	Id	Dolo	Pmz2	P100grn	Mz1	Mz2
Genotypes	36	236^{*}	0.003	2.35^{**}	9.24^{*}	0.004*	0.08^{**}	0.27^{ns}	36.2^{**}	91.3^{ns}	17.5^{ns}
Rep (Loc)	4	362^{*}	0.004^{ns}	1.62^{ns}	8.19^{ns}	0.0005^{ns}	0.06^{*}	0.02^{ns}	20.7^{ns}	163.4^{ns}	1.5^{ns}
Loc	1	201495^{**}	0.022^{**}	43.61^{**}	565^{**}	0.007^{*}*	1.39^{**}	41.5^{**}	2.7^{ns}	330.8^{ns}	3512^{**}
Gen x Loc	36	198.6^{ns}	0.002^{ns}	1.23^{ns}	3.4^{ns}	0.0002^{ns}	0.03^{ns}	0.26^{ns}	13.5^{ns}	76.9^{ns}	16.1^{ns}
Error	144	144.7	0.0001	0.85	5	0.0002	0.02	0.2	14.6	93.7	12.9
CV (%)		10.7	8.3	5.9	7.4	1.7	5.1	101.1	11.1	22.1	87

FV= source of variation; Gl= degrees of freedom; Loc = locations; Rto= grain yield; Pmz1= main weight of cobs; Pmz= cob weight; Pgr= grain weight; Dmz= cob diameter; Lmz= cob length; DFM= days to male flowering; DFF= days to female flowering; AsinF= floral asynchrony; apta= plant height; Amz= cob height; apta/Amz= cob position; Nhil= number of rows; Ghil= number of grains per row; Id= shelling rate; dolo= diameter olote; Pmz2= weight of secondary cobs; P100grn= weight of one hundred grains; Mz1= number of primary cobs; Nmz2= number of secondary cobs; CV= coefficient of variation; *= significant at 0.05; **= significant at 0.01; ns, not significant.

Between locations, the differences were associated with the differentiated environmental effect, although the Gen x Loc interaction showed that genotypes have stability (Table 1), a desirable characteristic for genetic selection and improvement. González-Huerta *et al*. (2008) and Velázquez-Cardelas *et al*. (2018a, b) demonstrated the heterogeneity of the environment in the ecological area of the High Valleys of the Central Table, as well as its limitations to choose the superior genotypes, but in the present study, since Gen x Loc was not significant for most of the variables evaluated, mestizos with high yield potential could be identified, as well as making inferences about the combinatorial aptitude of the lines that formed them.

Principal component analysis (ACP) and graphical dispersion analysis (ADG)

The ACP and ADG were performed with the average value of the characteristics evaluated through both locations because Gen x Loc was not significant. It was observed that the first two main components described 48% of the total variation (Figure 1). Iezzoni and Pritss (1991) and Maji and Shaibu (2012) point out that both main components are sufficient to adequately represent the variation of the evaluated variables.

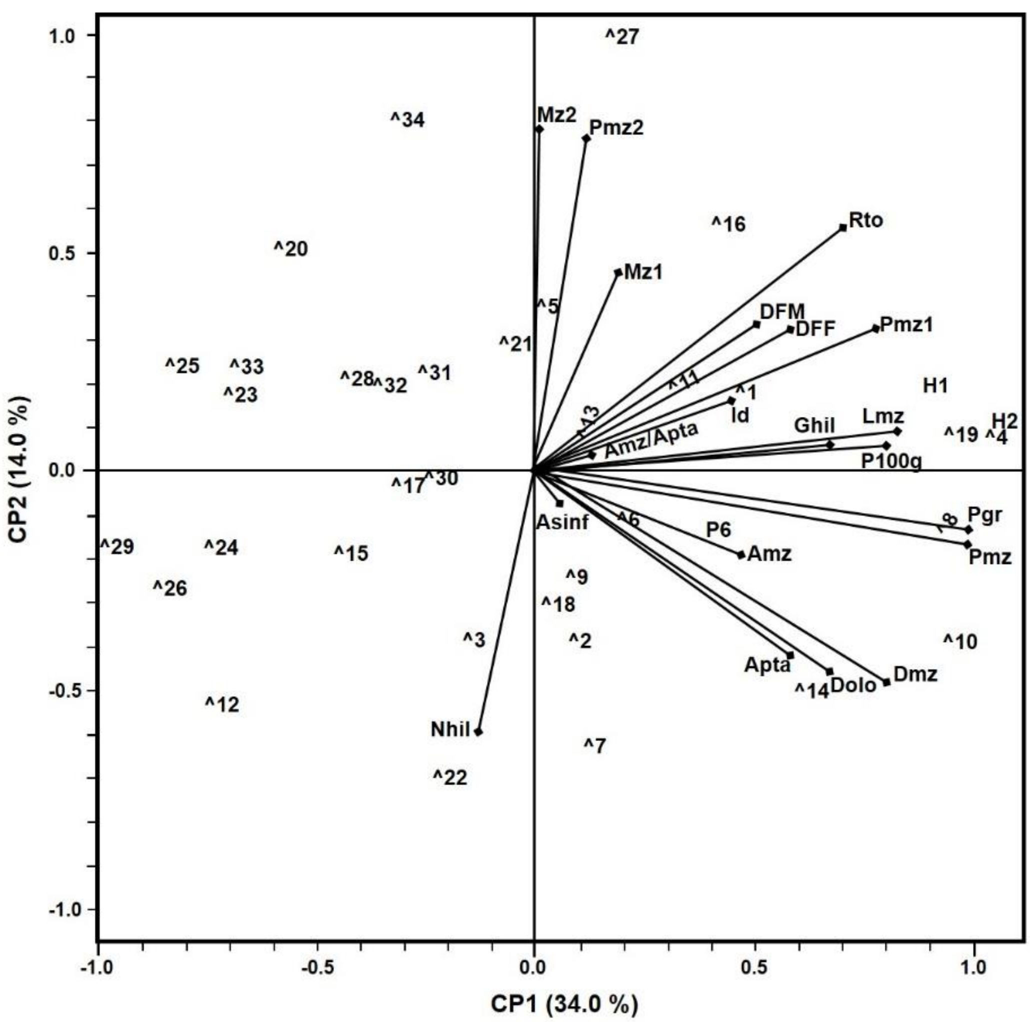

Figure 1. Biplot for 34 corn mestizos and 20 characteristics evaluated using the first two main components (CP1 and CP2).

Although Rto was the most relevant feature in the explanation of the variation and the main attribute to improve, it was considered that also other characteristics such as Pmz1, Pgr, Pmz, Dmz, Lmz, P100grn and Mz2 were decisive for the explanation of the variation. They were also useful to discriminate against mestizos and to identify outstanding lines, through their behavior.

Based on the length of the vector of the characteristics evaluated (Figure 1), in addition to Rto, the selection was made considering only three variables: Pmz1, Pgr and Pmz2; Yan and Frégeau-Reid (2008); Maji and Shaibu (2012) made a similar simplification of the number of variables and pointed out that the reduction of dimensionality through ACP has the benefit of explaining the variation without omitting necessary and important information for the selection of interesting phenotypes. In the present study, it was confirmed that ACP represented a useful tool for genotype selection in a genetic improvement program.

Based on the defined characteristics, 16 mestizos were classified as outstanding and the rest had a lower behavior than the witnesses (P6, H_1 and H_2). The outstanding mestizos were located in quadrants I and II, where prominent expressions of some were observed in some particular characteristic (Figure 1): ^27 in Pmz2, ^8 in Pgr, ^16 in Rto.

When comparing the mestizos with P6 it was found that only ^1, ^4, ^5, ^8, ^10, ^11, ^14, ^16, ^19 and ^27 exceeded it or had a similar behavior, so It is inferred that they showed a desirable expression in: Rto, Pmz1, Pmz, Dmz, Lmz, Pgr, Nmz2 and Pmz2 (Figure 1). Based on the expression of a tester, Velázquez-Cardelas *et al*. (2018) showed that native populations have genes that can contribute to improving productive potential. The mestizos located in quadrants III and IV (Figure 1) were not outstanding, as they showed limited phenotypic expression and negative interaction between the lines that formed them and P6.

The H_1 and H_2 hybrids (7.6 and 8 t ha^{-1}, had similar behavior to the outstanding mestizos (8.5 to 6.2 t ha^{-1}). The mestizos ^4, ^16, ^19 and ^27 exceeded in Rto to H_2, therefore, they can be used as unconventional hybrids. These results agree with González-Huerta *et al*. (2008); Velázquez-Cardelas *et al*. (2018a, b) who demonstrated that native populations have productive potential in the ecological area of Central High Valleys of Mexico.

The analysis allowed an integral classification of the lines to discriminate more than 60% of them, the upper and inferred lines, of superior combinatorial aptitude were those that in combination with P6 showed the superior phenotypic expression: mestizos ^1, ^4, ^5, ^8, ^10, ^11, ^14, ^16, ^19 and ^27.

The use of ACP and ADG made this process practical and simple, as it allowed to easily visualize the interrelations with the mestizos in the biplot. Although field evaluation and in-depth knowledge of the characteristics of genotypes by the plant breeder is essential, it was verified that the use of graphical dispersion analysis is advantageous for the identification of outstanding parents.

Conclusions

The selection of genotypes through the use of the multivariate analysis applied to the mestizos allowed the selection of the lines with superior combinatorial aptitude in a simple and effective way, together, the analysis of graphic dispersion allowed to synthesize the variation without omitting important information for the selection of the genotypes, in addition to that it was possible, within the outstanding ones, to associate them with some characteristic of interest for the improvement and to which their outstanding expression was due.

The selected mestizos had high productivity and agronomic-morphological characteristics that are of importance for a new breeding program, and with possibilities of being used as unconventional experimental hybrids.

Cited literature

Gabriel, K. R. 1971. The biplot graphic display of matrices with applications to principal components analysis. Biometrika. 58(3):453-467. Doi: 10.2307/2334381.

González, H. A.; Vázquez, G. L. M.; Sahagún C. J. y Rodríguez, P. J. E. 2008. Diversidad fenotípica de variedades e híbridos de maíz en el Valle de Toluca-Atlacomulco, México. Revi. Fitotec. Mex. 31(1):67-76.

Hallauer, R. A.; Carena, M. J. and Miranda Filho, J. B. 2010. Handbook of plant breeding. Quantitative genetics in maize breeding Vol. 6. Ed. Springer. 663 p. Doi 10.1007/978-1-4419-0766-0.

Iezzoni, F. A. and Pritts, P. M. 1991. Application of principal component analysis to horticultural research. HortScience 26(4):334-338. http://hortsci.ashspublications.org/content/26/4/334.fullpdf+html.

Jenkins, M. T. 1929. Correlation studies with inbred and crossbred strains of maize. J. Agric. Res. 39(9):677-721. https://doi.org/10.31274/rtd-180813-15931.

Jugenheimer, R. H. 1981. Maíz. Variedades mejoradas, métodos de cultivo y producción de semillas. Ed. Limusa. 841 p.

Maji, A. T. and Shaibu, A. A. 2012. Application of principal component analysis for rice germplasm characterization and evaluation. J. Plant Breed. Crop Sci. 4(6):87-93. Doi: 10.5897/JPBCS11.093.

Rohlf, F. J. 2009. NTSYSpc: numerical taxonomy system. Ver. 2.21h for Windows. Exeter Software. Setauket, New York. 31 p.

SAS Institute. 2004. SAS/STAT User's Guide: Version 9.2 SAS Institute, Inc. Cary, North Carolina, USA. 1689 p.

Velázquez-Cardelas G. A.; González-Huerta, A.; Pérez-López, D. J. y Castillo-González, F. 2018b. Análisis de híbridos comerciales y mestizos de maíz formados con germoplasma de INIFAP y del CIMMYT. Rev. Mex. Cienc. Agríc. 9(3b):615-627.

Velázquez-Cardelas, G. A.; González-Huerta A; Pérez-López, D. J. y Castillo-González, F. 2018a. Comportamiento de mestizos de maíz en tres localidades del Centro de México. Rev. Mex. Cienc. Agríc. 9(6):1217-1230.

Yan, W. and Frégeau-Reid, J. 2008. Breeding Line Selection Based on Multiple Traits. Crop Sci. 48(2):417-423. Doi:10.2135/cropsci2007.05.0254.