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Preliminary study to determine phenotypic differences and sample size in Cacahuacintle corn

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Abstract

This study was carried out in spring-summer of 2016 in six communities of the municipality of Calimaya de Díaz González, State of Mexico to sample land of cooperating farmers and estimate phenotypic differences in plant and corn dimensions in the Cacahuacintle breed. In 30 plots, of one hectare each, distributed in the Municipal District, San Lorenzo Cuauhtenco, Santa María Nativitas, San Marcos de la Cruz, San Diego La Huerta and Zaragoza de Guadalupe, 50 plants and corn per producer were considered at random (almost 21 000 data). Simple statistics and values of n were calculated with two methodologies. With a principal component analysis, the relationship between producers, communities and variables was analyzed. The most outstanding results showed that there are important phenotypic differences in all characteristics. The values of n would allow sampling plots where Cacahuacintle is planted in that municipality, with method 1, only the population size should be known and a value assigned to the sampling error should be chosen. With the application of method 2 or other complementary techniques, fewer plants could be sampled than with method 1 but the arithmetic mean, standard deviation, standard error, coefficient of variation or proportions between two of these should be known. In the four quadrants of the biplot, at least three of the five cooperating farmers in each locality were grouped. There was a positive and significant correlation between the number of quality leaves for wrapping tamales (NHT) with the number of grains and rows of grain in the corn, this trend was also observed between the first and the length and diameter of the spine. Thus, NHT could be used as an indirect selection criterion to increase the size of the corn.

Keywords: Cacahuacintle race, High Valleys of central Mexico, plots of cooperating farmers, random sampling.

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Introduction

In Mexico, farmers contribute to the conservation and generation of genetic diversity in situ by selecting cobs and grains in their best creoles; through the morphological variants that have been presented by natural selection, mutation, introduction, recombination and geographic isolation, they are still creative and innovative when recombining them with each other or with materials of other races to increase this diversity (Wellhausen *et al*., 1951; González *et al*., 2006; Valdivia *et al*., 2010; Romero *et al*., 2018).

The Mexican breeds used for the production of corn and pozole are Ancho, Bofo, Bolita, Blanco de Sonora, Cacahuacintle, Elotes Occidentales, Gordo, Harinoso de ocho hileras, Jala and Tabloncillo, among others; In the first, farmers have practiced selection to increase grain size and quality, as well as totomoxtle, which have been favored by the environments that predominate in their ecological niche (Romero *et al*., 2018).

The Dulcillo del Norte races of rough grain and Elotero de Sinaloa are grown for tortillas, and their corn is delicious and sweeter. In the state of Hidalgo, the creoles corn is preferred for their sweetness and in Guanajuato the conical or western corn, of red and blue grains, stand out for their excellent tortillas of good color, softness and more anthocyanins. In Chiapas, the prices of a corn bought in the field vary from $1.00 to $2.50 and come from improved varieties or hybrids; their corn is long, with more rows of grain and up to 13.8 °Brix (Aguilar *et al*., 2006; Coutiño *et al*., 2010).

The localities and the years of evaluation associated with the Mexican central plateau contribute to the differences observed in producer lands or in trials established in the experimental stations (Reynoso *et al*., 2014). In the Toluca Valley, State of Mexico, both factors or their combination, soil types, altitude, temperature and precipitation, hail and frost are the main components of their heterogeneity (González *et al*., 2006; González *et al*., 2008; González *et al*., 2010). The Cacahuacintle race is marketed mainly for the production of corn and as a blunt grain for the elaboration of pozole; its grain yields in commercial and experimental sowing vary from 2.5 to 10.3 t ha^{-1}, but its production as a blunt grain is 2 000 to 4 000 kg ha^{-1}, with a price between $2 and just over $9.50 kg^{-1} in the time of greatest demand. Up to 20 000 quality corn can be produced in 50 000 plants ha^{-1} (Ramos and Gerón, 1998; Aguilar *et al*., 1999; González *et al*., 2006; Arellano *et al*., 2010).

Since the 50's several features have also been improved in this special use corn. In experiments established in farmers' plots the height of the plant varied from 2.24 to 2.47 m, the height of the cob from 1.25 to 1.67 m, the length of the cob is between 13.21 and 14. 43 cm, between 12 and 14.22 rows have been recorded of grain, the diameter of the cob is between 5.41 and 5.67 cm and the weights of olote and grain per cob range from 21.5 to 26.1 g and from 127.7 to 146.3 g, respectively, with yields of 4 066 to 6 379 kg ha^{-1} . However, this breed has little adaptability in other municipalities of the Mexican center, has a late biological cycle and is very susceptible to stem and root acame, and to the cob and grain rot caused by *Fusarium* spp., among others (González *et al*., 2006, 2007, 2008, 2010).

Cacahuacintle and Palomero Toluqueño are parents of the Conical race, and the latter and Tuxpeño gave rise to the Chalqueño racial complex (Wellhause *et al*., 1951), the former has also been used to form corn varieties and could be an excellent progenitor of forage varieties (Franco *et al*., 2015). Currently there are no improved or hybrid varieties and farmers are the owners of the empirical knowledge related to their technology (Aguilar *et al*., 1999; González *et al*., 1999). There are no data on sample sizes and little has been published about its variability, especially in dimensions of the spine and its relationship with performance and performance components (González *et al*., 2006, 2008).

Simple statistics are used in the majority of sampling methodologies for quantitative variables (Cochran, 1998; Pérez, 2005), hence the importance of obtaining preliminary data on their variability that allows us to reliably recommend how many farmers, plants, corncobs and cobs should considered (Gónzalez *et al*., 2006; González *et al*., 2008). Thus, the objectives of this study were to sample producer lands by plant and corn characteristics, propose sample sizes with two methodologies and define some relationships between agronomic variables.

Materials and methods

Description of the experimental area

This municipality is located 17 km south of Toluca. The municipal seat is located at 19° 09' 30''north latitude and 99° 37'17'' west longitude, at 2 650 meters above sea level. Its subhumid temperate climate has an annual average of 12.8 °C, minimum and maximum of -4 and 26 °C. Its Andosol soils are derived from volcanic ash, acidic pH (between 3.8 and 6) and rest on a volcanic substrate of loose pumicite (tepojal), between 40 and 100 cm. It is made up of San Diego La Huerta (SD), Cabecera Municipal (CM), San Lorenzo Cuauhtenco (SL), Santa María Nativitas (SM), Zaragoza de Guadalupe (ZG), San Marcos de la Cruz (SMC), San Bartolito, San Andrés Ocotlán and Concepción Coatipa (Ramos and Gerón, 1998; González *et al*., 1999; González *et al*., 2006).

Genetic material

In this study, the 30 producers were considered shown in Table 1.

Table 1. Cooperating farmers and participating communities.

Producer	Location	Abbreviation
Leobardo Jasso	Calimaya	CM
Francisco Bobadilla	Calimaya	CM
Fabián Espinoza	Calimaya	CM
Benjamín Rosas	Calimaya	CM
José Manjarrez	Calimaya	CM
Sergio Medina	San Lorenzo Cuauhtenco	SLC
Federico Colín	San Lorenzo Cuauhtenco	SLC

Producer	Location	Abbreviation
Salvador Delgado	San Lorenzo Cuauhtenco	SLC
Edilberto Carmona	San Lorenzo Cuauhtenco	SLC
Gregorio Jasso	San Lorenzo Cuauhtenco	SLC
Sergio Sánchez	San Diego la Huerta	SDH
Esteban López	San Diego la Huerta	SDH
Miriam Salazar	San Diego la Huerta	SDH
Amelia Robles	San Diego la Huerta	SDH
Tomas Mendoza	San Diego la Huerta	SDH
Roque Bobadilla	San Marcos de la Cruz	SMC
Santiago Bobadilla	San Marcos de la Cruz	SMC
Doroteo Carmona	San Marcos de la Cruz	SMC
Leónides Bobadilla	San Marcos de la Cruz	SMC
Silvio Carmona	San Marcos de la Cruz	SMC
Roberto Rosas	Zaragoza de Guadalupe	ZG
Ariel Colín	Zaragoza de Guadalupe	ZG
Fidel Jasso	Zaragoza de Guadalupe	ZG
Juan Rosas	Zaragoza de Guadalupe	ZG
Jorge Colín	Zaragoza de Guadalupe	ZG
Melitón Muciño	Santa María Nativitas	SMN
Amando Alegría	Santa María Nativitas	SMN
Edén Corrales	Santa María Nativitas	SMN
Francisco Alegría	Santa María Nativitas	SMN
Rubén Corrales	Santa María Nativitas	SMN

Preliminary sample size

It was considered that: a) the value of N is known, but there is no information on n; b) random sampling without replacement will be applied to record simple statistics in r quantitative variables; c) the six locations are representative of that municipality; and d) the choice of plants with complete competence was made in 400 m^2 of the center of one ha.

The first methodology was proposed by Rendon and Cervantes (1991) and is based on:

$$n \geq \frac{N}{(N-1)b_i^2+1} \qquad (i = 1, 2, 3, \ldots, r)$$

Where: N and n are farmers and plants and corn considered, bi fluctuates from zero to one and is the proportion of the standard deviation (σ_i) for each of r variables (the smaller the more reliable, but the demand for more time and human resources and financial). This equation does not depend on unknown parameters and because of its simplicity it can be used to generate tables of n at different values of N and bi; its application leads to:

a) For N= 2 350 farmers (González *et al*., 1999) and bi= 0.2

$$n \geq \frac{N}{(N-1)b_i^2+1} = \left[\frac{N}{(N-1)b_i^2+1}\right] = \left[\frac{2350}{(2349)(0.20)^2+1}\right] = 24.74$$

b) In commercial planting of 50 000 ha^{-1} plants

$$n \geq \frac{N}{(N-1)b_i^2+1} = \left[\frac{N}{(N-1)b_i^2+1}\right] = \left[\frac{50000}{(49999)(0.20)^2+1}\right] = 24.98$$

N and n were approximate to 30 farmers and 50 plants and corn per farmer (almost 21 000 data) and the bi values used in the preliminary sampling were 0.05, 0.075 and 0.1.

Preliminary calculation of n with InfoStat

In this, the value is obtained to estimate a population average or proportion with a confidence of precision determined by the user. It also allows calculating n to detect, in a variance analysis of a fixed effects model in monofactorial experiments, a difference between means of groups or populations as small as specified by the user, as well as the sample size to estimate the difference between two populations (Balzarini *et al*., 2008; Di Rienzo *et al*., 2008). To estimate n the following is used.

$$n \geq \left(\frac{2Z_{1-\frac{\alpha}{2}}\,\sigma}{c}\right)^2$$

Where: σ is the standard deviation, c is the amplitude required for the confidence interval (1-α) % for the population mean, c can be chosen arbitrarily or expressed as a fraction ‘f’ of the sample mean (c= Ȳf) . In this study f took values of 0.05, 0.075 or 0.1.

Data register

They were quantified (CIMMYT, 1999; González *et al*., 2011): female flowering (FF; days), plant and corn heights (AP, AM; cm), stem diameter (DT; cm), length (LR), diameter (DR) and rachis weight (PR) (cm, cm, g), length (LE; cm) and corn diameter (DE; cm), rows of grain (NH), corn weight (PE; g) and quality leaf for wrapping tamales (NHT).

Statistical analysis

Simple statistics were obtained with the statistical analysis system (SAS) version 6.03 for Windows and with InfoStat (2008). The procedures are described in Gómez and Gómez (1984). An analysis of main components was also made with 30 farmers and 12 variables and another with six locations and those variables (Sánchez, 1995; González *et al*., 2010).

Results and discussion

Simple statisticians

The female flowering (FF) varied from 85 to 125 days, its mean and range were 103.47 and 40 days. Male flowering and FF are positively and significantly correlated and so are with physiological maturity; they are a reliable indicator of the biological cycle of Cacahuacintle but differ in part from that published by Arellano *et al*. (2010); González *et al*. (2006, 2008).

In Table 2 shows the heights of plant and corn (AP and AM) ranged from 1.26 to 3.9 m and from 1 to 2.9 m, their averages were 2.67 and 1.61 m. Both averages are similar to those recorded by González *et al*. (2006) and by Arellano *et al*. (2010). The stem diameters (DT) varied from 2.5 to 4.6 cm, their average was 3.07 cm, these are primary components of the grain and forage yield in maize of the Mexican center (Franco *et al*., 2015). In creoles, positive and significant correlations have been observed between them, but with higher plants there is more acame and cob rot and less grain yield (González *et al*., 2007; Arellano *et al*., 2010).

Table 2. Simple statistics for Cacahuacintle corn planted in farmers' lands of the municipality of Calimaya, state of Mexico. 2016 data.

Variable	n	Min	Max	Mean	S^2	DE	EE	CV	As	Ku
FF	1 460	85	125	103.47	78.92	8.89	0.23	8.59	0.24	-0.77
AM	1 460	1	2.9	1.61	0.06	0.25	0.01	15.4	0.93	2.91
AP	1 460	1.26	3.9	2.67	0.12	0.35	0.01	12.97	-0.28	1
DT	1 460	2.5	4.6	3.07	0.21	0.46	0.01	13.84	0.74	0.12
NH	1 460	6	26	12.08	3.52	1.88	0.05	15.54	0.27	3.13
DCH	1 460	4.6	9.7	7.16	0.50	0.71	0.02	9.88	-0.16	0.17
DM	1 460	3.6	7.7	5.89	0.33	0.57	0.02	9.76	-0.24	0.22
NG	1 460	11	40	24.75	17.6	4.2	0.11	16.95	-0.28	0.52
NHT	1 460	0	7	2.92	0.78	0.88	0.02	30.19	0.69	1.27
DR	1 460	1.5	2.1	1.85	0.04	0.12	0.31	6.41	-0.28	-0.39
LR	1 460	3	30	13.09	16.59	4.07	0.11	31.13	0.45	0.38
LE	1 460	7.1	30	20.38	6.7	2.59	0.07	12.71	-0.14	1.31
PE	1 460	100	999	530.44	18498	136.06	3.56	25.65	0.29	0.23
PR	1 460	5	90	27.2	188.94	13.75	0.36	50.55	0.72	0.61

FF= flowering; AM and AP; corn and plant heights; DT= stem diameter; NH= number of rows; DCH and DM= diameters with leaves and corn; NG and NHT= number of grains and leaves for tamale; DR and LR= rachis diameter and length; LE and PE= corn length and weight; PR= spine weight; n sampled plants; min, max, minimum and maximum; S^2= variance; DE and EE= standard deviation and error; CV= coefficient of variation; As= asymmetry; Ku, Kurtosis.

The number of rows (NH) varied from 6 to 26, with an average of 12.08. In other NH studies, it ranged from 8 to 20, with an average of 14. Cacahuacintle cobs with six rows had not been detected and with only 26 in Palomero Toluqueño (González *et al*., 2006; González *et al*., 2008). In the first case perhaps sterility or insects damaged two rows of grain.

The diameter of corn with leaves (DCH) varied from 4.60 to 9.70 cm, with an average of 7.16 cm. The corn diameters (DM) were located between 3.6 and 7.7 cm, with an average of 5.81 cm. Both are components of the corn's visual quality; the best ones will be commercialized between $0.50 and just over $2.00 per piece (Aguilar *et al*., 2006; González *et al*., 2006).

The number of grains in a row of corn (NG) ranged from 11 to 40, with an average of 24.75. Grains and cobs per plant and grain weight are reliable indicators of the productivity of a cultivar (Andrade *et al*., 2000). Arellano *et al*. (2010) recorded between 0.63 and 1.28 cobs per plant in Cacahuacintle, with weights of 100 seeds between 33 and 67 g. With 12 rows per cob there would be between 132 and 480 grains. If 80% of 50 000 ha^{-1} plants produce cob there would be between 5 280 000 and 19 200 000 grains, if all the grains of the cob are used as seed, they could be planted between 88 and 320 ha.

The number of quality leaves for wrapping tamales (NHT) ranged from 0 to 7, with an average of 2.92. In times of greatest demand, a profit of up to $3.00 per chosen plant is obtained. In this and other surrounding municipalities there are people specialized in exploiting this activity to supply the local markets of the Toluca Valley. There are no studies in this breed to determine whether NHT could be considered as an indirect selection criterion.

Spinal diameters (DR) ranged between 1.5 and 2.1 cm, with an average of 1.85 cm. The length of the spine (LR) varied from 3 to 30 cm, with an average of 13.09 cm. Spine weights (PR) differed from 5 to 90 g, with a central value of 27.2 g. It would be desirable to define whether these are important in the translocation of assimilated leaves and stems to the corn or the cob to use them as criteria for indirect selection in plant breeding and seed production.

The length of the corn (LE) ranged from 7.1 to 30 cm, its average was 20.38 cm. Corn weights with totomoxtle (PE) varied from 100 to 999 g, with an average of 530.44 g (Table 2). These results are mainly attributed to the efficient empirical improvement that farmers have made and the application of better technological packages. Both are very important to obtain a better price, in other regions of Mexico, a corn bought in the field costs from $1.00 to $2.50 at the time of greatest demand (Aguilar *et al*., 2006; Arellano *et al*., 2010; Coutiño *et al*., 2010). Long cobs, 8 and 12 rows, would be used for the production of pozole and those between 14 and 16 would be marketed as corn (Aguilar *et al*., 1999; González *et al*., 2006), but the corn of the Jala breed should be considered up to 60 cm (Aguilar *et al*., 2006). In corn diameter, with and without totomoxtle, its dimensions are larger than those recorded in the other four races of High Valleys of Central Mexico (González *et al*., 2007; González *et al*., 2008).

Principal component analysis

The results observed in Figure 1 confirm that in the municipality of Calimaya there are very heterogeneous environmental conditions; there are altitude gradients from 2,610 to more than 3 000 m (Aguilar *et al*., 1999; González *et al*., 2006; Arellano *et al*., 2010). These differences are not observable in the Tenango del Valle-Toluca-Atlacomulco region, except in its mountainous parts (González *et al*., 2008, 2011). Calimaya's agriculture is rainfed, so temperature, humidity, hail and frost, together with the heterogeneity of its soils, have contributed to its differentiation (Aguilar *et al*., 1999; González *et al*., 1999). Although there are few, little genetic variability has been estimated in this breed, which supports the previous results (González *et al*., 2006; González *et al*., 2008).

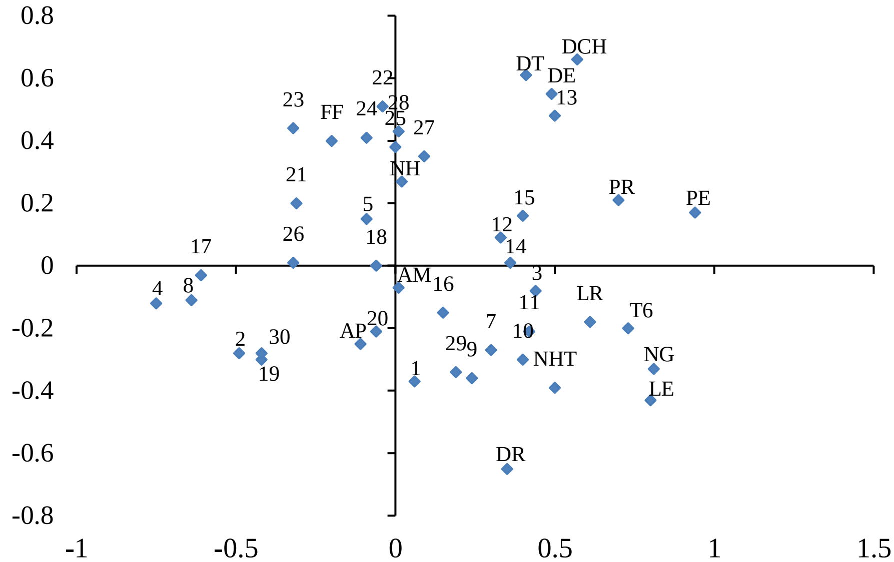

Figure 1. Biplot for cooperating producers (in number) of the municipality of Calimaya and variables evaluated (in letters) in Cacahuacintle corn.

The other components that contribute to this differentiation are the empirical genetic improvement that the farmers have made, the diversity of technological packages that they apply and the different agronomic management they provide to their plots (Aguilar *et al*., 1999; González *et al*., 1999). In trials conducted on farmers' lands it has been estimated that the genetic variability in Cacahuacintle varied from 15.9 to 50.2% in plant and cob heights, length and rows of the cob and grain yield, but in cob diameter, pot weights and there were negative estimates of grain per plant and volumetric (González *et al*., 2006).

Regarding the variables, several groups (G's) were detected: in G1 FF and NH were identified, in G2 the stem, corn and corn leaf diameters were observed; in G3 the rachis and corn weights appeared. In G4, rachis length and diameter stood out, number of quality leaves for wrapping tamales, number of grains per row and length of corn, in G5, plant and cob heights were associated (Figure 1). González *et al*. (2006) concluded that grain yield had a greater relationship with plant and cob heights, and length, diameter, and weights of grain and pot per cob.

The previous results also suggest stratifying the sampling area to reduce the environmental differences that exist between locations, as González *et al*. (2006); González *et al*. (2008); Arellano *et al*. (2010); Romero *et al*. (2018).

The interrelations shown in Figure 2 have been verified in the Cacahuacintle creole evaluation experiments in Calimaya (González *et al*., 2006; González *et al*., 2008a, b), but there are no antecedents for the dimensions of the spine. González *et al*. (2011) concluded that the dimensions of the spine were positively and significantly correlated with grain yield in maize of the Conical breed. These results also suggest that the collection of creoles where Cacahuacintle predominates could be used to form a balanced compound that could be used to conserve, increase and use its genetic diversity in situ, or cross these compounds with other breeds of flour grain, with subsequent improvement by selection and hybridization.

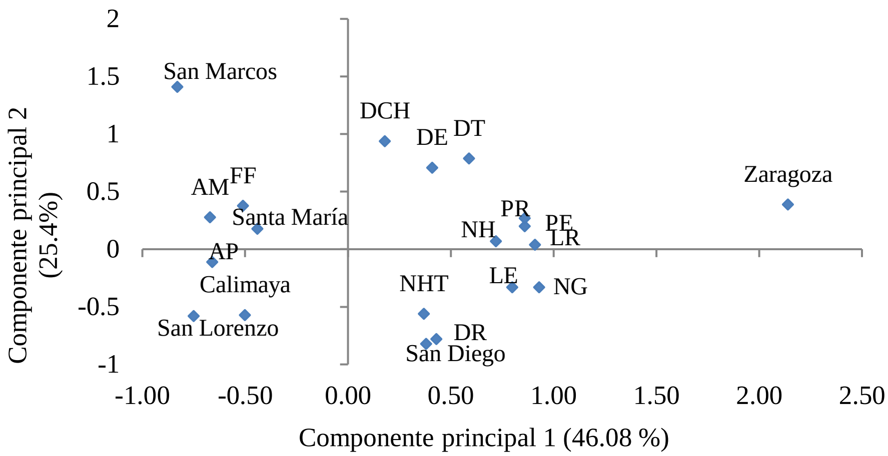

Figure 2. Biplot between communities of the municipality of Calimaya and variables evaluated in Cacahuacintle corn.

Sample sizes proposed

With method 1, n= 396 when bi= 0.05; yes bi= 0.075 or 0.1, n= 175 or 100. With method 2, when bi= 0.05, n varies from 45 (FF) to 7588 (DR), but with bi= 0.075 or 0.1 should be evaluated between 20 and 3 372 or between 11 and 1897 plants or corn (Table 3). The previous results allow us to verify that method 1 is simpler. With it, the same number of plants and corn is sampled in each variable at the same value of bi, nor are population means and variances required, among other simple statistics (Rendón and Cervantes, 1991; Cochran, 1998; Pérez, 2005).

Table 3. Sample sizes proposed to characterize Cacahuacintle corn for the production of corn on farmland in the municipality of Calimaya, State of Mexico, based on two methodologies. 2016 data.

Variable	Mean	DE	E1	E2	E3	F1	F2	F3
FF	103.47	8.89	396	175	100	45	20	11
AM	1.61	0.25	396	175	100	148	65	37
AP	2.67	0.35	396	175	100	105	46	26
DT	3.07	0.46	396	175	100	892	396	223
NH	12.08	1.89	396	175	100	150	66	37
DCH	7.16	0.71	396	175	100	104	46	26
DM	5.89	0.57	396	175	100	226	100	56
NG	24.74	4.23	396	175	100	179	79	44
NHT	2.92	0.88	396	175	100	558	248	139
DR	1.85	0.12	396	175	100	7 588	3372	1897
LR	13.09	4.07	396	175	100	596	264	149
LE	20.38	2.59	396	175	100	2 844	1264	711
PE	530.44	136.06	396	175	100	408	181	102
PR	27.2	13.75	396	175	100	1 576	700	394

However, for the characteristics that present little variability, it could lead to choosing very large sample sizes, such as in FF, AM, AP, NH, DCH, DM, and NG, since with the methodology 2 a value of n smaller is proposed to 60. If there are budgetary, time or qualified personnel restrictions, the values of n provided by both methodologies could be combined. Consideration should also be given to the fact that in that municipality the environmental conditions, genetic variability, technological packages and the different agronomic management that farmers apply will lead to variability, regardless of the method and technique of sampling (González *et al*., 2006; González *et al*., 2008; Arellano *et al*., 2010).

The variables were defined in Table 1. E1, E2, E3, are the maximum permissible errors (%) for method 1; F1, F2, F3, are the products between the arithmetic mean with the values of bi, at 0.05, 0.075 and 0.1 respectively and correspond to method 2.

The calculation of sample sizes with other methodologies, based on the preliminary values presented in this study, could provide better unbiased estimates of population parameters, particularly if a stratification of the communities in the upper part is performed and considering the different technological packages that producers use. The use of genetic and experimental designs, for better control of environmental heterogeneity and to divide genetic variability, would also be very useful.

Conclusions

There was significant phenotypic differentiation in all the variables evaluated. To sample plots in Cacahuacintle it is desirable to stratify the municipality of Calimaya to reduce the heterogeneity between their locations. With method 1, for bi= 0.05, 0.075 or 0.1, n= 396 in all variables.

With method 2 should be evaluated from 11 to 7588 plants or corn, depending on the products between the arithmetic mean with the values of bi, at 0.05, 0.075 and 0.1 respectively and the sampled variable. The use of genetic and experimental designs would help to make the sampling techniques used more efficient because they exercise greater control of environmental heterogeneity.

At least four of the five cooperating farmers were grouped in the four quadrants of the biplot. There was a positive and significant correlation between number of quality leaves for wrapping tamales (NHT) with number of grains and rows of grain in the corn, there was also between the first and length and diameter of the spine. It is suggested to use NHT as an indirect selection criterion to increase the size of the corn.

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