



Inbreeding depression simulation in popcorn cultivars to estimate the effective population size for germplasm conservation

Emmanuel Arnhold^{1*}, Derly José Henriques da Silva², Odilon Lemos de Mello Filho³, and José Marcelo Soriano Viana⁴

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ABSTRACT - Nowadays the corn variability is preserved mainly in germplasm banks. A population maintained in a germplasm bank can lose genetic variability when regenerations are performed with an inadequate effective population size. We therefore aimed at an estimation of the effective population size that minimizes alterations in the plant means of ear height, prolificity index, weight of 100 grains, grain yield and expansion capacity after ten regenerations in six popcorn populations. Six populations and their respective selfed generations were appraised in complete blocks with two replications. Yield was the trait with the most pronounced inbreeding depression. Population Rosa-Claro presented the strongest depression for yield (94.3%) and was used to simulate different effective sample sizes. It was concluded that an effective sample size of 200 unrelated non-inbred plants is enough to minimize inbreeding depression after ten regenerations, in all analyzed traits in the six populations.

Key words: population, plant genetics, genetic variability.

INTRODUCTION

The conservation of the genetic patrimony of a country and the maintenance of active germplasm banks is of strategic importance, mainly for breeding of species of agronomic, pharmacologic and industrial interest, besides avoiding genetic drift caused mainly by the deforestation of areas of great biodiversity.

Corn is a native American species and there is evidence of its domestication 8,000 to 10,000 years ago

(Paterniani and Campos 1999). Native populations cultivate different types of it across most part of the American continent. The species was closely linked to the food, tradition and culture of peoples such as Mayan, Inca, Aztec and American Indians (Mangelsdorf 1974).

The conservation of the corn genetic variability is presently mainly administrated in active germplasm banks. According to Crossa (1989), the main purpose of a germplasm bank is to preserve the variability of a

¹ Universidade Federal do Maranhão, Centro de Ciências Agrárias e Ambientais (CCAA), BR 222, Chapadinha-MA, C. P. 9, 65.550-000, Brasil. *E-mail: emmanuelarnhold@yahoo.com.br

² Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV), 36.570-000, Viçosa, MG, Brasil

³ Embrapa Soja, Rua Lauro Melo, 14, Bairro Parque Real, C. P. 180, 78.740-351, Rondonópolis, MT, Brasil

⁴ Departamento de Biologia Geral, UFV

cultivated species. Crossa et al. (1994) further stated that a germplasm bank curator's main objectives are to acquire, maintain, distribute, characterize, regenerate, preserve, evaluate, and to use the genetic diversity of a cultivated and its related wild species.

However, a population conserved in a germplasm bank can lose genetic variability, because according to Crossa (1989) and Crossa and Vencovsky (1994), the effectiveness of the regeneration of collections is affected by factors such as sample size, gene oscillation and seed viability. Regeneration is necessary to recover the germination and the vigor of a certain access and, according to Crossa et al. (1993), one of the objectives of access regeneration is to maintain at least one copy of each allele of the original population.

The sample size used in the regeneration depends on the number of alleles and their frequency and the genetic structure of a population (Crossa 1989, Crossa et al. 1993). Pinto et al. (2000) concluded however that the genetic structure of the analyzed populations to estimate the appropriate number of S1 progenies for the recurrent selection in corn did not influence the sample size.

According to Crossa (1989) and Falconer (1989), small samples lead to allele fixation and loss by genetic drift; in other words, due to random fluctuation that occurs naturally in the population. When the sample size is large, the regeneration is costly and when it is too small, rare alleles are lost (Crossa et al. 1993, Crossa et al. 1994). Crossa (1989) also comments that one of the consequences of the use of small samples is the decrease of heterozygosity. Therefore, besides losing rare alleles, the regeneration of a population in very small samples also tends to inbreeding depression, which can be irreversible and cumulative if such small samples are used in future regenerations as well.

According to Cruz (2005) the phenomenon of inbreeding is result of the mating of related individuals and selfing. It can influence the means of a population and the similarity of the derived lines.

In this sense, the question arises as to which would be the sample size to be used in the regeneration of a population so the genetic properties of this populations are maintained. Crossa and Vencovsky (1994) commented that the effective population size (N_e) is a measure of the genetic characterization of an access.

The concept of effective population size (N_e) was introduced by Wright (1931). In a simplified way it refers

to the number of unrelated non-inbred plants that compose the sample and which really participate in the formation of the next generation. Differently, the physical size of the sample (n) expresses the total number of individuals of the sample. Therefore, the estimate of the effective population size for different populations would be an alternative to recommend a sample size to be used in the regeneration of a certain access, to prevent the decrease of heterozygosity and alterations in the allele frequency.

This study aimed to estimate the effective population size that minimizes cumulative inbreeding depression after ten regenerations in six popcorn populations for the trait that is most sensitive to inbreeding depression.

MATERIAL AND METHODS

Selfings involving the populations Amarelo, Beija-Flor, Branco, Rosa-Claro, Roxo, and Viçosa were performed. All plants came from the Genetics Section - Active Germplasm Bank of the Corn Breeding Program, of the General Biology Department of the Federal University of Viçosa. The parents and inbreeding populations ($F = 1/2$) were evaluated in Viçosa, state of Minas Gerais, Brazil, in the following crop season. The experimental was installed in a complete random block design with two replications. Each plot consisted of two 5m rows spaced 0.9m apart. Five plants m^{-1} were left after thinning, resulting in a density of approximately 55.555 plants ha^{-1} .

The traits analyzed per plot were plant medium height (PH); and ear medium height (EH) in meters; prolificity index (PI); weight of a hundred grains (WHG) in grams; grain yield (GY) in $kg ha^{-1}$; and expansion capacity (EC) in $mL g^{-1}$.

Grain weight data were corrected to 14.5% standard moisture, based on the wet weight. For the determination of the population expansion capacity, 25 ml grains were used in an American electric popcorn pumper (Hot Air Popcorn Pumper H7340), of Proctor Silex. The grains were filled in when the popcorn pumper temperature had reached 100 °C. The time of evaluation was of about one minute.

Six variance analyses were performed to detect significant differences of the analyzed traits between each population and their respective inbred populations (S_1).

The inbreeding depression (ID) for each trait was obtained according to Vencovsky and Barriga (1992), by the difference of each population mean from its respective self-pollinated generation (S_1).

Different effective sizes were simulated, with the objective of estimating the effective size that minimizes the expected inbreeding depression after ten regenerations. The population and the trait in which the effect of the inbreeding depression was most pronounced were selected for this procedure.

The following formula was used (Falconer 1989):

$$\mu_1 = \mu_0 - ID / N_e$$

where

μ_1 = mean of the S_1 generation;

μ_0 = mean of the population;

$ID = \mu_0 - \mu_1 = \sum p_i q_i d_i$ = inbreeding depression;

N_e = effective size

where $\Delta_F = \frac{1}{2} N_e$

RESULTS AND DISCUSSION

The differences between population and S_1 population means were significant for all traits by the F test at 5% probability in the Amarelo, Viçosa and Rosa-Claro populations only (Table 1).

A positive effect of dominance was observed for the three populations in relation to plant height, ear height and grain yield, since the mean of the self-pollinated population was lower than the mean of the original population (Table 1). In the same Table the prolificity index showed negative effect of dominance in the Amarelo population and positive in the other populations where the differences were significant. The trait weight of a hundred grains had a negative dominance effect in the Branco population and positive in the others where the differences were significant. The expansion capacity had positive and negative dominance effects, in agreement with results obtained by Scapim et al. (2002).

The only trait where the differences were significant in all populations was grain yield (Table 1). This was also the trait where the inbreeding depression (ID) was highest, and the Rosa-Claro population had the highest percentage of inbreeding depression (94.6%). We therefore decided to just simulate the values of effective population size, based on the inbreeding

depression obtained for grain yield of the Rosa-Claro population.

Pacheco et al. (2002) verified inbreeding depression for yield in corn populations, from S_0 to S_1 (mean of 10 environments), ranging from 34.6% (CM-01) to 59.2% (CM-30), with a mean of 49.1%. These values of inbreeding depression were generally lower than the values we obtained for the populations under study; however, the authors analyzed normal open-pollinated elite corn varieties, while unimproved popcorn populations were evaluated in our study. Pacheco et al. (2002) also mentioned that the inbreeding depression was stronger in populations with a wider genetic base which had never been exposed to self-pollination. In this sense, less inbreeding depression can be expected in populations where breeding narrowed the genetic base, which is the case with the open-pollination elite varieties.

With effective sizes of 10, 20, 40, 60, 100, 200, 300, 400, and 600 plants, the inbreeding depression estimated for regeneration was 9.46, 4.73, 2.37, 1.58, 0.95, 0.47, 0.32, 0.24, and 0.16% respectively. After one generation, even when using an effective sample size of only 10 plants, the expected inbreeding depression is low (9.46%). However, the inbreeding effect is cumulative and the expected inbreeding should therefore be observed over a larger number of generations.

When increasing the effective size, there is a decrease in the expected inbreeding depression (Table 2). However, very high values of effective sizes make the regenerations very expensive. With samples of larger effective sizes (300, 400 and 600) the expected decrease in the estimate of grain yield mean is very low after ten generations (Table 2). For these three samples, an inbreeding depression of 3.11, 2.34 and 1.57% is expected, respectively, for effective sizes of 300, 400 and 600. With an effective sample size of 100 plants, the regenerations would be much less costly, but the inbreeding depression higher (9.07%). Effective sample sizes with less than 60 plants are not recommended since the expected inbreeding depression would be over 15% or more than 500 kg ha⁻¹ (Table 2). Omolo and Russel (1971) found significant differences in the population means of regenerations with 80 plants versus 500 plant samples. However, the mean difference was very small and the authors concluded that samples of 80 plants can be used in the regeneration of the analyzed

Table 1. Means, inbreeding depression (ID) and inbreeding depression in percentage (ID%) for the seven traits[†] under study in the six populations and their respective S1 generations

Population	PH	EH	PI	WHG	GY	EC
Branco	1.07**	0.50	0.79	11.76**	326.8**	16.12
Branco (S1)	0.97	0.46	0.68	15.02	54.1	16.39
ID	0.10			-3.26	272.7	
ID%	9.30			-27.73	83.44	
Roxo	1.61	0.74	0.67**	14.11	1597.0**	19.43**
Roxo (S1)	1.24	0.58	0.43	12.77	354.8	16.94
ID			0.24		1242.2	2.49
ID%			36.14		77.78	12.80
Beija-Flor	1.59	0.67	0.83**	15.30**	1571.4**	14.66**
Beija-Flor (S1)	1.53	0.69	0.48	12.15	281.7	19.60
ID			0.35	3.15	1289.7	-4.94
ID%			42.24	20.60	82.1	-33.76
Amarelo	1.55**	0.74**	0.60**	16.38**	1100.6**	24.39**
Amarelo (S1)	1.39	0.68	0.65	13.12	458.7	18.34
ID	0.16	0.06	-0.05	3.26	642.0	6.05
ID%	10.29	8.05	-8.37	19.89	58.3	24.79
Viçosa	1.58**	0.74**	0.87**	19.59**	1729.0**	20.53**
Viçosa (S1)	1.46	0.68	0.78	13.55	430.5	24.44
ID	0.12	0.06	0.09	6.05	1299.0	-3.91
ID%	7.88	8.72	10.22	30.87	75.1	-19.04
Rosa-Claro	1.72**	0.72**	1.20**	19.78**	3516.8**	23.86**
Rosa-Claro (S1)	0.95	0.45	0.66	15.59	189.3	18.77
ID	0.77	0.26	0.54	4.18	3327.5	5.09
ID%	44.64	36.80	45.04	21.14	94.6	21.35

** : means of the populations and their respective S₁ populations are statistically different, at 1% probability by the F test. PH: plant height (m), EH: ear height (m), PI: prolificity index, WHG: Weight of a hundred grains (g), GY: grain yield (kg ha⁻¹), and EC: expansion capacity (mL g⁻¹)

populations, since a certain degree of inbreeding depression is tolerated.

The use of very small samples or samples with very small effective sizes would reduce the costs and labor for regenerations; however, the inbreeding depression would be very pronounced. With an effective sample size of 10 plants, a mean grain yield of 1301.60 kg ha⁻¹ would be expected after 10 regenerations, which

corresponds to 37.01% of the original mean (3516.80 kg ha⁻¹). The expected inbreeding depression for an effective sample size of 10 plants over 10 regenerations would be 2215.20 kg ha⁻¹ in the mean grain yield. Using an effective size of 20 plants, the expected inbreeding depression on grain yield would be 1350.07 kg ha⁻¹ after ten regenerations, and the expected mean 61.59% of the original mean.

Table 2. Simulation of the expected grain yield estimate (GY) and its respective percentage in relation to the original mean (GY%) in different effective sample sizes (N_e) in ten regenerations, considering the Rosa-Claro population

Regenerations	GY (N_e 10)	GY%	GY (N_e 20)	GY%	GY (N_e 40)	GY%
1	3184.08	90.54	3350.45	95.27	3433.64	97.63
2	2882.82	81.97	3191.95	90.76	3352.42	95.33
3	2610.05	74.22	3040.95	86.47	3273.12	93.07
4	2363.10	67.19	2897.09	82.38	3195.70	90.87
5	2139.51	60.84	2760.03	78.48	3120.11	88.72
6	1937.08	55.08	2629.46	74.77	3046.31	86.62
7	1753.80	49.87	2505.06	71.23	2974.25	84.57
8	1587.86	45.15	2386.55	67.86	2903.90	82.57
9	1437.63	40.88	2273.65	64.65	2835.21	80.62
10	1301.60	37.01	2166.09	61.59	2768.14	78.71
Regenerations	GY (N_e 60)	GY%	GY (N_e 100)	GY%	GY (N_e 200)	GY%
1	3461.37	98.42	3483.55	99.05	3500.19	99.53
2	3406.79	96.87	3450.59	98.12	3483.63	99.06
3	3353.06	95.34	3417.95	97.19	3467.15	98.59
4	3300.19	93.84	3385.61	96.27	3450.75	98.12
5	3248.15	92.36	3353.57	95.36	3434.43	97.66
6	3196.93	90.90	3321.84	94.46	3418.18	97.19
7	3146.51	89.47	3290.41	93.56	3402.01	96.74
8	3096.89	88.06	3259.28	92.68	3385.91	96.28
9	3048.06	86.67	3228.44	91.80	3369.89	95.82
10	2999.99	85.30	3197.90	90.93	3353.95	95.37
Regenerations	GY (N_e 300)	GY%	GY (N_e 400)	GY%	GY (N_e 600)	GY%
1	3505.74	99.68	3508.51	99.76	3511.28	99.84
2	3494.68	99.37	3500.21	99.53	3505.75	99.68
3	3483.66	99.06	3491.93	99.29	3500.22	99.53
4	3472.67	98.74	3483.67	99.06	3494.70	99.37
5	3461.72	98.43	3475.43	98.82	3489.19	99.21
6	3450.80	98.12	3467.21	98.59	3483.69	99.06
7	3439.92	97.81	3459.01	98.36	3478.19	98.90
8	3429.07	97.50	3450.83	98.12	3472.71	98.75
9	3418.25	97.20	3442.67	97.89	3467.23	98.59
10	3407.47	96.89	3434.52	97.66	3461.76	98.43

With an effective population size of 10 plants, the expected inbreeding depression after ten regenerations is very low (4.63%). Effective sample sizes of 300, 400 and 600 plants would reduce the expected inbreeding depression after ten regenerations by only 1.52, 2.29 and 3.06% respectively. Therefore, it would not be justified to use effective samples sizes of over 200 in order to minimize the inbreeding depression.

Omolo and Russel (1971) analyzed different sample sizes in five regenerations in two normal corn populations. The 200 plant sample was the smallest that did not present significant inbreeding depression for

yield, compared to the 500 plant sample. The authors mentioned that they changed from 500 over to 200 plant samples in the regeneration of these populations. Pinto et al. (2000) also recommended 200 plants for a recurrent selection program with corn, based on the grain yield of two study populations.

However, the trait and the population used to estimate the inbreeding depression were the ones with the strongest inbreeding depression, so smaller effective sample sizes might be suitable in the regeneration of other populations, considering all analyzed traits, without leading to significant alterations

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in the mean after ten generations. In this sense, according to Crossa (1989) and Crossa et al. (1993), the sample size used in the regeneration depends on the number and frequency of alleles and on the genetic structure of a population.

CONCLUSION

An effective sample size of 100 to 200 unrelated non-inbred plants was enough to minimize the expected inbreeding depression after ten regenerations in all

analyzed traits in the six populations. An effective sample size of 200 plants was the most appropriate.

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Simulação da depressão endogâmica em milho-pipoca visando estimar o tamanho efetivo para conservação de germoplasma

RESUMO - Atualmente a conservação da variabilidade do milho é feita principalmente em bancos de germoplasma. Se as regenerações são realizadas a partir amostras com tamanho efetivo inadequado, uma população conservada em um banco de germoplasma pode perder variabilidade. Portanto, objetivou-se estimar o tamanho efetivo populacional que minimiza alterações nas médias de altura de planta e espiga, índice de prolificidade, peso de cem grãos, produtividade de grãos e capacidade de expansão, após dez regenerações, em seis populações de milho-pipoca. Estas populações e suas gerações autofecundadas foram avaliadas em blocos completos, com duas repetições. Produtividade foi a característica com maior depressão endogâmica. A depressão foi mais pronunciada na produtividade da população Rosa-claro (94,3%), sendo utilizada para simular diferentes tamanhos efetivos. Concluiu-se que uma amostra que contenha tamanho efetivo de 200 plantas não endogâmicas é suficiente para minimizar a depressão endogâmica, após dez regenerações, em todas as características analisadas nas seis populações.

Palavras-chave: Conservação de germoplasma; tamanho efetivo.

REFERENCES

- Cruz DC (2005) **Princípios de genética quantitativa**. Editora UFV, Viçosa, 394p.
- Crossa J, Taba S, Eberhart SA, Bretting P and Vencovsky R (1994) Practical considerations for maintaining germplasm in maize. **Theoretical and Applied Genetic** **89**: 89-95.
- Crossa J and Vencovsky R (1994) Implications of the variance of effective population size on the genetic conservation of monoecious species. **Theoretical and Applied Genetic** **89**: 936-942.
- Crossa J, Hernandez CM, Bretting P, Eberhart SA and Taba S (1993) Statistical genetic considerations for maintaining germplasm collections. **Theoretical and Applied Genetic** **86**: 673-768.
- Crossa J (1989) Methodologies for estimating the sample size required for genetic conservation of outbreeding crops. **Theoretical and Applied Genetics** **77**: 153-161.
- Falconer DS (1989) **Introduction to quantitative genetics**. 3rd ed., Longman Scientific & Technical, Harlow, 438p.
- Mangelsdorf PC (1974) **Corn: Its origin evolution and improvement**. Harvard University Press, Massachusetts, 261p.
- Pacheco CAP, Santos MX, Cruz CD, Parentori SN, Guimaraes PED and Vieira PA (2002) Inbreeding depression of 28 maize elite open pollinated varieties. **Genetics and Molecular Biology** **25**: 441-448.
- Paterniani E and Campos MS (1999) Melhoramento do milho. In: Borém A (ed.) **Melhoramento de Espécies Cultivadas**. Editora UFV, Viçosa, p.429-485.

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Pinto RMC, Lima Neto FP and Souza Junior CL (2000) Estimativa do número apropriado de progênies S_1 para a seleção recorrente em milho. **Pesquisa Agropecuária Brasileira** **35**: 63-73.

Scapim CA, Pacheco CAP, Tonet A, Braccini AL and Pinto RJB (2002) Análise dialélica e heterose de populações de milho-pipoca. **Bragantia** **61**: 219-230.

Omolo E and Russel WA (1971) Genetic effects of population size in the reproduction of two heterogeneous maize populations. **Iowa State Journal Science** **45**: 499-512.

Vencovsky R and Barriga P (1992) **Genética biométrica no fitomelhoramento**. Revista Brasileira de Genética, Ribeirão Preto, 486p.

Wright S (1931) Evolution in Mendelian populations. **Genetics** **16**: 97-159.