PHENOTYPIC RECURRENT SELECTION TO IMPROVE PROTEIN QUALITY IN NON-OPAQUE MAIZE POPULATIONS

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ABSTRACT: Maize (*Zea mays* L.) protein is considered to be of low quality due to low levels of the essential lysine and tryptophan amino acids. An alternative to solve this problem is to use the opaque-2 gene, which improves the level of these amino acids, but has negative pleiotropic effects on agronomic characters. A phenotypic recurrent selection scheme was carried out in two non-opaque maize populations to verify the possibility of improving their protein quality without using this gene. Four cycles were completed and a 20% selection intensity for tryptophan content in the kernels was used in two populations, IG-1 and IG-2. The original and the four-cycle populations were evaluated in three locations for agronomic traits. For protein and tryptophan content, a separated trial was carried out because plants of the plots were hand-pollinated. No increase in tryptophan content was observed in the IG-2 population, whereas IG-1 presented a small increase (0.70% per cycle). The ratio tryptophan/protein increased 1.26% per cycle in IG-1 and the protein content did not increase in both populations. The ESALQ-VD2-opaque check was superior in relation to both populations for protein quality, as expected, even after completion of four selection cycles. The kernel yield (2.5% per cycle) prolificacy, plant and ear heights, decreased with selection cycles, as a correlated response to selection. Phenotypic recurrent selection in non-opaque maize was not able to increase, at reasonable rates, the protein quality of maize kernels.
Key words: tryptophan, correlated response, maize breeding

SELEÇÃO RECORRENTE FENOTÍPICA PARA MELHORAMENTO DA QUALIDADE PROTÉICA EM POPULAÇÕES DE MILHO NÃO OPACO

RESUMO: A proteína do milho (*Zea mays* L.) é considerada como de baixa qualidade, pois apresenta nível reduzido dos aminoácidos essenciais lisina e triptofano. Uma alternativa para contornar esse problema consiste na introdução do gene opaco-2, que eleva a quantidade desses aminoácidos, embora apresente efeitos pleiotrópicos negativos em caracteres agronômicos relacionados à produtividade. O objetivo deste trabalho foi verificar a viabilidade de melhorar a qualidade protéica do milho com uso de Seleção Recorrente Fenotípica (SRF), sem introdução do gene opaco. Foram realizados quatro ciclos de SRF em duas populações, IG-1 e IG-2, para o teor de triptofano nos grãos, com 20% de intensidade. Os quatro ciclos seletivos e as populações originais foram avaliados para caracteres agronômicos em três locais. Para avaliação da qualidade protéica, foi conduzido um experimento com controle da polinização, para se evitar o efeito de xênia. Houve pequeno aumento nos níveis de triptofano para IG-1 (cerca de 0,70% por ciclo) e ausência de alteração em IG-2. Ocorreu aumento na relação triptofano/proteína para IG-1 (1,26% por ciclo) e os teores de proteína não se alteraram para as duas populações. A qualidade protéica da testemunha ESALQ VD2-opaco foi superior a das populações mesmo após a realização dos quatro ciclos. Como resposta correlacionada à seleção, houve redução na produção de grãos (2,50% por ciclo), prolificidade, altura da planta e altura da espiga. O baixo ganho, associado às alterações desfavoráveis em caracteres agronômicos, indica que este método de seleção possivelmente não é eficiente para elevar a qualidade protéica.
Palavras-chave: triptofano, resposta correlacionada, melhoramento do milho

INTRODUCTION

Maize protein is considered to be of low quality as it contains low levels of the essential lysine and tryptophan amino acids. This may cause nutritional deficiencies when the maize is used as an exclusive protein source, without the addition of supplements (Glover & Mertz, 1987). Genotypes containing the opaque-2 gene (o_2o_2), present higher levels of lysine and tryptophan than the normal genotypes (O_2O_2), which suggests advantages in the use of the opaque maize in human diets and in rations for monogastric animals (Mertz et al., 1964; Graham et al., 1980; Glover & Mertz, 1987; Bjarnason & Vasal, 1992).

However, opaque-2 genotypes present kernels with low density, low productivity, increase in the moisture of the kernel during harvest, and broken endosperm, leading to losses during harvest, handling and storing. Therefore, the opaque cultivars have low commercial value (Paez & Zuber, 1973; Glover & Mertz, 1987; Vasal et al., 1993a). This fact led to the development of materials called QPM (quality protein maize), which are

opaque genotypes with a vitreous endosperm (Zuber & Helm, 1972; Bjarnason, 1990; Pixley & Bjarnason, 1993). Much research has been developed with QPM, especially by the CIMMYT (Magnavaca & Mertz, 1992; Villegas et al., 1992; Bockholt et al., 1992; Vasal et al., 1993b; Vasal & Hallauer, 1994; Bertechini et al., 1999).

An alternative for this problem is using recurrent selection to improve the protein quality of normal maize (non-opaque) by increasing the levels of essential amino acids (Zuber & Helm, 1972). The protein and lysine content usually present high values for heritability in normal genotypes (Choe et al., 1976; Goertz et al., 1978), and the lysine and tryptophan contents are positively correlated (Villegas et al., 1984). Based on this, satisfactory results from the use of this method should be expected.

However, reports on recurrent selection in normal maize showed conflicting results. Zuber & Helm (1972) reported increases of 30 to 48% in the percentage of lysine in kernels, without presenting alteration in their protein content. Deutscher and Zuber in 1984, through personal communication to Glover & Mertz (1987), reported small increases in lysine content, but with a substantial reduction in grain yield, which made unfeasible the total increase of the productivity for lysine. Glover (1976) did not observe increases of lysine levels in normal maize populations submitted to recurrent selection.

This article reports the results of four cycles of phenotypic recurrent selection to improve the protein quality of the kernels in two non-opaque maize populations, as well as alterations in various important agronomic traits.

MATERIAL AND METHODS

The two populations submitted to phenotypic recurrent selection are called IG-1 and IG-2, with orange flint and yellow dent kernels, respectively, both of which present intermediate cycle, without the opaque-2 gene. They were obtained in Piracicaba, SP, Brazil.

An area with 2,000 plants (50,000 plants ha^{-1}) was planted for each population, from which 800 plants were selected and were crossed in pairs generating 400 ears. The ears that presented an insufficient number of kernels were discarded. This brought the number down to 250 ears, of which 20 kernels from the intermediary region were removed to determine the tryptophan and protein contents, based on the methodology by Hernandes & Bates (1969).

A 20% intensity of selection for tryptophan content was applied resulting in 50 selected ears. From each one, 40 seeds were removed and mixed up, coming to a total of 2,000 seeds, which were used for the recombination and beginning of a new cycle. Four phenotypic recurrent selection cycles were carried out using the same procedure.

The original population and their four selective cycles were evaluated in experiments carried out in two environments, in Piracicaba, SP, Brazil, during the agricultural year of 1991/1992. The VD2-opaque ESALQ variety, with yellow dent kernels, late cycle and o_2o_2 genotype was used as a check, in a total of 11 entries. The plots were made up of 5 m long rows, spaced 0.9 m between rows and 0.20 m between plants, with 25 plants per plot after thinning ($\approx$55,000 plants ha^{-1}). The experimental design consisted of randomized complete blocks with five replications. Tryptophan (TRP%) and the protein (PROT%) contents of the kernels were evaluated. To avoid the xenia effect, hand pollination to control pollen within each plot was carried out, obtaining thus approximately 20 pollinated ears for each one. A 10-kernel sample, from the intermediary region of each ear, was taken for laboratory analysis giving a total of 200 kernels.

To evaluate the correlated response to selection in important agronomic traits, experiments were installed using a randomized complete block design with 10 replications in other three environments of Piracicaba, following the same procedures except hand pollination. Data of grain yield (GY), prolificacy (PROL), plant (PH) and ear (EH) heights of five plants from each plot, number of kernels per ear (NKE), number of kernels per row (NKR) and number of rows (NR), were also taken from five random ears of each plot.

Joint analyses of variance were carried out based on the following mathematical model (Steel & Torrie, 1980):

$$y_{ijk} = m + t_i + l_j + (tl)_{ij} + b_{k(j)} + e_{ijk},$$

y_{ijk} = observed value for the character for treatment i, location j, block k; m = mean; t_i = effect of treatment i, fixed (i = 1 to 11, representing 5 cycles for IG-1, 5 for IG-2 and the ESALQ VD2-opaque check); l_j = effect of location j, random; $(tl)_{ij}$ = interation effect between the treatment i and the location j, random; $b_{k(j)}$ = effect of block k in the location j, random; e_{ijk} = experimental error, $e_{ijk} \cap N(\mu, \sigma^2)$.

In situations where a difference was found between the treatments, the 10 degrees of freedom (DF) of this source of variation were orthogonally decomposed in differences between the IG-1 cycles (4 DF), IG-2 cycles (4 DF) and the contrast between IG-1, IG-2 and the ESALQ VD2-opaque check (2 DF). After this orthogonal decomposition, the adjustments of a linear regression model, considering the cycles as independent variables, were tested (Sokal & Rohlf, 1995). Next, the parameters of this regression were estimated for: intercept (a) and the slope of the regression line (b). The slope of the regression line measures the alteration in the character per selective cycle, or better, genetic gain (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

The average values for tryptophan content (TRP) for the four selective cycles indicated that there was a tendency for linear increase with recurrent selection for the IG-1 population (b%=0.68), with an accumulated gain of 2.4% in the fourth cycle. For the IG-2 population, the values throughout the selective cycles were slightly higher than that of the original population, causing an accumulated increase of only 0.56% (b%=0.05, not significant). For both populations the averages of the fourth cycle were inferior in relation to the check. The protein content (PROT) did not change in both populations, presenting the same contents as the check. For tryptophan content in the protein (TRP/PROT) a tendency to increase was only observed in the population IG-1, which was expected as this TRP population increased and the PROT remained constant. After the fourth selection cycle, the accumulated gain in IG-1 for this trait was 4.20%, where the two populations in all the cycles differed from the check (Tables 1 and 2).

The estimatives of the regression parameters (Table 2) showed that there is a tendency of response between the selective cycles for TRP only for IG-1. This gain should be considered satisfactory (0.68% per cycle), taking into consideration the easy application of the phenotypic recurrent selection. However, gains of this magnitude imply in the necessity of having many selective cycles until their levels of tryptophan are the same as the check (approximately 8 cycles, if the gain is constant). There were no adjustments for linear regression for PROT in any of the populations. The TRP/PROT relation showed the same behavior as TRP, with linear adjustment only for IG-1. The increase was significant for the IG-1, for the TRP/PROT character, with a gain of 1.26% per cycle in relation to the original population, where 15 cycles were necessary, under constant gain, to obtain the check levels.

The reductions in GY were 2.21% and 3.75% per cycle, for IG-1 and IG-2 populations respectively, resulting in a reduction of approximately 10% for IG-1 and 15% for IG-2 after four cycles. These values could be considered high due to the importance of the trait and as they imply in the reduction of the productivity of tryptophan. Although there was only a linear adjustment among IG-1 cycles for PROL, both populations presented reductions of high magnitude, with values of 1.43% and 2.42% per cycle for IG-1 and IG-2, respectively (Tables 3 and 4). This was already expected as this trait is usually positively correlated with GY (Souza Jr. et al., 1985). The reduction in this character was approximately 5% in IG-1 and 10% in IG-2 after four cycles. There were linear reductions of 1.15% and 1.42% per cycle in PH and 1.41% and 1.59% in EH, for IG-1 and IG-2, respectively. These reductions can be considered favorable as the smaller heights imply in a smaller percentage of broken and lodging plants and can be added to the results of mass selection to reduce size, normally carried out in breeding programs. The reduction after four cycles was around 5%.

Table 1 - Estimatives of mean values for each selective cycle (μ) for tryptophan content (TRP), protein content (PROT) and tryptophan content in the protein (TRP/PROT) for kernels of the IG-1 and IG-2 maize populations, and the ESALQ-VD2-opaque check.

Population	Cycle	Trait					
		TRP		PROT		TRP/PROT	
		μ	$(\mu / \mu_0) . 100$[1]	μ	$(\mu / \mu_0) . 100$	μ	$(\mu / \mu_0) . 100$
		% x 10^{-2}	------ % ------	-------------- % ---------------		% x 10^{-1}	%
IG-1	0	7.99	100.00	11.23	100.00	7.05	100.00
	I	8.03	100.44	11.09	98.75	7.03	99.79
	II	8.18	102.37	11.31	100.71	7.14	101.30
	III	8.18	102.37	11.03	98.22	7.32	103.88
	IV	8.19	102.40	11.12	99.02	7.34	104.20
IG-2	0	8.42	100.00	11.23	100.00	7.37	100.00
	I	8.51	101.08	11.27	100.36	7.42	100.68
	II	8.44	100.30	10.94	97.42	7.70	104.49
	III	8.45	100.38	10.92	97.24	7.66	103.99
	IV	8.47	100.56	11.24	100.09	7.46	101.26
Check		9.61		11.26		8.38	
Mean		8.41		11.15		7.44	
LSD[2]		0.94		0.73		0.67	
CV[3] (%)		7.51		4.37		8.78	

[1]Mean in relation to the cycle zero (μ_0).
[2]Least significant difference (5%).
[3]Coefficient of variation.

Table 2 - Estimates of parameters of linear regression: intercept (a), slope of the regression line (b) and coefficient of determination (R^2) for tryptophan content (TRP), protein content (PROT) and the tryptophan/protein relationship (TRP/PROT), for the IG-1 and IG-2 maize populations.

Population	Parameter	Trait		
		TRP	PROT	TRP/PROT
		------------------ % ------------------		
IG-1	a	0.0800**	11.4600**	0.7000**
	b	0.0005*	-0.0699	0.0088*
	b%=(b/a).100	0.6750	-0.6099	1.2571
	R^2	0.7968	0.5812	0.8855
IG-2	a	0.0845**	11.4143**	0.7437**
	b	0.0001	-0.0594	0.0043
	b%=(b/a).100	0.0473	-0.5204	0.5782
	R^2	0.0270	0.1748	0.2076

*,**$P < 0.05$ and $P < 0.01$, respectively, by t test.

The gains in the tryptophan content were not high enough to justify the use of phenotypic recurrent selection to increase the protein quality in normal maize. This was not expected, since the results of the literature, with selection and genetic gain estimatives to increase the levels of lysine, amino acid also associated to protein quality and positively correlated to tryptophan, have been promising (Zuber & Helm, 1972; Choe et al., 1976; Goertz et al., 1978; Villegas et al., 1984). These small or non-existing gains can be due to the inefficacy of the breeding method used, or to the fact that the populations are not responsive to selection, which is due to their genetic variances and to heritability that they present in the trait. It is presumed that IG-1 would be more responsive than IG-2, as it presents linear alteration of the cycle 0 to cycle VI for TRP.

There are few estimates for heritability coefficients in the literature for TRP in normal maize populations. Goertz et al. (1978) obtained low heritability estimate for tryptophan (27%), which can explain the inefficacy of phenotypic recurrent selection. However, estimates of high magnitude are found in opaque maize populations for the lysine and tryptophan content (Motto, 1979; Beaver et al., 1985), which indicate that the presence of the gene opaque-2 should alter the genetic base of this character. Our results suggest that more elaborate breeding methods, with progenie evaluation, should be used.

The alterations for GY were of great magnitude, as a result of response correlated to selection. Similar results were obtained for Deutscher and Zuber, cited by Glover & Mertz (1987), who also found a reduction in grain yield after selection to increase the lysine content. The other evaluated agronomic traits, EH/PH (relative position of the ear), NKR and NR, did not present alterations during the selection cycles, so that it can be presumed that they are not correlated to tryptophan

content (results not shown).

The gain with selection is due to genetic variability for the tryptophan content of the populations, where there might not have been a noticeable gain if this variability was small. To answer these questions, experiments based on some adequate designs to estimate genetic parameters should be carried out.

The IG-2 was slightly higher than IG-1 in relation to protein quality, measured by the tryptophan content and by the relation protein/protein (Table 1). It is not possible to affirm if this is due to the fact that the IG-2 population presents dent kernels and the IG-1 population flint kernels, as there is nothing about this in the literature. However, for many agronomic traits, the kernel type can imply in differences between the populations.

The protein content was not significantly altered, but some traits, for which selection was not oriented for, present great changes during the cycles. Thus, the genetic correlations between these traits and the tryptophan content should be very high. The grain yield presented the greatest reduction when compared to the others, followed by prolificacy. Another hypothesis is that the increase of the levels of tryptophan, although slight, could cause some physiological disturbance in the plants, with reduction in grain yield and in other traits. This phenomenon has been described for the lysine amino acid indicating that it acts like an inhibitor for enzymes involved in the metabolism of the aspartate (Dotson et al., 1990; Frisch et al., 1991). The inhibition of the aspartate kinase, first enzyme of this metabolic via, appears when there is a lack of intermediary substrates for the synthesis of methionine, limiting the proteic synthesis, with adverse results for the development of endosperm (Brochetto-Braga et al., 1992). As the content of this amino acid is positively correlated to that of tryptophan, the selection can have caused the inhibition. The same physiological mechanism should also act for tryptophan.

For the other components of grain yield, there was a decrease only for PROL, which should therefore be negatively correlated to TRP. As NKR and NK did not present this response to selection, it should be possible to minimize the reduction in GY by means of selection to increase these traits, together with that carried out for protein quality. Another alternative should be to also carry out selection to increase PROL, which has high heritability and is positively correlated to grain yield (Souza Jr. et al., 1985). The use of selection indexes can also be useful, as they allow the combination of traits in a single parameter to decrease the negative effect of the correlated response.

Nevertheless, the reduced gains with selection, associated to alterations in important agronomic traits, seem to indicate the inefficacy of phenotypic recurrent selection to improve protein quality of maize, as an alternative for the introduction of the opaque-2 gene, since results with QPM are very promising (Bjarnason, 1990;

Table 3 - Estimatives of mean values for each selective cycle (μ) for grain yield (GY), prolificacy (PROL), plant height (PH) and ear height (EH), for the IG-1 and IG-2 maize populations, and the ESALQ VD2- opaque check.

		Trait							
		GY		PROL		PH		EH	
Population	Cycle	μ	$(\mu / \mu_0) . 100^1$	μ	$(\mu / \mu_0) . 100$	μ	$(\mu / \mu_0) . 100$	μ	$(\mu / \mu_0) . 100$
		Mg ha^{-1}	%		%	cm	%	cm	%
IG-1	0	5.15	100.00	1.06	100.00	207	100.00	109	100.00
	I	5.02	97.48	1.06	100.00	205	99.03	109	100.00
	II	4.81	93.40	1.03	97.17	202	97.58	106	97.25
	III	4.83	93.79	1.00	94.34	199	96.14	104	95.41
	IV	4.68	90.87	1.01	95.28	198	95.65	104	95.41
IG-2	0	4.98	100.00	0.99	100.00	206	100.00	108	100.00
	I	4.92	98.80	1.00	101.01	204	99.03	106	98.15
	II	4.65	93.37	1.00	101.01	197	95.63	104	96.30
	III	4.55	91.37	0.95	95.95	194	94.17	102	94.44
	IV	4.22	84.74	0.89	89.90	196	95.15	102	94.44
Check		4.97		1.07		241		144	
Mean		4.80		1.01		204		109	
LSD^2		0.67		0.10		11.0		8.61	
CV^3(%)		13.48		10.76		5.06		7.47	

^{1}Mean in relation to the zero cycle (μ_0).
^{2}Least significant difference (5%).
^{3}Coefficient of variation.

Table 4 - Estimatives of parameters of linear regression: intercept (a), slope of the regression line (b) and coefficient of determination (R^2) for grain yield (GY, Mg ha^{-1}), prolificacy (PROL), plant height (PH, cm) and ear height (EH, cm) for the IG-1 e IG-2 maize populations.

Population	Parameter	Trait			
		GY	PROL	PH	EH
		Mg ha^{-1}		---------- cm ----------	
IG-1	a	5.12**	1.06**	207.00**	110.00**
	b	-0.12**	-0.02*	-2.00**	-2.00*
	b%=(b/a).100	-2.21	-1.43	-1.15	-1.41
	R^2	0.92	0.84	0.97	0.89
IG-2	a	5.04**	1.02**	205.00**	108.00**
	b	-0.19**	-0.03	-3.00*	-2.00**
	b%=(b/a).100	-3.75	-2.42	-1.42	-1.59
	R^2	0.95	0.68	0.79	0.94

*,** $P < 0.05$ and $P < 0.01$, respectively, by t test.

Pixley & Bjarnason, 1993; Lambert & Chung, 1995).

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