DIALLELIC ANALYSIS FOR LYSINE AND OIL CONTENTS IN MAIZE GRAINS

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ABSTRACT: Six S_5 lines of maize, with differences for lysine and oil contents in grains, were used to carry out a complete series of diallelic crosses. The resulting 15 hybrids were grown in a field at two nitrogen levels (10 and 130 kg N ha^{-1}). The general combining ability (GCA) and specific combining ability (SCA) were obtained by using the method 4, model I of Griffing for grain yield, and grain lysine and oil contents. Significant ($p < 0.001$) interaction was observed between GCA and N levels for grain yield, indicating the selection of different lines for each N level. This interaction was not significant for lysine content, but there were significant effects of GCA for this trait at both N levels ($p < 0.1$). Significant effects were not observed for GCA or SCA for oil content, however a positive correlation was observed between lysine and oil contents in the hybrids, in the lines and even in the control cultivars. The results indicate the effectiveness of selection for lysine content, irrespective of N level, in the studied non-opaque maize lines, and the possibility of achieving both high lysine and oil content in grains.
Key words: cereal, opaque, nitrogen, proteins, near infrared

ANÁLISE DIALÉLICA PARA CONTEÚDOS DE LISINA E ÓLEO EM GRÃOS DE MILHO

RESUMO: Seis linhagens S_5 de milho, com diferenças para conteúdos de óleo e de lisina nos grãos, foram usadas para realizar uma série completa de cruzamentos dialélicos. Os 15 híbridos resultantes foram cultivados em campo com dois níveis de nitrogênio (10 e 130 kg N ha^{-1}). A capacidade geral de combinação (CGC) e a capacidade específica de combinação (CEC) foram obtidas com o método 4, modelo I de Griffing para produtividade de grãos e concentração de lisina e óleo nos grãos. Interação significativa ($p < 0.001$) foi observada entre CGC e níveis de N para produtividade de grãos, indicando seleção de diferentes linhagens para cada nível de N. Esta interação não foi significativa para conteúdo de lisina, mas existiu efeito significativo ($p < 0.1$) da CGC nos dois níveis de N para esta variável. Os efeitos de CGC e CEC foram não significativos para conteúdo de óleo, contudo, foi observada correlação positiva entre conteúdos de lisina e óleo nos híbridos, nas linhagens e mesmo nas cultivares utilizadas como controle. Os resultados indicam a efetividade da seleção para conteúdo de lisina nas linhagens não opaco de milho estudadas, independente dos níveis de N, e a possibilidade de atingir concomitantemente maiores níveis de óleo e lisina nos grãos.
Palavras-chave: cereal, opaco, nitrogênio, proteínas, infravermelho próximo

INTRODUCTION

Cereal grains contribute to over 60% of the total world food production. In spite of being among the most important species cultivated in the world and serving as a source of dietary calories and protein, maize grains are poor in both oil content (O'Quinn et al., 2000; Romanelli & Milan, 2005) and quality protein (Varisi et al., 2007), since their predominant grain storage proteins, the zeins, are deficient in lysine and tryptophan (Azevedo et al., 1997; Ferreira et al., 2006; Landry & Delhaye, 2007) The study of nitrogen (N) metabolism has been a major factor to help understanding amino acids metabolism (Medici et al., 2004, 2005; Lea & Azevedo, 2006, 2007; Samborski et al., 2008). Different strategies have been used to improve these traits by either independent or concomitant approaches. Maize has been improved for high oil content alone (Adams & Jensen, 1987; Thomison et al., 2003), or both high oil and high lysine contents

(O'Quinn et al., 2000), using traditional plant breeding methods, such as the diallelic crosses (Misevic et al., 1989). However, the main approach to improve maize quality protein has been the use of the *opaque-2* gene (*o2*) (Azevedo et al., 2006). This mutant gene confers high lysine content to maize endosperm, but has pleiotropic negative effects on agronomic traits. *Opaco-2 modifier* genes can overcome the adverse effects of the *o2* gene, resembling normal maize in kernel phenotype and agronomic performance, and holding superior quality protein. These kind of modified *o2* genotypes are generally called "quality protein maize" (QPM). The protein quality of QPM is close to that of skim milk, resulting in improved human health in developing countries (Prasanna et al., 2001).

The studies of opaque and floury maize mutants have demonstrated important effects of these mutations on enzymes involved in the metabolic aspartate pathway, dry matter, storage protein fractions and amino acid compositions (Azevedo et al., 2003, 2004a,b; Pompeu et al., 2006; Landry et al., 2005; Varisi et al., 2007). Another approach for achieving high lysine content in maize seeds is the breeding by recorrent selection using non-opaque maize populations (Garcia & de Souza, 2002). The aim of this work was to evaluate the combining ability for lysine and oil contents and the phenotypic association between these traits in non-opaque maize.

MATERIAL AND METHODS

Six S_5 lines of maize (*Zea mays* L.), developed in Seropédica, state of Rio de Janeiro, Brazil (22º44' S; 43º42' W), and their 15 possible hybrids were used. Two controls were also used which were obtained from Embrapa Milho e Sorgo, Brazil. One control was the cultivar BR 106 and the other was the population Sintetico Elite NT, which had been selected for high yield on low N soils (Santos et al., 1998). Lines 2, 3, 4 and 10, were derived from the commercial hybrid AG 311 (Agroceres seed company, Brazil) whereas the lines 5 and 6 were originated from the commercial hybrid AG 302 of the same company. The contrasting responses to N supply for these lines are reported in Medici et al. (2005), which show that the severe N deficiency caused a reduction in grain yield in lines 2, 3, 5, and 10, prolificacy (ears per plant) in lines 2, 3, 4, and 5, grain N content in all lines (except line 10), and total plant N in lines 2, 3, and 5. On the other hand, this severe N deficiency also caused an increase in 100-kernel weight in line 2 and anthesis-silking interval in lines 3 and 5.

The experiments for lines and hybrids were conducted side by side in Anhembi, State of São Paulo, Brazil (22°45' S, 48°00' W) in 2001. The soil type was an Oxisol and the soil analysis of the first 20 cm depth showed pH - 4.4; organic matter - 26 g dm^{-3}; P - 63 mg dm^{-3}; K - 5.5 $mmol_c$ dm^{-3}; Ca - 140 $mmol_c$ dm^{-3}; Mg - 7 $mmol_c$ dm^{-3}; Al - 6 $mmol_c$ dm^{-3}; H + Al - 42 $mmol_c$ dm^{-3}.

The experimental design for the lines and for hybrids was factorial of genotypes, by two levels of N supply (10 and 130 kg N ha^{-1} as urea) in a randomized complete block design with six replicates. In the experiment for lines, the two maize controls were used. The plots consisted of one-row plot 5 m long, spaced at 0.9 m, at a within-row distance of 0.2 m between plants. According to Storck et al. (2007), this is an adequate sample size.

Grain yield was recorded after the ears had been dried to a uniform moisture level (12%) and adjusted for dry weight. The lysine and oil contents of grains were determined using single kernel near infrared spectroscopy analysis (Rascón-Cruz et al., 2004), using the equipment INFRATEC IRIS 1255 (FOSS TECATOR AB, Sweden). These data were submitted to analyses of variance and Tukey test (α = 0.05). Phenotypic correlation between oil and lysine was also performed.

The general combining ability (GCA) and specific combining ability (SCA) information were obtained by using the method 4, model I analysis of Griffing (1956). The interactions between combining abilities and N levels was obtained according to Vencovsky & Barriga (1992), calculating the square sum of the interaction as the square sum for low N plus the square sum for high N minus the square sum for the average of these N levels.

RESULTS AND DISCUSSION

The maize lines exhibited differences for lysine and oil content in the grains (Table 1). The diallelic analysis exhibited a greater additive than non-additive effect for the evaluated traits (Table 2). This analysis also exhibited significant interaction between GCA and N levels for grain yield, indicating the selection of different lines for each N level. This interaction was not significant for lysine content, but there were significant effects of GCA for this trait at both N levels. Significant effects were not observed for GCA or SCA for oil content (Table 2). Positive correlations between lysine and oil content in the grains of lines, hybrids and controls at high and low N were also observed (Figure 1).

Breeding for high lysine content will be successful, but it could decrease the grain yield, since there was no coincidence between the lines with high lysine content and high grain yield (Table 3). The small increase in lysine with substantial decrease in grain

Table 1 - Grain yield, lysine and oil content of the six maize lines and their hybrids. The values of lysine and oil content are averages between low and high N levels.

Lines and hybrids	Grain yield (high N)	Grain yield (low N)	Oil	Lysine
	g per plant		%	
2	17.0 b	8.1 c	6.46 c	0.296 d
3	14.9 b	6.5 c	9.12 a	0.388 a
4	15.9 b	10.3 c	8.58 ab	0.354 b
5	15.4 b	7.8 c	5.91 c	0.305 cd
6	27.9 a	23.2 a	7.23 bc	0.323 cd
10	25.8 a	17.7 b	9.34 a	0.347 bc
2 × 3	57.9 a	33.6 ab	6.41 bcd	0.277 bcde
2 × 4	58.4 a	36.2 ab	5.72 d	0.261 cde
2 × 5	57.6 a	32.0 ab	6.79 bcd	0.254 cde
2 × 6	51.3 a	22.5 bc	5.71 d	0.270 bcde
2 × 10	58.3 a	34.4 ab	7.67 abc	0.278 bcde
3 × 4	13.8 b	12.5 c	8.54 a	0.368 a
3 × 5	53.9 a	39.3 a	7.71 ab	0.277 bcde
3 × 6	51.0 a	33.2 ab	8.01 ab	0.314 b
3 × 10	50.5 a	31.4 ab	7.55 abc	0.301 bc
4 × 5	52.2 a	37.6 ab	5.95 cd	0.247 e
4 × 6	44.0 a	29.5 ab	6.97 abcd	0.291 bcd
4 × 10	49.8 a	32.9 ab	7.09 abcd	0.283 bcde
5 × 6	45.2 a	27.4 abc	6.29 bcd	0.271 cde
5 × 10	49.6 a	33.0 ab	7.24 abcd	0.273 bcd
6 × 10	46.3 a	29.5 ab	7.84 ab	0.301 bc

Means followed by the same letter, within lines or hybrids, do not differ (Tukey test, $\alpha = 0.05$).

Table 2 - Analysis of variance: probabilities for effects of general combining ability (GCA) and specific combining ability (SCA) at high (HN) and low (LN) nitrogen levels and interaction with these levels (HN × LN) on traits.

	GCA			SCA		
Trait	HN	LN	HN × LN	HN	LN	HN × LN
Grain yield	***	**	***	***	***	NS
Grain lysine content	*	*	NS	NS	NS	NS
Grain oil content	NS	NS	NS	NS	NS	NS

***, **, *, NS - Significant at the 0.001, 0.01 and 0.1 levels, and not significant at 0.1 level respectively.

yield, by recurrent selection for lysine was reported in the work of Garcia & de Souza (2002).

Despite the differences among the lines for oil content, their combining abilities were not significant, but this was, at least in part, due to an elevated environmental effect on this trait, since the coefficient of variation for oil content was 17.4%, while this value for lysine content was 10.2%.

The phenotypic positive correlations between lysine and oil contents in lines, hybrids and control cultivars maize indicated that it is possible to achieve both high lysine and oil contents, which is further supported by previous reports. Working with transgenic maize exhibiting reduction in some zeins, Huang et al. (2006) reported higher lysine and oil contents when compared to the wild type. On the other hand, the plant breeding for high oil maize seeds was achieved with a small increase in lysine content (Adams & Jensen, 1987; Thomison et al., 2003). Another indication about the association between lysine and oil in maize seeds is the successful improvement of maize with both high lysine and high oil (O'Quinn et al., 2000). Furthermore, Lou et al. (2005) studying the effect of the *o2* mutation on different genetic backgrounds, reported the highest increase oil content in the recessive homozygote (*o2o2*), which also exhibited the highest lysine content.

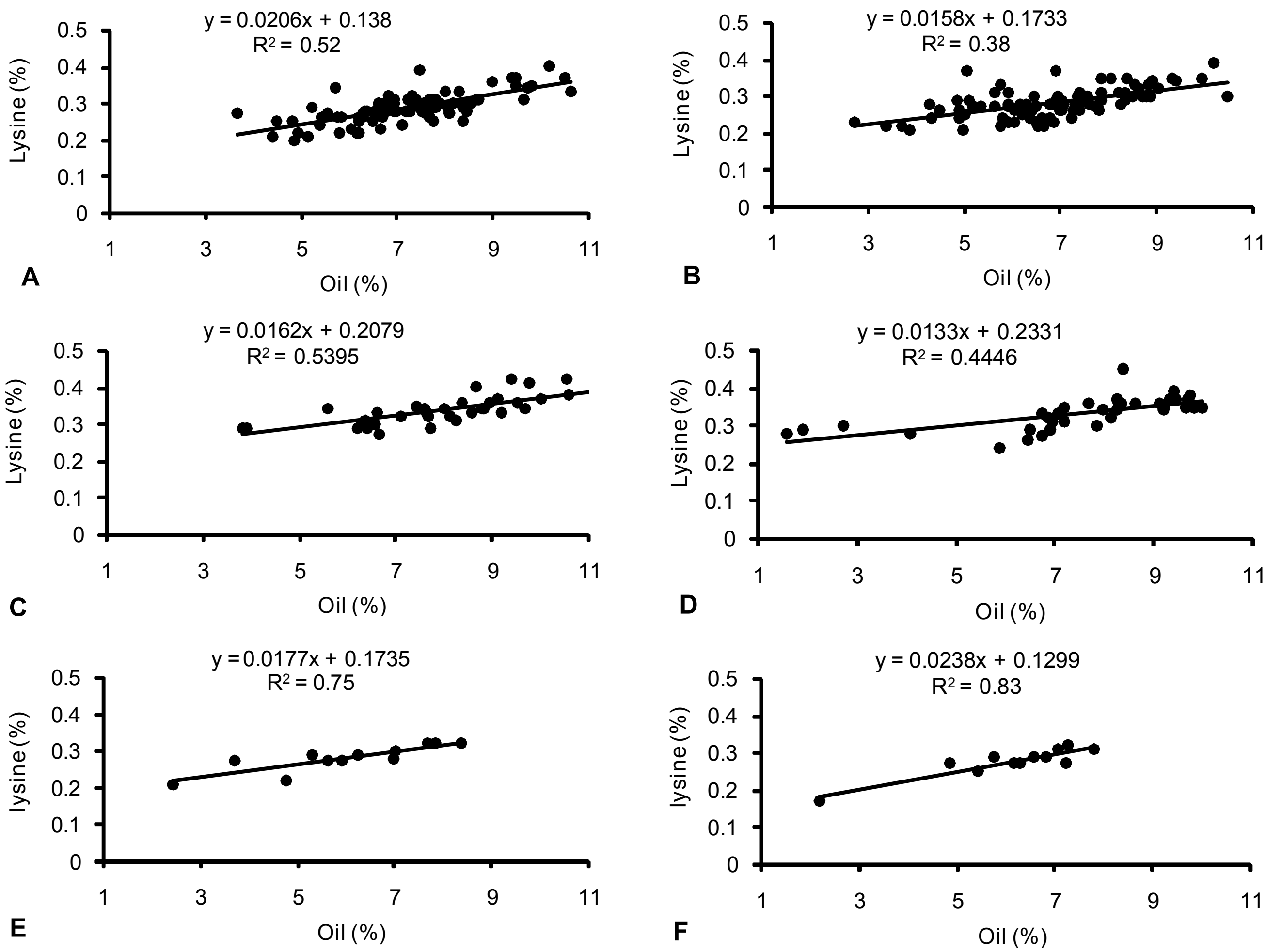

Figure 1 - Correlation between lysine and oil contents in grains of hybrids (A and B), lines (C and D) and maize controls (E and F) under high (A, C and E) and low (B, D and F) nitrogen supply.

Table 3 - Effects of general combining ability at high (HN) and low (LN) nitrogen levels for grain yield and grain lysine content.

Trait	Grain yield		Grain lysine content	
	HN	LN	HN	LN
	--- g per plant ---		--------- % ---------	
2	8.68	1.26	-0.009	-0.032
3	-5.43	-1.91	0.026	0.031
4	-7.13	-2.27	0.006	0.008
5	3.54	3.90	-0.036	-0.014
6	-1.61	-2.89	0.004	0.009
10	1.95	1.90	0.009	-0.002

What could be the link between lysine and oil in the seeds? A putative link could be the response to stress, since the expression of many genes related to physiological stress is increased in all high lysine maize mutants studied by Gibbon & Larkins (2005), and the oil synthesis in the seed can be also increased by abscisic acid (ABA), one of the stress hormones of plants (Finkelstein & Somerville, 1989; Holbrook et al., 1992). The report by Kim et al. (2004) showed that rice mutants, induced by gamma-ray irradiation, also exhibited both elevated lysine production and increased activity of stress related enzymes.

Seed oilbodies are simple organelles comprising a matrix of triacylglycerol surrounded by a phospholipid monolayer embedded and covered with unique proteins called oleosins, which are important for controlling oilbody structure and lipid accumulation (Siloto et al., 2006). The study of oleosins also supports the putative relation between ABA and oil accumulation in seeds, since some reports indicate positive effects of ABA, salt or osmotic stress on oleosins accumulation during seed development (Holbrook et al., 1991, 1992; Vanrooijen et al., 1992; Keddie et al., 1994; Williams & Tsang, 1994; Naot et al., 1995; Buchanan et al., 2005).

Despite these reports indicating positive effects of ABA or stress on oil and lysine accumulation, there are also some reports indicating the opposite relationship between ABA and lysine. The expression of the Arabidopsis bifunctional lysine-ketoglutarate reductase/ saccharopine dehydrogenase enzyme, which contains the first two linked enzymes of lysine catabolism, was strongly enhanced by ABA or sugar starvation (Stepansky and Galili, 2003; Stepansky et al., 2005). Therefore, further studies are necessary to understand the role of ABA on lysine accumulation in cereals and other plants, such as Arabidopsis.

The results from the present work indicate that it is possible to increase lysine content in the studied non-opaque maize lines, and also the possibility of achieving both high lysine and oil content in grains by plant breeding.

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