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Diallel analysis of maize inbred lines for grain yield, oil and protein content.

Amir José Klein Werle[1], Fernando Rafael Alves Ferreira[1], Ronald José Barth Pinto[1*], Claudete Aparecida Mangolin[1], Carlos Alberto Scapim[1] and Leandro Simões Azeredo Gonçalves[1]

Abstract – *This study aimed to investigate the combining ability and heterotic effects on grain yield (GY), oil content (OC) and crude protein (CP) in tropical and temperate maize lines. Hybrids and inbred lines were evaluated in a complete diallel scheme without reciprocals, in two experiments in randomized blocks with three replications. The partitioning of the sum of squares for general (GCA) and specific (SCA) combining ability indicated that both additive and non-additive effects were involved in the genetic control of the studied traits. The estimates of the quadratic components showed predominance of non-additive genetic effects in the trait control. However, for OC and CP, the non-additive effect had a clearly negative influence on the hybrid combinations. The cross L_6 x L_{10} was considered promising, because it associated RG positively with BP and OC.*

Key words: *Zea mays L.; combining ability, heterosis; Griffing method, plant breeding.*

INTRODUCTION

Maize (*Zea mays* L.) is considered one of the major cereals in the world, with a production of 55.4 million tons of grain on an area of 12.7 million hectares in Brazil (FAO 2013). The economic importance of maize is due to the broad possibilities of use, in fresh or processed form. However, the development of increasingly productive maize hybrid varieties resulted in a loss of nutritional value, especially with decreases in protein and oil, due to the negative correlation with yield (Uribelarrea et al. 2004, Zhang et al. 2008, Bueno et al. 2009).

Starch, protein and oil content are the most important chemical storage components of maize. Maize kernels contain 70-75% starch, 8-10% protein and 4-5% oil (Boyer and Hannah 2001). The maize proteins can be divided into classes according to their solubility. In maize, the concentration of the α-zein protein is highest, representing 50-60% of the total protein. Although the α-zein is poor in essential amino acids such as lysine and tryptophan, the protein fractions in grains are not constant and can vary according to the grain type and be changed by mutant genes (Prasanna et al. 2001).

Among the different mutant genes that modify the maize

grain quality, the opaque -2 gene is one of the best known. Its presence causes a reduction in α-zein synthesis, thus resulting in an increase in β-zein fractions, an easier-to-digest protein that makes these amino acids more readily available. However, the low seed density and soft texture of this mutant type are associated with some undesirable agronomic traits, including susceptibility to pests and to organisms causing cob rots, a lower drying rate, and a higher incidence of mechanical damage to grain at harvesting (Oliveira et al. 2004, Gibbon and Larkins 2005). With few exceptions, these mutants were not developed commercially by private companies, although several studies are designed to mitigate these problems (Gupta et al. 2009, Gutiérrez-Rojas et al. 2010). As a result of this research, we developed a new maize type called quality protein maize (QPM), which combines the nutritional excellence of maize opaque-2 with the grain structure of the commercial cultivars (Badu-Apraku and Lum 2010).

In animal feed, a high oil concentration in maize kernels is desirable due to the use as feed grains, since the calorific value of oil is higher than that of starch. Moreover, in human food, maize oil is well-accepted for cooking, because of the high content of unsaturated fatty acids, resulting in low

[1] Universidade Estadual de Maringá (UEM), Departamento de Agronomia, Avenida Colombo 5790, Maringá, 87.020-900, PR, Brazil
[2] Universidade Estadual de Londrina (UEL), Departamento de Agronomia, Rodovia Celso Garcia Cid, PR 485 km 380, Campus Universitário, Londrina, 86.057-970, PR, Brazil. *E-mail: rjbpinto@uem.br

cholesterol levels when metabolized (Mangolin et al. 2004). However, as the increase in the seed oil content is usually accompanied by a decrease in seed mass, the reduction in grain yield is a limiting factor to a more the widespread use of hybrid maize with high oil content (Misevic et al. 1989).

The genetic variability for grain quality traits is considered low in temperate maize germplasm (Osorno and Carena 2008). In this context, cooperation efforts between public and private bodies involved in the project Maize Germplasm Improvement (GEM) include the introduction of tropical germplasm to broaden the genetic base.

The introduction of tropical into temperate plant germplasm can not only broaden the genetic base of breeding programs, but also increase the adaptability of new hybrids (Fan et al. 2003). However, little effort has been made to assess the grain quality in tropical lines and their combining ability with temperate lines. Accordingly, the purpose of this study was to investigate the effect of combining ability and heterosis of tropical and temperate maize lines on the grain, oil and protein content.

MATERIAL AND METHODS

The plant material consisted of 12 parental lines and their non-reciprocal hybrids. The parents belong to a group of elite lines of an association between the State University of Maringá and Syngenta Seeds Ltd. The origin and other characteristics of the lines used are shown in Table 1.

The experiments of parent and hybrid evaluation were carried out private properties in Cascavel and Mauá da Serra, Paraná, according to the normally used cultural practices for maize cultivation under a high agro-technical level. The experiments were evaluated in a complete randomized block design with three replications, where each plot consisted of two 6-m long rows. Rows were spaced 0.6 m and plants 0.20 apart, with a total area of 7.2 m^2.

The following traits were evaluated: i) grain yield (GY), from harvest with mechanical harvester equipped with a weighing system and moisture gauge, after moisture correction to 13% and conversion from g plot^{-1} to kg ha^{-1}; ii) percentage of crude protein (CP), using the Kjeldahl method (Compêndio Brasileiro de Alimentação Animal 2009), and iii) oil content (OC), using the Soxhlet method (Compêndio Brasileiro de Alimentação Animal 2009). The data of CP and OC were obtained from the analysis performed at the Laboratory of Animal Nutrition, Federal University of Paraná.

The GY, CP and OC data were subjected to analysis of variance (F test). Based on the analysis of variance, the sum of squares of the treatments was partitioned into general and specific combining ability (GCA and SCA, respectively), using method 2, in a fixed model, as defined by Griffing (1956). The average heterosis percentage among the variables was also analyzed, using software Genes (Cruz 2013) for all analyses.

RESULTS AND DISCUSSION

The effect of genotypes (lines + hybrids) was statistically significant for all traits, indicating a wide variability among the treatments (Table 2). The environments differed with regard to responses for grain yield and crude protein, but not for oil content. According to Jellum and Marion (1966), the oil content is much more related to the genotypes than the environment and is considered a trait with high heritability. The effects of the genotype-environment interaction were only significant for grain yield, indicating a differentiated genotype performance in response to environmental changes.

The mean CP of the parental lines was 10.28% for tropical and 9.53% for temperate lines. For the hybrids, the means of the groups of tropical x tropical, temperate x temperate and tropical x temperate crosses were 8.91, 8.88 and 8.73%, respectively. For oil content, the mean percentages of the lines were 4.26% for tropical and 4.25% for temperate.

Table 1. Characteristics of 12 maize inbred lines, used in the maize breeding program of the State University of Maringá and Syngenta Seeds Ltda

Lines	Cycle	Adaptation	Group	Origin
L$_1$	Super early	Temperate	Dent	Southern dent
L$_2$	Super early	Temperate	Flint	B73
L$_3$	Super early	Temperate	Dent	Southern dent
L$_4$	Very early	Temperate	Flint	Lancaster
L$_5$	Very early	Tropical	Flint	Pop Suwan
L$_6$	Early	Temperate	Dent	Stiff Stalk Synthetic
L$_7$	Early	Temeprate	Dent	Stiff Stalk Synthetic
L$_8$	Early	Tropical	Dent	Tuxpeño
L$_9$	Early	Tropical	Dent	Tuxpeño
L$_{10}$	Early	Temperate	Flint	Non-Stiff Stalk Synthetic
L$_{11}$	Early	Tropical	Dent	Tuxpeño
L$_{12}$	Early	Tropical	Flint	Pop Suwan

The means of the cross groups tropical x tropical, temperate x temperate and tropical x temperate were 3.93, 4.29 and 4.12%, respectively. For grain yield, the means of the combinations tropical x tropical, temperate x temperate and tropical x temperate were 8,651.85, 10,310.32 and 9,761.59 kg ha^{-1}, respectively.

The partition of the sum of squares of genotypes in the sum of squares for general (GCA) and specific (SCA) combining ability showed that both GCA and SCA were significant, indicating that additive and non-additive effects were involved in the genetic control of these traits (Table 2). There were no significant interactions for GCA x Environments and SCA x Environments for protein and oil, indicating that the genetic effects remained constant in the tested environments. On the other hand, the interaction was significant for grain yield, requiring individual analysis of GCA and SCA for each environment.

The estimates of the quadratic components indicated

Table 2. Estimates of mean squares of corn genotypes and environments, general and specific combining ability (GCA and SCA) and of the error, estimates of quadratic components of the effects of combining ability and coefficient of variation (CV) for crude protein (PB), oil content (OC) and grain yield (GY)

Sources of Variation	df	Mean Square		
		CP	OC	GY
Blocks/locations	4	1.40*	1.28*	530862.49*
Genotypes (G)	77	1.53**	1.07**	25239565.22**
GCA	11	1.30*	1.06*	18207529.56**
SCA	66	1.57**	1.07**	26411580.30**
Locations (L)	1	6.34**	0.03	448061.38*
G x L	77	0.39	0.38	1382224.21*
GCA x L	11	0.34	0.27	1354158.81**
SCA x L	66	0.40	0.40	1386906.24**
Error	308	0.30	0.49	67341.22
Mean		8.96	4.16	8871.57
CV(%)		6.17	16.84	9.28
Quadratic components				
Locations – Cascavel				
GCA		0.014	0.0165	350117.89
SCA		0.440	0.4490	7129149.93
Locations – Mauá da Serra				
GCA		0.034	0.0144	348510.44
SCA		0.407	0.0554	6770045.87

* and ** significant at 1 and 5% probability, respectively.

Table 3. Estimates of the effects of general combining ability for crude protein (CP) oil content (OC) and grain yield (GY), evaluated in 12 maize inbred lines in a complete diallel scheme without reciprocals

Parents	Evaluated traits			
	CP	OC	GY	
			Cascavel	Mauá da Serra
L$_1$	0.043	0.112	325.49	196.077
L$_2$	-0.139	0.042	301.71	194.865
L$_3$	-0.32	-0.063	495.02	618.361
L$_4$	-0.054	0.008	-0.154	163.439
L$_5$	-0.065	-0.262	300.67	925.608
L$_6$	0.199	0.239	736.58	441.114
L$_7$	-0.039	0.026	516.69	278.553
L$_8$	-0.015	-0.195	-415.817	-372.832
L$_9$	-0.016	-0.097	-25.415	-340.699
L$_{10}$	0.039	0.004	-445.874	-883.663
L$_{11}$	0.094	0.097	-376.345	-142.395
L$_{12}$	0.273	0.088	-1412.573	-1078.428

a predominance of the non-additive genetic effects for all traits (Table 2). However, it is clear that for crude protein and oil content the non-additive effect influenced the hybrid combinations negatively. This result was corroborated by other studies that indicate a negative correlation of yield increase with the CP and OC (Prasanna et al. 2001, Oliveira et al. 2006).

The best lines for CP, L_{12} (0.273) and L_6 (0.199), had a good overall performance in the test environments (Table 3). Line L_6 had the highest positive GCA for oil content, with 0.239, followed by the lines L_1, L_{11} and L_{12} with 0.112, 0.097 and 0.088, respectively. Note that the lines L_6 and L_{12} obtained the best GCA values for CP and OC. However, only line L_6 obtained positive values of GCA for grain yield, ranking first in Cascavel and third in Mauá da Serra. In Mauá da Serra, lines L_5 and L_3 had the highest GCA values, with 925.61 and 618.36, respectively.

With regard to the specific combining ability (S_{ij}) of the hybrids derived from the crosses tropical x tropical (L_5 x

L_{12}), temperate x temperate (L_1 x L_3, L_2 x L_4, L_2 x L_{10}, L_3 x L_7, L_4 x L_7, L_6 x L_{10} and L_7 x L_{10}) and tropical x temperate (L_1 x L_{11}, L_2 x L_5, L_3 x L_9, L_6 x L_9, L_7 x L_8 and L_7 x L_9) these
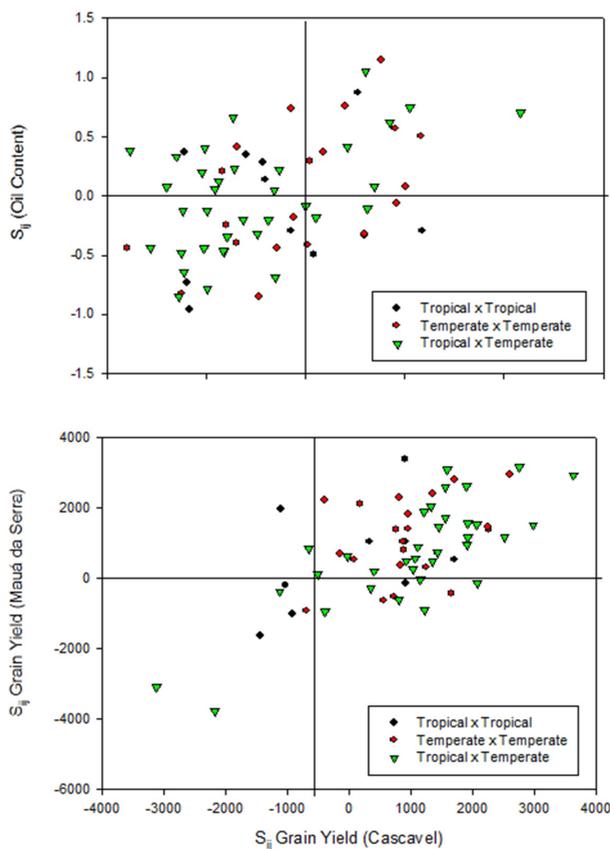

Figure 1. Estimates of the effects of specific combining ability for crude protein, oil content and grain yield evaluated in 12 maize inbred lines in a complete diallel scheme without reciprocals.
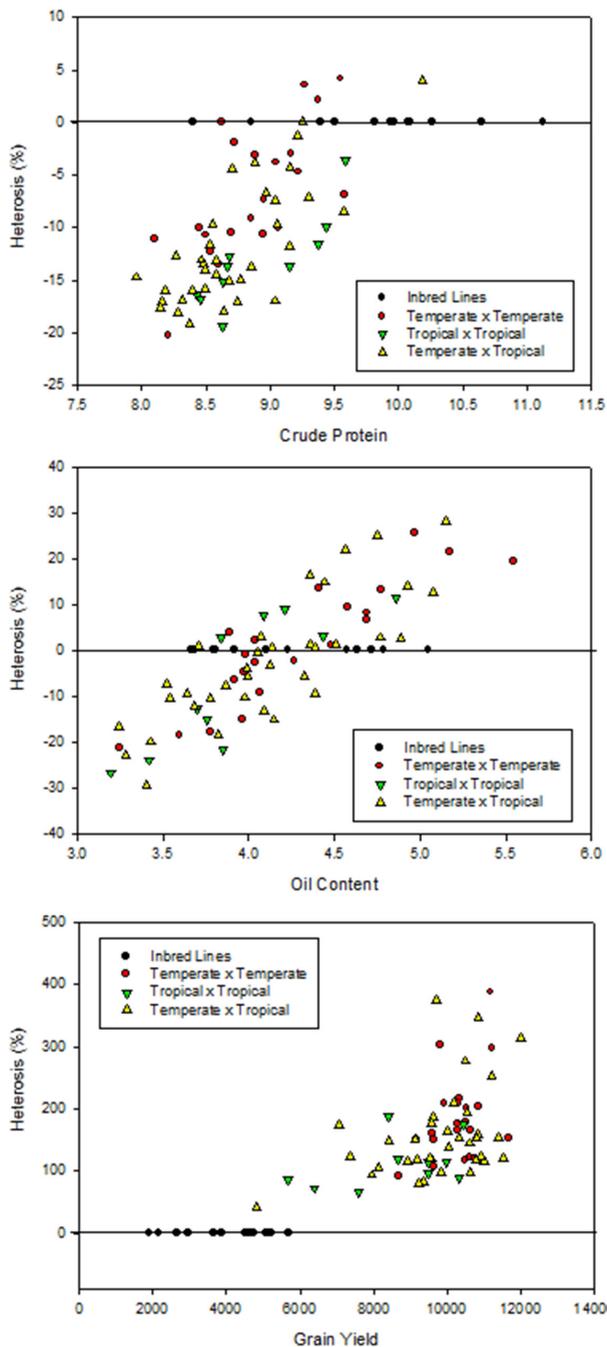

Figure 2. Heterosis percentage in relation to the percentage of crude protein, oil content and grain yield of 64 corn hybrids evaluated in a complete diallel scheme without reciprocals.

S_{ij} values were positive for crude protein and oil content (Figure 1). However, according to the estimates of the mean effect of GCA, only the lines L_6, L_{10}, L_{11} and L_{12} were higher for both traits, since the obtained values for CP and OC were positive and high. Thus, only the combinations L_1 x L_{11}, L_6 x L_9, L_2 x L_{10} and L_6 x L_{10} are promising, since they have at least one superior parent for the average effect of GCA for the assessed traits.

The hybrids L_1 x L_{10} (temperate x temperate) and L_1 x L_{12}, L_4 x L_8 and L_2 x L_8 (tropical x temperate), has excellent grain yields, with highest S_{ij} in both environments (Figure 1.) In Cascavel, the S_{ij} values for GY of the hybrids L_1 x L_{11}, L_6 x L_9, L_2 x L_{10} and L_6 x L_{10} reached -1118.52, 406.16, 970.84 and 2267.46, respectively. In Mauá da Serra, the values were -376.97, 203.61, 1804.44 and 1433.18, respectively.

Figure 2 presents the results of heterosis percentage in relation to CP, OC and GY for 64 maize hybrids evaluated in a complete diallel scheme without reciprocals. The study of the possible manifestation of heterosis for CP showed a positive heterosis percentage for only four hybrids: L_1 x L_7, L_6 x L_{10} and L_7 x L_{10} (temperate x temperate group) L_1 x L_{11} (temperate x tropical groups). Among these hybrids, only L_1 x L_7 obtained a positive heterosis value (0.11). For OC, 31 hybrids had positive heterosis, ranging from 0.39 to 28.16%. The OC was highest in the hybrids L_6 x L_{10}, L_2 x L_6, L_7 x L_9 and L_1 x L_{11}, with 5.54, 5.17, 5.14 and 5.07%, respectively, which had positive heterosis values.

For grain yield, heterosis ranged from 40.50 to 386.46%, mainly for the hybrids L_2 x L_8, L_3 x L_7, L_5 x L_7, L_6 x L_8, and L_1 x L_{10}, with a GY of 12008.55, 11698.56, 11525.36, 11402.13, and 11240.26 kg ha^{-1}, respectively. The combinations L_1 x L_{11} and L_6 x L_{10}, with positive heterosis for CP and OC, reached GY of 8125.24 and 10645.97 kg ha^{-1}, respectively. Therefore, L_6 x L_{10} was considered a promising hybrid because it associated grain yield positively with crude protein and oil content.

Análise dialélica de linhagens de milho para rendimento de grãos, teores de óleo e proteína

Resumo - *O presente trabalho teve como objetivos investigar a capacidade combinatória e o efeito heterótico de linhagens tropicais e temperadas sobre o rendimento de grãos (RG), teor de óleo (TO) e proteína (PB). Os híbridos e as linhagens foram avaliados a partir de um dialelo completo, sem recíprocos, em dois experimentos delineados em blocos casualizados com três repetições. O desdobramento da soma de quadrado de genótipos em soma de quadrados para capacidade geral (CGC) e específica (CEC) de combinação e a subsequente constatação de significância de CGC e de CEC evidenciaram que tanto efeitos aditivos quanto não-aditivos estiveram envolvidos no controle genético das características avaliadas. Pelas estimativas dos componentes quadráticos, houve superioridade dos efeitos genéticos não-aditivos no controle das características. Contudo, percebe-se que para PB e TO o efeito não-aditivo influenciou negativamente as combinações híbridas. O cruzamento L_6 x L_{10} foi considerado promissor, pois associou positivamente RG com PB e TO.*

Palavras-chave: *Zea mays L., capacidade combinatória, heterose, método Griffing, melhoramento de plantas.*

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