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VARIABILITY AND GENETIC EFFECTS FOR TRYPTOPHAN AND METHIONINE IN COMMERCIAL MAIZE GERMPLASM

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ABSTRACT - Maize (*Zea Mays* L.) is a major food and feed crop; however, maize proteins are nutritionally imbalanced due to low levels of certain essential amino acids, including tryptophan and methionine. The objectives of this study were (1) to determine the variability in methionine and tryptophan levels present in commercial maize inbred lines, (2) to characterize the genetic groups of commercial maize breeding germplasm for their methionine and tryptophan content, (3) to estimate general combining ability, specific combining ability, and reciprocal genetic effects for tryptophan and methionine content in this germplasm. Seventy-six inbred lines representing nine different genetic groups (B14/B73 – 5 lines, B14/B73/Iodent – 16 lines, B73 – 16 lines, OH43/W153R – 6 lines, OH43/Iodent – 4 lines, Mo17 – 10 lines, European Flint – 8 lines, Plata – 6 lines, Unrelated – 5 lines) were evaluated. There was significant variability among the genetic groups and among the inbreds in some of the groups. A six-parent diallel mating design was completed with three parents selected for low metionine methionine and three parents selected for high methionine levels. A second diallel mating design was carried out with three parents selected for high tryptophan levels and three parents selected for low tryptophan levels. Analysis of the diallel crosses revealed significant general and specific combining ability effects, as well as reciprocal effects. These effects were of greater magnitude in the methionine diallel than in the tryptophan diallel. These studies suggest that methionine should respond better to selection than tryptophan. Maize breeders may be able to exploit these genetic effects to develop hybrids with elevated levels of tryptophan or methionine.

KEY WORDS: Amino acids; Diallel; Nutrition; Inbred lines; Hybrids.

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INTRODUCTION

Maize is a major food and feed crop in many parts of the world. For this reason, improving the nutritional quality of maize is an important objective. While total protein content in maize varies, in monogastric diets, maize is nutritionally limited by deficiencies in lysine, methionine and tryptophan. Thus in monogastric diets the levels of lysine, tryptophan and methionine have greater impact on the nutritional value than the level of total protein. Supplementation of these diets to make up for these deficiencies increases the cost of feed, particularly in the cases of tryptophan and methionine for which inexpensive supplements are lacking. Since the discovery that the *opaque-2* mutation confers increased levels of nutritionally limiting amino acids (MERTZ *et al.*, 1964), maize breeding research aimed at improving amino acid balance has focused on the use of this mutation, ultimately resulting in the development of agronomically adapted germplasm with improved amino acid balance (VASAL, 2001). This approach has not been used extensively in commercial breeding programs, possibly because *opaque-2* is recessive and its beneficial effects would be masked by wild-type pollen, and possibly because of the large amount of effort required to overcome the detrimental pleiotropic effects of the *opaque-2* mutation. An alternative approach to using *opaque-2* for improving amino acid balance is to use traditional recurrent selection strategies to improve breeding germplasm. In order to do this effectively, it is important to understand the genetic mechanisms controlling amino acid content.

Studies have been conducted with the objective of characterizing grain amino acid levels in maize varietal trials. DOTY *et al.* (1946) concluded the level of the amino acids present in maize kernel protein was related to the genetic constitution of hybrids

examined. AGUIRRE *et al.* (1953) studied the lysine and methionine content of maize and reported genetic variation in the methionine content, confirming the findings of DOTY *et al.* (1946). TELLO *et al.* (1965) investigated racial and varietal trends affecting the nutritive value of maize. By sampling a collection of varieties from Mexico, Central America, and the Caribbean, TELLO *et al.* (1965) concluded lysine content is a racial characteristic. ZUBER *et al.* (1975) reported very little differences in mean lysine content among dent, floury, popcorn, and flint types of maize. None of the accessions representing these four groups had a greater lysine content than an *opaque-2* variety. GOERTZ *et al.* (1978) studied 158 entries from an Andean maize collection and found while there was an association between protein concentration and grain type (floury vs. flinty), there was no corresponding improvement of percentage of lysine in the dry matter of grain.

Relatively little information is available about the genetic control of amino acid composition in commercial germplasm. In addition to classification by race and different types of maize, commercial breeders utilize a more specific classification of the germplasm for breeding purposes. Maize germplasm in private breeding companies is commonly classified by the population, inbred line, or cross from which they are derived (MBS GENETICS, 2002). Thus modern inbreds are classified as B73, Mo17, OH43/Iodent, B14/B73/Iodent, OH43/W153R, B14/B73 or European Flint-type. While these groups of inbreds and their hybrid combinations have been rigorously evaluated for their agronomic performance, little is known about their amino acid content.

The diallel mating design is particularly well suited to characterizing genetic effects of inbred lines and their hybrid combinations. With appropriate analysis of the data from a diallel mating design, information can be obtained about the general combining ability (GCA), specific combining ability (SCA), reciprocal genetic effects and heterosis (GRIFFING, 1956). The concepts of GCA and SCA are useful to plant breeders in characterizing inbred lines in crosses (HALLAUER and MIRANDA, 1981). GCA is defined as the average performance of a line in hybrid combinations and pertains solely to the inbred lines included in the diallel. SCA is defined as the expected performance of a specific hybrid combination when compared to the average performance of the parent inbred lines (SPRAGUE and TATUM, 1942). A complete diallel, where all possible crosses and parents are included, was used in this study to elucidate the genetic effects controlling methionine and tryptophan levels in maize grain.

The objectives of this study were (1) to determine the variability in methionine and tryptophan levels present in commercial maize inbred lines, (2) to characterize the genetic groups used by private maize breeding programs for their methionine and tryptophan content, (3) to estimate the possible genetic effects controlling tryptophan and methionine levels including general combining ability, specific combining ability, and reciprocal effects. Completion of these objectives will facilitate evaluation of the potential for breeding for tryptophan and methionine levels using currently available commercial germplasm.

MATERIALS AND METHODS

Plant material

Seventy-six different maize inbreds representing the following genetic groups were screened for their content of methionine and tryptophan: B14/B73, B14/B73/Iodent, B73, OH43/W153R, OH43/Iodent, Mo17, European Flint, Plata, and Unrelated (Table 1). Seeds for each entry were collected from several sources and from different years. These seeds were selected and were planted in the diallel studies.

A subset of these inbred lines was selected and used to construct two complete diallels, hereafter referred to as the tryptophan diallel and the methionine diallel, each with six parents. Each parent was selected from a different genetic group defined in Table 1 such that the three parents with the highest amino acid levels and the three parents with lowest amino acid levels were used. The choice of whether a high or low inbred line was chosen from a specific group was made arbitrarily. Parents for the methionine diallel were chosen similarly, except they were selected on the basis of their methionine content. Two inbred lines, PSI101 and PSI301, were common to both diallels as they had high levels of both tryptophan and methionine. Table 2 provides a description of the parental inbred lines included in the two six-parent diallels. The inbred lines are proprietary and have been coded for confidentiality purposes. Each parental line was crossed to each other line in the diallels, and reciprocal crosses were made in each case. Each parental line was also self-pollinated. Both tryptophan and methionine levels of the resulting cross- and self-pollinated kernels were determined, giving two measurements for each ear produced in each diallel (e.g., tryptophan diallel analyzed for tryptophan, tryptophan diallel analyzed for methionine, methionine diallel analyzed for methionine, and methionine diallel analyzed for tryptophan).

Field procedures

To produce grain for the diallel analysis, all inbred parent lines were planted in 12.5 m rows containing 30-kernels in the Pau Seeds breeding nursery near Boone, Iowa, in the summer of 2002. Plants were hand-pollinated. One plant in each row was self-pollinated to produce grain used for analysis of the inbred,

TABLE 1 - *Description of the different genetic groups in terms of agronomic traits.*

Genetic Group	Characteristics
B14/B73	Seed parent – Larger ear type – Average kernel row number - Dented kernel type –Average root strength – Good stalk efficiency- Lower tolerance to leaf diseases – Fast dry down – Average plant integrity late season
B14/B73/Iodent	Similar to previous group – Iodent enhances earlier flowering and faster dry down – Late season plant intactness is also improved.
B73	Seed parent – Large girthy ear type – Dented kernel – Average test weight – Taller plant stature - Very good stalk and root strength – Good stay green but susceptibility to the European Corn Borer – Later flowering, slower dry down.
OH43/W153R	Pollinator – Determined ear type – Softer kernel texture – High stress tolerance (heat, drought) – Shorter plant stature – Average root quality – Good leaf diseases tolerance – Earlier flowering and fast dry down.
OH43/Iodent	Pollinator – Semi-determined ear type – Improved test weight over OH43/W153R – Medium plant size – Good stalk strength – Early flowering and very efficient dry down – Very good plant integrity in late season.
Mo17	Pollinator – Longer, slender ear type with good flex – Harder kernel texture (more flinty, more round in grade other than B73's) and higher test weight – Taller plant height – Good stalk strength and average root efficiency – Late flowering and slower dry down – Good stay green and leaf diseases tolerance – Better tolerance to the European Corn Borer.
European Flint	Pollinator – Small ear, few kernel rows – Vitreous round kernel type (flint), higher test weight – Very early flowering and very early grain set in the fall but slow dry down after black layer – Good cold tolerance at emergence.
Plata	Pollinator used in the U.S. - Late Argentine-based germplasm – Flinty kernel texture – Very high test weight – Late flowering and very slow dry down in U.S. temperate conditions – Very good stalk quality and average root strength – Good tolerance to diseases and ear damaging insects.
Unrelated	Mix of varied origins above.

TABLE 2 - *Description of the parent inbred lines included in the diallels with the genetic group in which they belong and the trait for which they were selected.*

Diallel	Parent inbred line	Genetic group	Trait
Tryptophan	PSI101	B14/B73	High tryptophan
	PSI301	B73	High tryptophan
	PSI701	Mo17	High tryptophan
	PSI801	OH43/W153R	Low tryptophan
	PSI401	B14/B73/Iodent	Low tryptophan
	PSI001	Unrelated	Low tryptophan
Methionine	PSI101	B14/B73	High methionine
	PSI702	Mo17	High methionine
	PSI301	B73	High methionine
	PSI402	B14/B73/Iodent	Low methionine
	PSI601	OH43/Iodent	Low methionine
	PSI002	Unrelated	Low methionine

and other plants in the same row were used as male pollen donors or as females to produce one ear containing the F_1 grain that was analyzed. All ears were harvested individually.

Preparation of samples for analysis of methionine and tryptophan levels

Each ear of maize was air dried, shelled, and packaged individually. From each ear, five randomly selected whole kernels were ground using a Wiley Mill with a 40-mesh screen. Each ground sample was stored in an Eppendorf tube. With the cap of the tube open, the samples were dried for four hours at 65°C, after which the tubes were stored in ambient conditions. Each sample was considered one entry in the experiment. The other entries were two checks, B101 and an *opaque2* (*o2*) inbred, each in duplicate, and six standards, each in triplicate. The B101 inbred was chosen as a check because it has high levels of methionine (HALLAUER and WRIGHT, 1995). The o2 inbred was used as a check for high tryptophan. Commercially-prepared, pure amino acids were used as standards in concentrations of 5, 20, 35, 60, 75, 100 mM for methionine and 0, 100, 240, 300, 480, 600 µM for tryptophan.

Amino acids were measured using a randomized complete block design that included the experimental entries in one 96-well microtiter plate. The analysis was replicated on three plates with a different randomization of the entries on each plate (i.e. three blocks).

Protein hydrolysis

Ten milligrams of each ground sample and check were weighed into the well of a V-bottom, 96-well microtiter plate. Each sample in the plate was subjected to enzymatic hydrolysis using Pepsin. To each well, 200 µL of 0.1 mg/mL Pepsin solution in a KCl-HCl pH2 buffer was added. The plate was sealed, covered with a lid and placed in a 37°C shaking incubator for approximately 15 hours. After the incubation period, the plate was centrifuged at 3000 rpm for 20 minutes, after which the supernatant was removed for further analysis.

Amino acid assays

The method for the determination of tryptophan is a modified version of the one originally described by SASTRY and TUMMURU (1985). Twenty microliters of hydrolysate or standard was transferred directly into the wells of a flat-bottom, 96-well assay plate. The plates were sealed between operations to prevent evaporation. For each plate, the assay solution consisted of 9.5 mL of concentrated HCl, 250 µL 2.5% Thioglycolic Acid (TGA), and 250 µL 10% sucrose. After the assay solution changed from clear to a yellow color, eighty microliters of this solution was added to the hydrolysate in the assay plate. The plate was then agitated for three minutes, after which the optical density at 510 nm was determined immediately with a microplate reader.

The method used for the determination of methionine in maize kernels is similar to the microbiological method described by WRIGHT and ORMAN (1995). An auxotrophic strain of *Escherichia coli*, P4x (JACOB and WOLLMAN, 1961), was used in this assay. The inoculum was prepared in M9 media supplemented with 10 µL of 1 mg/mL methionine solution per 5 µL M9 media solution (MANIATIS *et al.*, 1982) and grown to late log phase. Ten mL of hydrolysate or a standard was transferred directly into a flat-bottom, 96-well assay plate. The plates were sealed between operations to prevent evaporation. To each well, 100 µL of M9 media and 2 µL of the inoculum were added. The plate was sealed, covered with a lid, and placed in a 37°C shaking incubator for seven hours. After the incubation period, the plates were shaken on a plate shaker for three minutes and the 595 nm light scattered by the sample was determined using a microplate reader.

These methods for analysis of tryptophan and methionine are not particularly accurate with respect to standard methods, but are highly repeatable, rapid and inexpensive, making them well suited to ranking and comparing large numbers of samples.

TABLE 3 - *Average methionine and tryptophan content (g per 100 g dry weight) of the genetic groups and the checks.*

		Tryptophan		Methionine	
Genetic Group	**No. of entries**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
B101 (Met check)	5	.	.	0.1021 a	.
o2 (Trp check)	5	0.0602 a	.	.	.
B14/B73	5	0.0418 b	0.00526 **	0.0856 b	0.0135 **
Mo17	10	0.0412 b	0.00396 *	0.0721 b	0.0148 **
B14/B73/Iodent	16	0.0400 b	0.00667 **	0.0721 b	0.0137 **
European Flint	8	0.0387 b, c	0.00351	0.0725 b	0.0119 **
B73	16	0.0381 b, c	0.00628 *	0.0801 b	0.0160 **
Unrelated	5	0.0375 b, c	0.00464 **	0.0745 b	0.0109 **
OH43/Iodent	4	0.0365 b, c, d	0.00393 *	0.0557 c	0.00737 **
OH43/W153R	6	0.0328 c, d	0.00444 **	0.0764 b	0.00900 **
Plata	6	0.0304 d	0.00461 **	0.0689 b, c	0.00497 *

[1] Asterisks indicate statistically significant variation within the inbreds of a genetic group as determined by single factor ANOVA at the level of α=0.05 (*) or α=0.01 (**).

The letters a, b, c, and d represent different Dunett's groupings. Entries followed by the same letter are not statistically different (α=0.05).

TABLE 4 - *Mean tryptophan content (g per 100g dry wt) for two complete diallels. Each diallel includes six maize inbred lines from different genetic groups and their corresponding reciprocal F_1 crosses.*

Tryptophan Diallel

♀	♂						
	PSI101	PSI301	PSI701	PSI801	PSI401	PSI001	Mean
PSI101	**0.2412**	0.2162	0.2222	0.2458	0.2513	0.1985	0.2268
PSI301	0.2165	**0.2582**	0.2210*	0.2464	0.2304	0.2330	0.2316
PSI701	0.2375	0.2272	**0.2555**	0.2259	0.2238	0.2363	0.2301
PSI801	0.2184	0.2257	0.2050	**0.2338***	0.2152	0.2054	0.2139
PSI401	0.2147	0.2210*	0.2314	0.1887	**0.2080**	0.2307	0.2164
PSI001	0.2231	0.2204	0.2046	0.2007	0.1926	**0.2063**	0.2083
Mean	0.2220	0.2224	0.2158	0.2215	0.2227	0.2208	
						Grand mean	0.2210
						Parent mean	0.2338

* Missing data point represented by the overall mean of either the parents or the crosses.

Methionine Diallel

♀	♂						
	PSI101	PSI702	PSI301	PSI601	PSI002	PSI402	Mean
PSI101	**0.2412**	0.2245	0.2162	0.2164	0.2570	0.2490	0.2326
PSI702	0.2382	**0.2055**	0.2034	0.1926	0.2278	0.2088	0.2142
PSI301	0.2165	0.1933	**0.2582**	0.2106*	0.1973	0.2106*	0.2024
PSI601	0.1695	0.1880	0.1991	**0.2239**	0.1971	0.2106*	0.1884
PSI002	0.2042	0.1940	0.1869	0.2132	**0.2156**	0.2473	0.2091
PSI402	0.2351	0.1964	0.2024	0.1975	0.2149	**0.1945**	0.2093
Mean	0.2127	0.1992	0.2016	0.2049	0.2188	0.2350	
						Grand mean	0.2106
						Parent mean	0.2232

* Missing data point represented by the overall mean of either the parents or the crosses.

Statistical analyses

In the survey of commercial inbred lines, to characterize the variation among inbreds in each genetic group, the triplicate tryptophan or methionine values for each inbred in a given group were used to conduct single-factor ANOVAs for each amino acid. Variation between genetic groups was characterized with ANOVAs for each amino acid using the mean tryptophan or methionine level of each inbred, with the inbreds grouped by the genetic groups described in Table 1. This variation was characterized further using Dunnetts's test to compare the mean tryptophan or methionine values of each inbred group at the level of significance $\alpha = 0.05$.

In the diallel analysis, each of the 36 ears produced was considered a treatment, and the mean value of the three measurements for each amino acid was considered an independent variable in the analysis of variance. To identify factors with significant contributions to the variation in tryptophan and methionine content, an analysis of variance of the diallel crosses was conducted for the following sources of variance in the model: Parents, Parents vs. Crosses, Crosses, GCA, SCA, Reciprocal effects, and the Experimental error. GCA, SCA, and the reciprocal effects were calculated for each six-parent diallel using Griffing's experimental method 3, model I, which includes both reciprocal and non-reciprocal F1 crosses but not the self-pollinated parental lines (GRIFFING, 1956). For this model, the restrictions imposed on the combining ability effects are (1) the sum of the estimates of the GCA effects for all parents in the diallel are constrained to zero and (2) the sum of the estimates of the SCA effects for all crosses are constrained to zero. There is no restriction on the estimates of the reciprocal effects.

TABLE 5 - *Mean methionine content (g per 100g dry wt) for two complete diallels. Each diallel includes six maize inbred lines of distinctly different genetic groups and their corresponding reciprocal F_1 crosses.*

Tryptophan Diallel

♀	♂						
	PSI101	PSI301	PSI701	PSI801	PSI401	PSI001	Mean
PSI101	**0.1000**	0.0897	0.0842	0.0881	0.1028	0.0744	0.0878
PSI301	0.0917	**0.1052**	0.0938*	0.1179	0.0972	0.0980	0.1012
PSI701	0.0819	0.0888	**0.0828**	0.0945	0.0792	0.0978	0.0884
PSI801	0.0949	0.0978	0.0943	**0.0932***	0.0995	0.0955	0.0964
PSI401	0.0871	0.0938*	0.0761	0.0824	**0.0846**	0.0884	0.0835
PSI001	0.1003	0.1142	0.1039	0.0950	0.1102	**0.1117**	0.1047
Mean	0.0912	0.0976	0.0896	0.0956	0.0978	0.0908	
						Grand mean	0.0938
						Parent mean	0.0969

* Missing data point represented by the overall mean of either the parents or the crosses.

Methionine Diallel

♀	♂						
	PSI101	PSI702	PSI301	PSI601	PSI002	PSI402	Mean
PSI101	**0.1000**	0.0943	0.0897	0.0898	0.1146	0.0893	0.0955
PSI702	0.1137	**0.0901**	0.0852	0.0904	0.0876	0.0986	0.0951
PSI301	0.0917	0.1019	**0.1052**	0.0822*	0.0716	0.0822*	0.0884
PSI601	0.0640	0.0605	0.0668	**0.0693**	0.0632	0.0822*	0.0636
PSI002	0.0844	0.0765	0.0776	0.0795	**0.0786**	0.0811	0.0798
PSI402	0.0727	0.0722	0.0758	0.0633	0.0633	**0.0666**	0.0695
Mean	0.0853	0.0811	0.0790	0.0808	0.0801	0.0897	
						Grand mean	0.0822
						Parent mean	0.0850

* Missing data point represented by the overall mean of either the parents or the crosses.

RESULTS

Evaluation of inbred lines within genetic groups

In order to survey the variability for tryptophan and methionine levels in commercial inbred lines, seventy-six inbred lines representing the nine genetic groups (Table 1) were analyzed (Table 3). Each group contained at least four lines. The purpose of the study is to rank and compare tryptophan and methionine content of the commercial inbred lines to facilitate selection of lines to include in the diallels. Methionine and tryptophan concentrations are reported on a dry weight basis in grams per 100 grams of sample. The inbred lines B101 and *o2* were included in the analysis to serve as checks for high methionine and tryptophan, respectively. Statistically significant variation was identified among the inbreds within some of the groups and between the means of some of the genetic groups (Table 3). This variation should be interpreted with caution because the grain that was analyzed was produced in different environments. The genetic group B14/B73 had the highest mean values for tryptophan and methionine. However, these values were significantly different than the high methionine and

high tryptophan checks. The genetic group Plata had the lowest mean value for tryptophan and the second lowest mean value for methionine. The B101 and *o2* checks had significantly higher levels of methionine and tryptophan, respectively, than any of the genetic groups.

Analysis of complete diallels

To characterize the genetic effects associated with the tryptophan and methionine levels in maize, we used the data from the survey of commercial inbred lines to select parents for two six-parent diallels, one designed to examine the genetics of methionine and the other designed to analyzed the genetics of tryptophan levels (Table 2).

We determined the methionine and tryptophan content of the grain produced by the self-pollinated parents and the F_1 crosses generated in the two six-parent diallels. The mean tryptophan and methionine content for all of the entries in the diallels are presented in Tables 4 and 5, respectively. In both diallels, the mean of all parents was higher than the mean all crosses. In the tryptophan diallel analyzed for tryptophan, the parents selected for their high tryptophan content had tryptophan levels higher than the parents selected for their low tryptophan content. Similarly in the methionine diallel analyzed for methionine, the parents selected for their high methionine content had methionine levels higher than the parents selected for their low methionine content. This relationship did not hold for the methionine levels in the tryptophan diallel or for the tryptophan levels in the methionine diallel.

Results of the ANOVA of factors contributing to the variances of the methionine and tryptophan values are presented in Table 6. This analysis was conducted on the mean values of the three repeated measures, and mean squares were adjusted accordingly. The test for parents versus crosses showed significant differences ($\alpha = 0.05$) between the means of the parents and that of the crosses for tryptophan but not methionine in both diallels. In all diallels, the means among Parents and among Crosses showed significant differences, confirming the variability for tryptophan and methionine content in the lines chosen for this study. Statistically significant genetic effects including the general combining ability (GCA), specific combining ability (SCA), and reciprocal effects made significant contributions to the model in both diallels for both traits.

The estimates of the GCA effects, the SCA effects, and the reciprocal effects for tryptophan diallel analyzed for tryptophan and methionine diallel analyzed for methionine are reported in Table 7. The methionine diallel analyzed for methionine had more significant estimates of the genetic effects than for the tryptophan diallel analyzed for tryptophan, the standard errors for the estimates of the GCA, SCA and reciprocal effects are 0.003528, 0.005987, and 0.007729, respectively. For the methionine diallel analyzed for methionine, the standard errors for the estimates of the GCA, SCA and reciprocal effects are 0.001445, 0.002453, and 0.003166, respectively.

In the tryptophan diallel, PSI301, a high tryptophan parent and a derivative of the B73 genetic group, had the only significant ($\alpha = 0.01$) and positive estimate of the GCA effect for tryptophan. In the methionine diallel, PSI101, a derivative of the B14/B73 genetic group, and PSI702, a derivative of Mo17, both high methionine parents, had the highest significant ($\alpha = 0.01$) estimates for GCA effect for methionine. Lines selected as high methionine parents had positive GCA effects in the methionine diallel and lines selected as low methionine parents had negative GCA effects in the methionine diallel. The same trend is observed for tryptophan in the tryptophan diallel.

Because the sum of the individual estimates for the SCA effect were constrained to zero, a positive and statistically significant estimate for the SCA effect represents the best hybrid combination in the diallel for its methionine or tryptophan levels. In the tryptophan diallel, crosses between parents selected for high tryptophan all had negative or non-significant SCA effects for tryptophan, as did crosses between parents selected for low tryptophan. The largest SCA effect was found in a cross in which one parent was selected for high tryptophan and the other for low tryptophan (PSI301 x PSI801). Most crosses in which one parent was selected for high tryptophan and the other for low tryptophan had positive estimates for the SCA effect. In the methionine diallel, two out of three crosses between parents selected for high methionine had positive estimates for the SCA effects for methionine. The largest SCA effect was found in a cross in which one parent was selected for high methionine and the other for low methionine (PSI101 x PSI002), though all other crosses in which one parent was selected for high methionine and the other was selected for low methionine had negative or non-significant estimates for the SCA effect. The estimates for the SCA effect for the low methionine x low methionine combinations were either not significant or positive and significant.

TABLE 6 - *Analyses of variance for methionine and tryptophan content of self and F_1 seed produced by six maize inbred lines in two diallel mating designs.*

			Trait	
			Methionine	Tryptophan
Diallel	**Sources of variation**	**DF**	**Mean Squares**	**Mean Squares**
Tryptophan	Parents	5	0.00013176 ****	0.00050858 ***
	Parents vs. Crosses	1	0.0000305 ns	0.00082518 **
	Crosses	29	0.00010381 ****	0.00023716 **
	GCA	5	0.00020645 ****	0.00023601 *
	SCA	9	0.00008144 ****	0.00022646 *
	Reciprocal Effects	15	0.00008302 ****	0.00024397 **
	Experimental Error	79	0.00002144	0.00011947
Methionine	Parents	5	0.00025701 ****	0.00054884 ***
	Parents vs. Crosses	1	0.00003836 ns	0.00078584 **
	Crosses	29	0.00019761 ****	0.00040134 ****
	GCA	5	0.00047164 ****	0.00097261 ****
	SCA	9	0.00007026 ****	0.00027643 *
	Reciprocal Effects	15	0.00018268 ***	0.00028587 **
	Experimental Error	74	0.00002005	0.00014479

*, **, ***, **** Significant at α=0.10, α=0.05, α=0.01, α=0.001 respectively; ns, not significant.

According to Griffing's analysis, the sum of the estimates for the reciprocal effects is not constrained to zero. A positive estimate implies that the combination in which a specific parent is used as a female has higher trait values than in the same combination in which the parent is used as a male, and the converse is true for a negative estimate for the reciprocal effect. In the tryptophan diallel analyzed for tryptophan, no high tryptophan x high tryptophan combinations had significant estimates for the reciprocal effect. Three high tryptophan x low tryptophan combinations had negative and significant estimates for the reciprocal effect. All low tryptophan x low tryptophan combinations had negative, significant estimates for the reciprocal effect for tryptophan,. The low tryptophan x low tryptophan combination PSI001 x PSI401 had the most negative estimate for the reciprocal effect, followed by the high tryptophan x low tryptophan combination PSI401 x PSI101. In the methionine diallel analyzed for methionine, two of three high methionine x high methionine combinations had positive and significant estimates for the reciprocal effect. The highest negative and significant estimates for the reciprocal effect were found in high methionine x low methionine combinations. Two of the low methionine x low methionine combinations had negative and significant estimates for the reciprocal effect; the other had a positive and significant estimate for the reciprocal effect.

DISCUSSION

There is interest in developing maize with increased content of methionine and tryptophan because low levels of these amino acids limit the nutritional value of the grain. To increase the levels of these amino acids using breeding methods common to hybrid breeding programs, it is important to understand the variability in amino acid levels within the available germplasm. To characterize the variability in commercial germplasm, tryptophan and methionine levels were determined for seventy-six commercial inbred lines representing nine genetic groups. Significant variability was found among the genetic groups, so that it may be possible to conduct direct selection for tryptophan and methionine levels.

TABLE 7 - *Estimates for the genetic effects for tryptophan in the Tryptophan diallel (A) and for methionine in the Methionine diallel (B). The estimates for the general combining ability (GCA) are included in the diagonal of the table (bold). The estimates for the specific combining ability (SCA) are included below the diagonal. The estimates for the reciprocal effects are included above the diagonal. The first three parents were chosen for their high levels of tryptophan or methionine and the last three parents were chosen for their low levels of tryptophan or methionine.*

A. Tryptophan Diallel

♀	♂					
	PSI101	PSI301	PSI701	PSI801	PSI401	PSI001
PSI101	**0.00429**	0.00015	0.00765	–0.01370 *	–0.01830 *	0.01230
PSI301	–0.01492 **	**0.00599** **	0.00310	–0.01035	-0.00470	–0.00630
PSI701	0.00144	–0.00601	**0.00313**	–0.01045	0.00380	–0.01585 *
PSI801	0.01091 *	0.01316 *	–0.00458	**–0.00408**	–0.01325 *	–0.00235
PSI401	0.00898	–0.00002	0.00474	–0.01370 *	**–0.00126**	–0.01905 **
PSI001	–0.00641	0.00779	0.00441	–0.00578	–0.00001	**–0.00807** *

*, ** Significant at α=0.05 and α=0.01, respectively.

B. Methionine Diallel

♀	♂					
	PSI101	PSI702	PSI301	PSI601	PSI002	PSI402
PSI101	**0.01028** **	0.00970 **	0.00100	–0.01290 **	–0.01510 **	–0.00830 **
PSI702	0.00416 *	**0.00737** **	0.00835 **	–0.01495 **	–0.00555 *	–0.01320 **
PSI301	–0.00212	0.00365	**0.00034**	–0.00770 **	0.00300	-0.00320
PSI601	–0.00557 *	–0.00411 *	0.00197	**–0.01001** **	0.00815 **	–0.00945 **
PSI002	0.00985 **	–0.00469 *	–0.00512 *	0.00198	**–0.00282** *	–0.00890 **
PSI402	–0.00632 **	0.00100	0.00162	0.00572 *	–0.00202	**–0.00516** **

*, ** Significant at α=0.05 and α=0.01, respectively.

In addition to determining the variability of the trait, we also wanted to determine the genetic factors controlling levels of tryptophan and methionine. The analyses of variance of the diallels showed that the GCA makes the largest contribution to the model, with a more pronounced contribution in the methionine diallel than in the tryptophan diallel. As SPRAGUE and TATUM (1942) demonstrated, the GCA is attributable primarily to additive effects. Additive effects reflect the average effects of genes. This notion is important to plant breeders when selecting breeding methods because selection strategies can be designed to take advantage of GCA effects. Successful recurrent selection consists of selection of individuals with favorable alleles, thus high estimates of GCA effects, and incorporating them into the next cycle of selection. From the estimates of the GCA effects, we observed that in the tryptophan diallel and the methionine diallel, the highest and significant estimates for the GCA effect were found in the respective high parent. Whether a maize breeder wants to establish a recurrent selection program for higher levels of tryptophan or methionine or develop hybrids with higher levels of tryptophan or methionine, an inbred line with a higher estimate for the GCA effect should be used to realize the potential of a successful hybrid combination.

In all diallels, with the exception of methionine diallel analyzed for methionine, the SCA and the reciprocal effects contribute about equally to the model, but to a lower extent than the GCA. The SCA is attributable to non additive genetic effects such as dominance, which is due to intra-allelic interactions, and epistasis, which is due to inter-allelic interactions. To the plant breeder, a high estimate of the SCA effect for two specific parents indicates a good hybrid combination. As these estimates pertain to the crosses in these diallels only, further investigation may be required to reveal patterns in SCA effects that are exploitable by breeders.

The estimates for the reciprocal effect determine the direction a cross should be made in order to obtain the highest levels of methionine or tryptophan in a hybrid combination. We observed that those estimates of the reciprocal effects for high x low combinations that had significant estimates were all negative. This suggests that high x low combinations in which the high parent is used as the male had higher levels of methionine or tryptophan. Also, all high x high crosses had positive reciprocal effects, although these were only significant in the methionine diallel.

Heterosis, or hybrid vigor, is a very important concept for plant breeders, especially in breeding cross-pollinated crops. In the analyses of variance, the test of Parents versus Crosses is an indication of heterosis. Interestingly, the Mean Squares for this test were found significant ($\alpha = 0.05$) in the tryptophan diallel analyzed for tryptophan and in the methionine diallel analyzed for tryptophan, but the crosses had less tryptophan than the parents. In hybrid breeding programs, it will be important to overcome this trend in order to maximize the level of tryptophan in the grain. The test Parents versus Crosses did not yield significant differences in the methionine diallel analyzed for methionine and tryptophan diallel analyzed for methionine.

The genetic effects associated with tryptophan levels were smaller than those associated with methionine levels. This may be because there was less variability in tryptophan levels in maize germplasm or because there was more error in the analytical method used to quantify tryptophan in maize kernels. Comparison of the standard errors computed for the estimates of the genetic effects clearly shows that the precision of the assays was different. Overall, the standard errors for the tryptophan assay were twice as great as for the methionine assay.

This study offers guidelines to breeders interested in using commercial germplasm to develop improved populations or hybrids with increased levels of tryptophan and methionine. The distinct variability in tryptophan and methionine levels among the genetic groups suggests that breeders can recycle inbred lines and maintain genetic variability. This may allow the possibility of enhancing the levels of tryptophan or methionine in breeding populations. Identification of hybrid combinations with elevated methionine and tryptophan can be based somewhat on the methionine and tryptophan levels of the parental inbred lines, but it will require evaluation of the amino acid levels of the hybrid grain to verify that amino acid levels are indeed improved.

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