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Grain composition and amino acid content in maize cultivars representing 80 years of commercial maize varieties

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Abstract
In order to determine how modern hybrids have impacted grain composition and amino acid content of the corn crop, we characterized a set of cultivars that were widely grown in different eras from the 1920s through 2001. Grain composition exhibited clear trends with time, with protein decreasing and starch increasing. The effects of different plant densities were examined. The grain protein content of modern hybrids responds to plant density and environment differently than the protein content of older varieties. These differences are consistent with a model in which protein content is modulated by different growth conditions. These differences may explain, in part, the mechanism by which modern hybrids maintain yield in different environments, i.e. reduction of protein content in stressful environments frees resources that are used to maintain yield. We examined the content of the nutritionally limiting essential amino acids lysine, methionine and tryptophan in grain of these cultivars. On a per tissue mass basis, the levels of these amino acids dropped with time while on a per protein basis, their levels were not significantly changed. We conclude that the development of modern hybrids has resulted in maize with reduced protein content, but the nutritional quality of this protein has not changed.

Keywords
Protein quality, Plant densities, Nutritional quality, Essential amino acids

Disciplines
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GRAIN COMPOSITION AND AMINO ACID CONTENT IN MAIZE CULTIVARS REPRESENTING 80 YEARS OF COMMERCIAL MAIZE VARIETIES

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ABSTRACT - In order to determine how modern hybrids have impacted grain composition and amino acid content of the corn crop, we characterized a set of cultivars that were widely grown in different eras from the 1920s through 2001. Grain composition exhibited clear trends with time, with protein decreasing and starch increasing. The effects of different plant densities were examined. The grain protein content of modern hybrids responds to plant density and environment differently than the protein content of older varieties. These differences are consistent with a model in which protein content is modulated by different growth conditions. These differences may explain, in part, the mechanism by which modern hybrids maintain yield in different environments, i.e. reduction of protein content in stressful environments frees resources that are used to maintain yield. We examined the content of the nutritionally limiting essential amino acids lysine, methionine and tryptophan in grain of these cultivars. On a per tissue mass basis, the levels of these amino acids dropped with time while on a per protein basis, their levels were not significantly changed. We conclude that the development of modern hybrids has resulted in maize with reduced protein content, but the nutritional quality of this protein has not changed.

KEY WORDS: Protein quality; Plant densities; Nutritional quality; Essential amino acids.

INTRODUCTION

Maize production in the U.S. has increased at a rate of 1-2% per year on a per acre basis since about 1930 (TRACY *et al.*, 2004). In order to determine the reasons for this gain, experiments have been conducted using public and private (RUSSELL, 1974; DUVICK, 1977) cultivars that were widely grown in different decades. By evaluating these "era cultivars" in common environments, it has been estimated that greater than 70% of the yield gain is due to genetic improvement (DUVICK, 1984; RUSSELL, 1984). These studies suggest a mechanism for the observed increase in production through breeding. By evaluating the era cultivars in low plant densities commonly used in the 1930s and in the high plant densities used today, it became clear that part of the gain in productivity is due to adaptation to higher plant densities. At low plant densities, grain yield is not significantly different in modern hybrids than in older ones, while at higher plant densities, modern hybrids yield considerably more than older cultivars (DUVICK, 1977).

The set of largely private germplasm used in these studies consists of two widely grown open pollinated cultivars and a series of hybrids released by Pioneer Hi-bred International that were widely grown between 1930 and 2001. This set of cultivars is called the era hybrids and has been characterized with respect to many factors involved in determining agronomic performance (DUVICK *et al.*, 2004).

While changes in agronomic performance in the course of developing modern hybrids have been extensively studied, less attention has been given to changes in grain quality during this process. The majority of the effort spent in developing maize cultivars has been devoted to improving the agronomic characters leading to grain yield. Modifications to grain quality are essentially unintended effects; however, it is desirable to know the magnitude and direction of these effects as they are likely to be magnified in future breeding efforts. Nutritional quality is particularly important because most of the maize grain that is produced is used for food or feed. It has been reported that grain protein decreased on average 0.3% per 10 years, while grain starch increased on average 0.3% per 10 years and

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grain oil did not change significantly in the era hybrids (DUVICK *et al.*, 2004). Among the main nutritional limitations of maize grain are contents of the essential amino acids lysine, methionine and tryptophan. While it has been reported that levels of these amino acids are correlated with protein content (MILLER *et al.*, 1950), the correlation of tryptophan with non-zein protein is higher that with total protein (FREY *et al.*, 1949). Thus, increasing total protein may lead to increasing levels of essential amino acids but decreased quality of protein as defined as levels of essential amino acids on a per total protein basis. Consistent with this are several analyses of grain from the Illinois long-term protein selection experiment that conclude high protein populations have a greater proportion of zeins than low-protein populations (reviewed in (BELOW *et al.*, 2004). It is therefore interesting to examine the protein quality of the era hybrids to determine if the reported decrease in protein content over the development of modern hybrids has been accompanied by a change in protein quality.

The objectives of this study were to characterize differences in cultivars that were grown widely in different eras with respect to grain composition. We expanded on the earlier study (DUVICK *et al.*, 2004) by examining the constituents protein, oil, and starch in different plant densities. We also report levels of nutritionally limiting amino acids tryptophan, lysine, and methionine to identify changes that have occurred to the amino acid balance of grain.

MATERIALS AND METHODS

Experimental design

The 45 cultivars listed in Table 1 were produced in 2003 at two locations (Woodland, CA and Johnston, IA). At each location, each hybrid was produced at two planting densities: 90,000 plants/Ha (high density) and 44,500 plants/Ha (low density). Each plot consisted of 2 rows which were allowed to open pollinate. The Iowa location was produced with normal rainfall. The California location receives essentially no rainfall during the growing season and is irrigated. At this location, drought stress treatments were imposed by withholding irrigation prior to and during flowering or during the grain fill period. The three environments were managed as separate experiments, due to the need to block drip irrigation plumbing. Thus, the two locations provide three separate environments in which the experiment was conducted. Within each experiment, 2 replicates of the density main plots, each containing all genotype entries as split plots, were established. Density main plots were randomized within each experiment and hybrid split plots were independently randomized within each replicate of the density main plots.

Grain analysis

Plots in Iowa were harvested by hand and grain from several ears from each plot was bulked and used for analysis. Bulk grain representing each plot in California was harvested with a combine equipped for grain sampling. Samples were dried to about 10% moisture. Each sample was analyzed with NIR to predict protein, oil and starch content using a Perstorp 6500 NIR spectrometer (Foss North America, Eden Prairie, Minnesota) equipped with a sample transport module and natural product cell. Samples are reported on a zero percent moisture basis.

Amino acid content was determined on a 40 kernel sub-sample using a microbial assay (SCOTT *et al.*, 2004) to measure the content of tryptophan, methionine and lysine. Assays were carried out in 96-well plates, with each rep randomized as a block and analyzed on three plates. Thus, six 96-well plates were used for the experiment.

Data analysis

The amino acid data were analyzed using a mixed linear model as follows:

$$y_{ijklmn} = \lambda_i + \rho_{ij} + \gamma_{jk} + \alpha_{jkl} + \beta_{jkm} + \delta_n + \lambda\delta_{in} + \psi\lambda_i + \psi\delta_n + \psi\lambda\delta_{in} + \eta_p + \eta\delta_{np} + \eta\lambda_{ip} + \varepsilon_{ijklmn}$$

y_{ijklmn} = response
λ_i effect environment i
ρ_{ij} = effect of replicate j within environment i
γ_{jk} = effect of plate k within replicate j
α_{jkl} = effect of row l on plate jk
β_{jkm} = effect of column m on plate jk
δ_n = effect of density n
$\lambda\delta_{in}$ = environment by density interaction
ψ = year of hybrid release (continuous)
$\psi\lambda_i$ = year by environment interaction
$\psi\delta_n$ = year by density interaction
$\psi\lambda\delta_{in}$ = year by environment by density interaction
η_p = effect of hybrid p
$\eta\delta_{np}$ = hybrid by density interaction
$\eta\lambda_{ip}$ = hybrid by environment interaction
ε_{ijklmn} = residual

Plates, rows, and columns are laboratory variables for the 96-well plates used in the microbial amino acid analyses only. Rows and columns represented the rows and columns of individual cells on the plates. The environment variable was for the two environments in California (two different water treatments) and the Iowa environment.

Year was fit as a continuous variable (covariate) along with year by environment, year by density, and year by environment by density interactions. The analysis of protein, oil, and starch data was done with by NIR, and did not involve 96-well plates, thus the model for those traits did not have plate, row within plate, or column within plate. When samples were assigned to plates for amino acid analysis, all the entries in replicates labeled "one" in all three environments were randomly ordered and assigned to three plates. Likewise, all samples assigned to replicates labeled "two" across the three environments were randomly assigned to a second set of three plates. Therefore, in the nesting of effects in the model, replicates are nested within environments due to the field design, but due to the lab design, plates are nested within the replicate variable but not within environ-

ments (plate effects were specific to the laboratory). All effects in the model were considered fixed except hybrids, hybrid by environment interaction, and hybrid by density interaction. Variances were estimated by restricted maximum likelihood (SAS proc mixed). Hybrid by environment by density interaction was not significant, and was dropped for all traits. Hybrid by environment and hybrid by density interaction was not significant for amino acid traits and was dropped for those traits. All main effects and interactions not involving the covariate effect of year were tested for significance with type-I F-tests. Effect of years and interactions of year with densities and environments were tested with type III F-tests. Degrees of freedom were computed according to Satterthwaite approximations as implemented in SAS proc mixed. Generalized least squares estimators of regression coefficients and their standard errors for the change in trait value per year and their standard errors were presented for appropriate effects based on significance of the type III hypothesis tests. The magnitudes of the residual terms in the models were between 35 and 71% of the magnitude of the total variance for each trait. These F-test results are summarized in Table 2.

RESULTS AND DISCUSSION

A set of 45 cultivars that were each grown widely at some time between 1920 and 2001 were used in this study (Table 1). The majority of these cultivars are commercial hybrids released by Pioneer Hi-bred International. Each cultivar was grown in three different environments differing in their water availability, and in two different plant densities. Within each water experiment, a split plot treatment design

TABLE 1 - *Hybrids used in this study and a year that they were widely grown.*

Cultivar	Year	Cultivar	Year
Krug YD	1920	3571	1968
Reid YD	1920	3334	1969
351	1934	3388	1970
307	1936	3517	1971
322	1936	3366	1972
317	1937	3301A	1974
330	1939	3541	1975
336	1940	3382	1976
340	1941	3377	1982
339	1942	3378	1983
344	1945	3475	1984
352	1946	3379	1988
350B	1948	3417	1990
347	1950	3394	1991
301B	1952	3489	1994
354	1953	3335	1995
329	1954	33G26	1998
354A	1958	33P67	1999
3618	1961	34B23	1999
3206	1962	34M95	2001
3306	1963	34N44	2002
3376	1965	34H31	2002
3390	1967		

TABLE 2 - *Hypothesis tests.*

	Composition (%)			Amino acids (g/100g tissue)			Amino acids/Protein		
Effect[1]	Oil	Protein	Starch	Lysine	Methionine	Tryptophan	Lysine	Methionine	Tryptophan
Rep	n.s.[2]	n.s.	*	n.s.	**	**	n.s.	**	**
Plate(Rep)				**	**	**	**	**	**
Row(Plate*Rep)				**	**	**	**	**	**
Colm(Plate*Rep)				**	**	**	**	**	**
Environment	n.s.	**	**	**	**	**	**	**	**
Density	n.s.	**	**	**	**	n.s.	**	n.s.	**
Environment*Density	n.s.	**	n.s.	n.s.	*	n.s.	*	*	n.s.
Year	**	**	**	**	*	**	n.s.	n.s.	n.s.
Year*Environment	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year*Density	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year*Environment*Density	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Row, Colm and Plate are laboratory variables used for the measurement of amino acid levels and not in the analyses of protein, oil or starch.

[2] ** P<0.01; * P<0.05.

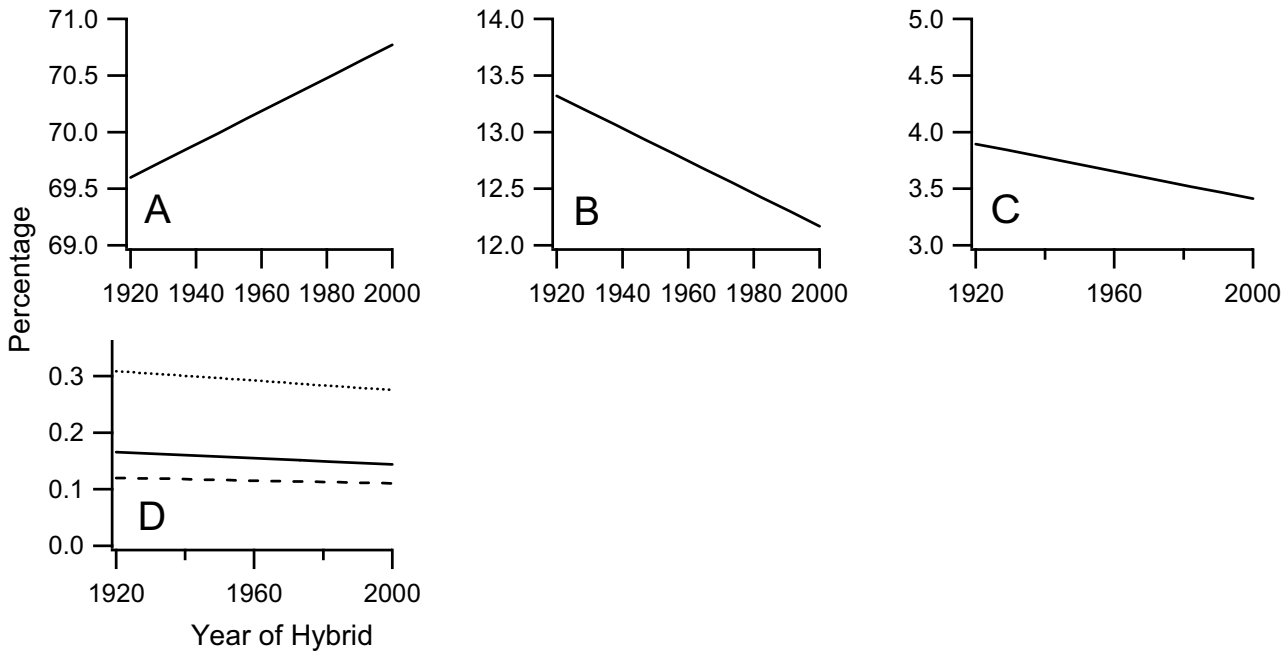

FIGURE 1 - Grain composition of the era hybrids produced averaged across environments and plant densities. Lines are fit to data plotted as percentage of the tissue mass of each component. A. Starch; B. Protein; C. Oil; D. Amino acids; dotted line, lysine; solid line, tryptophan; dashed line, methionine. Slopes and standard errors for these data are presented in Table 3.L.

was used with density as the main plot and entry as the split plot. Two replicates of each treatment and cultivar were grown in each experiment. Each water treatment was established as a separate experiment. The combination of imposed drought stress and different plant densities resulted in production environments that were quite different. Because drought stress has been shown to result in decreased yields with increased protein content (LILBURN *et al.*, 1991), we anticipated that these environments should provide an opportunity to observe environmental effects on the traits examined. Data were collected on protein, oil and starch content as well as the content of the nutritionally limiting amino acids methionine, tryptophan and lysine. Amino acid levels were expressed both on the basis of tissue mass and total protein content to provide information about the total amino acid content and the protein quality, respectively.

TABLE 3 - *Slopes of regression of trait values on years.*

Trait	Slope ± Standard Error
Oil	-0.006 ± 0.002
Protein	-0.014 ± 0.003
Starch	0.015 ± 0.003
Lysine	-0.00042 ± 0.00013
Methionine	-0.00012 ± 0.00005
Tryptophan	-0.00027 ± 0.00006

Effect of environment on grain composition

Environment had a significant effect on all traits except oil (Table 2). The magnitudes of these changes were up to .03% of the tissue mass. A study of commercial hybrids revealed that total protein content and total sulfur amino acids were elevated with a concomitant decrease in grain yield when these hybrids were produced in drought conditions (LILBURN *et al.*, 1991). This result is consistent with our study. The well-watered environment had lower mean protein values than the two drought stress environments (Fig. 2B). The significant environmental effects observed in our study may be a manifestation of the extreme differences in production environments imposed during plant growth and the fact that two of our environments involved drought stress.

Plant density had a significant effect on all traits except oil, methionine/per protein, and tryptophan

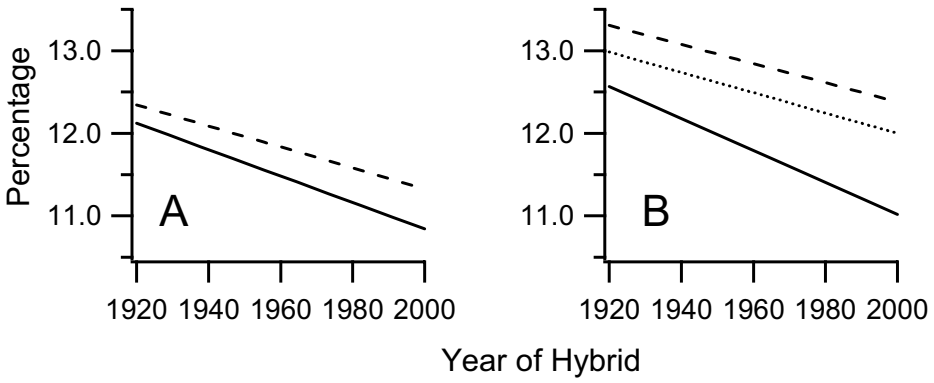

FIGURE 2 - Change in protein content with time. A. Dashed line, low plant density; solid line, high plant density. B. Dashed line, drought stress at flowering; dotted line, well watered; Solid line, drought stress at grain fill. Slopes and standard errors for these data are presented in Table 4.

TABLE 4 - *Slopes ± standard errors of regression of protein content on year of hybrid.*

		Averaged across environments	Averaged across plant densities
Density	High	-0.016 ± 0.0030	
	Low	-0.013 ± 0.0030	
Environments	IA		-0.012 ± 0.0034
	CA Stress at grain fill		-0.019 ± 0.0034
	CA Stress at flowering		-0.012 ± 0.0034

TABLE 5 - *Slopes ± standard errors of regressions of oil content on year for different treatments.*

	Environments		
	IA	CA stress at fill	CA stress at flowering
High Density	-0.0079 ± 0.0024	-0.0070 ± 0.0024	-0.0062 ± 0.0023
Low Density	-0.0014 ± 0.0024	-0.0044 ± 0.0024	-0.0093 ± 0.0023

(Table 2). Protein content averaged 0.6% higher and starch content averaged 0.3% lower in low plant densities. The effect of plant density on amino acid content was mixed, with methionine being higher and lysine being lower at low plant density. Protein quality was decreased at low plant density in the two significant cases lysine/protein and tryptophan/protein. Thus, while low plant density allows accumulation of more protein, lysine and tryptophan are underrepresented in this protein, resulting in lower quality.

Changes in grain composition with year of hybrid release

The year that the hybrids were widely grown had a significant effect on protein, oil and starch content (Table 2). Protein decreased and oil decreased with time, and starch increased with time (Table 3, Fig. 1). This is similar to a previous report (Duvick *et al.*, 2004), in which protein decreased with hybrid year while starch went up. Our values for protein and starch are slightly lower than the values of 0.03% per year previously reported. In contrast to our results, the previous study reported no change in oil content.

Lysine, tryptophan, and methionine all had significant year effects when expressed as acid content per tissue mass, while the same amino acids when expressed on a per protein basis did not (Table 1). The results for amino acid per tissue mass are illustrated in Fig. 1 and Table 3. Like total protein content, the content of amino acid per tissue mass de-

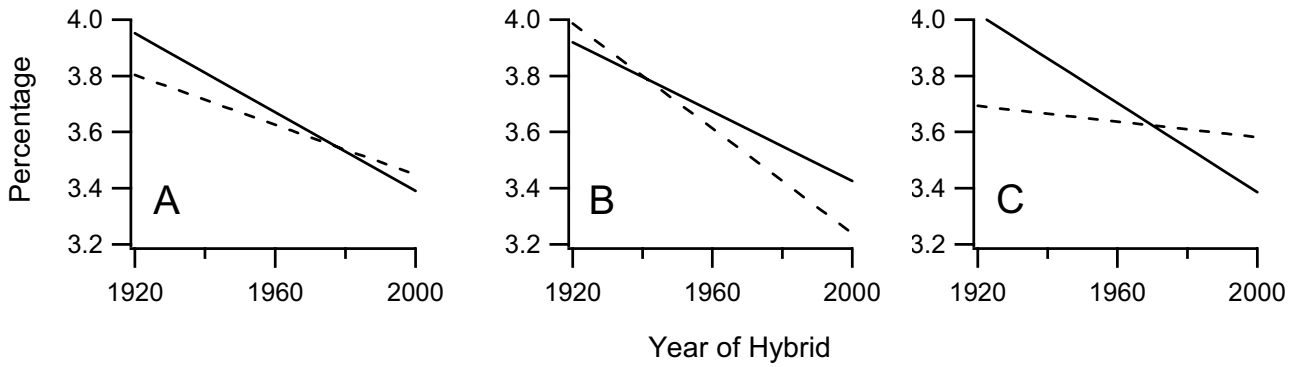

FIGURE 3 - Change in Oil content of the era hybrids over time. Dashed line, low plant density, Solid line, high plant density. A. Drought stress at grain fill. B. Drought stress at flowering. C. Well watered. Slopes and standard errors for these data are presented in Table 5.

creased with year of hybrid release. The fact that amino acid per protein content did not have a significant release year effect (in contrast to amino acid per tissue mass) suggests that composition of hybrids has changed over time, while the quality of the protein (defined as methionine, lysine or tryptophan per protein) has not changed in a statistically detectable way.

The test of significance of the Year*Density effect can be used to identify significant differences between high and low plant density in the change in trait values with time. These effects were only significant for protein content (Table 2). The significant Year*Density effect for protein content indicates that the protein content of modern hybrids is reduced more in high plant density than the protein content of older hybrids (Table 4, Fig. 2). It has been reported that the yield of modern hybrids is reduced less by increased plant density (DUVICK *et al.*, 2004). This greater reduction in protein content in modern hybrids may be a compensating mechanism that allows these varieties to maintain their yield in high density conditions. Reduced protein content may free resources that can be used by the plant to maintain yield.

The test of significance of the Year*Environment effect can be used to identify significant differences between environments in the change in trait values with time. Like Year*Density, the Year*Environment effect was only significant for protein content (Table 2, 4, Fig. 2). This indicates that the protein content of modern hybrids responds differently to different environments than that of older hybrids.

The test of Environment*Density can be used to identify significant differences in response across all release years for the interaction between the environment and density treatments. This effect was only significant for oil content (Table 1). The effect of plant density on oil content varied with environment. The mean oil content was higher in high plant densities. The Year*Environment*Density effect can be used to determine if the slopes of trait vs. time regression lines are effected differently in different environment-density combinations. This effect was only significant for oil content. Some environments had larger plant density effects than others (Table 5, Fig. 3). This can be seen by comparing the difference in slopes of the two lines in the well watered case to the difference in slopes of the lines in the stress cases.

While clear trends are observable in grain composition in over the course of development of the era hybrids, the magnitude of the changes are small and on the order of magnitude of changes attributed to environmental effects. At plant densities used today and with low environmental stresses, these changes have resulted in grain with higher starch content and lower content of protein with unchanged quality. These changes may be favorable for some end uses and unfavorable for others.

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