MinnesotaCorn
RESEARCH & PROMOTION COUNCIL

FINAL REPORT

PROJECT TITLE: Variability in Oil Composition in Maize
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ABSTRACT

A major focus in corn breeding to date has been on increasing yield with little attention paid to compositional properties of the grain. Corn grain is composed of different tissues with different compositional properties including the oil rich embryo, the endosperm that is comprised of a matrix of protein and starch molecules, and the pericarp that is rich in fiber. Studies looking at compositional properties in corn have largely focused on properties of the endosperm tissue due to its importance to a number of different industries (e.g. fresh and canned sweet corn markers, masa based products such as chips and tortillas, etc.). In contrast, very little is known about variation in oil among diverse maize lines. Reports have shown high variability in total oils, which has been linked in large part to the size of the embryo, but an in depth look at the variability on oil characteristics has not been done. Understanding the variation that exists in oil characteristics is an important step in determining the possibility of developing higher value products such as renewable food, fuel, plastics, or other products. Here we conducted a detailed profiling of oil content for 140 unique samples that included a single replicate of 100 diverse inbred lines and 10 commercial hybrids grown in replicate across two environments to assess the range of natural variation and partition sources of variation impacting grain oil composition. These 140 samples were evaluated for 66 total traits including Omegas, Trans Fat, Saturated Fat, Unsaturated Fat, and Total Fat. This assessment of natural variation and partitioning of variation is an important first step in determining alternative uses of corn.

INTRODUCTION

Maize kernels are comprised of several main structures. The outer layer of the kernel is comprised of the pericarp and the bottom part of the kernel where there is no pericarp coverage is called the tip cap that is carried over after kernels are harvested from the cob. The pericarp is primarily made of hemicelluloses, such as arabinoxylans and xylans, and phenolics. Immediately underneath the pericarp is the aleurone, which is a thin layer of cells with the predominant function of mineral and enzyme storage. Beneath the aleurone is the endosperm that makes up the vast majority of the kernel by size and weight. The endosperm has two main components, the vitreous endosperm and the floury endosperm, with the vitreous endosperm surrounding the floury endosperm. The embryo is surrounded by the endosperm on three sides, and has a high fat and protein content. On average, maize kernels are ~83% endosperm, ~11% germ, ~5% pericarp, and ~1% tip, although the portions of these tissues as well as the total starch, protein, and oil content can vary greatly across genotypes, environmental conditions, and cultivation practices.

Corn breeding efforts to date have focused primarily on improving yield. Though, there have been efforts to improve compositional attributes for various applications. A notable example of breeding efforts for protein content in maize is the QPM germplasm. Quality protein maize was originally developed as cattle

feed with high lysine and tryptophan content. Another notable effort in breeding for both total protein and total oil content is the Illinois long-term selection experiment (http://mooselab.cropsci.illinois.edu/longterm.html). This experiment, which initially started as a divergent selection experiment for high and low protein and high and low oil, has been running for over 100 cycles with continuous observed improvement. With regards to the selection for high oil, a doubling in oil content has been observed within this very narrow set of germplasm, indicating a polygenic nature to the control of total oil content in maize. Much less is known about the genetics controlling variation in oil properties, or even the degree to which variation in oil properties exists within the broader diversity of corn.

The goal of this research was to assess the extent of variation that exists for oil properties in a broad set of germplasm that is representative of variation within corn as well as to assess variation that exists within elite germplasm that has undergone extensive selection for increase yield, potentially resulting in a bottleneck of variation in other aspects of the grain such as oil properties. To do this, utilized a gas chromatography assay to look at 66 different parameters of the fat and oil in the grain on a total of 140 samples. This study provides critical foundational information on the potential of pushing corn into a value added space based on oil content and properties, and the degree to which modern elite germplasm is positioned for this effort.

OBJECTIVE AND GOAL STATEMENTS

Objective 1: Determine fat and oil profiles of 100 spectrally diverse temperate corn inbred lines.
Objective 2: Determine fat and oil profiles from 10 current commercial hybrids grown in a replicated field trials (n=40 samples).

MATERIALS AND METHODS

For both objectives we worked with Eurofins, the world leader in food, environment, pharmaceutical and cosmetic products testing to performing their comprehensive assay that quantifies Omega 3's, 6's, 9's, Trans Fat, Saturated Fat, Unsaturated Fat, and Total Fat. In this assay a sample is first hydrolyzed with acid. Fat is then extracted, and ethers are evaporated. The fat is then saponified and derivatized, and the sample is analyzed on a gas chromatograph. The following 66 parameters are measured: C4:0 (Butyric Acid), C6:0 (Caproic acid), C8:0 (Caprylic acid), C10:0 (Capric acid), C11:0 (Undecanoic acid), C12:0 (Lauric Acid), C14:0 (Myristic acid), C14:1 (Myristoleic acid), C15:0 (Pentadecanoic acid), C15:1 (Pentadecenoic acid), C16:0 (Palmitic Acid), C16:1 Omega 7, C16:1 Total (Palmitoleic Acid + isomers), C16:2 (Hexadecadienoic Acid), C16:3 (Hexadecatrienoic Acid), C 16:4 (Hexadecatetraenoic Acid), C17:0 (Margaric Acid), C17:1 (Heptadecenoic Acid), C18:0 (Stearic Acid), C18:1 (Vaccenic acid), C18:1 Omega 9 (Oleic Acid), C18:1, Total (Oleic Acid + isomers), C18:2 Omega 6 (Linoleic Acid), C18:2, Total (Linoleic Acid + isomers), C18:3 Omega 3 (Alpha Linolenic Acid), C18:3 Omega 6 (Gamma Linolenic Acid), C18:3, Total (Linolenic Acid + isomers), C18:4 Omega 3 (Octadecatetraenoic Acid), C18:4 Total (Octadecatetraenoic Acid), C20:0 (Arachidic Acid), C20:1 Omega 9 (Gondoic Acid), C20:1 Total (Gondoic Acid + isomers), C20:2 Omega 6, C20:2 Total (Eicosadienoic Acid), C20:3 Omega 3, C20:3 Omega 6, C20:3, Total (Eicosatrienoic Acid), C20:4 Omega 3, C20:4 Omega 6 (Arachidonic Acid), C20:4, Total (Eicosatetraenoic Acid), C20:5 Omega 3 (Eicosapentaenoic Acid), C21:5 Omega 3 (Heneicosapentaenoic Acid), C22:0 (Behenic Acid), C22:1 Omega 9 (Erucic Acid), C22:1 Total (Erucic Acid + isomers), C22:2 Docosadienoic Omega 6, C22:3 Docosatrienoic, Omega 3, C22:4 Docosatetraenoic Omega 6, C22:5 Docosapentaenoic Omega 3, C22:5 Docosapentaenoic Omega 6, C22:5 Total (Docosapentaenoic Acid), C22:6 Docosahexaenoic Omega 3, C24:0 (Lignoceric Acid), C24:1 Omega 9 (Nervonic Acid), C24:1 Total (Nervonic Acid + isomers), Total Omega 3 Isomers, Total Omega 5 Isomers, Total Omega 6 Isomers, Total Omega 7 Isomers, Total Omega 9 Isomers, Total Monounsaturated Fatty Acids, Total Polyunsaturated Fatty Acids, Total Saturated Fatty Acids, Total Trans Fatty Acids, Total Fat as Triglycerides, Total Fatty Acids. The minimum detection limits vary for each of the traits from 0.02% to 0.04%.

To identify the 100 diverse lines inbred lines for this analysis we screen a previously developed set of diverse inbred lines (n=~500) that were selected based on the criteria that they flower and mature in a Midwest growing environment and have sufficient agronomic properties for use in field trials (Hansey et al., 2011). The 100 lines that maximize spectral diversity based on absorbance of ~50 wavebands using near infrared reflectance (NIR) spectroscopy were identified from this larger panel. The variation in reflectance data is a direct result of variation in compositional properties of the grain, as NIR reflectance is a property of the chemical bonds that exist in a sample. By selecting on maximum variation in reflectance data we are maximizing compositional variation in a cost effect subset of the lines. A single replicate for each of these 100 spectrally diverse genotypes were subjected to the above assay.

We grew 10 current elite hybrids (DKC36-28RIB, DKC32-12RIB, DKC45-65RIB, DKC61-80RIB, DKC49-72RIB, DKC40-45RIB, DKC50-87RIB, DKC40-77RIB, LH227 x LH295, B73 x Mo17) in replicate in St. Paul, MN at the Minnesota Agricultural Experiment Station and in Waseca, MN at the Southern Research and Outreach Center. In St Paul, plots were 4-row plots that were 19.5 feet feet center-to-center with 30 inch row spacing between rows. In Waseca, plots were 2-row plots that were 25 feet center-to-center. Grain harvested from these 40 plots (10 genotypes x 2 replicates x 2 locations) were subjected to the assays described above.

RESULTS AND DISCUSSION

Determine fat and oil profiles of 100 spectrally diverse temperate corn inbred lines.

From a set of highly diverse inbred genotypes we identified the 100 individuals that maximized compositional diversity by maximizing variation in previously obtained spectral profiles (Figure 1; Burns et al., 2021). These genotypes are primarily yellow dent corn inbred lines, and thus have the highest relevance to current elite U.S. corn germplasm. These 100 compositionally diverse samples were assayed for 66 traits related to fat and oil composition. Of these 66 traits, only 14 were observed above the minimum detection limit in at least one sample (Figure 2). For total fat as triglycerides and total fatty acids a wide range of variation was observed (Figure 2 and Table 1). However, for many of the traits that were quantified above the minimum detection limit, there was very little observed variance among this panel of 100 spectrally diverse samples.

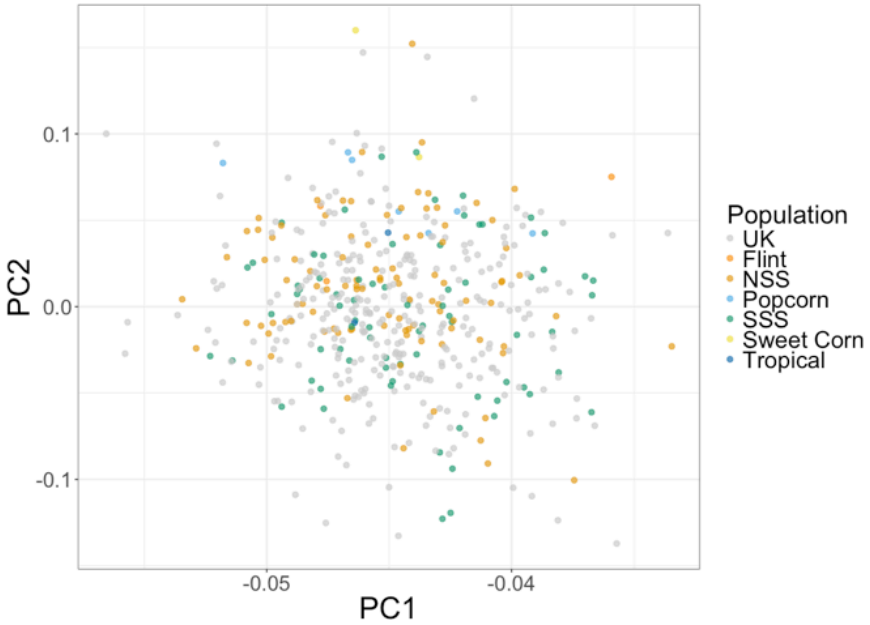

Figure 1. Principal component analysis of absorbance values from ~50 wavebands in the near infrared spectrum for a panel of 500 diverse inbred lines.

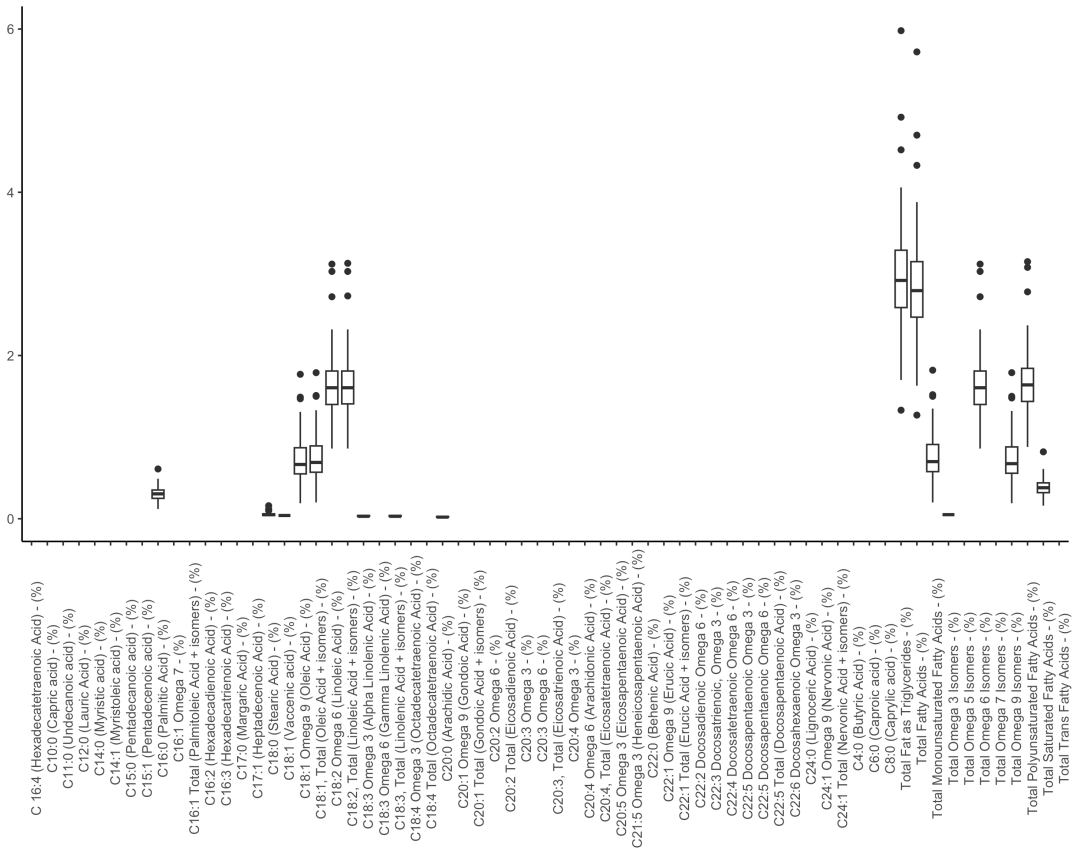

Figure 2. Variation in abundance of 66 fat and oil traits in a panel of 100 spectrally diverse inbred lines. Traits with no box plot presented had no individuals with observed abundance above the minimum detection limit.

Table 1. Summary statistics for traits with at least one sample that had a value above the minimum detection limit for the trait in a panel 100 spectrally diverse inbred lines.

Trait	Mean	Standard Deviation	Min	Max	Coefficient of Variation
C16:0 (Palmitic Acid) - (%)	0.312	0.080	0.12	0.61	25.487
C18:0 (Stearic Acid) - (%)	0.052	0.020	0.03	0.16	38.841
C18:1 (Vaccenic acid) - (%)	0.029	0.001	0.03	0.04	3.779
C18:1 Omega 9 (Oleic Acid) - (%)	0.726	0.263	0.19	1.77	36.299
C18:1, Total (Oleic Acid + isomers) - (%)	0.747	0.266	0.20	1.79	35.555
C18:2 Omega 6 (Linoleic Acid) - (%)	1.638	0.367	0.86	3.12	22.422
C18:2, Total (Linoleic Acid + isomers) - (%)	1.644	0.368	0.86	3.13	22.355
Total Fat as Triglycerides - (%)	2.959	0.630	1.33	5.98	21.278
Total Fatty Acids - (%)	2.830	0.602	1.27	5.72	21.281
Total Monounsaturated Fatty Acids - (%)	0.759	0.269	0.20	1.82	35.396
Total Omega 6 Isomers - (%)	1.639	0.367	0.86	3.12	22.425
Total Omega 9 Isomers - (%)	0.734	0.266	0.19	1.79	36.167
Total Polyunsaturated Fatty Acids - (%)	1.677	0.370	0.88	3.15	22.082
Total Saturated Fatty Acids - (%)	0.390	0.100	0.16	0.82	25.513

Determine fat and oil profiles from 10 current commercial hybrids grown in a replicated field trials.
Similar to the 100 spectrally diverse inbred lines, for the majority of the traits there were no samples that had a concentration above the minimum detection limit (Figure 3). Within the commercial hybrids 15 traits were observed above the minimum detection limit in at least one sample (Figure 3 and Table 2). Again, substantial variation was observed for total fat as triglycerides and total fatty acids, but also for a number of other traits related to Omega 6/Linoleic Acid (e.g. C18:2 Omega 6 (Linoleic Acid), C18:2 Total (Linoleic Acid + isomers), Total Omega 6 Isomers) and Total Polyunsaturated Fatty Acids. Because the hybrid entries were grown in replicate across two environments we were also able to calculated the repeatability for these each trait in this experiment. Due to the nature of the experimental design and entries being evaluated we could not calculate heritability. However, repeatability provides an upper bounds to the heritability of a trait. In this experiment, the repeatability for most of the traits was quite high, ranging from 0.688 – 0.874 for all of the traits expect C18:3 Omega 3 (Alpha Linolenic Acid) and C18:3 Total (Linolenic Acid + isomers). These two traits with relatively low repeatability also had relatively very low overall abundance in the hybrids and had no samples in the inbred lines in which a concentration above the minimum detection limit was observed

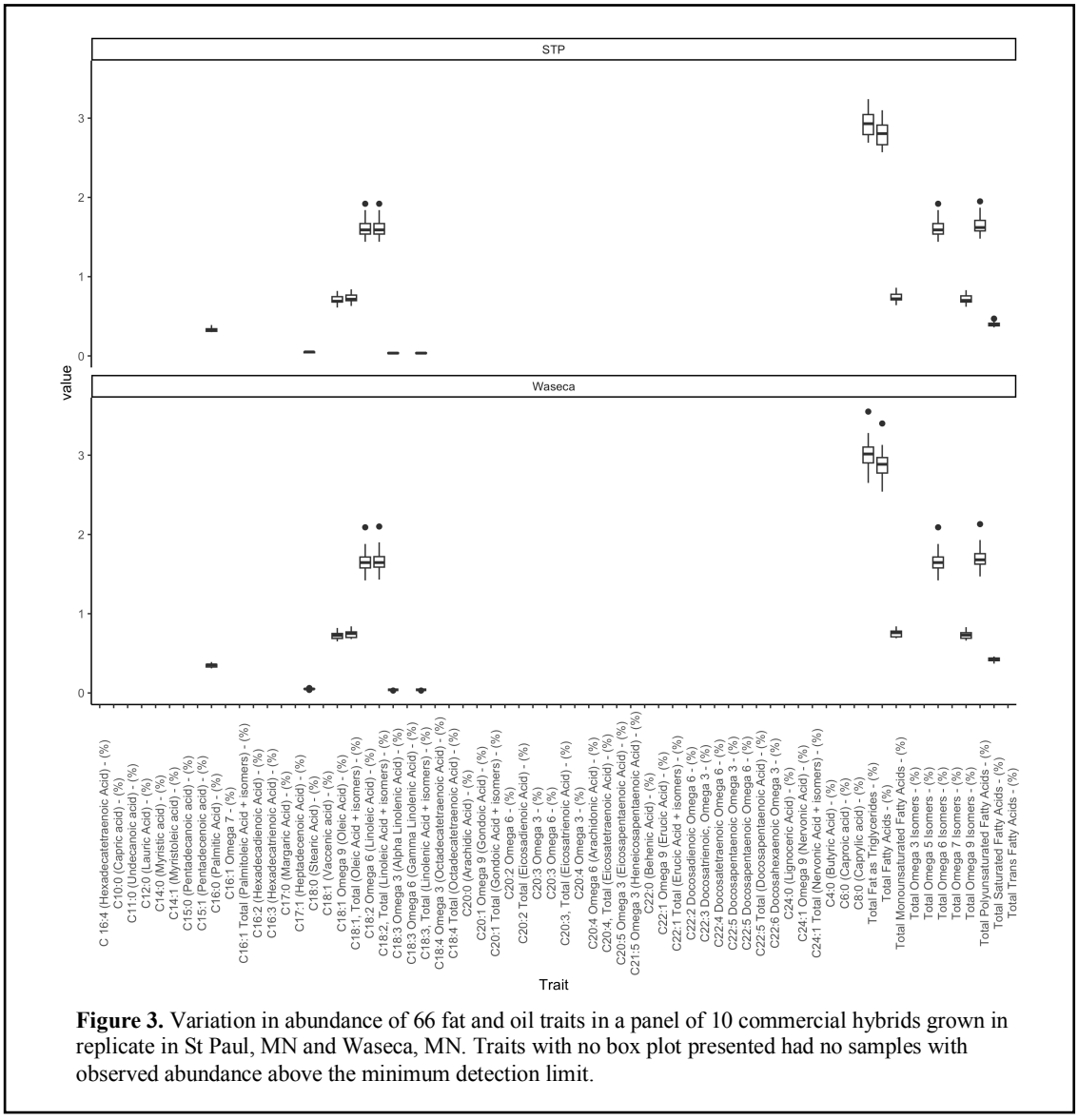

Figure 3. Variation in abundance of 66 fat and oil traits in a panel of 10 commercial hybrids grown in replicate in St Paul, MN and Waseca, MN. Traits with no box plot presented had no samples with observed abundance above the minimum detection limit.

Table 2. Summary statistics for traits with at least one sample that had a value above the minimum detection limit for the trait in a panel of 10 commercially relevant hybrids grown in a replicated field trial in two environments with two replicates per environment.

Trait	Mean	Standard Deviation	Min	Max	Coefficient of Variation	Repeatability
C16:0 (Palmitic Acid) - (%)	0.338	0.027	0.30	0.39	7.919	0.738
C18:0 (Stearic Acid) - (%)	0.049	0.006	0.04	0.06	12.830	0.739
C18:1 Omega 9 (Oleic Acid) - (%)	0.718	0.051	0.61	0.82	7.143	0.705
C18:1, Total (Oleic Acid + isomers) - (%)	0.738	0.052	0.63	0.84	7.103	0.688
C18:2 Omega 6 (Linoleic Acid) - (%)	1.643	0.143	1.42	2.09	8.732	0.871
C18:2, Total (Linoleic Acid + isomers) - (%)	1.646	0.145	1.43	2.10	8.784	0.874
C18:3 Omega 3 (Alpha Linolenic Acid) - (%)	0.037	0.005	0.03	0.04	12.140	0.316
C18:3, Total (Linolenic Acid + isomers) - (%)	0.037	0.005	0.03	0.04	12.140	0.316
Total Fat as Triglycerides - (%)	2.976	0.184	2.65	3.55	6.184	0.731
Total Fatty Acids - (%)	2.846	0.176	2.54	3.40	6.175	0.731
Total Monounsaturated Fatty Acids - (%)	0.748	0.053	0.64	0.86	7.099	0.702
Total Omega 6 Isomers - (%)	1.643	0.144	1.42	2.09	8.735	0.869
Total Omega 9 Isomers - (%)	0.725	0.052	0.62	0.83	7.191	0.700
Total Polyunsaturated Fatty Acids - (%)	1.682	0.143	1.47	2.13	8.483	0.870
Total Saturated Fatty Acids - (%)	0.412	0.030	0.36	0.47	7.356	0.710

CONCLUSIONS

There is relatively limited natural variation in the composition of fats and oils in current elite maize hybrids or in a panel of diverse inbred lines that have demonstrated spectral, and therefore are compositionally diverse for some grain traits. The variation in the inbred lines that is reflected in the spectral variation is more likely to be coming from differences in the fiber, starch, and protein content and composition based on the range of variation observed for most of the fat and oil traits. There are some traits that show relatively low repeatability and are therefore more impacted by environmental variation. However, for the most part, the observed oil traits had relatively high repeatability across the two testing environments, indicating a strong genetic component to the observed variation in the commercial hybrids and support the possibility of making genetic improvements through breeding efforts for these traits.

EDUCATION, OUTREACH, AND PUBLICATIONS

This work has not yet been disseminated through peer reviewed publications.

REFERENCES

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