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Abstract

The general public has shown increasing interest in consuming oils that are beneficial to health. Thus, oil from corn (*Zea mays* L.), along with most major edible oils, has been the target of genetic alterations to improve the fatty acid composition. The fatty acid profile goals vary, depending upon the intended use for the oil. We have targeted the development of corn oil with 1) low total saturated fatty acids, 2) high total saturated fatty acids, and 3) mid-oleic acid, in each case via two different sources: exotic germplasm identified through an international program to broaden the corn germplasm base and introgression of a wild, grassy relative of corn, *Tripsacum* (*Tripsacum dactaloides* L). We have been successful at identifying corn lines with each of the noted traits.

Keywords

Agronomy, NCRPIS

Disciplines

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Comments

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Feature

Improving the fatty acid composition of corn oil by using germplasm introgression

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Summary

*The general public has shown increasing interest in consuming oils that are beneficial to health. Thus, oil from corn (*Zea mays L.*), along with most major edible oils, has been the target of genetic alterations to improve the fatty acid composition. The fatty acid profile goals vary, depending upon the intended use for the oil. We have targeted the development of corn oil with 1) low total saturated fatty acids, 2) high total saturated fatty acids, and 3) mid-oleic acid, in each case via two different sources: exotic germplasm identified through an international program to broaden the corn germplasm base and introgression of a wild, grassy relative of corn, *Tripsacum (Tripsacum dactaloides L.)*. We have been successful at identifying corn lines with each of the noted traits.*

Introduction

Corn oil, a by-product of the wet-milling industry, is found mainly in the germ of corn seeds. Typical dent corn oil has the following fatty acid composition: 61.9% linoleic acid, 24.1% oleic acid, 11.0% palmitic acid, 2.0% stearic acid, 0.7% linolenic acid, and traces of other fatty acids, with about 13% total saturated fats. Corn oil, however, can have wide variations in fatty acid compositions. For example, we previously evaluated 418 corn hybrids and 98 corn inbreds grown in Iowa, finding fatty acid ranges as follows: palmitic acid, 6.7–16.5%; palmitoleic acid, 0.0–1.2%; stearic acid, 0.7–6.6%; oleic acid, 16.2–43.8%; linoleic acid, 39.5–69.5%; linolenic acid, 0.0–3.1%; and arachidic acid, 0.0–1.0% (1). Similar ranges in fatty acids were observed in high-yielding populations grown in four countries with temperate climates (2) chosen from materials in the Latin American Maize Project (LAMP). Some of these lines had unusual fatty acid compositions, including one with only 8.3% total saturates and another with 20.2% total saturates. Corn oil, along with many other major vegetable oils, such as canola, soybean, and sunflower oils, recently has been the target of genetic alteration to improve the fatty acid composition, with several goals in mind. A main driving force for changing fatty acid composition of oilseeds, by either plant breeding techniques or by transgenic means, has been an increasing recognition of the health implications. For example, many nations around the world have banned the sales of foods containing *trans* fats arising from the process called hydrogenation, suggested a restricted amount of *trans* fats for consumption, or begun mandatory labeling of foods containing *trans* fatty acids.

The purpose of hydrogenation is to increase oxidative stability and/or the solids content of an oil to enhance its functionality in foods. The process of hydrogenation results in increased saturation of an oil, along with a change in conformation of some of the naturally occurring *cis* double bonds to *trans* double bonds.

For example, unhydrogenated oils contain no *trans*-18-carbon monounsaturated isomers, whereas, after partial hydrogenation, canola oil contains ~33%, soybean oil contains ~27%, and sunflower oil contains ~56% of the isomer. *Trans*-18-carbon monounsaturated isomer levels of 59.1% have been reported in hydrogenated corn oil and 26.6% in corn oil margarine. *Trans* fatty acids also are found naturally in fats from ruminant animal sources such as beef, milk, and cheese. In March 2003, Denmark was the first country to officially suggest restricting *trans* fatty acids in the diet. They adopted legislation restricting *trans* fatty acids in oils and processed foodstuffs containing fats and oils as ingredients to <2%, but exempted naturally occurring *trans* fatty acids in animal fats. In the United Kingdom, a major retailer banned sales of processed foods containing hydrogenated fats on November 24, 2005. Canada began mandatory labeling of *trans* fatty acids for processed foods on December 12, 2005, followed closely by the United States, who began mandatory labeling of *trans* fatty acids in foods on January 1, 2006.

Thus, our goals for corn seed modifications are to provide suitable fatty acid compositions in the extracted edible oils to allow for 1) oxidative and frying stability while providing good flavor without hydrogenation, 2) good quality salad oil with high polyunsaturated and low saturated fatty acid levels, and 3) enough solids to make margarines and shortenings without hydrogenation (Table 1). Thus, the respective nutrition-related goals for altering the fatty acid composition of corn oil include: 1) increased monounsaturates, specifically oleic acid, to reduce total plasma cholesterol without reducing the high-density lipoprotein (HDL)-cholesterol level, 2) low total saturates to reduce plasma cholesterol, and 3) high total saturates to manufacture margarines and shortenings without *trans* fats, which raise low-density lipoprotein-cholesterol and lower HDL-cholesterol.

Because of the limited variability for value-added traits in agronomically improved adapted germplasm, biotechnology or

mutagenesis techniques are typically used to alter these traits. We have successfully used alternative approaches to alter corn traits and to broaden the genetic base, by introgressing adapted germplasm with useful genes from germplasm developed through the Germplasm Enhancement of Maize (GEM) Project and from Tripsacum. Our past research has shown that we can inbreed exotic corn lines with adapted lines to enhance particular traits, often obtaining lines with wider ranges of these values than in those of the parents. Introgression of Tripsacum with Corn-Belt lines has produced lines with greater percentages of oleic acid and/or reduced percentages of saturated fatty acids than either of the parent lines. Further crossing of these introgressed lines to other Corn-Belt inbreds creates new lines with altered fatty acid profiles and good agronomic traits.

Aims of the study

The objectives of this paper are to report our progress in specialty corn-line evaluation and development for grain composition, including oil quality. Fatty acid profiles of oils from the new lines show promise in providing industry with oils to achieve oil-stability and nutrition-related goals.

Methods

Oil extracted from the germ can be analyzed on a gas chromatograph for its fatty acid methyl ester composition. We have devised a quick set-up for this process (1). Germs from single kernels of corn are removed from the kernel. The germ is hydraulically pressed and hexane-extracted to remove oil. After methylation, fatty acid methyl esters are injected onto a gas chromatograph for a total analysis of the fatty acid profile. The targeted values for lines in our breeding program are lines with <6% low total saturated oil, >17% high total saturated oil, and 50–65% oleic acid for a mid-monounsaturated oil, all for different end-use applications (Table 1).

In genetically diverse open-pollinated populations, we inbreed at least one generation before evaluation. Although there are still genetic differences in the fatty acid profiles among individual kernels, replanting single kernels from the line producing an unusual fatty acid composition will supply enough materials for preservation during the next growing year. Self pollination along with evaluation eventually allows trait fixation.

Genetic diversity in corn germplasm

Many thousands of indigenous corn populations have been collected and deposited in gene banks. It is likely there exists great variation for grain quality traits, such as oil quality, but these populations have rarely been evaluated for these traits, and subsequently used to improve modern corn hybrids. Very little data impacting grain or feed quality has been collected on corn accessions, but that which has been collected suggests significant variability for these traits in corn genetic resources (2–4). Genetic variability for a given trait is important for the crossing, inbreeding, and selection of a breeding program to be successful in moving the trait to economically important levels.

Table 1. Targeted values of oil quality for food and industrial applications.

Fatty acid target	Goal	Potential application
High saturated fatty acids	30%	<i>Trans</i> fatty-acid-free margarine/shortening
Low saturated fatty acids	<6%	Low-saturated-fat labeling on food products
Mid-oleic acid	50-65%	Heart-healthy, stable cooking and frying oil

Limited variability for oil quality, however, especially for mid-oleic corn, is found in present-day hybrids and elite breeding materials (1). Breeding involves crossing a good genotype by another good genotype to select a better genotype, and in the process genetic variation often decreases, especially for traits not under selection. Corn breeders have been very successful in selecting for high yield of grain, but in the process have restricted their breeding efforts to a limited set of total available genetic variation, and through selection have reduced variation even further. It is not likely that most of the favorable alleles needed to improve oil quality would be present in the small array of germplasm selected by breeders for improved grain yield. To overcome this problem, we have evaluated and used exotic germplasm, and breeding lines introgressed with exotic and wild relative germplasm, to identify and develop lines with improved oil quality.

Genes from exotic germplasm

Our first survey of fatty acid profiles in exotic germplasm evaluated lines developed from a self pollination (S_1) of 20 populations included in LAMP, an international project to evaluate agronomic characteristics of corn accessions in U.S. and Latin American germplasm banks for use in breeding programs (2). We found wide ranges of fatty acid composition, and lines with unusual values. The extreme variation in this set of materials was for low and high total saturated fatty acids of 8.3% to 20.2%. The highest value for oleic acid was 46.1%, a value within the range sometimes found in adapted corn breeding materials. The highest yielding populations from LAMP were used in the GEM project to cross to elite commercial inbreds, from which lines with increased genetic variation for useful traits were developed in a cooperative relationship between the public and private corn breeding sectors (5). We evaluated fatty acid profiles of a LAMP population, FS8A(T), also known as PI 53662, and a small sample of lines developed from its breeding cross with two elite inbreds. Wide variation was found, in particular for oleic acid (Table 2).

Because our results were so encouraging in the small sample, we decided to practice more intensive inbreeding and selection in this breeding cross to develop lines with improved profiles of fatty acids. We hypothesized we would find even better results by developing larger numbers of lines. Over 500 S_1 lines were made in 2003, of which 88 were selected on the basis of agronomic performance and advanced to S_2 lines. Although the S_2 lines had wide variation in their fatty acid profiles, the values were not as extreme as found earlier. The range for total saturated fatty acids was 11.3–17.8%, showing that likely progress in developing lines with high or low profiles would be successful (Table 2). The range in oleic acid was only 20.0–46.2%, and progress to reach desired levels of mid-oleic acid would likely be dif-

Table 2. Fatty acid profiles in the original LAMP population, GEM lines derived from a 25% exotic breeding cross, and breeding lines developed from the GEM lines.

Entry	16:0	18:0	18:1	18:2	Total Saturates
LAMP population					
FS8A(T)	12.6	1.9	46.1	38.7	14.6
GEM lines					
FS8A(T):1801-1	11.9	2.2	60.6	24.6	14.0
FS8A(T):1801-5	12.9	1.7	17.8	66.5	14.6
FS8A(T):1801-47	16.9	4.2	36.2	41.8	21.0
FS8A(T):1801-71	12.1	4.4	40.9	41.9	16.5
FS8A(T):1801-55-4	12.5	1.1	38.6	46.9	13.6
Breeding lines					
FS8A(T):N1801-207-1	10.0	1.3	32.2	55.8	11.3
FS8A(T):N1801-191-1	16.5	1.4	21.7	59.6	17.8
FS8A(T):N1801-324-1	12.5	1.4	20.0	65.2	14.0
FS8A(T):N1801-113-1	12.8	2.0	46.2	38.2	14.8

difficult if not impossible with these results. The value of 60.6% found in the earlier sample was measured on individual kernels. Although kernels on an S_1 ear differ genetically, it would still be likely to find other kernels with high values and plant the seed if only part of the embryo was used for the fatty acid analysis. Because the line with the highest oleic value in the larger S_2 sample was 46.2%, it would be necessary to either analyze the previous S_1 generation, resample the breeding cross by making another set of S_1 lines, or cross lines with the higher oleic acid values to lines from other germplasm with higher values in the hope of getting a transgressive segregant with higher values than both parents.

The results for developing these corn lines with oils having high total saturates are encouraging, but the slight reduction in ranges of the extreme values means we will need to work harder to obtain our goals. The reduction in the S_2 lines for mid-oleic acid may have several possible causes. Perhaps if we had analyzed the 500 S_1 lines we may have found more extreme values.

The intense agronomic selection from the S_1 to the S_2 generation may have eliminated the lines of interest for percentage of oleic acid. The environment may have played a role in the results we obtained. Environmental factors have been reported to influence the composition of seeds, resulting in quality changes. Factors such as temperature, soil type, planting date, year, and location can play an important role. More work is needed to illustrate the relationship between environment and properties of corn. More work also is needed to screen sources of the GEM materials so work can focus on the best breeding crosses for a particular trait of interest, such as mid levels of oleic acid.

Genes from *Tripsacum*

Our greatest success to date in improving oleic acid levels has come from introgressing Corn Belt lines with *Tripsacum* germplasm. Thus, most of our effort at increasing this fatty acid has been with these materials. *Tripsacum* is a wild and grassy relative of corn with a typical fatty acid profile of 6.4% palmitic, 1.7% stearic, 34.3% oleic, 57.6% linoleic and 0.8% linolenic acids. *Tripsacum* is a perennial native grass found in the central, eastern and southern United States and is grown as a nutritious forage crop for cattle.

In our current work, four recovered introgressed *Tripsacum* lines were selected, #5 and #13 with mid-oleic acid levels, and #88 and #92 with high total saturated fatty acids (Table 3). They were crossed with Corn Belt lines, W153R, for the mid-oleic acid lines, and with A632 and B73 for the high total saturated fatty acid lines. The resulting S_1 and S_3 backcrosses grown in a randomized complete block design with two replications at two locations in Ames, IA, produced lines averaging as high as 52.1% oleic acid for the mid-oleic acid lines, and as high as 18.8% total saturated fatty acids for that target (Table 3). For these materials, the derivative lines were higher in oleic or saturated fatty acids depending on the donor than either of the parents. Thus, there

Table 3. Means of fatty acid profiles in the *Tripsacum dactyloides* L. materials and crosses (6).

Entry	16:0	18:0	18:1	18:2	18:3	Total Saturates
High Oleic Acid						
Line 1 Germplasm development						
Female (Corn Belt line W153R)	8.4	2.1	33.8	54.0	1.7	10.4
Male (Tripsacum Introgressed #5)	10.2	3.5	42.5	42.9	0.9	13.7
Male (Tripsacum Introgressed #13)	7.9	2.3	43.0	45.7	1.1	10.2
S_1 generation (W153R x #5)#13	8.4	2.3	41.6	46.5	1.2	10.7
S_3 generation (W153R x #5)#13	8.1	2.2	52.1	36.7	0.9	10.3
High Saturated Fatty Acids						
Line 1 Germplasm Development						
Female (Corn Belt line B73)	9.7	1.6	26.2	61.2	1.3	11.3
Male (Tripsacum Introgressed #88)	13.7	3.4	35.2	46.5	1.0	17.1
S_1 Generation (B73 x #88)	12.1	2.1	30.2	54.3	1.2	14.2
S_3 Generation (B73 x #88)	13.7	2.4	33.6	49.0	1.3	16.0
Line 2 Germplasm Development						
Female (Tripsacum Introgressed #92)	13.1	2.8	36.2	47.0	1.0	15.9
Male (Corn Belt line A632)	9.1	1.5	23.1	64.5	1.9	10.6
S_1 Generation (#92 x A632)	12.7	3.1	28.2	55.1	1.1	15.7
S_3 Generation (#92 x A632)	14.1	3.2	31.7	50.0	1.0	17.3
Line 3 Germplasm Development						
Female (Tripsacum Introgressed #88)	13.7	3.4	35.2	46.5	1.0	17.1
Male (Corn Belt line A632)	9.1	1.5	23.1	64.5	1.9	10.6
S_1 Generation (#88 x A632)	13.7	2.2	26.7	56.4	1.0	15.9
S_3 Generation (#88 x A632)	16.1	2.7	29.3	51.0	1.0	18.8

is even greater potential to further improve the lines by enhancing these fatty acid compositions. These new corn lines will be useful for breeding additional corn for oil quality traits.

From the survey of fatty acid compositions in the *Tripsacum*-introgressed germplasm we concluded that there is great potential in this germplasm to make additional gains in breeding for mid-oleic and high total saturated fatty acids, as well as new corn lines with unique combinations that have functional importance, such as a combination of mid-oleic and high stearic acids in the same line. By investigating the variation in fatty acid composition introduced into corn lines from the *Tripsacum* we were able to develop corn lines with improved oil quality.

Conclusions

New corn lines having oils with modified fatty acid composition continue to be developed through our breeding programs. By analyzing corn lines developed from crosses of Corn Belt commercial inbreds with elite exotic materials available through the USDA Germplasm Enhancement of Maize (GEM) program we identified lines with mid-oleic acid (60.6%) and high total satur-

ated fatty acid (21.0%) compositions, but these fatty acid percentages were not as extreme when we tried to replicate or improve the results by selfing in the same breeding cross using a larger sample size. Corn lines developed from crossing Corn Belt hybrids with *Tripsacum* germplasm produced lines with a mid-oleic acid level of 52.1% and a high total saturated fatty acid level of 18.8% in the S_3 generations, suggesting promise of even greater levels in succeeding generations. Developing new breeding crosses using exotic corn germplasm and *Tripsacum*-introgressed lines may yield even wider ranges of genetic diversity for fatty acid profiles.

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