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Susan A. Duvick

United States Department of Agriculture

Linda M. Pollak

Iowa State University

Jode W. Edwards

United States Department of Agriculture

Pamela J. White

Iowa State University, pjwhite@iastate.edu

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Abstract

Breeders need sources of genes for altering the fatty acid content of oil in maize (*Zea mays* L.) that are not available in Corn Belt germplasm. Previously we determined lines developed from maize introgressed with genes from *Tripsacum dactyloides* had useful variation for fatty acid composition. We conducted this study to validate the variation, thereby showing that the trait could be transferred to Corn Belt inbreds using traditional plant breeding methods to create maize lines with altered fatty acid composition useful for an oil quality breeding program. Based on their fatty acid profiles, maize lines were selected from an open pollinated population that was introgressed with genes from *Tripsacum dactyloides*. These introgressed lines were both self-pollinated and backcrossed to Corn Belt lines while undergoing selection for various fatty acid compositions. The parental lines and S1 and S3 progeny from the backcrosses were compared to commercial Corn Belt hybrids and inbreds in an experiment using a randomized complete block design with two replications at two locations near Ames, Iowa. The plants were hand pollinated and hand harvested. The fatty acid compositions were analyzed by using Gas Chromatography to characterize the fatty acid methyl esters made from the oil of five individual kernels from each ear. The relative amounts of the two types of fatty acids of interest, a monounsaturated fatty acid, (oleic acid) and saturated fatty acids (palmitic and stearic acids), were greatly increased by selection breeding within the *Tripsacum* introgressed germplasm. New oil products with more healthful fatty acid compositions and products with reduced trans fats can be developed from these new lines.

Keywords

Zea mays, Oil quality, Germplasm development, Fatty acids separation

Disciplines

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Comments

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ALTERING THE FATTY ACID COMPOSITION OF CORN BELT CORN THROUGH TRIPSACUM INTROGRESSION¹

S.A. Duvick^{2,*}, L.M. Pollak³, J.W. Edwards³, P.J. White⁴

² USDA-ARS Plant Introduction Station Research Station, Ames, Iowa, 50011, USA

³ USDA-ARS Corn Insects and Crop Genetics Research Unit, Department of Agronomy, Iowa State University, Ames, IA 50010, USA

⁴ Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50010, USA

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ABSTRACT - Breeders need sources of genes for altering the fatty acid content of oil in maize (*Zea mays* L.) that are not available in Corn Belt germplasm. Previously we determined lines developed from maize introgressed with genes from *Tripsacum dactyloides* had useful variation for fatty acid composition. We conducted this study to validate the variation, thereby showing that the trait could be transferred to Corn Belt inbreds using traditional plant breeding methods to create maize lines with altered fatty acid composition useful for an oil quality breeding program. Based on their fatty acid profiles, maize lines were selected from an open pollinated population that was introgressed with genes from *Tripsacum dactyloides*. These introgressed lines were both self-pollinated and backcrossed to Corn Belt lines while undergoing selection for various fatty acid compositions. The parental lines and S₁ and S₃ progeny from the backcrosses were compared to commercial Corn Belt hybrids and inbreds in an experiment using a randomized complete block design with two replications at two locations near Ames, Iowa. The plants were hand pollinated and hand harvested. The fatty acid compositions were analyzed by using Gas Chromatography to characterize the fatty acid methyl esters made from the oil of five individual kernels from each ear. The relative amounts of the two types of fatty acids of interest, a monounsaturated fatty acid, (oleic acid) and saturated fatty acids (palmitic and stearic acids), were greatly increased by selection breeding within the *Tripsacum* introgressed germplasm. New oil products with more healthful fatty acid compositions and products with reduced trans fats can be developed from these new lines.

KEY WORDS: *Zea mays*; Oil quality; Germplasm development; Fatty acids separation.

¹ This paper is respectfully dedicated to Dr. Donald N. Duvick with admiration for his many accomplishments in maize genetics and breeding.

* For correspondence (fax: +1 515 294 9359; e.mail: sduvick@iastate.edu).

INTRODUCTION

Maize oil has many uses and good flavor, but its nutritional quality would be improved by altering the fatty acid composition. WEBER (1978, 1987) looked at the fatty acid make up of the germ, endosperm, pericarp, tip cap, root and leaf and found the germ contains approximately 80% of the kernel lipid. The maize inbreds in Weber's study, had an average fatty acid composition of 11% palmitic (C16:0, a saturated fatty acid), 2% stearic (C18:0, a saturated fatty acid), 24.1% oleic (C 18:1, a monounsaturated fatty acid), 61.9% linoleic (C18:2, a polyunsaturated fatty acid), and 0.7% linolenic (C18:3, a polyunsaturated fatty acid). WHITE and WEBER (2002) noted altering the fatty acid content of maize oil by increasing the oleic acid composition would enhance oxidative stability. Oleic acid has only one unsaturated bond susceptible to oxidation compared to two or more unsaturated bonds found in polyunsaturated fatty acids linoleic and linolenic. The increased oleic acid also would provide a more healthful fatty acid composition that could decrease coronary heart disease (MATTSON and GRUNDY, 1985). Developing maize oil with other arrangements of altered fatty acid compositions also would be beneficial. For example, increased saturated fatty acid composition (palmitic, C16:0 and stearic, C18:0) of the oil would allow food manufacturers to produce margarines without hydrogenation and the subsequent formation of undesirable trans fatty acids. To address health-conscious consumers' demands for unsaturated, trans-free spreads for their diets, manufacturers are looking for new sources of natural oils with increased oleic and saturated fatty acids.

Corn Belt maize from the U.S. was shown by BEADLE *et al.* (1965) to have very little variation for fatty acid content. For example, they evaluated 103 samples of refined maize oils produced commercial-

ly over a period of 2.5 years and found that the linoleic acid content averaged 55.5% and 93% of these values were within two units of this value. Plant breeders can use traditional plant breeding methods to develop new maize lines with altered fatty acid compositions if the available germplasm has sufficient genetic variation for the fatty acid traits. JELLUM (1970) surveyed the fatty acid profiles of 144 plant introductions representing 52 foreign countries and 33 open-pollinated varieties from various regions of the United States. He found a wide range of fatty acid values, 6-22% for palmitic, 0.6-15% for stearic, 14-64% for oleic, and 19-71% for linoleic acid. DUNLAP *et al.* (1995a) analyzed the fatty acid profiles of 418 maize hybrids and 98 maize inbreds from adapted elite germplasm and 1000 exotic maize lines (DUNLAP *et al.*, 1995b). These exotic lines showed a wider range for fatty acids than the adapted elite germplasm but the variation was deemed to be too narrow to make selections for altering oleic acid composition. PONELEIT *et al.* (1965) researched the inheritance of linoleic and oleic acids in two related parental maize lines, R84 and Illinois High Oil, and the segregating generation from the cross of the two lines. They found that the low linoleic is dominant to high and low oleic is recessive to high. DE LA ROCHE *et al.* (1971) also studied the inheritance of linoleic and oleic acids in the same two maize lines, R84 and IHO, and confirmed their hypothesis that the *ln* locus controlled the relative amounts of oleic and linoleic acids in these lines. They suggested that a single gene with incomplete dominance controlled 20% of the difference of oleic and linoleic acids with a considerable maternal effect in the unrelated lines C103 and R84. But in the progeny of other unrelated lines, C103 and IHO, they found evidence that two loci are responsible for the relative oleic and linoleic content. In a later study, WIDSTROM and JELLUM (1975) studied six maize inbreds and found the inheritance of palmitic, oleic, and linoleic acid was controlled by additively whereas additive and dominance provided equal explanations of variation for stearic acid. JELLUM and WIDSTROM (1983) studied the inheritance of stearic acid in inbreds with 2% stearic, plant introductions with 10% stearic, their crosses and progeny and backcross progeny and found evidence supporting a single recessive gene for high stearic and the segregation in the progeny indicated one or more modifying genes had some effect on stearic acid content. PLEWA and WEBER (1975) measured palmitic, stearic, oleic and linoleic acids in diploid and Monosomic embryos to determine the

dosage effect of chromosomes on the fatty acid content of maize embryo lipids. They found a Monosomic group (group 2) had higher palmitic and stearic acid contents than in the diploid plants and this finding supported the idea that since palmitic is a precursor to stearic and stearic is a precursor of oleic acid that if the desaturation of oleic to linoleic were impaired, there should be an increase in palmitic and stearic acids. SUN *et al.* (1978) investigate the mode of inheritance for palmitic and stearic acids in two diverse maize lines, their cross, progeny and backcross and reciprocal backcross. They concluded the inheritance of stearic followed the single gene model but palmitic inheritance was less clear but probably followed single gene Mendelian inheritance. PLEWA and WEBER (1975) measured palmitic, stearic, oleic and linoleic acids in diploid and Monosomic embryos to determine the dosage effect of chromosomes on the fatty acid content of corn embryo lipids. They found a Monosomic group (group 2) had higher palmitic and stearic acid contents than in the diploid plants and this finding supported the idea that since palmitic is a precursor to stearic and stearic is a precursor of oleic acid that if the desaturation of oleic to linoleic were impaired, there should be an increase in palmitic and stearic acids. WIDSTROM and JELLUM (1984) found evidence of genes on the long arm of chromosome 5 that have a major effect on oleic and linoleic acids in maize germ oil in inbred line GE82. Also they suggested that in inbred X-187 a recessive gene on the long arm of chromosome 4 controls high linoleic acid. PAMIN *et al.* (1985) used recurrent breeding methods in 89 interpopulation full-sib families to increase oil quantity while retaining the desired fatty acid composition. Their selection criterion included high oil, high total unsaturated fatty acids and high linoleic content. ALREFAI *et al.* (1995) used restriction fragment length polymorphism (RFLP) to measure the number and location of quantitative trait loci in 200 S1 lines derived from a cross between IHO and ILO. They found 15 RFLP loci on 12 chromosomal regions associated with the amount of palmitic acid, 17 loci on 10 regions were associated with stearic, 12 loci on eight regions related to oleic and linoleic acids, and 17 loci in eight regions related to linolenic acid. They also noted a positive correlation between increased oil content and oleic acid.

In addition to native and exotic germplasm as sources of genes for altering the fatty acid profile of maize in a breeding program, there are potentially new genes from wild relatives of maize.

Tripsacum dactyloides (Tripsacum), a warm weather perennial grass native to eastern and southwestern United States and South America, is a wild relative of maize. BURKHART *et al.* (1994) measured the fatty acids in *Tripsacum dactyloides* in 23 populations (2N to 4N ploidy) from 13 environments. They found Tripsacum oil had low saturated, high oleic and low linolenic fatty acids. This composition is desirable for an edible oilseed crop. EARLIER *et al.* (1973) analyzed the fatty acid variation in Tripsacum and found a range for C16:0 (palmitic acid) of 7.7 to 19.3%; C18:0 (stearic acid) was 0.9 to 4.2%; C18:1 (oleic acid) was 26.0 to 41.7%; C18:2 (linoleic acid) was 47.6 to 62.7%; and C18:3 (linolenic acid) was present in trace amounts.

Tripsacum can be hybridized and backcrossed to maize to form introgressed hybrids. MAGUIRE (1960) showed segments of Tripsacum chromatin were successfully transferred to the maize chromosome. In their studies of maize evolution, HARLAN and DE WET (1977) investigated introgressing genes from Tripsacum into maize. They postulated since Tripsacum is adapted to many diverse growing regions and climates it must hold a vast array of genetic variability and could be useful for diversifying the

maize genome. They investigated 54 combinations of derivatives of maize x Tripsacum hybrids and were able to recover stable maize lines.

GRAY and NEWELL (1973) also found by backcrossing the Tripsacum introgressed maize lines several times to maize they were able to generate balanced maize genomes. These plants emerged as recovered maize.

BERGQUIST (1977) found dominant resistance to six maize diseases in BC₈ (backcrossed to maize 8 times) populations of Tripsacum x maize hybrids including anthracnose, fusarium stalk rot, northern maize leaf blight, southern maize leaf blight, common rust and Stewart's bacterial blight.

DE WET *et al.* (1969, 1972, 1974b, 1977) and HARLAN and DE WET (1977) made interspecific crosses between *Zea mays* L. and tetraploid *Tripsacum dactyloides* (L.). They observed barriers to crosses between *Zea* and Tripsacum could be overcome by selecting compatible parents. He and his colleagues were able to make populations from open pollinated, Tripsacum-introgressed maize crosses. They used *Z. mays* as the female parent and *T. dactyloides* as the male parent.

DUVICK *et al.* (2003) selected maize lines intro-

TABLE 1 - Germplasm and sources.

Line	Pedigrees	Source	Reason for inclusion
#5	Recovered Parental line	Hawaii 1997 and Puerto Rico 1994/95	Elevated oleic acid
#13	Recovered Parental line	Iowa 1997 and Puerto Rico 1994/95	Elevated oleic acid
#88	Recovered Parental line	Iowa 1994 and 1997	Elevated palmitic and stearic
#92	Recovered Parental line	Iowa 1994 and 1997	Elevated palmitic and stearic
A632	Corn Belt Stiff Stalk Inbred line	Iowa 1994	Stiff Stalk Heterotic group
B73	Corn Belt Stiff Stalk Inbred line	Iowa 1994	Stiff Stalk Heterotic group
Mo17	Corn Belt Non-Stiff Stalk Inbred line	Iowa 1997	Non-Stiff Stalk Heterotic group
W153R	Corn Belt inbred	Iowa 1997	Other Heterotic group
#88 x A632	S1 of Breeding Cross	Iowa 1994	Elevated palmitic and stearic
B73 x #88	S1 of Breeding Cross	Iowa 1994	Elevated palmitic and stearic
#92 x A632	S1 of Breeding Cross	Iowa 1994	Elevated palmitic and stearic
(W153R x #5)#13	S1 of Breeding Cross	Iowa 1995	Elevated oleic, reduced saturated fatty acids
S3 of #88 x A632	S3 Derivative line	Hawaii 1997	Elevated palmitic and stearic
S3 of #92 x A632	S3 Derivative line	Hawaii 1997	Elevated palmitic and stearic
S3 of B73 x #88	S3 Derivative line	Hawaii 1997	Elevated palmitic and stearic
S3 of (W15R x #5)#13	S3 Derivative line	Hawaii 1997	Elevated oleic, reduced saturated fatty acids
Public Hybrid 1	Corn Belt Hybrid	Iowa 1994	Typical Corn Belt fatty acids
Commercial Hybrid 1	Corn Belt Hybrid	Circa 1998	Typical Corn Belt fatty acids
Commercial Hybrid 2	Corn Belt Hybrid	Circa 1998	Typical Corn Belt fatty acids

TABLE 2 - Means of relative fatty acid contents (%)^a for each pedigree.

Group	16:0	18:0	18:1	18:2	18:3	SATS ^b
High Oleic						
Line 1 Germplasm development						
Female (Corn Belt line W153R)	8.4a	2.1a	33.8a	54.0d	1.7d	10.4a
Male (Tripsacum Introgressed #5)	10.2b	3.5b	42.5b	42.9b	0.9a	13.7b
Male (Tripsacum Introgressed #13)	7.9a	2.3a	43.0b	45.7c	1.1bc	10.2a
S1 generation (W153R x #5)#13	8.4a	2.3a	41.6b	46.5a	1.2c	10.7a
S3 generation (W153R x #5)#13	8.1a	2.2a	52.1c	36.7a	0.9ab*	10.3a
High Saturated Fatty Acids						
Line 1 Germplasm Development						
Female (Corn Belt line B73)	9.7a	1.6a	26.2a	61.2c	1.3ab	11.3a
Male (Tripsacum Introgressed #88)	13.7c	3.4b	35.2b	46.5a	1.0a	17.1d
S1 Generation (B73 x #88)	12.1b	2.1a	30.2a	54.3b	1.2ab	14.2b
S3 Generation (B73 x #88)	13.7c	2.4a	33.6b	49.0a	1.3b	16.0c
Line 2 Germplasm Development						
Female (Tripsacum Introgressed #92)	13.1c	2.8b	36.2c	47.0a	1.0a	15.9b
Male (Corn Belt line A632)	9.1a	1.5a	23.1a	64.5d	1.9b	10.6a
S1 Generation (#92 x A632)	12.7bc*	3.1b	28.2ab	55.1c	1.1c	15.7b
S3 Generation (#92 x A632)	14.1b	3.2b	31.1b	50.0b	1.0a	17.3b
Line 3 Germplasm Development						
Female (Tripsacum Introgressed #88)	13.7b	3.4c	35.2c	46.5a	1.0a	17.1c
Male (Corn Belt line A632)	9.1a	1.5a	23.1a	64.5d	1.9b	10.6a
S1 Generation (#88 x A632)	13.7b	2.2ab	26.7ab	56.4c	1.0a	15.9b
S3 Generation (#88 x A632)	16.1c	2.7b	29.3b	51.0b	1.0a	18.8d
Corn Belt Hybrids						
Public	10.2a	1.6a	23.6a	63.5b	1.1a	11.8a
Commercial 1	9.8a	1.8a	22.7a	64.3b	1.3a	11.6a
Commercial 2	9.5a	1.4a	38.5b	49.5a	1.1a	10.9a

^a Fatty acids are identified according to number of carbon atoms and number of double bonds: palmitic acid, 16:0; stearic acid, 18:0; oleic acid, 18:1; linoleic acid, 18:2; and linolenic acid, 18:3.

^b Sats refers to the combined values of 16:0 and 18:0. Values with different letters in a column within a line germplasm group are significant at $p \leq 0.05$.

* Standard errors of the means varied among the individual values thus assignment of significance between numbers of the same value are not always the same.

gressed with genes from *Tripsacum dactyloides* from one of these populations for several combinations with altered fatty acids through several cycles of selection and evaluation. Altered fatty acid lines were developed contained either high oleic, low total saturates or high total saturates. This study was undertaken to validate the effect of crossing the progeny derived from a population of maize introgressed with *Tripsacum dactyloides* to Corn Belt inbred lines on the fatty acid profiles of the maize oil. We evaluated four parental lines introgressed with *Tripsacum* and the offspring of backcrossed Corn Belt inbred lines.

MATERIALS AND METHODS

Germplasm development

In this study, new maize lines were developed from a population of maize introgressed with *Tripsacum dactyloides* created by De Wet and Harlan in the 1960's and 1970's (DE WET *et al.*, 1969, 1972; DE WET and HARLAN, 1974b; HARLAN and DE WET, 1977; STALKER *et al.*, 1977). Of the Recovered Introgressed Lines maize lines, 95 self-pollinated ears were tested for fatty acid content and categorized as high oleic (3 lines), high polyunsaturated (5 lines), and high total saturated fatty acids (6 lines). The seed from the selected lines were planted in a crossing block in the field nursery. Several individual plants from each category self pollinated or crossed to a plant from inbred lines of A619, A632, B14A, B73, H99, Mo17, Oh43, and W153R known as the Corn

Belt inbred lines. At each generation of self-pollination, the Recovered Introgressed Lines and lines derived from crossing the introgressed lines to Corn Belt inbred lines were tested and selected based on their altered fatty acid profiles. At each generation the fatty acid profiles of the lines were compared to fatty acid profiles of the Corn Belt lines grown as checks in the same nursery. Not all of the useful germplasm from the introgressed lines and backcrosses to Corn Belt inbreds could be used for this study because of the size and scope of the project. Four recovered introgressed lines were selected for this study, #5 and #13 with high oleic acids and #88 and #92 with high total saturated fatty acids, along with their Corn Belt crosses to A632, B73 and W153R, their derivative S₃ backcrosses, two commercial hybrids, one public hybrid (B73 x Mo17), and the inbred lines of A632, B73, Mo17, and W153R (Table 1). In order to have enough seed for multiple plantings, seed of the same generations but from different seasons were combined (Table 1) for the parental lines. Breeding crosses and progeny were planted in two locations with two replications in a randomized complete block at the Iowa State University Agronomy and Sorensen farms near Ames, Iowa.

Although it might have been preferable to sample more environments or years, only two were used because the maize had to be hand pollinated and therefore needed to be close to Ames, In addition, previous studies by JELLUM and MARION (1966) and PONELEIT and BAUMAN (1970) found that oil quality traits were more affected by genotype than the planting date, location and or year. The ears from each genotype were hand harvested, dried, shelled, and monitored in cold storage until analyzed.

Laboratory analysis

The fatty acid content of the maize material was measured by following a modified method as described by DUNLAP *et al.* (1995a) originally designed for soybeans (HAMMOND, 1991). For this method individual maize kernels (five from each ear) were selected from the central portion of the ear as suggested by WHITE and WEBER (2003) to minimize the variance of oil content based on position on the ear (JELLUM, 1967; LAMBERT *et al.*, 1967) and placed in individual wells of a 50 well aluminum crushing plate. The opposing top plate with pegs the diameter of the wells was fitted onto the bottom plate and placed on the platen

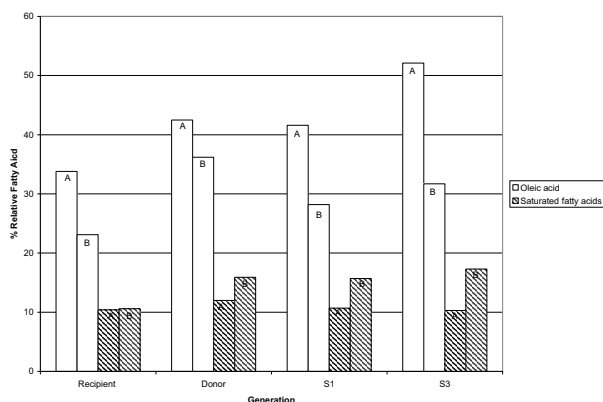


FIGURE 1 - Fatty acid comparison for two corn lines named High Oleic Acid (A) and High Total Saturated Fatty Acids (B).

of a hydraulic press. The samples were pressed to 40,000 psi effectively crushing the kernels and exposing the germ and endosperm. To extract the oil, hexane (1ml) was added to each well of the crushing plate, covered with a glass pane and steeped overnight at room temperature. The next day, 200 μ L of the oil and hexane solution were transferred into 1ml auto sampler vials.

To prepare fatty acid methyl esters the oil samples in the auto sampler vials had, 500 μ L of 1N sodium methylate in methanol added and allowed to react at room temperature for two hours with occasional shaking. The reaction was stopped by adding 150 μ L of water and the vials were topped off with hexane.

Fatty acid separation and identification (HAMMOND, 1991) was accomplished by injecting one μ L of the sample onto a 15 m Durabond-23 capillary column (J&W Scientific, Deerfield, IL.) with an internal diameter of 0.25 mm and a film thickness of 0.25 μ and a flame ion detector and integrator in a Hewlett Packard 5890 series II gas chromatograph (Hewlett Packard, Avondale, PA). The oven temperature was set at 220°C and the column and injection ports were set at 250°C. The peak integration and area were compared against GLC 64 standards (Nu Prep Check, Elysian, MN) to identify and quantify the relative fatty acids.

Data analysis

Data were analyzed using a mixed linear model to account for multiple levels of random variation. A separate analysis was done for each of the five fatty acids. Locations, replicates within locations, and genotypes were fixed effects. Seed sources within genotypes, rows within seed sources, ears within rows, and kernels within ears were all random effects. Kernels within ears constituted the residual (error) source of variation. Variance components were estimated using Restricted Maximum Likelihood. Best Linear Unbiased Estimators were subsequently obtained for the mean values of each of the five fatty acids for each of the 19 experimental pedigrees. Standard errors were computed for each least squares mean and p-values computed using contrasts for all paired comparisons of interest. Individual least squares means for each pedigree were required because there was considerable imbalance in the sample sizes in the data for the random sources of variation. The number of seed sources varied from one to 16 for introgression pedigrees with a total of 72 seed sources across the 19 introgression and check pedigrees. The number of rows planted per seed source varied from one to eight with an average of four. The number of ears used per row varied from one to 13 with an average of 3.4. The number of kernels per ear that were evaluated was very consistent with almost all ears having five kernels.

RESULTS

The recovered parental introgressed lines were distinctively different from the Corn Belt lines in their fatty acid profiles (Table 2). The lines #5 and #13 were selected at each generation for their high oleic acid values. The resulting mean value was 42.8% Oleic acid. When crossed to W153R which had an inherent higher oleic acid percentage (33.8% oleic acid) than the other Corn Belt inbred lines (mean of 23.6% oleic acid), there appears to be het-

TABLE 3 - Standard error of relative fatty acid contents^a for each pedigree.

Name	16:0	18:0	18:1	18:2	18:3	SATS ^b
W153R	0.96	0.57	2.96	2.89	0.19	0.79
#5	0.27	0.15	0.86	0.84	0.06	0.22
#13	0.49	0.29	1.52	1.48	0.10	0.40
S1 of (W153R x #5) #13	0.43	0.25	1.30	1.27	0.08	0.35
S3 of (W153R x #5)#13	0.60	0.34	1.90	1.86	0.15	0.49
B73	0.96	0.57	2.93	2.87	0.19	0.79
#88	0.56	0.33	1.71	1.67	0.12	0.46
S1 of B73 x #88	0.67	0.40	2.04	1.99	0.13	0.55
S3 of B73 x #88	0.32	0.18	1.01	0.99	0.08	0.26
#92	0.49	0.29	1.52	1.49	0.11	0.40
A632	1.02	0.58	3.20	3.13	0.23	0.83
S1 of #92 x A632	0.95	0.56	2.89	2.82	0.19	0.78
S3 of #92 x A632	0.33	0.19	1.02	1.00	0.07	0.27
#88	0.56	0.33	1.71	1.67	0.12	0.46
A632	1.02	0.58	3.20	3.13	0.23	0.83
S1 of #88 x A632	0.58	0.34	1.79	1.75	0.13	0.47
S3 of #88 x A632	0.50	0.29	1.55	1.52	0.12	0.41
S1 Public Hybrid	0.96	0.57	2.92	2.86	0.21	0.79
S1 Commercial Hybrid 1	0.97	0.57	2.99	2.92	0.21	0.80
S1 Commercial Hybrid 2	0.96	0.57	2.93	2.87	0.21	0.79
Mo17	0.96	0.57	2.92	2.86	0.19	0.79

^a Fatty acids are identified according to number of carbon atoms and number of double bonds: palmitic acid, 16:0; stearic acid, 18:0; oleic acid, 18:1; linoleic acid, 18:2; and linolenic acid, 18:3.

^b Sats refers to the combined values of 16:0 and 18:0.

erosis because the S₁ generation had a mean of 42% oleic acid, which is higher than the mid-parent value of 39%. There was another increase in heterosis in the S₃ generation of the derivatives resulting in a value of 52% oleic acid. The standard errors were much larger for the Corn Belt material than for the recovered introgressed parental lines, the crosses with the Corn Belt inbreds and the derivative lines (Table 3).

Additionally, because the breeding cross and derivative lines of parents #5, #13 and W153R were not selected for higher or lower total saturated fatty acids it is interesting that those values remained steady over the generations (Fig. 1). Total saturated fatty acid values remained within a range of 10 to 12%, values considered to be normal for maize.

The lines selected for elevated total saturated fatty acids, #88 and #92 recovered introgressed parents, had an average of 16.5% total saturated fatty acids compared to the Corn Belt inbreds, A632, B73 and Mo17, which had 11% total saturated fatty acids

(Table 2). There seemed once again to be heterosis in the hybrids because the S₁ generation had an average value of 15% and the S₃ generation had an average of 17% total saturated fatty acids. The greatest standard error for total saturated fatty acids (sats) (Table 3) was found in the Corn Belt inbreds, A632, B73 and Mo17, Public Hybrid, Commercial Hybrid 1 and Commercial Hybrid 2, with 0.8 standard error of the mean (SEM) compared to the *Tripsacum* introgressed lines, #88 and #92, with 0.4 SEM, the S1 derivatives with 0.5 SEM and the S3 derivatives with 0.3 SEM. The germplasm selected for studying the high total saturated fatty acids had crosses made both ways (with the *Tripsacum* introgressed parent as the female, #88 x A632 and #92 x A632, and the *Tripsacum* introgressed parent as the male, B73 x #88). The total saturated fatty acid values were slightly greater with the *Tripsacum* introgressed parent as the female with 16% saturated fatty acids for introgressed as female versus 14% with

the Corn Belt inbreds as the female for S₁, and 18% total saturated fatty acids with the *Tripsacum* introgressed parent as the female versus 16% with the Corn Belt parent as the female for S₃. There were essentially no differences among the public hybrid, B73, or Mo17, with 12% total saturated fatty acids, and the two commercial hybrids with 11% total saturated fatty acids. WEBER (1987), in a survey from 1975, found that A632 (67% linoleic) and Mo17 (68% linoleic) were estimated to be used in 22% of hybrids produced, are readily recognized, and useful reference checks for Corn Belt inbreds.

Our general conclusion is that there is great potential in this germplasm to make additional gains in breeding for high oleic and high total saturated fatty acids as well as new maize lines with unique combinations that have nutritional importance such as a combination of high oleic and high stearic acids in the same line. Developing new breeding crosses using exotic maize germplasm and *Tripsacum* introgressed lines may yield even wider ranges of genetic diversity within and among fatty acid profiles.

Tripsacum introgressed maize lines have a wider range for nutritionally important fatty acids than Corn Belt dent maize lines. By investigating the variation in fatty acid composition introduced in to maize lines from the *Tripsacum* we were able to develop maize lines with improved oil quality.

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