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Fatty Acid Patterns of Genetically Modified Cry3Bb1 Expressing *Bt*-Maize MON88017 and Its Near-Isogenic Line

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Fatty acid (FA) profiles of the *Bt*-maize line MON88017 expressing the Cry3Bb1 protein and its near-isogenic line DKC5143 were examined. Plant compartments under study included leaves taken from different internodes and roots. Sample preparation involved pressurized liquid extraction (PLE) of the biomass, transmethylation of the extracted lipids to give fatty acid methyl esters (FAMEs), and finally GC-MS analysis. The essential quality parameters for the FA profiles included total FA and sum of saturated FA, as well as double-bond index (DBI). FA profiles of the roots—characterized by high concentrations of homomorphic FA including palmitic and stearic acid, along with low concentrations of polyunsaturated surrogates—revealed high similarity between the genetically modified and the near-isogenic line. In contrast, FA profiles of the leaves showed significant differences: higher total FA concentrations and higher DBI were observed for the near-isogenic line. This was overwhelmingly associated with lower concentrations of α-linolenic acid (18:3ω3,6,9ccc) in the genetically modified leaf samples. These differences were particularly pronounced for leaves taken from the fourth elongated, above-ground internode. Given the large reported variability in the population of maize lines, MON88017 and its near-isogenic line can be regarded as equivalent with regard to their fatty acid profiles, despite the differences observed for the leaves. Further experiments are needed to assess whether the genetic modification of *Bt*-maize plants might induce unintended effects with regard to FA profiles.

KEYWORDS: ***Bt*-maize; MON88017; fatty acids; GC-MS coupling; pleiotropic effects**

INTRODUCTION

Assessments of the food safety and nutritional value of both human food and animal feed derived from genetically modified (GM) crops require detailed knowledge of the plant composition (*1*). Possible pleiotropic effects caused by the integration and expression of the genomic sequences introduced into genetically modified crops need to be assessed with regard to changes in the nutritional value and digestibility, as well as other factors, such as resistance against abiotic and biotic stressors. Most recently, lignin patterns of the genetically modified *Bt*-maize line MON88017 expressing the Cry3Bb1 protein to combat the Western corn rootworm *Diabrotica virgifera virgifera* (LeConte; Coleoptera: Chrysomelidae) and of the corresponding near-isogenic line DKC5143 were studied (*2*). The patterns were detailed at the molecular level using analytical thermochemolysis of leaf and root samples taken from a field-release site. In general, they proved to be virtually identical for the GM and near-isogenic lines, indicating a substantial equivalence of the two.

The objective of this work was to study the fatty acid (FA) patterns of root and leaf samples isolated from the GM line MON88017 and its near-isogenic counterpart. FAs, the key components of all living cells, are among the constituents that define macroscopic plant characteristics, including forage digestibility and feed intake, as well as health and stress tolerance (drought, salinity, and temperature as well as attacks by saprophages and herbivores) (*3*, *4*). They can be regarded as

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important indicators in assessing the substantial equivalence of GM plant lines to their near-isogenic counterparts. The comparison between and thus the establishment of equivalence of these two was the focus, because it is generally hypothesized that genetic modification has unintended effects on plant characteristics.

Two independent methods to elucidate FA profiles were used. Freeze-dried samples were first subjected to exhaustive pressurized solvent extraction (PLE), as detailed in Poerschmann and Carlson (*5*), followed by transmethylation of the organic extracts to yield volatile fatty acid methyl esters (FAMEs) amenable to GC-MS analysis. Thermochemolysis as used in the study of the lignin patterns (*2*) was applied as the second method, because it results in the simultaneous release of FAMEs. However, the significance and scope of the thermoanalytical approach were restricted to saturated and monounsaturated fatty acid surrogates, because di- and polyunsaturated fatty acids are prone to decomposition when this harsh treatment is used (*6*).

MATERIALS AND METHODS

Chemicals and Maize Samples. The genetically modified maize line MON88017 along with its near-isogenic counterpart DKC5143 (both from Monsanto Co., St. Louis, MO) were harvested at a field-release research site in southern Germany. Two kinds of leaves ("old" and "young"), as well as root samples were used, as in the lignin pattern study (*2*). Old leaves were taken from the fourth elongated, above-ground internode, whereas young leaves were taken from the fourth internode from the top of the stem. All plants grew under identical conditions and were harvested at the same stage of development at approximately BBCH 75 (*7*), in order to balance out potential differences in the intracellular FA patterns during plant development as indicated by Feussner and Wasternack (*8*). Ten randomly selected plants of each variety were harvested. The roots and leaves of the harvested maize plants were cut, homogenized, and freeze-dried. Roots were washed cautiously with distilled water to remove adhering soil matrix. After freeze-drying, samples from each plant of a given variety were combined and carefully homogenized. Four equal freeze-dried subsamples (about 2 mg each) of each plant compartment belonging to each maize line were subjected to the preparation scheme detailed below.

Identification of FAMEs was performed using an authentic standard FAME mix (FAME mix C_8−C_{22}, Supelco, Munich, Germany; catalog no. 18920-1; common shorthand designation of FAMEs used throughout the text). Isotopically labeled internal standard 2,2′-d_2-palmitic acid was also purchased from Supelco. Pesticide residue-grade chloroform and methanol used as solvents in PLE, as well as *n*-hexane used in the extraction of FAMEs, were from Merck (Darmstadt, Germany). Solvents were degassed prior to use in PLE. Trimethylchlorosilane was purchased from Supelco.

PLE and Transmethylation. Twenty milligram aliquots of the freeze-dried samples were subjected to PLE using an ASE 200 accelerated solvent extraction system (Dionex, Idstein, Germany). The extraction was performed using a chloroform/methanol (1:1 v/v) solvent mixture at 110 °C and two extraction cycles of 10 min each (experimental details are given in ref *5*). The combined extracts were spiked with isotopically labeled internal standard (1000 μg g^{-1} referred to dry biomass). The extracts were dried with sodium sulfate, and the volume was reduced to about 500 μL using a Turbo-Vap II evaporator (Zymark, Idstein, Germany). Preparation of FAMEs using a trimethylchlorosilane/methanol mixture (1:9 v/v) was performed according to the method of Poerschmann et al. (*9*). A 1 μL aliquot of FAMEs in *n*-hexane was subjected to GC-MS analysis. Extractions were performed twice for each sample, and each extract was analyzed twice by GC-MS (see below). The corresponding data points from these four runs were averaged. Generation of FAMEs by thermochemolysis is described in Poerschmann et al. (*2*).

GC-MS Coupling. An Agilent HP 6890 GC operated in splitless injection mode and equipped with an HP 5973B mass selective detector (MSD) was used. Data acquisition was performed in full-scan mode.

Table 1. FA Profiles (as FAMEs) of the Genetically Modified and the Near-Isogenic Maize Root Samples (Concentration in Micrograms per Gram Referred to Dry Biomass)[a]

		compartment	
FAME	ion[b] (amu)	genetically modified	near-isogenic
Σ FAME (C_{12-20})	74, 55, 67, 79	2860	2765
Σ saturated FAME (C_{12-20})	74	2250	2140
DBI	74, 55, 67, 79	0.52	0.48
Σ odd-numbered	74 (256, 284)	124	99
lauric (12:0)	74 (214)	59	38
myristic (14:0)	74 (242)	116	126
C_{15}-branched	74 (256)	∼25	∼20
pentadecanoic (15:0)	74 (256)	57	45
palmitic (16:0)	74 (270)	1200	1060
heptadecanoic (17:0)	74 (284)	40	33
stearic (18:0)	74 (298)	545	720
Σ 18:1	55 (296)	410	350
linoleic (18:2)	67 (294)	305	285
linolenic (18:3)	79 (292)	51	38
arachic (20:0)	74 (326)	39	37
3-OH-butyric	43 (104)	33	21
benzoic	105 (122)	39	24
malonic	101 (132)	67	54
levulinic	43 (130)	7430	7080
methyl maleate + methylene succinate	127 (158)	205	215
malic[c]	103 (162)	325	300
aconitic	153 (216)	285	275

[a] Average of four measurements; RSDs for FAMEs and functionalized methyl esters between 4.5 and 13% (significantly higher data scatter for surrogates of lower abundance, including 15:0 and 17:0). [b] Quantification ion (in parentheses: molecular weight of the methyl ester in D). [c] Sum of the enantiomers (L-isomer prevailing).

Quantification of FAMEs was performed using McLafferty ions of *m*/*z* 74 amu for saturated, *m*/*z* 55 amu for monounsaturated, *m*/*z* 67 amu for diunsaturated, and *m*/*z* 79 amu for triunsaturated FAMEs. The *m*/*z* 76 amu McLafferty ion of the deuterated palmitic acid served as the calibration ion. The ions used to quantitate the individual functionalized acids are listed in **Table 1**. Abundant sterols, including β-sitosterol, were quantitated using their molecular ions in relation to the abundance of the *m*/*z* 76 amu ion. The GC-MS analysis parameters were the following: 30 m × 0.25 mm × 0.25 μm DB-5 fused silica capillary column (Agilent, Waldbronn, Germany) for thermochemolysis, and 30 m × 0.25 mm × 0.25 μm SolGelWax (SGE, Darmstadt, Germany) for PLE-derived FAMEs; initial temperature, 40 °C, 2 min hold, linear ramp at 12 °C min^{-1}, final temperature, 290 °C, with a 5 min hold applied in both cases.

Data Analysis and Statistics. Statistical data were calculated on the basis of the plant compartments of the two different plant lines (genetically modified versus near-isogenic). If not stated otherwise, data of four replicates were used. The data listed in the tables represent arithmetic means ± standard deviations (SD). The normality of the distribution of the data was tested using the Kolmogorov−Smirnov test. Prior to the analysis, the data were $\log(n_i + 1)$ transformed to ensure homogeneity of the variances. Treatment differences between the mean values of the functional parameters were evaluated by a one-way analysis of variance (ANOVA), followed by the Student−Newman−Keuls test of significant differences at $\alpha = 0.01$ (SigmaStat 2.0, SPSS Inc., Chicago, IL).

Multivariate statistics were calculated using principal component analysis (PCA) using the statistical software package SPSS 10.0 (SPSS Inc.), as described by McSpadden et al. (*10*). PCA computes a compact and optimal description of the data set. The first principal component is the combination of variables that explains the greatest amount of variation. The second principal component defines the next largest amount of variation and is independent of the first principal component. There can be as many possible principal components as there are variables (*11*). In this study, the data set was condensed into two principal components (PC 1 and PC 2). Prior to statistical analysis, all

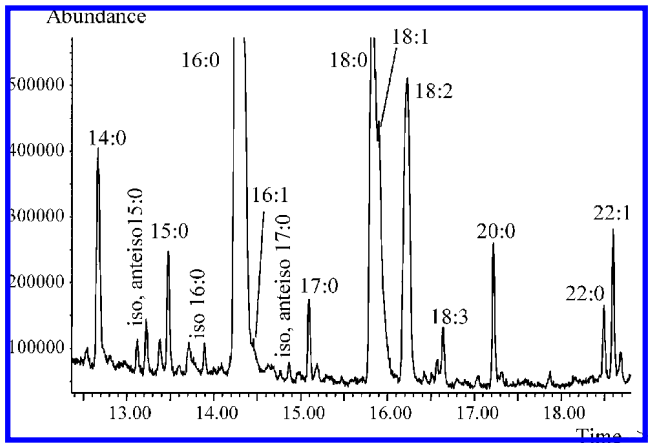

Figure 1. Fatty acid pattern (as FAMEs) of the genetically modified plant root compartment. Peaks: shorthand designation (see text). Data presentation: sum of selected ions *m*/*z* 74 amu (indicative of saturated FAMEs), *m*/*z* 55 amu (monounsaturated), *m*/*z* 67 amu (diunsaturated), and *m*/*z* 79 (polyunsaturated). GC column stationary phase: SolGelWax.

data were log-transformed [$y_i = \log(n_i + 1)$] to ensure homogeneity of the variances (*12*). To compare the FA patterns between each other, the double-bond index (DBI) was used. This index proved to be very useful in characterizing the nutritional value of plants as food and feed, as well as the environmental fate of the FAs, and is defined as (*13*)

$$\mathrm{DBI} = [(1 \times \%\ \text{monoen}) + (2 \times \%\ \text{dien}) + (3 \times \%\ \text{trien})]/\Sigma(\%\ \text{saturated FAs})$$

RESULTS

Fatty Acid Profiles of Roots. The FAME pattern of the root compartment of the genetically modified maize line MON88017 is illustrated in **Figure 1**. The use of a polar stationary phase (SolGelWax) allowed the separation of FAMEs of identical alkyl chain lengths according to the degree of alkyl chain unsaturation (e.g., retention sequence of the C_{18}-cluster: stearic 18:0 < oleic 18:1 < linoleic 18:2 < linolenic 18:3). As expected, a strong even-over-odd discrimination was evident. The most abundant surrogates included homomorphic palmitic, myristic, and stearic acids, as well as monounsaturated oleic acid (18:1ω9c) and diunsaturated linoleic acid (18:2ω6,9 cc). Detailed consideration of the monounsaturated 18:1 peak cluster provided strong evidence that oleic acid was accompanied by *cis*-vaccenic acid (18:1ω7c), the latter known to be of bacterial origin (*14*).

The results of fatty acid pattern determination for the root samples indicated that the total FA concentrations were very similar for both varieties, with 2860 and 2765 μg g^{-1} for MON88017 and the near-isogenic line, respectively (**Table 1**). The same held true for the corresponding FA profiles.

The data listed in **Table 1** demonstrate that the degrees of unsaturation of the alkyl chains were very similar for both lines (0.52 and 0.48 for the genetically modified and the near-isogenic line, respectively). In addition to the FAs, a variety of functionalized aliphatic acids, including nonvolatile di- and tricarboxylic acids, could be detected. Overall, levulinic acid was the most abundant analyte. Its concentration significantly exceeded that of the most abundant FA, palmitic acid (**Table 1**). The relatively high concentrations of "citric acid cycle" metabolites, including malic and (*trans*-) aconitic acid, were striking.

In addition to FAs, resin acids, typically formed in response to wounding (*15*), could also be detected. The most abundant among them was dehydroabietic acid. Its average concentration was 16 μg g^{-1} (referred to dry biomass) for the roots and 21 μg g^{-1} for the leaves (no difference between old leaves and young leaves). No differences between the genetically modified and the near-isogenic lines could be observed.

Table 2. Concentration of Homomorphic Acids in Roots As Determined by Thermochemolysis (Micrograms per Gram Referred to Dry Biomass)[a]

root	myristic	palmitic	stearic	Σ (14:0, 16:0, 18:0)
genetically modified	180	1190	470	1840
near-isogenic	205	1020	490	1715

[a] RSDs for FAMEs between 9 and 17%; average of four measurements. Quantification by dividing the abundance of the McLafferty ion *m*/*z* 74 amu of the target acids by that of the deuterated standard (*m*/*z* 76 amu).

Table 3. FA Profiles (as FAMEs) of the Genetically Modified and Near-Isogenic Maize Leaf Samples (Concentration in Micrograms per Gram Referred to Dry Biomass)[a]

	compartment			
FAME	genetically modified old	near-isogenic old	genetically modified young	near-isogenic young
Σ FAME (C_{12-22})	10450	13260	13220	14270
Σ saturated FAME (C_{12-22})	5345	5975	6210	6490
DBI	2.69	3.55	3.17	3.40
Σ odd-numbered	138	148	150	147
lauric	414	415	430	505
myristic	550	530	435	395
C_{15}-branched	13	20	20	25
pentadecanoic	64	65	60	55
palmitic	3475	4040	4630	4810
palmitoleic	51	75	110	105
heptadecanoic	56	60	65	65
stearic	430	465	390	430
Σ 18:1	95	90	90	100
linoleic	420	490	625	780
linolenic	4460	6630	6075	6650
arachic	148	150	75	75
behenic	135	140	100	105
22:1	60	65	50	95
malonic	205	265	180	200
levulinic	4600	4610	18800	22350
methyl maleate + methylene succinate	630	730	720	770
glutar[b]	175	230	260	230
malic	800	795	680	590
aconitic	1735	1830	2230	2355
β-sitosterol	~70	~75	~40	~35

[a] Average of four measurements; RSDs for FAMEs and functionalized methyl esters between 4.0 and 9.5%. [b] Glutar: abbreviation for 3-hydroxy-2-methylglutaric acid dimethyl ester.

The results of the thermochemolysis experiments (**Table 2**) confirmed the results obtained by the PLE preparation scheme in principle. Again, a slightly enhanced total concentration of saturated FAMEs was evident (compare **Tables 1** and **2**), but the differences were within the 10% data scatter, which can be considered the discrimination interval for field trials to reveal differences in quality parameters. Concentration data for stearic acid as given in **Table 1** scattered to a higher degree due to poor resolution between stearic acid, on the one hand, and the monounsaturated 18:1 FAME-cluster, on the other hand, which hampered the integration of ion traces. Higher concentration data for myristic acid obtained by thermochemolysis (see **Table 2**) as compared to PLE concentration data (see **Table 1**) were due to minor interferences when using root samples in the thermochemolysis approach.

Fatty Acid Profiles of Leaves. Table 3 lists the FA profiles of the leaf samples under study. In general, higher total FA concentrations along with higher DBI values were evident in comparison to root samples (**Tables 1** and **3**). Moreover, there

Table 4. Concentration of Homomorphic Acids in Leaves As Determined by Thermochemolysis (Micrograms per Gram Referred to Dry Biomass)[a]

leaf	myristic	palmitic	stearic	Σ (14:0, 16:0, 18:0)
genetically modified "old"	510	3110	345	3965
near-isogenic "old"	555	3450	405	4410
genetically modified "young"	450	3600	330	4380
near-isogenic "young"	415	3650	340	4405

[a] RSD for FAMEs between 6 and 15%; average of four measurements.

were significant differences between the total FA concentrations of the genetically modified and the near-isogenic lines. Total FA concentrations of the GM samples were lower by ~27% for the old leaves and by ~8% for the young leaves compared to the near-isogenic samples. In the old leaf compartment, the sum of saturated $C_{12}-C_{22}$ FAMEs was lower by ~11% for the genetically modified line; thus, the overall 27% reduction in the total FA concentration was chiefly due to unsaturated FA surrogates.

In contrast to the FA profiles of the roots, the DBI for the near-isogenic variety was higher in comparison to the corresponding GM variety. This statement is true for the old leaf plant compartment in particular. The DBI of the FAs from the old leaf compartment of the genetically modified line was lower by ~32% compared to the corresponding value for the near-isogenic line. High abundances of α-linolenic acid chiefly accounted for the high degree of unsaturation. Similar findings were obtained for Novelis T (MON810)/Nobilis and Valmont T (Bt176)/Prelude varieties (unpublished results). The DBI values of the FAs from the leaves of these Cry1Ab expressing genetically modified lines amounted to 71% (Novelis T) and 82% (Valmont T) of those obtained for their corresponding near-isogenic lines (Nobilis and Prelude, respectively).

Among the dicarboxylic and functionalized acids, a significant increase in levulinic acid concentration was evident when turning from the old to the young leaf compartment. A similar, though less pronounced, trend could be observed for aconitic acid.

cis-Vaccenic acid, accompanying the more abundant oleic acid, was again detected in both lines at concentrations ranging from 9 to 14% referred to those of oleic acid. Thus, the assumption that microorganisms or soil particles sorbed onto the roots contributed significantly to the *cis*-vaccenic acid levels in this plant compartment was obviously not valid. Characteristic FAs of *Bacillus thuringiensis* serovar *kurstaki* strain HD-1, including i-13:0, 17:1ω5c, and 17:1ω10c as obtained from whole-cell cellular analysis (*16*), could not be identified in any compartment of the genetically modified maize under study.

As with the roots, the results of thermochemolysis experiments (**Table 4**) confirmed the results obtained by the PLE preparation scheme (**Table 3**). Slightly enhanced total concentration of saturated FAMEs was again evident, but the difference was within the 10% data scatter.

ANOVA analysis at $\alpha = 0.01$ for all identified fatty acids under study, including the homomorphic acids, revealed no significant differences between the genetically modified and the near-isogenic samples. This held true for all three compartments under study (root, old leaf, and young leaf). Although the total fatty acid concentration proved to be significantly different for both sample types, differences between single FAs proved to be marginal. Multivariate statistics using PCA carried out with the data set of 27 fatty acid concentrations (**Figure 2**) and the second PCA of four homomorphic acids (**Figure 3**) showed clear differentiation into two clusters with every PCA. The first cluster was formed by roots of the genetically modified and

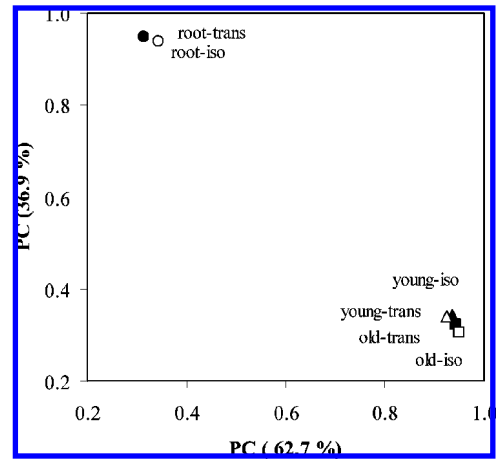

Figure 2. PCA ordination plot of roots, old leaves, and young leaves from the genetically modified and the near-isogenic plant compartments considering the 27 identified fatty acids listed in **Table 1**.

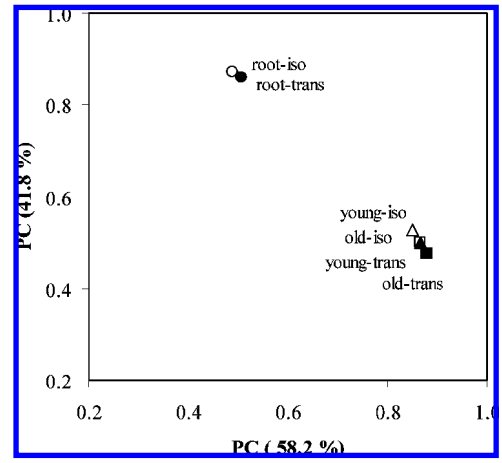

Figure 3. PCA ordination plot of roots, old leaves, and young leaves from the genetically modified and the near-isogenic plant compartments considering the homomorphic myristic, palmitic, and stearic acids.

the near-isogenic samples, and the second cluster was made up of old and young leaves of both kinds of samples. The evaluated data sets of fatty acids and homomorphic acids accounted for 99.5 and 99.9% of the total PCA variances.

DISCUSSION

Fatty Acid Profiles of Roots. Fatty acid profiles of roots in both the *Bt*-maize and its near-isogenic line proved to be very similar, pointing to a lack of a pleiotropic effect on fatty acids and thus indicating equivalence. This was also observed for other genetically modified lines: whole plant FA analyses of *Bt*-maize TC6275 expressing the Cry1F insecticidal crystal protein to combat European corn borer revealed no significant differences between this variety and its corresponding near-isogenic line (*17*). These results were confirmed in principle for other genetically modified maize lines expressing the Cry1Ab protein (*18, 19*).

FA analysis of root samples collected in field trials should generally be considered with caution, however. As detailed above, oleic acid was accompanied by *cis*-vaccenic acid, which confirms the findings of Miyatani et al. (*20*) obtained for conventional maize roots. The "bacterial" *cis*-vaccenic acid could have originated from microorganisms sorbed onto the roots, thus contributing to biased results. However, because the plots with the genetically modified and the near-isogenic lines

were adjacent to each other in the field trial experiment, this contribution was assumed to be similar for both lines; hence, the comparison should not have been biased. Likewise, odd-numbered and branched surrogates including iso- and anteisoisomers should also be considered with caution because of soil particles that adhered to the root matrix. The particles could not be removed quantitatively without affecting the target lipids. Further experiments with hydroponic cultures should be able to overcome these limitations.

Levulinic acid is known to regulate protein metabolism, the amino derivative being essential for chlorophyll synthesis. It is considered a precious chemical with great potential, sometimes called a "new green platform chemical" (*21*). Levulinic acid is currently produced from wheat. To the best of our knowledge, its generation from maize has not been detailed yet. Root exudates were not considered as sources of malic and aconitic acids, because such carbon sources are known to be utilized easily by soil microorganisms (*22*). This assumption was supported by the fact that oxalic and tartaric acids, known to be common in root exudates (*23*), could not be detected.

Fatty Acid Profiles in Leaves. The combined findings obtained for the leaves of the MON88017 and DKC5134 maize lines, as well as the results of earlier studies (Novelis T/Nobilis, Valmont T/Prelude), indicated that the insertion of the genetic elements coding for the *Bt*-proteins Cry1Ab or Cry3Bb1 and controlling their expression influenced the FA synthesis. The decrease in the total FA concentration in the GM lines and the higher degree of saturation of the GM FA patterns were expected to contribute to the reduction of chemical reactivity in soils. Dinel et al. (*24*) found the reactivity to be reduced by ~30% for the genetically modified lines based on cumulative carbon dioxide evolution from soils incubated with *Bt*-maize (*Cry1Ab* gene) and near-isogenic maize. This might have been due to, among other reasons, the higher lignin content and lower lipid concentration in the genetically modified maize line.

A higher DBI should result in higher turnover rates in soils after harvesting and plowing in, which in turn could influence the nematode populations. Findings in Griffiths et al. (*25*), according to which smaller nematode populations were observed at sites with Cry1Ab expressing *Bt*-maize, could be explained by the FA patterns. Modified FA profiles in *Zea mays* L. also resulted in altered FA profiles in animal tissues, as exemplified by cows (*26*).

Differences in FA profiles between a genetically modified and a near-isogenic line can be regarded as significant only when they exceed the natural variability of the universe of the currently available conventional lines. As described in a study detailing the fatty acid contents of grain derived from MON88017 and a range of conventional lines (*27*), there is an enormous variance for the fatty acid contents in the current population of conventional maize lines. More research is needed, evaluating different methodological approaches for FA analyses, the natural variability in maize (conventional and genetically modified), and looking at different aspects of fatty acid biosynthesis, composition, content in different plant compartments, and their influence on the biotic and abiotic environment.

Given the large variability in the population of maize lines, MON88017 and its near-isogenic line can be regarded as equivalent with regard to their fatty acid profiles, despite the differences observed for the leaves.

The very high concentrations of levulinic acid in the young leaf compartments of the plants (specimen collected from the top of the maize stem) were considered to be especially promising for potential extraction of this useful chemical from maize. Levulinic acid is known to occur in pigments of young leaf mitochondria with high photosynthetic activity. Thus, its concentration is higher in young leaves compared to old leaves with lower photosynthetic activity.

Further methodological work regarding fatty acids might be focused on (i) the differentiation of fatty acids into lipid classes (a promising preparation scheme for lipid class fractionation by means of sequential PLE was described recently (*5*) and could be applied for the characterization of maize lines) and (ii) clarifying whether insertion of novel genetic elements results in stress-like response of plants. It has been established that herbicides and metalloid species can change the FA profiles of maize plants, as can biological stress including pathogen attack. FAs are considered to be useful indicators of plant response, in particular, because they regulate the expression of defense-related genes (*3*).

To better understand potential unintended effects of genetic modification, further plant constituent classes should be studied, including sterols, hydroxy acids, and carbohydrates. Preliminary quantitation of sterols (**Table 3**) pointed to a similarity between the concentrations of the most abundant sterol, β-sitosterol, in the leaves from the genetically modified and the near-isogenic lines (campesterol and stigmasterol of second lower abundance were not considered in the study). Thus, insertion of the genetic elements into MON88017 is not expected to interfere with the mevalonate pathway to synthesize sterols. Long-chain ω-hydroxy acids as constituents of cutin (serving as a barrier to pathogenic attacks and to prevent water loss) and suberin (ensuring low water permeability and likewise serving as a barrier to microbial attacks), both of which are sources of aliphatic moieties in soil, should be considered. Preliminary results obtained for the Novelis T (MON810)/Nobilis and Valmont T (Bt176)/Prelude pairs (*28*) indicated that the total concentrations of $C_{16}-C_{26}$ ω-hydroxy acids released by saponification of PLE extracts were significantly higher for the genetically modified lines. This was accompanied by an abundance shift toward longer chains, in particular ω-OH-C_{22} and ω-OH-C_{24}. Whether these changes are of real biological significance remains unclear, though.

Carbohydrates chiefly determine turnover rates in soil. Breakdown products of carbohydrates as determined by thermochemolysis (*29*) include levoglucosan, levoglucosenone, 1,2,4-trimethoxybenzene, furfural, C_6-meta saccharinic acids, and others. Sophisticated carbohydrate analysis to address pleiotropic effects, including ion chromatography coupled with amperometric detection, seems to be an interesting approach to elucidate unintended effects on plant metabolism and also to assess natural variability between different maize cultivars.

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