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ARTICLE

Influence of the Input System (Conventional versus Organic Farming) on Metabolite Profiles of Maize (*Zea mays*) Kernels

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Maize (*Zea mays*) kernels grown conventionally and organically, respectively, were investigated using a gas chromatography/mass spectrometry (GC/MS)-based metabolite profiling methodology. By analysis of three cultivars grown at two locations with different input systems and at a third location where both organic and conventional farming were applied, the impact of the growing regime on the metabolite spectrum should be put into the context of natural variability. The applied analytical approach involved consecutive extraction of freeze-dried maize flour and subsequent subfractionation. Approximately 300 compounds from a broad spectrum of chemical classes were detected, of which 167 were identified. The metabolite profiling data were statistically assessed via principal component analysis (PCA) and analysis of variance (ANOVA). The PCA demonstrated that the observed separations were mainly due to genetic differences (cultivars) and environmental influences. The different input systems (conventional/organic) only led to minor differentiations. ANOVA and quantification of selected constituents confirmed these observations. Only three metabolites (malic acid, *myo*-inositol, and phosphate) were consistently different because of the employed input system if samples from all field trials were considered.

KEYWORDS: Metabolite profiling; GC/MS; *Zea mays*; conventional farming; organic farming; input system

INTRODUCTION

With the arising ecological awareness in the 1980s, farmers and consumers started to look for alternatives to conventional farming. Rather than intensively applying mineral fertilizers and relying on chemical plant protection, organic farming is based on minimal use of off-farm inputs and ecologically friendly management practices (*1*, *2*). The share of organically farmed area has continuously increased over the last 2 decades, in particular in Europe and North America (*3*). Surveys indicate that many consumers purchase organic foods because of the perceived health and nutrition benefits (*4*), although a recent systematic review found no evidence for a difference in nutritional quality between organically and conventionally produced foods (*5*).

The rising interest in this field is also reflected by an increased scientific activity; from 1993 to 2008, an 8-fold increase of scientific publications concerned with "organic farming" can be observed (*6*). Many of these publications deal with the impact of organic farming practice on soil parameters, such as organic matter (*7*, *8*), biodiversity and vitality (*9*), or pH (*10*). Others focus on the influence of input regimes and tillage systems (*11*, *12*) on yield. From a food quality point of view, parameters, such as protein content, nutrient levels (*13*, *14*), and minerals (*15*), have been thoroughly investigated.

A more comprehensive approach for the assessment and evaluation of a broad spectrum of crop constituents, complementing the above-described targeted studies, is envisaged by the application of the so-called "omics" techniques. For example, the impact of different amounts and forms (organic and inorganic) of nitrogen supply on the gene expression level in the wheat endosperm has been investigated (*16*). Many of the genes showing differential expression in this study are known to participate in nitrogen metabolism and storage protein synthesis. Other studies involved proteomics approaches. A comparison of the protein compositions of potato tubers subjected to organic and mineral-based fertility management practices suggested an increased stress response in organic farming (*17*). In wheat, 16 "diagnostic" proteins with potential to afford a signature to prove authenticity of organic wheat were proposed (*18*).

In addition to transcriptomics and proteomics, metabolomic-based approaches should also be suitable to reflect the impact of different input systems on crops, because metabolites can be considered as the ultimate response of organisms to processes regulating metabolism (*19*). Gas chromatography coupled with mass spectrometry (GC/MS) proved to be one of the most robust technologies for metabolite profiling (*20*). At present, there is only one example for the application of this approach to organically farmed crops; the analysis of 52 polar metabolites in one wheat grain variety grown under organic and conventional farming practices detected only moderate differences (*21*).

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Table 1. Metadata on Agronomy 2004−2006

										fertilization			plant protection		
	sowing date	harvest date	temperature (°C)[a]	rain (mm)[b]	soil index[c]	N[d] (kg/ha)	P_2O_5[d] (mg/100 g)	K_2O[d] (mg/100 g)	pH[d]	fertilizer	(kg/ha)[e]	date	herbizide/ treatment	(L/ha)	date
						Season 2004									
Frankendorf (conventional farming)	April 21, 2004	Oct 19, 2004	14.0	501	80	104	9	16	7.1	NK	30	April 21, 2004	Artett	2.8	May 12, 2004
										NK	80	June 06, 2004	Spectrum	1.4	May 12, 2004
Schönbrunn (organic farming)	April 22, 2004	Sept 19, 2004	14.7	403	52	53	22	31	7.5				currycomb		May 13, 2004
													roller hoe		May 25, 2004
						Season 2005									
Frankendorf (conventional farming)	May 3, 2005	Oct 25, 2005	14.3	570	80	82	20	23	6.8	NK	30	May 03, 2005	Artett	2.8	June 2, 2005
										NK	100	June 13, 2005	Spectrum	1.4	June 2, 2005
Schönbrunn (organic farming)	May 12, 2005	Sept 27, 2005	14.8	482	52	[f]	17	19	7.6				currycomb		May 27, 2005
													currycomb		June 1, 2005
													roller hoe		May 6, 2005
													roller hoe		June 21, 2005
						Season 2006									
Scheyern (conventional farming)	May 12, 2006	Oct 25, 2006	15.2	387	63	79	11−20	11−20	6.1	NP	60	May 12, 2006	Motivell	0.9	June 12, 2006
										N	130	May 15, 2006	Certrol	0.8	June 12, 2006
Scheyern (organic farming)	May 12, 2006	Oct 25, 2006	15.2	387	64	70	11−20	11−20	6.0	[g]	[g]	[g]	roller hoe		May 22, 2006
													roller hoe		June 16, 2006
													roller hoe		July 1, 2006
													hoe		July 2, 2006

[a] Mean temperatures in May−October. [b] Sum of precipitation in May−October. [c] Productivity indicator "Ackerzahl". Relative yield in comparison to the best German site (Ackerzahl = 100) (*37*). [d] Soil samples (from 0 to −90 cm) taken in spring before application of fertilizer. [e] Total amount of fertilizer. [f] No data available. [g] Mustard grown in autumn of the preseason was ploughed into the ground as green manure.

The aim of this study was to investigate the metabolite profiles of maize (*Zea mays*) grown conventionally and organically using a methodology that was recently shown to be suitable to demonstrate variations in maize grain metabolite pools resulting from the interplay of environment, season, and genotype (*22*). By analysis of three cultivars grown at two locations with different input systems and at a third location where both organic and conventional farming were applied, the impact of the growing regime on the metabolite spectrum should be put into the context of natural variability.

MATERIALS AND METHODS

Plant Materials. Three maize (*Z. mays*) cultivars (Amadeo, KWS Mais GmbH, Germany; Lukas, Limagrain GmbH Edemissen, Germany; Flavi, Caussade Semences, Caussade, France) were grown in the season 2004 at two locations with different input regimes. At location Frankendorf (Bavaria, Germany), the crops were grown conventionally, and at location Schönbrunn (Bavaria, Germany), organic farming was employed (experiment Ia). The same procedure was repeated in the season 2005 (experiment Ib). For experiment II, samples from two plots with different input systems (conventional/organic) were obtained from one location Scheyern (Bavaria, Germany). Samples were obtained from field trials with totally randomized field plot design. Growing periods were as follows: Frankendorf, April 21−Oct 19, 2004 and May 3−Oct 26, 2005; Schönbrunn, April 22−Sept 19, 2004 and May 12−Sept 27, 2005; Scheyern, May 12−Oct 25, 2006. A total of 10 cobs were harvested from the two mid rows of each plot, and a subsample of 100 g kernels was taken for further processing. For each cultivar/location, three field replicates were analyzed in triplicate. The locations employing organic farming had been managed for at least 3 years according to the provisions laid down in Council Regulation 2092/1991 (*23*). Metadata on agronomy are provided in **Table 1**.

Sample Processing. Air-dried (30−40 °C, 3 days) maize kernels (10−15% moisture) were frozen in liquid nitrogen and immediately ground with a cyclone mill (Cyclotec, Foss, Germany) equipped with a 500 μm sieve. The flour was freeze-dried (ALPHA 1-4 LSC, Christ, Osterode, Germany) for 48 h. The moisture content of the resulting material (< 2%) was determined as the loss of weight by drying at 105 °C for 3 h. Freeze-dried flour samples were stored at −18 °C in tightly closed low-density polyethylene (LDPE) bottles (Kautex Textron, Bonn, Germany).

Metabolite Extraction and Sample Preparation for GC/MS Analysis. Extraction and fractionation of freeze-dried maize flour were performed as previously described (*22*). Lipids and polar compounds were consecutively extracted from the flour. After transesterification, the lipid extract was separated by solid-phase extraction into a fraction containing fatty acid methyl esters (FAMEs) and hydrocarbons (fraction I) and a fraction containing minor lipids, e.g., sterols and free fatty acids (fraction II). Selective hydrolysis of silylated derivatives was applied to separate the polar extract into a fraction containing silylated sugars and sugar alcohols (fraction III) and a fraction containing organic acids and amino acids (fraction IV). The four fractions obtained were analyzed by GC/MS. Fractions II and IV were silylated before GC analysis. The GC conditions were in agreement with previously described procedures (*22*).

Internal standards were tetracosane, 5α-cholestan-3-ol, phenyl-β-D-glucopyranoside, and *p*-chloro-L-phenylalanine, and retention time standards were hydrocarbons C11, C16, C24, C30, and C38.

Metabolite Identification by GC/MS Profiling. Metabolites were identified according to mass spectral data from custom (A, mass spectral data and retention times of reference compounds), public [B, mass spectral data and retention index of Golm Metabolome Database (*24*)], commercial [C, mass spectral data of NIST02 mass spectral library (*25*)] mass spectral libraries, and literature [E, (*26*); F, (*27*); and G, (*28*, *29*)]. For A−C, metabolites were denoted as identified if the similarity index was >750 on a scale of 0−1000; in addition, for A, a maximum relative retention time deviation of 0.1% and, for B, a maximum relative retention index deviation of 1.0% were required.

Statistical Analysis. Retention time matching was performed by use of Chrom*pare*, a self-tailored MS Excel tool, basically on the basis of Student's *t* test [(*30*); www.chrompare.com]. The tool is optimized for a comparison of chromatographic data, including automated retention time adjustment according to retention time standards. Data from triplicate analysis of each sample were averaged for further statistical analyses. Principal component analysis (PCA) and analysis of variance (ANOVA) were performed within Systat 11 (Systat Software, Inc., Richmond, CA). For PCA, data were autoscaled by the standard deviation of each variable [correlation matrix, (*31*)] to reduce the influence of metabolites with high abundance. For ANOVA, the model described in eq **1** was used for each analyte

$$v_{ik} = \mu + c_i + i_k + (ci)_{ik} + e_{ik} \quad (1)$$

where v_{ik} is the response for the *ith* cultivar ($n = 3$) and the *kth* location/input system ($n = 2$), μ is the overall mean, c_i is the effect of the *i*th maize cultivar, i_k is the effect of the *kth* location/input system, $(ci)_{ik}$ is the effect of the interaction between the *i*th maize cultivar and the *k*th location/input system, and e_{ik} is the random error, including error of field replicates. The significance level was set to $p < 0.01$ for all statistical comparisons.

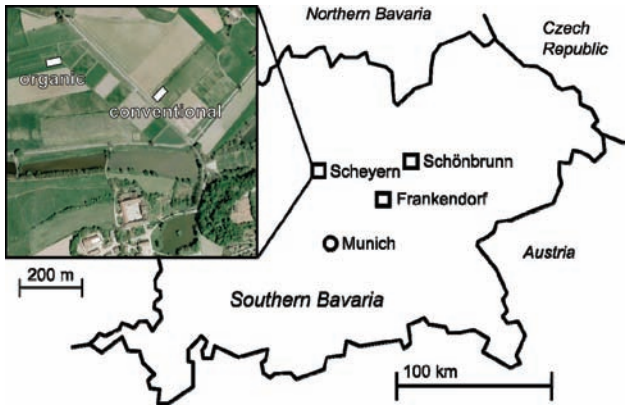

Figure 1. Geographical locations of the field trial sites Frankendorf, Schönbrunn, and Scheyern. Aerial photo: Bayerische Vermessungsverwaltung. Cartography: Kober-Kümmerly+Frey, Köln.

Table 2. Number of Statistically Significant Differences Obtained by ANOVA ($p < 0.01$) and Tukey's HSD ($p < 0.01$) of Metabolite Profiling Data from Fractions I (Major Lipids), II (Minor Lipids), III (Sugars and Sugar Alcohols), and IV (Organic Acids, Amino Acids, and Amines) of Three Maize Cultivars (Amadeo, Lukas, and Flavi) Grown in the Experiments Ia, Ib, and II

	experiment			
	Ia	Ib	II	consistent[a]
Location (Input System)				
compounds included	125	127	126	116
differences				
fraction I	7	3	1	0
fraction II	8	0	0	0
fraction III	10	11	3	1
fraction IV	11	3	10	1
total	36	17	14	2
differences (%)	29	13	11	2
Genetic Background (Cultivar)				
compounds included	125	127	126	116
differences				
fraction I	5	8	19	2
fraction II	12	14	17	7
fraction III	5	9	12	2
fraction IV	7	10	22	3
total	29	41	70	14
differences (%)	23	32	56	12

[a] Numbers of compounds consistently included for comparison and differences consistently detected as statistically significant in all experiments.

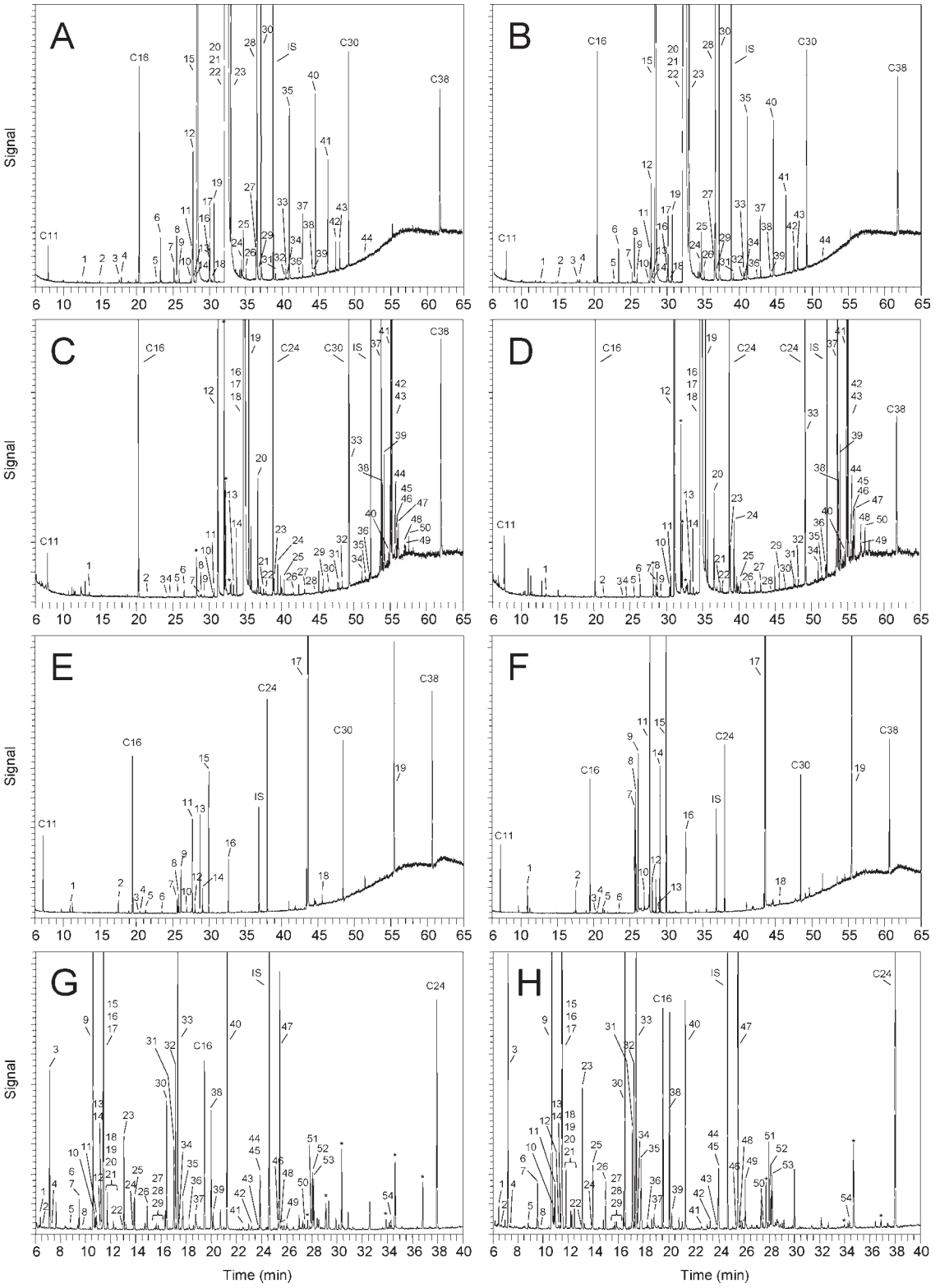

Figure 2. GC/MS total ion current chromatograms of metabolite profiling (A/B) fractions I (major lipids), (C/D) II (minor lipids), (E/F) III (sugars and sugar alcohols), and (G/H) IV (acids and amino acids) obtained by analysis of cultivar Lukas grown conventionally at location Frankendorf (left column) and organically at location Schönbrunn (right column) in season 2004. The peak numbers refer to the numbers in Table 3.

Differences were considered to be statistically significant if no interaction effect was observed, and the main effect was significant by ANOVA and after post hoc testing by Tukey's HSD.

RESULTS

Three cultivars (Amadeo, Lukas, and Flavi) were used in the study to evaluate the impact of input systems on metabolite profiles of maize kernels differing in genetic background. They were grown in the seasons 2004 (experiment Ia) and 2005 (experiment Ib) at two locations in Bavaria, one (Frankendorf) with conventional farming practices and the other (Schönbrunn) with organic farming practices. The two locations were approximately 30 km apart. To minimize environmental influences, a further experiment (II) was designed in which the three cultivars

Table 3. Maize Constituents Identified in Metabolite Profiling Fractions I−IV

fraction I			fraction II			fraction III			fraction IV		
number	compound	ident.[a]	number	compound	ident.[a]	number	compound	ident.[a]	number	compound	ident.[a]
	saturated FAME[b]			free fatty acids[c]			sugars and sugar alcohols[c]			acids[c]	
1	10:0	A	1	9:0	A	1	glycerol	A	1	lactic acid	A
4	12:0	A	2	12:0	A	2	erythritol	A	2	hydroxyacetic acid	A
6	14:0	A	3	13:0	A	3, 4, 5	arabinose	A	8	4-hydroxybutyric acid	C
9	15:0	A	6	14:0	A	6	ribitol	A	9	phosphoric acid	A
15	16:0	A	8	15:0	A	7, 8, 9	fructose	A	12	maleic acid	A
19	17:0	A	10	16:1	C	10, 12	galactose	A	14	4-aminobutyric acid	A
23	18:0	A	11	16:1 (9*Z*)	A	11, 15	glucose	A	17	succinic acid	A
26	19:0	A	12	16:0	A	13	mannitol	A	20	glyceric acid	A
30	20:0	A	13	17:0	A	14	sorbitol	A	21	fumaric acid	A
31	21:0	A	16	18:3 (9*Z*, 12*Z*, 15*Z*)	A	16	inositol	A	22	pyrrole-2-carboxylic acid	A
35	22:0	A	17	18:2 (9*Z*, 12*Z*)	A	17	sucrose	A	24	glutaric acid	A
37	23:0	A	18	18:1 (9*Z*)	A	18	trehalose	A	28	2-piperidinecarboxylic acid	C
40	24:0	A	19	18:0	A	19	raffinose	A	29	β-aminoisobutyric acid	A
43	26:0	A	21	19:0	A				30	malic acid	A
44	28:0	A	23	20:1 (11*Z*)	A				34	cinnamic acid	A
	unsaturated FAME		24	20:0	A				35	γ-aminobutyric acid	A
7	15:1 (10*Z*)	A	27	22:0	A				41	α-aminoadipic acid	A
11	16:1	C	28	23:0	A				42	*cis*-aconitic acid	B, C
12	16:1 (9*Z*)	A	30	24:0	A				45	3-glycerophosphoric acid	B, C
13	16:1 (9*E*)	A		fatty alcohols[c]					47	citric acid	A
17	17:1 (9*Z*)	A	9	16:0	A				51	*p*-cumaric acid	A
22	18:1 (9*Z*)	A	14	18:0	A					amino acids and amines[c]	
24	19:1 (10*Z*)	C	15	phytol	A				3	alanine	A
28	20:1 (11*Z*)	A	22	20:0	A				4, 18	glycine	A
33	22:1 (11*Z*)	A	26	22:0	A				5, 26	β-alanine	A
38	24:1 (15*Z*)	A	29	24:0	A				6	valine	A
10	16:2	C	32	26:0	A				7	norvaline	A
16	17:2	D	34	28:0	A				10	leucine	A
21	18:2 (9*Z*, 12*Z*)	A	49	32:0	D				11	ethanolamine	A
27	20:2 (11*Z*, 14*Z*)	A		hydroxy FAME[b,c]					13	alloisoleucine	A
32	22:2 (13*Z*, 16*Z*)	A	20	12-OH 18:1 (9Z)	A				15	isoleucine	A
20	18:3 (9*Z*, 12*Z*, 15*Z*)	A	25	9,12-OH 18:0	G				16	proline	A
	hydrocarbons			phenolic compounds[c]					23	serine	A
2	14	A	4	methyl *p*-hydroxy-cinnamate	A				25	threonine	A
3	15	A	5	methyl 3-methoxy-cinnamate	C				27	homoserine	A
5	17	A	7	methyl ferulate	A				31	pyroglutamic acid	A
8	18	A		sterols/stanols[c]					32	methionine	A
14	19	A	36	cholesterol	A				33	aspartic acid	A
18	20	A	37	campesterol	A				36	5-hydroxynorvaline	C
25	22	A	38	campestanol	A				37	threonic acid	A
29	23	A	39	stigmasterol	A				38	glutamic acid	A
34	25	A	40	Δ7-campestenol	E				39	phenylalanine	A
36	26	A	41	β-sitosterol	A				40	asparagine	A
39	27	A	42	sitostanol	A				43	putrescine	A
41	squalene	A	43	Δ5-avenasterol	A				44	glutamine	A
42	cholestane	C	44	gramisterol	F				46	citrulline	A
			45	Δ7-stigmastenol	F				48	ornithine	A
			46	cycloartenol	A				50	histidine	A
			47	Δ7-avenasterol	F				52	lysine	A
			48	24-methylene-cycloartanol	A				53	tyrosine	A
			50	citrostadienol	F				54	tryptophan	A
				tocopherols[c]						others[c]	
			31	δ-tocopherol	A				19	2,4-hydroxy-pyrimidine	C
			33	γ-tocopherol	B, C				49	adenine	A
			35	α-tocopherol	A						

[a] Identification according to A, mass spectral data and retention times of reference compounds; B, mass spectral data and retention index of Golm Metabolome Database (*24*); C, mass spectral data of NIST02 mass spectral library (*25*); D, mass spectral data; E, according to ref *26*; F, according to ref *27*; G, according to refs *28* and *29*. [b] Fatty acid methyl esters. [c] TMS derivatives of the respective compound.

were grown at one location (Scheyern), providing field plots for both conventional and organic farming at a distance of approximately 400 m (**Figure 1**).

The metabolite profiling methodology applied in this study is based on consecutive extraction of freeze-dried maize flour and subsequent subfractionation, resulting in four fractions including (I) major lipids, (II) minor lipids, such as free fatty acids and sterols, (III) sugars and sugar alcohols, and (IV) organic acids, amino acids, and amines. A total of approximately 300 distinct analytes were detected by GC/MS analysis. A comparison of mass spectral data and retention times to those of reference compounds or literature data resulted in the identification of 167 compounds (Table **3**). **Figure 2** provides an example of the respective total ion current chromatograms for cultivar Lukas

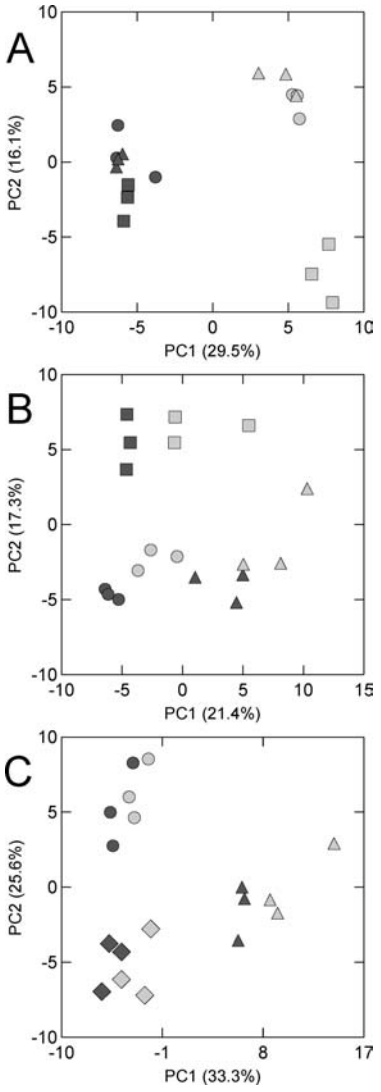

Figure 3. PCA of metabolite profiling data from fractions I−IV obtained by analysis of three maize cultivars (○, Amadeo; □, Lukas; and △, Flavi) grown at farming sites with different input regimes (black circles, black squares, and black triangles, conventional farming; gray circles, gray squares, and gray triangles, organic farming) in growing seasons (A) 2004 and (B) 2005 at locations Frankendorf (conventional farming) and Schönbrunn (organic farming) and (C) 2006 at location Scheyern (conventional and organic farming).

grown at locations Frankendorf (conventional farming) and Schönbrunn (organic farming) in 2004.

PCA. Metabolite profiling data obtained for the three cultivars (Amadeo, Lukas, and Flavi) grown in 2004 at the two locations with different input systems were subjected to statistical assessment via PCA to determine the major sources of variation. On the basis of the data from all metabolites covered in fractions I−IV, a clear separation according to farming locations/input systems was observed on the first principal component accounting for 29.5% of the variation (**Figure 3A**). At the location Schönbrunn (organic farming), the three cultivars formed one cluster, whereas at location Frankendorf (conventional farming), cultivar Lukas was differentiated on the second principal component (16.1% of the variation).

In the following season 2005, the clustering of the samples on the first two principal components (38.7% of the variation) was increasingly determined by differences between cultivars (**Figure 3B**). The effects of the farming location/input system were much less pronounced than in 2004.

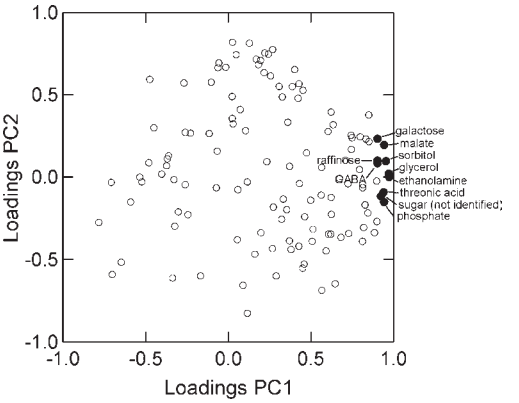

Figure 4. Factor loading scores of principal components 1 and 2 of PCA of metabolite profiling data of three cultivars grown in season 2004 at two locations [Frankendorf (conventional farming) and Schönbrunn (organic farming)] from fractions I−IV. Black circles represent compounds with the 10 highest absolute loading scores on principal component 1.

An even clearer impact of the genetic background became obvious from the data obtained for the three cultivars grown under different input systems at the same location. As shown in **Figure 3C**, the cultivars showed quite distinct clusters on the first two principal components of the PCA, explaining 58.9% of the variation. However, only small differences were observed between the samples obtained by conventional and organic farming.

ANOVA. ANOVA was performed for each of the three data sets to evaluate the number of differences because of locations/input systems and genetic background. In 2004, the levels of a total of 125 compounds were compared; post hoc testing (Tukey's HSD, $p < 0.01$) revealed 29% to be statistically significantly different for locations/input systems and 23% to be statistically significantly different for genotype (**Table 2**). In agreement with the clustering seen in the PCA, in 2005, the number of the statistically significant differences for locations/input systems was much lower (13% of 127 compounds), whereas the differences because of the influence of cultivars increased to 32%. At location Scheyern, where both conventional and organic farming were applied at the same site, the number of differences because of input systems decreased further to only 11%. The clear separation of cultivars seen in this experiment is reflected by 56% of the 126 compounds being statistically significantly different because of the genetic background.

Assessment of the different chemical classes revealed that the influence of locations/input systems was mainly reflected by statistically significant differences in the polar fractions III and IV, whereas differences between cultivars were found more in the lipophilic fractions I and II. In total, only 2 metabolites turned out to be consistently different over all three seasons (malic acid and *myo*-inositol) because of input system but 14 because of cultivar (**Table 2**).

Comparison of Relative Metabolite Levels. In 2004, the influence of locations/input systems was most prominent. To determine the metabolic sources of variation, loading scores of the first principal component of the PCA data were examined (**Figure 4**). Metabolites with the 10 highest absolute loading scores were quantified on the basis of relative signals. They all belonged to the polar fractions III and IV containing sugars, sugar alcohols, acids, and amines. In addition, the levels of *myo*-inositol were determined, because this metabolite was found to be consistently different by ANOVA over all seasons (**Figure 5**).

In the first growing season 2004 (experiment Ia), higher levels for these compounds were detected in the samples grown at the location Schönbrunn under the organic regime. All differences

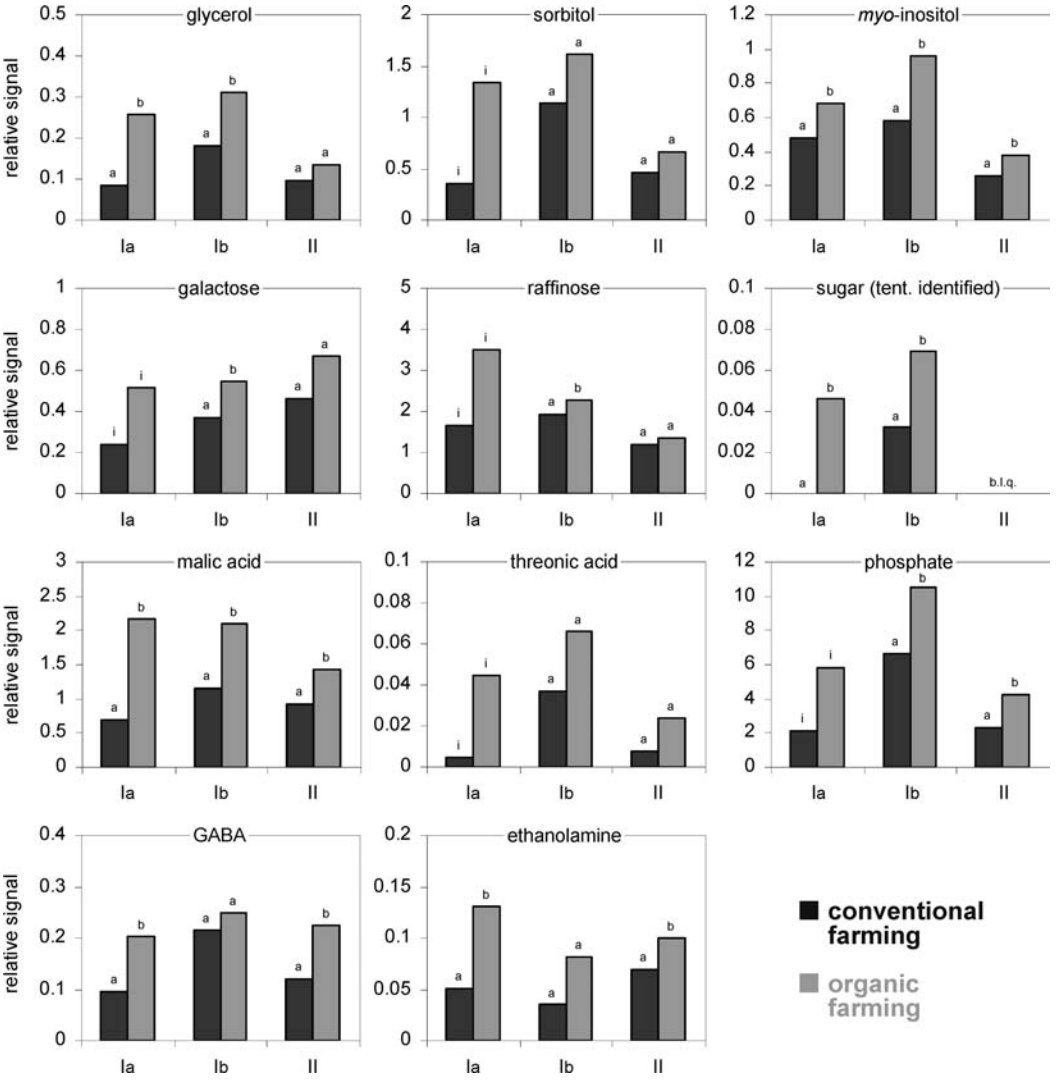

Figure 5. Comparison of locations with conventional and organic farming by semi-quantified levels of compounds selected according to the 10 highest loading scores on principal component 1 in experiment Ia and *myo*-inositol. Relative signals are calculated on the basis of the respective internal standard. Statistically significant differences (Tukey's HSD, $p < 0.01$) are indicated by different characters (a and b) within each experiment (Ia, Ib, and II). (i) Significant interaction of cultivar × location ($p < 0.01$).

were statistically significant ($p < 0.01$), or the lower value was below the limit of quantification. Although, the levels of some of the metabolites showed a significant ($p < 0.01$) interaction effect of cultivar and farming location, a closer look at the interaction diagrams revealed an ordinal interaction in these cases for the effect of locations/input systems, which allowed for a further evaluation of these results on the basis of individual cultivars (*32*). A comparison of the levels of these metabolites between conventional and organic practices by post hoc testing (Tukey's HSD, $p < 0.01$) resulted in significant differences for these compounds. Repetition of this trial in 2005 (experiment Ib) resulted in fewer statistically significant differences. The smallest differences were detected in experiment II, in which both input systems were applied at one location (Scheyern). With regard to all three sample sets, only malic acid, *myo*-inositol, and after post hoc testing, phosphate, turned out to be consistently different at all experiments.

Of the 14 differences consistently observed between cultivars over all three sample sets, 9 metabolites were identified and quantified (**Figure 6**). Differences were observed for compounds from all four metabolite profiling fractions I−IV.

DISCUSSION

The sequence of PCA plots obtained from the experiments Ia, Ib, and II (panels **A**−**C** of **Figure 3**) demonstrates that the observed separations are mainly due to the genetic differences (cultivars) and environmental influences; the different input systems (conventional/organic) only lead to minor differentiations. **Figure 3A** (experiment Ia) showed a strong separation of farming sites on the first principal component in 2004. At this point, it remained unclear whether this effect was due to the different input systems employed or only the different locations. The repetition in 2005 (experiment Ib, **Figure 3B**) also resulted in differentiations according to locations and/or input systems; however, the genetic background (cultivars) turned out to be the dominating contributor to the observed clustering. Finally, experiment II revealed that, if environmental influences are minimized by performing the trials at one location, only a very slight differentiation according to the input system is observed and the clustering pattern is mainly determined by the differences in cultivars (**Figure 3C**).

The clustering in **Figure 3A** may be explained by differences in nutritional supply and soil composition and influences in the local

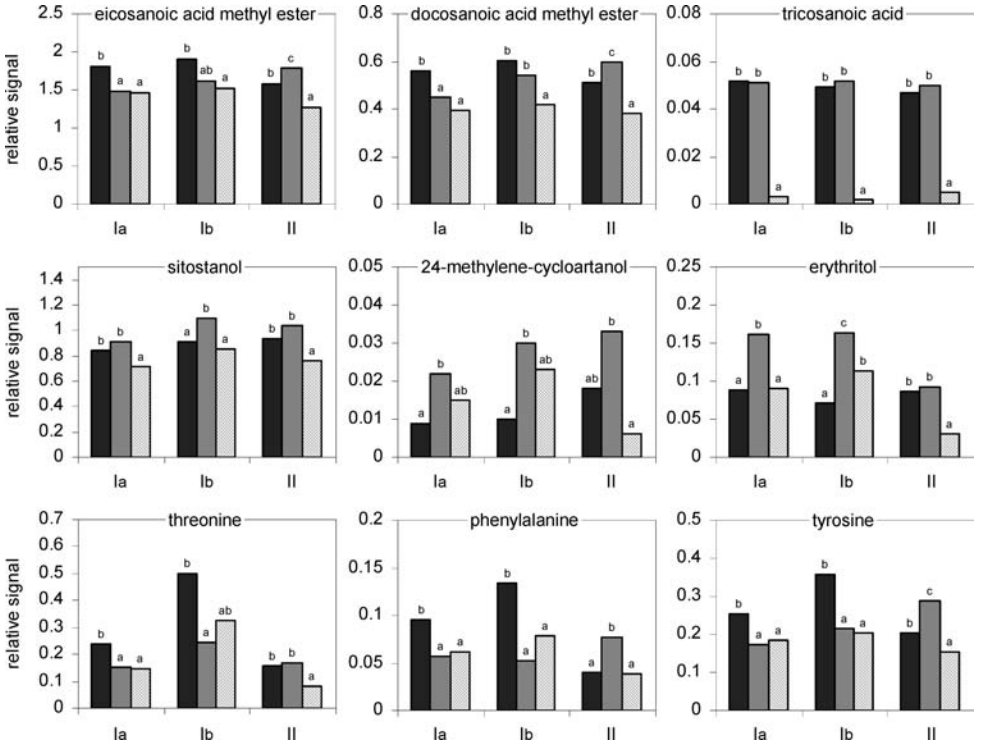

Figure 6. Comparison of three maize cultivars (black bars, Amadeo; dark gray bars, Lukas; light gray bars, Flavi) by semi-quantified levels of identified compounds that showed consistently different levels in three field trials (Ia, Ib, and II). Relative signals are calculated on the basis of the respective internal standard. Statistically significant differences (Tukey's HSD, $p < 0.01$) are indicated by different characters (a, b, and c) within each experiment.

microclimates. The location Frankendorf was characterized by a silty loam soil with a rather high soil index of 80, which represents 80% of the performance capacity of an "ideal" soil (*33*), whereas the location Schönbrunn had a more sandy loam soil with a soil index of only 52. In addition, although temperatures were more adequate at Schönbrunn, precipitation was much more abundant at the location Frankendorf and may have contributed to better plant growth at this location (**Table 1**). Additional support for the less favorable conditions in Schönbrunn is given by the clear separation of Lukas on principal component 2 at this location (**Figure 3A**). Lukas is known to be a robust cultivar under different environmental conditions from dryness to low temperature (*34*). Apparently, at location Frankendorf, the growing conditions met the requirements for all cultivars, resulting in one PCA cluster. Under the conditions in Schönbrunn, the more robust cultivar Lukas behaved differently from Amadeo and Flavi. In 2005, the precipitation was higher than in 2004 at both locations (**Table 1**) and obviously reached a sufficient amount to ensure similar growth behavior of the maize plants at both farming sites. However, a differentiation of locations/input systems was still observable within the clusters of each cultivar.

Evaluation of metabolite profiling data by ANOVA confirmed the observations made by PCA; most differences between locations and/or input systems were found in 2004, less in 2005, and only a few number of differences between input systems conducted at the same location in 2006. The decrease in statistically significant differences because of locations/input systems and the simultaneous increase because of the genetic background (cultivar) from field trials Ia/Ib to II reflects the setup of these experiments. The extent of changes seen because of the factors genetics and environment is in the same order of magnitude as observed for maize kernel metabolites in a previous study employing the same metabolite profiling methodology (*22*).

Considering the broad range of low-molecular-weight constituents analyzed by the applied GC/MS metabolite profiling approach, the number of consistent differences identified owing to input system is relatively small; only for malic acid, *myo*-inositol, and phosphate were higher levels determined for maize grown at organic farming sites in all three experiments. For two of these metabolites, similar effects are known from other studies. A metabolite profiling approach analyzing 51 polar metabolites in wheat grown at different input practices also reported higher levels of *myo*-inositol at growing sites with organic farming compared to the respective conventional site (*21*). *myo*-Inositol plays important functional roles in various physiological routes involved in, for example, seed desiccation, osmoregulation, and stress response (*35*). At this point, the data available do not allow for a reasoned answer why this metabolite might be consistently changed in organically grown crops. Phosphate is one of the most important plant constituents that affect growth and metabolism (*36*). The increased levels of phosphate observed in the organically grown maize samples in this study are in agreement with higher levels of phosphate reported in various other organically grown plants (*2*, *5*).

In conclusion, the application of a comprehensive metabolite profiling approach allowed for the investigation of the effect of conventional and organic farming management practices on maize metabolites from different chemical classes ranging from lipophilic to polar. The assessment of impact factors on metabolic variation, such as genotype, farming location, and growing season, enabled the evaluation of differences in light of natural variation. The results of this study suggest that genotype and environment are the major contributors to differentiations seen in metabolite profiles of maize kernels. The application of different input systems had only a small impact on the metabolites covered by the applied analytical approach. The few consistent differences

seen between maize grown conventionally and organically are in agreement with phenomena previously observed for organically grown crops.

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