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Metabolite Profiling of Germinating Rice Seeds

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A metabolite profiling approach based on gas chromatography−mass spectrometry (GC-MS) was used to investigate time-dependent metabolic changes in the course of the germination of rice. Brown rice kernels were soaked and incubated for a total of 96 h under ambient conditions. Samples taken during the germination process were subjected to an extraction and fractionation procedure covering a broad spectrum of lipophilic (e.g., fatty acid methyl esters, hydrocarbons, fatty alcohols, sterols) and hydrophilic (e.g., sugars, acids, amino acids, amines) low molecular weight rice constituents. Investigation of the obtained fractions by GC resulted in the detection of 615 distinct peaks, of which 174 were identified by means of MS. Statistical assessment of the data via principal component analysis demonstrated that the metabolic changes during the germination process are reflected by time-dependent shifts of the scores, which were similar for the three rice materials investigated. Analysis of the corresponding loadings showed that polar metabolites were major contributors to the separation along the first principal component. Relative quantifications based on standardized peak heights revealed dynamic changes of the metabolites in the course of the germination.

KEYWORDS: Metabolite profiling; rice; *Oryza sativa* L.; germination; GC-MS

INTRODUCTION

Metabolites are the end products of cellular processes and represent the ultimate reflection of the response of biological systems to genetic or environmental changes (*1*). Accordingly, "unbiased" approaches to metabolite analysis have been developed in recent years, providing tools that complement other untargeted techniques to analyze gene products, such as transcriptomics or proteomics (*2*). Metabolomics, that is, the measurement of all metabolites in systems under given conditions, is an extremely challenging goal requiring the interplay of various complementary techniques. Metabolite profiling can be considered as one of the most pragmatic approaches presently applied. It aims at extracting, separating, and analyzing a spectrum of metabolites as broad as possible from complex matrices in an effective and reproducible way (*3*). Among the various technology platforms established for metabolite profiling, the coupling of capillary gas chromatography and mass spectrometry (GC-MS) proved to be one of the most robust methodologies, in particular, for a comprehensive analysis of primary plant metabolites (*4*).

Metabolite profiling has been applied to assess genotypic and phenotypic diversity in plants. Comparative investigations of breeding systems, for example, conventional versus genetically modified crops (*5*), or the impact of induced mutations on crop metabolites (*6*) have been conducted. Moreover, the influence of farming practices and environmental impacts on crops have been investigated (*7*). Metabolite profiling techniques have also been employed to follow the development of plant systems, for example, the metabolic changes in *Arabidopsis* seeds (*8*) and strawberry fruits (*9*). For rice, metabolomic data have been used to investigate changes during tillering (*10*), to reveal metabolic modulation in foliage (*11*), and to phenotype natural variants (*12*).

One example for an important stage in the development of plants is the germination of seeds. This phase in the life cycle of a plant is characterized by a combination of various catabolic and anabolic processes. Distinct and time-dependent alterations in metabolite levels are to be expected, and metabolite profiling should be a suitable analytical tool to provide a comprehensive

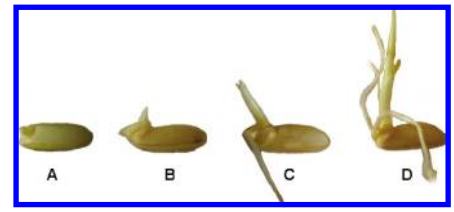

Figure 1. Germinated brown rice after a soaking period of 24 h (**A**) and after incubation times of 48 h (**B**), 72 h (**C**), and 96 h (**D**).

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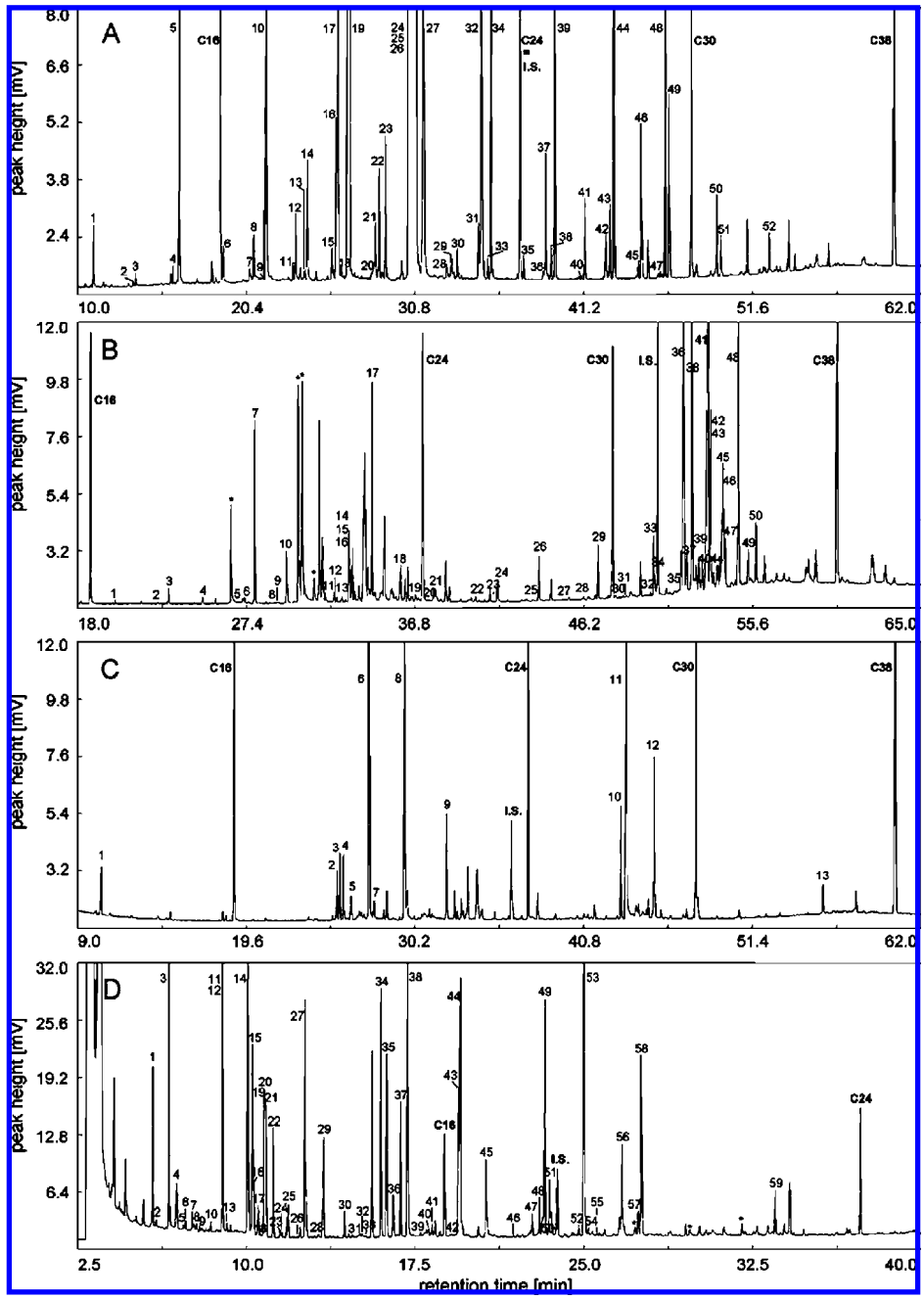

Figure 2. CG-FID chromatograms of fraction I (**A**), fraction II (**B**), fraction III (**C**), and fraction IV (**D**) obtained from germinated rice II32B-ge. I.S.: internal standards tetradecane (**A**), 5α-cholestane-3β-ol (**B**), phenyl-β-D-glucopyranoside (**C**), and *p*-chloro-L-phenylalanine (**D**). C16, C24, C30, C38: retention time standards; identification of peaks is given in **Tables 1**−**3**. Residual FAME in fraction II and residual sugars in fraction IV are marked with asterisks.

picture of these changes. Therefore, the aim of this study was to apply metabolite profiling based on GC-MS on germinating seeds.

Brown rice kernels were selected as example. Germinated brown rice has been commercially available in Japan since 1995. Germination has been considered to enhance the texture and to improve the nutritional value of brown rice (*13*). Targeted analyses have demonstrated that the germination of rice is accompanied by a spectrum of significant changes in metabolite contents (*13*−*15*). A proteomics-based investigation of rice seeds (*16*) during germination has also been performed. However, no metabolite profiling-based investigations of the germination process have been described. The objectives of this study were to analyze a broad spectrum of low molecular weight compounds covering a wide range of chemical classes in germinating rice materials, to test the applied GC-MS approach for its suitability to reflect the germination process by a time-dependent clustering based on multivariate analysis, and to quantify constituents and follow their dynamic changes during the germination.

MATERIALS AND METHODS

Chemicals. Internal standards (tetracosane, 5α-cholestan-3β-ol, phenyl-β-D-glucopyranoside, *p*-chloro-L-phenylalanine) and retention time standards (undecane, hexadecane, tetracosane, triacontane, octatriacontane) were purchased from Fluka (Taufkirchen, Germany). Reference compounds were obtained from VWR International (Darmstadt, Germany), Fluka (Taufkirchen, Germany), Sigma-Aldrich (Steinheim, Germany), Cognis (Illertissen, Germany), and Roth (Karlsruhe, Germany). All other reagents and solvents were of analytical grade.

Table 1. Compounds Identified in Fraction I in Germinated Rice II32B-ge

no.	compd	ident[a]	no.	compd	ident[a]	no.	compd	ident[a]
saturated FAME[b]			unsaturated FAME			hydrocarbons		
1	C10:0	A	**8**	C14:1	A	**2**	C14	A
3	C11:0	A	**12**	C15:1	A	**4**	C15	A
5	C12:0	A	**16**	C16:1	C	**9**	C17	A
6	C13:0	A	**17**	C16:1*cis*	A	**13**	C18	A
10	C14:0	A	**21**	C17:1	A	**18**	C19	A
14	C15:0	A	**24**	C18:1	A	**22**	C20	A
19	C16:0	A	**28**	C19:1	B	**29**	C22	A
23	C17:0	A	**32**	C20:1	A	**33**	C23	A
27	C18:0	A	**37**	C22:1	A	**38**	C25	A
30	C19:0	A	**42**	C24:1	A	**40**	C26	A
34	C20:0	A				**43**	C27	A
35	C21:0	A	**7**	C14:2	C	**45**	C28	A
39	C22:0	A	**11**	C15:2	C	**46**	squalene	A
41	C23:0	A	**15**	C16:2	C	**47**	cholestane	C
44	C24:0	A	**20**	C17:2	C	**48**	C29	A
49	C26:0	A	**25**	C18:2	A	**50**	C31	A
51	C28:0	A	**31**	C20:2	A			
52	C30:0	A	**36**	C22:2	A			
			26	C18:3	A			

[a] Identification according to (A) mass spectrometric data and retention time of reference compound, (B) mass spectrometric data and retention index of the Golm metabolome database, and (C) NIST 02 MS library. [b] Fatty acid methyl esters.

Rice Material. Rice seeds II32B-ge had been obtained by mutation breeding from the hybrid rice maintainer II32B; seeds MH-ge2 and MH-ge3 originated from the rice cultivar Minghui 86. The seeds were planted during the 2005 winter season at Hainan, China.

Sample Preparation. Rough rice grains were manually dehulled by means of a wooden rice dehuller. The brown rice seeds were soaked in tap water at 30 °C for 24 h. After the soaking period, the seeds were placed on moist filter paper in Petri dishes and incubated at 30 °C for 72 h. Samples were taken after 0 h (brown rice before soaking), 24 h (soaked brown rice), 48, 72, and 96 h. Samples were immediately frozen in liquid nitrogen and ground with a cyclone mill equipped with a 500 μm sieve (Cyclotec, Foss, Germany). The flour was freeze-dried for 48 h and stored at −18 °C until analysis.

Metabolite Profiling. Extraction and fractionation of freeze-dried rice flour were performed in accordance with previously described procedures (*6*). Lipids and polar compounds were consecutively extracted from the flour. Lipids were transesterified in methanol and

Table 3. TMS Derivatives of Compounds Identified in Fractions III (Sugars and Sugar Alcohols) and IV (Acids, Amino Acids, and Amines) in Germinated Rice II32B-ge

no.	compd	ident[a]	no.	compd	ident[a]
sugars and sugar alcohols			amino acids and amines		
1	glycerol	A	**3**	alanine	A
2, **3**, **4**	fructose	A	**9**	2-aminobutyric acid	A
5, **7**	galactose	A	**10**	β-alanine	A
6, **8**	glucose	A	**11**	valine	A
9	*myo*-inositol	A	**12**	norvaline	A
10, **11**	sucrose	A	**15**	leucine	A
12	trehalose	A	**16**	ethanolamine	A
13	raffinose	A	**18**	γ-aminobutyric acid	A
			19	isoleucine	A
acids			**20**	proline	A
1	lactic acid	A	**22**	glycine	A
2	glycolic acid	A	**27**	serine	A
5	3-hydroxypropanoic acid	C	**29**	threonine	A
6	pyruvic acid	A	**30**	β-alanine	A
7	β-hydroxybutyric acid	A	**31**	homoserine	A
8	3-methyl-2-hydroxybutyric acid	C	**33**	β-aminoisobutyric acid	A
13	γ-hydroxybutyric acid	C	**35**	pyroglutamic acid	A
14	phosphoric acid	A	**36**	methionine	A
17	maleic acid	A	**37**	aspartic acid	A
21	succinic acid	A	**38**	γ-aminobutyric acid	A
24	glyceric acid	A	**39**	5-hydroxynorvaline	C
25	fumaric acid	A	**42**	2-aminopimelic acid	C
26	pyrrole-2-carboxylic acid	A	**43**	glutamic acid	A
28	glutaric acid	A	**44**	phenylalanine	A
32	2-piperidinecarboxylic acid	C	**45**	asparagine	A
34	malic acid	A	**46**	α-aminoadipic acid	A
40	threonic acid	A	**48**	putrescine	A
41	3-phenyl lactic acid	A	**49**	glutamine	B
47	*cis*-aconitic acid	C	**52**	citrulline	A
50	3-glycerophosphoric acid	B	**54**	ornithine	A
51	2-aminoethylphosphoric acid	C	**56**	histidine	A
53	citric acid	A	**57**	lysine	A
			58	tyrosine	A
others			**59**	tryptophan	A
4	2-pyrrolidinone	A			
23	2,4-hydroxypyrimidine	C			
55	adenine	A			

[a] Identification according to (A) mass spectrometric data and retention time of reference compound, (B) mass spectrometric data and retention index of the Golm metabolome database, and (C) NIST 02 MS library.

Table 2. TMS Derivatives of Compounds Identified in Fraction II in Germinated Rice II32B-ge

no.	compd	ident[a]	no.	compd	ident[a]	no.	compd	ident[a]
free fatty acids			fatty alcohols			sterols and triterpenic alcohols		
1	C12:0	A	**7**	C16:0	A	**34**	cholesterol	A
2	C13:0	A	**12**	C18:0	A	**36**	campesterol	A
4	C14:0	A	**13**	phytol	A	**37**	campestanol	A
6	C15:0	A	**19**	C20:0	A	**38**	stigmasterol	A
8	C16:1	C	**22**	C22:0	A	**39**	Δ^7-campestenol	*23*
9	C16:1 (*cis* 9)	A	**26**	C24:0	C	**41**	β-sitosterol	A
10	C16:0	A	**29**	C26:0	A	**42**	sitostanol	A
11	C17:0	A	**33**	C28:0	A	**43**	Δ^5-avenasterol	A
14	C18:3	A	**40**	C30:0	D	**44**	gramisterol	*24*
15	C18:2	A	**50**	C32:0	D	**45**	Δ^7-stigmastenol	*24*
16	C18:1	A				**46**	cycloartenol	A
17	C18:0	A	phenolic compounds			**47**	Δ^7-avenasterol	*24*
18	C19:0	A	**3**	methyl *p*-hydroxy-cinnamate	A	**48**	24-methylene-cylcoartanol	A
20	C20:1	A	**5**	methyl ferulate	A	**49**	citrostadienol	*24*
21	C20:0	A	tocopherols					
23	C22:1	A	**28**	δ-tocopherol	A			
24	C22:0	A	**30**	γ-tocopherol	B			
25	C23:0	A	**32**	α-tocopherol	A			
27	C24:0	A						
31	C26:0	B						
35	C28:0	A						

[a] Identification according to (A) mass spectrometric data and retention time of reference compound, (B) mass spectrometric data and retention index of the Golm metabolome database, (C) NIST 02 MS library, and (D) MS data.

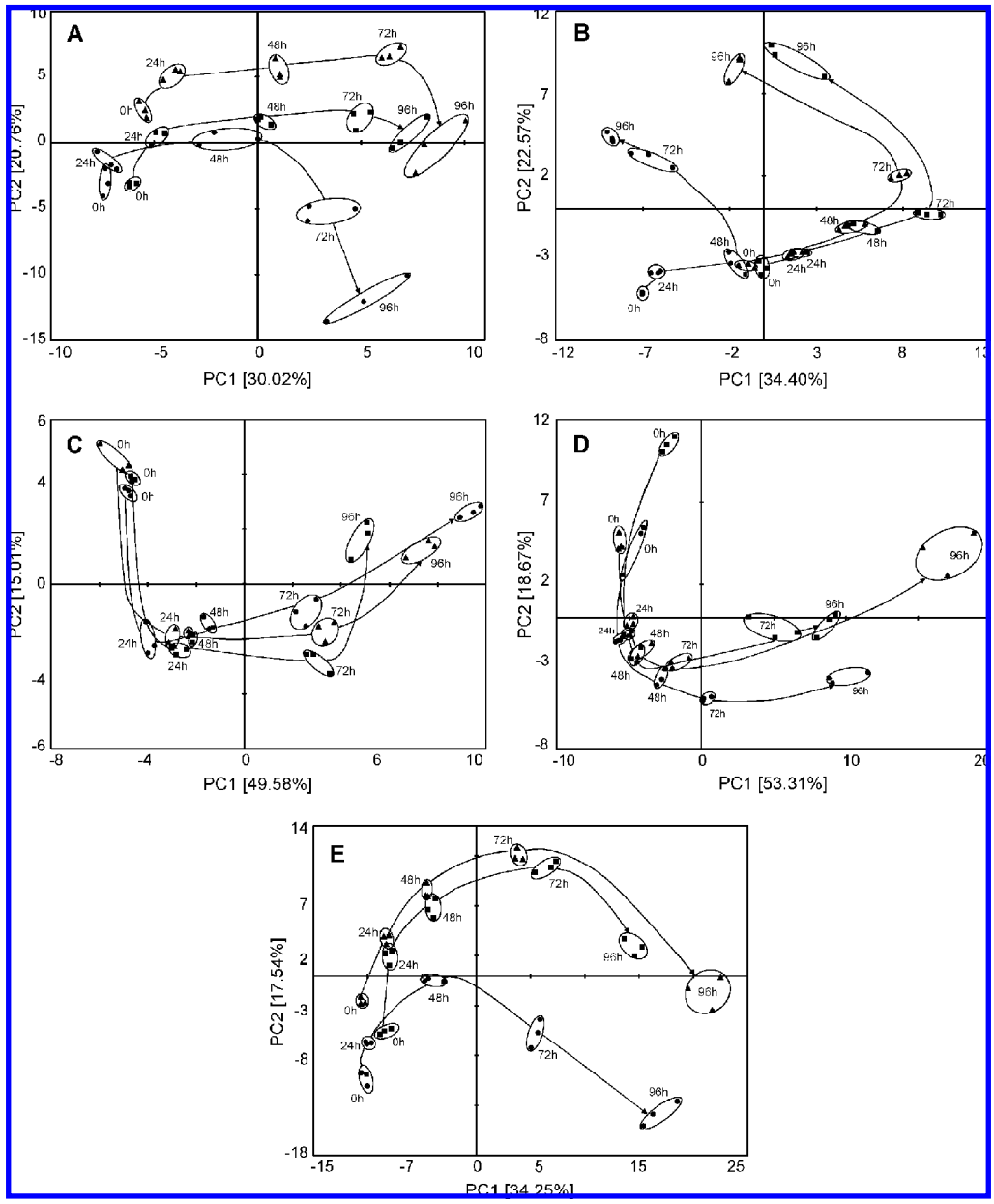

Figure 3. PCA of standardized GC-FID metabolite profiling data of fractions I (**A**), II (**B**), III (**C**), and IV (**D**) and of combined fractions I−IV (**E**) in the course of germination (0, 24, 48, 72, and 96 h) of II32B-ge (●), MH-ge2 (▲), and MH-ge3 (■).

subsequently separated by solid phase extraction into a fraction containing fatty acid methyl esters (FAME) and hydrocarbons (fraction I) and a fraction containing minor lipids, for example, 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 gas chromatography (GC-FID, GC-MS). Fractions II and IV were silylated before GC analysis. The GC conditions were in agreement with previously described procedures (*6*). Rice constituents were identified by comparing retention times and mass spectra with those for reference compounds and by comparing mass spectra with the entries of the mass spectra libraries NIST02 and the Golm metabolome database (*17*).

Statistical Analysis. Rice samples were analyzed in triplicate. GC-FID data were acquired and integrated using Chrom-Card 2.3 (Thermo Electron, Italy). Peak heights and retention times were exported to Chrom*pare* 1.1 (*18*) for standardization and consolidation of the data. Principal component analysis (PCA) was performed using XLSTAT 7.5.2 (Addinsoft, France).

RESULTS AND DISCUSSION

Three brown rice samples (II32B-ge, MH-ge2, and MH-ge3) were subjected to germination. The morphological changes of the rice kernels observed upon soaking and incubation are shown in **Figure 1**. The initial enlargements of the rice embryos and the subsequent formations of shoots and roots are in good agreement with changes described for germinated rice grains (*19*). By definition, germination of seeds begins with water uptake (imbibition) by the quiescent seed and ends with the start of elongation by the embryonic axis, usually the radicle.

Extraction and Fractionation. Samples taken at different time points during the germination process were immediately freeze-dried and ground. Freeze-dried flour was subjected to the extraction and fractionation scheme previously described for rice grains (*6, 20*). The approach is comparable to other strategies with regard to the extraction of metabolites with solvents differing in polarity and subsequent derivatization (*21, 22*). However, additional transesterification−solid phase extraction

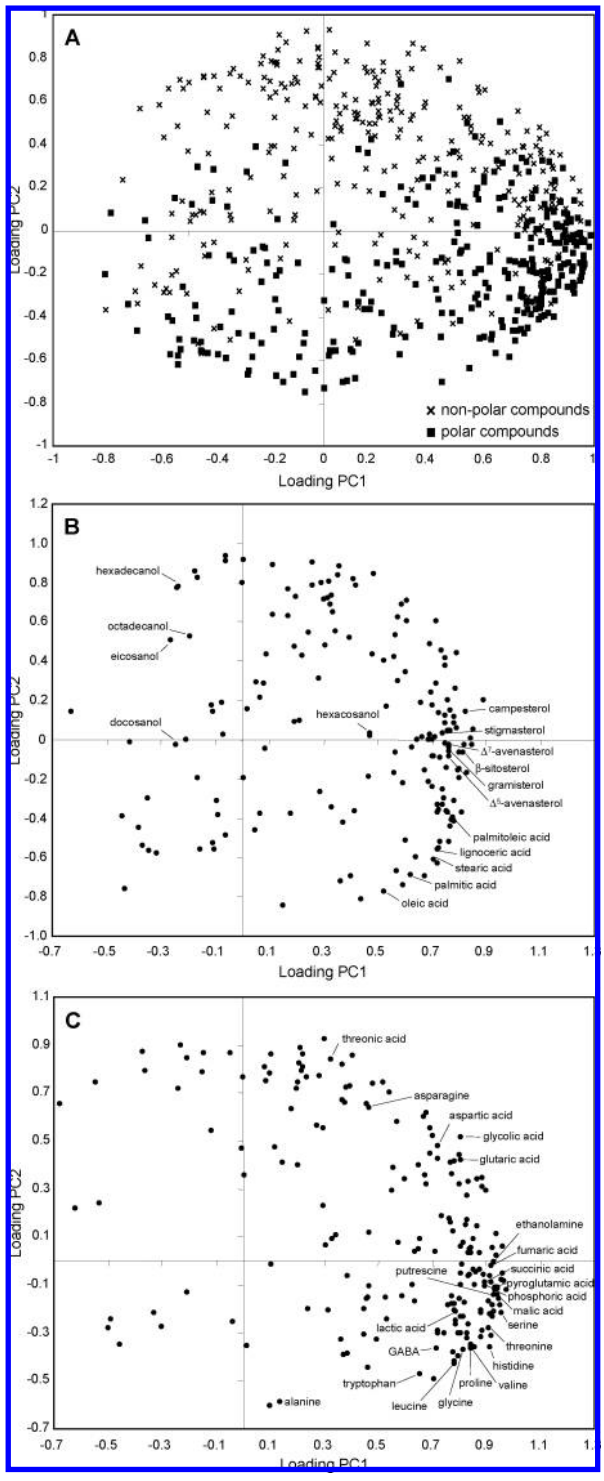

Figure 4. Loading plots of standardized GC-FID metabolite profiling data from nonpolar and polar compounds (fractions I−IV) (**A**), minor lipids (fraction II) (**B**), and acids, amino acids, and amines (fraction IV) (**C**).

(lipids) and selective hydrolysis of silylated derivatives (polar compounds) are applied to separate major from minor constituents. This procedure results in four fractions containing fatty acid methyl esters and hydrocarbons (fraction I), free fatty acids, alcohols, and sterols (fraction II), sugars and sugar alcohols (fraction III), and acids, amino acids and amines (fraction IV).

Capillary Gas Chromatography. The fractions were analyzed by capillary gas chromatography. GC-FID analysis resulted in the detection of a total of 615 peaks in the course of the germination of the brown rice. As examples, the four fractions obtained from the germinated rice II32B-ge are shown in **Figure 2**. GC-MS analysis enabled the identification of 174 peaks (**Tables 1−3**).

Principal Component Analysis. For consolidation of the raw data, peak heights and corresponding retention times were exported to Chrom*pare*, a software tool developed for comparative analysis of metabolite profiling data (*18*). Chrom*pare* automatically corrects retention time shifts on the basis of retention time standards and standardizes peak heights on the basis of internal standards added before the fractionation process. The consolidated data were statistically assessed using PCA. Scores plots obtained for the four single fractions and the combined four fractions are shown in **Figure 3**. The metabolic changes are reflected by time-dependent shifts of the scores for the first two principal components PC1 and PC2. For the nonpolar fractions, the first two principal components explained 51% of the total variation in fraction I (**Figure 3A**) and 57% in fraction II (**Figure 3B**). An even more pronounced variance was covered by the first two principal components for the polar fractions: 65% in fraction III (**Figure 3C**) and 72% in fraction IV (**Figure 3D**), respectively. In the scores plot based on the combined metabolite profiling data from all four fractions a variance of 52% was covered by PC1 and PC2 (**Figure 3E**). Similar coverages of variance have been reported in other metabolite profiling studies (*5, 7*).

All three rice samples showed similar score patterns depending on the time of germination. However, a differentiation between II32B-ge, derived from the hybrid rice maintainer II32B, and the two Minghui 86-derived rice lines MH-ge2 and MH-ge3 was observed.

Analysis of the corresponding loadings taking into account the data of all 615 peaks detected in fractions I−IV resulted in the loading plot shown in **Figure 4A**. Polar metabolites were found to be major contributors to the separation along the first principal component, whereas predominantly nonpolar metabolites were responsible for the separation along the second principal component, indicating more pronounced changes of polar metabolites during the germination process. Panels **B** and **C** of **Figure 4** show examples of the loading plots obtained for fractions II and IV.

Relative Quantifications. Quantitative comparisons were based on standardized peak heights. **Figures 5** and **6** show changes observed for representatives of different classes of metabolites in the course of the germination process.

Fatty Acid Methyl Esters. The fatty acid methyl esters detected in fraction I result from transesterification of the lipid extract and reflect the total contents and the fatty acid compositions of the rice triglycerides. After 96 h of germination, slightly decreased contents of fatty acid methyl esters were observed for II32B-ge (−32%) and MH-ge2 (−20%), whereas the level in MH-ge3 remained almost unchanged (**Figure 5A**). A decrease of the crude lipid content during germination by 25% has been reported for barley (*25*). However, it was noted that the degree of lipolysis is greatly influenced by the germination conditions, for example, temperature, moisture, and germination time.

Hydrocarbons. Heptacosane, octacosane, nonacosane, and squalene were major representatives of the hydrocarbons detected in fraction I. The peak heights determined for these metabolites significantly increased in the course of the germination (**Figure 5A**). Hydrocarbons are known constituents of starch lipids in rice (*26*). Germination of rice involves cytolytic and amylolytic degradation of rice starch, which might result in an improved extraction of grain lipid constituents associated with polysaccharides.

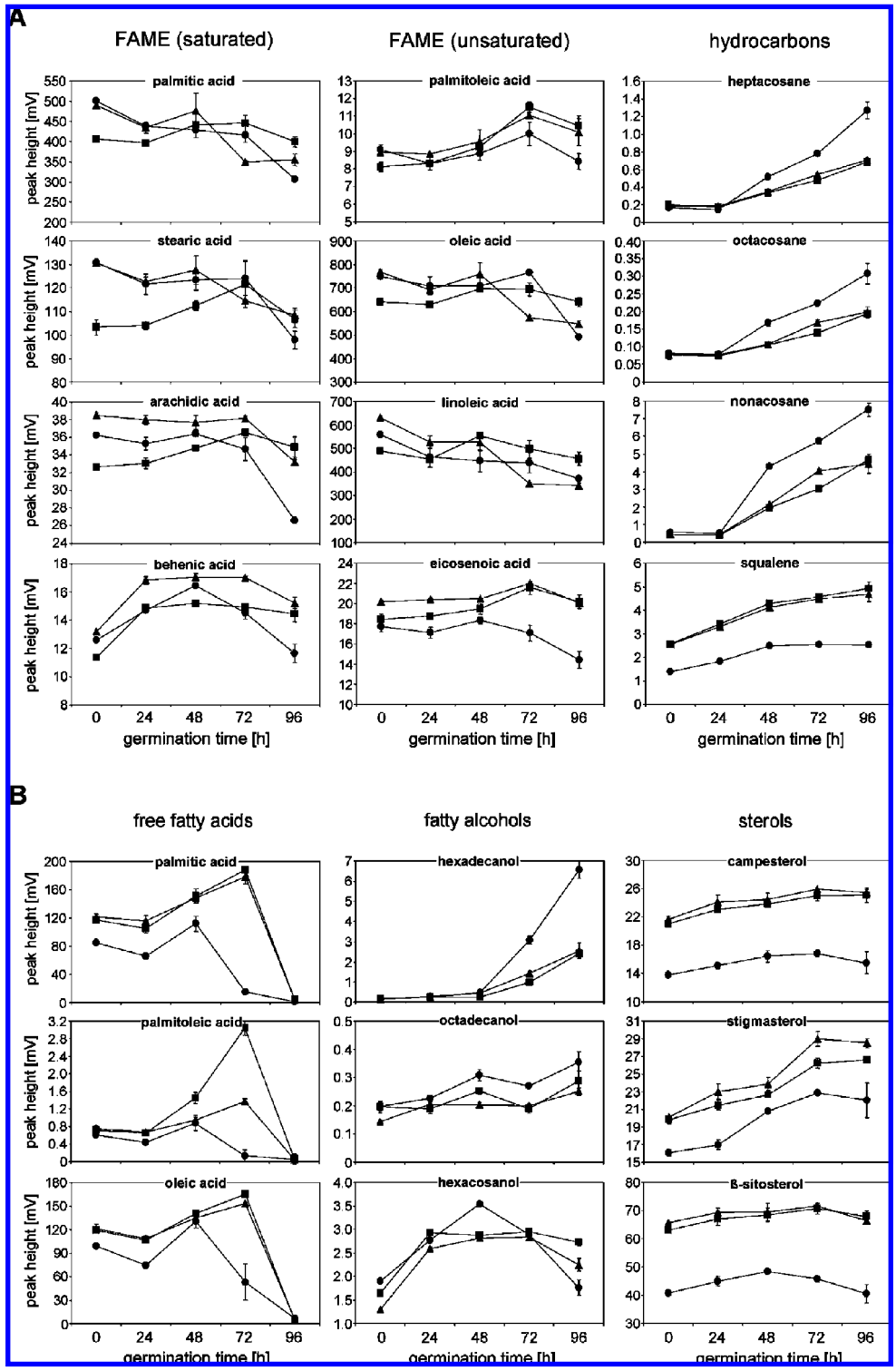

Figure 5. Standardized peak heights (mean ± confidence interval, $n = 3$) for selected fatty acid methyl esters (FAME; saturated and unsaturated) and hydrocarbons obtained from fraction I (**A**) and free fatty acids, fatty alcohols, and sterols obtained from fraction II (**B**) in the course of germination of II32B-ge (●), MH-ge2 (▲), and MH-ge3 (■).

Free Fatty Acids/Fatty Alcohols. Compared to brown rice, germinated rice incubated for 96 h exhibited significantly decreased contents of free fatty acids (on average, −93%). As shown in **Figure 5B** for palmitic, palmitoleic, and oleic acid, concentrations of free fatty acids increased in the initial stage of the incubation, but decreased rapidly after 72 h. In contrast, contents of fatty alcohols, for example, hexadecanol, octadecanol, and hexacosanol, were shown to be increased in germinated rice compared to ungerminated rice (**Figure 5B**).

Sterols. The overall changes in sterol levels were relatively small. Germination resulted in slightly increased concentrations in germinated rice ranging from +6% in II32B-ge to +12% in MH-ge3, respectively. Changes observed for the three major sterols campesterol, stigmasterol, and β-sitosterol are shown in **Figure 5B**. In soybeans these sterols exhibited a more pronounced increase (on average, +30%) after 120 h of germination (*27*). Germination of tobacco seeds resulted in changes of the three major sterols similar to those seen in the present study (*28*).

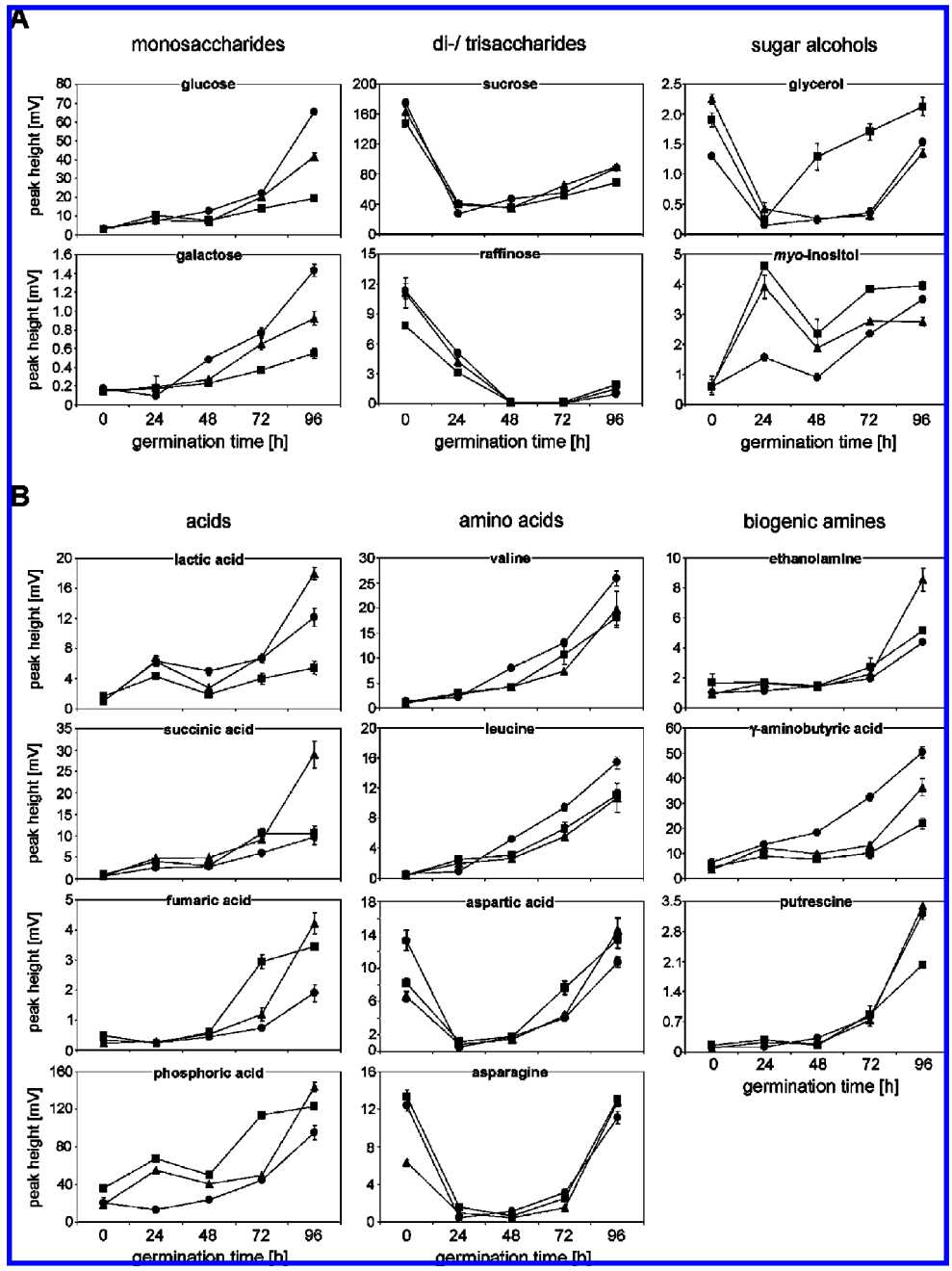

Figure 6. Standardized peak heights (mean ± confidence interval, $n = 3$) for selected sugars and sugar alcohols obtained from fraction III (**A**) and acids, amino acids, and amines obtained from fraction IV (**B**) in the course of germination of Il32B-ge (●), MH-ge2 (▲), and MH-ge3 (■).

Sugars. Compared to nonpolar metabolites, the changes observed for polar constituents were much more pronounced. Levels of monosaccharides (e.g., glucose, galactose) increased significantly in the course of the germination, whereas those of di- and trisaccharides (e.g., sucrose, raffinose) were drastically reduced (**Figure 6A**). The data are in agreement with results obtained by targeted analyses of sugars in germinated barley (*25*). Analysis of rice starch during a germination period of 7 days revealed a decrease by −43%, whereas an up to 40-fold increase in the level of reducing sugars was observed (*14*). Similarly to the lipid degradation, starch degradation and accumulation of reducing sugars in germinating rice were shown to be greatly influenced by the incubation conditions (*29*). As shown for glycerol and *myo*-inositol, no consistent patterns were observed for sugar alcohols (**Figure 6A**).

Acids. In general, the levels of acids detected in fraction IV significantly increased during the germination (**Figure 6B**). Investigation of organic acids in barley during malting also revealed increased acid levels after 6 days of germination (*30*). However, the overall changes were much less pronounced than those observed in the present study.

Amino Acids. The levels of most amino acids increased significantly in the course of the germination. Representative examples (leucine, serine) are shown in **Figure 6B**. Only for two amino acids (aspartic acid and asparagine) were U-shape patterns observed. Significantly increased amino acid levels in different rice kernel fractions have been observed after a soaking period of 4 h (*31*). Increased contents of amino acids (up to +800%) have also been reported for germinated wheat (*32*). Previous investigations of changes in the amino acid levels revealed a strong influence of the germination conditions (*15, 29, 32*).

Amines. Changes observed for ethanolamine, γ-aminobutyric acid (GABA), and putrescine are shown in **Figure 6B**. A 5.0−10.5-fold increase in GABA content was observed for the

germinated rice material. Increased contents of GABA, the biogenic amine of glutamic acid, is of particular interest because of its health-promoting impacts on blood pressure and sleeplessness and suppression of liver damage (*13*). In soaked and germinated brown rice 2.3−24.7-fold increases in GABA contents have been reported (*13, 15, 33, 34*). Accumulation of GABA during soaking and germination was shown to vary greatly depending on the rice cultivar (*33*) and the germination temperature (*29*).

The concentration of glutamic acid was shown to be significantly decreased (−76%) in germinated brown rice (*13, 15*). In contrast to these findings, the glutamic acid contents in the three analyzed brown rice kernels consistently increased during the incubation process. However, incubation conditions had been shown to have a significant impact on the changes of glutamic acid contents (*15*).

Compared to ungerminated rice, the levels of putrescine significantly increased in the germinated rice, particularly in the last stage of the incubation. Comparably to the findings for GABA, the levels of the precursor amino acid ornithine were found to be significantly increased rather than decreased in the germinated brown rice. Putrescine, the precursor for the polyamines spermidine and spermine, was reported to play an important role in cellular plant processes (*35*). However, it is also discussed to potentiate the toxicity of other amines and may act as a spoilage indicator (*36*). To initiate germination, the brown rice samples investigated in this study were soaked in ordinary tap water. Therefore, changes of putrescine concentration may be a result of not only endogenous but also exogenous factors. Soaking of rice for 24 h at 35 °C was shown to increase the aerobic plate count by a factor of 10^4 (*15*).

The data obtained demonstrate the suitability of the described metabolite profiling technique to follow metabolic changes in a complex plant matrix. Owing to inherent features of the approach, for example, choice of extraction solvents, derivatization steps, or volatilities of derivatives, the type of metabolites covered is to some extent predetermined and not fully "unbiased". However, the metabolites stem from a wide range of chemical classes, and the number of compounds detected and identified is in an order of magnitude comparable to those reported for other GC-MS-based metabolite profiling studies (*10, 37*). In addition to representatives of primary plant metabolism, nutritionally relevant metabolites are covered. They range from the lipophilic sterols to the polar amino compounds GABA and putrescine. The applied methodology is suitable to cover those metabolites shown to form a basis for metabolic phenotyping of rice variants using a GC-MS approach (*12*). The metabolic changes observed during the germination of rice seeds also confirm the potential of metabolite profiling techniques to reveal distinct metabolic switches as indicated for the development and germination of *Arabidopsis* seeds (*8*).

Further studies are required to determine whether the time-dependent score patterns observed for the three rice materials investigated in this study are also valid for other varieties and incubation conditions. Ideally, a set of biomarker metabolites could be developed representing the germination period, as shown for the initiation and early growth of rice tillering (*10*). Such marker metabolites might also be correlated to the contents of nutritionally important metabolites claimed to be responsible for the advantageous properties of germinated rice (*13*).

Finally, the metabolite profiling approach might be incorporated as an additional tool in a comparative safety assessment approach as has been discussed for genetically modified crops because of its potential to increase the probability of detecting unintended effects (*38*).

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