JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 5499−5505 **5499**

Chemical Characterization of Interspecific Hybrids between *Allium cepa* L. and *Allium kermesinum* Rchb.

JÖRG STORSBERG,[†] HARTWIG SCHULZ,*[,†] MICHAEL KEUSGEN,[§] FADI TANNOUS,[§] KLAUS J. DEHMER,[#] AND E. R. JOACHIM KELLER[#]

Federal Centre for Breeding Research on Cultivated Plants, Institute for Plant Analysis (BAZ), Neuer Weg 22/23, D-06484 Quedlinburg, Germany; Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, D-53115 Bonn, Germany; and Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

Interspecific hybridization between wild and cultivated species of the genus *Allium* has been performed to generate plant material possessing biochemical properties of both parental plants. These cross-breeding experiments should lead to *Allium* plants with higher amounts of valuable constituents. The chemical characterization of interspecific hybrids between *A. cepa* and *A. kermesinum* is described on the basis of their sulfur-containing constituents and secondary metabolites. In addition, the hybrid character has been proven by random amplified polymorphic DNA (RAPD) analysis of the progenies obtained from the crosses. It has been shown that the distribution of the cysteine sulfoxides as well as the volatile secondary metabolites in the hybrids is not uniform. The profiles are mainly determined by the paternal wild species *A. kermesinum*. It has been ascertained that the gas chromatography profiles of the hybrids show increasing amounts of unsymmetrical substituted oligosulfides, which are known to be physiologically active substances. On the basis of statistical calculations, three different types of hybrids can be separated. The chemical analysis of cysteine sulfoxides and volatile sulfur-containing substances is shown to be a useful tool for breeding purposes as it allows an effective selection with regard to optimal distribution and amount of valuable constituents.

KEYWORDS: ***Allium*** **hybrids; RAPD analysis; SPME-GC analysis, cysteine sulfoxides; aroma profiles**

INTRODUCTION

In all times mankind has used several species of the genus *Allium* as food, spice, or herbal remedy. Some of these species have been cultivated, such as garlic (*Allium sativum*) or onion (*Allium cepa*). Today, their value for human health care is one of the most important aims of research, and up to now many applications of *Allium* species are known for the use of phytopharmaceutical preparations. Sulfur-containing constituents, which are mainly responsible for the pharmacological properties, due to some enzymatic reactions also cause the characteristic smell and taste of these plants. Starting with odorless, nonvolatile cysteine sulfoxide derivatives, such as (+)-*S*-(2-propenyl)-L-cysteine sulfoxide (alliin) or (+)-*S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin), which in the presence of the enzyme alliinase undergo reaction to alk(en)yl thiosulfinates, *Allium* plants generate the corresponding alk(en)yl (poly)sulfides as the main aroma compounds, released on injury (*1*−*3*). These substances are physiologically active and exhibit a widespread possibility of application, as there are antibiotic and antitumor properties, especially for stomach cancer protection (*2*, *4*). Investigations on various wild species of the genus *Allium* have shown that some of these species contain higher amounts of the aroma precursors (cysteine sulfoxides) than the cultivated species. Therefore, it was assumed that breeding experiments involving wild species and onion may increase the yields of volatile sulfur compounds of the hybrids in comparison to the maternal plant *Allium cepa*. As a side effect, there exists the possibility to obtain hybrids with new aroma profiles possessing both potent pharmacological and new flavoring properties. During recent years several of these experiments have been carried out (*5*), and also the subject of this work is to develop a new hybrid generated from crossings of *Allium kermesinum* and *A. cepa*. In this study we present the results of this hybridization demonstrated by the total amounts of cysteine sulfoxides and the aroma profiles in comparison to the parent plants.

MATERIALS AND METHODS

Reagents. All chemicals unless otherwise mentioned were purchased from Merck (Darmstadt, Germany) or Fluka (Neu-Ulm, Germany); if necessary, they were purified according to standard procedures. Authentic reference samples for gas chromatography (GC) and mass

* Address correspondence to this author at Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Pflanzenanalytik, Neuer Weg 22/23, D-06484 Quedlinburg, Germany [telephone +49(0)3946 47231; fax +49 (0)3946 47234; e-mail h.schulz@bafz.de].

[†] Federal Centre for Breeding Research on Cultivated Plants

[§] Institute for Pharmaceutical Biology.

[#] Institute of Plant Genetics and Crop Plant Research (IPK).

10.1021/jf049684a CCC: $27.50
Published on Web 07/30/2004

spectrometry (MS) were obtained from Oxford Chemicals, Harlepool, U.K. (allyl mercaptan, methyl propyl sulfide, diallyl sulfide, dipropyl sulfide, allyl methyl sulfide, allyl propyl sulfide, allyl propyl disulfide, allyl methyl disulfide, diallyl disulfide, diallyl trisulfide, dimethyl trisulfide, allyl propyl trisulfide, allyl methyl trisulfide), Symrise GmbH & Co. KG, Holzminden, Germany (dipropyl disulfide, dipropyl trisulfide), and Merck (dimethyl disulfide). *Taq* polymerase was obtained from Eppendorf (Hamburg, Germany), decamer primers were purchased from Operon (Alameda, CA), and the 100 bp DNA ladder was purchased from Invitrogen (Carlsbad, CA). Synthetic L-(+)-alliin and homologous sulfoxides were prepared following procedures already described (*6*, *7*). Briefly, l-cysteine was S-alkylated followed by oxidation of the sulfur atom. L-(±)-alliin was separated into the two diastereomers by fractional recrystallization.

Plant Material. The investigated *Allium* species *A. cepa* L. (culture variety Stuttgarter Riese) and *A. kermesinum* Rchb. as well as the resulting hybrids were cultivated under comparable conditions at the Institute of Plant Genetics and Crop Plant Research (IPK) (Gatersleben, Germany). Hybrids were generated by hand pollination and in vitro cultures (embryo rescue) (*8*). The successful hybridization was proven by randomly amplified polymorphic DNAs (RAPDs). All plants were harvested in late summer and autumn.

Sample Preparation. *Headspace Solid-Phase Microextraction Gas Chromatography (HS-SPME-GC) Analysis.* Several bulbs of the same sample were cut into small pieces (~1−2 mm). One gram of the freshly minced sample was transferred into a 20 mL headspace vial and combined with 5 mL of water. Then the sample suspension was tempered at 32 °C for 30 min until most of the enzymatic processes were finished. Then, further enzymatic activities were inhibited by adding 10.0 μL of sulfuric acid (0.1 M). Volatile substances, built under these conditions, were adsorbed on a SPME syringe [poly(dimethylsiloxane)-coated fiber, film thickness = 100 μm] for 10 min at 32 °C and immediately inserted to the injection port of the gas chromatograph.

HPLC and Biosensor. For analyses, 0.2−0.8 g (depending on the cysteine sulfoxides content) of an exactly weighed sample was heated for 10 min in 20 mL of methanol under reflux. The obtained residue was transferred to a mortar. After crushing, the material was resuspended in the same methanol for further extraction with the addition of 20 mL of water (*7*). The resultant extract was filtered, and the residue was washed three times with 3 mL of methanol. The combined filtrates were evaporated to dryness under reduced pressure and stored at −20 °C before further use. For biosensoric analyses, the obtained residue was redissolved in phosphate buffer (pH 7.0, 0.06 M, plus 0.17 M sodium chloride) to give a final volume of 25 mL. This solution was diluted 20 times (phosphate buffer) for the analysis of the genuine ammonium content and was diluted 40 times for the analysis of cysteine sulfoxides (final volumes = 25 mL).

For HPLC analyses, the residue was redissolved in phthaldialdehyde (OPA) derivatization reagent to give a final volume of 5 mL (*7*). Volumes of 15 μL each were analyzed after 30 min of incubation in the dark.

GC Analysis. GC analyses were performed on a Hewlett-Packard chromatograph model HP 5890 series II equipped with an FID and a 60 m × 0.25 mm i.d. HP-Innowax fused silica capillary column with a 0.5 μm bonded PEG phase. Volatiles were thermally desorbed from the SPME fiber for 2 min at 250 °C in the injection port and analyzed at the following conditions: injector temperature, 250 °C; detector temperature, 280 °C; H_2 flow rate, 1 mL/min; linear oven temperature program, from 35 to 220 °C at a rate of 10 °C/min; split ratio, 1:40. Mass spectrometry analyses were carried out on a Hewlett-Packard gas chromatograph (HP 5890 series II) using the same GC column as mentioned above, coupled with a mass spectrometer HP MSD model 5972. Operating conditions were as follows: carrier gas (He) velocity, 1 mL/min; ionization voltage, 70 eV; ion source temperature, 190 °C; scan range, 27−300 amu; libraries used for identification, Wiley 138 and NBS 75 K. The identification of the detected GC signals was confirmed by cochromatography and mass spectra of authentic standards. Linear retention indices of the volatile sulfur components were calculated against C_7−C_{20} *n*-paraffins as references according to the method of van den Dool and Kratz (*9*); the obtained results are presented in **Table 1**. The (*E*) and (*Z*) isomers of propyl 1-propenyl disulfide were distinguished relating to the published data of Brodnitz et al. (*10*) as well as Kallio and Salorinne (*11*).

Table 1. Relative Retention Time Indices of Volatile Sulfur Components Detected in *Allium* Species and Hybrids

compound	retention time	retention index
propyl mercaptan	5.51	845
2-propenyl mercaptan	6.14	891
methyl 2-propenyl sulfide	7.16	976
dipropyl sulfide	8.87	1093
dimethyl disulfide	9.04	1105
2-propenyl propyl sulfide	9.52	1137
di-2-propenyl sulfide	10.13	1177
methyl propyl disulfide	11.49	1271
dimethyl thiophene	11.81	1294
(*Z*)-methyl 1-propenyl disulfide	12.02	1308
methyl 2-propenyl disulfide	12.22	1322
(*E*)-methyl 1-propenyl disulfide	12.35	1332
dipropyl disulfide	13.61	1421
dimethyl trisulfide	13.88	1442
(*Z*)-1-propenyl propyl disulfide	14.12	1460
2-propenyl propyl disulfide	14.31	1474
(*E*)-1-propenyl propyl disulfide	14.42	1482
di-2-propenyl disulfide	14.99	1526
(*E*)-1-propenyl 2-propenyl disulfide	15.07	1533
methyl propyl trisulfide	15.86	1595
methyl 2-propenyl trisulfide	16.62	1658
dipropyl trisulfide	17.56	1738
2-propenyl propyl trisulfide	18.24	1797

RAPD Analysis. After DNA extraction using a modified Doyle and Doyle protocol (*12*, *13*), RAPD PCRs were carried out in 15 μL assays containing 50 ng of template DNA, 0.4 unit of *Taq* polymerase, 0.4 μM decamer primer (OP-A19, OP-D03, OP-D11, OP-F05, OP-G11), 0.1 mM of each dNTP, 2.0 mM magnesium chloride, and 10 ng of BSA per assay in a buffer supplied by the manufacturer. Amplifications were performed after an initial denaturing step (5 min, 95 °C) in 40 cycles under standard RAPD conditions (*14*): 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C, followed by a final 5 min polymerization step at 72 °C. Reaction products were separated by electrophoresis on 1.5% agarose gels, with a 100 bp DNA ladder being used as a molecular weight marker.

Cysteine Sulfoxide Analysis. Quantitative analyses was performed on a Shimadzu LC-4A liquid chromatograph equipped with a Chromatopac C-R3A integrator and a Spherimarge 80-ODS2 RP column (5 μm particle size; 250 × 4 mm with integrated guard column) operating at a constant flow rate of 1.0 mL/min. Detection was carried out using a UV detector set at 335 nm.

Best results for the analysis of cysteine sulfoxides were obtained using a procedure described by Krest et al. (*7*). A solvent gradient starting at 19% acetonitrile in phosphate buffer (pH 6.5, 0.05 M) for between 10 and 20 min, up to 27% at 50 min and 30% after 55 min was most suitable for the analysis of cysteine sulfoxides. Peaks were identified by cochromatography with standards as well as by comparison with samples after alliinase digestion (selective degradation of cysteine sulfoxides). Isoalliin was isolated from an authentic sample of *A. cepa*. Standards of cysteine sulfoxides were synthesized following the procedures formerly described (*6*, *7*). The quantitative HPLC determination was calibrated over the range of 0.01−0.4 mg/mL cysteine sulfoxides (injection volume = 15 μL). Standards were derivatized in the same manner as described above.

Determination of free ammonia and enzymatically formed ammonia was determined by a flow-through apparatus (*15*). Ammonia in equimolar amounts was obtained from cysteine sulfoxides by immobilized alliinase. Alliinase was isolated, stabilized, and purified according to the methods already described (*16*, *17*). Garlic powder was homogenized, and a crude alliinase preparation was obtained by precipitation with ammonium sulfate or by ultrafiltration. Ultrafiltered alliinase was used for standard experiments. Alliinase was stabilized with 10% sucrose, 0.17 M sodium chloride, and 25 mM pyridoxal-5′-phosphate (P-5′-P) dissolved in phosphate buffer (pH 7, 60 mM).

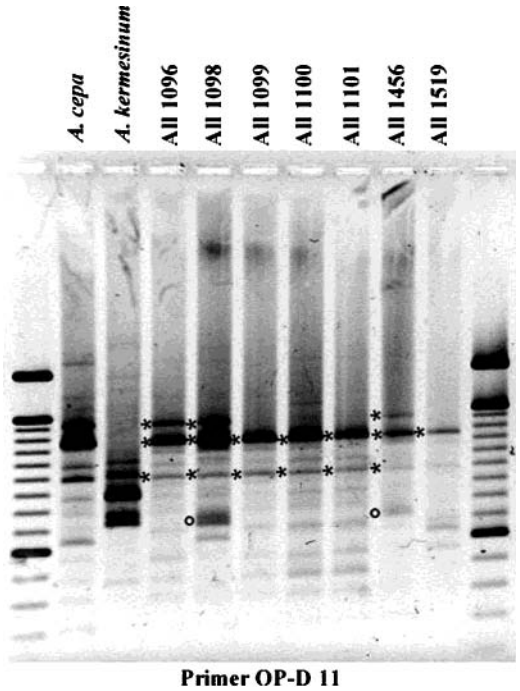

Figure 1. Random amplified polymorphic DNA (RAPD) analysis using OP-D11 as a primer.

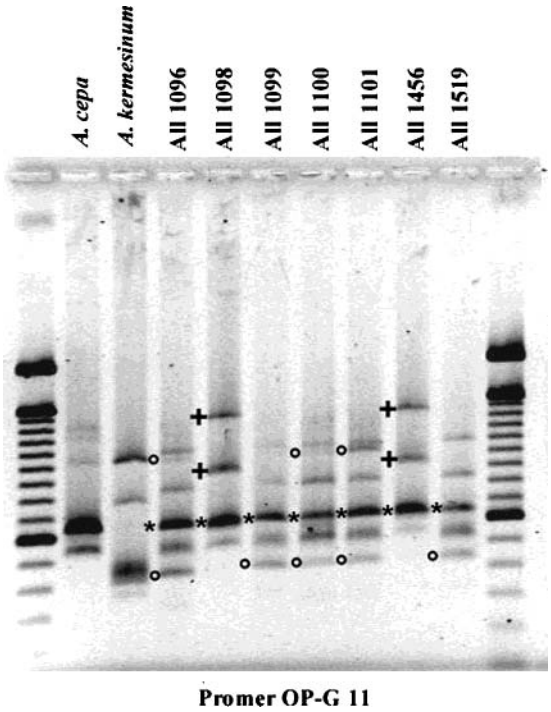

Figure 2. Random amplified polymorphic DNA (RAPD) analysis using OP-G11 as a primer.

Further purification was done by gel filtration followed by affinity chromatography. Immobilization of alliinase on a ConA/agarose carrier (Sigma, ord. no. C 7555). Briefly, the carrier was filled into a cartridge (60 μL) and rinsed with buffer. A solution containing alliinase was then pumped through this cartridge. Immobilized alliinase may be also renewed. Elution of the enzyme was performed with buffer containing mannose followed by rinsing with a glucose solution and buffer. The carrier may be newly loaded with alliinase as described above. For analysis of cysteine sulfoxides, the cartridge was operated with phosphate buffer (pH 7.0, 0.02 M containing 0.17 M sodium chloride). Analysis of cysteine sulfoxides by HPLC and the biosensor was repeated at least three times.

Statistical Calculations. The statistical calculations were performed with Statistica 6.1 by StatSoft Inc. 1984−2002 (Tulsa, OK).

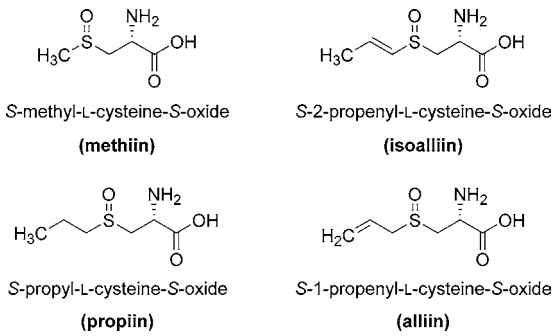

Figure 3. Structures of the four cysteine sulfoxides occurring in species of the genus *Allium*.

RESULTS AND DISCUSSION

RAPD Analysis. To prove the hybrid character of the progenies obtained from the crosses (performed using a well-defined mother plant of *A. cepa* and a population of pollinators derived from one accession of *A. kermesinum* of the collection), RAPD patterns of the parents and their putative hybrid offsprings were compared.

Besides by bands from the maternal parent *A. cepa* (*), several of the offspring are characterized by +hybrid bands also present in the examined individual plant of the *A. kermesinum* pollinator population All 1246 (°). Additional nonmaternal bands in the offspring (+), which most likely originate from other genotypes within the All 1246 population, also prove the hybrid status of the progeny (**Figures 1** and **2**).

Cysteine Sulfoxides. Four cysteine sulfoxides are known to be genuine in *Allium* species; they are *S*-methyl-L-cysteine-*S*-oxide (methiin), *S*-2-propenyl-L-cysteine-*S*-oxide (alliin), *S*-propyl-L-cysteine-*S*-oxide (propiin), and *S*-1-propenyl-L-cysteine-*S*-oxide (isoalliin) (**Figure 3**).

In the maternal plant of the hybrids, *A. cepa*, the main cysteine sulfoxide is isoalliin, accompanied by methiin and propiin; alliin is present only in traces (*18*). Contrary to that, the distribution of cysteine sulfoxides in the wild species and paternal plant, *A. kermesinum*, is determined by alliin and methiin along with smaller amounts of propiin and isoalliin (**Figure 4**).

There have been produced six hybrids from one *A. cepa* line and a population of *A. kermesinum*, and these plants have been investigated by HPLC analysis to determine their cysteine sulfoxide pattern. The distribution of the four cysteine sulfoxides in *Allium* species is clearly based on their genetics (*19*). Due to the heterocygousity in the genus *Allium* in general and due to the population character of the paternal parent *A. kermesinum* in this special case, the distribution of the cysteine sulfoxides in the hybrids is not uniform. There is a more or less strong influence from the wild species, shown by the presence of alliin, the main cysteine sulfoxide in *A. kermesinum*, in every hybrid cysteine sulfoxide pattern. This influence varies from very low amounts of alliin in hybrid 3 to hybrid 6, in which alliin is the leading cysteine sulfoxide compound (**Figure 4**).

The total amount of cysteine sulfoxides has been determined in the fresh material of the investigated plants using a cysteine sulfoxide biosensor (*15, 16*). In comparison to the HPLC analysis, this method is much faster (high-throughput method) and leads to the same results of total cysteine sulfoxides. Contrary to distribution of the cysteine sulfoxides, here the

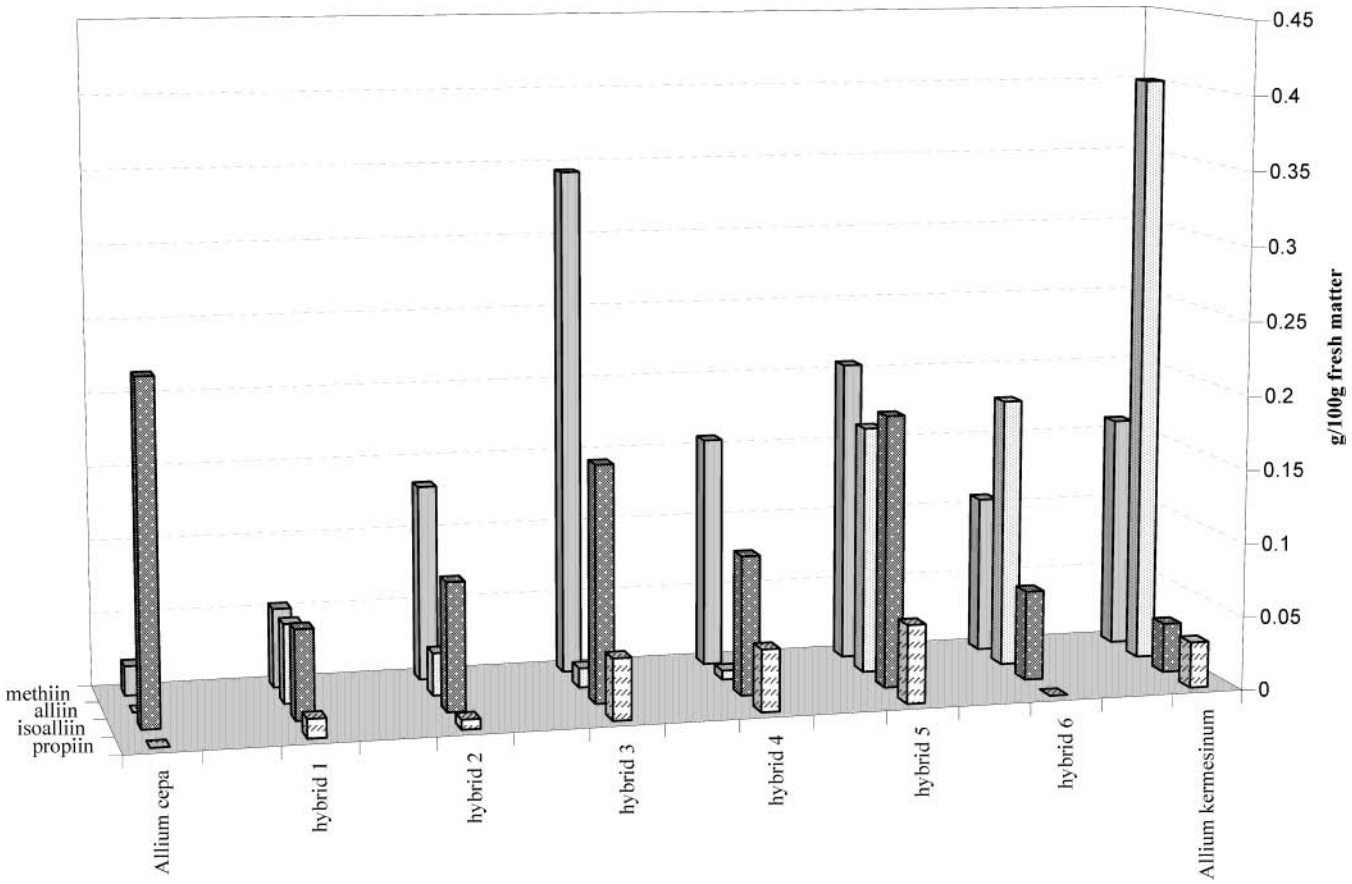

Figure 4. Distribution of cysteine sulfoxides in *A. kermesinum* and different *A. cepa* × *A. kermesinum* hybrids.

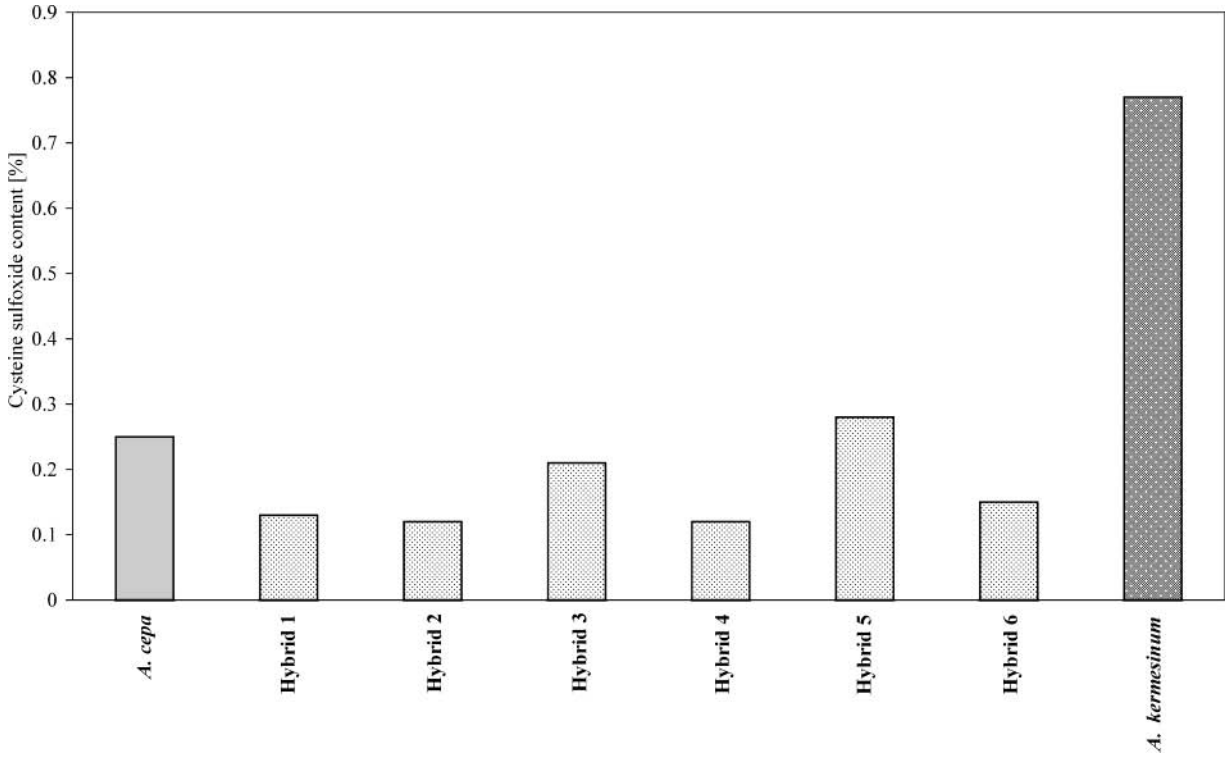

Figure 5. Total cysteine sulfoxide content found in different hybrids (*A. cepa* × *A. kermesinum*) and in the related parental species.

genetic influence of the paternal species *A. kermesinum*, which shows a high total amount of cysteine sulfoxides, seems to be not determining. Only one of the six analyzed hybrids presents a higher content of total cysteine sulfoxides than the maternal species *A. cepa* (**Figure 5**).

Aroma Profiles. Due to an enzymatic reaction with alliinase, the cysteine sulfoxides were metabolized, finally resulting in volatile sulfurous compounds such as mercaptans, sulfides, and disulfides (**Figure 6**). The pattern and distribution of these compounds is found to be specific for the individual species

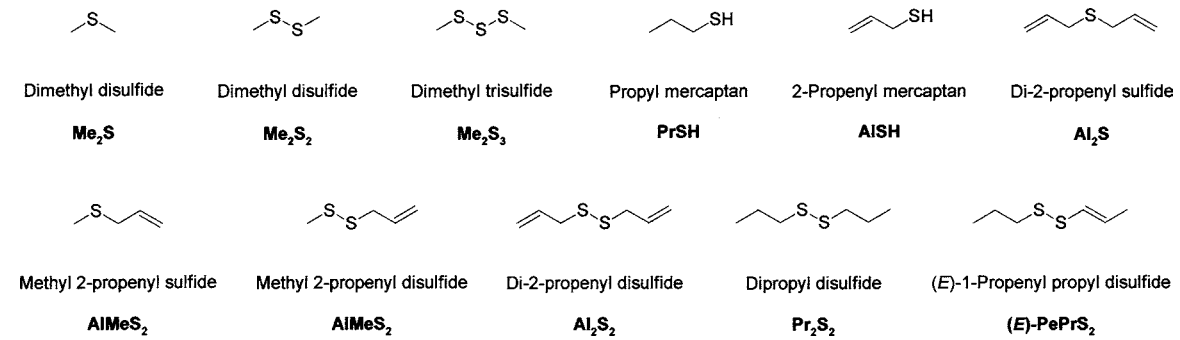

Figure 6. Structures of some representative volatile sulfur compounds occurring in the aroma profiles in species of the genus *Allium*. Me = methyl; Pr = *n*-propyl; Pe = 1-propenyl; Al = 2-propenyl or allyl.

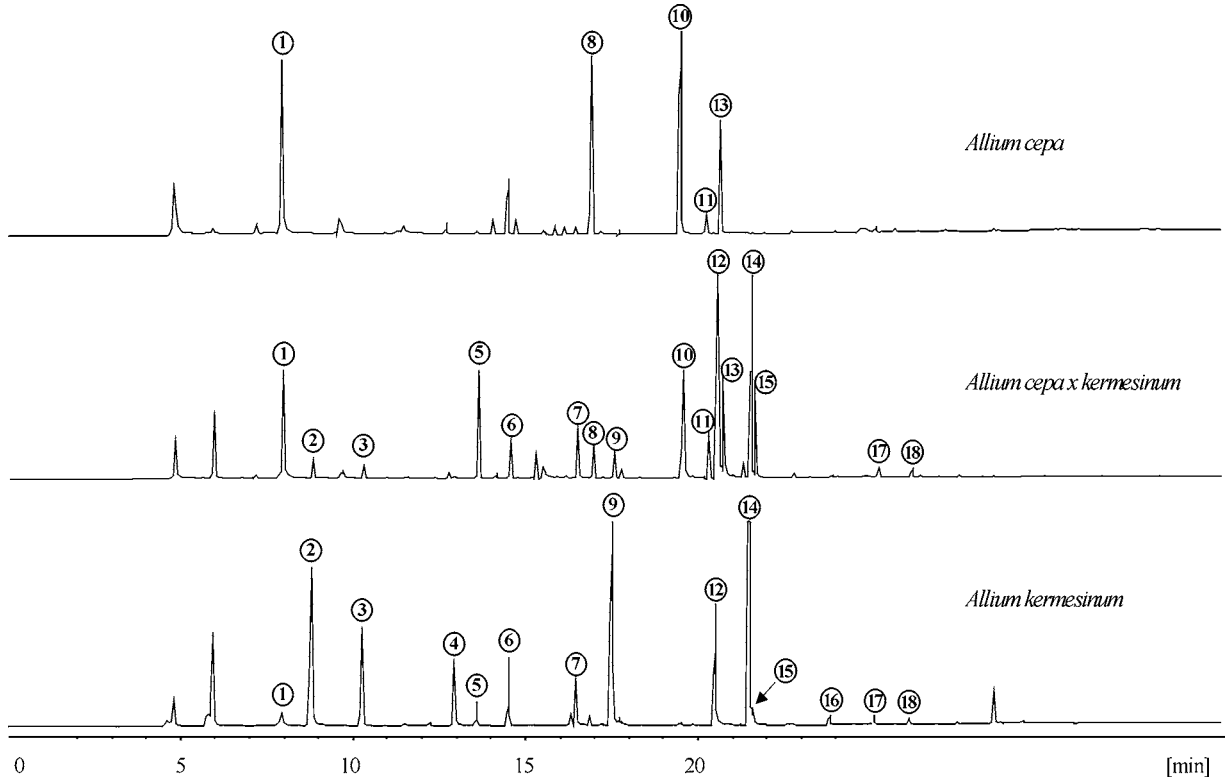

Figure 7. Comparison of the headspace solid-phase microextraction (SPME) gas chromatograms obtained from a hybrid plant and the related parental species. 1 = PrSH; 2 = AlSH; 3 = AlMeS, 4 = Me_2S_2; 5 = AlPrS; 6 = Al_2S; 7 = $MePrS_2$; 8 = dimethyl thiophene (DMT); 9 = $AlMeS_2$; 10 = Pr_2S_2; 11 = (*Z*)-$PePrS_2$; 12 = $AlPrS_2$; 13 = (*E*)-$PePrS_2$; 14 = Al_2S_2; 15 = (*E*)-$AlPeS_2$; 16 = $AlMeS_3$; 17 = Pr_2S_3; 18 = $AlPrS_3$.

(*19*). The leading sulfur-containing aroma compounds are the disulfides with respect to the corresponding cysteine sulfoxides. Therefore, the aroma profile of *Allium* species belonging to the alliin type, such as garlic, is determined by diallyl disulfide, whereas for species of the propiin/isoalliin type, such as onion, the main volatile compound is dipropyl disulfide.

Figure 7 presents the HS-SPME chromatograms of the parental species and a representative hybrid for a synoptic comparison. The main compounds in the chromatogram of *A. cepa* are propyl mercaptan, dimethyl thiophene, dipropyl disulfide, and the two isomeric forms of propyl 1-propenyl disulfide. Due to the occurrence of all four cysteine sulfoxides, the chromatogram of *A. kermesinum* shows 3 times more different volatile sulfur-containing metabolites. Here the leading compounds are allyl mercaptan, allyl methyl sulfide, allyl methyl disulfide, and diallyl disulfide, thus reflecting the proportions of the corresponding precursors.

As expected, with regard to the cysteine sulfoxide pattern, the chromatogram of the hybrid plant shows the highest number of sulfurous volatiles, with the leading compounds allyl propyl disulfide and diallyl disulfide. Every compound detected in the GC profile of the parent plants is also present in the chromatogram of *A. cepa* × *A. kermesinum*. It is remarkable that especially the not symmetrically substituted sulfides and disulfides, such as allyl propyl sulfide and allyl propyl disulfide, occur at higher amounts in the hybrids compared to the profiles of the parental species. Although these substances have not been detected in human cells after consumption of *Allium* plants up to now (*21*), their pharmacological and toxicological properties are partially, depending on the compound, well-known. As an example, allyl methyl disulfide is proven to cause a cholinesterase inhibition (*21*) and allyl propyl disulfide possesses hemolytic properties.

As shown in **Figure 8**, the significant differences in the cysteine sulfoxide pattern also cause significantly different profiles of the volatile sulfur compounds within the six investigated hybrids.

These profiles were calculated on the basis of the obtained gas chromatography results. Only the identified sulfur compounds, which usually amount to 90% of the total GC response,

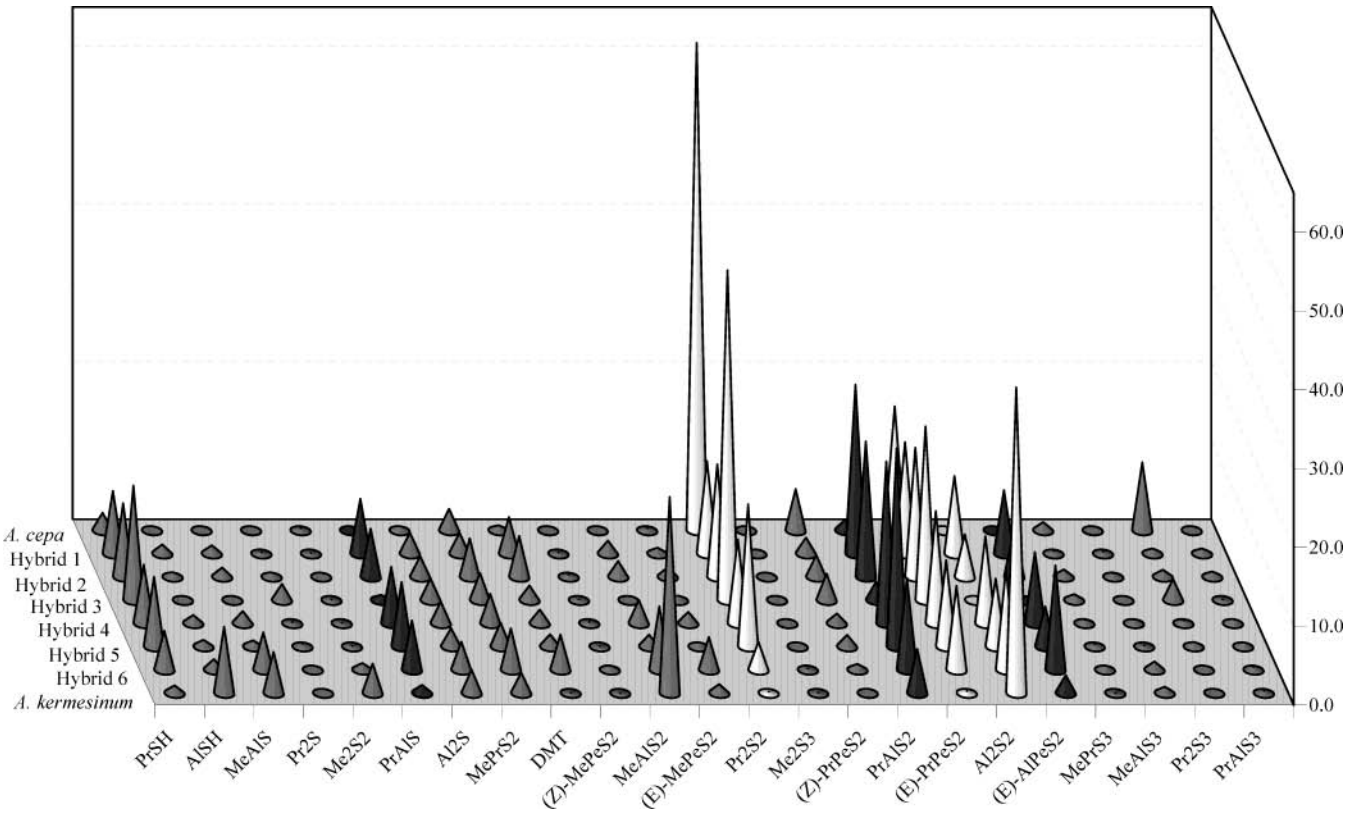

Figure 8. High-resolution gas chromatograms of six *Allium* hybrids in comparison to their parental species.

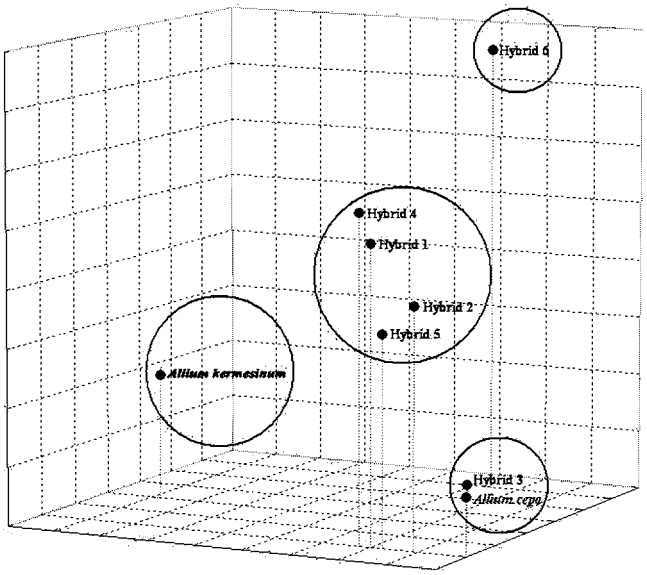

Figure 9. Principal component analysis (PCA) plot of *A. cepa* × A. *kermesinum* calculated on the basis of their volatile sulfurous compounds.

were considered. From a statistical calculation based on these GC profiles, there can be distinguished three groups within the analyzed *A. cepa* × *A. kermesinum* plants. The applied principal component analysis (PCA) shows that there is one hybrid very close to the aroma profile of the maternal species *A. cepa*, another group of four hybrids in a cluster between both parents, and at last one outstanding hybrid (**Figure 9**). As already mentioned in the discussion of the distributions of the cysteine sulfoxides, also the profiles of the volatile sulfur compounds are influenced by the heterocygousity within the genus *Allium*.

Conclusion. It is known that the distribution of cysteine sulfoxides in *Allium* species is predominantly determined by the genetics. The properties of pattern and amount of these constituents can partially be transferred in cross-breeding experiments. With the aim to generate plants with higher amounts of valuable constituents, we have carried out cross-breeding experiments using *A. kermesinum* as the paternal plant and the cultivated species *A. cepa*. The wild species *A. kermesinum* is known to show usually high amounts of cysteine sulfoxides with a garlic-like pattern determined by alliin. The experiments performed in this study show clearly that the hybridization does not lead to uniform profiles of sulfur compounds, probably due to the heterocygousity within the genus *Allium*. Using HS-SPME-GC analysis for the volatile compounds and HPLC analysis for the cysteine sulfoxides in combination with statistic algorithms, it is possible to very rapidly characterize single plants resulting from breeding experiments with respect to their valuable substances. Besides the opportunity of a moderate increase in the total amount of cysteine sulfoxides in comparison to the maternal species *A. cepa*, it has been also found that the aroma profiles of the obtained hybrids show unusually high amounts of unsymmetrically substituted oligosulfides, which are known to possess special physiological activity.

Therefore, the combination of fast chemical and statistical analyses is shown to be a useful tool for the support of breeding experiments.

ACKNOWLEDGMENT

We thank Oxford Chemicals (Harlepool, U.K.) and Symrise GmbH & Co. KG (Holzminden, Germany) for providing several reference samples. We also thank Thea Allner and Sylvia Zimmerman for the maintenance and multiplication of the plant material in vitro and in the field.

LITERATURE CITED

(1) Block, E. Die Organoschwefelchemie der Gattung *Allium* und ihre Bedeutung für die organische Chemie des Schwefels. *Angew. Chem.* **1992**, *104*, 1158−1203.

(2) Koch, H. P.; Lawson, L. D. *Garlic: The Science and Therapeutic Application of Allium sativum L.; Related Species*; Williams and Wilkins: Baltimore, MD, 1996; pp 1−227.

(3) Sendl, A. *Allium sativum* and *Allium ursinum*: Part 1. Chemistry, analysis, history, botany. *Phytomedicine* **1995**, *1*, 323−339.

(4) Gao, C. M.; Takezaki, T.; Ding, J. H.; Li, M. S.; Taijima, K. Protective effect of *Allium* vegetables against both esophageal and stomach cancer: a simultaneous case-referent study of high-epidemic area in Jiangsu province, China. *Jpn. J. Cancer Res.* **1999**, *90*, 614−621.

(5) Keusgen, M.; Schulz, H.; Glodek, J.; Krest, I.; Krüger, H.; Herchert, N.; Keller, J. Characterization of Some *Allium* Hybrids by Aroma Precursors, Aroma Profiles, and Alliinase Activity. *J. Agric. Food Chem.* **2002**, *50*, 2884−2890.

(6) Koch, I.; Keusgen, M. Diastereoselective synthesis of alliin by an asymmetric sulfur oxidation. *Pharmazie* **1998**, *53*, 668−671.

(7) Krest, I.; Glodek, J.; Keusgen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* **2000**, *48*, 3753−3760.

(8) Keller, E. R. J.; Schubert, I.; Meister, A. Interspecific crosses of onion with distant *Allium* species and characterization of the presumed hybrids by means of flow cytometry, karyotype analysis and genomic *in situ* hybridization. *Theor. Appl. Genet.* **1996**, *92*, 417−424.

(9) van den Dool, H.; Kratz, P. A. Generalization of the Retention Index System including Linear Temperature Programmed Gas−Liquid Partition Chromatography. *J. Chromatogr.* **1975**, *11*, 463−471.

(10) Brodnitz, M. H.; Pollock, C. L.; Vallon, P. P. Flavor components of onion oil. *J. Agric. Food Chem.* **1969**, *17*, 760−763.

(11) Kallio, H.; Salorinne, L. Comparison of onion varieties by headspace gas chromatography−mass spectroscopy. *J. Agric. Food Chem.* **1990**, *38*, 1560−1564.

(12) Doyle, J. J.; Doyle. J. L. Isolation of plant DNA from fresh tissue. *Focus* **1990**, *12*, 13−15.

(13) Dehmer, K. J.; Hammer, K. Taxonomic status and geographic provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP data. *Genet. Res. Crop Evol.* **2004**, *51*, 551−558.

(14) Williams, J. G. K.; Kubelik, A. R.; Livka, K. J.; Rafalski, J. A.; Tingey, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuleic Acids Res.* **1990**, *18*, 6531−6535.

(15) Mana, H.; Spohn, U. Rapid and selective determination of ammonium by fluorimetric flow injection analysis. *Fresenius' J. Anal. Chem.* **2000**, *366*, 825−829.

(16) Milka, P.; Krest, I.; Keusgen, M. Development of an Alliin Biosensor. *Pharmazie* **1999**, *54*, 435−438.

(17) Milka, P.; Krest, I.; Keusgen, M. Immobilization of alliinase on porous aluminum oxide. *Biotechnol. Bioeng.* **2000**, *69*, 344−348.

(18) Schulz, H.; Krüger, H.; Keusgen, M. Verteilung der Aromastoffe sowie der entsprechenden Präkursoren in interspezifischen *Allium*-Hybriden. *Lebensmittelchemie* **2001**, *55*, 37−38.

(19) Storsberg, J.; Schulz, H.; Keller, E. R. J. Chemotaxonomic classification of some *Allium* wild species on the basis of their volatile sulfur compounds. *J. Appl. Bot.* **2003**, *77*, 106−162.

(20) Amagase, H.; Petesch, B. L.; Matsuura, H.; Kasuga, S.; Itakura, Y. Intake of Garlic and Its Bioactive Components. *J. Nutr.* **2001**, *131*, 955S−962S (supplements).

(21) Liu, L. J.; Yeh, Y. Y. Inhibition of cholesterol biosynthesis by organosulfur compounds derived from garlic. *Lipids* **2000**, *35*, 197−203.

Received for review February 25, 2004. Revised manuscript received June 4, 2004. Accepted June 6, 2004. The financial support of the Federal Ministry of Education and Research (BMBF), Grant 03 I 3916 B (Innoregio-Program, Rephyna e.V.), is gratefully acknowledged.

JF049684A