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Sensory Characteristics and Volatile Profiles of Parsley (*Petroselinum crispum* [Mill.] Nym.) in Correlation to Resistance Properties against Septoria Blight (*Septoria petroselini*)

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ABSTRACT: Sixteen different genotypes of parsley, including two cultivars, six populations, and eight inbred lines, were investigated regarding their sensory characteristics in relation to the volatile patterns and resistance to *Septoria petroselini*. The sensory quality was determined by a combination of profile analysis and preference test, whereas the volatile patterns were analyzed by headspace-SPME-GC of leaf homogenates with subsequent nontargeted data processing to prevent a possible overlooking of volatile compounds. The more resistant genotypes are characterized by several negative sensory characteristics such as bitter, grassy, herbaceous, pungent, chemical, and harsh. In contrast, the contents of some volatile compounds correlate highly and significantly either with resistance (e.g., hexanal and α-copaene) or with susceptibility (e.g., *p*-menthenol). Some of these compounds with very strong correlation to resistance are still unidentified and are presumed to act as resistance markers.

KEYWORDS: sensory evaluation, volatiles, GC-MS, SPME, resistance marker

INTRODUCTION

Parsley (*Petroselinum crispum* [Mill.] Nym.) is widely used as a pot herb, both fresh and dry. All parts of the plant including leaves, stems, and roots are usable. The plant is known for both an outstanding and unique flavor and bioactive secondary metabolites.[1] The essential oil is applied in the food industry and as a fragrance in perfume manufacturing. The sensory quality as well as the composition of volatile compounds of freshly harvested, dried parsley and essential oil has been studied in the past.[2−5] A total of around 80 volatiles have been identified, of which a smaller number of 17 odorants show a relatively high aroma impact. The flavor of freshly harvested and cut parsley leaves is caused mainly by *p*-mentha-1,3,8-triene, myrcene, 2-sec-butyl-3-methoxypyrazine, myristicin, linalool, (*Z*)-6-decenal, and (*Z*)-3-hexenal. Apart from these results, knowledge exists about the variability of essential oil volatile patterns in cultivars of the taxon *P. crispum*,[6] but up to now most of accessions conserved in gene banks are not characterized with regard to sensory characteristics as well as metabolite contents.

In parsley production the plants may be affected by a number of diseases.[7,8] One of the most important is septoria blight caused by *Septoria petroselini* (Lib.) Desm. The fungus is seedborne and may also survive in plant debris and on up to now unknown volunteer plants.[9] Under favorable environmental conditions, the disease can spread rapidly, affecting both yield and quality by characteristic necrosis on leaf and stalks. Because of its seedborne nature, the disease can potentially develop on crops under any production system and may cause collapse of quality and high economical losses.[10]

It is a known that aroma active compounds such as C6 components deriving from the LOX pathway also are characterized by bioactivities which may be connected with resistance activities against fungi.[11−13] However, so far the interrelations between aroma compounds and their functionality in resistance mechanisms are widely unknown, not only in parsley. In the paper of Hoberg et al.[14] this problem was demonstrated for the first time on parsley by human sensory investigations of 44 genotypes with a high variation in their resistance levels against *S. petroselini*. The results show that no simple and straight correlation exists between sensory quality and resistance properties against this disease. Nevertheless, most of the susceptible genotypes were located in a cluster corresponding with high consumer preference or, vice versa, more or less resistant genotypes are of lower sensory quality. Unfortunately, until now these findings were not supported by metabolic analyses.

Therefore, the aim of this research is to combine the results of sensory investigation with volatile analysis as well as quantitative acquisition of the plants' susceptibility to *S. petroselini*. To get clear results plant materials from a worldwide gene bank collection, inbred lines, and standard cultivars were included in the measurements. The used material, totaling 16 genotypes, shows a broad response variation to fungal diseases in the field.

MATERIALS AND METHODS

Chemicals. The compound (*E*)-2-hexenal was obtained from Merck, Darmstadt, Germany. The chemicals α-and *β*-pinene, sabinene, *β*-myrcene, limonene, terpinolene, *β*-caryophyllene, and *β*-ionone were from Carl Roth GmbH, Karlsruhe, Germany. All other chemical standards were obtained from Sigma-Aldrich Co., Inc. (Milwaukee, WI).

Plant Material. Details of the investigated 16 genotypes are summarized in Table 1. Four populations with resistance to *S. petroselini*

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Table 1. Plant Material and Resistance Properties as Area under the Disease Progression Curve (AUDPC)

no.	genotype/ cultivar	comment	AUDPC (counts)
1	P09/504/0	population with resistance	69.33
2	P09/504/1-S_3*[a]	resistant line S_3	41.33
3	P09/504/1-S_3	resistant line S_3	50.17
4	P09/516/0	population with resistance	80.67
5	P09/516/2-S_3*	resistant line S_3	63.17
6	P09/516/2-S_3	resistant line S_3	28.33
7	P09/656/0	resistant standard population	21.00
8	P09/662/0	population with resistance	92.17
9	P09/701/0	population with resistance	116.00
10	P09/701/1-S_3*	susceptible line S_3	207.83
11	P09/701/1-S_3	susceptible line S_3	207.33
12	P09/701/2-S_3*	resistant line S_3	180.83
13	P09/701/2-S_3	resistant line S_3	191.17
15	cv. 'Gigante d'Italia'	standard cultivar, susceptible	271.33
16	P09/510/0	susceptible standard population	230.17
GP1−GP5	cv. 'Gruene Perle'	standard cultivar, 5 repetitions	196.00

[a] S_3*, mixture of S_3 sister lines because of limited seeds.

were included (no. 1, 4, 8, and 9) as well as eight S_3 inbred lines (self-pollinated successive in three generations, no. 2, 3, 5, 6, 10, 11, 12, and 13). Resistance reaction shows a quantitative action. In all resistant populations and inbred lines symptoms of disease occur, but later and an overall lower level than in susceptible populations. Highly susceptible lines 10 and 11 segregated from resistant population 9. Population 8 is the resistant standard and 16 is the susceptible standard. Two cultivars common in commercial production were included (no. 15, 'Gigante d'Italia', and no. GP1−GP5, 'Grüne Perle'). All populations were selected from the German Federal *ex situ* Gene Bank at Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK) at Gatersleben, Germany.

Plant Cultivation and Sampling. Resistance to *S. petroselini* was tested by natural infection. The experimental field showed a natural infection pressure of a high level. Seeds were sown in Quedlinburg, Germany, on April 3, 2009, in four rows of 0.23 m distance and 1.5 m length. For each trial plot margins of cv. 'Grüne Perle' was sown at both sides. The field experiment was designed with three randomized replications. Harvest of leaves occurred three times (July 10, August 20, and October 5, 2009).

Assessment of Infection Rate. Estimate of *S. petroselini* infection were carried out six times (June 13 and 27, July 8, August 19, September 30, and November 19, 2009). For harvest period, scoring occurs just before cutting. The rating scale used for field plots consists of level 0 (without any lesion), level 1 (sporadically and very small spots, most plants are free of symptoms, lesions < 1%), level 3 (clear symptoms on epigynous leaves, infestation in clusters or constant and low infestation across trail lot, lesions < 5%), level 5 (strong infestation within the older epigynous leaves, younger leaves only with partial symptoms, no continuously strong infection across the whole trail plot, lesions < 20%), level 7 (also young topmost leaves show strong infestation, most of the plants are infected, lesions > 20%), and level 8 (clearly stronger infection than level 7, lesions > 50%).

On the basis of the infection ratings for every population or inbred line, a mean area under the disease progress curve (AUDPC) was estimated for progression of infection according to the procedure of Moll et al.[15]

Sensory Assessment. For sensory testing fresh parsley leaves were used. The plants were harvested and directly delivered to the analytical laboratory. Before use, stems were removed and the leaves were washed and minced with a knife. The sensory panel consisted of 12 continuously trained members. The sensory attributes were trained using standard cultivars. Additionally, the sensory attributes 'sweet', 'sour', and 'bitter' were practiced using solutions of sucrose, citric acid, and caffeine, respectively, for threshold and recognition training. Before testing, the sensory profile was developed by collecting descriptors using standard cultivars by the same panel in previous sessions. The profile finally used, consisting of 27 descriptors, is identical with that of Hoberg et al.[14] The samples were served in covered glass bowls. During the sensory test in a first round all of the primary odor descriptors were checked from the headspace, whereas the taste and retronasal impressions were sampled in a second round of testing. At the end of each sensory protocol the testers were asked to give a rating of the preference on a nine-stage scale. All samples were tested with two technical repetitions except the standard cultivar cv. 'Gruene Perle', which was measured additionally with five agronomical repetitions (10-fold testing).

Gas Chromatographic Analysis. Washed, fresh leaves of parsley were homogenized at room temperature for 1 min in a Waring Blendor (high speed) together with a NaCl solution (20% w/v). The ratio of leaf weight to the volume of NaCl solution was 1: 3 w/v. The homogenate was filtered using gossamer. For each sample, two headspace vials containing 4 g of solid NaCl for saturation were filled with a 10 mL aliquot of the supernatant and sealed with a magnetic crimp cap including septum.

For automated headspace-SPME-GC a 100 μm polydimethylsiloxane fiber (Supelco, Bellefonte, PA) was used. After an equilibration time of 10 min at 35 °C (300 rpm), the extraction of volatiles persisted for 15 min at 35 °C. Desorption was 2 min in splitless mode and 3 min with split at 250 °C. An Agilent Technologies 6890 GC equipped with an MPS2 autosampler from Gerstel (Mühlheim, Germany), an HP-INNOWax column (0.25 mm i.d., 30 m length, and 0.5 μm film thickness), and FID was used for chromatography. Carrier gas was hydrogen with a flow rate of 1.1 mL/min. The temperature program was the following: 45 °C (5 min), from 45 to 210 °C at 5 K/min, and 15 min at 200 °C. The volatiles were identified by parallel running of selected samples on a GC-MS (EI mode, m/z range from 35 to 350 amu) with library search (NIST and MassFinder) and by retention indices as well as coelution of authentic references (selected compounds). All samples, except the standard cv. 'Gruene Perle', were run with one agricultural and two technical repetitions. The standard cultivar was measured with altogether five agricultural and two technical repetitions (10-fold measurement).

Data Processing. The commercial software Chromstat version 2.6 by Analyt Müllheim (Germany) was used for data processing by pattern recognition (nontargeted or holistic analysis approach). Data input for pattern recognition are raw data from the percentage reports (retention time/peak area data pairs) performed with the software package Chemstation by Agilent. Using Chromstat, the chromatograms were divided into 111 time intervals, each of which represents a possible peak (substance) occurring in at least one chromatogram of the analysis set. The peak detection threshold was set to the 10-fold value of background noise. The output of pattern recognition and data export was an Excel database comprising the areas of the 111 peaks (mean of two technical repetitions) for finally 16 genotypes.

RESULTS AND DISCUSSION

Resistance to Septoria Blight. The development of infection with *S. petrosilini* in the field trial is depicted in Figure 1 as the

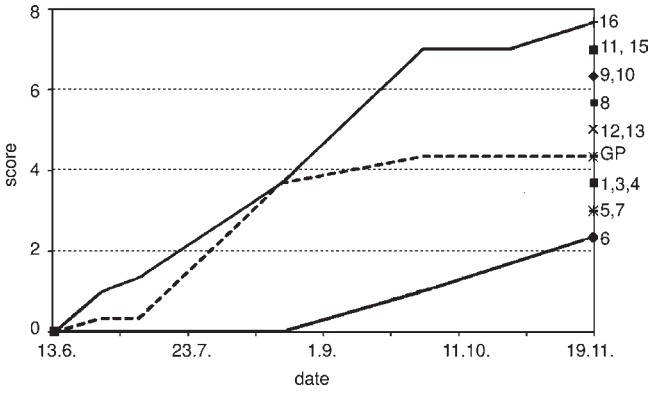

Figure 1. Development of infection rate depicted as disease progress curve. Area under Disease progression curve (AUDPC), value over time. *x*-axis, −156 days; *y*-axis, scoring level from 0 (without infection) to 8 (strong infection). The figure represents the characteristics of the typical resistant genotype (6) and typical susceptible genotype (16) disease progression as well as end levels. The dotted line is the standard cultivar for flavor (GP, 'Gruene Perle').

AUDPC over time. To simplify the demonstration characteristic of the most resistant line (no. 6), the standard cultivar 'Gruene Perle' (GP) and the most susceptible genotype (no. 16) are demonstrated over a time of 156 days. The progression curves of all other genotypes are located between the two extremes. At the right axis the end points of the AUDPC value are marked for all of the harvested genotypes.

The reaction of resistant parsley genotypes to the pathogen *S. petroselini* indicates a quantitative acting resistance. Also, resistant genotypes are affected by *S. petrosilini*, but the symptoms occur at a later time and at a lower level (no. 6) in comparison with susceptible genotypes (no. 16). The standard cultivar 'Gruene Perle' (GP) is characterized by a medium susceptible infestation.

Sensory Profile, Preference Test, and Susceptibility. Both the sensory parameters and the susceptibility to *S. petroselini* vary in a wide range in the selected genotypes. Figure 2 demonstrates results from the profile analysis and a preference test. The sensory profile (Figure 2a) of the most preferred sample (GP2) in comparison to the most disliked one (P13) shows

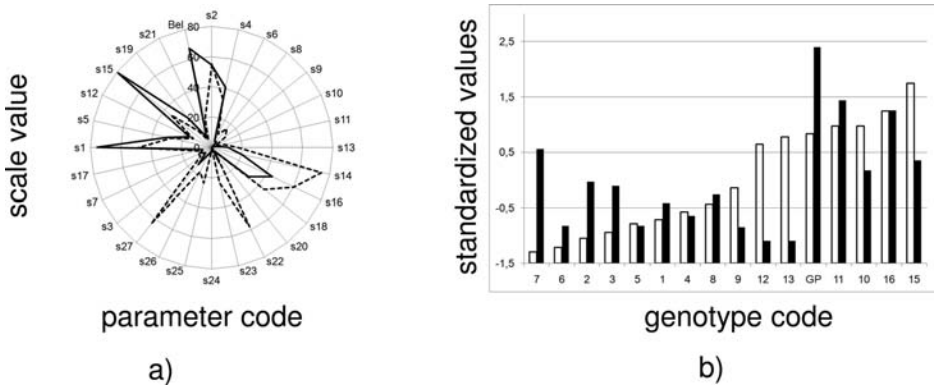

Figure 2. Sensory quality and susceptibility against *Septoria* displayed in standardized values. (a) Sensory profile of the most preferred (GP2) and the most disliked genotype (P13). Sensory parameters: (A, odor) s1 (typical parsley), s2 (green, grassy), s3 (lemon-like), s4 (herbaceous), s5 (spicy), s6 (hay-like), s7 (sweetish), s8 (pungent), s9 (chemical), s10 (musty), s11 (sourish); (B, taste) s12 (sweet), s13 (sour), s14 (bitter); (C, retronasal perception) s15(typical parsley), s16 (green, grassy), s17 (lemon-like), s18 (herbaceous), s19 (spicy), s20 (hay-like), s21 (sweetish), s22 (pungent), s23 (chemical), s24 (musty), s25 (sourish); (D, mouthfeel) s26 (metallic), s27 (harsh); (E) Bel (preference). (b) Results of preference test in comparison to the *Septoria* susceptibility: black bars, preference; white bars, AUDPC value.

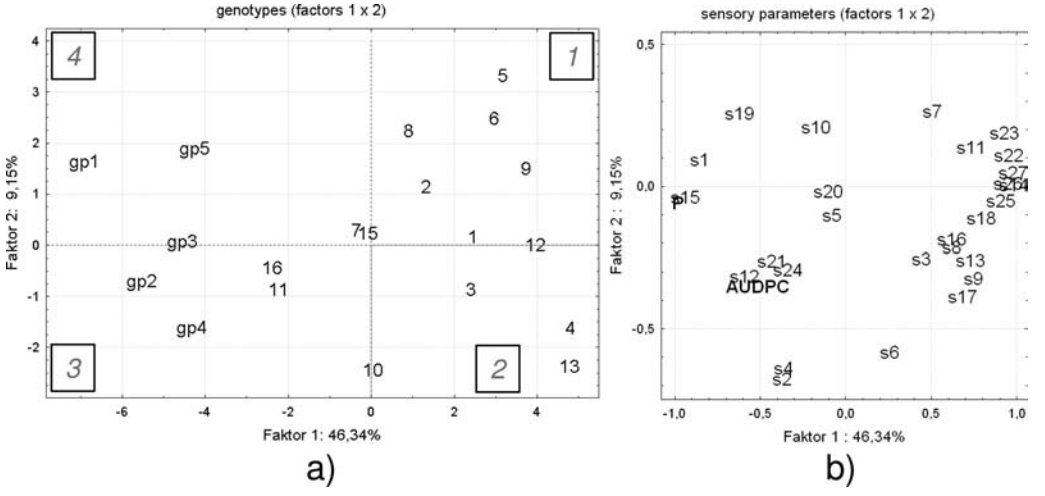

Figure 3. Principal component analysis using sensory characters and susceptibility, the area under disease progress curve (AUDPC): (a) genotype plot; (b) parameter plot. Parameter nomenclature conforms to Figure 2. P, preference.

clear differences in the majority of the characteristics. The preferred sample in Figure 2a is characterized by higher values in positive parameters (such as s1, s5, s12, s15, and s21), which are connected with sensations such as 'typical', 'spicy', or 'sweetish'. In contrast, sample P13 shows higher values in negatively perceived parameters (especially 'bitter', 'grassy', 'herbaceous', 'pungent', 'chemical', and 'harsh'). The preference is located between a value of 2.0 and 6.6 on the 9-stage scale, whereas the susceptibility measured as AUDPC shows values between 21.00 and 271.33. The parameter preference in correlation to *S. petroselini* susceptibility (AUDPC) is illustrated in Figure 2b. Obviously no narrow relationship exists between these two parameters. The correlation coefficient between the two parameters was +0.41, indicating no significance on a level of $p < 0.05$.

For data reduction and graphical presentation a principal component analysis (PCA) was performed with a data set containing the sensory parameters (27 characters) and susceptibility values (AUDPC). In Figure 3 the two-dimensional plots of the genotypes and the parameters are shown. On the left side of the plot (Figure 3a) the highly susceptible genotypes are located in quadrants 3 and 4, whereas the more resistant types are located on the right side in quadrants 1 and 2. In the plot of the parameters (Figure 3b) the preference (P) is located in the neighborhood of the sensory characters with positive connotations such as 'typical parsley' (retronasal, s15; and pronasal, s1) and 'spicy' (retronasal, s19). In contrast, several parameters with negative sensory descriptions are located in a cluster opposite to high values of preference (e.g., 'bitter' (taste, s14), 'metallic' (mouthfeel, s26), 'burning/harsh' (mouthfeel, s27), and 'sourish/silage' (retronasal, s25)). These findings are in accordance with results from screening a high number of gene bank accessions published by Hoberg et al.[14] Thus, in simple terms, parsley genotypes with higher resistance quality against *S. petroselini* are characterized by some negative sensory characters and lower preference.

Volatile Profiles and Susceptibility. By using the nontargeted approach for volatile analysis altogether 111 conjoint peaks were detected in the set of analyses. Of this number 15 substances were identified conclusively and 15 tentatively. The compound list is summarized in Table 2. A PCA based on the results of volatile analysis and *S. petroselini* susceptibility (AUDPC) is depicted in Figure 4. In analogy to Figure 3 the susceptible and resistant genotypes are located in two loose, but separated, clusters (Figure 4a). Again, all susceptible genotypes are assembled in quadrants 3 and 4, whereas the more resistant types are assigned in quadrants 1 and 2. In the parameter plot in Figure 4b, the 30 individual volatiles are spread over the parametric space. In the direct neighborhood of the susceptibility parameter AUDPC the compounds γ-terpinene (a10), *p*-menthenol (a22), sesquiphellandrene (a24), (*Z*)-3-hexenol (a13), and myristicine (a29) are arranged. Close neighborhood can be interpreted in such a way that high concentrations of the mentioned compounds are connected with high susceptibility. Opposite to this, the compounds (*E*)-2-hexenal (a9), α-copaene (a17), and hexanal (a3) as well as further compounds from the right side of the parameter plot maybe correlated with more or less resistance against *S. petroselini*.

The findings from PCA are supported by a correlation analysis including all 111 detected volatiles and AUDPC. The individual correlation coefficients (K) on the significance level of $p < 0.05$ are given in Table 2. In addition to the identified compounds (conclusively and tentatively), eight unidentified peaks are included. Only unknowns with a significant correlation coefficient greater than an absolute value of 0.60 are represented in the table, whereas a further 11 unknowns with significant but lower values were neglected. Significant correlations with coefficients between 0.88 and 0.72 were found for the following peaks (in decreasing order): unknown (u3), hexanal (a3), *p*-menthenol

Table 2. Excerpt of 111 Volatile Peaks of Parsley Homogenates Detected by Headspace-SPME

no.[a]	substance	RI[b]	identified by[c]	refs	K[d]
a1	α-pinene	1030	MS, RI	5	−0.02
a2	camphene	1070	MS	5	−0.28
a3	hexanal	1090	MS	5	−0.85*
a4	β-pinene	1116	MS, RI	5	0.02
a5	sabinene	1130	MS, RI	5	0.08
u1	unknown	1165			−0.72*
a6	β-myrcene	1170	MS, RI	2, 3, 5	−0.64*
a7	limonene	1208	MS, RI	5	−0.34
a8	β-phellandrene	1225	MS	2, 3, 5	0.52*
a9	(*E*)-2-hexenal	1232	MS, RI	25	−0.61*
u2	unknown	1240			0.63*
a10	γ-terpinene	1263	MS, RI	5	0.59*
a11	ocimene	1270	MS	5	−0.12
a12	terpinolene	1301	MS, RI	5	0.46*
u3	unknown	1330			−0.88*
u4	unknown	1365			−0.63*
a13	(*Z*)-3-hexenol	1402	MS, RI	5	0.54*
a14	*p*-mentha-1,3,8-triene	1425	MS	2, 3, 5	0.34
a15	2-methylcoumarane	1448	MS	*e*	0.40
a16	dimethylstyrene	1454	MS, RI	24	−0.36
u5	unknown	1505			0.63*
a17	α-copaene	1510	MS	5	−0.73*
a18	linalool	1565	MS, RI	2, 3	−0.35
u6	unknown	1600			0.72*
a19	β-caryophyllene	1628	MS, RI	5	0.48*
a20	terpinen-4-ol	1630	MS, RI	23	0.60*
u7	unknown	1633			−0.67*
a21	γ-elemene	1635	MS	5	−0.69*
a22	*p*-menthenol	1705	MS	*e*	0.76*
a23	dimethylanisole	1715	MS	*e*	−0.56*
u8	unknown	1755			0.71*
a24	sesquiphellandrene	1765	MS	*e*	0.66*
a25	*p*-cymenol	1825	MS	23	0.53*
a26	β-ionone	1912	MS, RI	*e*	−0.72*
a27	carotol	1965	MS	23	−0.44
a28	elemicin	1980	MS	23	0.05
a29	myristicine	2140	MS, RI	2, 3, 5	0.41
a30	apiole	2345	MS	5	0.53*
	sum of 111 compounds				0.43

[a] a, identified or tentatively identified volatiles; u, unidentified peak. [b] RI, retention index, calculated by coelution of a boiling point sample. [c] MS, identification by MS library search (NIST and MassFinder); RI, additional identification by coelution of authentic references. [d] Correlation coefficient between the realtive concentration of individual substances and the values of susceptibility (AUDPC). *, significant on a level of $p < 0.05$. [e] In the literature no confirmation was found for parsley.

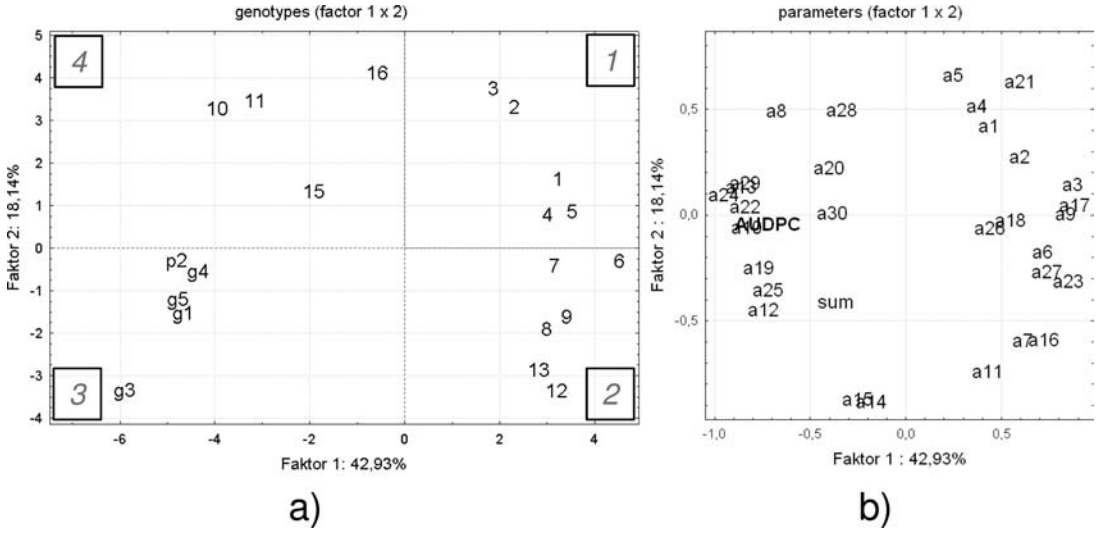

Figure 4. Principal component analysis using data of volatile analysis and susceptibility, the area under disease progress curve (AUDPC): (a) genotype plot; (b) parameter plot. Parameter nomenclature conforms to Table 2. P, preference.

(a22), α-copaene (a17), β-ionone (a26), and three additional unknowns (u1, u6, and u8). The sum of all 111 volatile metabolites shows no significant correlation (K = 0.43) with the susceptibility measurements. The unknown compound u3 has the highest and significant value with −0.88. The negative algebraic sign shows that high concentrations of this compound correlate with a high resistance against *S. petroselini*.

The identified compounds mentioned above belong to plant volatile organic compound (PVOCs) derived from three different biosynthetic pathways: so-called green leaf volatiles (GLV or LOX products), terpenoids, and carotenoid-derived metabolites. These pathways are important sources for volatiles included in signaling and defense mechanisms of plants.[16,17] The GLVs hexanal (a3) and (*E*)-2-hexenal (a9) are known to have direct fungicidal activities,[11−13,18] which is also in accordance with findings from Kishimoto in *Arabidopsis*.[19] Terpenoids (isoprenoids) constitute the largest group of PVOCs with a multitude of functional rules in plants.[20] Table 2 comprises several compounds that belong to the monoterpenoid as well as to the sesquiterpenoid group. Some terpenoids occurring in parsley also show a positive correlation to resistance (Table 2) and have been known for a long time as constituents in plant extracts or essential oils with high fungicidal activity: for example, β-myrcene (a6), β-caryophyllene (a19), and γ-elemene (a21).[21] Finally, the carotinoid-derived metabolite β-ionone inhibits the sporulation and growth of fungi in tobacco plants, cantaloupe melons, and maize roots.[22]

The nontargeted (or holistic) approach for volatile analysis uses as much as possible chromatographic information of all analytic runs conducted in the whole experiment. This is done by processing all peaks detectable above the threshold (defined as 10-fold of noise). In this way also unidentified compounds were included in the correlation with the resistance attribute, demonstrated as the AUDPC value. Using a common strategy with preliminary peak allocation to identified substances, valuable information would be lost by overlooking the unknown peaks, but further efforts on substance identification have to be done in the future to identify the interesting unknowns.

The results, demonstrated in this work, support the hypothesis that volatile metabolites are not only responsible for aroma impressions but also included, directly or indirectly, in resistance mechanisms of *S. petroselini*. The used correlation analysis does not reveal the manner of action (induced or constitutive mechanism) of the volatiles but gives the possibility to define metabolites as resistance markers. For this purpose peaks with significant positive as well as negative correlations to resistance are usable. In terms of genetics the correlation is of quantitative manner due to the fact that between susceptible and resistant genotypes concentration differences between some volatiles exist. Real "qualitative" events, that is, the appearance of "new" peaks in comparisons of susceptible and nonsusceptible genotypes, do not occur. The correlation between aroma and resistance attributes is an example of the multiple bioactivity of volatile metabolites in the context of sensory (aroma) and resistance quality.

In parallel to the paper of Hoberg et al.,[14] also in this work the highest preference values were found for susceptible types and vice versa. However, no significant correlation between negative sensory attributes and high resistance exists. In the results of Hoberg et al. some outliers were found in the totality of 44 gene bank accessions. This is the chance for plant breeders to select genotypes with the combination of acceptable resistance (or tolerance) to *S. petroselini* and coexistent high sensory quality.

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ABBREVIATIONS USED

AUDPC, area under the disease progress curve in counts; EI, electron ionization; GC-MS, gas chromatography—mass spectrometry; *m*/*z*, mass-to-charge ratio; PVOC, plant volatile organic compounds; PCA, principal component analysis.

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