DIVERSITY OF VOLATILE PATTERNS IN A GENE BANK COLLECTION OF PARSLEY (PETROSELINUM CRISPUM [MILL.] NYMAN)

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Abstract

A collection of 219 accessions mostly provided by the German Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany was cultivated in field. The patterns of volatile metabolites were determined effectively by a rapid, non-targeted analysis approach in leaf homogenates. The used method is a combination of an effective sample preparation and a non-targeted data processing. First results show that the investigated accessions belong to two different basic chemotypes. The two types differ mainly in volatiles deriving from the lipoxygenase pathway ((Z)-3-hexenal, (E)-2-hexenol), the phenylpropane compound apiole and minor terpenes like β-myrcene and γ-terpinene. The relationship of the volatile patterns to common essential oil analysis as well as properties like resistance and sensory quality (profile analysis and reference test) will be investigated in future.

Introduction

Parsley (Petroselinum crispum [Mill.] Nyman.) is widely used as a pot-herb, both fresh and dry. The essential oil is applied in food industry and as a fragrance in perfume manufacturing. The composition of volatile compounds of freshly harvested and dried parsley as well as the essential oil has been studied in the past (1). In contrast, the diversity of the volatile metabolites in different cultivars and accessions is widely unknown. Therefore a collection of 219 accessions mostly provided by the German Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany was cultivated in field. The patterns of volatile metabolites were determined effectively by a rapid, non-targeted analysis approach in leaf homogenates. By this way in principle the area of all peaks of a chromatogram set above a threshold are detectable. Overlooking of unexpected substances caused by a high diversity of the volatile metabolites is prevented using this non-targeted method. In parallel molecular analysis using AFLP, SRAP, RAPD- and dpRAPD-markers were performed to discover the genetic relationship of the different accessions.

Experimental

Plant material. A collection of 219 accessions mostly provided by the German Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research in
Gatersleben, Germany was cultivated in field. The plant material was stored until analysis at -86 °C.

**Sample preparation.** After thawing 1 mass part of parsley and 3 volume parts of a 20 % (w/v) sodium chloride solution were homogenized for 1 minute in a Waring blender. The homogenate was filtered using gossamer. For each sample, four headspace vials containing 4 g solid NaCl for saturation were filled with a 10 ml aliquot of the supernatant, sealed with a magnetic crimp cap including septum.

**Automated headspace-SPME-GC.** 100-μm-polydimethylsiloxane-fibre (Supelco, Bellefonte, PA, USA); equilibration time 10 min at 35 °C (300 rpm); extraction 15 min at 35 °C; desorption 2 min splitless and 3 min with split at 250 °C; GC Agilent Technologies 6890) equipped with MPS2 autosampler from Gerstel (Mühlheim, Germany) and FID; column HP INNOWax, 0.25 mm ID, 30 m length and 0.5 μm film thickness; carrier gas hydrogen 1.1 ml/min; temperature programme 45 °C (5 min), from 45 to 210 °C at 5 K/min and 15 min at 200 °C. All samples were run in triplicate. The volatiles were identified by parallel running of mass spectrometric analysis (GC/MS) and by retention indices.

**Marker analyses.** DNA of the parsley genotypes was isolated according to Porebski (2). RAPD analysis followed the protocol of Williams (3), dpRAPD-analysis according to Budahn (4) and SRAP-analysis corresponding to Li (5). The amplification products were separated on denaturing PAAGE (Sequigene GT, BIO-RAD, 38 x 50 cm). Geles were silver stained according to Bassam (6). AFLP amplification followed Vos (7) and separation and detection using Licor 4300S. Clear and strong bands were recorded as 1/0 matrix. The distance analysis was realized with NTSYSpc (Exeter Software) in the UPGMA modus.

**Figure 1.** 3D-plot of a principal component analysis (PCA) of altogether 219 genotypes using 133 peaks. The visualization shows two clearly distinct clusters. Cluster **a** contains 132 genotypes and cluster **b** 87, respectively. **Software: Statistika 7.1 by StatSoft.**
Results

To prevent overlooking of qualitative changes (new compounds) a so called non-targeted or holistic analysis approach with pattern recognition of chromatograms was used (8,9,10). Data input for pattern recognition by the commercial software CHROMStatTM 2.6 were percentage reports (retention time/peak area data pairs). This kind of data processing consider all detectable peaks of an analysis set (in this experiment around 600) without peak allocation or identification. Using CHROMSTATTTM, the chromatograms were divided into 133 time intervals, each of which represent a possible peak (substance) occurring in at least one chromatogram of the analysis set. A modified principal component analysis (PCA) was performed to visualize the results and check the reproducibility of GC analyses. Substance identification of genetically interesting peaks was done by parallel GC/MS runs of identical samples.

The results of analysis of volatile metabolites as well as the distance analysis based on molecular markers showed that the 219 genotypes split clearly into two groups. Regarding metabolites the two groups differ mainly in volatiles deriving from the lipoxygenase pathway ((Z)-3-hexenal, (E)-2-hexenol), the phenylpropane compound apiol and minor terpenes like β-myrcene and γ-terpinene. (Z)-3-hexenal (green-grassy) and β-myrcene (parsley-like) are known as character impact compounds of the typical parsley aroma (1). Also apiol is characterised by a parsley-like odour whereas γ-terpinene smells like wood, terpene and lemon. Root parsley genotypes clustered into the smaller group (cluster b) and all genotypes with curled leaves, except one, belong to the other group (cluster a). Genotypes with smooth leaves can be found in both groups. The allocation of genotypes to the two clusters shows only 10 mismatches (5 %) in comparison between the metabolomic and molecular marker data.

Figure 2. Analysis of genetic distance using AFLP, SRAP, RAPD and dpRAPD markers. For comparison celery was included to enhance the statistical power.
These results indicate that all investigated accessions split into two groups on the base of the lipoxygenase activity. The grouping does not correlate with leaf shape (curled or smooth) which is one of the most noticeable trait in parsley. The relationship of the volatile patterns with common essential oil analysis, properties like resistance and sensory quality (profile analysis and reference test) will be investigated in future.

References