Secondary Metabolites in the Rhizomes of Diploid and Tetraploid Gingers (Zingiber officinale Roscoe)

NISHIKAWA Kazutaka*1, ISHIMARU Kanji*2, FUJIOKA Toshihiro*3, GOTO Masahiro*4, IMAHORI Yoshihiro*5 and TANAKA Norie*6

* 1 Faculty of Health and Living Sciences Education, Naruto University of Education, 748 Nakashima, Takashima, Naruto-cho, Naruto, Tokushima 772−8502
* 2 Faculty of Agriculture, Saga University, I Honjo-machi, Saga 840−8502
* 3 Faculty of Pharmaceutical Sciences, Fukuoka University, 8−19−1 Nanakuma, Jonan-ku, Fukuoka 814−0180
* 4 Faculty of Home Economics, Kobe Women’s University, 2−1, Aoyama, Higashi-ku, Kobe, Hyogo 654−8585
* 5 Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1−1 Gakuen−cho, Nakaku, Osaka 599−8531
* 6 Project Management Office, The University of Tokushima, 2−1 Minamijosanjima-cho, Tokushima 770−8506

The rhizomes of diploid and tetraploid gingers (Zingiber officinale Roscoe) were studied for their composition of volatile and pungent compounds. The tetraploid ginger was derived from the diploid ginger by in vitro colchicine techniques. GC and GC-MS analyses revealed that the volatile compounds in the diploid and tetraploid gingers exhibited a similar compositional pattern. The pungent compounds were also isolated from the tetraploid ginger rhizomes and their structures were identified as 6-gingerol and 6-dehydroparadol by chemical and spectroscopic evidence. The HPLC analysis showed that the composition of pungent compounds in tetraploid ginger was not so different from that of diploid ginger. Therefore, tetraploid ginger showed a potential alternative to diploid ginger, since tetraploid ginger has several advantages over diploid ginger such as pollen fertility, germinability, and larger size.

(Received Sep. 18, 2013 : Accepted Feb. 17, 2014)

Key words : Zingiber officinale Roscoe, diploid ginger, tetraploid ginger, volatile compound, pungent compound

Ginger (Zingiber officinale Roscoe, 2n = 22) is a monocotyledonous herbaceous plant belonging to the family Zingiberaceae. It is an important commercial species, and has been used as a source of spice and medicine in Africa, Asia, and America since ancient times. The rhizomes of ginger contain both the characteristic flavor and pungency of the spice. The volatile compounds of ginger have been found to possess various pharmacological properties, including antioxidant, anti-inflammatory, and antimicrobial activities. What gives ginger its pungency are gingerols and shogaols, which consist of a homologous series of alcohols, each containing a phenolic group. Additional studies have identified ginger’s antioxidant, antiulcer, and antiallergic effects. On the other hand, Adaniya et al. succeeded in inducing tetraploid ginger (2n = 44) using in vitro colchicine techniques. This tetraploid ginger demonstrates enhanced pollen fertility and germinability, and tends to grow substantially larger than the diploids in both plant and rhizome size. However, there is little information about the secondary metabolites in the rhizomes of tetraploid ginger. As part of the search for natural food and medicine sources, the present study investigated the secondary metabolites, i.e., volatile and pungent compounds, of tetraploid ginger.

Materials and Methods

1. Plant materials

The Koganesato ginger strain was used in this experiment. The tetraploid ginger was derived from the diploid ginger by in vitro colchicine treatment and the chromosome number was checked according to the methods of Adaniya et al.
2. Quantitative determination of volatile compounds by GC and GC-MS

The fresh rhizomes were washed to remove any soil, peeled, and sliced. The sliced rhizomes were grated with a food cutter and filtered with gauze. The slurry (9 ml) was extracted with 1 ml of n-hexane with stirring at 3,000 rpm for 15 min. After the extract (hexane fraction) was filtered and dried over anhydrous sodium sulfate, the solvent was removed by rotary vacuum evaporator at 40°C. The each sample was subjected to GC (Shimadzu GC-14A, FID) analysis; column: DB-1 capillary column (0.25 mm i.d. × 30 m, 0.25 mm film thickness, J & W Folsom, CA, USA), carrier gas: nitrogen at a flow rate of 1.8 ml/min, column temperature: 50°C (2 min) → 3°C/min → 230°C (10 min), and detector temp.: 250°C. GC-MS was carried out on a Shimadzu GC-17A equipped with a Shimadzu QP-5000 under the same GC conditions. Identification of the volatile compounds was based on GC relative retention index (RI), co-injection with authentic compounds, and computer matching of the mass spectra against a library of spectra built up from authentic compounds, by comparison of fragmentation patterns of the mass spectra with likely authentic compounds.

3. Extraction and isolation of pungent compounds

The lyophilized rhizomes (37.47 g) of tetraploid ginger were mashed and extracted at room temperature with CHCl₃ (400 ml × 4) and MeOH (400 ml × 4) (Fig. 1). The extract, after concentration under reduced pressure, was subjected to Sephadex LH-20 (4.5 cm i.d. × 23 cm) column chromatography and eluted by H₂O increasing amount of MeOH and then by CHCl₃ to afford four fractions (Fr s. 1-4). Fr. 3 was applied on Preparative C18 125 Å (H₂O-MeOH: 1:0→0:1 and MeOH-CHCl₃: 1:0→0:1) and Sephadex LH-20 (H₂O-MeOH: 1:0→0:1 and MeOH-CHCl₃: 1:0→0:1) column chromatography to produce Compound 1 (196.5 mg). Fr. 4 was purified by Sephadex LH-20 (MeOH-CHCl₃: 1:0→0:1) column chromatography to give Compound 2 (32.2 mg). Compounds 1 and 2 were identified as [6]-gingerol and [6]-dehydroparadol, respectively, by comparison of the spectroscopic data (¹H- and ¹³C-NMR) with those in the references (Fig. 2).

4. Quantitative determination of pungent compounds by HPLC

The lyophilized rhizomes (approximately 20 mg) of both diploid and tetraploid ginger were mashed and extracted with EtOH-H₂O (1:1) for 16 h at room temperature. After filtration through a filter (0.22μm, Millipore, USA), each extract was subjected to HPLC analysis. The HPLC analytical conditions were as follows: column: TSK-gel ODS 80Ts (4.6mm i.d. x 250 mm), mobile phase: MeOH-H₂O (9:1, in 40 min), flow rate: 0.4 ml/min, column temperature: 40°C, detection: 280 nm, Rt (min): [6]-gingerol (1) (8.79) and [6]-dehydroparadol (2) (9.46), and zingerone (3) (7.55). Zingerone (3) was purchased from Sigma-Aldrich Co., Ltd. Analytical HPLC was performed on the instrument equipped with a photodiode array UV-VIS detector (Gulliver, Jasco, Japan).

Results and Discussion

The total peak area of the gas chromatograph of diploid and tetraploid gingers was almost the same. The aroma of ginger is characteristic, and many volatile compounds such as terpenoids and sesquiterpenoids have been identified in various samples of ginger. In this experiment, seventeen volatile compounds were positively identified in both
diploid and tetraploid gingers (Table 1). Monoterpenoids found included five monoterpenic hydrocarbons (α-pinene, camphene, myrcene, β-pinene, and terpinolene) and seven oxygenated monoterpenes (linalool, citronellol, borneol, decanal, neral, geranial, and geranyl acetate). Sesquiterpenoids included three sesquiterpene hydrocarbons (α-zingiberene, α-farnesene, and β-bisabolene) and two oxygenated sesquiterpenes (nerolidol and farnesol).

A number of volatile compounds identified from the diploid and tetraploid gingers were nearly the same as those of the most recent reports\(^\text{[10-13]}\). As can be seen in Table 1, the total peak area (54~59%) of sesquiterpenoids was greater than that (ca. 21%) of monoterpenoids. This result corresponded with the results of Connell and Jordan\(^\text{[13]}\). In both diploid and tetraploid gingers, the major compounds were three sesquiterpene hydrocarbons. In particular, the peak area of α-zingiberene, which has an odor unique to ginger, was the most pervasive (32~34%).

Furthermore, Connell and Jordan reported that freshly cut rhizomes possessed a ‘citrus-like’ aroma, and identified compounds producing this ‘citrus-like’ odor as geranial (citral \(a\)) and neral (citral \(b\))\(^\text{[12]}\). In this study, the ‘citrus-like’ odor compounds geranial and neral were also detected (a total citral content: 3.9~4.4%), but none of the samples exhibited a ‘citrus-like’ aroma. It seemed that geranial and neral were present in lower concentrations due to differences in the storage and cultivation of the rhizomes. Linalool (a floral and rosy odor), camphene (a camphoraceous odor), and geranyl acetate (a unique compound in fresh ginger rhizomes) were also identified\(^\text{[10-13]}\). In both gingers, the content of geranial was higher than that of geranyl acetate, probably due to the oxidation of ginger rhizomes during storage. A comparison of the volatile compounds of the two gingers showed few quantitative differences, specifically in the content of borneol, decanal, and farnesol. Furthermore, no difference in volatile flavors was sensed through direct odor or taste observations.

Therefore, it was considered that the composition of volatile compounds in diploid and tetraploid gingers exhibited a similar compositional pattern.

Ginger rhizomes also contain such pungent substances as gingerols and shogaols\(^\text{[7]}\). Since there have been few reports concerning the pungent compounds found in the rhizomes of tetraploid ginger, the pungent compounds in the rhizomes of tetraploid ginger were investigated here. As illustrated (Fig. 1), the extracts of the rhizomes were applied to Sephadex LH-20 and Preparative C18 columns to give rise to two compounds. Compounds 1 and 2 were identified as [6]-gingerol and [6]-dehydroparadol, respectively, by comparison with reference compounds\(^\text{[20-23]}\) (Fig. 2). The main pungent compound, [6]-gingerol (1), was contained in both diploid and tetraploid gingers. [6]-Dehydroparadol (2), structurally related to the gingerols and the shogaols, was also isolated and identified in the tetraploid ginger for the first time.

As the natural pungency of ginger is known to be derived from mixtures of gingerols, we investigated the relative contributions of compounds 1, 2 and the related paradol, zingerone (3) (Fig. 2), in the rhizomes of diploid and tetraploid gingers. Among the three compounds examined, [6]-gingerol (1) was the major compound in both diploid and tetraploid gingers (diploid, 0.74% DW; tetraploid, 0.64% DW) (Table 2). [6]-Dehydroparadol (2) was detected at a scant 0.07~0.09% DW, and zingerone (3) was hardly detected. This result is consistent with the report that zingerone (3) is not a natural

---

**Table 1** Composition of volatile compounds from diploid and tetraploid ginger rhizomes (peak area, %)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rt (min)</th>
<th>Diploid</th>
<th>Tetraploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>(7.35)</td>
<td>1.34 ± 0.01a</td>
<td>1.28 ± 0.07a</td>
</tr>
<tr>
<td>Camphene</td>
<td>(7.75)</td>
<td>5.74 ± 0.38a</td>
<td>4.96 ± 0.29a</td>
</tr>
<tr>
<td>Myrcene</td>
<td>(9.40)</td>
<td>0.60 ± 0.04a</td>
<td>0.51 ± 0.03a</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>(10.78)</td>
<td>6.49 ± 0.64a</td>
<td>7.27 ± 1.40a</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>(13.55)</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>Linalool</td>
<td>(13.97)</td>
<td>0.07 ± 0.01a</td>
<td>0.06 ± 0.01a</td>
</tr>
<tr>
<td>Citronellal</td>
<td>(15.98)</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>Borneol</td>
<td>(16.39)</td>
<td>0.54 ± 0.06a</td>
<td>0.15 ± 0.03b</td>
</tr>
<tr>
<td>Decanal</td>
<td>(18.39)</td>
<td>0.81 ± 0.20b</td>
<td>1.95 ± 0.17a</td>
</tr>
<tr>
<td>Neral</td>
<td>(19.71)</td>
<td>0.26 ± 0.05a</td>
<td>0.26 ± 0.06a</td>
</tr>
<tr>
<td>Geranial</td>
<td>(21.10)</td>
<td>4.17 ± 0.41a</td>
<td>3.67 ± 0.49a</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>(26.58)</td>
<td>0.87 ± 0.19a</td>
<td>0.90 ± 0.28a</td>
</tr>
<tr>
<td>α-Zingiberene</td>
<td>(32.03)</td>
<td>34.40 ± 0.86a</td>
<td>31.71 ± 1.42a</td>
</tr>
<tr>
<td>α-Farnesene</td>
<td>(32.58)</td>
<td>11.77 ± 0.51a</td>
<td>10.59 ± 0.42a</td>
</tr>
<tr>
<td>β-Bisabolene</td>
<td>(33.38)</td>
<td>12.57 ± 0.26a</td>
<td>11.34 ± 0.62a</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>(34.39)</td>
<td>0.33 ± 0.04a</td>
<td>0.42 ± 0.02a</td>
</tr>
<tr>
<td>Farnesol</td>
<td>(40.82)</td>
<td>0.14 ± 0.01b</td>
<td>0.20 ± 0.02a</td>
</tr>
</tbody>
</table>

tr: peak area less than 0.005% (almost undetected).
Each value is the mean±SE for 5 samples.
Values in the the table not showing different superscript letters are significantly different at \( p < 0.05 \) by Student’s \( t \)-test by using the statistical analysis system.
compound of ginger rhizomes but an artifact derived from gingerols. The content of pungent compounds in diploid ginger was not significantly different from that in tetraploid ginger. In direct taste and scent observations, we did not sense any difference in pungency.

In this study, the volatile and pungent compounds from rhizomes of tetraploid ginger were investigated for the first time. In a review article, Dhawan and Lavania showed that the productivity of secondary metabolites was enhanced in many cases of induced polyploids[10]. However, our results indicated that the secondary metabolites in the rhizomes of tetraploid ginger were quite similar to those of diploid gingers, presenting only some minor quantitative differences. The induced tetraploid ginger has several advantages over diploid ginger, such as enhanced pollen fertility, germinability and size[6-10]. In terms of supplementing human diets with these secondary metabolites, the rhizomes of tetraploid ginger should be useful for the field of pharamaceutics, as well as foods. Further studies are required regarding the secondary metabolites of tetraploid ginger.

Acknowledgement This study was supported by a subsidy from the Urakami Foundation.

References
13) NISHIMURA, O.: Identification of the characteristic odorants in fresh rhizomes of ginger (Zingiber officinale Roscoe) using aroma extract dilution

Table 2 Concentrations of pungent compounds extracted from diploid and tetraploid ginger rhizomes (% as dry weight of the ginger)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diploid</th>
<th>Tetraploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]-Gingerol</td>
<td>0.74±0.07</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td>[6]-Dehydroparadol</td>
<td>0.07±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Zingerone</td>
<td>tr</td>
<td>tr</td>
</tr>
</tbody>
</table>

tr: peak area less than 0.005% (almost undetected). Each value is the mean±SE for 5 samples.