Cytological studies on three species of Gastrodia, myco-heterotrophic orchids

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ABSTRACT. Cytological studies on three species of Gastrodia, myco-heterotrophic orchids, were carried out. Gastrodia gracilis had the chromosome number of 2n=22. The chromosome complement at resting stage showed the complex chromocenter type, and the metaphase chromosomes showed a bimodal karyotype in length consisted of two large chromosomes of 2.7 μm and 20 small ones varied from 2.1-1.1 μm. Gastrodia nipponica had the 2n=24 chromosomes showing the intermediate type between the simple chromocenter type and the complex chromocenter type at resting stage, and a gradual karyotype at metaphase chromosomes varied from 2.2-1.1 μm. Gastrodia flavilabella showed the 2n=26 chromosomes showing the intermediate type between the simple chromocenter type and the complex chromocenter type at resting stage, and a gradual decrease in size from the largest to the smallest chromosomes at metaphase varied from 1.6-0.9 μm. Thus, the different chromosome numbers existed in Gastrodia; 2n=22 in G. gracilis, G. confusa (Aoyama and Tanaka 1986) and G. pubilabiata (Nakata 2004); 2n=24 in G. nipponica; 2n=26 in G. flavilabella; 2n=36 in G. elata (Aoyama and Tanaka 1986), G. angusta (n=18, Liang 1984) and G. javanica (Aoyama and Yokota 2011).

KEYWORDS: Chromosome number, Gastrodia, Karyotype, Myco-heterotrophic, Orchidaceae

Thirty-six species are recognized in the myco-heterotrophic genus Gastrodia R.Br., tribe Gastrodieae, subfamily Epidendroideae, family Orchidaceae (Govaerts 2003). They are distributed widely in tropical Asia and extend from Africa to Australia and Japan. Among them, only four species has been studied cytologically. We came across three species of the genus in the natural habitat. In this paper, karyotype analysis in G. gracilis, G. nipponica and G. flavilabella were dealt with, and their karyotypes were compared with those of the other species previously reported.

MATERIALS AND METHODS

Gastrodia gracilis Blume (Japanese name: Nayo-tenna) is distributed in the southwest Japan and the central Taiwan. This species grows rarely in Cryptomeria forest. Three plants of the species used in this study were collected at an altitude of 170 m in the northern area of Nagasaki Prefecture, Kyushu District, Japan in late June, 2011 (Fig. 1A).

Gastrodia nipponica (Honda) Tuyama (Japanese name: Haruzaki-yatsushiroran) is distributed in the southwest Japan and Taiwan. This species grows rarely in evergreen forest and bamboo forest. Two plants of the species used in this study were collected at an altitude of 130 m in the eastern area of Kochi Prefecture, Shikoku District, Japan in early May, 2011 (Fig. 2A).

Gastrodia flavilabella S. S.Ying is endemic to the central Taiwan. This species grows rarely in the meadow on the periphery of the mountainous forest. Three plants of the species used in this study were collected at an altitude of 1220 m in the central area of Lugu, Nantou County, Taiwan in late July, 2011 (Fig. 3A).

For observations, young flower buds were collected from their inflorescences. The ovaries were cut into 1-2 mm round slices. They were immersed in 2 mM 8-hydroxyquinoline at 15°C for 4 h, and were fixed in Farmer’s solution at 5°C for 24 h. Fixed materials were hydrolyzed in a 1:2 mixture of 45% acetic acid and 1N HCl at 60°C for 30 sec, stained in 2% aceto-orcein, and squashed on glass-slides following the Aoyama and Yokota’s procedure (2011).

The classification of the chromosome types at resting and mitotic prophase stages followed Tanaka (1977). Terminology of chromosome morphology at mitotic metaphase on the basis of the position of the centromere followed Levan et al. (1964).

RESULTS AND DISCUSSION

Gastrodia gracilis Blume, 2n=22. The chromosome feature at resting stage of the species was of the complex chromocenter type (Fig. 1B). The chromosomes at mitotic prophase formed early-condensed segments that were located in the proximal and interstitial regions (Fig. 1C).

The chromosome number of 2n=22 was uniformly counted in nine cells of the integument tissues at mitotic metaphase (Fig. 1D). Two chromosomes in the complement were 2.7 μm and the other 20 chromosomes varied gradually in length from 2.1-1.1 μm (Fig. 1E). The total chromosome length was 35.8 μm, and its average length was 1.6 μm. Among the 22 chromosomes, 19 were median centromeric, and three were submedian centromeric. Thus, this species showed a bimodal karyotype in length and a symmetric karyotype in centromeric position.

The chromosome number of 2n=22 found here for G.
Gracilis was same as that of G. confusa (Aoyama and Tanaka 1986) and G. pubilabiata (Nakata 2004).

Gastrodia nipponica (Honda) Tuyama, 2n=24. The chromosome features at resting stage were intermediate between the simple chromocenter type and the complex chromocenter type (Fig. 2B). The chromosomes at mitotic prophase formed early-condensed segments that were usually located in the proximal region (Fig. 2C).

The chromosome number of 2n=24 was uniformly counted in seven cells of the integument tissues at mitotic metaphase (Fig. 2D). The chromosomes in the complement varied gradually in length from 2.2-1.1 μm (Fig. 2E). The total chromosome length was 34.5 μm, and its average length was 1.4 μm. Among the 24 chromosomes, 22 were median centromeric, and two were submedian.

Fig. 1. Gastrodia gracilis Blume. A. Flowering inflorescence in a natural habitat (photographed by Y. Kawachino). B. Resting chromosomes. C. Prophase chromosomes. D. Metaphase chromosomes showing 2n=22. E. Chromosome alignment by length at mitotic metaphase. Bars indicate 10 μm for B-D and 2 μm for E.

Fig. 2. Gastrodia nipponica (Honda) Tuyama. A. Flowering inflorescence in a natural habitat. B. Resting chromosomes. C. Prophase chromosomes. D. Metaphase chromosomes showing 2n=24. E. Chromosome alignment by length at mitotic metaphase. Bars indicate 10 μm for B-D and 2 μm for E.

Fig. 3. Gastrodia flavilabella S.S.Ying. A. Flowering inflorescences in a natural habitat. B. Resting chromosomes. C. Prophase chromosomes. D. Metaphase chromosomes showing 2n=26. E. Chromosome alignment at mitotic metaphase by length. Bars indicate 10 μm for B-D and 2 μm for E.
centromeric. Thus, this species showed a gradual karyotype in length and a symmetric karyotype in centromeric position.

The chromosome number of 2n=24 found here for G. nipponica was newly recorded in this genus.

Gastrodia flavilabella S.S. Ying. 2n=26. The chromosome features at resting stage were intermediate between the simple chromocenter type and the complex chromocenter type (Fig. 3B). The chromosomes at mitotic prophase formed early-condensed segments that were located in the proximal region (Fig. 3C).

The chromosome number of 2n=26 were uniformly counted in six cells of the integument tissues at mitotic metaphase (Fig. 3D). The chromosomes in the complement varied gradually in length from 1.6-0.9 μm (Fig. 3E). The total chromosome length was 29.5 μm, and its average length was 1.1 μm. Among the 26 chromosomes, 15 were median centromeric, ten were submedian centromeric, and rest one was subterminal centromeric. Thus, this species showed a gradual karyotype in length and a symmetric karyotype in centromeric position.

The chromosome number of 2n=26 found here for G. flavilabella was newly recorded in this genus.

Comparison of chromosome characters in Gastrodia Chromosome characters of three species studied here were summarized and compared with those of four species previously reported (Table 1). They were differed from each other in chromosome numbers, largest and smallest chromosome lengths, average chromosome lengths, total chromosome lengths and karyotypes at resting stage and metaphase chromosome.

Among four species with common external character of the rapid elongated flower stalk after fertilization, three species such as G. gracilis, G. confusa and G. pubilabiata were karyomorphologically more closely similar in 2n=22 chromosomes, chromosome sizes, and karyotypes at resting stage and metaphase chromosomes than those in G. nipponica excepting total chromosome length. The large chromosome commonly found in three species was heterogeneous in their 2n=22 chromosome complements. If it was thought that the one large chromosome was derived by the fusion of two chromosomes, the chromosome number was converted to be 2n=24, of which was equal to that of G. nipponica. Thus, the structure changes of chromosome complements might be occurred in their specific evolution.

Among the three species which do not have elongated flower stalk, G. elata and G. javanica showed karyomorphological similarity (Aoyama and Yokota 2011), while the karyotype of G. flavilabella differed from that of G. elata and G. javanica regarding chromosome number and sizes and metaphase karyotype.

Thus, Gastrodia showed the cytological polymorphism with three different karyotypes. Further cytological investigation in the genus and allied genera are necessary to clarify and justify the phylogeny of the subtribe Gastrodiinae.

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LITERATURE CITED

Table 1. Comparison of chromosome characters in seven species of Gastrodia.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome number (2n)</th>
<th>Chromosome length max.-min. (μm)</th>
<th>average (μm)</th>
<th>total (μm)</th>
<th>Karyotype at resting*</th>
<th>Karyotype at metaphase</th>
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<td>G. gracilis</td>
<td>22</td>
<td>2.7-1.1</td>
<td>1.6</td>
<td>35.8</td>
<td>complex bimodal</td>
<td>present study</td>
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<td>3.4-1.0</td>
<td>1.6</td>
<td>34.4</td>
<td>complex bimodal</td>
<td></td>
<td></td>
</tr>
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<td>G. pubilabiata</td>
<td>22</td>
<td>3.5-1.0</td>
<td>-</td>
<td>-</td>
<td>complex bimodal</td>
<td></td>
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<tr>
<td>G. nipponica</td>
<td>24</td>
<td>2.2-1.1</td>
<td>1.4</td>
<td>34.5</td>
<td>intermediate gradual</td>
<td></td>
<td>present study</td>
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<tr>
<td>G. flavilabella</td>
<td>26</td>
<td>1.6-0.9</td>
<td>1.1</td>
<td>29.5</td>
<td>intermediate gradual</td>
<td></td>
<td>present study</td>
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<tr>
<td>G. elata</td>
<td>36</td>
<td>3.0-1.0</td>
<td>1.4</td>
<td>49.4</td>
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<td></td>
<td>Aoyama &amp; Tanaka 1986</td>
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<tr>
<td>G. javanica</td>
<td>36</td>
<td>2.0-0.8</td>
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<td>42.7</td>
<td>intermediate bimodal</td>
<td></td>
<td>Aoyama &amp; Yokota 2011</td>
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*complex; the complex chromocenter type, simple; the simple chromocenter type, intermediate; intermediate type between the simple chromocenter type and the complex chromocenter type.