A Cytogenetic Study on the F_1 Hybrid of *Chrysanthemum makinoi* $\times$ *Ch. vulgare*

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Chrysanthemum makinoi is endemic to Japan, while *Ch. vulgare* is widely distributed in Europe and extends into the northern regions of Hokkaido, Japan. The two species differ greatly from each other in external morphology as well as ecological characteristics. Leaves of *Ch. makinoi* are ovate and one-lobed, while those of *Ch. vulgare* are oblong and bi-pinnatisect. Flower heads of *Ch. makinoi* have well developed ray flowers and flowers in late October, while those of *Ch. vulgare* are without ray flowers and flowers in July. According to Kitamura (1940), the two species fall into different sections of the genus. Chromosome number of the two species were reported to be $2n=18$ (Tahara 1914-1915, Shimotomai 1933, 1937). Karyotype of the two species were reported by Tanaka (1959b) and Tanaka and Shimotomai (1961). The chromosomes of *Ch. makinoi* were smaller than those of *Ch. vulgare*. *Ch. makinoi* had two satellite chromosomes, while *Ch. vulgare* had three satellite chromosomes. In the present investigation cytogenetic observations on the F_1 hybrids of the two species were made, to individuate the cytogenetically homologous chromosomes between two species.

Materials and methods

Clones of *Ch. makinoi* were collected from a foothill in Ushita, Hiroshima. Clones of *Ch. vulgare* were collected from a foothill in the vicinity of Marburg, Germany, and sent to the writers by the Marburg Botanical Garden. Reciprocal cross pollinations were made in late October, 1956, between the first flower heads of *Ch. makinoi* and the last flower heads of *Ch. vulgare*.

Somatic chromosomes were observed in root tips treated with 8-oxyquinoline, fixed in 45% acetic acid and stained with aceto-orcein. Meiosis was studied in PMC's fixed in 30% acetic acid and squashed in aceto-orcein or aceto-carmin. This method was described in the previous reports (Shimotomai *et al.* 1956, Tanaka 1959a).

Observations

Reciprocal crosses of *Ch. makinoi* $\times$ *Ch. vulgare* were successful when *Ch. makinoi* was used as the seed bearing plant. From approximately 1,000 cross pollinations 45 achenes were obtained. The germination of achenes was poor. Only nine of the 45 achenes germinated. Five of the nine seedlings were albino and died at an early stage of development. Four F_1 hybrids survived and flowered in early September.

The morphological characteristics of F_1 hybrids were more or less intermediate to those of the parental species. Stems of the F_1 hybrids were about

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200 cm in length, while those of *Ch. makinoi* were about 50 cm and those of *Ch. vulgare* were about 150 cm. Leaves of the F_1 hybrids were oblong, 8–10 cm long, 5–7 cm wide, and pinnatilobed, while those of *Ch. makinoi*

Fig. 1 a-c. Leaves of *Ch. makinoi* (a), *Ch. vulgare* (c) and their F_1 hybrid (b). $\times 2/5$.

were ovate, 3.0–5.0 cm long, 3.0–5.0 cm wide, and one-lobed, and those of *Ch. vulgare* were oblong, 15–25 cm long and 7–10 cm wide, and bipinnatifid (Fig. 1). Flower heads of the F_1 hybrids had white and short ray flowers curled and arranged irregularly, while those of *Ch. makinoi* had long white ray flowers and those of *Ch. vulgare* were without ray flowers (Fig. 2). All of the F_1 hybrids were found to be sterile.

Somatic chromosomes in F_1 hybrids. The F_1 hybrids had $2n=18$ chromosomes. Chromo-

somes of the parental species were distinguished by the differences in length, by the position of the centromere and by the satellites (Figs. 3 and 5). Chromosomes of *Ch. vulgare* were about 1.2–1.5 times longer than those of *Ch.*

Fig. 2 a-c. Flower heads of *Ch. makinoi* (a), *Ch. vulgare* (c) and their F_1 hybrid (b). $\times 4/5$.

makinoi. The chromosomes of parental species were found without any amphiplastic alteration in F_1 hybrid showing preservation their size difference.

The morphology of the chromosomes of *Ch. vulgare* was as follows: Chromosome 1_v : This was the longest chromosome of the complement and had a median centromere. Chromosome 2_v : This was the second longest chromosome and had a submedian centromere. Chromosome 3_v : This was the third longest chromosome and had a median centromere. Chromosome 4_v : This was one of the medium sized chromosomes and possessed satellites on the short arm. The centromere of this chromosome situated in a sub-

median position. Chromosome 5_v: This was one of the medium chromosomes and had a median centromere. Chromosome 6_v: This was one of the medium chromosomes and had a submedian centromere. Chromosome 7_v: This was one of the smaller chromosomes and possessed satellites on the short arm. The centromere of this chromosome situated in a subterminal position. Chromosome 8_v: This was one of the shorter chromosomes and possessed

Figs. 3 and 4. Somatic and meiotic chromosomes of the F₁ hybrids of *Ch. makinoi* × *Ch. vulgare*. Parental chromosomes can be distinguished by the difference in length and width and by the position of centromeres. The chromosomes of *Ch. makinoi* were designated by letter m and those of *Ch. vulgare* by letter v. 3, somatic chromosomes of the F₁ hybrid (Text-figure 5). 4, meiotic chromosomes in a PMC at diakinesis of the F₁ hybrid. 1II+16I. The bivalent was 1_m–1_v. Five univalents, 4_m, 8_m, 4_v, 7_v and 8_v, were found to have satellites and attached to nucleoli (Text-figure 6). ×1200.

satellites on the short arm. The centromere of this chromosome situated in a subterminal position close to the terminal region. Chromosome 9_v: This was one of the shorter chromosomes and had a median centromere.

The morphology of the chromosomes of *Ch. makinoi* was as follows: Chromosome 1_m: This was the longest chromosome of the complement and

had a median centromere. Chromosome 2_m : This was the second longest chromosome of the complement and had a centromere situated in a submedian position close to the subterminal region. Chromosome 3_m : This was the third longest chromosome and had a median centromere. Chromosome 4_m : This was one of the medium sized chromosomes and possessed satellites on the short arm. The centromere of this chromosomes situated in a subterminal position. Chromosome 5_m : This was one of the medium chromosomes and had a median centromere. Chromosome 6_m : This was one of the medium chromosomes and had a submedian centromere. Chromosome 7_m : This was

Figs. 5 and 6. Photomicrographs of somatic and meiotic chromosomes of the F_1 hybrids of *Ch. makinoi* $\times$ *Ch. vulgare*. 5, somatic chromosomes of the F_1 hybrid. 6, meiotic chromosomes in a PMC at diakinesis of the F_1 hybrid. $1_{II} + 16_I$. $\times 900$.

one of the smaller chromosomes and had a submedian centromere. Chromosome 8_m : This was one of the smaller chromosomes and possessed satellites on the short arm. The centromere of this chromosome situated in a subterminal position. Chromosome 9_m : This was one of the smaller chromosomes and had a submedian centromere.

Meiotic chromosomes in F_1 hybrids.

Meiosis in all of the F_1 hybrids showed similar meiotic chro-

mosome behaviour. Meiosis was irregular with very poor pairing of chromosomes. At both diakinesis and metaphase I, two types of configurations, $1_{II} + 16_I$ and 18_I , were observed (Table 1). Most PMC's showed $1_{II} + 16_I$ (Figs. 4 and 6).

Chromosomes of parental species were distinguishable through the differences in

length and by the position of the centromere (Fig. 4). Chromosomes of *Ch. vulgare* were longer than those of *Ch. makinoi*. At diakinesis five satellite

Table 1. Frequencies of chromosome configurations at metaphase I in the PMC's of the F_1 hybrid of *Ch. makinoi* $\times$ *Ch. vulgare*

Configurations	Number of PMC's
$1_{II} + 16_I$	46
18_I	21

chromosomes, 4_v, 7_v, 8_v, 4_m and 8_m, all univalents, were found attached to nucleoli through the arms possessing the satellites. The satellites of these chromosomes also attached to the nucleoli.

The bivalent was rod-shaped. In a few PMC's the bivalent showed matrix connection. Chromosomes of the bivalent were unequal in length, and both had a centromere in the median position. They were identified as chromosomes 1_v and 1_m, the longest chromosome of the parental species. Since only this bivalent was observed in F₁ hybrids, it can be concluded that only the longest chromosomes of both parental chromosome complements are homologous.

At anaphase I chromosomes of the bivalent separated toward the poles. Univalents, on the other hand, remained at the equatorial plate. At late anaphase I some of the univalents moved to the poles, while others lagged near the equatorial plate. At telophase I all chromosomes transformed into several round particles of chromatin. The chromatin particles disappeared at late telophase I. All PMC's after telophase I were without nuclei. No second meiotic division was seen. Anthers of tube flowers were without pollen.

Summary

1. An F₁ hybrid (2n=18), *Chrysanthemum makinoi* (2n=18) × *Ch. vulgare* (2n=18), was reported.

2. At meiosis in the F₁ hybrid 18 univalents or one bivalent and 16 univalents were formed at both diakinesis and metaphase I.

3. The chromosomes of the bivalent were determined to be the longest chromosomes of the parental species.

Literature cited

- Kitamura, S. 1940. Compositae Japonicae. Pars Secunda. Memoirs Coll. Sci. Kyoto Imp. Univ., Ser. B, 15(3), Art. 9: 258-446.
- Shimotomai, N. 1933. Zur Karyogenetik der Gattung *Chrysanthemum*. Jour. Sci. Hiroshima Univ., Ser. B, Div. 2, 1(3): 1-100.
- 1937. Chromosomenzahlen bei einigen Arten von *Chrysanthemum*. Zeitschr. ind. Abstamm. u. Vererb. 74: 30-33.
- , Tanaka, R., Masumori, S. and Ishiguro, M. 1956. Über die Polyploidie und geographische Verbreitung bei *Chrysanthemum japonense* Nakai. Bot. Mag., Tokyo 69: 515-518. (Japanese with German résumé).
- Tahara, M. 1914-1915. Cytological studies on *Chrysanthemum*. Bot. Mag., Tokyo 29(336): 491-493, (337-339): 6, 7, 9, 15, (340): 48-50. (Japanese with English résumé).
- Tanaka, R. 1959a. On the speciation and karyotypes in diploid and tetraploid species of *Chrysanthemum*. I. Karyotypes in *Chrysanthemum boreale* (2n=18). Jour. Sci. Hiroshima Univ., Ser. B, Div. 2, 9: 1-16.
- 1959b. ditto. II. Karyotypes in *Chrysanthemum Makinoi*. (2n=18). ibid. 9: 17-30.
- and Shimotomai, N. 1961. Cytogenetic studies on the F₁ hybrid of *Chrysanthemum lineare* × *Ch. nipponicum*. Zeitschr. f. Vererb. 92: 190-196.