KARYOTYPE ANALYSIS OF NATURAL POPULATIONS OF *TRILLIUM TSCHONOSKII**

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Two tetraploid species ($2n=20$) are known in the genus *Trillium* from Japan, *i.e.*, *T. tschonoskii* Maxim. and *T. apetalon* Makino. The present paper deals with the former species. The genome constitution of this plant is presented as K_2K_2TT , the two diploid progenitors K_2K_2 and TT being extinct (Haga 1951, 1956; Haga and Kurabayashi 1953).

The patterns of H-segments in the cold treated chromosomes revealed the profound karyotypic variations in genome K_1 of a diploid species *T. kamschaticum* ($2n=10$, K_1K_1), the genomes K_1 and K_2 being closely homologous (Haga 1951). The genome complex K_2T making *T. tschonoskii* ($2n=20$, K_2K_2TT) is contained also in *T. hagai* ($2n=15$, K_1K_2T and $2n=30$, $K_1K_1K_2K_2TT$) and *T. miyabeianum* ($2n=20$, K_2TSU), but no chromosomal variation is found in K_2T , in spite of the analysis of 59 plants of triploid and two plants of hexaploid *T. hagai* (Haga 1956), and 16 plants of *T. miyabeianum* (Kurabayashi and Saho 1957). As to *T. tschonoskii*, however, the karyotype of only a single plant has been reported (Haga and Kurabayashi 1953). The present paper reports further karyotype analysis of 14 plants of *T. tschonoskii* from three main islands of Japan, *i.e.*, Hokkaido, Sikoku and Kyushu, and describes two distinct karyotypes.

MATERIALS AND METHODS

Plants were collected from the following localities: Hayakita, Hokkaido (June, 1964); Ashoro, Hokkaido (July, 1963); Mt. Tsurugi, Shikoku (June, 1963); and Mt. Aso, Kyushu (June, 1963). They were maintained in the garden of Hikosan Biological Laboratory, until the present observations were made in June-July, 1965. Intact plants with roots were chilled at 0°C for 3-4 days, the root-tips were fixed with La Cour 2BE and Feulgen stain was applied. In making temporary preparations of chromosomes, root-tips were squashed in a mixture (distilled water 50 ml, gum Arabic 30 g, chloral hydrate 200 g, and glycerin 16 ml; cf. Beeks 1955) diluted by the usual aceto-carmin. The above squash-mounting medium was personally suggested by Dr. U. Nur, University of Rochester, to whom the writers are grateful.

OBSERVATION AND DISCUSSION

The somatic complement of *T. tschonoskii* consists of four sets of five chromosomes,

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A-E. So far, 14 plants were examined as to the patterns of H-segments: two types, 1 and 2, were distinguished in each of A, C, and E, three types in D, 1, 2 and 3, but no morphological differentiation was found between the two pairs of B's (Figs. 1 and 2). All 14 plants were revealed to be homozygous allotetraploids of the constitution of karyotype-1 or karyotype-2. In the following tabulation chromosome pairs were arranged presumably following genome alignment K_2/T .

Fig. 1. Somatic chromosome complement of *T. tschonoskii* from Ashoro, Hokkaido. $\times 1050$.

Fig. 2. Somatic chromosome complement of *T. tschonoskii* from Mt. Aso, Kyushu. Above K_2K_2 , below TT. $\times 1050$.

Chromosome	A	B	C	D	E	Refer to
Karyotype-1	1.1/2.2	1.1/1.1	1.1/2.2	1.1/3.3	1.1/2.2	(Fig. 1)
Karyotype-2	1.1/2.2	1.1/1.1	1.1/2.2	2.2/3.3	1.1/2.2	(Fig. 2)

A set of A2, B1, C2, D3, and E2 is very similar to a set of genome T tentatively sorted out from the genome complex K_2T by Haga and Kurabayashi (1953). On this assumption the other set A1, B1, C1, D1 and/or D2, and E1 represents genome K_2 .

Karyotype-1 is found in plants from Hayakita and Ashoro, Hokkaido, and Mt. Tsurugi, Shikoku, but karyotype-2 is restricted to Mt. Aso, Kyushu:

Locality	Hayakita	Ashoro	Tsurugi	Aso	Total
Karyotype-1	2	2	1	—	5 plants
Karyotype-2	—	—	—	9	9 plants

Though the sample was very small, 14 plants in total, two important features were revealed; (i) in spite of dimorphism in chromosome D of K_2 , D1 and D2, there was no case of heterozygous pair, 1.2, and (ii) karyotypes were different at different localities.

The karyotype of *T. tschonoskii* from Siraoi, Hokkaido, described earlier (Haga and Kurabayashi 1953; Haga 1956) is different in some respects (chromosomes B, C, and E of genome K_2) from the ones described in the present paper. However, an important point is consistent, *i.e.*, the karyotype is homozygous. Taking into account the earlier papers, three karyotypes are conceivable in genome K_2 . This suggests that one of the parent species K_2K_2 was chromosomally polymorphic. At the time and at the places the species K_2K_2 hybridized with the other diploid species TT, some populations of K_2K_2 might be fixed to a homozygous karyotype and some others of K_2K_2 to another homozygous karyotype. Fixation of a population to a single homozygous karyotype has been reported in the existing diploid *T. kamtschaticum* (Haga and Kurabayashi 1954). The fixed type of chromosome of the species K_2K_2 might be different among the populations as inferred from the case of *T. kamtschaticum* (Haga and Kurabayashi 1954; Haga (1969)). Thus hybridization and chromosome doubling gave rise to allotetraploids *T. tschonoskii*, which are homozygous but the karyotypes are different at different localities.

SUMMARY

The karyotypes of *Trillium tschonoskii* ($2n=20$, K_2K_2TT) collected from four localities of Japan Islands have been studied by means of differential reactivity of H-segments of the chromosomes to low temperature.

In all 14 plants examined, 10 pairs of chromosomes were found to be homozygous. But the karyotype of plants from Mt. Aso (Kyushu) was different with regard to chromosome D from that of the plants from the other three localities, Hayakita and Ashoro in Hokkaido and Mt. Tsurugi in Shikoku.

The above findings suggested that one of the parental, now extinct, diploid species ($2n=10$, K_2K_2) might be differentiated into homozygous local populations. The other extinct homozygous diploid species ($2n=10$, TT) had hybridized with K_2K_2 at different localities to give rise to the present *T. tschonoskii*.

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