Chromosomal variation in the K_2T genome complex of polyploid *Trilliums*

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ABSTRACT

Chromosomal variation in the tetraploid species *Trillium tschonoskii* ($4x=20$, K_2K_2TT) from a population in Hokkaido and three populations in Kyushu was analyzed by the patterns of H-segments which were revealed by cold treatment of the chromosomes. Chromosomal variation in the K_2T genome complex in the interspecific hybrid, *T. × miyabe anum* ($4x=20$, K_2TSU), was also analyzed. A total of 21 different chromosome types were distinguished in the K_2T complex, i. e., A1-A2 in chromosome A, B1-B4 in B, C1-C6 in C, D1-D4 in D, and E1-E5 in E. All were assortable into two series of homologous chromosomes, K_2^* and T^* . K_2^* is either the genome K_2 or T in each of the chromosomes A to E. The same holds true for T^* also. Since K_2^* and T^* were both polymorphic in all chromosomes, A to E, it is valid that both the genomes K_2 and T are chromosomally polymorphic. Consequently, it is certain that both extinct diploid progenitors, K_2K_2 and TT, are chromosomally polymorphic.

1. INTRODUCTION

Five species and three hybrids are known in Japanese trilliums, and their genomic constitutions have been determined by a series of previous studies (cf. Haga 1937, 1974; Haga *et al.* 1974; Uchino 1980). Chromosomal variations in the three genomes, K_1 , S, and U, have been thoroughly investigated by the patterns of H-segments, and profound chromosomal polymorphism in each of these three genomes has been found. In regard to the remaining two genomes, K_2 and T, our knowledge is still fragmentary (Haga 1956; Haga and Kurabayashi 1953; Uchino 1975; Watanabe and Kayano 1971). In the present paper, an attempt has been made to elucidate in detail the features of chromosomal variation in the K_2T genome complex in *T. tschonoskii* Maximowicz ($4x=20$, K_2K_2TT) and a hybrid *T. × miyabe anum* Tatewaki ($4x=20$, K_2TSU).

2. MATERIALS AND METHODS

For cytological preparations, the flowers of *T. tschonoskii* were collected at random from the natural populations tabulated below and the names of the localities are abbreviated in parentheses.

Region and locality	No. of plants examined
Hokkaido:	
Shikotsu (Sk), Ishikari Shicho	9
Kyushu:	
Kugino (Kg), Aso Gun, Kumamoto Pref.	58
Takamori (Tm), Aso Gun, Kumamoto Pref.	56
Gokanoshō (Gk), Yatsushiro Gun, Kumamoto Pref.	61

The Kugino (Kg) population is the same one as the Aso described by Watanabe and Kayano (1971). The Kg and Takamori (Tm) populations were situated ca. 16 km distance from each other in the Aso region. The Gokanoshō (Gk) population was located ca. 38 km distant from Kg and ca. 47 km from Tm.

T. × miyabe anum (10 plants; Uchino 1975) were collected by the Trillium Research Group headed by the late Dr. M. Kurabayashi from three localities in the south-eastern region of Hokkaido, Biratori, Shoya, and Satsunai, which were separated by 75–112 km. After being collected, they were preserved in Trillium Garden of Hokkaido University. The author is particularly indebted to this group for their cooperation.

Cytological preparations were made according to the method described in previous papers (Kayano and Watanabe 1970; Uchino 1980).

3. RESULTS AND DISCUSSION

(1) *Classification of chromosome types.*

The five chromosomes constituting a genomic set in trilliums were designated according to length as A, B, C, D, and E, A being the longest and E the shortest. Different types of patterns of H-segments were distinguished in everyone of the five chromosomes, A–E, of the K_2T complex. These different types were numbered, *e.g.*, A1 and A2 chromosome A, B1, B2, B3, and B4 in B, and so on (Fig. 1; Uchino 1975).

There were 2 different types in chromosome A, 4 in B, 6 in C, 4 in D, and 5 in E, *T. tschonoskii* and *T. × miyabe anum* inclusively (Fig. 1). Finer distinctions could be made between the patterns of the H-segments, as evident in the case of chromosome B. Types B1 and B4 were different in the length of the H-segment. The segment was longer in B1 than in B4. This difference was manifested repeatedly in many cells in which B1 and B4 coexisted as homoeologous chromosomes, B1·B1/B4·B4. The slash mark separates homoeologous pairs and the decimal, homologous chromosomes (Fig. 2). The difference between B1 and B4 was not so pronounced but consistently evident in all cells observed in all individual as well as in all cells observed in different individuals. The same differences in the length of H-segments were also found in chromosome types, *i.e.*, among C1, C2, and C4, between C5 and C6,

Fig. 1. Chromosome types in the K_2T genome complex in *T. tschonoskii* and *T. x miyabe anum*. Types observed only in *T. x miyabe anum* are indicated by the superscript m. B3 and E4, not found in *T. tschonoskii* of this study, were observed in the species discussed in the previous report (Haga and Kurabayashi 1953). $\times 1,200$.

Fig. 2. Somatic metaphase in the ovular tissue of *T. tschonoskii* ($4x=20$, K_2K_2TT) from the Kugino (Kg) population. Type B1 and B4 differ with respect to length of the H-segment in the long arm. $\times 1,200$.

between D1 and D3, and between E4 and E5 (Fig. 1).

Distinctions among different chromosome types were based simply on morphological differences in the patterns of H-segments. Thus, in certain instances, homoeologous chromosomes could be indicated by the same numbering. For example, a homoeologous pair of chromosome B in karyotype 3 of *T. tschonoskii* was indicated as B-1.1/1.1, where the slash separates the homoeologous chromosomes and decimal, homologous chromosomes (cf. Uchino 1985). This is important in the following descriptions.

Chromosome A (Fig. 1, A1-A2): A1 has two H-segments, one on each arm, and A2, one H-segment on one of the two arms. In A1, one of the two H-segments is very short and slit-like.

Chromosome B (Fig. 1, B1-B4): One H-segment was seen in B1, B2, and B4, on the long arm in B1 and B4 and on the short arm in B2. B4 could be distinguishable from B1 by its very short slit-like H-segment. B3 has two H-segments, one on each arm; the H-segments on the long and short arms were almost the same length.

Chromosome C (Fig. 1, C1-C6): The short arms of chromosome C revealed no H-segment except C4, but six types were distinguished by long arms. C3 had one H-segment. C1, C2, and C4 each had two H-segments. In C2 and C4, two H-segments were short or slit-like, while the proximal H-segment in C1 was conspicuously long. Both two H-segments on C2 were of the same length, but in C4, the proximal H-segment was slit-like. Three H-segments were evident in C5 and C6, but the types differed from each another in the pattern of H-segments.

Chromosome D (Fig. 1, D1-D4): Four types were identified by the long arms. A long H-segment appearing between two dot-like segments made D2 distinctive. D1 and D3 differed in the length of their proximal H-segments and the number and size of the dot-like segments. D4 had three distinct H-segments.

Chromosome E (Fig. 1, E1-E5): No H-segment was found in E2. E3 had one H-segment on the short arm. The short arm of E1 was distinctive in having H-segment and dot-like segment. Two H-segments were in E4 and E5, one on the short arm and the other on the long arm. The H-segment of the long arm of E5 was always longer than that of the long arm of E4.

Consequently, 21 types could be distinguished among the five chromosomes, A-E, of the K_2T complex. This range of chromosomal variation is much less compared to that known in the SU genome complex (cf. Haga *et al.* 1974) and the genome K_1 (cf. Fukuda and Kozuka 1958; Haga and Kurabayashi 1954).

(2) Frequency of chromosome types.

In the tetraploid *T. tschonoskii* ($4x=20$, K_2K_2TT), five componental chromosomes, A-E, are each comprised of four members. An analysis of 9 plants

Table 1. *Frequency of various types in chromosomes A to E in the K₂T genome complex*

Chromosome type	<i>T. tschonoskii</i>				<i>T. miyabeianum</i> **
	Sk	Kg	Tm	Gk	
A1	18	116	112	122	10
A2	18	116	112	122	10
Total	36	232	224	244	20
B1	36	206	192	142	18
B2	—	—	—	—	1
B3	—	—	—	—	1
B4	—	26	32	102	—
Total	36	232	224	244	20
C1	18	116	88	6	—
C2	18	116	112	122	7
C3	—	—	24	116	10
C4	—	—	—	—	1
C5	—	—	—	—	1
C6	—	—	—	—	1
Total	36	232	224	244	20
D1	18	—	—	—	6
D2	—	116	112	122	—
D3	18	116	112	122	10
D4	—	—	—	—	4
Total	36	232	224	244	20
E1	20	115	112	122	—
E2	16	116	112	122	5
E3	—	—	—	—	9
E4	—	—	—	—	5
E5	—	—	—	—	1
Total	36	231*	224	244	20

* A chromosome deficient for short arm is excluded.

** Ten plants from three distantly separated localities in Hokkaido.

showed the total number of each of these chromosomes to be 36, *i.e.*, Sk. The total number was 232 from an examination of 58 plants, *i.e.*, Kg. The total number was 224 and 244 for 56 and 61 plants examined, *i.e.*, Tm and Gk (Table 1). The four members in each chromosome, A-E, constitute a homoeologous pair, *e.g.*, A-1·1/2·2, B-1·1/4·4, etc. But, extinction of the diploid progenitor K₂K₂ and TT made it impossible to determine the genome to which a particular

chromosome belonged. And thus, the chromosomes were assorted simply on the basis of the patterns of H-segments.

Chromosome A: A1 and A2 were observed in equal frequency, halving the total frequency in all four populations, Sk, Kg, Tm, and Gk, in *T. tschonoskii* and in *T. × miyabeanum*. This indicates that half of the total comes from one of the genome of the K_2T complex, and another half the other genome of the complex. This means that the chromosome type was fixed for A1 and A2 in the respective genome, K_2 and T, and the genomes were comprised of no other types. A1 was assigned to K_2^* and A2 to T^* simply for the sake of convenience (Table 1).

Chromosome B: All 36 B chromosomes were of B1 in Sk. Thus, 18 ($=36/2$) B1s come from the genome K_2 and another 18 from the genome T. In Kg, Tm, and Gk in *T. tschonoskii*, as well as *T. × miyabeanum*, B1 exceeds half the total frequency, thus indicating the presence of B1 in both K_2 and T. K_2^* includes B1 and B4, and B1 is a member of T^* in *T. tschonoskii*. In *T. × miyabeanum*, assuming 10 out of 18 B1s come from T^* , then K_2^* comprises B1, B2, and B3 (Table 1). Here, a homologous series with less chromosomal variation was assigned to T^* , and the more variable series to K_2^* in each chromosome, B-E.

Chromosome C: C2 was fixed in all four populations of *T. tschonoskii*, exactly halving the total frequency, i.e., K_2^* . The remaining halves were 18 ($=36/2$) C1s and 116 ($=232/2$) C1s in Sk and Kg, respectively. And the remaining halves were 88 C1s + 24 C3s ($=112$) in Tm and 6 C1s + 116 C3s ($=122$) in Gk. Consequently T^* comprises C1 and C3 in *T. tschonoskii*. In *T. × miyabeanum*, 10 C3s come from T^* . Then K_2^* is variably composed of C2, C4, C5, and C6 (Table 1).

Chromosome D: D3 was fixed in all four populations, Sk, Kg, Tm, and Gk, in *T. tschonoskii* and also in *T. × miyabeanum*, i.e., T^* . Then K_2^* is polymorphic for D1, D2, and D4 (Table 1). It should be pointed out that the homologous pair was D-1.1/3.3 in Sk, and D-2.2/3.3 in Kg, Tm, and Gk in *T. tschonoskii*.

Chromosome E: E2 was fixed in Kg, Tm, and Gk, i.e., T^* , and E1 was fixed at the same time in the above three populations. In Sk, however, the frequency of E1 was greater than half of the total frequency, indicating the presence also of E1 in T^* . T^* is thus comprised of E1 and E2 in *T. tschonoskii*. In *T. × miyabeanum*, E4 and E5 were already sorted in K_2^* (Uchino 1975) and so E3 is present in both K_2^* and T^* .

Summarizing the above descriptions, the following series of homologous chromosomes or homologous series is tabulated.

A	K_2^* 1	B	K_2^* 1 2 3 4	C	K_2^* 2 4 5 6	D	K_2^* 1 2 4	E	K_2^* 1 3 4 5
	T^* 2		T^* 1		T^* 1 3		T^* 3		T^* 1 2 3

The homologous series K_2^* and/or T^* are mutually independent among the different chromosomes, A-E, with no relation to the genomes K_2 and T . K_2^* may actually represent a homologous series of the genome K_2 , or a homologous series of the genome T . The same holds true for T^* . Except for chromosome A, K_2^* is polymorphic, indicating one of the genomes, K_2 or T , of the K_2T complex to be polymorphic. T^* is polymorphic in chromosomes C and E and, with respect to them, the genomes K_2 and T are both polymorphic. Thus, the extinct diploid progenitors, K_2K_2 and TT , must be chromosomally polymorphic.

(3) *Distribution of chromosome types.*

The chromosome types A1, A2, B1, C2, D3, E1, and E2 are fixed in all four populations of *T. tschonoskii*; Sk, Kg, Tm, and Gk (Table 1). These types were also found in other populations in Hokkaido and Shikoku, though the number of plants in which they were observed was very few, 1-9 plants (cf. Watanabe and Kayano 1971). These types were also found in *T. × miyabe anum* (Table 1). They quite possibly represent widespread standard types which had been present in the diploid progenitors, K_2K_2 and TT , both now extinct.

Two types B4 and D2 were found with high frequency in three populations, Kg, Tm, and Gk, in Kyushu, but not in Hokkaido and Shikoku (cf. Watanabe and Kayano *loc. cit.*, 5 plants; the present paper, 9 plants). These types were not found in *T. × miyabe anum* (Table 1). On the other hand, D1 was found in all 14 plants from Hokkaido and Shikoku, but not in the populations in Kyushu (cf. Table 1, Watanabe and Kayano *loc. cit.*). D1 was also seen in *T. × miyabe anum*. It thus seems likely that *T. tschonoskii* differs in its progenitor diploids in Kyushu and other regions.

Seven types seen in *T. × miyabe anum*, were not present in *T. tschonoskii*, i.e., B2, C4, C5, C6, D4, E3, and E5 (cf. Fig. 1). It is possible that these types are (1) very rare in *T. tschonoskii*, (2) are restricted to localities where the hybrid plants are raised, or (3) are new variants arisen in hybrid plants. The decision as to which possibility is applicable is open to question.

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