CYTOGENETIC STUDIES IN THE TRIBE TRITICEAE
I. A POLYHAPLOID PLANT OF *AGROPYRON TSUKUSHIENSE* VAR.
TRANSIENS OHWI FOUND IN A STATE OF NATURE¹⁾

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Ag. tsukushiense (Honda) Ohwi var. *transiens* (Hack.) Ohwi, a common grass growing as a weed in fields and along road-sides, is widely distributed in Japan, China, Manchuria and Korea. This species is hexaploid ($2n=42$). Elucidation of the genome combination of this species is important from the viewpoint of the genomic structure of Asiatic species of the genus.

A spontaneous polyhaploid plant of the species, i.e. a haplohexaploid, was found in a valley of the hilly neighborhood of Misima, Shizuoka-Ken. It is well known that polyhaploids furnish critical information concerning the genome constitution of polyploid species. Therefore, a morphological and cytological investigation of the polyhaploid plant was undertaken. The results are given in this paper.

MATERIALS AND METHODS

In 1962 a polyhaploid plant, $2n=21$, of *Ag. tsukushiense* var. *transiens* was found in a small valley of the hilly neighborhood of Misima, on a small path at the uppermost edge of the hilly fields of the valley where this hexaploid species occurs in large numbers together with *Ag. ciliare* Franchet, *Arundinaria pygmaea* (Miq.) Mitford var. *glabra* (Makino) Ohwi, *Miscanthus sinensis* Anders., and other weeds and shrubs. The spikes and a clone of the haploid were collected and the latter was transplanted into the experimental field. Two seeds were obtained from 23 spikes. They were assumed to have been produced by pollination with the pollen of hexaploid plants growing near by. The two seeds gave two plants in 1963, one a monosomic ($2n=41$; cult. no. 6348-2) and the other a disomic, i.e. a complete hexaploid ($2n=42$; cult. no. 6348-1). Both plants were used for a morphological and cytological comparison with the haploid and with two strains of *Ag. tsukushiense* var. *transiens* collected in Misima, cult. nos. 6374 and 6380. Also a strain of hexaploid *Ag. humidum* Ohwi et Sakamoto collected in Misima (cult. no. 6388) was employed in the crosses. Pollen fertility was

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assessed after staining the pollen grains by diluted aceto-carmin. Materials for cytological observation were fixed in Farmer's solution (3 absolute ethanol: 1 glacial acetic acid). Aceto-carmin stain technique was used in all cytological preparations. Microphotographs were made from temporary slides.

RESULTS

The morphological characteristics of the polyhaploid in comparison with the monosomic plant and a hexaploid strain are summarized in Table 1. Typical spikes and spikelets of the plants are shown in Figs. 1 and 2. The whole plant, top internode, flag leaf, spike and floral parts of the polyhaploid are significantly shorter than those of the monosomic plant and the hexaploid strain. The number of spikelets per spike is also smaller. Accordingly, the polyhaploid plant had the appearance of a miniature of the hexaploid species. However, it produced a larger number of tillers. As to the morphology of the disomic obtained from the polyhaploid, it was the same as that of the hexaploid species.

Table 1. Morphological characteristics of the polyhaploid, the monosomic and a hexaploid strain of *Ag. tsukushiense* var. *transiens*

Characters	Polyhaploid	Monosomic (cult. no. 6348-2)	Hexaploid strain (cult. no. 6380)
No. of tillers	28.0	21.0	17.2
Plant height (cm)	73.7	99.0	102.6
Length of top internode (cm)	36.3	52.1	50.2
Length of flag leaf (cm)	7.5	13.0	13.9
Length of spike (cm)	13.5	20.3	20.3
No. of spikelets per spike	15.7	17.9	18.5
Length of glume (mm)	4.1	8.4	7.9
Length of lemma (mm)	7.9	11.1	10.6
Length of awn (mm)	14.0	21.1	20.0
Length of palea (mm)	7.9	11.0	10.5
Length of seed (mm)	—	6.2	5.6

Pollen fertility and seed fertility from self-pollination of the polyhaploid, the monosomic, the disomic and two hexaploid strains are given in Table 2. The polyhaploid seems to be almost completely sterile. Of about 4,000 pollen grains examined only 4 were morphologically normal and the rest were all abortive. The monosomic plant showed considerably lower pollen- and seed-fertility, 28.5% and 53.3%, respectively, as compared with the disomic plant and the hexaploid strains.

The polyhaploid was crossed by the disomic, two hexaploid strains and a strain of *Ag. humidum*. The seed set and number of disomic vs. monosomic plants in cross combinations involving the polyhaploid as the female parent are given in Table 3. The crossing success amounted to 0.7–1.6%. The hybrid seeds were all normal and

Fig. 1. Typical spikes of the polyhaploid, the monosomic, the disomic and a hexaploid strain of *Ag. tsukushiense* var. *transiens* ($\times 0.4$)

- a. polyhaploid
- b. monosomic (cult. no. 6348-2)
- c. disomic (cult. no. 6348-1)
- d. hexaploid strain (cult. no. 6380)

Fig. 2. Spikelet, glume, lemma, palea and seed (from left to right) of the polyhaploid, the monosomic, the disomic and a hexaploid strain ($\times 1.2$)

- a. polyhaploid
- b. monosomic (cult. no. 6348-2)
- c. disomic (cult. no. 6348-1)
- d. hexaploid strain (cult. no. 6380)

Table 2. Pollen- and seed-fertility of the polyhaploid, the monosomic, the disomic and two hexaploid strains of *Ag. tsukushiense* var. *transiens*

Strains	Pollen fertility				Seed fertility		
	Observed number	Normal	Abnormal	%	No. of florets	No. of seeds	%
Polyhaploid	3,932	4	3,928	0.001	970	0	0
Monosomic (cult. no. 6348-2)	2,853	814	2,039	28.53	584	311	53.25
Disomic (cult. no. 6348-1)	3,152	2,474	678	78.49	278	213	76.61
Hexaploid strain (cult. no. 6374)	—	—	—	—	443	279	62.98
Hexaploid strain (cult. no. 6380)	—	—	—	—	734	477	64.99

Table 3. Seed set and number of disomic and monosomic in cross combinations involving the polyhaploid as the female parent

Cross combinations	No. of florets crossed	No. of seeds set	Crossing success (%)	No. of seeds sown	No. of seeds germinated	No. of plant matured	Disomic plants	Monosomic plants
Polyhaploid × Disomic (cult. no. 6348-1)	494	5	1.01	5	5	4	4	0
Polyhaploid × Hexaploid strain (cult. no. 6374)	368	6	1.63	6	5	4	4	0
Polyhaploid × Hexaploid strain (cult. no. 6380)	4,018	28	0.69	28	22	19	17	2
Polyhaploid × <i>Ag. humidum</i> (cult. no. 6388)	140	0	0	—	—	—	—	—

showed good germination. Out of 27 plants matured, only two were monosomic and the rest were all disomic (Table 3). These two monosomic plants were obtained only from the cross between the polyhaploid and a hexaploid strain, cult. no. 6380.

Chromosome pairing at MI of PMCs of the polyhaploid, the monosomic and a hexaploid strain is given in Table 4. Of 442 cells examined of the polyhaploid, 84% showed 21_I (Fig. 3a, b) and 14% had $1_{II}+19_I$ (Fig. 3c). All bivalents were associated loosely with a terminal chiasma as shown in Fig. 3c, d. Rarely 1_{III} or 1_{IV} was found.

Table 4. Chromosome pairing at MI of PMCs of the polyhaploid, the monosomic and a hexaploid strain of *Ag. tsukushiense* var. *transiens*

Chromosome pairing	No. of cells observed	%
Polyhaploid :		
21_I	369	83.5
$1_{II}+19_I$	61	13.8
$1_{III}+18_I$	2	0.5
$2_{II}+17_I$	8	1.8
$1_{IV}+17_I$	1	0.2
$3_{II}+15_I$	1	0.2
Total	442	100.0
Monosomic (cult. no. 6348-2) :		
$20_{II}+1_I$	164	97.0
$19_{II}+3_I$	5	3.0
Total	169	100.0
Hexaploid strain (cult. no. 6380) :		
21_{II}	132	97.8
$20_{II}+2_I$	3	2.2
Total	135	100.0

Fig. 3. Chromosome pairing at meiosis of the polyhaploid, the monosomic and a hexaploid strain ($\times 1,440$)

a. polyhaploid MI: 21_I b. polyhaploid late MI: 21_I c. polyhaploid MI: $1_{II}+19_I$
 d. polyhaploid MI: $2_{II}+17_I$ e. polyhaploid An abnormal pollen quartet f. polyhaploid
 An abnormal pollen quartet g. monosomic (cult. no. 6348-2) MI: $20_{II}+1_I$ h. hexaploid
 strain (cult. no. 6380) MI: 21_{II}

The average chromosome pairing in the polyhaploid was $0.002_{IV} + 0.005_{III} + 0.18_{II} + 20.62_I$. Two abnormal pollen quartets of the polyhaploid are shown in Fig. 3e, f.

In the majority of cells chromosome pairing of the monosomic was $20_{II} + 1_I$ (Fig. 3g). All bivalents were tightly paired. In almost all of meiotic figures at MI of a hexaploid strain 21_{II} were found (Fig. 3h). No multivalents occurred either in the monosomic or in the hexaploid strain.

DISCUSSION

The stems, leaves and floral parts of the polyhaploid of *Ag. tsukushiense* var. *transiens* are characterized by miniature-like dimensions as compared with the hexaploid strains (Table 1). This is a well-known characteristic of haploids or polyhaploids, as recently reviewed by Katayama and Nei (1964). Growth and flowering of the polyhaploid plant in the experimental field were normal. A marked characteristic of this plant was the vigorous tillering; clonal propagules were easily produced by separation of tillers. In 1963, 0.5% colchicine solution was applied to the basal parts of tillering clones of the polyhaploid using Sears' method* (Sears 1941), and three fertile spikes were produced yielding 71 well-developed seeds.

Complete sterility of the polyhaploid is attributable to the very weak pairing between the 3 genomes and, consequently, high frequency of univalents at MI as shown in Table 4. However, crosses involving the polyhaploid as the female parent were successful and about 1% of florets pollinated set normal seeds (Table 3). Of 27 hybrid plants obtained from the crosses, two plants were monosomic.

The genome structure of polyploid species has been estimated from chromosome pairing at MI of interspecific or intergeneric hybrids involving the species in question. However, this method of genome determination is usually inconclusive, if no diploid species are available as analysers. In many cases it is almost impossible to distinguish autosyndetic from allosyndetic pairing, according to the type of polyploidy of the species involved, either auto-, autoallo-, segmental autoallo- or allopolyploidy. In doubtful cases polyhaploid plants provide critical information concerning the genome structure of polyploid species (Dewey 1961). As shown in Table 4, no bivalents were found in 84% of the polyhaploid cells of *Ag. tsukushiense* var. *transiens*, and only one was observed in 14% of cells examined. Average chromosome pairing per cell was $0.0_{IV} + 0.0_{III} + 0.2_{II} + 20.6_I$, which indicates very low frequency of multivalent formation. Chromosome pairing of the monosomic and of the hexaploid strains was $20_{II} + 1_I$ and 21_{II} , respectively, in the majority of cells. From these results it is concluded that *Ag. tsukushiense* var. *transiens* is an allohexaploid whose genome constitution comprises three different genomes.

* The author is indebted to Dr. M. Muramatsu for the technical help in the application of this method.

Matsumura (1941, 1942) reported detailed cytological observations of two inter-specific hybrids, *Ag. ciliare* ($2n=28$) $\times$ *Ag. tsukushiense* var. *transiens** and *Ag. tsukushiense* var. *transiens** $\times$ *Ag. cristatum* ($2n=14$). In the former hybrid, Matsumura (1941) observed the average chromosome pairing, $0.4_{IV}+0.0_{III}+12.5_{II}+8.4_I$, with the range of bivalent formation from 10 to 14_{II} , and the mode at 14_{II} . From the result he derived the genome formula, II KK LL, for *Ag. tsukushiense* var. *transiens* (Matsumura 1948). The present study clearly supports his view. In the latter hybrid, *Ag. tsukushiense* $\times$ *Ag. cristatum*, Matsumura (1942) obtained $0.0_{IV}+0.1_{III}+2.2_{II}+19.5_I$ as the average chromosome pairing, $(0-5)_{II}$ as the range of bivalent formation with $(2-3)_{II}$ as the mode. Considering from his and the present author's results, it is assumed that the genome of diploid *Ag. cristatum* is different from those found in *Ag. tsukushiense* var. *transiens*, though it seems to be partially homologous with one of the *tsukushiense* genomes.

Among the genera of the tribe Triticeae, *Agropyron* and *Elymus* comprise many polyploid species. Of 65 species of *Agropyron* whose chromosome numbers were examined, tetraploidy was reported in 45, hexaploidy in 18 and diploidy only in 10 species (Sakamoto 1964). In such genera as *Agropyron*, it is assumed that the type of polyploidy is complicated and the many polyploid species are of very complex origin (Stebbins 1947). At present, cytological observations of polyhaploids in *Agropyron* have been reported only for two species, *Ag. desertorum* (Dewey 1961) and *Ag. intermedium* (Dewey 1962). The pertinent cytological data and the type of polyploidy of these two species are summarized in Table 5 together with those obtained from the present investigation. According to Table 5, *Ag. desertorum* is an autotetraploid, *Ag. intermedium* is a segmental autoallohexaploid and *Ag. tsukushiense* is an allohexaploid species. Polyhaploid plants of *Ag. desertorum* and *Ag. intermedium* allowed the elucidation of the genome structure of both owing to the cytological examination of the

Table 5. Cytological data on polyhaploids and type of ploidy in three species of *Agropyron*

Species	Polyhaploid plant no.	Somatic chromosome no. of polyhaploid	Range of bivalents	Mode of bivalents	Average chromosome pairing at MI	Type of ploidy	Reference
<i>Ag. desertorum</i> (4x)	1	15	5-7	7	$0.3_{III}+6.6_{II}+1.1_I$	autotetraploid	Dewey (1961)
	2	14	6-7	7	$7.0_{II}+0.1_I$		
	3	14	$1_{IV}+5_{II}$	5	$1.0_{IV}+5.0_{II}$		
	4	14	6-7	7	$7.0_{II}+0.1_I$		
<i>Ag. intermedium</i> (6x)	1	21	0-7	2-3	$0.1_{III}+3.1_{II}+14.5_I$	segmental autoallohexaploid	Dewey (1962)
<i>Ag. tsukushiense</i> (6x)	1	21	0-3	0	$0.0_{IV}+0.0_{III}+0.2_{II}+20.6_I$	allohexaploid	

* Matsumura used for the specific name of this species the designation *Ag. semicostatum*.

hybrids (Dewey 1961, 1962). The genus *Agropyron* appears to comprise many species displaying various types of polyploidy of complex origin. Thus, to determine them is a fascinating study made accessible due to the occurrence of polyhaploids.

SUMMARY

Ag. tsukushiense var. *transiens*, a weed growing in fields and along road-sides, is a very common hexaploid species ($2n=42$), widely distributed in Japan, China, Manchuria and Korea. In a valley of the hilly neighborhood of Misima, a spontaneous polyhaploid plant ($2n=21$) of the species was found. It was smaller than the hexaploid strains, but its tillering was very vigorous. In natural condition it yielded two seeds which were assumed to have been produced by pollination with the pollen of hexaploid plants growing in its proximity. The two seeds gave two plants, one a monosomic ($2n=41$) and the other a disomic ($2n=42$).

Pollen fertility of the polyhaploid, the monosomic and the disomic was 0.001, 28.5 and 78.5%, and seed fertility was 0, 53.3 and 76.6%, respectively. The polyhaploid was crossed by the disomic and by two hexaploid strains, and the crossing success amounted to 0.7–1.6%.

As to chromosome pairing at MI of PMCs, the polyhaploid showed 21_I in 84% and $1_{II}+19_I$ in 14% of 442 cells observed. In the majority of cells chromosome pairing in the monosomic was $20_{II}+1_I$. From the result it is concluded that *Ag. tsukushiense* var. *transiens* is an allohexaploid whose genome constitution comprises three different genomes. Summarizing the cytological studies of polyhaploids in *Agropyron*, the types of polyploidy in this genus are assumed to be various and complex.

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