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CYTOGENETIC STUDIES ON INTERGENERIC HYBRIDS BETWEEN TWO *AGROPYRON* SPECIES AND *HORDEUM BULBOSUM* (4x)[1)]

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INTRODUCTION

In the genome analysis of polyploid species, polyhaploid plants of the species in question provide critical information concerning genome structure. In hybrids between several species of the tribe Triticeae and *H. bulbosum,* chromosomes of *H. bulbosum* are eliminated and consequently haploid plants of the female parent are produced. This technique called "the bulbosum method" is useful for the production of haploids in the tribe Triticeae. So far, we have obtained polyhaploids of *Aegilops crassa* (6x) by this method (Shigenobu and Sakamoto, 1977).

In order to obtain polyhaploids in the genus *Agropyron,* nine species were pollinated by *H. bulbosum* (4x). Among progenies obtained by embryo culture, six plants did not show the elimination of any chromosomes derived *H. bulbosum* and were intergeneric hybrids between *A. repens* (6x) or three ecotypes of *A. tsukushiense* (6x) and *H. bulbosum* (4x) (Shigenobu and Sakamoto, 1981).

Out of six hybrids obtained, two plants from *A. repens* or *A. tsukushiense* CT-2 × *H. bulbosum* HB-1 (4x) (2n = 34 or 2n = 35) were examined morphologically and cytologically. As the genome relationships between *A. tsukushiense* and *H. bulbosum* (4x) was discussed in a previous paper (Shigenobu and Sakamoto, 1981), the present one mainly reports the morphological and cytogenetic studies of intergeneric hybrids between *A. repens* and *H. bulbosum* (4x).

MATERIALS AND METHODS

Crosses and embryo culture were carried out at the Plant Germ-plasm Institute. The techniques have been described in detail by Shigenobu and Sakamoto (1981).

RESULTS

Intergeneric hybrid between *A. repens* and *H. bulbosum* HB-1 (4x)

This hybrid (2n = 34) decreased in somatic chromosome number from 35 to 34 during vegetative growth, indicating the elimination of one chromosome. This hybrid was vigorous and produced numerous tillers, but did not head for three years after it was produced. As shown in Table 1, this hybrid was shorter than the parent

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plants, it started to flower earlier in the year than its parents and it was completely sterile with small and nondehiscent anthers. The length of the top internode, flag leaf, and spike was shorter than the parent plants. The average number of rachis nodes per spike in 18 spikes produced was 20.8. The hybrid was clearly less vigorous than the parents showing negative heterosis.

TABLE 1. Quantitative characters of the hybrid between *A. repens* and *H. bulbosum* HB-1 (4x) and the parents

Characters	Parental strains		Hybrid
	A. repens	*H. bulbosum* HB-1 (4x)	*A. repens* × HB-1 (2n=34)
Date of first flowering	24th May	13th May	4th May
Plant height (cm)	65.4	114.5	42.4
Length of top internode (cm)	32.7	46.5	18.0
Length of flag leaf (cm)	11.7	13.1	10.1
Length of spike (cm)	12.1	15.3	7.1

The spike morphology of the hybrid resembled the female parent rather than being intermediate between the parents (Fig. 1). The spike of *Agropyron* consists of solitary spikelets at each node of the rachis, while that of *H. bulbosum* is composed of three spikelets with a single floret at each rachis node. The spike of the hybrid has 1, 2, or 3 spikelets at each rachis node with an average of 1.3 spikelets. Bulbous

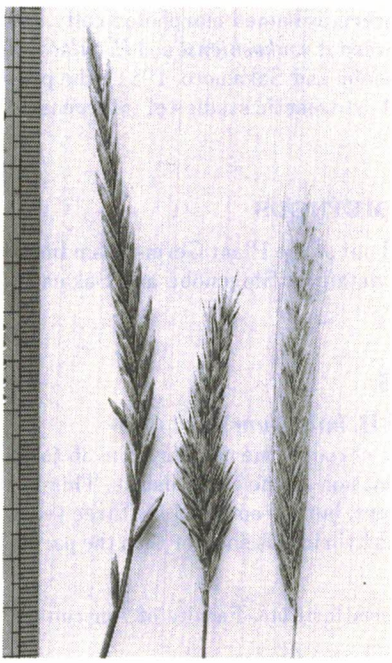

FIG. 1. Spikes of *A. repens*, *H. bulbosum* HB-1 (4x) and their intergeneric hybrid plant (2n=34). Left : *A. repens*, Center : *A. repens* × *H. bulbosum* HB-1 (4x), Right : *H. bulbosum* HB-1 (4x).

swelling at the base of the culm, which is characteristic of the pollen parent, was also observed in the hybrid, although the degree of swelling was less than in the pollen parent.

A reduction of chromosome number was observed in most of PMCs as well as some root-tip cells of the hybrid. The chromosome number and chromosome pairing at MI of PMCs of the hybrid is shown in Table 2. Of 88 cells examined, the majority (86.4%) showed a reduction of from one to six chromosomes in the same anther. Cells having 31 and 30 chromosomes, indicating the elimination of three and four chromosomes, were observed at high frequencies 26.1% and 35.2%, respectively. This hybrid formed various numbers of bivalents and univalents and, occasionally, one quadrivalent or trivalent at MI. Of 264 bivalents observed, 210 (79.5%) were rod-shaped.

The total amount of chromosome pairing was calculated; quadrivalents and trivalents were counted as two bivalents and one bivalent + one univalent, respectively (Sakamoto and Muramatsu, 1966). The number and frequency of bivalents according to this method are given in Table 3. The total amount of chromosome pairing showed a wide range from one to seven bivalents. Cells having two and three bivalents were observed at the rather high frequencies of 28.4% and 31.8%, respectively. The average number of the total bivalent formation per cell was 3.1.

Chromosome pairing at MI of PMCs of *A. repens* showed 21_{II} and $1_{IV} + 19_{II}$ in 88 (77.2%) and 22 (19.3%) cells, respectively, among 114 PMCs examined. $1_{III} + 19_{II} + 1_{I}$, $2_{IV} + 17_{II}$ and $1_{IV} + 18_{II} + 2_{I}$ were also found in two (1.8%), one (0.9%), and one (0.9%) cells, respectively. The average chromosome pairing was $0.22_{IV} + 0.02_{III} + 20.52_{II} + 0.04_{I}$. Of 717 bivalents observed, 616(85.9%) were ring-shaped.

Chromosome pairing at MI of PMCs of *H. bulbosum* HB-1 (4x) formed various numbers of quadrivalents and bivalents and, occasionally, one or two trivalents together with some univalents at MI. Only one sexivalent was found in a cell. The average chromosome pairing was $0.007_{VI} + 1.7_{IV} + 0.043_{III} + 9.9_{II} + 1.2_{I}$. Of 1,379 bivalents observed, 1,051 (76.2%) of were ring-shaped. In spite of varying multivalent formations this strain showed relatively high pollen fertility (75.3%).

Intergeneric hybrid between *A. tsukushiense* CT-2 and *H. bulbosum* HB-1 (4x)

The growth of this hybrid (2n = 35) was somewhat weak in spite of good tillering. Morphological characteristics of this hybrid have been described by Shigenobu and Sakamoto (1981).

A reduction of chromosome number was observed in most of PMCs as well as some root-tip cells of the hybrid. The chromosome number and chromosome pairing at MI of PMCs of the hybrid is shown in Table 4. Of 42 cells examined, the majority (76.2%) showed a reduction from one to six chromosomes in the same anther. Cells having 32 chromosomes, indicating the elimination of three chromosomes, were observed at a maximum frequency of 57.1%. This hybrid formed various numbers of bivalents and univalents and, occasionally, one quadrivalent or trivalent at MI. Of 229 bivalents observed, 138 (60.2%) were rod-shaped.

The total amount of chromosome pairing was calculated as above, and the number and frequency of bivalents according to this method are given in Table 5. The total amount of chromosome pairing showed a wide range from two to eight bivalents. Cells having five and six bivalents were observed at a high frequency of 23.8% and 28.6%, respectively. The average number of the total bivalent formation

TABLE 2. The chromosome number and chromosome pairing at MI of PMCs of the hybrid between *Agropyron repens* (6x) and *Hordeum bulbosum* HB-1 (4x)

Chromosome number	No. of cells observed	Chromosome pairing				Frequency	%
		IV	III	II	I		
2n = 34	12			2	30	3	3.4
				3	28	4	4.5
				4	26	2	2.3
				5	24	3	3.4
2n = 33	4			1	31	1	1.1
				2	29	1	1.1
				3	27	1	1.1
				4	25	1	1.1
2n = 32	11			2	28	4	4.5
				3	26	3	3.4
			1	4	21	1	1.1
				6	20	1	1.1
				7	18	2	2.3
2n = 31	23			1	29	3	3.4
				2	27	6	6.8
				3	25	9	10.2
			1	2	24	2	2.3
				4	23	1	1.1
			1	3	22	1	1.1
				5	21	1	1.1
2n = 30	31			1	28	3	3.4
				2	26	8	9.1
				3	24	7	8.0
				4	22	9	10.2
		1		2	22	1	1.1
				5	20	1	1.1
				6	18	1	1.1
			1	5	17	1	1.1
2n = 29	6			2	25	3	3.4
				3	23	2	2.3
			1	3	20	1	1.1
2n = 28	1			5	18	1	1.1
Total	88						99.4

TABLE 3. Frequency of total bivalents in the hybrid between *Agropyron repens* (6x) and *Hordeum bulbosum* HB-1 (4x)

No. of total bivalents	No. of cells observed	Frequency %
1	7	8.0
2	35	28.4
3	28	31.8
4	16	18.2
5	7	8.0
6	3	3.4
7	2	2.3
Total	88	100.1

TABLE 4. The chromosome number and chromosome pairing at MI of PMCs of the hybrid between *Agropyron tsukushiense* CT-2 (6x) and *Hordeum bulbosum* HB-1 (4x)

Chromosome number	No. of cells observed	Chromosome pairing				Frequency	%
		IV	III	II	I		
2n = 35	10			2	31	2	4.8
				4	27	1	2.4
				5	25	2	4.8
				6	23	1	2.4
				7	21	2	4.8
			1	6	20	1	2.4
				8	19	1	2.4
2n = 34	1		1	5	21	1	2.4
2n = 33	6			4	25	1	2.4
				5	23	1	2.4
				6	21	1	2.4
				7	19	2	4.8
				8	17	1	2.4
2n = 32	24			3	26	2	4.8
				4	24	3	7.1
		1		5	22	5	11.9
				3	22	1	2.4
				6	20	9	21.4
				7	18	2	4.8
				8	16	2	4.8
2n = 29	1		5	19	1		2.4
Total	42						100.4

TABLE 5. Frequency of total bivalents in the hybrid between *Agropyron tsukushiense* CT-2 (6x) and *Hordeum bulbosum* HB-1 (4x)

No. of total bivalents	No. of cells observed	Frequency %
2	2	4.8
3	2	4.8
4	5	11.9
5	10	23.8
6	12	28.6
7	7	16.7
8	4	9.5
Total	42	100.1

per cell was 5.5.

Chromosome pairing at MI of PMCs of *A. tsukushiense* CT-2 showed 21_{II} in most cells (30 cells, 93.8%) examined, and $20_{II} + 2_{I}$ were found in two cells (6.2%). The average chromosome pairing was $20.94_{II} + 0.12_{I}$. Of 670 bivalents observed, 635 (94.8%) were ring-shaped.

DISCUSSION

From the observation of chromosome pairing on interspecific hybrids involving *A. repens* (Dewey, 1964) and in a polyhaploid of *A. repens* forming three to seven bivalents with the average chromosome pairing : $0.02_{III} + 5.59_{II} + 9.71_{I}$ (Dewey, 1974), it was proposed that *A. repens* is a segmental autoallohexaploid with two closely related genomes and a third distinctly different one (Dewey, 1974), whose genome formula was designated $S_1\ S_1\ S_2\ S_2$ X X (Dewey, 1976). *H. bulbosum* HB-1 (4x) used as the male parent in these hybrids zero to four quadrivalents and one or two additional tri- or sexivalents with an average 1.7 quadrivalents per cell.

Judging from these results, the autosyndesis of genomes derived from both *A. repens* and *H. bulbosum* HB-1 (4x) in the intergeneric hybrid between *A. repens* and *H. bulbosum* HB-1 (4x) is expected to form three to twelve bivalents per cell. However, the formation of a maximum seven bivalents with an average 3.1 total bivalent formation in this hybrid was lower than expected. This may mean no homology among the chromosome complements of three genomes of *A. repens* and two of *H. bulbosum*. The lower frequency of bivalents in this hybrid might be due to the elimination of some chromosomes in most PMCs (86.4%), although it is not known from which parent they were derived.

In the previous paper (Shigenobu and Sakamoto, 1981) it was suggested that there was a lack of segmental homology among the chromosome complements of three genomes of *A. tsukushiense* and two of *H. bulbosum*. This conclusion was supported by the present results in the hybrid between *A. tsukushiense* CT-2 and two of *H. bulbosum* HB-1 (4x).

Cytological studies dealing with selective elimination of *H. bulbosum* chromosomes have been undertaken mainly in interspecific hybrids of *Hordeum*. So far, it has suggested that the control over selective chromosome elimination resides in

the genome balance between *vulgare* and *bulbosum* chromosomes or some heritable factors involved in *H. bulbosum* (Subrahmanyam and Kasha, 1973; Fukuyama and Kurozumi, 1977). However, the present results can not be explained either by the genome balance hypothesis that stable hybrids are obtained by increasing the bulbosum genomes or by heritable factors in *H. bulbosum*.

Cells with varying numbers of chromosomes and chromosome instability in mitotic and meiotic tissues were observed in several interspecific or intergeneric hybrids involving *Hordeum* (Kasha ans Sadasivaiah, 1971; Lange, 1971; Humphrey, 1978; Mujeeb *et al.*, 1978; Fedak and Nakamura, 1982). In the present study, two hybrids between *A. repens* or *A. tsukushiense* CT-2 and *H. bulbosum* HB-1 (4x) showed chromosome instability in mitotic and meiotic tissues. These observations suggest the possibility that some seedlings obtained in the previous paper (Shigenobu and Sakamoto,1981) show aneuploidy or/and chromosome instability; therefore, they might not develop into plants.

SUMMARY

Instead of obtaining polyhaploids in the genus *Agropyron* by "the bulbosum method", intergeneic hybrids between two *Agropyron* species and *Hordeum bulbosum* (4x) were produced. Out of six hybrids obtained, two from *A. repens* (6x) or *A. tsukushiense* CT-2 (6x) × *H. bulbosum* HB-1 (4x) (2n=34 or 2n=35) were examined morphologically and cytologically. These hybrids were shorter than their parent species but had numerous tillers. They were completely sterile. The spike morphology resembled the female parent rather than an intermediate between the parents. Several characteristics of the pollen parent were also observed in these hybrids.

In these hybrids a reduction of chromosome number was observed in most PMCs as well as some root-tip cells. The chromosome pairing at MI of PMCs in *A. repens* × *H. bulbosum* suggests no homology among the chromosome complements of three genomes of *A. repens* and two of *H. bulbosum*.

These results indicate that the genome balance hypothesis can not be applied to intergeneric hybrids between *Agropyron* and *H. bulbosum*.

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