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Effectiveness of ph Gene in Inducing Homoeologous Chromosome Pairing in Agrotricum*

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Summary. We observed pairing, when the *ph* gene was present, between wheat (*Triticum aestivum* L. em. Thell.) chromosome 4B, and an *Agropyron intermedium* (Host) Beauv. chromosome (Ai) carrying a gene resistant to wheat streak mosaic (WSM). In a monosomic addition polyhaploid [2n = 22 = 19' + 5B' (*ph*) + 4B'+ Ai'], we recorded an average of 4.1 bivalents and 0.3 trivalents per cell. Induced homoeologous pairing was most effective when both 5B chromosomes carrying *ph* gene were present. Our data suggest that chromosome 4B of wheat and the *Agropyron* chromosome (Ai) carrying a gene for resistance to WSM are homoeologous and that it is possible to use either *ph* mutant or nullisomic 5B stock to induce genetic recombination between the two chromosomes.

Key words: Wheat - *Agropyron* - Wheat Streak Mosaic

Introduction

A primary obstacle for transferring genes from wheat-related species to wheat (*Triticum aestivum* L. em. Thell.) is the lack of pairing between homoeologous chromosomes, because the long arm of wheat chromosome 5B carries a dominant gene (*Ph*) that restricts synapsis to homologous chromosomes only (Okamoto 1957; Riley 1958; Riley and Chapman 1958; Sears and Okamoto 1958). To facilitate interspecific gene transfer in wheat, three methods have been suggested: (1) ionizing radiation, (2) induced homoeologous pairing and subsequent crossing over and genetic recombination by removing or suppressing chromosome 5B effect, and (3) misdivisions of two univalents followed by reunion of two telocentrics from different univalents (Sears 1972). Among the three techniques, induced genetic recombination appears to be the most desirable because only a segment of chromatin may be involved in crossing over and it involves only homoeologous chromosomes. With the availability of plants carrying recessive *ph* alleles or deletion of *Ph* locus (Wall, Riley and Chapman 1971; Sears 1975), gene transfer involving wheat and wheat-related species will become a realistic approach (Wang and Liang 1977a).

In this paper we show the effectiveness of using the *ph* mutant in inducing homoeologous pairing between wheat chromosome 4B and an *Agropyron* chromosome carrying a gene for resistance to wheat streak mosaic virus.

Materials and Methods

'Chinese Spring' monosomic 5B plants were crossed, as female, to CI 15092 (Wells et al. 1973), which is a disomic substitution line in which a pair of *Agropyron intermedium* chromosomes are substituted for the wheat 4B pair. Progeny possessing the *Agropyron* chromosome (Ai) were identified by their resistance to wheat streak mosaic (WSM) and by microscopic examination for the presence of Ai chromosome. Resistant plants with 41 chromosomes selected from F_1 progeny were crossed, as female, to plants having *ph/ph* genes in chromosome 5B; their progeny were tested for reactions to WSM, and resistant plants with 41 chromosomes [19'' + 5B' (*ph*) + 4B' + Ai'] were selected. Those 41 chromosome plants were backcrossed as female to *ph/ph* plants and their progeny having the chromosome complement of 19'' + 5B''(*ph/ph*) + 4B' + Ai' were identified. In a separate cross involving plants of 20'' + telo $5B_L$' + telo $5B_S$' and 19''+ 5B' (*ph*) + 4B' + Ai', one offspring with 40 chromosomes and resistance to WSM was obtained.

Spikes from all plants resistant to WSM were fixed in Carnoy's B solution for 24 hours, pollen mother cells were stained in acetocarmine, and chromosome behavior was examined at metaphase I. Frequency of homoeologous pairing between chromosomes 4B and

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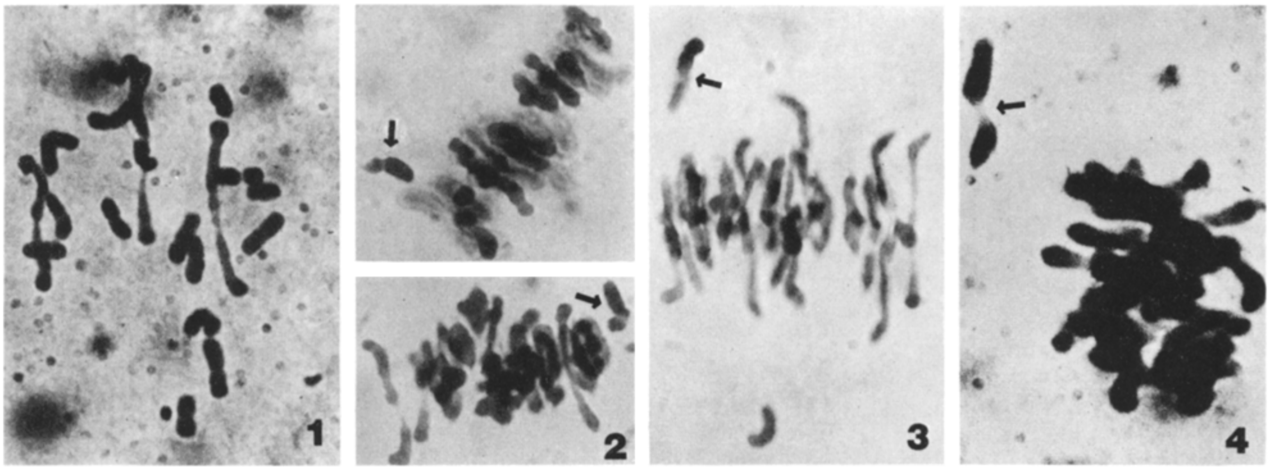

Fig.1. Metaphase I configuration in a polyhaploid plant (2n = 22 = 21' + Ai'), showing 3 bivalents and 16 univalents. Fig.2. Two metaphase I configurations showing a heteromorphic bivalent (arrowed) composed of wheat chromosome 4B and a telosomic chromosome, Ais, in a 19"+ 5B"(*ph/ph*) + 4B' + tAi'$_s$ plant. Fig.3. Metaphase I configuration in a 19" + 5B' (*ph*) + 4B' + Ai' plant, showing the homoeologous pairing between 4B and Ai (arrowed). Fig.4. Metaphase I configuration in the same plant as in Fig.3, but in which the heteromorphic bivalent formed by 4B and Ai had begun to desynapse (arrowed)

Ai in plants carrying one or two doses of *ph* allele was determined by (1) direct observation and (2) percentage of metaphase I cells not showing 4B and Ai as univalents in plants carrying *ph* gene minus the percentage of cells not showing 4B and Ai as univalents in plants carrying *Ph* genes. The percentage of cells not showing 4B and Ai as univalents in plants carrying *Ph* gene was used as a control in determining the homoeologous pairing between chromosomes chromosomes 4B and Ai.

Results and Discussion

A WSM resistant polyhaploid plant [2n = 22 = 19' + 5B' (*ph*) + 4B'+ Ai] was obtained (Fig.1) from a plant with chromosome constitution of 19" + 5B'(*ph*) + 4B' + Ai'. The origin of the polyhaploid appears to be tychoparthenogenetic, in which the egg occasionally develops into an individual without fertilization. The frequencies of homoeologous pairing in the polyhaploid plant and plants having different chromosome complement are presented in Table 1.

The resistant polyhaploid plant had an average of 4.1 bivalents (ranging from 0 to 8) and 0.3 trivalent (ranging from 0 to 1.0) per cell. The number of bivalents observed in the polyhaploid were comparable with that of the nullisomic 5B haploid of 'Holdfast' studied by Riley and Law (1965). The nullisomic 5B plant (19" + 4B' + Ai') had an average of 3.3 univalents and 18.1 bivalents per cell; the 42 chromosome plant with both 5B chromosomes carrying *ph* alleles had an average of 1.6 univalents and 18.4 bivalents plus other multivalents. Apparently induced homoeologous pairing was less frequent in nullisomic 5B plants than in plants with a pair of *ph* alleles. The higher frequency of homoeologous pairing in *ph/ph* plants might also have been attributable in part to the short arm of chromosome 5B, which carries genes promoting homoeologous pairing (Feldman 1966; Riley and Chapman 1967).

The frequency of homoeologous pairing between chromosomes 4B and Ai apparently relates to the dosage effects of the *ph* gene and perhaps the promoter gene in the short arm of chromosome 5B (Table 2). Because plants with 5B carrying *Ph/Ph* gene should not exhibit any homoeologous pairing, we considered the 18.6 % of meiotic cells not showing 4B and Ai as univalents as a controlling figure in calculating the homoeologous pairing frequency in plants with one and two doses of *ph* allele. Therefore, plants with chromosome constitution of 19" + 5B' (*ph*) + 4B' + Ai' should have had an estimated 7 % (25.6 % minus 18.6 %) pairing between 4B and Ai. Similarly, plants with two doses of *ph* allele had an estimated 16 % homoeologous pairing. The estimated figures were reasonably close to those observed directly.

Table 1. Frequency of homoeologous pairing in three types of plants differing in chromosome constitution

Chromosome constitution	No. cells examined	Average number per cell					
		Univ.	Biv.	Triv.	Quad.	Quin.	Hexa.
22=19'+5B'(*ph*)+4B'+Ai'	31	12.9	4.1	0.3	----	----	----
40=19''+4B'+Ai'	30	3.3	18.1	0.1	0.03	----	----
42=19''+5B''(*ph/ph*)+4B'+Ai'	36	1.6	18.4	0.6	0.30	0.05	0.03

Table 2. Homoeologous pairing between chromosome 4B of *Triticum aestivum* and Ai of *Agropyron intermedium* in plants differring in number of *ph* alleles

Chromosome complement	Estimated pairing between 4B and Ai				Observed 4B-Ai pairing		
	No. plants examined	No. cells examined	% cells without univ.	Estimated 4B-Ai pairing (%)	No. of plants	No. of cells	No. cells showing 4B-Ai pairing
19''+5B''(*Ph/Ph*)+4B'+Ai'	5	161	18.6	0	5	150	0
19''+5B'(*ph*)+4B'+Ai	2	328	25.6	7	6	180	5
19''+5B''(*ph/ph*)+4B'+Ai'	2	288	34.5*	16	4	120	13
19''+5B''(*ph/ph*)+4B'+Ai$_s$'	-	---	----	--	1	56	10

* Significantly different from 18.6 % at 0.05 level

As the short arm of chromosome Ai is known to carry a gene for resistance to WSM (Wang and Liang 1977b), we also analyzed the pairing frequency between chromosome 4B and Ai$_s$, finding that chromosomes 4B and Ai$_s$ were paired in 10 out of 56 (17.8 %) metaphase I cells (Table 2 and Fig.2). Similarly, Cauderon, Saigne, and Dauge (1973) recorded a 15 % homoeologous pairing involving an *Agropyron intermedium* telocentric chromosome, which is homoeologous to group 7 and carries stem rust resistance. In that desynapsis often occurred between chromosome 4B and the 2-armed Ai (Figs.3, 4), the pairing frequency between 4B and Ai presented in Table 2 could be lower than their actual frequencies.

Currently we are examining the progeny of those plants in which chromosomes 4B and Ai were paired for WSM reaction and for chromosome behavior to search for induced genetic recombination involving the WSM-resistant gene. The cytological evidence we observed indicates that Ai or Ai$_s$ was homoeologous to chromosome 4B of *T. aestivum* and that their pairing frequency was high enough in the presence of *ph/ph* gene to conclude that intergeneric gene transfer between *T. aestivum* and *A. intermedium* is feasible.

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