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Evolution

Chromosome constitution of polyploid wheats: Introduction of diploid wheat chromosome 4

(cytogenetics/progenitor/homoeologue)

M. WAZUDDIN AND C. J. DRISCOLL†

Department of Agronomy, Waite Agricultural Research Institute, University of Adelaide, South Australia, Australia

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ABSTRACT **Chromosome 4 of diploid wheat (chromosome *d4*) is not present in hexaploid wheat. This chromosome has been added to hexaploid wheat and observed not to pair meiotically with its 21 chromosomes. Also, chromosome *d4* compensates for Cornerstone male sterility, which involves a recessive mutation in chromosome arm 4AS. Chromosome *d4* has been separately substituted for chromosomes 4A and 4B. These two substituted hexaploid chromotypes have the entire genome of diploid wheat and may have agricultural significance. An alternative hypothesis of the evolution of polyploid wheats is proposed that involves the loss of chromosome *d4* and the retention of two versions of chromosome 4B at the early tetraploid stage.**

The generally accepted pattern of evolution of the polyploid wheats is that diploid wheat donated the A genome to tetraploid wheat by hybridization with the B-genome progenitor(s) and that the tetraploid hybridized with another diploid species to produce the hexaploid. Sax (1) reported the meiotic pairing in the F_1 of the diploid *Triticum monococcum* Linnaeus × the tetraploid *Triticum turgidum* Linnaeus and concluded, "At the time of the first reduction division about seven pairs of chromosomes are formed, leaving approximately 7 single chromosomes." However, later observations indicated fewer pairing configurations. Means of 5.6, 5.1, and 4.9 bivalents or trivalents were reported by Riley *et al.* (2) in hybrids of *Triticum dicoccoides* Körnicke × *Triticum thaoudar* Reuter, *Triticum aegilopoides* (Link) Balansa, and *T. monococcum*, respectively. Vardi (3) reported a mean of 5.5 bivalents or trivalents in the hybrid of *Triticum durum* Desfontaines × *Triticum boeoticum* Boisser, and Johnson and Dhaliwal (4) reported means of 5.9 and 6.1 bivalents or trivalents in the hybrid of emmer × *T. boeoticum* and *Triticum urartu* Tumanjan, respectively.

Conclusive identification of a single species donor of the B genome has not been possible despite the analysis and promotion of a number of individual species, such as *Aegilops bicornis* Jaubert et Spach by Sears (5), *Aegilops speltoides* Tausch by Riley *et al.* (2), *T. urartu* by Johnson and Dhaliwal (4), and *Aegilops sharonsis* Eig by Kushnir and Halloran (6). Alternatively, a more complex origin of the B genome involving more than one species has been proposed. Sarkar and Stebbins (7) suggested that a number of diploids similar to *A. speltoides* contributed the B genome.

Hexaploid wheat undoubtedly evolved from the hybridization of tetraploid wheat and *Aegilops squarrosa* Linnaeus, a diploid species with seven pairs of chromosomes designated the D genome, as demonstrated by Kihara (8) and by McFadden and Sears (9).

The seven chromosomes of hexaploid wheat that belong to the D genome were identified by Sears (10) by meiotic pairing patterns of chromosome-deficient hybrids of hexaploid wheat monosomics and tetraploid wheat. Those hexaploid wheat chromosomes, which were originally numbered XV to XXI, inclusively, were redesignated 1D to 7D, nonrespectively, by Sears (11).

Okamoto (12, 13) addressed the problem as to which of the remaining 14 chromosomes, which were originally numbered I to XIV, belong to the A genome and which belong to the B genome by determining the extent of meiotic pairing of telocentrics in hybrids of hexaploid wheat ditelocentric stocks and a synthetic tetraploid (*T. aegilopoides* × *A. squarrosa*). Thirteen of the 14 ditelocentric stocks, excluding ditelocentric IV, were available for this experiment. On the basis of pairing frequencies, seven telocentrics were recognized as members of the B genome and six telocentrics were recognized as members of the A genome. By default, chromosome IV was regarded as a member of the A genome. Chromosome IV had been shown by Sears (10) to be a homoeologue of chromosome VIII, which had been redesignated 4B; hence, chromosome IV was redesignated 4A by Sears (11).

The genome identity of chromosome IV was tested by Chapman and Riley (14), who concluded that it was a member of the A genome in that telocentric 4AS was reported to have paired in 48% of meiocytes of hybrids involving *T. thaoudar*. However, this conclusion was reversed by Miller *et al.* (15). Chapman *et al.* (16) observed no pairing of telocentrics in the hybrid of double ditelocentric 4AS × *T. urartu*, and Dvorak (17) observed no telocentric pairing in the hybrid of ditelocentric 4AS × *T. urartu*. Therefore, on chromosome pairing data chromosome 4A behaves as a B-genome chromosome.

Chromosome IV also morphologically resembles the B-genome chromosomes as shown by Gerlach (18) with N-banding and by Dennis *et al.* (19) with polypyrimidine-polypurine labeling. Chen and Gill (20) noted the similarity of N-banded chromosome 4A and an N-banded chromosome of *A. speltoides*, and they suggested that chromosome 4A should be redesignated 4B. Rayburn and Gill (21) reached the same conclusion after *in situ* hybridization studies.

All of the available evidence indicates that chromosome 4A is a B-genome chromosome. On that basis, either another chromosome that is currently regarded as a B-genome chromosome is an A-genome chromosome, or only six of the chromosomes of diploid wheat were retained by tetraploid wheat. Dvorak (22) argued that chromosome 4B is an A-genome chromosome and should be renumbered 4A; however, the evidence for this is not compelling, as chromosome 4B does not substantially resemble any of the diploid wheat chromosomes. In the crosses of the 14 critical double ditelocentrics of hexaploid wheat by *T. urartu*, as reported by Chapman *et al.* (16), 8 hybrids had no cells with telocentrics paired and the other 6 hybrids had telocentrics paired in

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†To whom reprint requests should be addressed.

37–83% of cells. These data indicate that only six chromosomes of diploid wheat are present in hexaploid wheat.

Search for the diploid wheat chromosome that is not present in hexaploid wheat was begun. The Cornerstone male-sterility mutant facilitated the extraction of this chromosome in that it was used as a means of identifying the diploid wheat chromosome bearing a male-fertility homoeoallele of the male-sterility mutant. Subsequently, this diploid wheat chromosome was separately introduced into hexaploid wheat in place of chromosomes 4A and 4B.

MATERIALS AND METHODS

The five diploid wheat species used in these experiments carry the following accession numbers: *T. urartu* (K1181), *T. monococcum* (K1182), *T. thaoudar* (K1183), *T. aegilopoides* (K1184), and *T. boeoticum* (K1185). The hexaploid wheat, *Triticum aestivum* Linnaeus, lines used were the Cornerstone male-sterility mutant line involving *ms1c* on chromosome arm 4AS (refs. 23 and 24) in a Federation/2*Pitic 62//Timgalen/3/2*Chinese Spring background and Chinese Spring monosomics 4A and 4B (ref. 10). Tetraploid Cornerstone (25) was also used as a bridging species between hexaploid Cornerstone and *T. monococcum* as difficulties were initially experienced in obtaining this hybrid.

Cornerstone was separately pollinated with the five diploid wheat species followed by three applications of 40 ppm gibberellic acid at 24-hr intervals. Sixteen-day hybrid embryos were excised and cultured on standard nutrient medium. The seedlings were subsequently transferred to soil in 6-inch (15-cm) pots, and plants were raised to the 3- to 4-tiller stage in a controlled-environment cabinet with 20/15°C day/night temperatures and a 14-hr day. At the 2- to 4-tiller stage the seedlings were treated with 0.10–0.25% colchicine (aqueous) plus 3% dimethyl sulfoxide by the aerated-root method for 4–4.5 hr. Some of the resultant amphiploids were backcrossed two or three times as the male parent to Cornerstone to produce addition lines. The male-sterile backcross individuals self-eliminated.

Monosomics 4A and 4B were pollinated by *d4* disomic addition lines in order to produce substitution lines in the F_2. N-banding (26) was used to determine the presence or absence of chromosomes 4A and 4B.

The self-fertility of various greenhouse-grown lines was expressed as the percentage of seed set in primary and secondary florets, excluding the two basal and the four apical spikelets. The control was euploid Chinese Spring.

Chromosome Designations. The following designations are used in this manuscript. Chromosomes I to XXI were the original numbers applied by Sears (10) to the wheat chromosomes. They were renumbered 1A to 7D, nonrespectively, by Sears (11). Chromosomes 4A and 4B are also referred to as $4B_1$ and $4B_2$, without respectivity. Chromosome 4 of diploid wheat is referred to as *d4* when no reference is made to an individual species, whereas chromosome 4 of *T. urartu*, *T. monococcum*, and *T. thaoudar* is referred to as *u4*, *m4*, and *th4*, respectively.

RESULTS

Hybridizations. The Cornerstone × diploid species hybridizations resulted in a range of seed set of 4–22% and an F_1 establishment range of 3–11% (Table 1). The hybrids usually showed fewer than seven pairing configurations per cell: 93–100% of cells had six or fewer configurations (Table 2). The modal number of configurations was six in hybrids involving *T. urartu* and *aegilopoides*, and five, four, and three in hybrids involving *T. boeoticum*, *T. thaoudar*, and *T. monococcum*, respectively. The low percentage of cells with seven or more configurations, which ranged from 0% to 7%, presumably include pairing events between B- and D-genome homoeologues. Trivalents were infrequent in that they occurred in a range of 2–8% of cells in the five hybrids, and no observed cell had more than one trivalent. The trivalents could have involved pairing between all three A-, B-, and D-genome homoeologues or between two A-genome homoeologues and a B- or D-genome homoeologue. The latter alternative probably accounted for the majority of trivalents, and on that basis and on the basis of their infrequency the trivalents have been pooled with the bivalents in Table 2. However, because of the fact that some bivalents involved homoeologues (one cell involving *T. urartu* had eight bivalents) and some trivalents may have involved homoeologues only, the pairing data shown in Table 2 represent an overestimate of the meiotic affinities of the A-genome of hexaploid wheat and the genome of each of the five diploid wheat species. The degree of representativeness of the five diploid taxa used in this experiment is not known; however, these data do not support an equivalence of the A genomes of diploid and hexaploid wheats. At most an equivalence of six chromosomes is supported.

Table 1. Seed set and hybrid establishment of crosses of Cornerstone hexaploid wheat × diploid wheat species

Male parent	No. florets pollinated	% seed set	% F_1 established of florets pollinated
T. urartu	868	16	11
T. monococcum	922	22	7
T. thaoudar	650	4	3
T. aegilopoides	858	14	9
T. boeoticum	663	8	3

Addition Lines. All five amphiploids have been observed at flowering and all are fertile. Hence, the genome of all of these diploid species can compensate for Cornerstone male sterility. The BC_1 heptamphiploid was also fertile in all cases; hence, a single dose of the genome of these diploid wheat species can compensate for Cornerstone male sterility. The amphiploids of two of these species, *T. urartu* and *T. thaoudar*, have been sufficiently backcrossed to Cornerstone to produce single-chromosome addition lines. Also, the amphiploid involving *T. monococcum* and the tetraploid bridging species has been sufficiently backcrossed to hexaploid Cornerstone to produce single-chromosome addition lines. A fertile monosomic addition line has been produced with all three species, and in all three cases the extra chromosome showed no N-banding and did not pair meiotically with the 21 bivalents (Fig. 1). Progeny of selfed monosomic addition lines have been observed in all three cases and the 42-chromosome plants were male sterile and the 43- and 44-chromosome plants were fertile; hence, the extra chromosome carries the male-fertility compensation factor(s). The extra chromosome undoubtedly came from diploid wheat and represents extraction of the diploid wheat chromosome that is not present in hexaploid wheat. On the bases that (*i*) hybrids of hexaploid wheat × diploid wheat

Table 2. Percentage of cells with various numbers of bivalents or trivalents in hybrids of Cornerstone hexaploid wheat × diploid wheat species

Male parent	No. hybrids	Total no. cells	Bivalents or trivalents						
			≤2	3	4	5	6	7	≥8
T. urartu	6	147	1	2	10	17	63	6	1
T. monococcum	8	243	28	32	22	14	4	0	0
T. thaoudar	4	102	17	18	33	20	11	1	0
T. aegilopoides	4	100	0	7	10	37	41	5	0
T. boeoticum	4	100	4	3	13	44	35	1	0

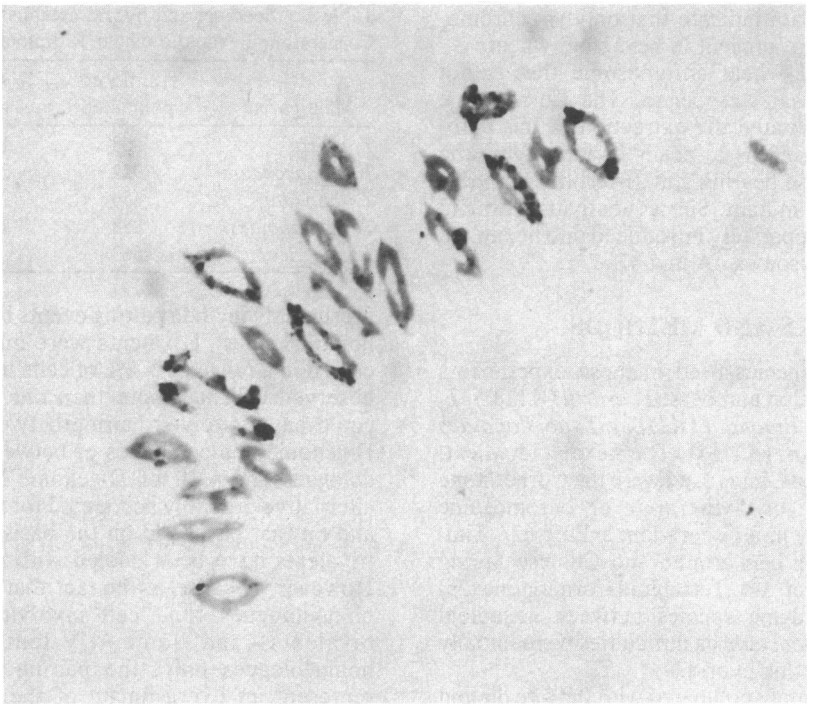

FIG. 1. N-banded metaphase I of the monosomic addition of chromosome *u4* to hexaploid wheat. The *u4* univalent is upper right and the 4A and 4B bivalents are the sixth from the left and right, respectively.

usually have six or fewer pairing configurations per cell, (*ii*) telocentrics of chromosome 4A do not pair with the chromosomes of diploid wheat, (*iii*) the extracted diploid wheat chromosome does not pair with the chromosomes of hexaploid wheat, and (*iv*) the extracted diploid wheat chromosome carries a homoeoallele of the Cornerstone 4AS male-sterility mutant, the extracted diploid wheat chromosome is regarded as chromosome 4 of diploid wheat and is designated *d4*.

The monosomic and disomic addition lines involving *u4*, *m4*, and *th4* are all highly fertile (Table 3). A number of telocentric and isochromosome addition lines have been isolated and observed to be either male sterile or highly fertile; hence, the fertility-bearing factor(s) is in one arm only of *d4*. Chromosome *d4* is approximately equal armed, and the fertility-bearing arm has been designated the α arm and the other the β arm. Monotelocentric *u4*α, *m4*α, and *m4*β, ditelocentric *u4*α and *m4*α, and monoisochromosome *m4*α, *u4*α, and *th4*β addition lines have been isolated.

Table 3. Seed set percentages of hexaploid wheat control and various chromosome *d4* addition and substitution lines

Chromotype†	No. plants	Total no. spikes	% seed set
Hexaploid wheat control	6	30	92
Addition lines			
u4'	6	29	98
u4"	6	33	89
u4α*t'*	3	13	89
u4α*t"*	3	15	90
u4α*i'*	4	17	93
m4'	5	20	98
m4"	5	24	90
m4α*t'*	4	19	84
m4α*t"*	4	18	94
th4'	1	7	93
th4"	2	9	86
Substitution lines			
u4' (4A)	1	7	67
u4" (4A)	1	7	88
u4' (4B)	1	6	35
u4" (4B)	1	3	42

†Symbols: ', monosomic; ", disomic; *t*, telocentric; *i*, isochromosome; (4A), substituted for 4A.

Substitution Lines. Monosomic and disomic lines with substitutions of chromosome *u4* for chromosomes 4A and 4B have been isolated. Absence of chromosome 4A or 4B was confirmed by N-banding in all cases. All four substitution lines have good vigor and are fertile. The fertility of the disomic 4A-substitution line is similar to that of euploid Chinese Spring wheat (Table 3 and Fig. 2).

From the few plants that have been observed the 4B-substitution lines appear to be less fertile than the 4A-substitution lines. The *Ms1c/ms1c* genotypes of the 4B-substitution plants are unknown and these genotypes may affect the fertility levels of these substitutions.

DISCUSSION

The meiotic observations on the *d4* addition lines demonstrate that the genome of the diploid wheats examined in this study differ from the A genome of hexaploid wheat in one major way—namely, one chromosome of the diploid is not present in the hexaploid. One explanation of this nonequivalence is that after incorporation of the A genome in tetraploid wheat one chromosome only of the A genome underwent considerable heterochromatic change so that it now N-bands differently and fails to recognize its original form in meiotic pairing. This explanation relies on major change in one chromosome and significantly less change in all of the other chromosomes of the same genome. Another explanation is that the donor of the A genome of polyploid wheat has a chromotype different to the diploids used in this experiment and is equivalent to the A genome of hexaploid wheat. This explanation relies on the extinction or the nondiscovery of such a diploid chromotype.

Alternative Hypothesis. This explanation is based on the assumption that all seven chromosomes of the type of diploid

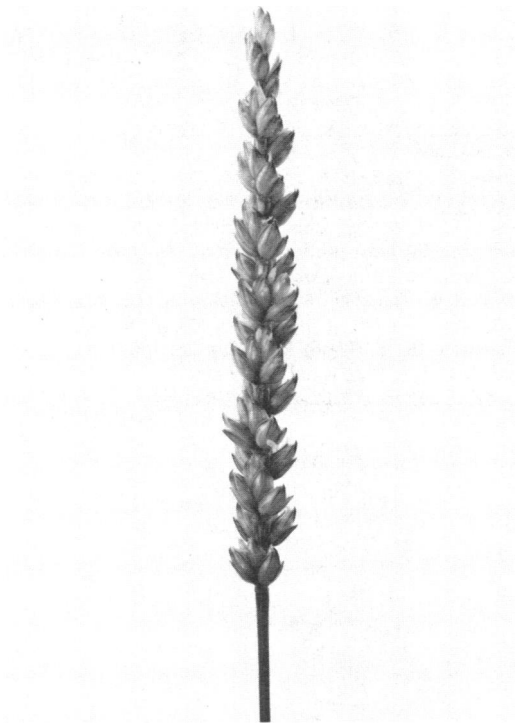

FIG. 2. Spike of the line with disomic substitution of chromosome *u4* for chromosome 4A.

wheat used in this study were initially present in the tetraploid, and one of these chromosomes (*d4*) was subsequently lost. The loss of *d4* could have been compensated for by the retention of two forms of its B-genome homoeologue, namely the chromosomes currently referred to as 4A and 4B.

The following theory is advanced, based on a modification of the Zohary and Feldman (27) concept of pivotal and differential genomes in the *Aegilops–Triticum* group. Their concept involves hybridization of tetraploid species that had one common genome and one genome that was only partly related. On hybridization between tetraploids of this type, the common genome would be fixed but the partially related genomes would segregate chromosomes and parts of chromosomes in subsequent generations. This would result in the evolution of tetraploids that had one genome of a diploid species and a second genome that does not equate to any diploid in terms of specific chromosome constitution. Zohary and Feldman consider that the B genome of tetraploid wheat arose in this manner. Sakar and Stebbins (7) suggested that wild tetraploid wheat evolved from the hybridization of raw amphiploids. The hybrid tetraploids would have had all seven chromosomes of diploid wheat as the pivotal genome and differential genomes that could have had two dissimilar homoeologues of chromosome *d4*. It is likely that the pairing-inhibitor mutation on chromosome 5B (refs. 28 and 29) had not occurred at such an early time, and multivalents formed in these primitive tetraploids. Quadrivalent formation between the two *d4* chromosomes and the two B-genome homoeologues could have resulted in exclusion of both the *d4* chromosomes and retention of both B-genome homoeologues. Mutation to the pairing-inhibitor allele on chromosome 5B could have rendered the two B-genome homoeologues incapable of meiotic pairing with each other and could have fixed the chromosome constitution that is found in extant *T. durum*.

A chromosome similar to chromosome 4A is present in *Triticum timopheevi* Zhukovsky. Feldman (30) observed telocentric pairing in over 27% of cells of the hybrid of *T. aestivum* ditelocentric 4A and a (*T. timopheevi–A. squarrosa*) amphiploid. Presence of a chromosome in *T. timopheevi* similar to chromosome 4A is also shown by the *in situ* studies of Rayburn and Gill (21). Hence, chromosome *d4* is probably not present in *T. timopheevi*, which is consistent with Wagenaar's conclusion that *T. timopheevi* probably originated from wild *T. dicoccoides* in their region of overlap in northern Iraq (31) and with Sachs' observation of 13.9 bivalents in the hybrid of *T. dicoccoides* Körnicke var. *nudiglumis* Nabalek × *T. timopheevi* Zhukovsky var. *typica* Zhukovsky (32). Meiotic analysis of the hybrid of *T. timopheevi* × *d4* ditelocentric addition lines would provide further evidence on this point.

On the above hypothesis chromosomes 4A and 4B may both be B-genome chromosomes and may be better referred to as $4B_1$ and $4B_2$, without respectivity. The primitive tetraploid with both $4B_1$ and $4B_2$ must have had an advantage in natural selection to have survived and dominated. The only evidence of this kind is the report of Joppa and Maan (33) of the substitution of chromosome *d4* of *T. boeoticum* for chromosome 4B of *T. durum*. This substitution was an extreme dwarf and male sterile.

The above hypothesis of the evolution of the polyploid wheats would mean that chromosome *d4* was never present in hexaploid wheat, as it was lost at the tetraploid level. It would also mean that the A-, B-, and D-genome progenitors donated six, eight, and seven chromosomes, respectively, to hexaploid wheat.

New Hexaploid Chromotypes. The separate substitutions of chromosome *d4* for each of chromosomes $4B_1$ and $4B_2$ represent new chromotypes of hexaploid wheat. On the above hypothesis they represent types that would have evolved if chromosome *d4* had not been lost during the evolution of the tetraploid wheats. They have seven chromosomes from each of the A-, B-, and D-genome progenitors. The potential importance of these hexaploid wheats in agriculture cannot be judged by the fact that the naturally evolved tetraploid wheat had a better survival ability than the tetraploid with the entire genome of diploid wheat. There are two reasons for this: (*i*) better survival would have meant a greater ability to produce viable offspring rather than yield, and the latter is the contemporary measure of success; and (*ii*) the competition occurred at the tetraploid level, not at the hexaploid level. Also, balanced combinations of alleles have been accumulated in the polyploid wheats of agriculture over a period of about 10,000 years; hence, considerable improvement of allelic combinations in the new hexaploid chromotypes would have to precede their meaningful evaluation for agricultural purposes. Breeding of the new chromotypes of hexaploid wheat would necessitate hybridization within one chromotype only to ensure automatic retention of that chromotype. Alternatively, selection could be imposed for a specific chromotype with the aid of N-banding.

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1. Sax, K. (1922) *Genetics* **7**, 513–552.
2. Riley, R., Unrau, J. & Chapman, V. (1958) *J. Hered.* **49**, 91–98.
3. Vardi, A. (1973) *Proc. Int. Wheat Genet. Symp.*, 4th, University of Missouri, Columbia, MO, Aug. 6–11, 1973, eds. Sears, E. R. & Sears, L. M. S. (Missouri Agric. Exp. Stn., Univ. Missouri, Columbia, MO), pp. 131–141.
4. Johnson, B. L. & Dhaliwal, H. S. (1978) *Am. J. Bot.* **65**, 907–918.
5. Sears, E. R. (1956) *Wheat Inf. Ser.* **4**, 8–10.
6. Kushnir, U. & Halloran, G. M. (1981) *Genetics* **99**, 495–512.
7. Sarkar, P. & Stebbins, G. L. (1956) *Am. J. Bot.* **43**, 297–304.
8. Kihara, H. (1944) *Agric. Hortic. (Tokyo)* **19**, 889–890.
9. McFadden, E. S. & Sears, E. R. (1944) *Rec. Genet. Soc. Am.* **13**, 26–27.

10. Sears, E. R. (1954) *Res. Bull. MO. Agric. Exp. Stn.* **572,** 1–59.
11. Sears, E. R. (1958) *Proc. Int. Wheat Genet. Symp.*, 1st, Aug. 11–15, 1958, ed. Jenkins, B. C. (Univ. Manitoba, Winnipeg, MB, Canada), pp. 221–229.
12. Okamoto, M. (1957) *Wheat Inf. Ser.* **5,** 7.
13. Okamoto, M. (1962) *Can. J. Genet. Cytol.* **4,** 31–37.
14. Chapman, V. & Riley, R. (1966) *Can. J. Genet. Cytol.* **8,** 257–263.
15. Miller, T. E., Shepherd, K. W. & Riley, R. (1981) *Cereal Res. Commun.* **9,** 327–329.
16. Chapman, V., Miller, T. E. & Riley, R. (1976) *Genet. Res.* **27,** 69–76.
17. Dvorak, J. (1976) *Can. J. Genet. Cytol.* **18,** 371–377.
18. Gerlach, W. L. (1977) *Chromosoma* **62,** 49–56.
19. Dennis, E. S., Gerlach, W. L. & Peacock, W. J. (1980) *Heredity* **44,** 349–366.
20. Chen, P. D. & Gill, B. S. (1983) *Proc. Int. Wheat Genet. Symp.*, 6th, Nov. 28–Dec. 3, 1983, ed. Sakamoto, S. (Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto Univ., Kyoto, Japan), pp. 39–48.
21. Rayburn, A. L. & Gill, B. S. (1984) *Can. J. Genet. Cytol.* **27,** 246–250.
22. Dvorak, J. (1982) *Can. J. Genet. Cytol.* **18,** 371–377.
23. Driscoll, C. J. & Barlow, K. K. (1976) *Induced Mutations in Cross-Breeding* (IAEA, Vienna, Austria), pp. 123–131.
24. Driscoll, C. J. (1977) *Crop. Sci.* **17,** 190.
25. Hossain, M. A. & Driscoll, C. J. (1981) *Can. J. Genet. Cytol.* **23,** 493–496.
26. Endo, T. R. & Gill, B. S. (1984) *Chromosoma* **89,** 361–369.
27. Zohary, D. & Feldman, M. (1962) *Evolution* **16,** 44–61.
28. Sears, E. R. & Okamoto, M. (1958) *Proc. Int. Congr. Genet.*, 10th, McGill University, Montreal, Aug. 20–27, 1958 (Univ. Toronto, Toronto), Vol. 2, 258–259.
29. Riley, R. & Chapman, V. (1958) *Nature (London)* **182,** 713–715.
30. Feldman, M. (1966) *Can. J. Gen. Cytol.* **8,** 144–151.
31. Wagenaar, E. B. (1966) *Evolution* **20,** 150–164.
32. Sachs, L. (1953) *Heredity* **7,** 49–58.
33. Joppa, L. R. & Maan, S. S. (1983) *Can. J. Gen. Cytol.* **24,** 549–557.