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AGROPYRON-WHEAT TRANSFERS INDUCED BY HOMOEOLOGOUS PAIRING[1]

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SUMMARY

Of 21 presumed transfers of leaf-rust resistance from the Agropyron chromosome (3Ag) of TAP 67 to chromosome 3D, one has proved not to be a transfer, three or probably four involve a chromosome or chomosomes other than 3D, and one apparently involves 3A or 3B as well as 3D and 3Ag. Cytogenetic tests and studies of pairing of the 3D/Ag transfer chromosomes with 3Ag and with telocentric 3Dα indicate that the 3Ag segments carrying the resistance gene are of substantial length in every case. The differences in male transmission (64.7% to 22.1%) appeared not to be closely related to the length of the 3Ag segment transferred. Homozygotes have been obtained for all 15 of the 3D/Ag transfers.

All 12 transfers of leaf-rust resistance from 7Ag of Agrus apparently involve 7D. Although a substantial segment of 7Ag has evidently been transferred in each case, three transfers showed significantly more than 50% male transmission. Two showed significantly less than 50%. The arm partially replaced by the 7Ag segment appears to be 7DL. Apparent homozygotes have been obtained for 11 of the 12 transfers.

The first successful use of induced homoeologous pairing for the transfer of a character to wheat was that of RILEY *et al.* (1968), who employed the genome of *T. speltoides* to cause a chromosome of *T. comosum* to pair with wheat chromosome 2D. They eventually derived a *comosum* chromosome with a 2D segment long enough to provide regular pairing with 2D. This chromosome conditioned resistance to the yellow-rust fungus.

By use of deficiency for chromosome 5B, I have been able to transfer resistance to *Puccinia recondita* from two different *Agropyron elongatum* chromosomes to wheat chromosomes (SEARS, 1972a, b). The two *Agropyron* chromosomes are those substituted for 3D in TAP 67 and 7D in Agrus. These substitution lines were first made monosomic-5B and were then pollinated by nullisomic-5B tetrasomic-5D, with the result that most offspring were nulli-5B, tri-5D, mono-3D (or -7D), and mono-3Ag (or -7Ag). In these plants homoeologous pairing of 3D or 7D with 3Ag or 7Ag occurred in about 30% of the cells. As a consequence of this pairing, numerous 3D/Ag and 7D/Ag transfer chromosomes carrying leaf-rust resistance were recovered. Substantial differences were found in the male transmission of these chromosomes (SEARS, 1972a).

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Additional data are now available on male transmission, and information has been obtained on the length and location of the transferred *Agropyron* segment, particularly in the 3D/Ag chromosomes.

TRANSFERS FROM 3AG TO 3D

The previous report (SEARS, 1972a) listed 21 presumed transfers obtained from a total of 299 offspring of nulli-5B, mono-3Ag, mono-3D. These were numbered from 1 to 21 in approximate accordance with their rank in pollen transmission during the first two generations.

Data from the third generation have established that supposed transfer #17 had the entire 3Ag rather than a transfer chromosome. Presumably the trivalent observed in the second generation was not a trisome but a translocation deficient for one chromosome. It thus involved two chromosomes instead of one, and the one univalent present in resistant plants was a 3Ag addition rather than a wheat monosome. This monosome reappeared in the third generation and was wrongly scored as a wheat monosome.

Data on male transmission in the third and fourth generations did not greatly change the rankings of the transfers concerned (Table 1). Three have shown significantly less than 50% male transmission. One has had substantially more than 50% transmission, but the excess is not significant at the 1% point.

TABLE 1. Male transmission and meiotic pairing of 3D/Ag transfer chromosomes.

Chrom. no.	No. male gametes	% transmission	Pairing with telo-3Dα		Pairing with 3Ag	
			No. cells	% pairing	No. cells	% pairing
1	51	64.7*	100	11.0	100	91.0
2	68	55.9	62	17.9	64	84.4
3	83	48.2	38	68.4	43	83.8
4	90	51.1	89	0.0	125	83.2
5	76	44.7	41	0.0	43	83.8
6	75	42.7			100	77.0
7	82	45.1	100	2.0	53	90.5
8	83	44.6	100	0.0	200	77.0
11	63	43.5	100	0.0	100	80.0
14	78	33.3**	200	69.5	76	72.3
15	65	38.5	100	1.0	100	75.0
16	63	42.9			43	69.8
18	84	30.9**	100	0.0	200	76.0
19	75	41.0	100	28.0	100	86.0
21	97	22.1**	29	0.0	98	46.9

*Different from 50% at the 5% point of significance.

**Different from 50% at the 1% point.

When Transfers 10, 13, and 20 were crossed with the 3Ag(3D) substitution line, the chromosome pairing showed in each case that the chromosome involved in the transfer was not 3D. Instead of the expected 21 bivalents, including one involving 3D/Ag and 3Ag, 19"+1'" +1' was regularly found. The trivalent must have involved the

transfer chromosome and its two partial homologues, 3Ag and presumably 3B or 3A, and the univalent must have been 3D (Fig. 1). This interpretation is supported by the fact that hybrids of Transfers 10 and 20 with ditelo-3Dα showed 96 and 97% pairing, respectively—about the amount expected if the 3Dα arm is intact in those lines. Male transmission of Transfers 10, 13, and 20 has been 39.2, 38.9, and 23.2%, respectively.

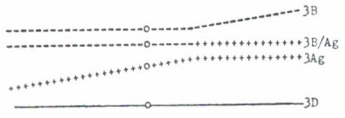


FIGURE 1. Assumed nature of Transfers 10, 13 and #20 from TAP 67 and their manner of pairing. The transfers may involve chromosome 3A instead of 3B.

Chromosome 3D was probably not involved in Transfer 9 either. From a cross of a heterozygote to mono-3D, a resistant, monosomic offspring was allowed to self, and a disomic was obtained. When this plant was crossed to the 3Ag(3D) substitution line, there was no pairing of the 3Ag chromosome, indicating that no transfer chromosome was present. Either this chromosome is not 3D, or univalent shift occurred in the cross of mono-3D x Transfer 9. This transfer had shown 44.1% male transmission.

Transfer #12 is now believed to be the result of exchange between 3D and not only 3Ag but also 3B or 3A. In the second generation a plant was obtained that had a trivalent and a univalent (in addition to a translocation chain-of-four). In the next two generations, crosses (as male) to normal gave rise to 11 resistant offspring that were analyzed cytologically, and every one had a trivalent and a univalent. Rarely the four chromosomes concerned formed a quadrivalent. That the univalent was 3D was demonstrated by a cross to mono-3D, from which the three resistant offspring with 41 chromosomes had 19"+1'". The seven with 42 chromosomes had 19"+1'"+1' (including one with 19"+1'"+t'). The chromosome bearing the *Agropyron* segment is evidently one that can pair occasionally with 3D but pairs more regularly with some bivalent, presumably 3A or 3B. Such a chromosome (Fig. 2) would have resulted if, in the nulli-5B, mono-3Ag, mono-3D plant, one arm of 3D paired and crossed over with 3Ag while the other arm underwent exchange with one of the two 3A (or 3B) chromosomes. Male transmission of Transfer 12 was 48.7%.

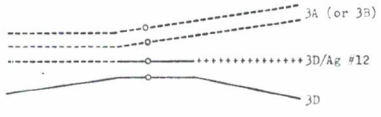
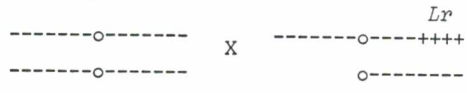


FIGURE 2. The suggested nature of Transfer #12 from TAP 67 and its pairing homologies.

In tests of 13 of the remaining 15 transfer chromosomes (Table 1), none paired as regularly with telocentric 3Dα as an intact 3D does (97%). At least part of 3Dα must be missing, then, in each of these 13 transfers. The fact that six showed no pairing at all with telo-3Dα seems to suggest that in these transfers the entire 3Dα arm is replaced by 3Agα. It is possible, however, that these chromosomes have a short, proximal segment of the 3Dα arm remaining. Such chromosomes might rarely form chiasmata with telo-3Dα, for wheat telocentrics show sharply decreased chiasma formation near the centromere (SEARS, 1973).

Genetic confirmation was obtained for the existence of differences in the distance of the exchange point from the centromere. From the preceding experiment, plants with chromosomes showing widely different amounts of pairing with telo-3Dα were crossed as males to euploid Chinese Spring and the offspring scored for resistance (Table 2).

TABLE 2. Results of experiment to determine distance of exchange point from centromere of 3D/Ag chromosomes



Chromosome no.	Transmission vs. 3D	Pairing with telo-3D	Gametes tested	Status of 3D in *Lr* segregants & no. plts. Complete	Telo (C.O.)	Both	Not determined	*Lr* transmission	Status of 3D in *lr* segregants & no. plts. Compl. (C.O.)	Telo	None	Not determined	C.O.
	%	%	No.					%					%
14	33	69.5	61	9	3	0	19	51	15	3	1	11	53.0
3	48	68.4	16	4	0	1	0	31	10	1	0	0	66.7
2	56	17.9	102	-	-	-	65	64	12	6	1	18	23.3
7	45	2.0	91	-	-	-	72	79	2	6	2	9	4.3
5	45	0.0	49	-	-	-	31	63	-	-	-	18	-

Where the transfer chromosome paired and crossed over rarely with 3Dα, more than half the offspring were expected to be resistant, because telo-3Dα's lack of 3Dβ would have caused selection in favor of the 3D/Ag chromosome, which carried the resistance gene. This expectation was met in the case of transfers 2, 5, and 7. With transfers 3 and 14, which had a high pairing rate, complete chromosomes were also expected to be favored, but up to half of these might have lost the *Agropyron* segment through crossing over and therefore failed to confer resistance. Thus, nearly equal numbers of resistant and susceptible offspring were expected, and they were found.

Transfer #5, with no pairing of 3D/Ag with 3Dα, gave a somewhat anomalous result, in that it was expected to have the highest frequency of resistant offspring but in fact had a substantially lower frequency than #7. However, the difference is of doubtful significance statistically; furthermore, cytological study of additional meiotic material might have revealed some pairing of #5. Alternatively, there may have been unusually high transmission of the non-crossover telocentric in this cross.

Cytological analysis of samples of the plants in the test confirmed the conclusions based on classification for resistance. In both the resistant and susceptible classes, most of the transmitted chromosomes were complete, but there were relatively more telocentrics among the susceptible offspring of #2 and #7. Presumably few or no telocentrics were present in the resistant offspring of these two or of #5.

Enough data were available to permit the calculation of the amount of crossing over between the centromere and the exchange points of 2, 3, 7, and 14. These were in the order expected, considering that the true value cannot exceed 50%. The crossover values are in every case higher than would be predicted from the chiasma frequencies, except that FU and SEARS (1973) have shown that substantial numbers of chiasmata present at diakinesis in bivalents of this type disappear by metaphase. Therefore, the present metaphase determinations presumably underestimated the actual chiasma frequencies.

Pairing of the 3D/Ag chromosomes with 3Ag itself was rather high in every case (Table 1). There was no support for the idea that a chromosome's rate of male transmission should be inversely related to the length (as judged by pairing ability) of its *Agropyron* segment, for the transfer chromosomes with essentially normal transmission had pairing as good as or better than those with the lowest transmission.

All of the 15 3D/Ag transfers have been obtained homozygous. Spikes of several are shown in Figure 3 in comparison with the control Chinese Spring. There are evidently differences among them, but to what extent these are due to 3D/Ag rather than to impurity of background is uncertain. That the differences from normal are in the direction expected for deficiency of chromosome 3D suggests that the transferred *Agropyron* segments do not fully compensate for the 3D segments they replace.

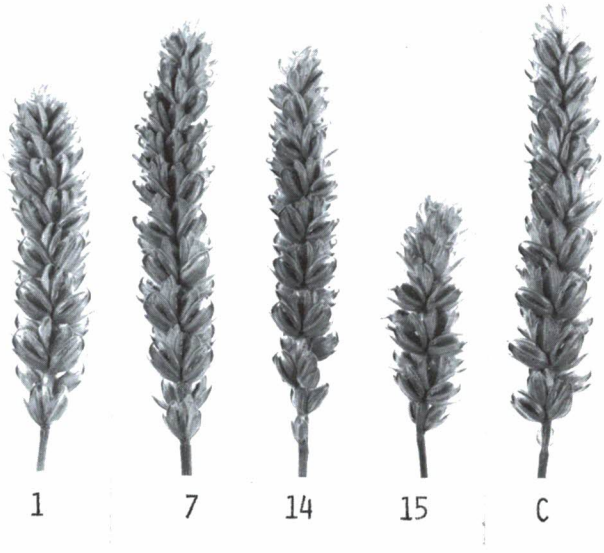


FIGURE 3. Spikes of representative transfers from the *Agropyron* chromosome of TAP 67 to chromosome 3D of Chinese Spring, and control (C).

TRANSFERS FROM 7AG TO 7D

As reported previously (SEARS, 1972a), 12 transfers were obtained from 138 offspring of nulli-5B, tri-5D, mono-7Ag, mono-7D. Male-transmission data for two generations showed two transfers (1 and 2) to be significantly higher than 50% and two (11 and 12) significantly lower than 50%.

The inclusion of data for two more generations (Table 3) adds two transfers (3 and 4) with more than 50% male transmission and one (10) with less than 50%, but casts doubt on the significance of the excess of #2 over 50%.

When five of the 7D/Ag chromosomes were combined with telo-7DS, the only 7D telocentric available, pairing was perfect, or nearly so, in every case (Table 3). Probably the exchanges that gave rise to the transfer chromosomes took place in the long arm of 7D, leaving 7DS intact.

TABLE 3. Male transmission and meiotic pairing of 7D/Ag transfer chromosomes.

Chrom. no.	No. male gametes	% trans-mission	Pairing with 7DS		Pairing with 7Ag	
			No. cells	% pairing	No. cells	% pairing
1	37	78.4**	24	100.0	121	87.6
2	77	62.7*	5	100.0	100	91.0
3	89	65.2**			100	93.0
4	62	69.8**	100	100.0	100	97.0
5	106	59.4	8	100.0	100	95.0
6	69	58.0	50	96.0	100	94.0
7	54	53.7			56	91.0
8	87	40.2				
9	97	49.5			100	87.0
10	133	41.4*				
11	59	22.0**				
12	57	28.1**			100	93.0

*Different from 50% at the 5% point.

**Different from 50% at the 1% point.

Pairing with 7Ag was also excellent with nearly every transfer chromosome, suggesting that a substantial segment of 7Ag had been transferred.

Homozygotes have apparently been obtained for 11 of the transfers (all but #11). There appear to be some differences among these (Fig. 4), but nearly all are vigorous and reasonably fertile.

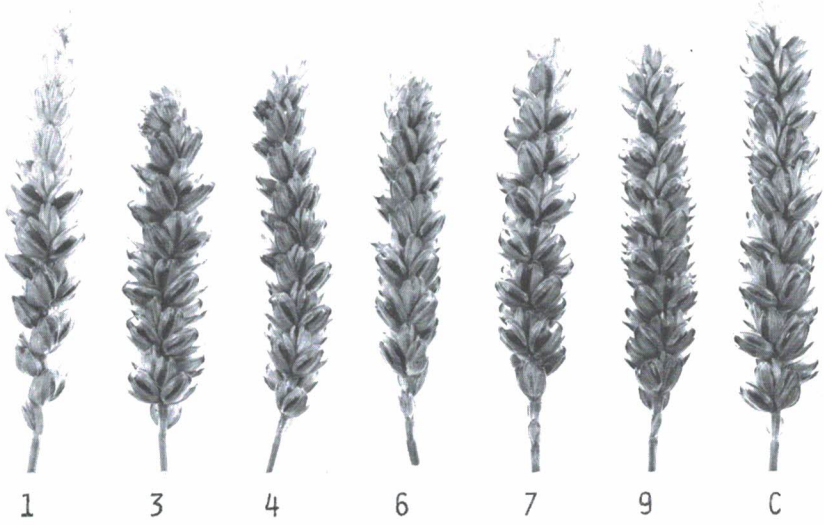


FIGURE 4. Spikes of representative transfers from the *Agropyron* chromosome of Agrus to chromosome 7D of Chinese Spring, and control.

The 7Ag chromosome carries a gene for stem-rust resistance (GOUGH and MERKLE, 1971), which could serve as a marker for a different region of the chromosome than that marked by the leaf-rust gene. However, the effect of the stem-rust gene is unfortunately not detectable in the Chinese Spring background of the present material.

DISCUSSION

For the two *Agropyron* chromosomes concerned, the induction of homoeologous pairing appears to be a much better way of effecting the transfer of the desired gene than the use of ionizing radiation. For 7Ag SHARMA and KNOTT (1966) analyzed 357 resistant offspring of irradiated plants and found only one transfer (to chromosome 7D) with normal male transmission. From the same chromosome induced to pair homoeologously, I recovered 12 transfers among only 138 plants, and 8 of the 12 appear to have male transmission that is normal or higher than normal. Sharma and Knott's transfer ('Agatha') produces an undesirably yellow flour (KNOTT, 1971). The present transfers have not yet been tested for flour color.

Because Agatha was produced by irradiation, there is no assurance that the transferred *Agropyron* segment is fully equivalent to the wheat segment replaced. KNOTT (1968) points out that somatic association of homoeologues would raise the frequency with which radiation-induced transfers involve homoeologues, but there seems little likelihood that radiation breaks would be at precisely equivalent points in the two homoeologues. It may therefore be a deficiency or duplication that results in Agatha's yellow flour, and the present transfers may then be free of this aberration.

We can assume that some alien chromosomes with very limited possibilities for crossing over with their wheat homoeologues can never give rise to fully satisfactory transfers through induced pairing. Radiation-induced transfers from such chromosomes might have a better chance for success, particularly if male transmission of the alien-addition monosome were low enough to permit efficient screening for the rare transfer of a small segment of the alien chromosome without loss of wheat chromatin. Also, the gene order in some homoeologues may differ in such a way that precise transfers through crossing over can never be successful, whereas imprecise transfers through radiation may have a reasonable chance of producing the right combination of genes.

Although the 3D/Ag transfers appear somewhat less promising than the 7D/Ag's, there is a chance that one or more will be better than 'Agent', a spontaneous 3D/Ag transfer from TAP 67 reported by SMITH *et al.* (1968). That R. A. McIntosh (personal communication) found no pairing of the Agent chromosome with telo-3Dα suggests that Agent may have more wheat chromatin replaced by *Agropyron* than several of the present transfers have. Because we can reasonably suppose that the transfer in Agent resulted from a rare spontaneous crossover, we can assume that this transfer is similar to or identical with one or more of the present group where the 3D/3Ag chromosome shows no pairing with telo-3D.

No firm conclusion can yet be drawn concerning the location of the 3Ag resistance gene. Transfers 7 and 15, with only 2% and 1% pairing, respectively, with telo-3Dα, pair reasonably well with the complete 3D. Therefore, with double crossovers presumably to be ruled out, they must have the entire 3Dβ arm and only a proximal part

of 3Dα. This would put the resistance gene in the proximal or middle portion of 3Agα. The various transfers would then have terminal 3Agα segments of different lengths replacing corresponding segments of 3Dα. The substituted segment would have to be relatively short in Transfers 4 and 14, which have good pairing with telo-3Dα (68.4% and 69.5%, respectively), but long enough to permit good pairing with 3Ag (83.2% and 72.3%, respectively). This supports a median location on 3Agα for the resistance gene. The six transfer chromosomes that failed to pair with telo-3D may well retain a short, proximal segment of 3Dα, as pointed out before.

Arguing against the idea that each 3Ag segment replaces a 3D segment of approximately equal length is the fact that some transfer chromosomes that paired well with 3Dα paired well with 3Ag also. There is some doubt, however, as to the significance of the relatively small differences observed in pairing with 3Ag. These differences could conceivably be due to environmental effects on chiasma formation or to examination being made at different sub-stages of MI.

Wherever the resistance gene is located in 3Ag, and assuming that crossing over between 3Ag and 3D can occur throughout their lengths, transfer chromosomes were to be expected that were all 3Ag except for a short terminal segment of one arm of 3D. Such chromosomes, which should pair very regularly with 3Ag, were not identified, but this does not necessarily mean that none occurred. They would presumably have paired very poorly with 3D and may therefore have been classified as intact 3Ag's and discarded.

Tests for pairing of the 3D/Ag chromosomes with telo-3Dβ, which is now available (through the generosity of R. A. McIntosh), should reduce the uncertainty concerning the make-up of some of them. Crosses between some of the transfer lines may also be revealing. Efforts to obtain telocentrics from 3Ag have thus far been unsuccessful.

Although crossing over can perhaps occur at various places along the length of 3D and 3Ag, double crossovers, which could have given rise to transfers with only a short, interstitial segment from 3Ag, may be so infrequent that none could have been expected in the present small sample. At any rate, no chromosome was obtained with the low pairing with 3Ag, high pairing with 3Dα, and good male transmission that would reasonably have been expected of this type of transfer. Advantage could perhaps be taken, however, of the possibility for crossing over to occur at more than one place between 3D and 3Ag. A transfer chromosome that has a substantial terminal segment of 3Ag (and all presumably do, since they all pair reasonably well with 3Ag), could be subjected to another round of induced homoeologous pairing, and a crossover might remove most of the 3Ag segment beyond the resistance gene.

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