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ANALYSIS OF WHEAT-AGROPYRON RECOMBINANT CHROMOSOMES*

E.R.SEARS

Agricultural Research Service, U.S.Department of Agriculture
and Agronomy Department, University of Missouri
Columbia, Mo 65201, U.S.A.

SUMMARY

Seventeen homoeologous-pairing-induced transfers of Puccinia triticina resistance (Lr24) from an Agropyron elongatum chromosome (3Ag) to wheat chromosome 3D were characterized as to the length and position of the transferred Agropyron segment. Pairing with telocentric chromosomes 3DL and 3DS and presence or absence of Agropyron genes for red seeds and glutamate oxaloacetate transaminase indicated that the segments are of at least five different lengths and that they replace terminal portions of 3DL of corresponding length. Two other transfers of Lr24 involve 3BL and are also terminal; and a third consists of a substantial segment of 3DS, a middle Agropyron portion, and a short, terminal segment of 3DL.

Eleven transfers of Lr19 from a different A.elongatum chromosome (7Ag) to wheat 7D were analyzed by their pairing with telo-7AgS and with a 12th transfer chromosome that does not include Lr19. The 11 transfer chromosomes all have the terminal part of 7AgL, and at least two have more than the entire arm. The 12th chromosome has the entire 7AgS and a proximal portion of 7AgL.

Transfer chromosomes with shorter Agropyron segments are being sought by subjecting those that already have the shortest segments to another round of homoeologous pairing, and by inducing pairing of telo-3AgL and -7AgL with 3D and 7D.

INTRODUCTION

An obvious way to enlarge the pool of genes available to wheat breeders for improvement of this important crop plant is to exploit the variability of its relatives. The genera *Aegilops* (now often placed in *Triticum*), *Agropyron*, *Secale*, and *Haynaldia* all have characters that would be useful if available in wheat. Many species of these genera cross rather easily with common wheat, and wider crosses, as with *Hordeum*, can be made using special techniques.

Once a wide cross has been made, the doubled or non-doubled F_1 can be backcrossed to wheat, and wheat-like lines can eventually be recovered that have one or more characters from the alien species. Unfortunately, the desired character is almost always accompanied by undesirable traits. This is because alien chromosomes do not ordinarily pair and cross over with wheat chromosomes, so at least one entire chromosome must be added to the wheat complement or substituted for a wheat chromosome in order to retain the desired gene.

X-rays have been successfully used to break the alien chromosome and allow a segment carrying the desired gene to be transferred to a wheat chromosome

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(1,2). Since the vast majority of the transfers thus obtained are reciprocal, with a terminal segment of the alien chromosome replacing a terminal piece of a wheat chromosome, almost all are deficient for critical wheat genes. About the only exceptions to be expected are where the segments concerned are very short, which is only possible if the desired alien gene is located near the end of its chromosome; or where the alien segment replaces a homoeologous one. Although the latter result seems to occur more often than expected on a random basis (3), it is still so rare that the recovery of irradiation-induced transfers is very laborious.

Fortunately, there is an alternative to induction of transfers by irradiation. The alien chromosomes do not fail to pair because of lack of homology with wheat chromosomes, but because their pairing is suppressed, mainly by a gene on chromosome 5B (4,5). When chromosome 5B is missing (or its effect suppressed), almost every alien chromosome is capable of pairing with three different wheat chromosomes. This of course provides an opportunity for crossing-over and consequent production of chromosomes that have only a segment of the alien chromosome—a segment replacing the homoeologous portion of the wheat chromosome concerned.

MATERIALS AND METHODS

Leaf-rust resistance was transferred to wheat from two different *Agropyron elongatum* chromosomes by means of induced homoeologous pairing (6,7,8). One of the *Agropyron* chromosomes used was the one substituted for wheat chromosome 3D in the line TAP67 produced in Oklahoma, and the other was a substitution for chromosome 7D of "Agrus", a line developed in Indiana. These *Agropyron* chromosomes, henceforth referred to as 3Ag and 7Ag, respectively, were each made heterozygous in plants nullisomic for chromosome 5B and trisomic for 5D. Each paired frequently with its D-genome homoeologue, although such pairing occurs rarely or not at all in the presence of 5B.

The homoeologous-pairing hybrids were crossed with euploid, and recombined chromosomes were recovered by selecting plants that were resistant to leaf rust (*Puccinia recondita* Rob. ex Desm.) but had no entire *Agropyron* chromosome. Twenty different transfers were identified from 3Ag and 12 from 7Ag.

For determining which wheat chromosome is involved in each transfer, standard methods of monosomic analysis were used. To characterize the transfer chromosomes, tests were made for frequency of pairing with the appropriate wheat telocentrics (except 7DL), the 3Ag and 7Ag chromosomes, and the *Agropyron* telocentrics (except 3AgS).

RESULTS

Transfers from 3Ag

The transfers were numbered from 1 to 21 on the basis of amount of male transmission from the heterozygote in early generations. (No. 17 proved to be notransfer). It was thought that greater transmissibility might be the result of a shorter *Agropyron* segment or a shorter replaced segment of wheat, but later work has largely failed to bear this out. The transmission data were presumably less reliable than if they had been obtained in later generations, when unrelated monosomes, trisomes, etc. had been eliminated.

It was expected that all the transfers would involve chromosome 3D, because 3D was monosomic in the nulli-5B plants and therefore free to pair with 3Ag. Chromosomes 3A and 3B, on the other hand, were disomic and therefore presumably

more likely to pair homologously than with 3Ag. Furthermore, the TAP67 substitution for 3D had occurred spontaneously, suggesting greatest homoeology of 3Ag with 3D. From the various crosses made and cytologically analyzed, it soon became clear that two transfers, Nos. 10 and 13, involve chromosome 3B instead of 3D, and No.12 involves both 3B and 3D.

For determining the composition of the 3D/3Ag transfer chromosomes, they were tested for pairing with 3Ag and with the telocentric chromosomes 3AgL, 3DL, and 3DS. Only the pairing with telo-3DL revealed differences among them. With 3Ag and telo-3AgL, all paired regularly, showing that each has a substantial portion of 3AgL. With telo-3DS, pairing was also excellent, indicating that much of 3DS is included. Together, the pairing with telo-3AgL and -3DS suggested that all the recombinants recovered had crossed over proximally to the leaf-rust gene, *Lr24*, so that at least the terminal portion of the long arm came from 3Ag and most or all of the other arm came from 3D.

Pairing with telo-3DL (Table 1) varied widely, from 0 to 70%, suggesting that some transfer chromosomes have a complete or nearly complete 3AgL arm, whereas others have a substantial, proximal portion of 3DL. Genetic data on crossing over with telo-3DL (Table 2) corroborate the pairing observations: Transfer chromosomes tested that pair frequently with 3DL showed little or no linkage of *Lr24* with the centromere, whereas those with little or no pairing with telo-3DL displayed very tight *Lr24*-centromere linkage.

TABLE 1. Male transmission of 3D/3Ag transfer chromosomes from heterozygotes, their pairing with telocentric 3DL, and their GOT constitution

Designation of transfer	Per cent transmission	Per cent pairing with telo-3DL	Kind of GOT gene
14	33	70	3D
3	48	64	3D
2	56	28	3D
19	41	28	3D
1	65	11	3D
4	51	1	3D
6	43	0	3D
9	44	-	3D
20	23	-	3D
7	45	1	3Ag
15	38	0.5	3Ag
5	45	0	3Ag
8	45	0	3Ag
11	43	0	3Ag
16	43	0	3Ag
18	31	0	3Ag
21	22	0	3Ag

TABLE 2. Degree of pairing of 3D/3Ag transfers with telo-3DL, and amount of crossing over between the *Agropyron* segment and the centromere

Designation of transfer	Per cent pairing with telo-3DL	Per cent crossing over	No.gametes tested
14	70	51.5	61*
3	64	66.7	16*
2	28	25.9	52.4*
4	1	2.0	51
6	0	1.4	70
7	1	4.1	78.8*
15	0.5	0.0	88

* Crossover data wholly (chromosomes 14, 3, 2) or partly (No. 7) from Sears (7). The amounts of crossing-over for Nos. 2 and 7 were recalculated to correct for telocentric crossover chromosomes not being detectable. Also, the effective number of gametes tested of Nos. 2 and 7 is given instead of the actual number, making allowance for the fact that only about half the potential recombinants in the 1973 table were actually examined cytologically.

Information concerning the enzyme glutamate oxaloacetate transaminase (GOT) has confirmed and extended the conclusions from pairing and crossing over (8). Production of this enzyme is controlled by homoeoalleles on chromosomes 3A, 3B, 3D and 3Ag. Three recognizably different isozymes are produced, one by 3A and 3Ag, one by 3B and 3D, and the third a hybrid between the other two. By the relative amounts that are produced of the three different isozymes, the dosages of the four loci can usually be deduced. For example, if the 3D allele is replaced by that of 3Ag, the isozyme that 3A and 3Ag jointly form increases fourfold in concentration and the 3B-3D isozyme decreases to one-fourth its normal amount.

It was thus easy to determine which *Got* allele a given transfer line has, and it turned out that nine of the 3D/3Ag transfers have the 3D allele and the other eight the 3Ag allele (Table 1, Fig. 1). Those that showed 11% or more pairing with telo-3DL all have the 3D allele, while the ones without any pairing with telo-3DL have the 3AG allele. Of the four transfers with only 1% or 2% telo-3DL pairing, two (Nos. 4 and 6) have the 3D allele and two (Nos. 7 and 15) have the 3Ag allele. From the crossover data for No. 7 (7), Hart *et al.* (8) concluded that the *Got* locus must be slightly more than 4.3 units from the centromere.

Two transfers, Nos. 10 and 13, involve chromosome 3B instead of 3D (Fig.1). Pairing with telo-3BS (90%+) and -3BL (0%) suggest that, as with the 3D/3Ag transfers, at least the terminal portion of the long arm is replaced by the corresponding part of 3Ag. The GOT data show that neither transfer has the 3Ag allele; therefore, both have less than the entire long arm of 3Ag. This is confirmed by the fact that a synapsis gene on 3BL is present in both transfers. The long arm of 3Ag evidently carries the same locus but has a less potent allele.

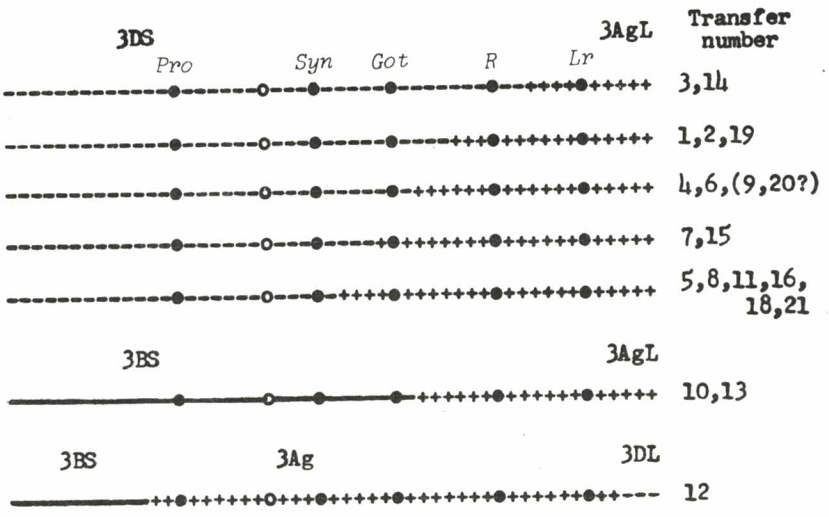

FIGURE 1. Constitution of *Triticum-Agropyron* transfer chromosomes, as estimated from gene content and pairing capacity. ---- = 3D chromatin, ++++ = 3Ag, ——— = 3B, o = centromere. The genes are: *Pro* = protein, *Syn* = synapsis, *Got* = glutamate oxaloacetate transaminase, *R* = red seed, *Lr* = leaf-rust resistance. *Syn* is placed to the left of *Got* because the latter is assumed to be near the exchange point in transfers #10 and #13 (the 3B portion of these chromosomes cannot extend much beyond *Got*, else they would be able to pair with telo-3BL; whereas no such restriction applies to *Syn*). Some allowance is made for the fact that wheat chromosomes most often pair near their ends, so that segments which pair regularly may be relatively short if they are terminal.

One transfer chromosome, No. 12, almost certainly is composed of a substantial terminal segment of 3BS (pairing 90% with telo-3BS), a central portion from 3Ag, and a short, terminal segment of 3DL (only about 3% pairing with telo-3DL). The 3Ag portion carries the *Lr24* and *Got* loci of the long arm, and a seed-protein locus of the short arm (9). This transfer chromosome must have arisen from simultaneous crossing-over of 3Ag with 3BS and 3DL. Unlike all the other crossovers in 3AgL, this one occurred distal to *Lr24*.

Transfers from 7Ag

Male transmission was also used as a basis for numbering the 7Ag transfer chromosomes, and again it proved to be somewhat misleading. There was very little selection against any except one of the transfers, indicating that the substitution of even large segments of 7Ag has little effect on pollen performance. In the early generations three transfers showed transmission significantly higher than 50%. No recent tests of transmission have been made.

All 12 transfers involve chromosome 7D, and in all but one it appears that a substantial part of 7Ag, including at least the terminal portion of the long arm, replaces the corresponding part of 7D (Fig. 2). The one exception is No. 11, which evidently has most or all of 7AgS, including the end, and little or none of 7AgL. As pointed out by R.A. McIntosh, No. 11 does not carry the major leaf-rust gene, *Lr19*, but instead an unnamed leaf-rust gene from 7AgS.

It also has a seed-protein gene from 7Ags (9) that is lacking in 10 of the other 11 transfers.

Although telo-7DL has not been available to test for pairing with the transfer chromosomes, their pairing with telo-7DS, -7AgL, and -7AgS has provided considerable information, as has the pairing of No. 11 with the others.

All but No.11 pair regularly with telo-7DS, and the seven tested pair regularly with telo-7AgL. Besides No. 11, only Nos. 4 and 6 pair with telo-7AgS (Table 3). From these data it is reasonable to conclude that transfers Nos. 4 and 6 have the entire 7AgL and a proximal segment of 7AgS - a short segment in the case of No. 4 and a substantial one in the case of No. 6. This conclusion accords with the fact that transfer No. 6 was the only one besides No. 11 found by Rodriguez-Loperena et al. (9) to have a 7AgS rather than 7DS protein allele. Probably the other nine transfers that have *Lr19* also have most or all of 7AgL, and some may have a proximal piece of 7AgS too short to support pairing with telo-7AgS.

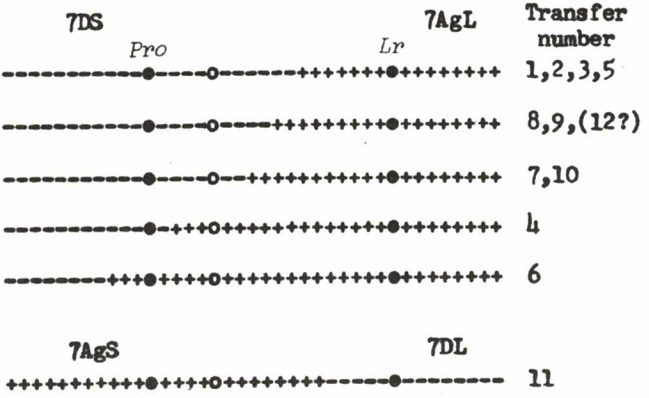

FIGURE 2. Constitution of 7D/7Ag transfer chromosomes, as estimated from gene content and pairing capacity. ---- = 7D chromatin, ++++ = 7Ag chromatin, o = centromere. The genes are: *Pro* = protein, *Lr* = leaf-rust resistance.

That these nine transfer chromosomes differ substantially in the length of their *Agropyron* segment is shown by the frequency with which they pair with the transfer No. 11 chromosome (Table 3). This frequency ranged from 0 to 21%. The fact that No. 6 showed essentially the same pairing with No. 11 (68%) as with telo-7AgS (66%) suggests that No. 11 has little or none of 7AgL. However, No. 4, which paired with telo-7AgS in only 7% of the microsporocytes, paired with No. 11 in 66 %, suggesting a substantial 7AgL component in No. 11. This would accord with the fact that both No. 10, which showed no pairing with telo-7AgS, and No. 7, which had little or no such pairing, paired about 20% with No. 11.

Evidently the shortest *Agropyron* segments are in transfers 1, 2, 3, and 5, which paired very little, or not at all, with No. 11. Which of these has the shortest *Agropyron* segment of all can probably be ascertained from their ability to pair with telo-7DL. Fortunately this telocentric is now available, having kindly been supplied by Dr. Eric Kerber.

TABLE 3. Male transmission of 7Ag/7D transfer chromosomes, and their pairing with telo-7AgS and transfer No. 11

Chromosome designation	Per cent transmission	Per cent pairing with	
		Telo-7AgS	Transfer #11
1	78	0	0
2	63	0	2
5	60	0	2
3	65	0	3
8	40	0	10
9	50	0	14
12	41	0	-
7	54	1?	19
10	41	0	21
4	70	7	66
6	58	66	68
11	22	98	ca.100

The *Agropyron* portion of transfer chromosome No. 11 evidently overlaps that of No. 1. Although no metaphase pairing of No. 11 with No. 1 was observed in 200 microsporocytes when both were monosomic, this did not preclude the occurrence of a small amount of pairing and crossing-over at an earlier stage, followed by precocious disjunction and subsequent univalent behavior, as noted by Fu and Sears (10). When the 1 x 11 hybrid was crossed (as male) to the 7Ag (7D) substitution line, one of four offspring showed 93.5% failure of two chromosomes to pair. One of these two chromosomes must have been the 7Ag chromosome and the other a recombinant between transfers No. 1 and No. 11. The second chromosome could not have been either of the parental chromosomes, both of which pair regularly with 7Ag, nor could it have been a normal 7D, introduced by contamination, for 7D does not pair at all with 7Ag in this genotype. It may be assumed, then, that Nos. 1 and 11 have a short *Agropyron* segment in common, and that crossing-over in that segment gave rise to a very largely 7D chromosome with only a short, interstitial segment, not carrying *Lr19*, from 7Ag. This recombined chromosome paired more frequently with 7Ag than expected, since it and 7Ag should have the same segment in common that Nos. 1 and 11 have.

Use of the same technique did not reveal any recombinants between Nos. 2 and 11 or 3 and 11, in spite of the fact that many more offspring (59 and 64) than from 1 x 11 were examined cytologically. However, with so little pairing of 2 and 3 with 11, very few recombinants were expected. With no recombinants recovered from 2 x 11 or 3 x 11, there is no way to decide whether their common segment is from 7Ag or 7D. Perhaps a comparison of Nos. 2 and 3 with No. 1 in ability to pair with telo-7DL will be illuminating.

DISCUSSION

One of the objectives of this project was to find out where the resistance genes are located in their respective chromosomes. This objective was only partially achieved. Each gene was found to be in the long arm. *Lr24* is evidently no closer to the centromere of 3Ag than about the middle of the arm, for exchanges occurred proximal to it that were nearly 50 crossover units from the centromere. All the transferred segments were terminal, and there is no way of knowing where *Lr24* is located in even the shortest of these—a segment that is still long enough to support regular pairing with the parent *Agropyron* chromosome. In Figure 1, *Lr24* is arbitrarily shown in the middle of the shortest 3Ag segment.

There is no indication that any of the 7Ag/7D transfers (except No. 11) have much less than the entire 7Ag long arm. This means that *Lr19* could be anywhere from fairly near the centromere to almost the distal end of the arm. In Figure 2 it is placed in the middle of the shortest 7Ag segment.

Even in those recombinants with the shortest *Agropyron* segment, then, this segment apparently constitutes a terminal piece of substantial length. However, this conclusion is based entirely on the ability of all the various transfer chromosomes to pair regularly with the original 3Ag or 7Ag. Since wheat chromosomes tend to have their chiasmata localized near the ends of their arms, the physical length of the shortest alien segments may be only a small fraction of the total length of the arm.

A second objective was to learn whether the relationship between the two *Agropyron* chromosomes and their wheat homoeologues is such that crossing over can occur at random along their lengths, or whether it is restricted to only one or a few places. At least a partial answer was obtained; it is clear that both alien chromosomes cross over with their homoeologues at a number of different places. The possibility that there are regions where exchanges cannot occur was by no means ruled out, however. In particular, crossovers near to the resistance gene on both sides were not recovered. No double crossovers were obtained, except one involving both 3D and 3B with 3Ag. Few would have been expected in a sample of the size involved, even if they occurred as freely between homoeologues as between homologues. Because of crossover interference, no doubles were expected close enough together to transfer only a small segment of *Agropyron* chromosome to the wheat chromosome.

A third objective, the most important one from a plant-breeding point of view, was to recover at least one transfer chromosome for both *Lr24* and *Lr19* that has no deleterious effects. Such effects could result from the action of other *Agropyron* genes brought along on the transferred segment, or from deficiency of important genes located on the wheat segment replaced. While insufficient testing of the transfers has been done to establish that all have unwanted characteristics, each does have an *Agropyron* segment of substantial genetic length; and the longer this segment, the greater the likelihood of a deleterious effect. All of the 7D-7Ag transfers tested, including those with the shortest 7Ag segment, have an *Agropyron* gene for yellow flour (J.Dvořák, personal communication).

There are two obvious methods for producing transfer chromosomes that carry *Lr24* or *Lr19* in a short *Agropyron* segment. The first is to select a 3Ag or 7Ag transfer that already has a relatively short *Agropyron* component and allow this to pair homoeologously with 3D or 7D. No matter where the resistance gene lies in the segment concerned, a crossover within the segment will give rise to a new chromosome still carrying the gene but with less alien chromatin (Fig. 3). This experiment is being carried out at Missouri by Miss Georgia Eizenga.

The second method of obtaining transfers of *Lr24* (and *Lr19*) that have little

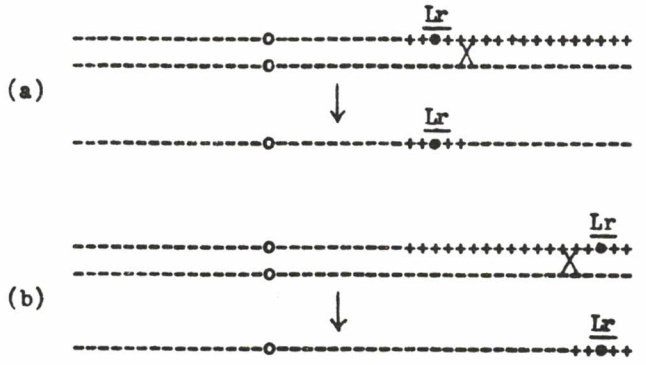

FIGURE 3. Expected consequence of crossing over between a transfer chromosome and the corresponding unchanged wheat chromosome. In (a) the resistance gene is assumed to be near the proximal end of the alien segment, in (b) near the distal end

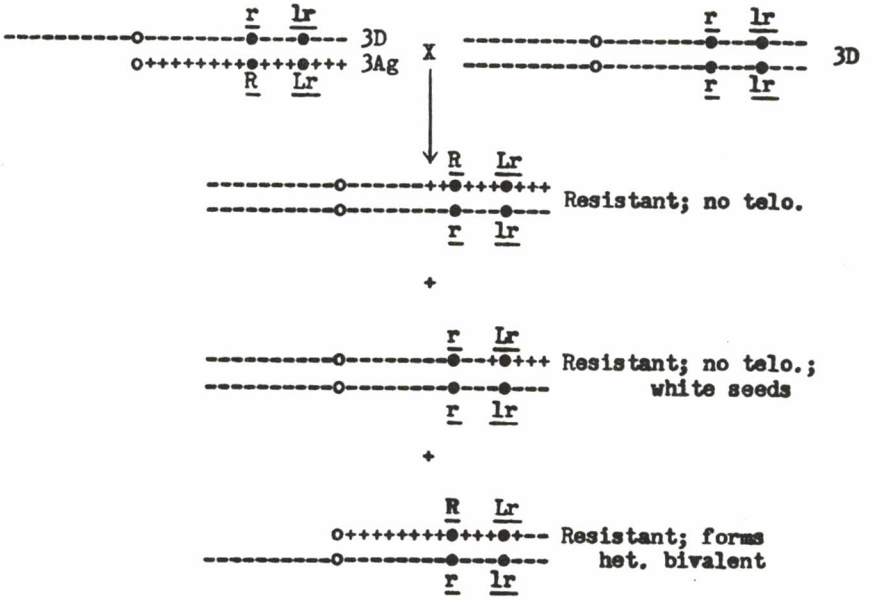

FIGURE 4. Results expected from use of normal pollen on *phph* plants heterozygous for a normal 3D chromosome, carrying the white-seed gene *r*, and a telocentric for the long arm of 3Ag.

associated *Agropyron* chromatin is to induce additional crossovers of 3D with 3Ag (and of 7D with 7Ag), anticipating that exchanges closer to the gene than any yet obtained will be recovered. Then the chromosome with the exchange closest on one side can be allowed to pair with the one having the closest exchange on the other side, and a recombined chromosome recovered that has a minimum amount of *Agropyron* chromatin.

Some improvements can be made on the original experiments. The critical plants need not be made nullisomic 5B but can simply be made homozygous for a homoeologous-pairing mutation that now exists (11,12). A telocentric for the *Agropyron* arm carrying the resistance gene can be used instead of the complete chromosome, thus making it easier to recognize the resulting transfer chromosomes (Fig. 4). Those with an exchange distal to the resistance gene will be telocentrics that should be able to pair with 3D (or 7D), and the frequency of this pairing will reveal the closeness of the exchange to the gene. Exchanges proximal to the gene will give rise to chromosomes that carry resistance but are not telocentric. On chromosome 3D the marker gene *r* (white seeds) proximal to the *Lr24* locus (R.A.McIntosh, personal communication) can be used to select for proximal exchanges that are close to *Lr24*.

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