Locating the alien chromatin segment in common wheat—*Aegilops longissima* mildew resistant transfers

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By use of the Chinese Spring *phl* mutant, recombination was induced between the 3S' short arm telosome of *Ae. longissima* and its group-3 wheat homoeologues. Transfer was thus obtained of chromosomal segments bearing the alien *Pm13* mildew resistance gene into several common wheat lines. To identify the wheat chromosome involved in each transfer, these were subjected to monosomic and C-banding analyses. Probably due to preferential 3B-3S' pairing, irrespective of the group-3 wheat chromosome present as monosome in the critical steps of the transfer work, in 213 of the cases 3B turned out to be the recipient chromosome, whereas 3D was the one involved in the remaining ones. Assessment of the residual pairing ability of the recombinant chromosomes in *F₁'s* between four 3B and three 3D transfer lines and their corresponding wheat ditelosomic as well as 3S' substitution lines, indicated about coincident values (about 40% pairing with DT3BS) in three of the 3B recombinants, a significantly different pattern in the fourth one (10%) and a more continuous variation among the 3D ones. An overall prevailing terminal location of the wheat-alien chromatin exchange points is tentatively hypothesized.

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In wheat, particularly the hexaploid *Triticum aestivum* L., well established chromosome engineering methodologies (SEARS 1972a, 1982) are applicable to the purpose of transferring into cultivated genotypes desired traits of alien origin. Genetically promoted recombination, particularly by use of wheat mutations for homoeologous pairing control genes, such as the *Phl* suppressor, ensures the greatest possible degree of refinement in targeting the recipient chromosome(s) and limiting the amount of alien chromatin introgressed. Several successful examples exist of such a non-conventional breeding approach (see, e.g., CEOLONI 1987; GALE and MILLER 1987), so far mainly resulting in the incorporation into wheat lines of disease resistance genes, but of course usable for the controlled transfer of any character of interest present in wild or cultivated wheat relatives.

A dominant gene for resistance to wheat powdery mildew, named *Pm13*, proved to be effective against a broad spectrum of the pathogen biotypes, spread in Italy and in several other countries (CEOLONI, unpubl.), has recently been transferred from *Ae. longissima* Schweinf. et Musch. into *Triticum aestivum* cv. Chinese Spring by *phl* mediated recombination (CEOLONI et al. 1988). In the transfer scheme adopted, the resistance source was represented by a ditelosomic addition line for the short arm of chromosome 3S' (3S'), previously proved to be homoeologous to the group-3 chromosomes of common wheat (CEOLONI 1983). This choice allowed to clearly identify pairing between the critical homoeologues and also restrict recombination to the critical arm only. Depending on whether recombination had taken place proximal or distal to the gene concerned and with the event occurring in meiocytes having a monotelosomic alien chromosome and a monosomic wheat homoeologue (3A, 3B or 3D, see CEOLONI et al. 1988), two types of recombinant products were expected and indeed recovered following a cross to euploid: “proximal” types, with complete chromosomes only, of which one carried an arm with the distal portion replaced by an alien segment, and “distal” ones, still having a telocentric but with a wheat terminal portion.

With a gametic recombination frequency averaging around 7%, proximal recombinants represented a 75% of the total (CEOLONI et al. 1988). Because they could be of immediate use for breeding, a detailed characterization of the 9 proximal
resistant transfers isolated out of a total of 12 has been undertaken.

In the present paper, data will be provided on the chromosomal location of the alien segment in each of such transfers, obtained through monosomic and C-banding analyses. A cytogenetic estimate of the amount of alien chromatin introgressed in some of them will also be given, as derived from meiotic metaphase I records of the residual pairing ability of every recombinant chromosome with its original wheat and alien homologues.

Materials and methods

As standard common wheat cv. Chinese Spring aneuploids, group-3 monosomics as well as 3B and 3D short arm ditelosomic lines were employed, both originally obtained from E. R. Sears. Ae. longissima chromosome 3S' substitutions into Chinese Spring for chromosome 3B and 3D, previously developed by C. Ceoloni, were also used.

The wheat–Ae. longissima mildew resistant recombinant lines analysed in the present study included six, named R1A, R2A, R3A, R4A, R5A, and R6A, in which 3A was the group-3 monosome in the critical steps of the transfer procedure (see Ceoloni et al. 1988). In two others, named R1B and R2B, 3B was instead in monosomic condition, whereas in the last, termed R1D, 3D was the monosomic wheat homoeologue.

Monosomic analysis

Chromosomal location of the alien chromatin segment carrying the Pm13 gene in each of the proximal transfer lines was firstly assessed through monosomic analysis and then verified with C-banding. Results deriving from both tests were coinciding, indicating that in 6 out of 9 proximal recombinants the wheat chromosome involved was 3B and in the remaining 3 it was 3D. In fact, all progenies were an acceptable fit to a ratio of 3 resistant:1 susceptible, except those involving chromosome 3B in R1A, R3A, R4A, R5A, R6A, and R1B and those involving 3D in R2A, R2B, and R1D, for which the relative incidence of susceptible plants was definitely lower (Table 1). This made possible, in all cases, discrimination of the critical cross from the noncritical ones. The conclusion was in each case considered unequivocal, even if in some of the critical F2's (namely in the case of R2A, R4A, R5A, R6A, and R1B, though, as expected, the susceptible phenotype was mostly accompanied by a 2n = 40, nullisomic chromosome constitution; see Materials and methods), plants with different chromosome numbers, mostly 2n = 41, were sporadically detected. This may be due to the not yet complete meiotic stability of the recombinant lines, which, at the time monosomic analysis was undertaken, still exhibited occasional univalents and multivalents. Besides this, both in the critical and noncritical F2's, and, as to the former ones, being particularly evident for R2A
Table 1. Segregation for mildew resistance and somatic chromosome number in F₂ progenies of group-3 wheat monosomic × Pm13 recombinant lines

| Recomb. line | Wheat monosome | F₂ progenies | Total plants | % susceptible | 2n susceptible plants | P (3:1) |
|--------------|----------------|--------------|--------------|---------------|-----------------------|---------|
| R1A          | 3A             | 45           | 22.2         | 40 41 42      | 0.50–0.75             |         |
|              | 3B             | 58           | 1.7          | 40            | <0.005                |         |
|              | 3D             | 34           | 29.4         | 40 41 42      | 0.50–0.75             |         |
| R2A          | 3A             | 75           | 24.0         | 40 41 42      | 0.75–0.90             |         |
|              | 3B             | 23           | 30.4         | 40 41 42      | 0.50–0.75             |         |
|              | 3D             | 26           | 11.5         | 40            | 0.10–0.25             |         |
| R3A          | 3A             | 29           | 31.0         | 40 41 42      | 0.50–0.75             |         |
|              | 3B             | 38           | 13.2         | 40 41 42      | 0.05–0.10             |         |
|              | 3D             | 32           | 28.1         | 41 42         | 0.50–0.75             |         |
| R4A          | 3A             | 55           | 27.3         | 40 41 42      | 0.50–0.75             |         |
|              | 3B             | 22           | 13.6         | 40 41 42      | 0.10–0.25             |         |
|              | 3D             | 39           | 28.2         | 41 42 43      | 0.50–0.75             |         |
| R5A          | 3A             | 17           | 23.5         | 41 42         | >0.90                 |         |
|              | 3B             | 46           | 13.0         | 40 42 42      | 0.05–0.10             |         |
| R6A          | 3A             | 55           | 27.3         | 40 41 42      | 0.50–0.75             |         |
|              | 3B             | 47           | 12.8         | 40 41 42      | 0.05–0.10             |         |
| R1B          | 3A             | 34           | 26.5         | 41 42         | 0.75–0.90             |         |
|              | 3B             | 56           | 12.5         | 40 41 42      | <0.05                 |         |
|              | 3D             | 45           | 24.4         | 40 41 42      | >0.90                 |         |
| R2B          | 3A             | 27           | 29.6         | 41 42         | 0.50–0.75             |         |
|              | 3B             | 35           | 26.6         | 40 41 42      | 0.50–0.75             |         |
|              | 3D             | 28           | 7.1          | 40            | <0.05                 |         |
| R1D          | 3A             | 53           | 24.5         | 40 41 42      | >0.90                 |         |
|              | 3B             | 56           | 26.8         | 40 41 42      | 0.75–0.90             |         |
|              | 3D             | 56           | 0            | –             | <0.005                |         |

*1 In these cases somatic chromosome numbers other than the expected 2n = 40 were sporadically detected. For explanation see text.

and R4A, an excess of susceptible plants was observed. This fact could be ascribed to a relatively low transmission rate of the recombinant chromosome (see Ceoloni et al. 1988), whose male but also female carrier gamete might have suffered a competitive disadvantage with respect to those bearing full wheat genotypes. Both reported distortions in the critical F₂ are indeed not infrequent when alien recombinant chromosomes are involved, which makes the final judgement actually based on the overall behaviour of the segregating progenies.

**C-banding**

Normal and recombinant 3B chromosomes were rather similar in their C-banding pattern, being so to this respect the short arm of *Ae. longissima* chromosome 3S¹ and its homoeologous arm of the B genome (Fig. 1a). However, the characteristic, prominent terminal heterochromatic band possessed by 3S’s, being much less intense in the case of 3B, where it showed consistently a comparable intensity to the adjacent more proximal one, allowed to clearly discriminate the two alternatives. In all cases the C-banding evidence confirmed the outcome of the monosomic analysis. In fact, all 3B recombinant lines exhibited a normal C-banded wheat complement except that for a 3B pair with a short arm telomeric band of clear 3S¹ derivation (Fig. 2a), and so did 3D recombinant lines, which obviously had a normal Chinese Spring C-banded karyotype except for the 3D pair (Fig. 2b). Indeed, differences between a normal and a recombinant 3D chromosome were even more clearcut. In all the three lines which monosomic analysis had shown to involve the D genome homoeologue, i.e., R2A, R2B, and R1D, a 3D pair with both an altered C-banding pattern and arm ratio was in fact clearly visible (Fig. 1b and 2b). The modification, which involved the terminal region of the short arm, does not seem to implicate the two
C-bands of normal 3DS, which, judging from their relative distance and intensity, look in fact apparently unchanged in recombinant 3D's. Indeed, it seems as if in these a further heterochromatic band, more distal than the most telomeric one of normal 3D, plus an appreciable amount of euchromatin (definitely more abundant than the very distal thin euchromatin stripe of a regular Chinese Spring 3D), both probably of 3S's origin, are present. Consequently, recombinant 3D chromosomes result much more metacentric than normal ones (Fig. 1b). This was true for all the above mentioned lines, though perhaps the amount of euchromatin between the two most terminal heterochromatic bands seemed maximum in R2A and minimum in R2B.

In trying to “quantify” these observations, measurements of defined chromosomal segments of the short arm of normal and recombined 3B and 3D were taken (Table 2). In the case of the 3B's, such a segment extended from the more distal centromeric heterochromatic band excluded (band 3Bp13 according to GILL 1987) up to the telomeric end, whereas for the 3D's it coincided with the entire short arm. No significant difference was detected between normal and recombined 3B's, though a tendency toward a slight increase determined by the presence of the terminal 3S's band was noticed in the latter ones (Table 2). As to the 3D comparison, the recombinant types exhibited an about 25 % increase in short arm length, which, as mentioned above, clearly altered the morphological characteristics of the original 3D. Such a test was probably not sensitive enough to discriminate among the three 3D recombinant chromosome types (that of R2A, R2B, and R1D). However, it looks interesting that the C-banding observation appears to be roughly confirmed by the meiotic pairing data (see ahead).

It seems quite evident that the chromosomal location emerging from the monosomic analysis and confirmed through C-banding, in several cases did not correspond with the somewhat expected involvement of the group-3 wheat chromosome, which was, in turn, monosomic in the critical steps of each of the transfer schemes (see CEOLONI et al. 1988). The lack of any 3A transfer could perhaps be simply due to chance, the total number of recombinants being relatively small. On the other side, the far most frequent involvement in recombination of 3B, which parallels the observed preferential pairing of the 3S's telosome with its closer homoeologue (CEOLONI et al. 1988), suggests that a higher cytogenetic affinity can represent a more important causative factor than the chromosomal condition (monosomic vs. disomic) of the putative pairing partners in determining their pairing and, thus, recombination patterns. Indeed, numerous evidences exist of pairing preferences occurring also between homologues (see, e.g., DvORAK and McGUIRE 1980; SANTOS et al. 1983; SUAREZ et al. 1988), the phenomenon thus being even more expected between structurally and genetically differentiated homoeologous chromosomes.

Table 2. Length in arbitrary units (a.u.) of defined chromosomal segments of the short arms of normal and recombined 3B and 3D

| Chromosome | No. of samples | Length (M ± SEM)² |
|------------|----------------|------------------|
| 3B         | 248            | 2.64 ± 0.029     |
| rec. 3B    | 304            | 2.73 ± 0.023     |
| 3D         | 171            | 2.05 ± 0.021     |
| rec. 3D    | 291            | 2.53 ± 0.021     |

¹ The measured segment in the case of the short arm of 3B extends from the more distal centromeric heterochromatic band excluded (band 3Bp13 in GILL 1987) up to the telomeric end, whereas for chromosome 3D it coincides with the entire short arm
² Observations performed on different recombinant lines per each type of chromosome revealed no significant difference between them; therefore the separate groups of records were pooled
Fig. 2 a and b. Partial C-banded somatic metaphases. A 3B (a) and a 3D (b) common wheat–Ae. longissima recombinant line. Asterisks indicate recombined chromosomes.
Evaluation of pairing ability of recombinant chromosomes

As one approach to evaluate the amount of alien chromatin bearing the Pm13 gene introgressed in the different proximal recombinants, metaphase I pairing was assessed in mildew resistant F1's between the transfer lines and their corresponding wheat ditelosomic line (ditelo-3BS or 3DS) as well as the 3S1 (3B) or 3S1 (3D) substitution line. This allowed to determine the residual pairing ability between every recombinant chromosome and each of its homologous counterparts (telo-3BS or 3DS and complete 3S1). Results are reported here of such a test performed on 7 out of 9 proximal recombinants (R3A and R5A excluded). In the case of the four 3B recombinants, the proximal wheat segment appeared to support very similar amounts of pairing with telo-3BS in RIA, R4A, and RIB (from 41.5 % in R1B to 42.4 % in R1A, Table 3), whereas the amount of such pairing was only 10.3 % in R6A. Pairing of the distal portion of the same recombinant chromosomes with the complete 3S1 confirmed this difference, being quite high for R6A (70.1 %) and significantly lower for the three others, these giving again about coincident values (from 47.4 % of R4A to 49.7 % of R1B).

Such a particular trend was not noticed in the case of the 3D transfer lines: out of the three, R2A showed the minimum pairing with telo-3DS (25.2 %) and R2B, the maximum (43.6 %). Values of pairing with 3S1 confirmed such a picture (Table 3).

The coinciding values exhibited by three out of the four 3B recombinant chromosomes lead to speculate about the possible existence of a recombinational “hotspot” in that region of 3B in which transition between alien and wheat chromatin occurred in the separate events involving R1A, R4A, and R1B. Indeed, a large body of evidence — and particularly clearcut are in this respect data recently derived from observations on mammalian genomes (STEINMETZ et al. 1987) — indicates that recombination does not occur at random but, instead, with unusually high frequency in certain areas, therefore being termed hotspots. In these areas, even though recombinational events are highly clustered, they do not occur at the same position, as seems to be the case for the three 3B recombinants of the present investigation. As a result of recombinational hotspots in the genome, genetic maps will not be completely congruent with physical maps, in that regions containing hotspots will appear unusually large, whereas those devoid will contract. Therefore, it will be particularly interesting to compare in the present system both types of maps. Indeed, preliminary data from an ongoing telocentric mapping experiment seem to confirm in terms of genetic map distances the MI pairing picture described here.

Certainly, the location of the wheat-alien chromatin breakpoint in the different recombinants cannot be deduced from the available data only. However, some considerations can be made. For instance, the residual pairing with complete 3S1 shown by those recombinant chromosomes which exhibited the highest pairing with the corresponding telosomes, approaching 50 % in the case of R1A, R4A, and R1B, would suggest that they possess a terminal 3S1 segment of considerable length. Yet, not considering the case of the 3B recombinants, where practically no information can be derived from the C-banding comparison, but analyzing that of R2B, whose recombinant 3D still supports a 35 % pairing with 3S1, the amount of distal alien chromatin apparently exceeding that of a normal 3DS appears relatively limited (Fig. 1b and 2b). If it is so for R2B, then R1A, R4A, and R1B should have an even shorter alien segment in the distal end of their short arms. Actually, an overall prevailing terminal-subtermi-

| Line | 3B transfer lines |  | 3D transfer lines |
|------|------------------|---|------------------|
| Pairing with 3BS | No. plants | No. cells | % pairing | No. plants | No. cells | % pairing |
| R1A | 3 | 166 | 42.4 | 1 | 55 | 49.1 |
| R4A | 4 | 307 | 42.1 | 2 | 143 | 47.4 |
| R1B | 2 | 166 | 41.5 | 2 | 93 | 49.7 |
| R6A | 2 | 175 | 10.3 | 2 | 91 | 70.1 |
| Pairing with 3S1 | | | | No. plants | No. cells | % pairing |
| R2A | 2 | 151 | 25.2 | 2 | 119 | 61.3 |
| R2B | 2 | 142 | 43.6 | 2 | 122 | 34.4 |
| R1D | 3 | 352 | 37.3 | 2 | 88 | 55.0 |
nal location of the exchange points would not be completely unexpected. In fact, the use of a telocentric as donor chromosome instead of a complete one could have shifted pairing and recombination more distally (see, e.g., SEARS 1972b, 1982). Also, more generally speaking, both in wheat and in several other plant species pairing and crossing-over appear to be preferentially located in distal regions (see, e.g., FLAVELL et al. 1984, for a review).

Conclusions

The successful transfer of *Ae. longissima* chromosomal segments into common wheat has provided lines resistant to powdery mildew thanks to the incorporation of the highly effective *Pm13* alien gene. To make the best use of these chromosomally engineered lines for breeding purposes, and also to have some knowledge of the pairing and recombination pattern characterizing the system under study, a thorough investigation is being carried out, of which some results have been presented here. A non-random occurrence of the pairing and recombination processes, both as to recipient chromosome of the *longissima* segment and, possibly, as to position of the exchange points along the arm, seems to come out from monosomic analysis, C-banding, and pairing patterns of the transfers analysed. However, to have a better estimate of the amount of alien genetic material introgressed in the various transfer products, telocentric mapping and in situ hybridization experiments are currently being performed, which, combined with the cytogenetic evidence, are expected to allow more soundly based conclusive considerations.

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