Structural changes of rye chromosome 1R induced by a gametocidal chromosome

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ABSTRACT

A certain chromosome of *Aegilops triuncialis* is known to cause chromosomal aberrations in common wheat. We studied how frequently the *Ae. triuncialis* chromosome induced chromosome mutations in chromosome 1R of rye which was substituted for chromosome 1B in a common wheat cultivar. Wheat-rye translocations and deletions in chromosome 1R were found in more than 10% of the plants examined, and most of them were stably transmitted in the subsequent generations. The possible use of this system for inducing wheat-alien translocations is discussed in relation to wheat breeding.

1. INTRODUCTION

Introduction of alien genes of agronomic importance from related wild species into cultivated forms is one of the targets of wheat breeding. To attain it, usually a series of alien chromosome addition or substitution lines of wheat are first produced, and then specific alien chromosome addition or substitution lines are treated with mutagens to induce translocations between the wheat and alien chromosomes. Also, they are crossed to a wheat stock lacking *Ph* gene to induce homoeologous pairing. Recently it was found that chromosome mutations frequently occur in the lines of common wheat with the addition of certain chromosomes, called gametocidal chromosomes, from different species of *Aegilops*, a wild genus related to wheat (Endo, 1988, 1990). When one of such *Aegilops* chromosomes is present in the sporophyte of common wheat, chromosomal structural aberrations, which are either lethal or sublethal, occur in the gametes lacking the *Aegilops* chromosome. If the gametes with chromosomal aberrations are func-

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tional, the resulting progeny will not suffer further chromosome mutations since they no longer possess the *Aegilops* chromosome. This study was designed to demonstrate that the *Aegilops* gametocidal chromosome also induces chromosome mutations in an alien (rye) chromosome added to or substituted in common wheat, leading to wheat-alien translocations. The results are reported in this paper.

2. MATERIALS AND METHODS

We used a common wheat cultivar Burgas 2, where a pair of wheat 1B chromosomes are substituted with a pair of rye 1R chromosomes, as the donor of an alien chromosome. To induce sublethal chromosome mutations we used a gametocidal chromosome from *Ae. triuncialis* which was added to a Japanese common wheat cultivar Norin 26. Although the *Ae. triuncialis* chromosome causes lethal chromosomal aberrations in the gametes lacking it in many other cultivars (Endo and Tsunewaki, 1975; Endo, 1978), chromosome 3B of Norin 26 has a dominant gene suppressing the lethal gametocidal action (Tsujiimoto and Tsunewaki, 1985).

Burgas 2 was crossed with the euploid and addition lines of Norin 26, respectively, to obtain the control F_1 ($20''+1B'+1R'$) and the critical F_1 ($20''+1B'+1R'+tr'$) (Fig. 1). These F_1 's were self-pollinated and the chromosome constitutions of the F_2 progeny were studied. In the F_2 progeny from the critical cross, three plants with disomic substitution for chromosome 1R and monosomic addition for the *Ae. triuncialis* chromosome ($20''+1R''+tr'$) were selected cytologically by C-banding. They were self-pollinated and backcrossed to euploid Norin 26, two of which showed normal seed-set. The normal fertility of these two plants indicated that they carried the dominant gene on chromosome 3B of Norin 26 which renders the gametocidal action of the *Ae. triuncialis* chromosome sublethal. Therefore, their progeny were further observed for chromosomal structural changes. The third plant, which showed a low seed-set, was not used since more likely it had lost the dominant gene on chromosome 3B of Norin 26.

Fig. 1. A scheme illustrating the induction of chromosome mutations in chromosome 1R by the *Ae. triuncialis* chromosome. 1B, 1R, and tr stand for wheat chromosome 1B, rye chromosome 1R, and the *Ae. triuncialis* chromosome, respectively.

Chromosome identification was conducted by modified C-banding and the N-banding techniques (Gill et al., 1990). Aberrant chromosomes involving 1R were further examined by fluorescence or non-fluorescence *in situ* hybridization using biotinylated probes of total rye genomic DNA and/or 18S.26S rDNA. *In situ* hybridization was conducted by the procedures described by Mukai et al. (1991, 1993b).

3. RESULTS AND DISCUSSION

Structural changes of the wheat and 1R chromosomes, with the exception of five 1R telosomes, were not detected in the selfed progeny of the control F_1 with $20''+1B'+1R'$. However, in the selfed progeny of the critical F_1 with $20''+1B'+1R'+tr'$, five translocations and one deletion, besides six telosomes, of chromosome 1R were found (Table 1, Figs. 2 and 3), in addition to many structural changes in the wheat chromosomes. Translocations and deletions of chromosome 1R also occurred in both the selfed and backcrossed progeny of the $20''+1R''+tr'$ plants; however, 1R telosome were not observed. This suggests that the 1R telosomes seen in the F_1 's originated from centromere misdivision of the 1R univalent. The structural aberrations of chromosome 1R, i.e., translocations and deletions, were found in 12% of the selfed progeny of the $20''+1B'+1R'+tr'$ plant, and in 13% and 10% of the selfed progeny and backcrossed progeny of the $20''+1R''+tr'$ plants, respectively.

All the 1R translocation chromosomes found in this study are shown in Fig. 2. Two chromosomes a and b occurred in the same plant, probably by a reciprocal translocation between chromosomes 5B and 1R. Chromosomes c and d also appeared in a single F_2 plant. Chromosome c had a part of the 1R short arm translocated to the 6B long arm, and therefore, had two nucleolus organizer regions. Chromosome d had the whole 1R short arm and a part of the 1R long arm to which possibly the distal long arm segment of chromosome 6B had been translocated. Presumably, the mutations in chromosomes c and d had occurred independently in the male and female gametes and were brought together by self-fertilization. Chromosomes e, f, and g involved the 1R short arm and wheat

Table 1. Frequencies of chromosomal structural changes involving 1R in the progeny of the plants with 1R and the *Ae. triuncialis* chromosome

Parental chromosome* constitution	Progeny	Germination rate	No. plants examined	No. 1R translocations	No. 1R deletions	No. 1R telosomes
$20''+1B'+1R'$ (control)	selfed	100%	29	0	0	5
$20''+1B'+1R'+tr'$	selfed	73	52	5	1	6
$20''+1R''+tr'$	selfed	95	76	7	3	0
$20''+1R''+tr'$	× Norin 26	91	31	1	2	0

* See a legend to Fig. 1.

Fig. 2. C-banded normal 1R and wheat chromosomes (top row) and wheat-1R translocations (a to m; k' is an N-banded chromosome). Arrows indicate the approximate translocation points; the translocation points of chromosomes f, g, k, and l were not studied. The horizontal line indicates the centromeric position. See the text for details.

chromosomes 2A, 4B, and 7B, respectively. Chromosomes h and i also included the 1R short arm but the wheat chromosomes involved were not identified. Chromosome j involved the whole-arm translocation of the 1B short and 1R long arms. Chromosome k possessed the 1R long arm and an unknown wheat chromosome segment since the terminal band in the short arm did not disappear after N-banding (see k'): The terminal bands of 1R are observed only by C-banding but not by N-banding. Also, the *in situ* hybridization analysis revealed that chromosome k lacks a nucleolus organizer region. Chromosomes l and m were the dicentric products of translocations between 6A and 1R and between 3B and 1R, respectively. It seems that the B genome chromosomes were involved in translocations more frequently than the other genome chromosomes.

Fig. 3. A partial metaphase cell subjected to fluorescence *in situ* hybridization using rye genomic DNA probe (green) and rDNA probe (red, indicated with *) simultaneously. The chromosome with two rDNA hybridization signals in both arms is chromosome c in Fig. 2, while the chromosome with one rDNA hybridization signal is chromosome d in Fig. 2. Their translocation break points are on the boundary between the bright- and pale-green chromosomal regions (indicated with arrows).

The arrows in Fig. 2 indicate the approximate translocation points, as were revealed by the fluorescence *in situ* hybridization using total genomic rye DNA as probe (Fig. 3). Translocations seem to occur throughout the 1R chromosome. The plants with these translocations, except chromosomes g and l, grew to maturity, and the translocations except chromosome m remained unchanged in the

Fig. 4. C-banded normal and deletion chromosomes of 1R. a' shows the *in situ* hybridization signal of rDNA probe in chromosome a. The horizontal line indicates the centromeric position. See the text for details.

subsequent progeny. Chromosome m produced various monocentric translocation chromosomes.

Fig. 4 shows all of the 1R deletions found in this study. The acrocentric morphology and the presence of the nucleolus organizer region, revealed by *in situ* hybridization analysis (see a' in Fig. 4), indicated that chromosome a carried an interstitial deletion in the 1R short arm. Chromosome b had a deficiency in the satellite, i.e., the entire terminal heterochromatic region had been lost. Chromosomes c and d had breakpoints in the short arm. Chromosomes e and f possessed the 1R short arm with only small proximal segments of the long arm. It may be possible to use these deletions for physically mapping genes on chromosome 1R.

Although the occurrence of structural changes in chromosome 1R in this study was frequent (13 translocations and 6 deletions in 159 plants examined), most of 1R translocations are probably genetically unbalanced and unsuitable for wheat breeding because the translocations occurred at random regardless of the homoeology between the chromosomes involved. As for alien chromosomes, like chromosome 1R, without evolutionary translocations relative to the wheat homoeologues, homoeologous recombination will give rise to better wheat-alien translocations. However, for alien chromosomes with structural rearrangements relative to their wheat homologues, i.e., all of the rye chromosomes except for 1R (Devos et al., 1993), their homoeologous recombination with wheat chromosomes may not be as high as for the alien chromosomes without evolutionary translocations. Moreover, the recombination products may be little better than random wheat-alien translocations. If so, the genetic chromosome-mutation inducing method described above might be more efficacious than the homoeologous-recombination inducing method in getting harmonious transposition of alien chromosomal segments into wheat chromosomes.

Once the basic wheat line, like the one with $20''+1R''+tr'$, is produced, it is easily maintained by cytological screening, and its progeny always yield chromosome mutations with no cumbersome treatment of mutagens. Furthermore, by backcrossing the basic line with the parental gametocidal addition line successively, like the one with $21''+tr''$, we can screen for plants with alien chromosome translocations and a gametocidal chromosome in every generation until we get favorable wheat-alien chromosomal rearrangements. Although in the present study we could find only the 1R translocations including the terminal C-bands, translocation of intercalary 1R chromosome segments would have been detected by the use of genomic *in situ* hybridization, as demonstrated by Mukai et al. (1993a) when a translocated rye chromatin segment specifying resistance to Hessian fly was detected.

At least three other *Aegilops* chromosomes are known to cause sublethal chromosome mutations in the same way as the *Ae. triuncialis* chromosome. One is the chromosome derived from *Ae. cylindrica* (Endo, 1988) and the other two

are from *Ae. speltoides* and *Ae. triuncialis* (a different chromosome from the one used in this study) (unpublished results). These three chromosomes exert their effect in the background genotype of the common wheat cultivar Chinese Spring. It appears that we can probably obtain structural changes in any alien chromosomes in common wheat by the use of such *Aegilops* chromosomes.

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