

A strategy for detecting chromosome-specific rearrangements in rye

E. Alvarez, C. Alonso-Blanco, R. García Suárez, J.J. Ferreira, A. Roca, and R. Giraldez

Abstract: To obtain translocations involving specific chromosomes in rye, a line in which chromosome 1R has large C-bands on its two telomeres but which lacks C-bands (or has very small ones) on the telomeres of the remaining chromosomes was used. About 6% of the plants produced using pollen from irradiated (1.2 krad (1 rad = 10 mGy)) spikes of this line possessed structural changes involving the labeled chromosome. These aberrations included translocations, ring chromosomes, isochromosomes, and telocentrics. It is concluded (i) that all nonlabeled chromosomes have the same probability of participating in reciprocal translocations with the labeled chromosome, 1R, and (ii) that most induced reciprocal translocations involved exchanges of chromosome segments of approximately equal length. The use of lines having the appropriate combination of telomeric C-bands improves the efficiency of obtaining reciprocal translocations involving specific chromosomes that could be used in the construction of detailed physical maps.

Key words: *Secale*, translocations, C-banding, x-ray irradiation.

Résumé : Afin d'obtenir des translocations impliquant des chromosomes spécifiques chez le seigle, une lignée dont le chromosome 1R possédait des grandes bandes C au niveau de ses télomères et dont les télomères des autres chromosomes étaient dépourvus (ou montraient peu) de bandes C a été employée. Environ 6% des plantes produites à l'aide du pollen provenant d'épis irradiés (1,2 krad (1 rad = 10 mGy)) de cette lignée montraient des changements structuraux impliquant le chromosome marqué. Ces aberrations comprenaient des translocations, des chromosomes en anneau, des isochromosomes et des chromosomes télocentriques. Il en a été conclu (i) que tous les chromosomes non-marqués ont la même probabilité de participer à des translocations réciproques avec le chromosome 1R marqué et (ii) que la majorité des translocations réciproques impliquaient des segments de taille semblable. L'emploi de ces lignées ayant la bonne combinaison de bandes C télomériques augmente l'efficacité de l'obtention de translocations réciproques impliquant des chromosomes spécifiques ce qui pourrait être exploité lors de la construction de cartes physiques détaillées.

Mots clés : *Secale*, translocations, révélation des bandes C, irradiation aux rayons x.

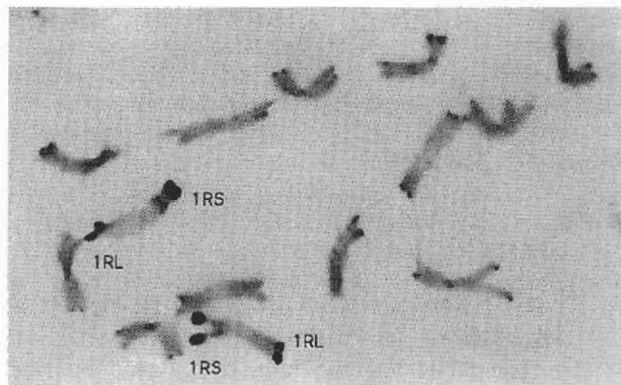
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Introduction

Translocations are the most extensively studied chromosome rearrangements in the Triticeae. They have been involved in the evolution of this tribe (Devos et al., 1993) and have been used in cytogenetic and physical mapping in association with other cytological markers (Alonso-Blanco et al. 1993; 1994a), and in situ hybridization (Alonso-Blanco et al. 1994b) and micro-isolation techniques (Sorokin et al. 1994).

In rye, a translocation set covering all seven chromosomes was constructed by Sybenga and Wolters (1972). This set was expanded by Ramulu and Sybenga (1985), and a few translocations of spontaneous origin were later described by Alvarez et al. (1994). However, the number of translocations in rye is much smaller than in maize (Longley 1961) or barley (Linde-Laursen 1988), in which a high degree of saturation for all chromosomes has been reached.

Fig. 1. Mitotic metaphase cell of line 1RSL. Chromosome 1R has large C-heterochromatin bands in both telomeres, the other chromosomes being practically free of such C-bands.



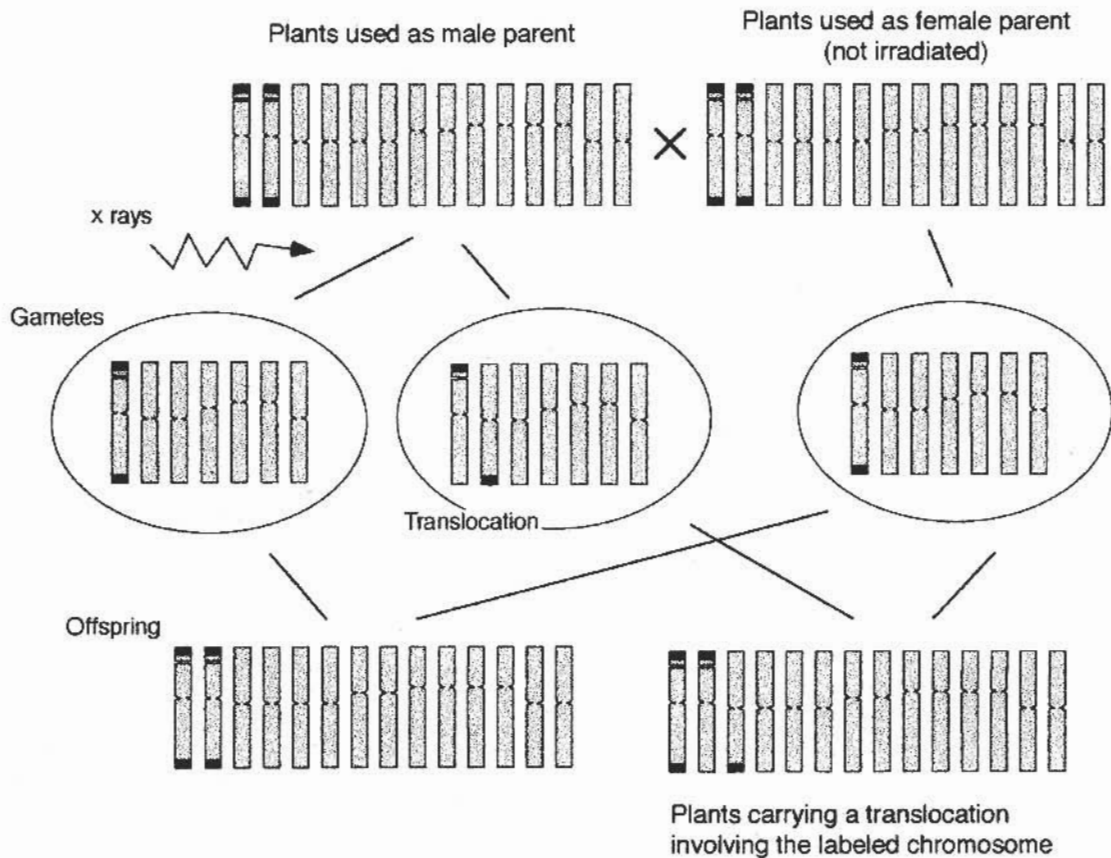
Chromosome rearrangements in rye can be efficiently induced by irradiation of pollen with x rays (Sybenga and Wolters 1972), but the identification of the translocations obtained can be difficult. Since most rye chromosomes show small differences in their C-band patterns, only reciprocal exchanges giving rise to size differences in the translocated

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Fig. 2. Diagram showing the experimental protocol realized. Spikes of line 1RSL were irradiated with x rays and the pollen was then used to pollinate nonirradiated spikes of the same line. From the relative positions of the large C-heterochromatin bands among the offspring, the presence of a translocation involving the labeled chromosome can be easily detected.



chromosomes can be unequivocally identified at mitosis. Plants carrying translocated chromosomes can also be identified by the presence of a quadrivalent at meiosis, but this requires the cultivation and laborious meiotic analysis of a large number of plants. In other plant species, such as maize and barley, the selection of translocation heterozygotes has been based on their semisterility resulting from meiotic irregularities. However, this criterion is not of use in rye, because translocation heterozygotes in this species tend to be rather fertile as a consequence of relatively frequent alternate orientation of the quadrivalent at meiosis (Sybenga 1995).

The aim of the research presented here was to induce chromosome rearrangements in rye by irradiating (x rays) spikes of a line with a labeled chromosome homozygous for C-bands on both telomeres and the remaining chromosomes deficient in C-bands on the telomeres. With this strategy, all translocations involving the labeled chromosome can be easily recognized in C-banded mitotic metaphases.

Materials and methods

Plant material

Chromosome rearrangements were induced by exposing spikes of a rye line (1RSL) near the beginning of anthesis to 1.2 krad (1 rad = 10 mGy) of x rays. The pollen was then used to pollinate non-

irradiated spikes of the same line. Chromosome 1R of line 1RSL has large C-heterochromatin blocks in both telomeres. Such C-bands are absent from the other chromosomes in this line (Fig. 1). The experimental procedure employed is shown in Fig. 2.

Different lines having large blocks of C-heterochromatin only in the telomeres of chromosome arms 3RL and (or) 2RS and a large interstitial C-band in 2RL were used to identify the nonlabeled chromosomes involved in some of the translocations obtained.

All plant materials were derived from crosses between lines kindly supplied by A. Lukaszewski (University of California, Riverside), in each of which only one chromosome arm was marked with a large telomeric C-band.

Mitotic and meiotic C-banding analysis of progeny

Root tips of germinated seeds were immersed in tap water at 0°C for 24 h to shorten the chromosomes and then fixed in acetic acid-ethanol 1:3 for at least 24 h. The fixed root tips were squashed in acetocarmine, destained in absolute alcohol, and C-banded (Giraldez et al. 1979). Squashing in acetocarmine avoids the cell breakage and chromosome dispersion that results from squashing in 45% acetic acid, and the resulting quality of the C-banding is sufficiently high to permit the identification of the large C-heterochromatin blocks.

Anthers having PMCs at metaphase I were fixed in acetic acid-ethanol 1:3, maintained in the fixative for 1-4 months at 3-4°C, squashed, and stained following the Giemsa C-banding technique described by Giraldez et al. (1979).

Table 1. Chromosome rearrangements observed among the plants obtained after pollen irradiation of line 1RSL.

Rearrangements involving the labeled chromosome 1R										Mean frequency of breaks in chromosome 1R ^a	Total number of plants analyzed		
Reciprocal translocations:				Loss of a telomeric C-band		Other changes (ring chromosomes, isochromosomes, telocentrics)	Rearrangements involving only nonlabeled chromosomes						
involving 1R and a submetacentric chromosome		involving 1R and an acrocentric chromosome		1Lt ⁻	1St ⁻		Reciprocal translocations	Other changes (deletions, telocentrics)					
2R	3R	7R	?	4R	5R	6R							
3	2	5	10	9	3	9	12	8	4	3	7	0.64	1089

^aIn 5 plants, chromosome 1R had two breaks (see text).

Fig. 3. Mitotic metaphase cells of plants with reciprocal translocations involving the labeled chromosome 1R. (a, b) Translocations involving chromosome 1R and a metacentric chromosome (2R, 3R, or 7R). (c) Translocation with the breakpoints located in the 1RL telomeric heterochromatin and in 4RL. (d) Translocation with the breakpoints located in the 1R satellite and in 5RL. (e) Translocation involving chromosomes 1R and 5R. (f) Translocation involving chromosomes 1R and 2R (see Fig. 4).

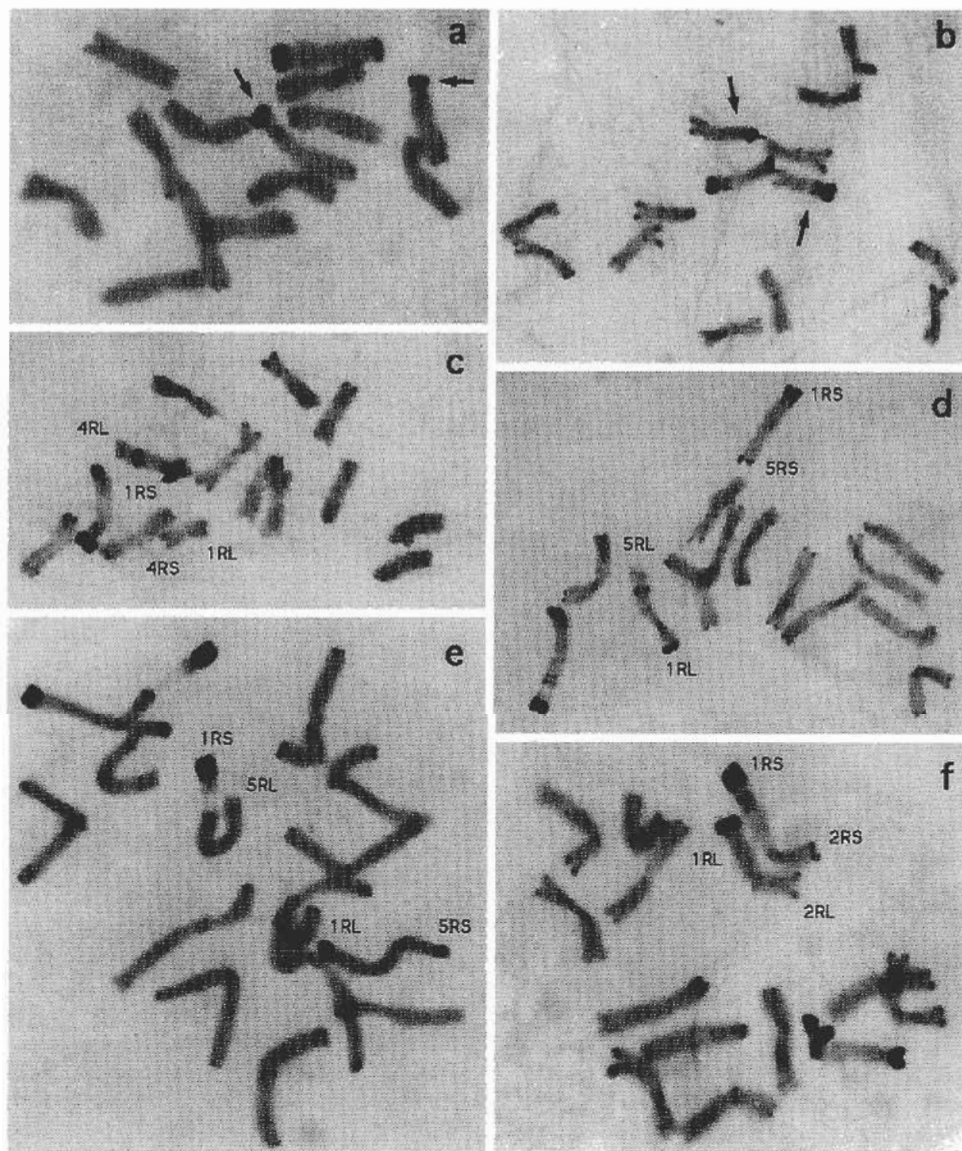


Table 2. The frequency of breaks in the different regions of the labeled chromosome 1R.

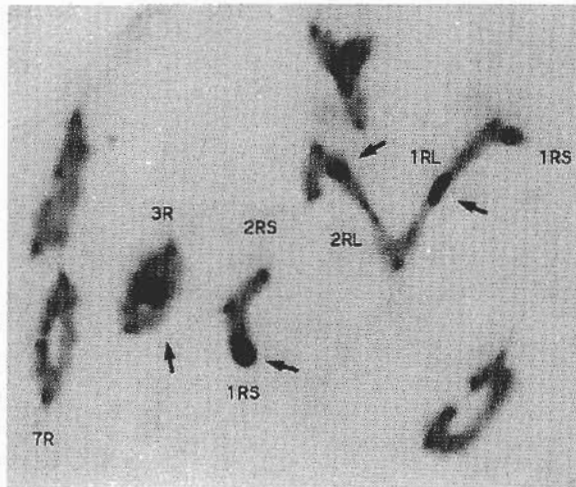
Rearrangement	Chromosome region						
	I	II	III	IV	V	III, IV, or V	VI
Deletion ^a	5						7
Ring chromosome ^b	3						3
Isochromosome + telocentric				1			
Nonreciprocal translocation		1	1		2		
Reciprocal translocation ^a	4	2	4		5	27	1
Total	12	3	5	1	7	27	11

Note: I, the telomeric C-band of the short arm (including the boundary between the heterochromatin and the euchromatin); II, the euchromatic region of the satellite; III, the short arm excluding the satellite; IV, the centromere; V, the euchromatic region of the long arm; and VI, the telomeric C-band of the long arm (including the boundary between the heterochromatin and the euchromatin).

^aTwo plants had a deletion and a reciprocal translocation.

^bRing chromosomes originated from two breaks at the boundaries of the telomeric C-heterochromatin blocks.

Fig. 4. Metaphase I cell of a plant obtained from the cross between the reciprocal translocation shown in Fig. 3f and a line having only two large blocks of C-heterochromatin, one in the telomere of chromosome arm 3RS and the second in an interstitial position in chromosome arm 2RL. The relative positions of the large C-bands (arrows) indicate that the translocation involves chromosomes 1R and 2R, the new telomere constitution of the translocated chromosomes being 1RS-2RS and 1RL-2RL.



Results

A total of 1859 seeds were produced using pollen from the irradiated spikes of line 1RSL, of which, 1113 germinated. The C-banded mitotic karyotypes were obtained for 1089 plants grown from these seeds. The chromosome rearrangements observed in these plants are summarized in Table 1. About 50% of the plants with chromosome rearrangements in mitosis flowered and were fertile.

Evidence of a reciprocal translocation involving the labeled chromosome 1R was found in C-banded mitotic karyotypes of 41 plants (Fig. 3). Meiosis was studied in 15 of these plants. A quadrivalent involving chromosome 1R was observed in all

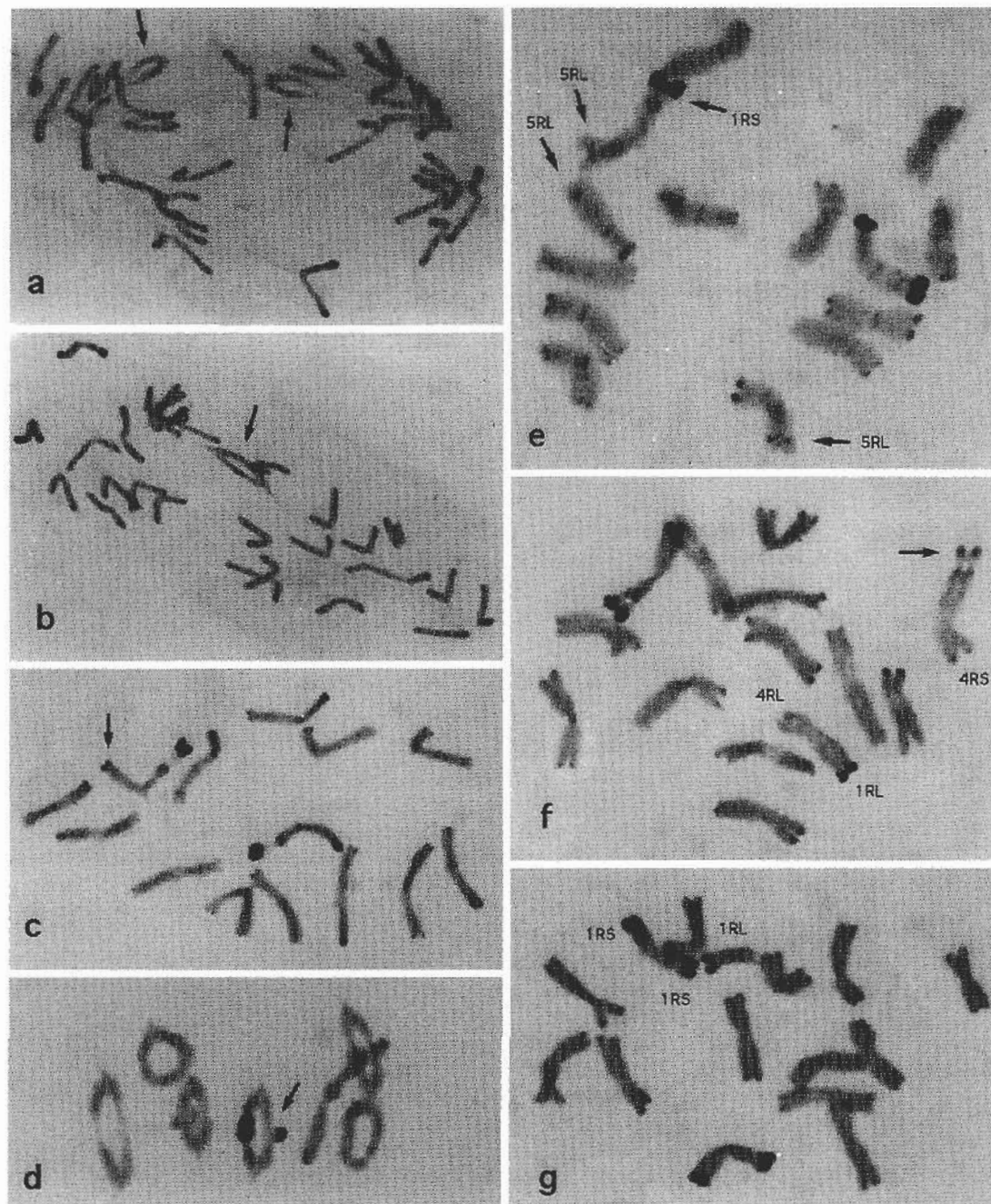
these plants at metaphase I. A second quadrivalent involving two nonlabeled chromosomes was observed in one plant.

Identification of the nonlabeled acrocentric chromosomes 4R, 5R, and 6R involved in reciprocal translocations with chromosome 1R was possible in all cases because of differences in C-banding patterns among these three chromosomes. However, identification of the submetacentric chromosomes 2R, 3R, and 7R was more difficult because of their similar C-banding patterns. Identification was achieved by analyzing metaphase I chromosomal configurations formed in plants derived from crosses between translocation lines in which these metacentric chromosomes were involved and lines in which only chromosomes 2R and 3R were labeled with large specific C-bands (Fig. 4). The frequency with which each nonlabeled chromosome was involved in reciprocal translocations with chromosome 1R is shown in Table 1.

In 20 plants, the C-heterochromatin block of the telomere of either the short (1RS) or the long (1RL) arm of the labeled chromosome 1R was completely or partially lost (Table 1). A more detailed analysis was carried out in 16 of these plants. It revealed that they were a heterogeneous group. Four plants (Fig. 5e) possessed nonreciprocal translocations probably originating from lesions produced in the last G2 phase and unequal chromatid segregation in the following second pollen mitotic division. Ten plants revealed single terminal deletions; seven of these (Fig. 5c) had the breakpoint in the telomeric heterochromatin and three (Fig. 5d) had the breakpoint in the boundary between euchromatin and heterochromatin. Two plants (Fig. 5f) had double-break rearrangements of 1R, including a heterochromatin deletion and a reciprocal translocation.

Two other rearrangements of the labeled chromosome 1R were found in four plants. In three of them, a ring chromosome was observed (Figs. 5a and 5b). These mutant chromosomes were the result of two breaks located at the boundaries between euchromatin and telomeric heterochromatin. The fourth plant possessed an isochromosome for the short arm of 1R and a telocentric chromosome for 1RL (Fig. 5g). Both chromosomes were probably derived from a single break within the centromere.

Fig. 5. (a, b) Mitotic anaphase cells of a plant carrying a 1R ring chromosome (arrows). In b there is evidence of a chromatid exchange being produced (arrow). (c) Mitotic metaphase cell of a plant showing a partial loss of the 1RL C-heterochromatin block (arrow). (d) Meiotic metaphase I cell of a plant showing a total deletion of the 1RL C-heterochromatin block (arrow). (e) Mitotic metaphase cell of a plant showing a nonreciprocal translocation. Three dosages of the distal segment of chromosome arm 5RL are present and one copy of the distal region of chromosome arm 1RL is lacking. (f) Mitotic metaphase cell of a plant showing evidence of two breaks in chromosome 1R, including a translocation with chromosome 4R and a deletion of the 1RS C-heterochromatin block (arrow). (g) Mitotic metaphase cell of a plant showing simultaneously a 1RS isochromosome and a 1RL telocentric chromosome.



The breaks in chromosome 1R occurred in six different regions: I, the telomeric C-heterochromatin block of the short arm (including the heterochromatin-euchromatin boundary); II, the euchromatic region of the satellite; III, the short arm excluding the satellite; IV, the centromere; V, the euchromatic

region of the long arm; and VI, the telomeric C-heterochromatin block of the long arm (including the heterochromatin-euchromatin boundary). Table 2 shows the distribution of breaks among these chromosome regions in 62 plants in which detailed mitotic and (or) meiotic analyses were carried out.

Discussion

In this work, only a small fraction of the possible rearrangements in chromosome 1R resulting from double breaks (3 ring chromosomes and 2 chromosomes with a telomeric heterochromatin loss and a translocation) would have been identified. With the protocol used, it is not possible to distinguish double-break rearrangements, such as paracentric inversions, small interstitial deletions, or interstitial translocations. However, probably all viable rearrangements arising from a single break in the labeled chromosome 1R (reciprocal translocations and terminal deletions) were detected. If it is assumed that with x-ray irradiation, exchange aberration events follow a Poisson distribution (Preston 1991), the detected mean frequency of breaks in the labeled chromosome (0.64%; Table 1) should be only slightly lower than the actual one. The distribution pattern of the breakpoints (Table 2) indicates that in all deletions, the breakpoints were localized in the telomeric C-heterochromatin or very close to it. Deletions of larger segments were probably lethal in heterozygotes. In about 10% of the 1R reciprocal translocations, the breakpoint was located in the telomeric C-heterochromatin blocks, a value that agrees with the relative lengths of these segments.

With respect to the frequency with which the different non-labeled chromosomes were involved in the 1R reciprocal translocations, the results (Table 1) are not significantly different from those expected under the hypothesis in which all non-labeled chromosomes have the same probability (1/6) of being involved ($\chi^2 = 3.537$; $0.5 > p > 0.3$; chromosomes 2R, 3R, and 7R grouped).

Since two nonhomologues are involved in the formation of a given reciprocal translocation, 21 different reciprocal translocations can be formed with the seven chromosomes of rye, chromosome 1R being involved in 6 of them. If all chromosomes have the same probability of participating in a given reciprocal exchange, the 43 1R translocations detected (including the two cases having both a reciprocal translocation and a deletion; Fig. 5f) would represent 6/21 of the total number of translocations produced. If this assumption is correct, approximately 107 translocations are expected between any two nonlabeled chromosomes. However, only three reciprocal translocations between two nonlabeled chromosomes (Table 1) were unequivocally detected, using alterations in length and (or) arm ratio of their metaphase chromosomes. This is in agreement with the results Gecheff (1996) obtained in barley, in which a large portion of the gamma-induced translocations resulted in the exchange of approximately equal chromosome segments. A similar trend was also observed in the 1R reciprocal translocations obtained here. Indeed, in a large number of these translocations (27 out of 43; Table 2), differentiation between breaks located in segments III, IV, and V could not be established, because the translocated chromosomes had lengths and arm ratios similar to those of the normal ones. This tendency can also be deduced from the observation that in the translocations having the breakpoint in the satellite, the length of this segment was not modified, or only slightly so (see Fig. 3d). In the production of a reciprocal translocation, two breaks are required, one in each of the chromosomes involved. If close proximity between the two breaks is necessary, the apparent excess of translocated segments that are similar in length could be the result of a nonrandom spatial

arrangement of the chromosomes in the interphase nucleus. A relic telophase arrangement of chromosomes (see Fussell 1984) would explain the tendency observed.

The irradiation-induced rye translocations obtained so far (Sybenga 1995) represent the small fraction of translocations involving changes in chromosome morphology. The use of lines having the appropriate combination of telomeric C-heterochromatin bands can be an efficient way of detecting almost all viable reciprocal translocations involving specific chromosomes, providing the possibility of reaching a greater degree of marker saturation, which can be of considerable interest in constructing detailed physical maps, among other possibilities. Plants homozygous or heterozygous for the translocations obtained can readily be recognized at mitosis and thus these translocations can be easily maintained.

Acknowledgments

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