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# Mapping of genes for male-fertility restoration in 'Pampa' CMS winter rye (*Secale cereale* L.)

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**Abstract** Hybrid rye breeding and seed production is based on the cytoplasmic male sterility (CMS)-inducing Pampa (P)-cytoplasm. For restoring male fertility in the hybrids, dominant, nuclear restorer genes are necessary. However, current pollinator lines are only partial restorers. Effective restorers were recently detected in the German inbred line L18 and in materials originating from the Argentinian rye cultivar Pico Gentario and an Iranian primitive rye accession called IRAN IX. $F_2$ populations were developed for each of these three restorer sources to map the responsible genes by means of RFLP (restriction fragment length polymorphism) markers. For this purpose, homo- and heterologous DNA probes were used leading to 101 polymorphic marker loci in total. For phenotypic evaluation, 100 to 134 randomly chosen plants from each of the populations were cloned and grown at two or three locations with two plants each. Segregation ratios of pollen fertility in the $F_2$ populations with L18 and IRAN IX were in accordance with a monogenic dominant inheritance. The segregation pattern for Pico Gentario indicated complementary gene action. Major dominant restorer genes were detected on chromosomes 1RS (L18) and 4RL (Pico Gentario, IRAN IX). The gene on 1RS explained 54% of the phenotypic variation and that on 4 RL 59% and 68% in the Pico Gentario and IRAN IX populations, respectively. Additionally, three minor genes from L18 were identified on chromosomes 3RL, 4RL and 5R. In the Pico Gentario population, a dominant modifier gene contributed by the female parent was found on chromosome 6R. This gene significantly enhanced the expression of the major restorer gene but on its own was not able to restore any degree of fertility. The map-distances between the major restorer loci and at least one flanking marker were small in all three $F_2$ populations (5–6 cM). In Pico Gentario an unfavorable linkage exists between the major restorer gene and a QTL for plant height. Since highly effective restorers are scarce in actual breeding populations, the major restorer genes detected on chromosomes 1 RS and 4RL should be introgressed into actual restorer lines. This is facilitated by using the closely linked molecular markers described.

**Keywords** CMS · Genetic resources · Hybrid rye · RFLP markers · Restorer genes

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## Introduction

In rye (*Secale cereale* L.) the first hybrid varieties were released in Germany in 1984. At present, 16 varieties are on the official list (Bundessortenamt 1999), occupying almost 60% of the total rye acreage. Some of these hybrids are also registered in Austria, Belgium, Denmark, Finland, France, The Netherlands, Norway and Sweden. In Sweden, Poland, Belorussia, Russia and Australia independent hybrid-rye breeding programs have been initiated. Commercial hybrid-seed production is based on the cytoplasmic-genic male sterility (CMS) induced by the Pampa(P)-cytoplasm from Argentinian primitive rye

(Geiger and Schnell 1970). The sterility is a maternally inherited trait characterized by degenerated anthers producing no, or only traces of, pollen. As in many other crops, the CMS of rye is associated with an alteration of the mitochondrial DNA (Tudzinsky et al. 1986). For restoration of male fertility, dominant, nuclear restorer genes are necessary. A first restorer line (L 18) was previously found shortly after the detection of the CMS source (Geiger 1972). However, this line had a very poor agronomic performance and, therefore, was neither used in any hybrid variety nor as a donor genotype for the development of improved pollinator lines.

Restorer genotypes for the P-cytoplasm occur at a frequency of 1–5% in European population varieties, but most of them only partially restore the hybrids leading to a pollen shedding of 45 to 72% compared to that of a population variety with normal (N) cytoplasm (Yuan 1995). The degree of fertility restoration mediated by such partial restorer lines considerably depends on environmental effects and seed-parent by pollinator-line interaction (Geiger and Miedaner 1996). Under rainy weather conditions at flowering, an incomplete male-fertility restoration favors infections of the pistils with ergot (*Claviceps purpurea*) spores and may cause considerable quality reductions.

The number of genes involved in restoration and their mode of action is still unclear. In experiments with line L18, Madej (1975, 1976) observed a segregation pattern fitting the hypothesis of two dominant restorer genes with complementary gene action. In contrast, Scoles and Evans (1979) found three dominant restorer genes with different levels of dominance and no epistasis. New, more effective restorer sources have recently been detected in an Argentinian population variety and an Iranian primitive rye accession (Geiger and Miedaner 1996). The first lines derived from these exotic populations revealed a higher and environmentally stable level of restoration in a preliminary test across three locations and four seed parents (Geiger and Miedaner 1996). These new sources of fertility restoration are presently used for the development of improved pollinator lines. However, transferring restorer genes by traditional backcrossing is costly and time consuming, because it requires extensive testcrossing to male-sterile seed parents in each backcross generation. Therefore, reliable genetic markers of fertility restoration would greatly facilitate and accelerate the breeding process.

Using translocation studies and isozyme and molecular-marker mapping, several restorer genes have been located in the rye genome. For the alternative CMS-inducing G-cytoplasm, one major gene and two modifying loci were located on chromosomes 4R, 3R and 6R, respectively, using primary trisomics of the rye variety Esto (Melz and Adolf 1991). The location of the gene on chromosome 4R was recently confirmed by RFLP (restriction fragment length polymorphism) markers (Börner et al. 1998). For line L 18, a major restorer gene for the P cytoplasm has been detected on chromosome 1R linked to the *Prx* (peroxidase)-7 locus with a recombination value of about 20% (Wricke et al. 1993).

In this study, we used RFLP markers (1) to map the restorer genes of line L18 and of two early generation inbred lines tracing back to Argentinian and Iranian rye populations, respectively, and (2) to study the mode of inheritance of these geographically diverse sources of male-fertility restoration.

## Materials and methods

### Plant materials

Three $F_1$ genotypes were produced by crossing male-sterile non-restorer (maintainer) genotypes in P-cytoplasm with restorer line L18 or a single male-fertile plant from each of the two restorer sources Pico Gentario and IRAN IX, respectively. Each of the three $F_1$ genotypes was selfed for the production of $F_2$ mapping populations. Since the three $F_1$ genotypes were each represented by just one single plant, expected gene frequencies should be 0.5 at all segregating loci as in ordinary $F_2$ populations (for more details see Miedaner et al. 1997). Line L18 was obtained within the Hohenheim hybrid-breeding programme and belongs to the Petkus gene pool. Pico Gentario and IRAN IX are self-incompatible rye populations from Argentina and Iran, respectively. Pico Gentario was obtained from the Botanical Garden of the Polish Academy of Sciences, Warsaw, Poland. IRAN IX is a population from Elburz-Karaj collected by Kuckuck (1956) and was provided by the gene bank of the ''Bundesforschungsanstalt für Landwirtschaft (FAL)'', Braunschweig, Germany. For brevity, the names of the restorer sources (L18, Pico Gentario, IRAN IX) will be used to designate the mapping populations in this paper.

From each $F_2$ population 131 (L18), 134 (Pico Gentario), or 100 (IRAN IX) single plants were randomly collected and cloned to produce five or seven plants per clone. One plant was used for collecting leaf samples for DNA extraction and the remainder for the evaluation of male fertility.

### Male-fertility assessment

For evaluation of male fertility, field trials were conducted at two or three locations (Table 1). The experiments were laid out in a randomized complete block design with two replicates and individual plants representing ''plots''. To minimize losses, plants were raised in the greenhouse and transplanted into the field by hand at the beginning of tillering in late autumn. Rows and plants within rows were spaced at about 20 $cm^2$.

The level of male fertility was assessed by visually scoring the size of the anthers and their degree of dehiscence on a 1 to 9 scale according to Geiger and Morgenstern (1975). Scores 1, 2, and 3 refer to non dehiscent, empty anthers with decreasing levels of degeneration, scores 4, 5, and 6 to partially male-sterile plants with about <10%, 11–50%, and >50% male–fertile anthers, respectively, and scores 7 to 9 to fully pollen-shedding anthers of increasing anther size. Score 9 corresponds to fully male-fertile plants in N-cytoplasm. Data analyses were based on averages of the ratings of two to three main tiller spikes per plant at middle and late flowering. These average anther scores are highly correlated ($r > 0.9$, $P = 0.01$) with the amount of pollen produced (Geiger et al. 1995). Additionally, the height of each plant was measured.

### RFLP assays

The plants used for collecting leaf samples were raised in the greenhouse. Immediately after cutting, leaf samples were frozen at –80 °C and afterwards lyophilized, ground in a mill, and stored at –80 °C until analysis. Genomic DNA was isolated from leaf mate-

**Table 1** Mapping populations, the number of $F_2$ plants, the environments used for assessing male-fertility scores, mapped probes, the average degree of polymorphism, and the total genome length

| Mapping population | No. of $F_2$ plants | Phenotypic data | | Marker data | | |
|---|---|---|---|---|---|---|
| | | Year | Locations[a] | No. of mapped probes | Level of polymorphism (%)[b] | Covered genome length (cM) |
| L18 | 131 | 1994 | EWE, OLI, HOH | 52 | 69 | 678 |
| Pico Gentario | 134 | 1994 | EWE, OLI | 55 | 88 | 903 |
| IRAN IX | 100 | 1995 | PET, BER | 21 | 81 | 267 |

[a] EWE = Eckartsweier near Kehl/Rhein, HOH = Stuttgart-Hohenheim, OLI = Oberer Lindenhof near Reutlingen, PET = Petkus near Berlin, BER = Bergen near Celle

[b] Percentage of polymorphic probes with at least one of the four restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hind*III) used

rial of each plant according to Saghai-Maroof et al. (1984) with minor modifications and digested with restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hind*III. RFLP analysis was carried out according to standard methods. Hybridization of membranes was performed with single- or low-copy genomic or cDNA probes of wheat, barley or rye. Wheat probes (*PSR*) were kindly provided by the John Innes Centre, Norwich, UK (Devos et al. 1993), barley probes (*MWG*) by the ''Bundesanstalt für Züchtungsforschung'', Grünbach, Germany (Graner et al. 1991), rye probes by the University of Hannover, Germany (*IAG*, Philipp et al. 1994), and the Institute of Genetics and Cytology, Academy of Sciences of Belorussia, Minsk, Belorussia (*SCB*, Korzun et al. 1998). The cDNA clone of the maize restorer gene *Rf2* (Cui et al. 1996) was shared by Dr. P. Schnable (Iowa State University, Ames). For analyzing populations L18 and Pico Gentario, probes were radioactively labeled with $^{32}$P-dCTP (Amersham, Braunschweig), for IRAN IX with Digoxigenin-dUTP and detected by means of the chemiluminescence antidigoxigenin-AMPPD protocol of Hoisington et al. (1994).

### Statistical analyses

Analyses of variance of the field data were computed for each location and combined across locations using standard procedures (Snedecor and Cochran 1989). Since the male-sterility data significantly deviated from normality, all statistical tests are only approximate for this trait. Data transformation did not lead to a more favorable distribution.

At each marker locus deviations from expected Mendelian segregation ratios (1:2:1, 3:1) and from an allele frequency of 0.5 were tested by the standard $\chi^2$ method (Snedecor and Cochran 1989). Because multiple tests were performed, appropriate Type-I error levels were determined by the sequentially rejective Bonferroni procedure (Holm 1979). Linkage maps were constructed for each population by applying the software package MAPMAKER V3.0 (Lincoln et al. 1993). Linkage between two markers was declared significant in the two-point analyses when the LOD score exceeded the threshold of 3.0. After determination of linkage groups and linear arrangement of the marker loci along the chromosomes, recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgan (cM) by Haldane's (1919) mapping function.

For each marker, a one-way analysis of variance (ANOVA) based on the entry means across locations was performed using the GLM procedure (SAS 1988) for each marker to test whether the phenotypic traits were significantly ($P$ = 0.01) different between marker classes. All individual plants of each marker class were designated as male sterile when the anther score was 1–3 or male fertile when the anther score exceeded 3.5. Owing to the varying numbers of individuals in each marker class, the Scheffé test (Snedecor and Cochran 1989) with an error probability of $P$ = 0.01 was used.

QTL analyses were performed with the software package PLABQTL (Utz and Melchinger 1996) using the regression approach of composite interval mapping (Haley and Knott 1992). A QTL with a LOD score ≥ 2.5 was declared significant. The phenotypic variance explained by a single marker (ANOVA) or marker interval (QTL mapping) was obtained from the square of the partial correlation coefficient ($R^2$). Estimates of total LOD score and total $R^2$ were computed by simultaneously fitting of all putative QTLs affecting the respective trait considering the interactions of all possible loci pairs. Estimates of QTL positions were obtained at the point where the LOD score assumed its maximum in the region under consideration (see Fig. 1).

## Results

### Level of polymorphism

The level of polymorphism detected by at least one of the restriction enzymes employed was high ranging from 69% to 88% (Table 1). The total genome length covered by these probes was 678 cM for L18, 903 cM for Pico Gentario, and 267 cM for IRAN IX. The latter population was only analyzed by those 21 probe-enzyme combinations that yielded markers with significant linkage to restorer loci in the other two populations.

### Phenotypic variation

Pollen-fertility means across locations were bimodally distributed in all three $F_2$ populations with peaks in the male-sterile and fully male-fertile classes (Table 2). None of the observed frequencies of male-fertility scores fitted to a normal distribution. The highest proportion of male-fertile plants was found in the population IRAN IX. The observed segregation ratios were in accordance ($\chi^2$ test) with a monogenic dominant inheritance of male-fertility restoration in case of populations L18 and IRAN IX. In Pico Gentario the best goodness-of-fit was reached when a two-gene model was applied with dominant and partially complementary inheritance (9:3:4 segregation ratio) of restoration.

For male-fertility restoration, significant differences existed between locations but genotype × location interactions were negligible in all three populations. Correspondingly, coefficients of correlation between environments were high ($r \geq 0.9$, $P$ = 0.01) throughout. Plant height was normally distributed in all three $F_2$ popula-

**Table 2** Frequency distribution (%) of mean male-fertility scores (1–9) in three $F_2$ mapping populations (classes with full male fertility are printed in bold) and $\chi^2$ tests for two Mendelian segregation ratios

| Mapping population | Male-fertility score[a] | | | | | | | | | | Tested segregation ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | **7** | **8** | **9** | Mean | 3:1[b] | 9:3:4[c] |
| | – – – – % – – – – | | | | | | | | | | – – – *P*[d] – – – | |
| L18 | 13 | 13 | 4 | 6 | 10 | 11 | **21** | **18** | **4** | 5.10 | 0.23 | 0.00 |
| Pico Gentario | 3 | 14 | 4 | 8 | 4 | 7 | **12** | **29** | **18** | 6.03 | 0.28 | 0.67 |
| IRAN IX | 1 | 6 | 11 | 1 | 5 | 1 | **2** | **4** | **69** | 7.35 | 0.11 | 0.00 |

[a] 1 = highly degenerated, non deshiscent, empty anthers; 9 = full-sized, abundantly pollen-shedding anthers
[b] Based on a one-gene model with male-fertility scores 4–9 being considered as male-fertile
[c] Based on a two-gene model with male-fertility scores 4–6 and 7–9 being considered as partially male-fertile and male-fertile, respectively
[d] *P* = probability level. Rejection of null hypothesis if $P < 0.05$

**Table 3** Chromosomal localization of RFLP markers significantly ($P = 0.01$) associated with male-fertility restoration in three $F_2$ populations and mean fertility scores (1–9) of the restorer, non-restorer, and heterozygous marker classes (estimates obtained by one-way analysis of variance). Data underlined refer to a restorer gene contributed by the female parent

| Mapping population | Chromosome | Marker | Marker class[a] | | |
|---|---|---|---|---|---|
| | | | Restorer | Heterozygous | Non-Restorer |
| | | | – Male-fertility score (1–9)[b] – | | |
| L18 | 1 RS | *PSR 596* | 5.9 a | 6.3 a | 1.8 b |
| | 3 RL | *PSR 1077* | 6.3 a | 5.6 a | 3.3 b |
| | 4 RL | *PSR 119* | 6.5 a | 4.5 b | 4.9 b |
| | 5 R | *PSR 929* | 6.1 a | 5.7 a | 4.1 b |
| Pico Gentario | 4 RL | *MWG 59* | 7.2 a | 7.2 a | 2.5 b |
| | 6 RL | *PSR 371* | 4.7 a | 6.6 b | 6.2 b |
| IRAN IX | 4 RL | *MWG 573* | 8.6 a | 8.5 a | 3.3 b |

[a] Different letters within a row designate significantly ($P \geq 0.05$) different means (Scheffé-Test)
[b] 1 = highly degenerated, non deshiscent, empty anthers; 9 = full-sized, abundantly pollen-shedding anthers

tions. For this trait, genotypic variance components were highly significant ($P = 0.01$) and coefficients of heritability were high in all populations, ranging from 0.80 to 0.92.

## Chromosomal localization, gene action and interaction

In mapping population L18, four restorer loci were detected by one-way analysis of variance (Table 3). According to the male-fertility scores for the three marker classes of the most closely linked markers, one of the restorer genes (on chromosome 1RS) can be considered as a major gene and the remainder as minor genes. Marker class differences indicated complete dominance of restoration for the major and two of the minor genes, and recessiveness of the remaining minor gene. In the Pico Gentario population, two markers were significantly associated with male fertility indicating that a major restorer gene is located on chromosome 4RL and a minor gene on 6RL. In the IRAN IX population, one marker on chromosome 4RL exhibiting dominant gene action was associated with male fertility.

These findings of the one-way analysis of variance agreed well with the results of composite interval mapping (Table 4). In the $F_2$ population L18 also four loci being significantly linked with male-fertility restoration were detected. About 54% of the phenotypic variation was explained by the markers co-segregating with the dominant restorer gene on chromosome 1RS. The three minor genes also showed high degrees of dominance. The only exception to the results of the one-way analysis concerned the minor restorer gene on chromosome 4R. This gene was positioned between markers *PSR 392* and *PSR 167* by interval mapping but was most closely linked to marker *PSR 119* by one-way analysis of variance. However, *PSR 392* and *PSR 119* have an estimated map distance of 3 cM only. In a simultaneous fit of all four intervals detected in the $F_2$ population L18, 66% of the total phenotypic variation could be explained. For chromosome 1, interval mapping identified the point where the LOD score assumes its maximum in close vicinity to the RFLP marker *PSR 596* (Fig. 1A).

In the Pico Gentario population, two marker intervals were significantly associated with male fertility. The locus on chromosome 4RL explained 59% of the phenotypic variation whereas the second locus on chromosome 6RL had only a minor effect on male-fertility restoration. The latter restorer allele was contributed by the CMS parent. The major restorer gene was almost completely dominant, the minor gene showed overdominance. For the major gene, the maximum LOD score was closely flanked by the markers *MWG 59* and *PSR 899* (Fig. 1B).

In the IRAN IX population, one marker interval on chromosome 4RL was closely associated with male fertility, explaining 70% of the phenotypic variation and exhibiting a high level of partial dominance. The respective marker interval was 5 cM (Table 4).

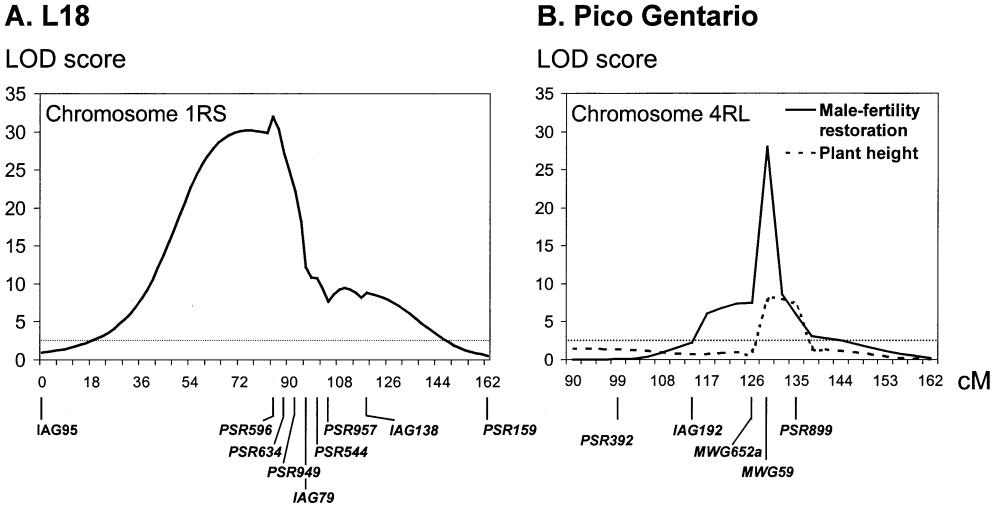


**Fig. 1A, B** LOD score curves and the respective markers for male-fertility restoration obtained by composite interval mapping. **A** Chromosome 1RS in the L18 population, **B** chromosome 4RL in the Pico Gentario population. The *broken line* refers to plant height. *Horizontal lines* mark a LOD score level of 2.5

**Table 4** Estimates of marker intervals, maximum LOD scores, portions of phenotypic variance explained by markers ($R^2$), and genetic effects of QTLs for male-fertility restoration (scores 1–9) in three $F_2$ populations. Negative additive effects refer to restorer genes contributed by the female parent. Estimates were obtained by composite interval mapping

| Mapping population | Chromosome | Marker interval | Length of marker interval (cM) | Max. LOD | $R^2$ [%] | Genetic effects | |
|---|---|---|---|---|---|---|---|
| | | | | | | Additive | Dominant |
| | | | | | | – – – 1 – 9[a] – – – | |
| L18 | 1 RS | *PSR 596 - PSR 634* | 4.6 | 32.0 | 53.7 | 2.0 | 2.0 |
| | 3 RL | *PSR 1077 - PSR 170* | 11.8 | 5.7 | 17.0 | 0.9 | 0.6 |
| | 4 RL | *PSR 392 - PSR 167* | 43.6 | 2.9 | 9.3 | 0.8 | 1.0 |
| | 5 R | *PSR 929 - PSR 911* | 15.8 | 3.3 | 10.6 | 0.4 | 0.5 |
| | Total[b] | | | | 65.8 | 3.4 | 3.0 |
| Pico Gentario | 4 RL | *MWG 59 – PSR 899* | 6.0 | 26.1 | 59.3 | 2.5 | 2.2 |
| | 6 RL | *PSR 371 - PSR 915* | 6.0 | 2.8 | 9.4 | –0.3 | 1.2 |
| | Total[b] | | | | 72.1 | | |
| IRAN IX | 4 RL | *PSR 899 - MWG 573* | 5.0 | 22.5 | 67.9 | 3.0 | 2.2 |
| | Total[b] | | | | 70.0 | | |

[a] 1 = highly degenerated, non deshiscent, empty anthers; 9 = full-sized, abundantly pollen-shedding anthers
[b] Estimates were obtained from a simultaneous fit of all QTLs considering interactions of all possible loci pairs

**Table 5** Two-way table of marker class means for male-fertility scores (1–9)[a] in the Pico Gentario population at RFLP marker loci MWG 59 and PSR 371 on chromosomes 4RL and 6RL, respectively; plant numbers are given in brackets, the shading highlights fully male-fertile classes. $M_a$ and $M_b$ designate marker alleles associated with restorer genes originating from the male and female parent, respectively

| Locus | Genotype | **Marker *MWG 59* (4 RL)** | | | Mean |
|---|---|---|---|---|---|
| | | $M_aM_a$ | $M_am_a$ | $m_am_a$ | |
| **Marker** | $m_bm_b$ | 4.6 (6) | 5.6 (10) | 3.5 (10) | 4.6 |
| ***PSR 371*** | $M_bm_b$ | 7.8 (17) | 8.0 (22) | 2.0 (9) | 5.9 |
| **(6 RL)** | $M_bM_b$ | 7.9 (7) | 7.1 (13) | 1.5 (6) | 5.5 |
| | Mean | 6.8 | 6.9 | 2.3 | 5.3 |

[a] 1 = highly degenerated, non deshiscent, empty anthers; 9 = full-sized, abundantly pollen-shedding anthers

Gene action at the two restorer loci mapped in the Pico Gentario population was further analyzed by a two-locus model (Table 5). In this analysis, each restorer locus is represented by its most-tightly linked RFLP marker. The marker class means revealed strong epistatic interaction of the complementary type. Full male-fertility restoration (mean male-fertility score ≥ 7.0) was only achieved in the presence of at least one restorer allele at each of the two loci. In the presence of the non-restorer allele in the homozygous state at the major restorer locus, the minor restorer gene had no significant effect, and in the presence of the non-restorer homozygote at the minor restorer locus, the major restorer gene could only partially restore fertility. This results in a 9:3:4 segregation pattern as confirmed by a goodness-of-fit test (Table 2).

To demonstrate the epistatic interactions between the two restorer genes, the frequency distributions of the male-fertility scores of single plants in the four marker-class combinations with both, one of each of the two, and no restorer gene, respectively, is presented in Fig. 2. Putative recombinants, i.e. single plants with a high male-fertility score but missing any restorer allele or vice versa, were excluded from this analysis. All plants without the major restorer gene from chromosome 4RL

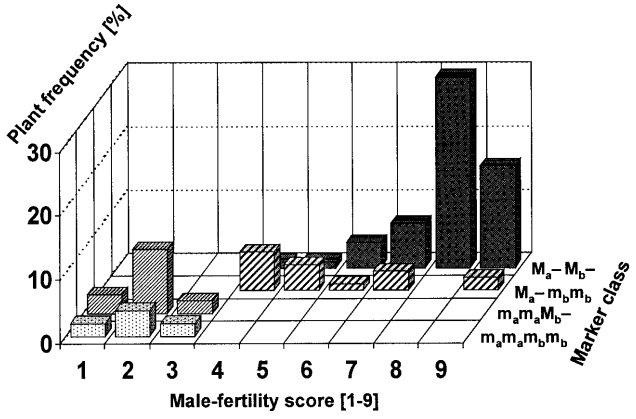


**Fig. 2** Frequency distributions of male-fertility scores (1–9, see Materials and methods) of single plants of the Pico Gentario population in four two-locus marker class combinations without putative recombinants; for explanation of marker allele symbols see Table 5

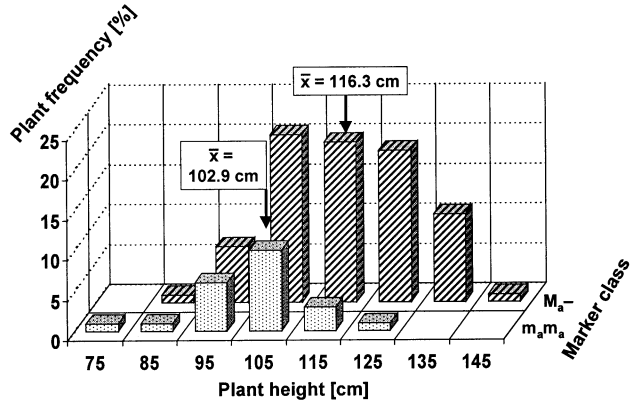


**Fig. 3** Frequency distributions of plant height (cm) of single plants in marker classes $M_a$– and $m_a m_a$ in the Pico Gentario population without putative recombinants; for explanation of marker allele symbols see Table 5

were totally male sterile. Plants possessing the foregoing restorer allele alone ranged in their male-fertility scores from 4 to 9 with no obvious peak, whereas all plants being homo- or heterozygous for both restorer genes showed male-fertility levels from 6 to 9 with two partially male-fertile exceptions only.

### Correlation between male-fertility restoration and plant height

In the Pico Gentario population one QTL for plant height was located in the same genomic region as the major restorer gene on chromosome 4. This QTL accounted for 24.2% of the phenotypic variation with a maximum LOD score of 8.1 (data not shown). The estimated map positions of this QTL and the restorer locus were only about 3 cM apart (Fig. 1B). As a consequence, plant height and male-fertility restoration were significantly associated across locations ($r$ = 0.48, $P$ = 0.01). To underline the importance of this linkage for selection, the distribution of plant height in the marker class without any restorer allele and in the marker classes possessing at least one major restorer allele are compared in Fig. 3. The homozyous non-restorer marker class had a significantly ($P$ = 0.01) lower mean ($\bar{x}$ =102.9 cm) than the marker class with the hetero- or homozygous restorers ($\bar{x}$ =116.3 cm). Both distributions showed similar ranges and standard deviations (11.2 and 12.1 cm, respectively) and did not deviate significantly ($P$ = 0.05) from normality. Similarly, in population L18 a QTL for plant height ($R^2$ = 15.5) was detected in the same region on chromosome 3 as the minor restorer gene, resulting in a phenotypic correlation between both traits of $r$ = 0.41 ($P$ = 0.01).

## Discussion

The improvement of male-fertility restoration in winter rye hybrids is a laborious and time-consuming process since assessing the character requires large-scale testcrossing programs and the male-fertility scores cannot be taken before flowering. In this situation, marker-assisted selection would be of great help to the breeder: the marker data is obtained from the candidates themselves (i.e. not from testcrosses) and the information is available before flowering allowing targeted inter- or backcrossing.

Only a small number of homologous markers was available in rye when we started our study (Philipp et al. 1994). Therefore we mainly used RFLP markers from other cereals. Most markers were highly polymorphic (Table 1) corresponding to the obligate outcrossing behaviour of winter rye. Mapping results were in accordance with the published wheat (Devos et al. 1993) and barley (Graner et al. 1991) maps in most instances (Glass 1997; Dreyer 2000). A great proportion of the probes detected more than one locus in rye. From a total of 115 probes used for screening polymorphisms among the parents of the $F_2$ Pico Gentario population only 31% detected a single locus, 43% two to five loci, and the remainder up to 14 loci (Dreyer 2000).

The chromosomal localization of the major restorer locus of L18 on chromosome 1RS by RFLP markers corresponds well with the previously published map position of this gene near isozyme locus *Prx 7* (Wricke et al. 1993). In this paper, additionally a weak association of male-fertility restoration with the banding pattern of the isozyme *Tpi 1* on chromosome 3R was found. This corresponds to the detection of a minor restorer locus on the same chromosome in our study. However, no map integrating the two marker positions is available at present. From our molecular data, it is not clear whether the restorer loci detected on chromosome 4RL in the Pico Gentario and IRAN IX populations are identical and, if so, whether restoration is caused by the same allele. To test this by a classical-genetic analysis, the restorer lines derived from Pico Gentario and IRAN IX were recom-

bined, the resulting $F_1$ was crossed with a male-sterile tester and the testcross progeny was analysed for restoration. Among 2060 single plants no recombinant could be found, i.e. all plants were fully male-fertile (Wilde and Miedaner, unpublished data). This indicates that both populations possess not only the same restorer gene but most likely also the same allele.

Interestingly, a major restorer gene (*Rfg 1*) for the alternative CMS-inducing G cytoplasm of rye was mapped about 37-cM distal to *PSR 899* (Börner et al. 1998), a marker that is closely linked to the major restorer locus of our populations Pico Gentario and IRAN IX (Table 4). Curtis and Lukaszewski (1993) also identified a gene on chromosome 4RL (*Rfc4*) that restored the CMS-inducing *Triticum timopheevi* cytoplasm in hexaploid wheat using wheat-rye addition lines. They located the gene about 16-cM distally from the telomere. In wheat, two other loci restoring the *T. timopheevi* cytoplasm, *Rf4* and *Rf6*, have been located on the short arms of chromosomes 6 A and 6B (Ma and Sorrells 1995; Ma et al. 1995), a region that is homoeologous to chromosome 4RL (Devos et al. 1993). The RFLP markers associated with *Rf4* and *Rf6* are about 22-cM apart or in close vicinity, respectively, to our major restorer gene in the Pico Gentario population as judged by the co-segregating RFLP marker *PSR899* in the consensus *Triticeae* linkage map of Marino et al. (1996). Together with our data, at least five restorer genes in rye and wheat for three phenotypically different CMS-inducing cytoplasms have been located on the long arm of rye chromosome 4 or the homoeologous wheat chromosomes 6A and 6B. Curtis and Lukaszewski (1993) found a further rye restorer gene for the *T. timopheevi* cytoplasm on chromosome 6RL where we located the minor restorer gene in the Pico Gentario population (Table 4). This minor restorer gene contributed by the female parent was not detected in the IRAN IX population. This might be due to the different female genotypes used for both mapping populations (Lo7-P × Lo6-N vs. Lo6-P for Pico Gentario and IRAN IX, respectively).

In the Pico Gentario population the restorer gene contributed by the female parent alone did not increase male fertility. This is in accordance with the fact that seed-parent lines are strictly selected for sterility maintenance (Geiger 1985). However, the presence of this gene significantly improved the level of male-fertility restoration caused by the major restorer gene (7.7 vs. 5.1). The presence of a ''silent'' restorer gene in a seed-parent line enhancing the efficacy of a major restorer gene of the pollinator line may explain the different levels of fertility restoration among crosses of a single restorer line with several seed-parent lines (Geiger and Miedaner 1996). Similar observations were made in male-sterile wheat lines carrying the CMS-inducing *T. timopheevi* cytoplasm. Genes modifying the expression of major restorer genes were detected by classical (Maan et al. 1984) as well as marker-assisted (Ma and Sorrells 1995) segregation analyses. In the latter case, the male-sterile wheat line carrying the modifier gene is considered to be easily restored. For the breeder this means that a hybrid may show an unsatisfactory level of male fertility if the seed parent is lacking the necessary modifier genes. It remains to be analysed at what frequency modifier genes occur in seed-parent lines and whether significant modifier effects will also occur if the pollinator line carries two major restorer genes such as the ones on chromosomes 1RS and 4RL described above. It is further unclear whether seed-parent lines with modifier genes show an environmentally less-stable sterility maintenance than those without. In maize, fertility restoration of the T cytoplasm is caused by the complementary action of two dominant genes, *Rf1* and *Rf2* (Laughnan and Gabay-Laughnan 1983). As in the Pico Gentario population, the presence of *Rf2* alone does not suffice for restoration. The *Rf2* locus has been mapped in maize in a position homoeologous to rye chromosome 4 (Wise and Schnable 1994) and was recently cloned (Cui et al. 1996). However, a cDNA probe of this gene hybridized with Pico Gentario DNA from the telomeric region of chromosome 4RS, more than 100-cM away from the map position of our major restorer gene on chromosome 4RL (Dreyer 2000).

The dominant inheritance of all major restorer loci detected in our study is an important pre-requisite for their use in practical hybrid rye breeding. Since satisfactory restorers are scarce among actual rye pollinator lines (Geiger and Miedaner 1997), it would be highly desirable to transfer the major restorer genes detected on chromosomes 1RS and 4RL into the current pollinator gene pool. This could be greatly facilitated by using the linked molecular markers described here for selecting the restorer alleles during the backcrossing process. Especially, the restorer locus on chromosome 4RL from Pico Gentario or IRAN IX would be worthwhile to introgress into the actual pollinator gene pool by repeated backcrossing. This gene is easier to select in segregating populations than the oligogenically inherited male-fertility restoration of L18 and has a much better restoration level (Geiger and Miedaner 1997). Fortunately, the map distances between the restorer loci and the nearest marker loci are small enough for a marker-based backcrossing.

The association between the restorer gene on chromosome 4RL and a major QTL for plant height in the Pico Gentario population contributed by the pollinator, however, impedes the transfer of this restorer gene into the pollinator gene pool. Figure 3 demonstrates that about half of the plants possessing the restorer allele would have to be discarded due to their tallness. Thus, much larger backcross populations would be needed to select for both fertility restoration and straw shortness. Markers would again be very useful to determine the desirable genotypes. For an implementation of marker-assisted selection in a practical breeding program PCR-based markers would be preferable. Efforts to develop such markers are under way.

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