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A map of rye chromosome *2R* using isozyme and morphological markers

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Summary. The segregation of isozymic loci for leaf peroxidases (*L2Per*) has been investigated in backcrosses and F_2 offspring of rye lines having purple seeds (*Ps*) and monstrosum ears (*mo*). The *Ps*, *L2Per-3b*, *mo*, and *L2Per-2* loci were linked. The *Ps* and *mo* loci have been previously located on the *2R* chromosome, and the *L2Per-3b* and *L2Per-2* loci have been located on the *2RS* chromosome arm. The results favor the gene order *Ps . . . L2Per-3b . . . mo . . . L2Per-2* or *Ps . . . mo . . . L2Per-3b . . . L2Per-2*. The position of the loci relative to the centromere is still not known, but the obtained results suggest that the *mo* locus could be located on the *2RS* chromosome arm. On the basis of previously reported linkage groups, the most probable arrangement of the loci located on chromosome *2R* is: *dw2 . . . Ps . . . (L2Per-3a . . . L2Per-3b . . . mo) . . . L2Per-2*. It has not been possible to know the position of *L2Per-4* loci (also located on *2RS* chromosome arm) relative to *L2Per-3a* and *L2Per-3b* loci.

Key words: *Secale cereale* L. – Linkage groups – Chromosome *2R* – Leaf peroxidases – Morphological markers

Introduction

In recent years, important progress has been made with the localization of genes on the chromosomes of rye (*Secale cereale* L.) in studies that involved wheat-rye chromosome substitutions, additions, and translocations. In these investigations, the location of genes for resistance to several wheat pathogens, as well as of a number of biochemical and molecular markers, such as isozymes, endosperm proteins, and repetitive DNA sequences, has been established (De Vries and Sybenga 1984).

Although several loci codifying for isozyme and morphological markers have been located on the rye chromosome arms (Bergman and Maan 1973; Tang and Hart 1975; Hart 1979; Rao and Rao 1980; Chojecki and Gale 1982; Salinas and Benito 1984a, b, 1985a, b; De Vries and Sybenga 1984; Schlegel and Mettin 1982; Schlegel et al. 1986), the corresponding genetic and cytogenetic maps are poorly developed. Linkage data are available to date for several morphological markers (De Vries and Sybenga 1984; Schlegel et al. 1986), for a number of isozymes and storage proteins (Singh and Shepherd 1984; Lawrence and Appels 1986; Benito et al. 1990a), and for isozymes and translocations (Figueiras et al. 1985, 1989; Benito et al. 1990b). However, the linkage relationships using isozymic and morphological markers simultaneously are still not available for rye.

In this paper we report on a map of the rye chromosome *2R* including morphological (purple seed and monstrosum ear) and isozyme markers (leaf peroxidases), and also review the linkage groups obtained using peroxidases in rye.

Materials and methods

Plant materials

The following crosses were analyzed in order to map the *2R* chromosome: four F_2-like progenies (named M1, M2, M3, and M4) between rye lines with purple seeds and lines with monstrosum ears, and one backcross between a line having purple seeds and another with normal seeds (named T1).

Electrophoresis

The following methods were used in order to map the *2R* chromosome. The electrophoresis of leaf peroxidases was performed

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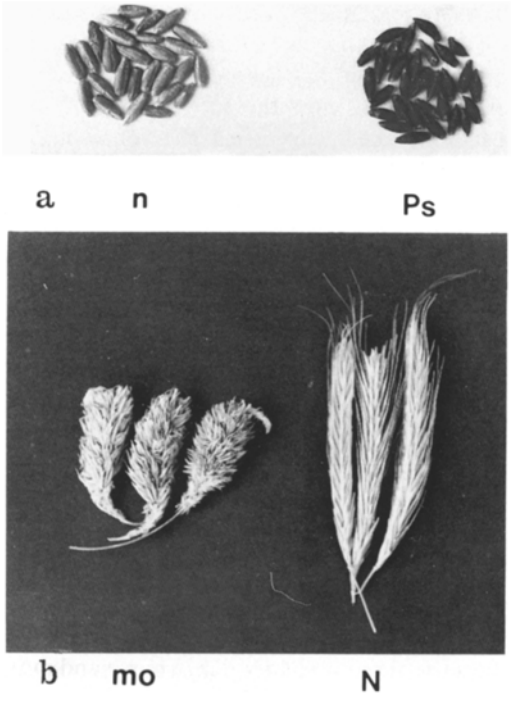

Fig. 1. **a** Phenotypes of the F_2-like parental rye lines with purple seed (*Ps*) and normal seed (*n*). Purple seed is a dominant mutant and normal seed is recessive (*Ps* > *n*). **b** Phenotypes of the F_2-like parental rye lines with monstrosum ears (*mo*) and normal ears (*N*). Monstrosum ear is a recessive mutant and normal ear is dominant (*N* > *mo*)

into horizontal 12% starch gels using the buffers and staining method described by Figueiras et al. (1985) and Selander et al. (1971). The electrode buffer was TRIS-citric acid (0.043 *M*, pH 7.0), the gel buffer was Histidine-ClH (0.006 *M*, pH 7.0), and the gels were electrophoresed at a constant voltage of 150 V for 5 h.

The genetic distances between the loci were estimated using the maximum likelihood method. In the backcross the distance = (recombinant/total progeny) × 100.

Results

The nomenclature used in this paper for the leaf peroxidase loci located on the *2R*S chromosome arm includes the letter of the tissue (*L2Per*).

The phenotypes of the F_2-like parental rye lines having purple seeds and monstrosum ears are shown in Fig. 1. The four F_2-like progenies studies (M1, M2, M3, and M4) segregated for *Ps* (purple seed), *mo* (monstrosum ear), and *L2Per-2* (leaf peroxidase) loci (Table 1). The backcross progeny analyzed (T1) segregated for *Ps*, *L2Per-2*, and *L2Per-3b* loci (Table 1). The *L2Per-2* and *L2Per-3b* loci showed null alleles and it is not possible to distinguish homozygotes for active alleles from heterozygotes for active and null alleles (Fig. 2). The leaf peroxidases exhibited monomeric behavior. The *Ps*, *mo*, and *L2Per-2* loci were linked in the four F_2-like progenies analyzed. The data from these four progenies (M1 + M2 + M3 + M4) can be pooled, since the χ^2 heterogeneity test was not significant at the 5% level (Table 1). The *Ps*, *L2Per-3b*, and *L2Per-2* loci were linked in the backcross (Table 1, Fig. 3a). The linkage relationships between the leaf peroxidase loci (*L2Per*) and the morphological markers (*Ps* and *mo*) are shown in Table 1 and Fig. 3.

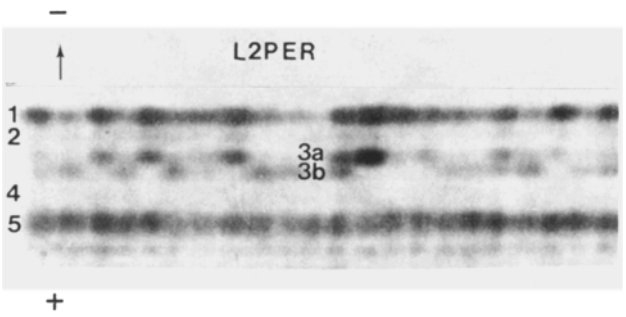

Fig. 2. The segregation of *L2Per-3a* and *L2Per-3b* leaf peroxidase loci in one F_2-like progeny. The leaf peroxidase loci present null alleles; therefore, it is not possible to distinguish homozygotes for active alleles from heterozygotes for active and null alleles

Table 1. Linkage data observed among the loci located on the *2R* chromosome

Loci	Distribution of progeny (phenotype)				χ^2 linkage	Distance (cMorgans)
	++	+−	−+	−−		
Pooled data of the four F_2-like progenies: M1 + M2 + M3 + M4						
mo, Ps	141	52	23	41	29.31***	31.01 ± 3.60
mo, LPer-2	157	55	34	33	12.73***	36.82 ± 3.79
Ps, LPer-2	118	46	63	32	0.85	–
Backcross T1						
Ps, L2Per-3b	1	29	30	20	25.68***	26.25 ± 4.92
LPer-2, LPer-3b	16	14	15	35	4.38*	36.25 ± 5.37
Ps, LPer-2	8	18	22	32	0.73	–

The peroxidase loci show null alleles, therefore, the phenotype − always indicates homozygosity for null alleles; the phenotype + indicates heterozygosity for active and null alleles in the backcross, homozygosity for active allele, and heterozygosity for active and null alleles in the F_2-like progenies. In the case of morphological markers, phenotype + indicates purple seeds or normal ears, and phenotype − indicates normal seed or monstrosum ears

* ($P < 0.05$)

*** ($P < 0.001$)

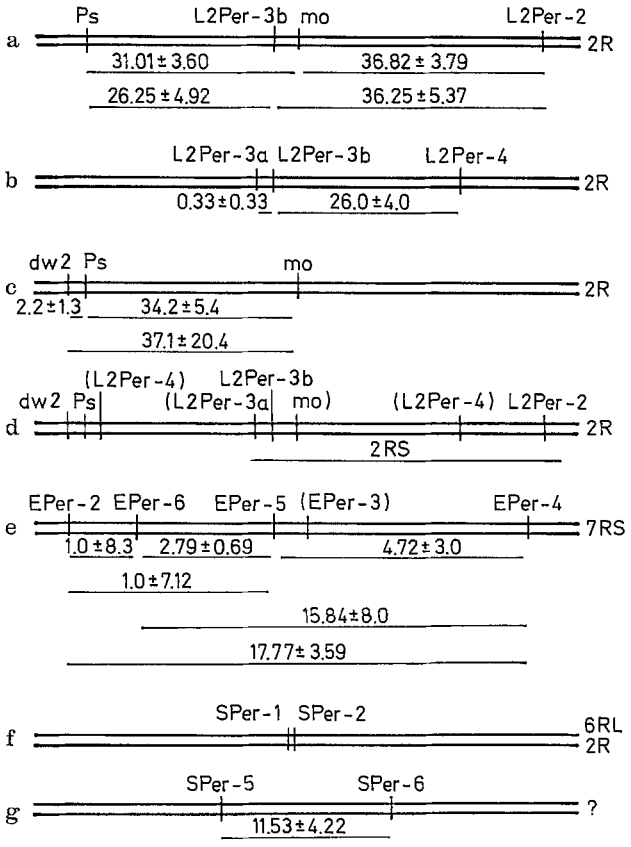

Fig. 3. **a** Most probable map of *2R* chromosome obtained in this work. **b** Linkage relationships between *L2Per* loci previously obtained by Figueiras et al. (1985) and Benito et al. (1990a). **c** Map of chromosome *2R* previously described by De Vries and Sybenga (1984). **d** Most probable arrangement for all the leaf peroxidase and morphological loci located on the *2R* chromosome (map obtained using all previous data simultaneously). Position of centromere unknown. The leaf peroxidase loci (*L2Per*) have been located on the *2RS* chromosome arm. The *L2Per-4* locus could be near the *L2Per-2* locus or near the *Ps* locus. The *L2Per-3a*, *L2Per-3b*, and *mo* loci could show alternative arrangements. **e** Linkage relationships observed between endosperm peroxidase loci (*EPer*) by García et al. (1982). The most probable location for these *EPer* loci is the *7RS* chromosome arm (Salinas and Benito 1984a). **f** and **g** Genetic distances obtained between embryo plus scutellum peroxidases (*SPer*) by García et al. (1982). The *SPer-1* and *SPer-2* loci have been located on chromosome *6R* of "Imperial" rye, the *6RL* chromosome arm of "King II" rye, and on chromosome *2R* of "Dakold" rye (Salinas and Benito 1984b). The chromosomal location of *SPer-5* and *SPer-6* loci is unknown

Discussion

The results obtained here are in agreement with previous results on the genetics of leaf peroxidases. The data from the four F_2-like progenies reveal that the *Ps*, *mo*, and *L2Per-2* are linked. The results favor the gene order: *Ps . . . mo . . . L2Per-2*. The distance obtained between *Ps* and *mo* loci (26.25 ± 4.92 cMorgans) was similar to that previously described (29.7 ± 3.5 cMorgans) by De Vries and Sybenga (1984). The most probable arrangement for the morphological loci mapped by De Vries and Sybenga (1984) on chromosome *2R* is: *dw2 . . . Ps . . . mo* (*dw2* = dwarf habit) (Fig. 3c). The data obtained from the backcross (T1) indicate that *Ps*, *L2Per-3b*, and *L2Per-2* loci are linked, suggesting the following sequence: *Ps . . . L2Per-3b . . . L2Per-2* (Table 1 and Fig. 3a). From all the data obtained in this work, the most probable arrangement proposed for these loci on chromosome *2R* would be: *Ps . . . L2Per-3b . . . mo . . . L2Per-2* (Fig. 3a).

However, because three point tests in which *Ps*, *mo*, and *L2Per-3b* are involved simultaneously have not yet been carried out and similar values have been obtained for the genetic distances (31.01 ± 3.60 cMorgans between *Ps . . . mo* and 26.05 ± 4.92 cMorgans between *Ps . . . L2Per-3b*), the alternative arrangement *Ps . . . mo . . . L2Per-3b . . . L2Per-2* remains possible. Previous data obtained by Figueiras et al. (1985) and Benito et al. (1990a) indicated that *L2Per-3a* and *L2Per-3b* loci were narrowly linked (0.33 ± 0.33 cMorgans) and *L2Per-3a* and *L2Per-4* loci (26.04 ± 4.0 cMorgans) were also linked (Fig. 3b). Because three point tests in which *L2Per-3a*, *L2Per-3b*, and *L2Per-4* loci are involved simultaneously are not available, two alternative arrangements could be suggested: *L2Per-3a . . . L2Per-3b . . . L2Per-4* or *L2Per-3b . . . L2Per-3a . . . L2Per-4*. The *L2Per-2* and *L2Per-3* loci have been located on the *2RS* chromosome arm (Salinas and Benito 1984b; Bosch et al. 1987). These results suggest that the *mo* locus could be located on the *2RS* chromosome arm. Therefore, the most probable arrangement for all the morphological and leaf peroxidase loci located on the *2R* chromosome would be: *dw2 . . . Ps . . . (L2Per-3a . . . L2Per-3b) . . . mo . . . L2Per-2*, where the *L2Per-4* locus is located near to *Ps* or near to *L2Per-2* loci (Fig. 3d). The position of the *L2Per-3a* and *L2Per-3b* loci relative to the *Ps* or *mo* loci still remains unknown. The chromosome arm location of the *Ps* locus, as well as the position of the genes relative to the centromere, were not determined.

The peroxidase isozymes are widely distributed among higher plants and the different tissues show diverse peroxidase patterns. In addition, the peroxidases observed in different tissues are controlled by structural genes located on other homoeologous groups or chromosomes (Benito and Pérez de la Vega 1979; Salinas and Benito 1984b; Bosch et al. 1987; Liu et al. 1990). The peroxidases from leaf (*L1Per* and *L2Per*), endosperm (*EPer*) and those from embryo plus scutellum (*SPer*) are characterized by monomeric behavior and the presence of null alleles. Moreover, in each different tissue several loci for peroxidases have been found, all of them having one active and one null allele. Triplicate sets for peroxidases have been described in hexaploid wheat on the homoeologous groups 1 (leaf peroxidases, *Per-1* = *L1Per*),

Table 2. Rye characters located on chromosome *2R*

Character	Chromosome/arm	References
Pgd-3	2RL	Salinas and Benito 1983
Pm2 Powdery mildew resistance	2RL	Driscoll and Jensen 1963 Lind 1982 Riley and Macer 1966
Gdh-1	2RS	Salinas and Benito 1983
L2Per-1, L2Per-2 L2Per-3a, L2Per-3b, L2Per-4	2RS	Salinas and Benito 1984b Figueiras et al. 1985 Bosch et al. 1986 Benito et al. 1990a
Rfc1 Male sterility restorer	2RS	Hossain and Driscoll 1983
Sec-2 Secalin	2R	Shewry et al. 1985
Ssp1 Salt-soluble protein	2R	Fra-Mon et al. 1984
LEst-2 Leaf esterase	2R	Schmidt et al. 1984
Ps Purple seed	2R	De Vries and Sybenga 1984
mo Monstrosum ear	2R	De Vries and Sybenga 1984
dw2 Dwarf habit	2R	De Vries and Sybenga 1984
Glu Beta-glucosidase	2R	May and Appels 1978
Asi Alpha-amylase subtilisin inhib.	2R	Hejgaard et al. 1984
Sod Superoxide dismutase	2R	Jaaska 1982
el Absent ligula	2R	Smirnov and Sosnichina 1984
Tyr Tyrosinase	2R	Zeven 1972
Lr2 Leaf rust resistance, 25	2R	Driscoll and Jensen 1963
An3 Anthocyanin	2R	Schlegel et al. 1986
an1b Anthocyanin-less	2R	Sturm et al. 1981 Schlegel et al. 1986
Pgd-2	1RL	Lawrence and Appels 1986
Mdh-1, Pgd-2	1R	Figueiras et al. 1985, 1989 Benito et al. 1990a

2 (leaf peroxidases, *Per-2* = *L2Per*), 3 (embryo plus scutellum peroxidases, *Per-3* = *SPer*), and 4 (endosperm peroxidases, *Per-4* = *EPer*).

The loci nomenclature used in hexaploid wheat is problematic when several loci are located on the same chromosome arm. In rye and also in wheat, several loci for peroxidases are shown to be linked; for this reason we preferred to use a loci nomenclature that includes the letter of the tissue, for example: *Per-1* = *L1Per*, *Per-2* = *L2Per*, *Per-3* = *SPer*, and *Per-4* = *EPer*. Five different *EPer* (from *EPer-2* to *EPer-6*) loci are linked (García et al. 1982; Rebordinos and Pérez de la Vega 1987) and are probably located on the *7R*S chromosome arm (Salinas and Benito 1984b; Fig. 3e); the *EPer-1* locus is probably located on the *4R*L chromosome arm (Salinas and Benito 1984b). Two *SPer* loci (*SPer-1* and *SPer-2*) are linked and have been located on the *6R*L chromosome arm of "King II" (Salinas and Benito 1984b), *6R* of "Imperial," and *2R* of "Dakold" rye (Fig. 3f). Another two *SPer* loci are linked (*SPer-5* and *SPer-6*) but their chromosomal location is still not known (Fig. 3g). Therefore, another characteristic of the peroxidase loci of each tissue is that they are closely linked. In Fig. 3 we present a summary of the linkage groups obtained in rye using peroxidases.

Other isozyme, protein, morphological, and resistance loci have been located on chromosome *2R* (Miller 1984; Schlegel et al. 1986; see Table 2). Salinas and Benito (1983) have located a locus for 6-Phosphogluconate dehydrogenase isozymes on the *2R*L chromosome arm; this locus was named *Pgd-2* in the paper of Figueiras et al. (1985) and, as a consequence, in the review of Schlegel et al. (1986). Lawrence and Appels (1986) and also Benito et al. (1990a) related the *Pgd-2* locus to the *1R*L chromosome arm. The *Mdh-1* (malate dehydrogenase) and the *Pgd-2* loci are linked (Figueiras et al. 1985, 1989) on chromosome *1R*; therefore the *Pgd* locus located by Salinas and Benito (1983) is a different locus located on the *2R*L chromosome arm and is not linked to the *Mdh-1* locus. We propose the following name for the *Pdg* locus that is located on the *2R*L chromosome arm: *Pdg-3* (Table 2).

Linkage studies are poorly developed in rye; several isozyme, morphological, and protein markers have been related to the *2R* chromosome, but respective linkage data are not yet available. The exploitation of linkages among different chromosomal markers (isozymes, proteins, morphologicals, C-bands, etc.), molecular markers (RFLPs), and agronomic traits can be intensified to improve rye cultivars and other Triticeae species.

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