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RFLP mapping of the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5 of rye (*Secale cereale* L.)

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Abstract An F_2 population was established for mapping the two dominant genes for dwarfness (*Ddw1*) and hairy peduncle (*Hp*) on chromosome 5R. The location of both genes was shown to be on the segment of chromosome 5RL which was ancestrally translocated and is homoeologous to *Triticeae* 4L. *Hp* co-segregated with the wheat gDNA probe WG199, localised in wheat on chromosomes 5AL, 4BL and 4DL. No segregation was observed between the traits hairy peduncle and hairy leaf sheath. The locus for *Ddw1* was found to map distally to *Hp/Xwg199* but proximal to the isozyme marker *β-amy-R1*. The genetical distances were 5.6 cM between *Hp/Xwg199* and *Ddw1* and 11.5 cM between *Ddw1* and *β-amy-R1*, respectively. The map position of *Ddw1* suggests that it is homoeologous to the wheat dominant dwarfing gene *Rht12*, present on chromosome 5AL and linked to *β-amy-A1*.

Key words Rye · RFLP · Genetic mapping · Dwarfing genes · Hairy peduncle

Introduction

Lodging is still one of the most consistant problems in rye. An improvement in resistance to lodging will reduce the application of growth regulators and, therefore, the costs of production. The main strategy for overcoming the problem is a reduction of plant height by the exploitation of dwarfing mutants. One of them, probably the best known in rye and so far the most frequently used, is the 'EM 1' mutant, which originated from the genebank collection of St. Petersburg and was first described by Kobyljanski in 1972. The dwarfism induced by this mutant is caused by a single dominant gene, originally named *Hl* (Humilus) and later renamed as *Dw1* (Dwarf 1) or *Ddw1* (Dominant dwarf 1) by Melz (1989). This gene was localised using trisomic analysis (Sturm and Engel 1980) on chromosome no. 2, which corresponds to chromosome 5R.

Börner and Melz (1988) studied the response of short-strawed rye plants, including the *Ddw1* mutant, to gibberellic acid and characterised this mutant as GA_3-sensitive. Another dwarfing mutant carrying the gene *ct2* on 5RL (De Vries and Sybenga 1984) was found to be GA_3-insensitive (Börner and Melz 1988). Whereas the locus for *ct2* has been mapped using RFLPs by Plaschke et al. (1993), who confirmed the localisation on 5RL, no mapping data is available for *Ddw1*. The present paper describes the tagging of the GA_3-sensitive dwarfing gene *Ddw1* and the gene for hairy peduncle (*Hp*), known to be located on the long arm of chromosome 5R (O'Mara 1951), using RFLP markers and one isozyme marker (β-amylase).

Materials and methods

Plant materials

A maping population was produced by crossing te line R1620 (tall, smooth peduncle) with R347/1 (dwarf, hairy peduncle). One single F_1 plant, heterozygous for *Ddw1* and *Hp*, was used to produce 140 F_2 seeds from which the embryo halves were germinated on filter paper and, after germination, transferred to pots and grown in the green house. The endosperm halves were used for the isozyme studies. To verify plant height where critical F_2 plants were intermediate in height six F_3 plants were analysed.

Analysis of morphological traits

The final plant heights of the 140 F_2 plants and six plants of each of the critical F_3 progeny were measured just before harvest. The character hairy peduncle was scored at flowering time. In addition a

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segregation for the trait hairy leaf sheath was observed and evaluated at the seedling stage.

DNA clones

A range of cDNA and genomic (g) DNA probes known to be located on *Triticeae* homoeologous group 5 and/or 4L were selected including six gDNA clones from *Pst*I and *Eag*I libraries of wheat (Heun et al. 1991; Devos et al. 1992), four gDNA clones from a wheat leaf *Pst*I library (Harcourt 1992), three cDNA clones from the wheat library described by Chao et al. (1989), two gDNA clones from a *Pst*I library of barley (Graner et al. 1991), one cDNA clone from barley (Anderson et al. 1992), one gDNA clone (Scb35) from a *Pst*I library of rye (Korzun et al. 1994), and one cDNA clone (*Tri a* III) from a gynoecium-specific library of wheat (Balzer et al. 1995).

DNA exctraction and digestion, Southern hybridization

Leaf DNA was extracted from 5 to 6 week-old seedlings by the procedure of McCouch et al. (1988). DNA was cut with the restriction enzymes *Hin*dIII *Dra*I, *Eco*RI and *Eco*RV. Restriction digests, gel electrophoresis, Southern transfer, probe labelling and filter hybridization were performed as described by Devos et al. (1992), except that denaturation of the labelled probe was by the addition of a 1/10 vol of 3 M NaOH.

β-Amylase analysis

Sample extraction, electrophoresis and visualisation were performed as described for wheat by Liu (1991). A half of an individual dry grain (endosperm end) was crushed in a microhammer mill and incubated in 50 µl of 20% sucrose solution containing 0.01 M DTT (DL-Dithiothreitol) at room temperature for 1 h. Flat-bed isoelectric focusing was carried out on 0.25-mm thick, 12-cm wide polyacrylamide gels containing 2% (w/v) ampholyte (Pharmalyte 4.2–4.9, Pharmalyte 4.5–5.4, Servalyte 4–6 in the ratio 1:1:1); 0.04 M L-glutamic acid and 0.1 M NaOH were used for anolyte and catholyte, respectively. Enzyme activity was visualised by soaking the gels in a 2% starch solution for 7 min. Then the starch solution was poured off and the gels were flooded with a solution of 1.5×10^{-3} M iodine, 3.5×10^{-3} M potassium iodide and 3% acetic acid.

Map construction

Linkage relationships and map distances in cM (Kosambi 1944) were estimated using the program MAPMAKER 2.0 supplied by E.S. Lander, Whitehead Institute of Biomedical Research, Cambridge/Mass., USA. Linkage groups were determined using pairwise analyses with a LOD threshold of 3.0. Multipoint analyses comparing candidate orders were used to determine the most likely map.

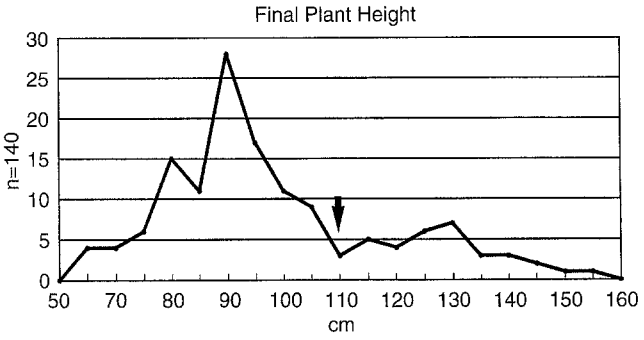


Fig. 1 F_2 segregation pattern for final plant height of the cross 'R1620' (*ddw1*) × 'R347/1' (*Ddw1*). The division of the phenotypic classes for the χ^2 test are marked by the *arrow*

Fig. 2 Phenotypes of adult plants of (from left to right): 2 × *Ddw1/.* and 2 × *ddw1/ddw1* genotypes

Results

Segregation of the morphological and isozyme loci

The 140 F_2 plants scored for final plant height could be classified into two groups (Fig. 1). In most cases the genotypes *ddw1/ddw1* (tall) could be clearly distinguished from the *Ddw1/.* (homozygous and heterozygous short) plants. However, although a 3:1 segregation was found from an arbitary division into dwarf and tall phenotypes (Fig. 2), it was not possible to classify all single plants in the F_2. Therefore, from critical F_2 plants having an intermediate plant height between 100 cm and 120 cm, F_3 progenies were analysed to reclassify the F_2 plants into heterozygous (segregating progeny) or homozygous tall (non-segregating progeny) genotypes.

As early as at the seedlings stage a segregation for the character hairy leaf sheath (Fig. 3 A) was observed and scored with 103 hairy leaf sheath:37 smooth leaf sheath seedlings ($\chi^2 = 0.15$; $P > 0.70$). Interestingly the same plants which had a hairy or smooth leaf sheath during early stages showed a hairy or smooth peduncle (Fig. 3 B) at the adult plant stage. Based on this co-

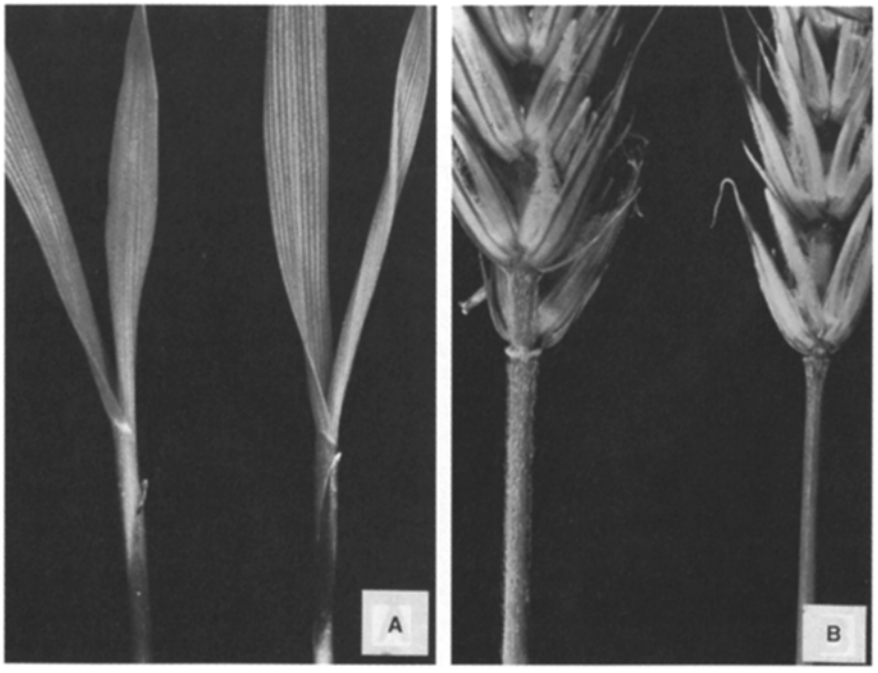


Fig. 3A, B Phenotypic expression of the traits. **A** Hairy leaf sheath at the seedling stage (left, hairy leaf sheath; right, smooth leaf sheath). **B** Hairy peduncle (left, hairy peduncle; right, smooth peduncle)

segregation it could be concluded, that the gene *Hp* determines both traits pleiotropically.

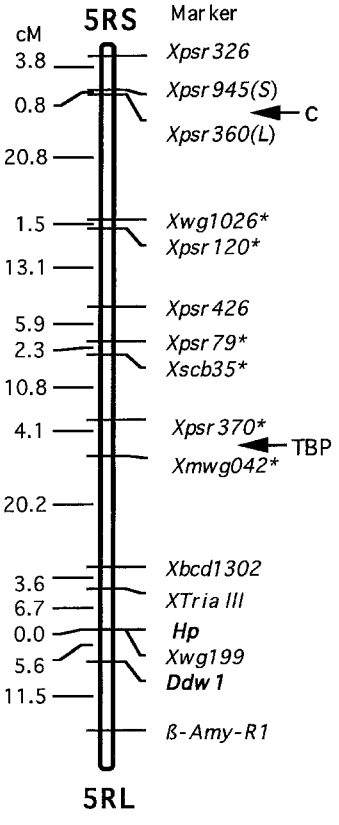
The segregation for the isozyme marker *β-amy-R1* was 31:75:34, which fitted the expected 1:2:1 ratio ($\chi^2 = 0.84$; $P > 0.50$) for monogenic inheritance.

RFLP analysis

Of the 18 probes hybridised to filters carrying DNA of the 140 progenies of the mapping population and digested with appropriate enzymes, 13 gave polymorphisms with at least one restriction enzyme and could therefore be mapped. The probes produced simple segregating hybridisation patterns which were easily scorable. In all 13 cases the segregation ratios conformed to an expected 1:2:1 ratio as tested by χ^2 ($P > 0.30$).

The 16 markers, including *Ddw1* and *Hp1*, cover a total distance of 110.5 cM (Fig. 4). The *Ddw1* locus maps between the isozyme locus *β-amy-R1* and the RFLP marker *Xwg199*, the latter showed co-segregation with *Hp*. Both morphological loci were found to be well separated from the translocation break point on the distal translocated segment of chromosome 5RL which is homoeologous to *Triticeae* 4L. In addition to the marker *Xwg199*, which was completely linked with *Hp*, there were three other markers proximal to *Ddw1* and *Hp/Xwg199* known to be located on *Triticeae* homoeologous group-4 chromosomes in wheat (*Tri a* III, Balzer et al. 1995; Börner et al., unpublished) and barley (*Xmwg042*, Graner et al. 1991; *Xbcd1302*, Anderson et al. 1992).

Fig. 4 Partial RFLP map of chromosome 5 of rye derived from the F_2 of the cross 'R1620' × 'R347/1' including the morphological genes *Ddw1* and *Hp*. Genetic distances are given in centimorgans (cM); *c* = centromere; *TBP* = 5RL/4RL translocation break point; * indicates preferred map locations with LOD differences < 2.5



Discussion

Based on the response to exogenously applied gibberellic acid there are, as in many other species, two categories – GA-sensitive or GA-insensitive – of dwarfing mutants in rye (Börner and Melz 1988; Börner 1991). The genetic analysis of the GA-insensitive dwarfs compared to GA-sensitive is much easier, because of the availibility of a GA seedlings test. The application of that test enables a qualitative distinction between the pheno-/geno-types. So far two loci for GA-insensitive dwarfing genes have been described in rye on chromosome 5R (*ct2*) and 7R (*ct1*), and both loci have already been RFLP mapped by Plaschke et al. (1993, 1995) using the seedlings test.

Much more difficult to detect and to study are the GA-sensitive dwarfing genes. No early generation physiological tests are available. As shown in Fig. 2, even at the adult plant stage, height it is difficult to distinguish qualitatively between the pheno-/genotypes and for plants having an intermediate height where only an additional F_3 analysis gives clear results. This, however, underlines the necessity for having an effective marker system, such as RFLPs or isozymes, available for selection. In the case of *Ddw1* the availability of a closely linked marker is the most important so that the dwarfing gene is not only of use in rye but can also be very sucessfully introduced into triticale (Wolski and Gryka 1994; Wolski et al. 1994). Both the RFLP marker *Xwg199*, with a distance of 5.6 cM, and the isozyme marker *β-amy-R1* (11.5 cM) could be used for early generation marker-aided selection. As both markers give 1:2:1 segregations they can be used in a selection program for the detection of homozygous short plants. Although the distance to *β-amy-R1* is slightly longer, this isozyme marker has the advantage that it will be easy and cheaper to handle by breeders.

The hairy peduncle ('hairy neck') has long been known to be associated with a particular rye chromosome and has been used as a phenotypic marker in wheat-rye hybrids or wheat-rye addition lines (e.g. Leighty and Tayor 1924; Kattermann 1935). In 1951 O'Mara located the gene for hairy peduncle on the long arm of chromosome I (5R) by meiotic studies of the wheat-rye addition line involving 5R. The distance to the centromere was calculated by Chang et al. (1973) and Chang (1975) to be at least 50 and 44.1–49.5 crossover units, respectively. The data fit with the map presented here, showing the gene *Hp* to be isolated from the centromere. Although there are at least four described genes controlling hairy peduncle and/or hairy leaf sheath, and both mono- and di- or tri-genic inheritance was observed by Melz (1987), the clear 3:1 segregation and co-segregation between hairy leaf sheath and hairy peduncle in the present F_2 population gave clear evidence that we are dealing with only one locus, pleiotropically responsible for both traits and linked to the dwarfing gene *Ddw1*. Surikov and Romanova (1978) described a monogenic dominant inheritance for hairy leaf sheath and a linkage to the gene for spring growth habit (*Sp1*) of 32.3%. After calculating a two-point linkage in the 'R1620' × 'R347/1' cross between *Hp* and *Xpsr426* (data not shown), the latter having been shown by Plaschke et al. (1993) to be closely linked with *Sp1* by 6 cM, a recombination frequency of 38.5% was obtained. Philipp et al. (1994) and Priyatkina et al. (1995) confirmed the localisation of a gene for hairy leaf sheath on chromosome 5R which was linked to the isozyme marker *ACO2*. The same isozyme marker was found to be linked to *Ddw1* (*Hl*) by Mikhailova et al. (1994). Referring to the paper of Kattermann (1935), published 60 years ago, it is surprising that the material he described already included a short-strawed rye inbred line, which in the F_4 produced plants that were nearly all either hairy peduncle, short or smooth peduncle, tall. There were very few tall plants with a hairy peduncle, which again fits with the close linkage between *Ddw1* (probably the same as Kattermann's dwarf) and *Hp* shown in the present study.

A comparison of the map presented here (Fig. 4) to the RFLP maps for chromosome 5R of Liu et al. (1992), Devos et al. (1993) and Plaschke et al. (1993) shows that, although the distances between common markers are different, the same order is present. The genes *Ddw1* and *Hp* are clearly located on the segment of chromosome 5RL which was translocated and shows homoeology to *Triticeae* 4L (Liu et al. 1992; Devos et al. 1993). Interestingly, Devos et al. (1993) also localised a dwarfing gene on the 4L segment of chromosome 5R in a similar position to *Ddw1*. That gene, however, was found to be partially recessive.

In wheat, Worland et al. (1994) have shown that the dominant GA-sensitive dwarfing gene *Rht12* known to be located on chromosome 5A (Sutka and Kovacs 1987) is located distally on the long arm of chromosome 5A. A tight linkage to the *β-Amy-A1* isozyme locus indicates that *Rht12* is present on the segment of 5AL which was ancestrally translocated from 4AL. Therefore, a homoeoallelic relationship seems to exist between *Rht12* (wheat) and *Ddw1* (rye).

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