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Comparative molecular mapping of GA insensitive *Rht* loci on chromosomes 4B and 4D of common wheat (*Triticum aestivum L.*)

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Abstract The two GA-insensitive dwarfing gene loci *Rht-B1* and *Rht-D1* were mapped using three F_2 populations, segregating for *Rht-B1c* (*Rht3*), *Rht-D1b* (*Rht2*) or *Rht-D1c* (*Rht10*). *Rht-B1c* was mapped on chromosome 4BS in the centromere region, distal and closely linked to the RFLP markers *Xpsr144* (11.9 cM) and *Xpsr584* (17.8 cM), but proximal to *Xmwg634* (30 cM). *Rht-D1c*, however, was found to be closely linked to the distally located markers *Xpsr921* (0.8 cM) and *Xmwg634* (1.5 cM). The homoeologous relationships between the GA-insensitive dwarfing genes within the *Triticeae* are discussed.

Key words Comparative genetic mapping · Dwarfing genes · GA insensitivity · Microsatellites RFLP · Wheat

Introduction

In wheat (*Triticum aestivum* L.) the GA-insensitive semi-dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) have been successfully utilised by plant breeders worldwide for more than three decades (new nomenclature following Börner et al. 1996). The yield advantage of wheats carrying the GA-insensitive semi-dwarfing genes is only partly due to the direct effect of the genes on plant height and increased lodging resistance. Field experiments analysing near-isogenic lines for GA-insensitive dwarfing genes clearly demonstrate that positive pleiotropic effects on the increased number of grains per spike result in higher yields under most environmental condition (Börner et al. 1993; Flintham et al. 1997). The more potent allele *Rht-B1c* (*Rht3*) has not been exploited in commercial varieties; however, F_1 progenies obtained from crosses with tall genotypes may be of significant interest for hybrid wheat breeders (Flintham and Gale 1997).

The Monosomic analyses of Morris et al. (1972), Gale et al. (1975) and Gale and Marshall (1976) revealed that *Rht-B1b* and *Rht-B1c* are located on chromosome 4B, with *Rht-D1b* on chromosome 4D. It was also shown, that *Rht-B1b* and *Rht-B1c* are allelic (Gale and Marshall 1976). By F_2 telocentric mapping McVittie et al. (1978) were able to position *Rht-B1b*/*Rht-B1c* 13 map units, and *Rht-D1b* 15 map units, from the centromere on the short arms of chromosomes 4B and 4D, respectively. In the early eighties Izumi et al. (1981) described a further GA-insensitive dwarfing gene on chromosome 4D present in the Chinese variety 'Ai-bian 1'. Telocentric mapping analysis (Izumi et al. 1983) revealed its location on the short arm of chromosome 4D with a distance of more than 50% recombination to the centromere. The gene was designated *Rht10* (new nomenclature *Rht-D1c*). However, test crosses between *Rht-D1b* and *Rht-D1c* gave clear evidence that the two *Rht* mutations, previously mapped with different distances from the centromere, are in fact allelic (Börner and Mettin 1988). Here we report molecular-mapping data for the *Rht* loci on both chromosomes 4B and 4D.

Materials and methods

Plant materials

Three F_2 mapping populations were established. A near-isogenic line of the variety 'Bersee', 'Bersee iso *Rht3*' (*Rht-B1c*), and 'Ai-bian 1'

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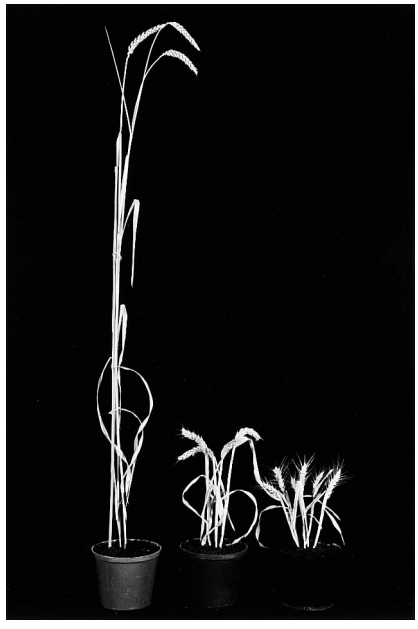

Fig. 1 Phenotypes of adult plants (from left to right) of 'Miro 808' and the two very potent dwarfs 'B3' (*Rht-B1c*) and 'Ai-bian1' (*Rht-D1c*)

(*Rht-D1c*) known to carry the very potent GA-insensitive dwarfing alleles on chromosomes 4B and 4D, respectively (Fig. 1), were crossed with the tall GA-sensitive Ukrainian variety 'Mironovskaya 808' ('Miro 808'). One single F_1 plant of each combination was used to produce 161 and 123 F_2 seeds, respectively. A third F_2 population was established by crossing 'Bersee iso *Rht2*' (*Rht-D1b*) with the GA-sensitive spelt (*Triticum spelta*) variety 'Schwabenkorn'. Here three F_1 plants were pooled to provide 112 F_2 seeds.

Target-trait analysis

All F_2 seeds were analysed, together with their parents, by applying the GA seedling test (Börner et al. 1987). After scoring seedling height at the three-leaf stage (measuring the distance between the stem base and the top of the second leaf sheath) the plantlets were planted into soil, vernalised, and grown on in the greenhouse. At harvest time the final plant height was measured. To verify the GA-response data, derived F_3 seedlings (15 per family) were re-tested, allowing a re-classification of the F_2 plants into homozygous (sensitive or insensitive) or heterozygous genotypes.

Molecular-marker analysis

DNA was extracted from fresh leaf material cut from F_2 seedlings, 4 weeks after transferring them from the GA test trays to the pots and before vernalisation. The procedures for DNA extraction, restriction digestion, gel electrophoresis, Southern transfer, probe labelling and filter hybridization were performed as described by McCouch et al. (1988) and Devos et al. (1992).

A selection of 14 RFLP probes, consisting of both cDNAs and genomic DNAs from various wheat, barley and oat libraries developed at the John Innes Centre, Norwich, UK (PSR clones), Cornell University, USA (BCD, CDO, WG clones), or the Institute for Resistance Genetics, Grünbach, Germany (MWG clones), known to be located on the *Triticeae* homoeologous group-4 chromosomes (mainly the short arms) were used.

The development of the microsatellite markers and PCR reactions were performed as described by Röder et al. (1995) and Plaschke et al. (1995 a). Fragment sizes were calculated using the computer program Fragment Manager Version 1.2 (Pharmacia) by comparing them with internal size standards. For mapping, the following wheat microsatellites (WMS) were chosen:

WMS113 – amplifying one locus (*Xgwm113*) on chromosome 4B
WMS149 – amplifying one locus (*Xgwm149*) on chromosome 4B
WMS165 – amplifying three loci (*Xgwm165*) on chromosomes 4A, 4B, 4D
WMS368 – amplifying one locus (*Xgwm368*) on chromosome 4B
WMS608 – amplifying one locus (*Xgwm608*) on chromosome 4D

The genotypes were scored for the individual plants at each locus to construct linkage maps with the MAPMAKER 2.0 computer program (Lander et al. 1987) using the Kosambi map-unit function.

Results

Analysis of GA-response and final plant height

The analysis of the two F_2 populations segregating for the strong dwarfing gene alleles *Rht-B1c* and *Rht-D1c*, located on chromosomes 4B and 4D respectively, gave a Mendelian segregation ratio of 3:1 for both shoot length after GA treatment (Fig. 2 A, C) and final plant height (Fig. 2 B, D), as tested by χ^2 ($P > 0.30$). No clear-cut segregation was observed in the F_2 populations segregating for the less-potent allele *Rht-D1b* (Fig. 2 E, F). However, subsequent GA-response testing of the F_3 families made it possible to re-classify the single F_2 plants as heterozygous (segregating progenies) or homozygous GA-sensitive or GA-insensitive (non-segregating progenies) genotypes. The segregation ratios fitted the expected 1:2:1 ratio for monogenic inheritance by the χ^2 test ($P > 0.30$).

Marker analysis

Of the 14 RFLP probes hybridised to filters carrying DNA of the three mapping populations, three, two or none were polymorphic, respectively, in the 'B3' × 'Miro 808', 'Ai-bian 1' × 'Miro 808' and 'B2' × 'Schwabenkorn' crosses, (Fig. 3), i.e. the level of polymorphic RFLP probes ranged from 0 to 25%. Only one common marker, *Xmwg634,* was found for the 'B3' × 'Miro 808' and 'Ai-bian 1' × 'Miro 808' populations. All segregating hybridisation patterns were simple (3:1 or 1:2:1) and easily scorable. Of the tested microsatellites one (WMS149) out of four (25%), one (WMS165) out of two (50%), and again one (WMS165) out of two (50%), were polymorphic and scorable for the 'B3' × 'Miro 808', 'Ai-bian 1' × 'Miro 808' and 'B2' × 'Schwabenkorn' crosses, respectively, and could be incorporated and mapped (Fig. 3).

The locus of *Rht-B1c* was mapped in the centromere region on the short arm of chromosome 4B and linked to *Xpsr144* and *Xpsr584* by 11.9 and 17.1 cM,

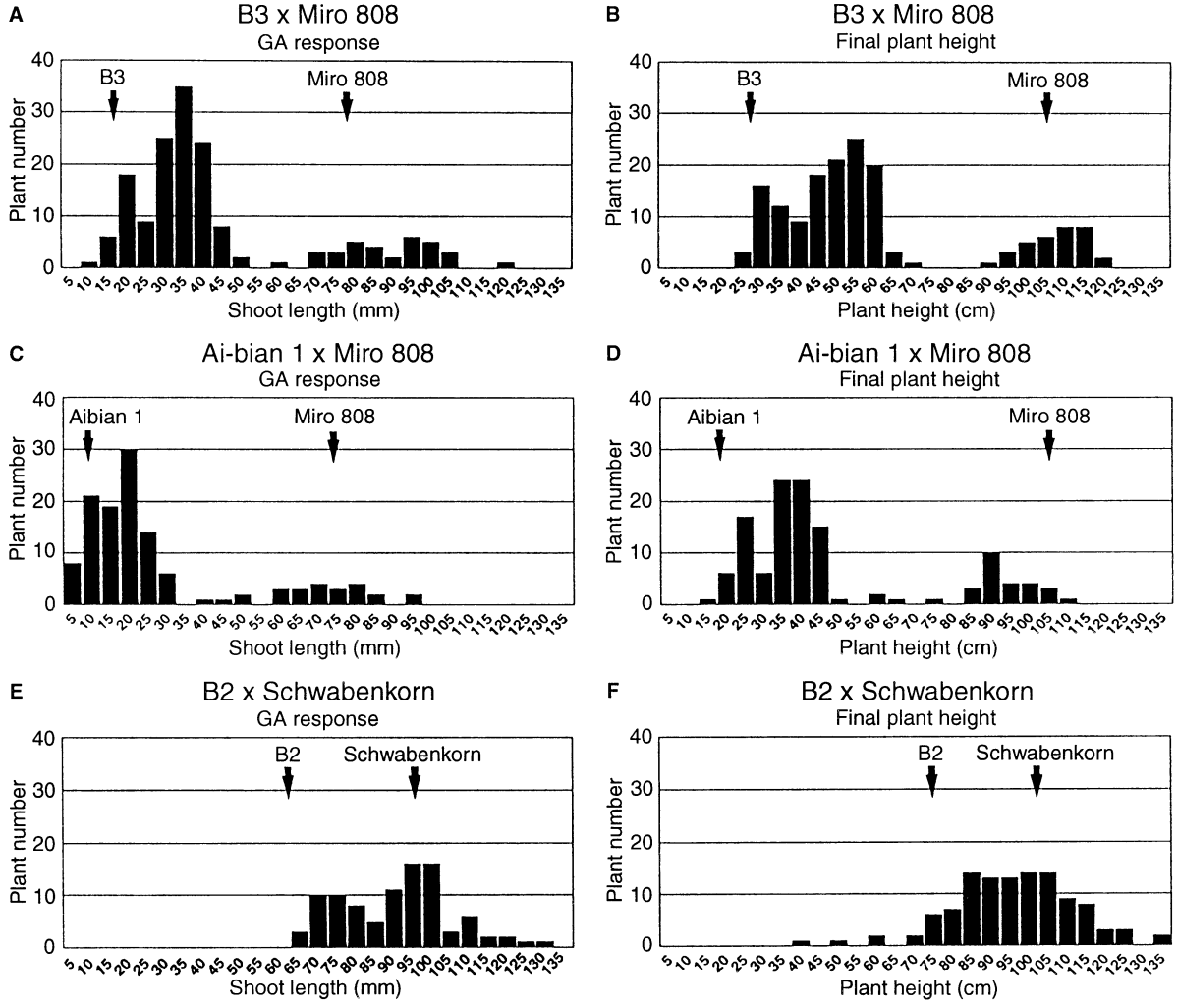

Fig. 2 F_2 segregation patterns for shoot length after GA_3 treatment (**A, C, E**) and final plant height (**B, D, F**) of the combinations 'B3' × 'Miro 808' (**A, B**), 'Ai-bian1' × 'Miro 808' (**C, D**) and 'B2' × 'Schwabenkorn' (**E, F**). The means of the parents are marked by the *arrows*

respectively. The RFLP marker *Xmwg634* was found to map distal to *Rht-B1c* with a distance of 30.6 cM. In contrast, *Rht-D1c* was closely linked to *Xmwg634* and *Xpsr921* by 1.5 and 0.8 cM, respectively. The distance to the microsatellite marker *Xgwm165*, which maps in the centromere region of chromosome 4DL (Röder et al., unpublished), is 28.0 cM. *Rht-D1b*, the less-potent allele on chromosome 4D, maps in the same region as *Rht-D1c*, 41.1 cM distal to *Xgwm165*.

Discussion

Gene mapping can be carried out quicker if information about the chromosomal location (in wheat mainly due to the utilisation of cytogenetic tester stocks) is

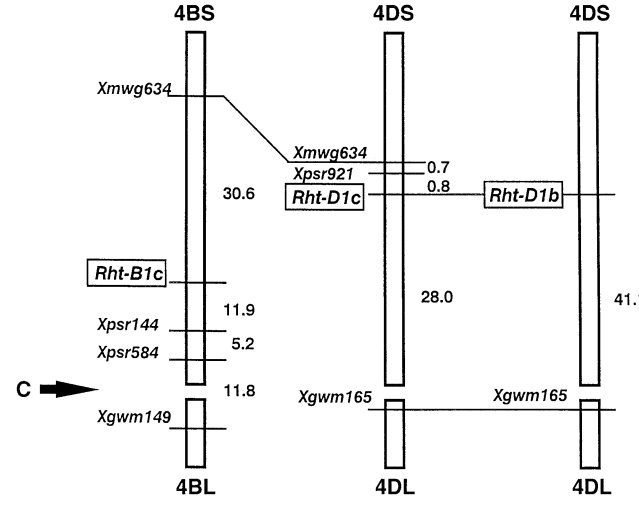

Fig. 3 Partial RFLP maps of chromosomes 4B and 4D derived from the F_2 populations of the crosses 'B3' × 'Miro 808', 'Ai-bian1' × 'Miro 808' and 'B2' × 'Schwabenkorn' (from left to right). Genetic distances are given in centimorgans (cM), c = centromere

already known. In the case of the GA-insensitive 'Norin 10' and 'Tom Thumb' dwarfing genes the application of the F_2 telocentric mapping technique even allowed an estimation of the distance (about 15 cM) of both loci to the centromeres of chromosomes 4B and 4D (McVittie et al. 1978). The same technique was used for the intrachromosomal mapping of the GA-insensitive 'Ai-bian 1' dwarfing gene, showing free recombination to the centromere of chromosome 4D (Izumi et al. 1983). A greater distance than about 15 cM was confirmed by the results of Bing-Hua and Jing-Yang (1986) describing a dominant gene for male sterility (*Ms2*) with a recombination frequency of 31% to the centromere and closely linked to the GA-insensitive *Rht* gene of 'Ai-bian 1' (Liu 1987).

The mapping data presented in the present paper confirm the location of *Rht-B1c* on chromosome 4B close to the centromere. The dwarfing gene was closely linked to *Xpsr144* and *Xpsr584*, the latter having been shown to be located on 4BS with a distance to the centromere of < 5cM (Gale et al. 1995). However, *Rht-D1c* was found to be closely linked to *Xpsr921*, which was placed at a distance of about 45 cM from the centromere on 4DS by Gale et al. (1995). The results indicate that either the two loci are not collinear, or else recombination in the region distal to *Rht-D1c* is strongly depressed in the 'Ai-bian 1' × 'Miro 808' mapping population.

The availability of a series of multiple alleles makes it possible to select those showing the strongest effect on the target trait. In this way the genetical analysis will be more efficient due to an easier scorability. In the present study, however, the less potent allele *Rht-D1b* was also included. This has previously been shown to be allelic to *Rht-D1c* (Börner and Mettin 1988), but linked by 13 cM to the centromere (McVittie et al. 1978). Although only one marker could be genetically linked to *Rht-D1b* it was possible to conclude that the dwarfing allele maps in the same position as *Rht-D1c*. The utilisation of *T. spelta* as one partner for the cross did not lead to the expected higher level of polymorphism. No RFLP marker was polymorphic for the D genome.

Within the *Triticeae* collinearity was shown for the two GA-sensitive dwarfing genes *Rht12* of wheat and *Ddw1* of rye (Korzun et al. 1996), for the genes determining vernalisation or the photoperiodic response in wheat, rye and barley (Börner et al. 1997), or for mutant loci determining the absence of ligules (*al*), waxless plants (*wa1*) and waxy endosperm (*Wx*) characters (Korzun et al. 1997). Surprisingly, no such relationship was found for the GA-insensitive dwarfing genes. In rye the two recessive GA-insensitive dwarfing genes *ct2* and *ct1* map on chromosomes 5RL and 7R, respectively (Plaschke et al. 1993, 1995 b). Although for both chromosomes 5R and 7R translocations of segments with homoeology to the *Triticeae* homoeologous group-4 chromosomes have been detected (Devos et al. 1993), both translocations involve a part of the long arm of the homoeologous group-4 *Triticeae* chromosome and not the short arm, which carries the *Rht* genes in wheat. In addition, it was shown that *ct2* maps proximal to at least five RFLP markers which were located on the long arms of the *Triticeae* homoeologous group-5 chromosomes, proximal to the translocation break point (Plaschke et al. 1993). In barley the GA-insensitive dwarfing gene *Rht-H1* was found to map in the centromere region of chromosome 2H (Börner and Korzun 1996), neither related to the *Rht* genes in wheat nor to the *ct* genes in rye. Generally, it can be concluded that within the *Triticeae* the GA-insensitive dwarfing mutants are due to lesions of non-homoeologous genes located on the homoeologous group-4 of wheat-5 and -7 of rye, and -2 of barley.

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