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Molecular mapping of two dwarfing genes differing in their GA response on chromosome 2H of barley

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Abstract The two recessive dwarfing mutants *gai* (*GA-ins*) and *gal* (*GA-less*), differing in their response to exogenously applied gibberellic acid (GA_3), were mapped in the centromere region and on the long arm, respectively, of the barley chromosome 2H. The gene *gai*, which determines reduced plant height and GA insensitivity pleiotropically, was found to co-segregate with the two RFLP markers *Xmwg2058* and *Xmwg2287*. Both markers are known to map close to the centromere. The GA-sensitive dwarfing gene *gal* was found to be linked to the three co-segregating RFLP markers *Xmwg581*, *Xmwg882* and *Xmwg2212* (proximal) and *XksuG5* (distal) by 3.6 and 9.5. cM, respectively. The distance between the two mutant loci was estimated to be about 55 cM. Homoeologous relationships between the dwarfing genes within the *Triticeae* are discussed.

Key words Dwarfing genes · GA insensitivity · Genetic mapping · RFLP · *Hordeum vulgare*

Introduction

The yield increases achieved in cereals during the past 60 years have been accompanied by steady decreases in straw length. Shorter plants remain resistant to lodging even under high-yielding growing conditions. Although plant height is generally known to be a quantitative character, major genes for reduced plant height have been identified in all main cereals.

On the basis of their response to exogenously applied gibberellins (GAs) major dwarfing gene mutants can be divided into GA-sensitive and GA-insensitive types. In GA-sensitive mutants (synthesis mutants) endogenous gibberellins are either absent or modified, whereas in GA-insensitive mutants any of the steps between the reception of the GA signal and the manifestation of the GA response (Reid 1986) may be influenced.

In cereal breeding programs both GA-insensitive and GA-sensitive dwarfing mutants have been exploited. In wheat, mostly GA-insensitive dwarfing genes have been introduced which reduce plant height and increase the number of grains per spike pleiotropically (Börner et al. 1993; Flintham et al. 1997). The two known GA-insensitive dwarfing gene loci *Rht-B1* and *Rht-D1*, originating from the Japanese variety ‘Norin 10’, were mapped on the short arms of chromosomes 4B and 4D, respectively, by Börner et al. (1997). In rye and barley breeding, mainly the GA-sensitive dwarfing genes *Ddw1* and *sdw1* (*denso*) are used. These genes have been mapped on chromosomes 5R of rye (Korzun et al. 1996) and 3H of barley (Barua et al. 1993; Laurie et al. 1993). *Ddw1* is also widely used in Triticale (Wolski and Gryka 1996). It is not yet clear whether GA-insensitive dwarfing genes will become as important in practical rye or barley breeding programs as in wheat. Recently, Ivandic et al. (1999) was able to map the GA-insensitive dwarfing gene *Dwf2* on the short arm of chromosome 4H at a position homoeologous to the *Rht-B1* and *Rht-D1* loci in wheat.

Here we report molecular mapping data for two recessive dwarfing gene mutants. Following the recommended rules for nomenclature and gene symbolization in barley three-letter locus symbols are used (Franckowiak et al. 1997), and the mutant genes are designated *gai* [formerly *GA-ins* (Favret et al. 1975) or *Rht-H1* (Börner and Korzun 1996; Ivandic et al. 1999); GA insensitive] and *gal* [formerly *GA-less* (Favret et al. 1975); GA sensitive] throughout the paper. From Favret et al. (1975) it was already known that *gai* is located on chromosome 2H and is associated with the *vrs* locus, determining the 2-row/6-row spike morphology character. Further analysis including both mutants indicated that *gai* and *gal* are linked with each other (40 cM) and are located on the short and long arm of chromosome 2H, respectively (Solari 1992).

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Materials and methods

Plant materials

The two dwarf mutants ‘Hv287’ (GA-insensitive) and ‘Hv288’ (GA-sensitive) were kindly provided by J.E. Flintham, John Innes Centre, Norwich, UK. Information about the origin of both lines was obtained from A.E. Martinez, INTA Castelar, Argentina. Based on this information and on Boother (1989) ‘Hv287’ and ‘Hv288’ were produced by backcrossing (twice) ‘M.C. 90’ (*gai*), induced by X-ray treatment, and ‘M.C. 96’ (*gal*), induced by EMS treatment, onto the cultivar ‘Triumph’. Both mutants were derived from ‘M.C. 20’. ‘M.C.’ is an abbreviation for ‘Mutante de Cebada’ (i.e. barley mutant).

Both dwarfs had two-rowed spikes and were crossed with the varieties ‘Betzes’ (2-rowed, tall) and ‘Monte Cristo’ (6-rowed, tall). One single F_1 plant of each combination was used to produce 108 and 132 F_2 seeds for the crosses involving ‘Hv287’ and ‘Hv288’, respectively.

Target trait analysis

The F_2 seeds of the crosses ‘Hv287’×‘Betzes’ and ‘Hv287’×‘Monte Cristo’ were analyzed together with their parents by applying the GA seedling test (Börner et al. 1987). After scoring the seedling heights at the three-leaf stage (measuring the distance between stem base and the top of the second leaf sheath) the plantlets were planted into soil and grown further in the greenhouse (Fig. 1). At harvest time the final plant height was measured. To verify the GA response data we re-tested derived F_3 seedlings (15 per family), thereby enabling a re-classification of the F_2 plants into homozygous (sensitive or insensitive) or heterozygous genotypes.

The F_2 progenies of the crosses ‘Hv288’×‘Betzes’ and ‘Hv288’×‘Monte Cristo’ were grown in the greenhouse without applying a GA test. Because ‘Hv288’ (Fig. 2) determines an extreme dwarfism the final plant height was used for scoring the F_2 populations. Again 15 F_3 seedlings per family were derived and re-tested, to enable re-classification of the F_2 plants.

At harvest time the F_2 populations of ‘Hv287’×‘Monte Cristo’ and ‘Hv288’×‘Monte Cristo’ were scored for the 2-row/6-row character.

Molecular marker analysis

DNA was extracted from fresh leaf material cut from 15 pooled F_3 seedlings per F_2 progeny (‘Hv287’×‘Monte Cristo’ and ‘Hv288’×‘Monte Cristo’) by the procedure of McCouch et al. (1988). The procedures for restriction digesting with enzymes *Hin*dIII, *Dra*I, *Eco*RI and *Eco*RV, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were as described by Devos et al. (1992).

A selection of 36 and 29 RFLP probes were used for analyzing the F_3 families of the ‘Hv287’×‘Monte Cristo’ and ‘Hv288’×‘Monte Cristo’ combinations, respectively. The probes, located on the

Fig. 1 Phenotypes of adult plants of 2× ‘Monte Cristo’ (left, wild type) and 2× ‘Hv287’ (right, *gai* mutant)

Fig. 2 Phenotypes of adult plants of 2× 'Monte Cristo' (left, wild type) and 2× 'Hv288' (right, *gal* mutant)

homoeologous group 2 *Triticeae* chromosomes, consisted of cDNAs and genomic DNAs from various wheat, barley, oat and rye libraries developed at the John Innes Centre, Norwich, UK (PSR clones), Cornell University, Ithaca, USA (BCD clones), Institute for Resistance Genetics, Grünbach, Germany (MWG clones), Kansas State University, Manhattan (KSU clones) or Institute of Applied Genetics, University of Hannover, Germany (IAG clones).

In addition to the RFLP probes the cleaved amplified polymorphic sequence (CAPS) marker mwgp2054, the sequence-tagged site (STS) marker mwgp2133 and the simple sequence repeat (SSR) HVM54 were used. Primer sequences and annealing temperatures for polymerase chain reaction (PCR) amplification of MWGP2054 and MWGP2133 are given in Ivandic et al. (1998). The restriction endonuclease *Taq*I was used for the generation of CAPS marker MWGP2054. PCR analysis of the SSR marker HVM54 was performed as described by Liu et al. (1996).

Individual F_2 plant genotypes were determined for each locus and linkage maps were constructed 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 F_2 histograms for seedlings length after GA_3 treatment of the crosses of 'Hv287'×'Betzes' and 'Hv287'×'Monte Cristo' provide clear evidence for a monogenic segregation in both progenies (Fig. 3A, B). The observed ratios fit the expected 1:3 ratio for a monogenic recessive inheritance of *gai* in the 'Betzes' and 'Monte Cristo' crosses (χ^2=0.049, P >0.80 and χ^2=0.197, P >0.60, respectively). Monogenic inheritance was confirmed by testing the GA response of the F_3 progenies, making it possible to re-classify the individual F_2 plants as heterozygous (segregating progenies) or homozygous (non-segregating progenies). The segregation ratios fit the expected 1:2:1 ratio (P >0.85).

When the final plant height measurements of the two F_2 populations segregating for *gal* was analyzed, a Mendelian segregation ratio of 1:3 was again observed for both crosses (Fig. 3C, D) as tested by χ^2 tests (P >0.90). Phenotypic scoring of F_3-families confirmed the expected 1:2:1 ratio for the 'Hv288×Betzes' and 'Hv288'×'Monte Cristo' crosses (P>0.60).

Marker analysis

For fine mapping, F_3 progenies of the crosses 'Hv287'×'Monte Cristo' and 'Hv288'×'Monte Cristo' were analyzed using a set of previously mapped restriction fragment length polymorphic (RFLP) markers. Of

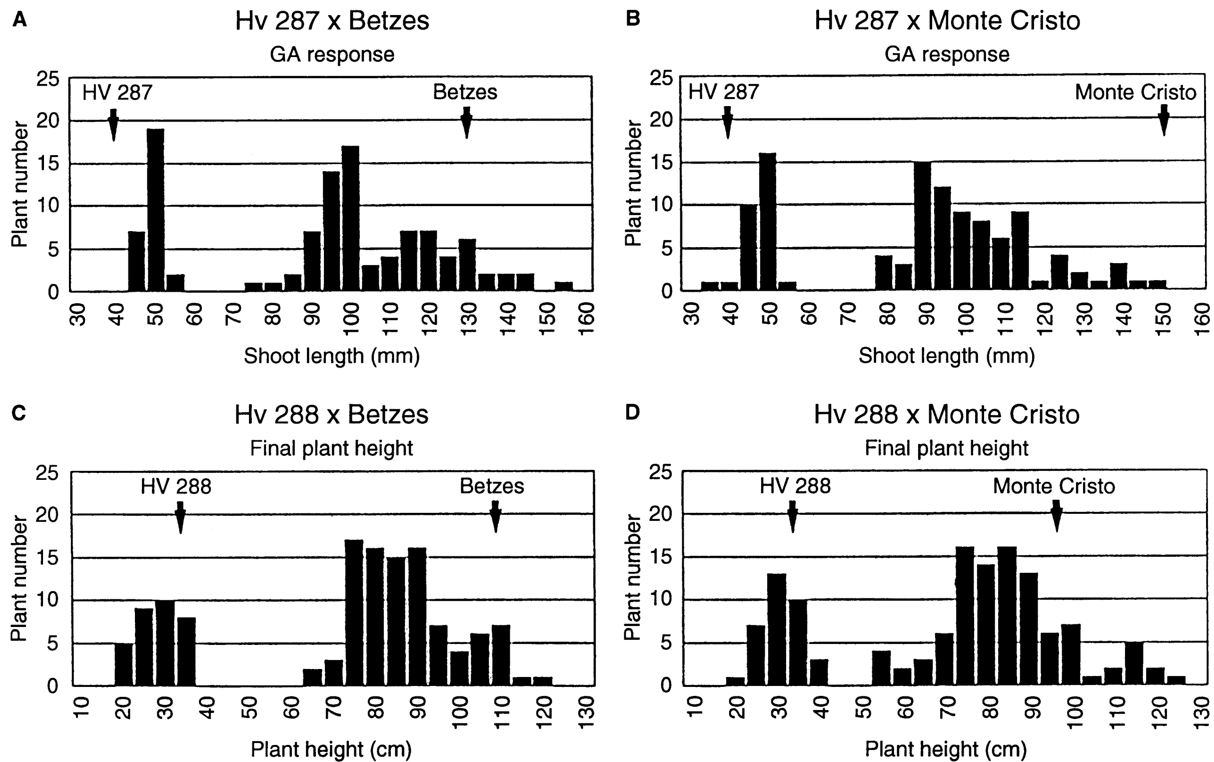

Fig. 3A–D F_2 segregation patterns for shoot length after GA_3 treatment (**A**, **B**) and final plant height (**C**, **B**) of the combinations 'Hv287'×'Betzes' (**A**), 'Hv287'×'Monte Cristo' (**B**), 'Hv288'×'Betzes' (C) and 'Hv288'×'Monte Cristo' (**D**). The means of the parents are marked by the *arrows*

the RFLP probes that hybridized to filters 26 were polymorphic in at least one mapping population, whereas 5 detected loci in both populations. Additional loci were detected by applying the CAPS marker, *Xmwgp2054*, the STS marker, *Xmwgp2133* and the SSR marker, *Xhvm54*, the latter being polymorphic in both populations. Because both dwarfing mutants were 2-rowed and 'Monte Cristo' was 6-rowed the mapping populations segregated for the gene *vrs1*, which could be integrated in both maps. As shown in Fig. 4 *gai* is located in the centromere region of chromosome 2HS and co-segregated with the 2 RFLP markers *Xmwg2058* and *Xmwg2287*. Flanking markers *Xmwg557* and *Xcmwg658* were located 1.8 cM distal and 1.8 cM proximal respectively to the GA-insensitive dwarfing gene. On the other hand, *gal* was mapped on chromosome 2HL, 3.6 cM distal to the co-segregating markers *Xmwg581*, *Xmwg882* and *Xmwg2212* and 9.5 cM proximal to *XksuG5*.

Discussion

It has been more than 20 years since Favret et al. (1975) described the two dwarfing mutants *gai* (*GA-ins*) and *gal* (*GA-less*) in barley and found the former to be located on chromosome 2 (2H). Although during the last decade much effort has been focused on the molecular mapping of agronomically important genes, including dwarfing genes, neither of these two has previously been located in relation to DNA markers.

Within the *Triticeae* molecular mapping data are available for five GA-insensitive dwarfing gene loci. Plaschke et al. (1993) were the first to map the GA-insensitive dwarfing gene *ct2* on the long arm of chromosome 5R of rye and *ct1* in the centromere region of chromosome 7R (Plaschke et al. 1995). In wheat the two multiallelic dwarfing gene loci *Rht-B1* and *Rht-D1* were mapped on the short arms of chromosomes 4B and 4D, respectively (Börner et al. 1997). In a comparable position on the short arm of chromosome 4H of barley Ivandic et al. (1999) mapped *Dwf2*, suggesting synteny of GA-insensitive dwarfing genes within the *Triticeae*. The data presented here provide evidence that in addition to homoeologous groups 4, 5 and 7, group 2 also carries GA-insensitive dwarfing genes. While the homoeologous group-4 genes show a (semi-) dominant inheritance the dwarf character of all other mutants is recessive.

Interestingly, Yang et al. (1995) described a partially dominant GA-insensitive dwarfing gene on the short arm of chromosome 2A of wheat. This gene, designated *Rht21*, was localized by applying nulli-tetrasomic and ditelosomic analysis. A homoeologous relationship between *gai* of barley and *Rht21* of wheat may eventually be indicated if comparative mapping data for the wheat gene become available.

From the much larger group of GA-sensitive dwarfing genes described only four have been mapped within the *Triticeae* using molecular markers. Two dominant genes, *Ddw1* and *Rht12*, were tagged in homoeologous regions

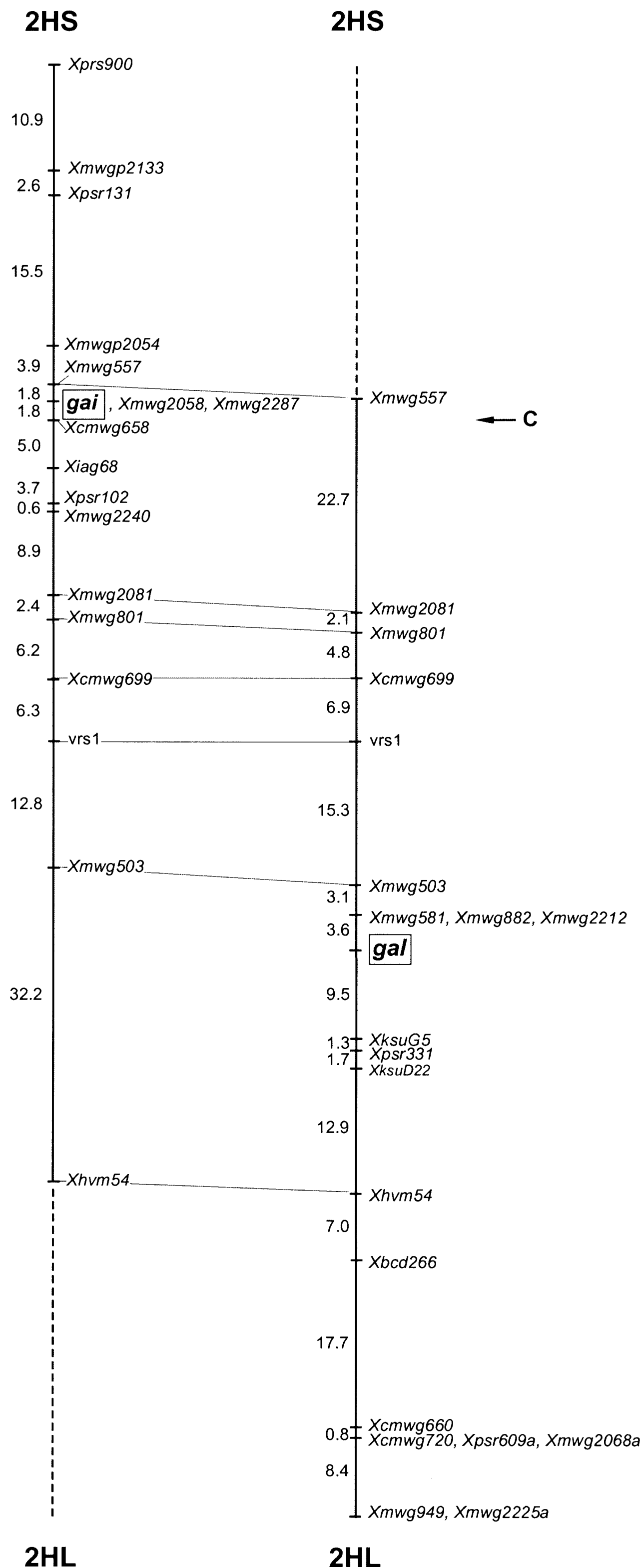

Fig. 4 Partial maps of chromosome 2H derived from the F_2 populations of the crosses 'Hv287'×'Monte Cristo' (*left*) and 'Hv288'×'Monte Cristo' (*right*) showing the map positions of *gai* and *gal*, respectively. Genetic distances are given in centimorgans (cM). *c*=centromere

on the distal parts of the long arms of chromosomes 5R (Korzun et al. 1996) and 5A (Korzun et al. 1997), respectively. Another dominant dwarfing gene of wheat – *Rht8* – was found to be located on chromosome 2DS about 35 cM from the centromere (Korzun et al. 1998), whereas in barley the recessive *sdw1* gene was mapped on the long arm of chromosome 3H (Barua et al. 1993; Laurie et al. 1993). The gene *gal*, investigated in the present paper, is the only GA-sensitive dwarfing gene that has been molecular-mapped on the long arm of the *Triticeae* group 2 chromosomes to date.

Two dwarfing genes – *sld2* and *hcm* – are integrated in the barley chromosome 2H linkage map for morphological markers published by Franckowiak (1997). Comparing their positions with those of the two loci mapped in the present paper, we were able to exclude allelic relationships. The gene *sld2* maps 18.2 cm distal to *eog* on 2HS, i.e. about 15 cM away from the centromere and is, therefore, not related to *gai*. The short culm-determining gene *hcm* is shown to be located on the long arm of chromosome 2H, but in contrast to *gal* proximal to *vrs1*.

The linkage of *Xmwg557* to *gai* observed in the 'Hv287'×'Betzes' cross (Börner and Korzun 1996) was confirmed in the 'Hv287'×'Monte Cristo' cross, however the map distance was significantly decreased (9.0+/-2.1 cM vs 1.8 cM). Although genotypic effects in recombination frequency have been reported for barley (Nilsson and Säll 1995) further analysis will be required to examine whether the discrepancy in map distances observed in the present study is due to genetic effects and, if so, to what extent it is restricted to the centromeric region of chromosome 2HL. A comparison of both maps shows that the two loci *gai* and *gal* are about 55 cM apart. This estimate is in accordance with a recombination frequency of 40% between *gai* and *gal* as was calculated by Solari (1992).

In addition to cereals GA-related dwarfing genes have been identified and mapped in various plant species including pea and *Arabidopsis* (for review see Bethke and Jones 1998). Regarding the latter, the isolation of *gai*, a GA-insensitive dwarfing gene, represents a first step toward understanding the molecular processes causing the dwarf phenotype, and there is preliminary evidence that this is a homolog for the *Rht* series of genes present on homoeologous group 4 of high-yielding semidwarf wheats (Peng et al. 1997; Gale and Devos 1998).

In this context, the high-resolution mapping of *gai* in barley will provide on one hand the basis for testing candidate genes from other species and, on the other hand, represents a starting point for the map-based isolation of this locus in barley. Moreover, the availability of closely linked markers, as they were identified in this study, provides an option for the rapid, marker-assisted introgression of the *gai* gene into various genetic backgrounds in order to further test its usefulness and potential in breeding for improved yield.

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