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Comparative mapping of a gibberellic acid-insensitive dwarfing gene (*Dwf2*) on chromosome 4HS in barley

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Abstract We report the genetic mapping of *Dwf2*, a dominant gibberellic acid (GA_3)-insensitive dwarfing gene which has been previously described to cause a very short growth habit in barley (*Hordeum vulgare*) mutant '93/B694'. Using RFLP and microsatellite markers we performed segregation analysis in an F_2 population comprising 86 individuals developed from a cross of '93/B694' (*Dwf2*) with 'Bonus M2' (*dwf2*). *Dwf2* was mapped on the short arm of barley chromosome 4H proximal to microsatellite marker *XhvOle* (5.7 cM) and distal to RFLP marker *Xmwg2299* (18.3 cM). The genetic localization of the *Dwf2* gene at a homoeologous position to the multiallelic *Rht-B1* and *Rht-D1* loci in wheat suggests synteny of GA-insensitive dwarfing genes within the *Triticeae*. Moreover, the extremely prostrate growth habit exhibited in barley '93/B694' (*Dwf2*) resembles that of wheat plants carrying the genes *Rht-B1c* (*Rht3*) or *Rht-D1c* (*Rht10*).

Key words *Hordeum vulgare* · Dwarfing genes · GA insensitivity · Comparative mapping · Microsatellites

Introduction

In cereals, the yield increases of the past decades have been achieved to a great extent by the incorporation of semi-dwarfing genes into the gene pool of the cultivated crops. In barley (*Hordeum vulgare*), the majority of modern cultivars carry the *denso* gene, which was located by Barua et al. (1993) and independently by Laurie et al. (1993) on the long arm of chromosome 3H. Using morphological markers Thomas et al. (1984) assigned the dwarfing gene GP*ert*, which is present in the European spring barley gene pool, to chromosome 5H. Börner and Korzun (1995) identified two recessive dwarfing genes on barley chromosome 2H that differ in their response to gibberellic acid (GA_3). One of these, *Rht-H1*, the GA_3-insensitive gene, was mapped in the centromeric region of 2H by close linkage to the restriction fragment length polymorphism (RFLP) marker *Xmwg557*.

In wheat, the GA-insensitive dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) have been exploited in breeding programs to develop high-yielding cultivars with reduced plant height. The corresponding loci, *Rht-B1* and *Rht-D1*, which are located on chromosomes 4BS and 4DS, respectively, carry a set of multiple alleles that give phenotypic expressions ranging from semi-dwarfism to extreme dwarf growth habit (Börner et al. 1996). To date, no homoeologous genes have been identified in other members of the *Triticeae* on chromosome 4S. In addition to barley chromosome 2H on which *Rht-H1* is located, rye chromosomes 5R and 7R are also known to carry GA-insensitive dwarfing genes, *ct2* and *ct1*, respectively, both mapped by Plaschke et al. (1993, 1995) using RFLP markers.

Falk (1994) described the GA_3-insensitive dwarfing gene *Dwf2*, which occurred as a spontaneous mutation in an anther culture-derived plant of the barley line 'H930-36', causing extreme dwarfism similar to that exhibited by wheat carrying *Rht-B1c*. The purpose of the study presented here was to investigate the allelic relationship between the two barley dwarfing genes *Dwf2* and *Rht-H1*. For comparative mapping of the *Dwf2* gene both microsatellite and RFLP markers

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previously shown to be linked to GA-insensitive dwarfing genes of wheat were used.

Materials and methods

Plant materials and analysis of traits

The barley GA_3-insensitive dwarfing mutant '93/B694' (*Dwf2*), kindly provided by D.E. Falk, University of Guelph, Ontario, Canada, was crossed with both the GA_3-insensitive dwarfing mutant 'Hv287' (*Rht-H1*) and the flowering-time mutant 'Bonus M2' (*dwf2*). The latter, kindly provided by L.W. Gallagher, University of California, Davis, USA, was used as the parent with normal plant height. One single F_1 plant of each combination was used to produce 128 and 86 F_2 seeds, respectively.

All F_2 seeds of the cross '93/B694' × 'Hv287' were studied together with their parents by use of a GA_3 seedling test (Börner et al. 1987). The length of the seedlings was determined at the three-leaf stage (measuring the distance between the stem base and the top of the second leaf sheath). For molecular mapping of *Dwf2*, all 86 F_2 individuals of the cross '93/B694' × 'Bonus M2' were grown in the greenhouse, and final plant height was measured at harvest time.

DNA and linkage analysis

Fresh leaf material was cut from young F_2 plants and used for isolation of genomic DNA following the procedure published by McCouch et al. (1988). Southern analysis and probe labeling were performed according to standard procedures (Devos et al. 1992) except that denaturation of the probes was achieved by adding a 1/10 volume of 3 *M* NaOH. Following digestion of the gDNA with restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII or *Dra*I, hybridization was carried out with a selection of RFLP probes originating from genomic libraries of barley (MWG; Institute for Resistance Genetics, Grünbach, Germany), wheat (PSR; John Innes Centre, Norwich, UK) and oat (CDO, WG; Cornell University, Ithaca, USA). All of the 16 RFLP probes used had been previously mapped on the *Triticeae* homoeologous group 4 chromosomes (Graner et al. 1991; Heun et al. 1991; Korzun et al. 1998). Polymerase chain reactions (PCRs) of the microsatellites HVM3, HVM13, HVM40, HVRCA and WMS165 were performed as described by Liu et al. (1996), Becker and Heun (1995) and Korzun et al. (1997a). A pair of primers was designed from the barley oleosin-1 (*Ole-1*) cDNA sequence, which contains simple sequence repeats (SSRs) (Aalen 1995). A product of approximately 220 bp in length was obtained after PCR amplification of HVOLE with the following primers: 5′ GAT GGA TGT CAG TCG GTC 3′ (forward); 5′ ATG AGC AGT AGT ACA ACT CTA AGC 3′ (reverse). The temperature profile was 5 min at 94°C, followed by 45 cycles of 30 s at 94°C, 30 s at 58°C and 1 min at 72°C. Segregation of SSR alleles was analyzed by electrophoresis on denaturing polyacrylamide gels (10%) followed by silver staining. Evaluation of multipoint linkage relationships and calculations of map distances were performed using the program MAPMAKER 2.0 (Lander et al. 1987) and the Kosambi function (Kosambi 1944).

Results

GA_3 seedling test

The analysis of the F_2 population developed by crossing the two GA-insensitive dwarfs '93/B694' (*Dwf2*) and 'Hv287' (*Rht-H1*) is shown in Fig. 1. The 128 seedlings scored for seedling length after GA_3-treatment could be divided into 100 GA-insensitive, 17 low-responding GA-sensitive and nine high-responding GA-sensitive plants. The segregation ratio fit the expected 12 : 3 : 1 ratio for a digenic inheritance caused by one dominant and one semi-dominant gene ($\chi^2 = 2.33$; $P > 0.30$). The detection of GA-sensitive plants in this progeny provided clear evidence that both dwarfing mutants are not allelic.

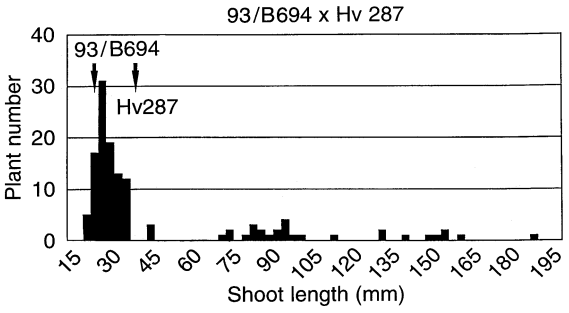

Fig. 1 F_2 segregation pattern for shoot length after GA_3-treatment of the cross '93/B694' (*Dwf2*) × 'Hv287' (*Rht-H1*). The means of the parents are indicated by *arrows*

Genetic mapping

The plant height measurements of the mapping population '93/B694' × 'Bonus M2' confirmed the dominant, monogenic mode of inheritance for the GA-insensitive dwarfing gene *Dwf2* as described by Falk (1994). As expected, a 3 : 1 segregation ratio of the parental phenotypes (Fig. 2) was observed in the F_2 population ($\chi^2 = 0.76$; $P > 0.30$).

Out of the 16 previously mapped RFLP probes originating from barley (6), wheat (8) and oat (2) genomic libraries 12 probes were found to be polymorphic between parents '93/B694' and 'Bonus M2' with at least one restriction enzyme. Nine RFLP markers were used for the construction of the linkage map. Figure 3 (left) shows the location of the *Dwf2* gene on the proximal region of the short arm of chromosome 4H. RFLP marker *Xmwg2299* could be mapped proximal from the GA-insensitive dwarfing gene at a distance of 18.3 cM. Linkage was also determined between *Dwf2* and markers *Xmwg634* and *Xmwg921*, which were located near the distal end of 4HS (33.1 cM and 34.3 cM, respectively). In addition to RFLP markers, four (out of six) microsatellite markers including one originating from the wheat genome (*Xgwm165*) could be mapped. While *Xgwm165* was located on the long arm of barley chromosome 4H at a distance of 39.6 cM from the dwarfing gene *Dwf2*, *Xhvm3* and *XhvRca* were mapped on chromosome 4HL closer to the centromere. The closest linkage to *Dwf2* (5.7 cM) was detected by microsatellite marker *XhvOle* (Fig. 4).

Segregation of all molecular markers, which covered a chromosomal segment of 73.9 cM, fit the expected 1 : 2 : 1 ratio ($P > 0.25$).

Fig. 2 Phenotypes of adult plants of barley mutant '93/B694' (*Dwf2*/*Dwf2*) (*left*) and 'Bonus M2' (*dwf2*/*dwf2*) (*right*)

Discussion

In addition to a mutant barley line carrying *Rht-H1*, a GA-insensitive dwarfing gene located on chromosome 2H, a second mutant showing no response to GA was detected in barley as a result of somaclonal variation (Falk 1994). This mutant, which carries a dominant GA-insensitive dwarfing gene (*Dwf2*), was of extremely short stature and resembled wheat plants carrying the very potent dwarfing genes *Rht-B1c* or *Rht-D1c* on chromosomes 4BS and 4DS, respectively. Based on these observations, the objective of the present work was to investigate whether wheat and barley possess related genetic systems, which would explain the similar phenotypic expression of dwarfing in the two species. Analysis of the allelic relationship between *Rht-H1* and *Dwf2* indicated clearly that the mutant traits are determined by different loci. To study the homoeologous relationships of the *Dwf2* gene with the dominant GA-insensitive dwarfing genes in wheat, we used a set of previously mapped markers for carrying out a comparative analysis. In particular, the linkage observed between *Xmwg634* and *Dwf2* (33.1 cM) enabled a direct comparison to be made with the chromosomal location of the *Rht-B1* and *Rht-D1* loci in wheat (Börner et al. 1997). Whereas a similar map distance (30.6 cM) was found between *Xmwg634* and the wheat dwarfing gene *Rht-B1c*, *Xmwg634* and the gene *Rht-D1c* were more closely linked (Fig. 3). Similarly, wheat RFLP marker *Xpsr921* was tightly linked to the *Rht-D1c* gene (0.8 cM), while it was mapped at a distance of 34.3 cM to the *Dwf2* gene.

In addition to the RFLPs, microsatellite marker *Xgwm165* proved useful for aligning the partial chromosome maps of wheat and barley. *Xgwm165*, which was originally developed in wheat, was placed on barley chromosome 4HL at a distance of 39.6 cM from the dwarfing gene *Dwf2*. This marker is known to be located at a distance of 28 cM and 41 cM from *Rht-D1c and Rht-D1b*, respectively (Börner et al. 1997), but data for the *Rht-B1* locus are not available. Analysis of *Xgwm165* was very helpful in the process of comparative mapping by identifying the chromosomal location of *Dwf2* at an early stage of this study. Further support for synteny between the genetic loci of wheat

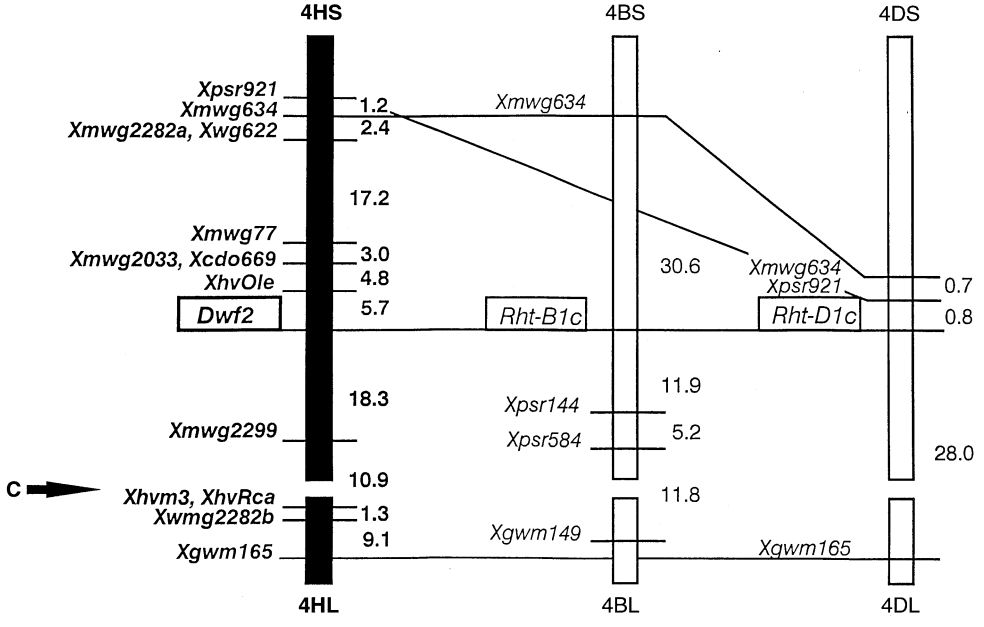

Fig. 3 Linkage map of a portion of barley chromosome 4H depicting the position of the GA-insensitive dwarfing gene *Dwf2* (*left*) in comparison to partial maps of chromosomes 4B and 4D of wheat carrying the genes *Rht-B1c* and *Rht-D1c*, respectively, both of which were mapped by Börner et al. (1997). Genetic distances are given in centiMorgans (cM). Short (*S*) and long (*L*) arms and centromeric region (*c*) of the chromosome are marked

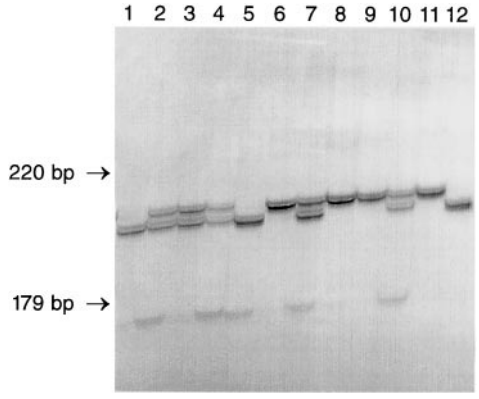

Fig. 4 Segregation pattern of barley microsatellite *XhvOle* used for mapping of GA-insensitive dwarfing gene *Dwf2* in the F_2 population '93/B694' × 'Bonus M2' (*Lanes 1–10* F_2 progeny), (*Lanes 11* and *12* crossing parents). Polymorphisms of simple sequence repeats were revealed by silver staining following polyacrylamide gel electrophoresis. The positions of molecular-weight marker bands (pGEM; Promega) are marked by *arrows*

and barley was then provided by RFLP analysis. In this context, it should be noted, that only a small minority of the simple sequence repeats derived from the wheat genome can be successfully amplified in barley or rye (Röder et al. 1995), indicating the high level of species-specificity of this marker system.

In conclusion, the colinearity of the molecular markers confirms the hypothesis that a homoeoallelic relationship exists between the GA-insensitive dwarfing genes of barley and wheat. To date, syntenic relationships between the dwarfing genes within the *Triticeae* have been demonstrated only with the dominant GA-sensitive dwarfing genes *Rht12* and *Ddw1*, which are located at the distal end of chromosomes 5AL in wheat and 5RL in rye, respectively (Korzun et al. 1997b).

In wheat, GA-insensitive dwarfing gene loci *Rht-B1* and *Rht-D1* on chromosomes 4BS and 4DS, respectively, carry series of multiple alleles which cause phenotypic expressions ranging from semi-dwarfing to extreme dwarfing habits. Therefore, it is conceivable that, in addition to *Dwf2*, other putative alleles will be identified and located at the newly mapped locus on barley chromosome 4HS. To gain an in-depth understanding of the mechanisms underlying the phenotypic variation at the molecular level, it will be necessary to isolate the multiallelic locus. In this respect, it is obvious that the barley genome is more accessible to the map-based cloning approach than the hexaploid genome of wheat.

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