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Molecular genetic mapping of dwarfing genes in oat

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Abstract Restriction fragment length polymorphism (RFLP) analysis provides a valuable tool for characterizing and understanding relationships among genes for useful traits in crop species, particularly in ones with complex genomes such as the hexaploid cultivated oat *Avena sativa* L. ($2n = 6x = 42$). Using Bulked Segregant Analysis (BSA) and F_2 RFLP linkage data, we mapped three dominant oat dwarfing loci to different regions of the oat genome. *Dw6*, in oat line OT207, is 3.3 ± 1.3 cM from the *Xumn145B* locus, which has not been placed on the hexaploid oat linkage map. *Dw7*, in line NC2469-3, is 4.3 ± 2.3 cM from *Xcdo1437B* and 33 ± 4.1 cM from *Xcdo708B*. This places *Dw7* to linkage group 22. *Dw8*, in the Japanese lines AV17/3/10 and AV18/2/4, mapped 4.9 ± 2.2 cM from *Xcdo1319A* in an AV17/3/10 × 'Kanota' F_2 population and 6.6 ± 2.6 cM from it in an AV18/2/4 × 'Kanota' population. This places *Dw8* to linkage group 3. Aneuploid analysis of markers linked to the dwarfing genes located *Dw6* on the smallest oat chromosome (chromosome 18) and *Dw7* on the longest satellited chromosome (chromosome 19). The RFLP markers closely linked to the three dwarfing genes identify distinct regions of the oat genome that contribute to plant height and they should be useful in characterizing new genetic sources of dwarfness in oat.

Key words *Avena sativa* · Dwarfing genes · RFLP · RFLP mapping · Bulked Segregant Analysis

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Introduction

One of the goals of oat breeding has been to reduce plant height in order to minimize the substantial lodging problems that affect grain quality and quantity. Because of its importance, plant height is a good candidate trait for restriction fragment length polymorphism (RFLP) mapping in oat.

Major dwarfing genes have been extensively used in developing semidwarf wheat and high-yielding rice varieties (Gale and Youssefian 1985). In oat, seven dwarfing genes have been genetically classified (Marshall and Murphy 1981). *Dw6* in line OT207 and *Dw7* in NC2469-3 have shown considerable potential in breeding programs (Federizzi and Qualset 1989; Farnham et al. 1990). A new locus, *Dw8*, was recently characterized among sources of dwarfness from Japan (Milach et al. 1997). Plants that carry a dwarfing allele at one of the *Dw6*, *Dw7*, or *Dw8* loci have a recognizable phenotype that is easy to score in a range of environments. This facilitates the molecular genetic mapping of these genes.

Recent development of the hexaploid oat RFLP linkage map (O'Donoughue et al. 1995) provides a new perspective for mapping important agronomic traits in

oat. The next step in using the map for breeding applications and for a better understanding of the oat genome is to locate important agronomic loci on the RFLP map.

Dwarfing genes have been recently mapped by molecular marker association in various small grain cereals. The *sd-1* semidwarfing gene was mapped on rice chromosome 1 (Cho et al. 1994), and the barley *denso* dwarfing gene was located on the long arm of chromosome 3H (Barua et al. 1993). Saghai Maroof et al. (1996) noted the potential orthology of the rice *sd-1* and the barley *sdw-b* genes, based on their common comparative map positions, and Van Deynze et al. (1995a) noted putative orthologous loci for dwarfing located on homoeologous chromosomes Triticeae 2L, rice 4, and maize 2 or 10. The mapping of dwarfing genes in oat is the first step in studying the relationships between them and dwarf mutants in other cereal species. The identification of mapped RFLP markers closely linked to the dwarfing genes will also be useful for genetically characterizing new sources of dwarfing genes in oat by allowing determination of the genomic location of a dwarfing gene in a single cross versus the need to testcross against all known dwarfing mutants to identify allelism or close linkage.

The location of major genes and quantitative trait loci (QTLs) on linkage maps using RFLP technology provides insights about the association between qualitative and quantitative gene loci. Robertson (1985) suggested that alleles with qualitative effects may represent the extreme of a spectrum of possible alleles at a locus. Paterson et al. (1995) noted the existence of both qualitative and quantitative loci at corresponding map positions among several of the cereals for traits that have been involved in the domestication of these species for grain production. The identification and location of qualitative and quantitative trait loci on RFLP linkage maps will be important for better understanding the basis of quantitative trait inheritance and searching for genomic regions of interest in crop breeding.

Distinct methods have been used to identify molecular markers associated with genes of interest in oat. Linkage associations between two oat crown rust resistance genes and RFLP markers were identified by Rooney et al. (1994b) from screening a series of early-generation backcross-derived lines using a large set of randomly selected RFLP markers. Bush et al. (1994) used oat diploid maps (O'Donoughue et al. 1992; Rayapati et al. 1994) to aid in producing backcross-derived near-isogenic lines (NILs). With these, RFLP marker linkages to three oat crown rust resistance genes were found. The NILs used in these experiments are effective tools for molecular mapping, but take time to develop. The Bulked Segregant Analysis (BSA) approach, as described by Michelmore et al. (1991), eliminates the need to generate NILs in that F_2s can be used to identify marker associations. The BSA approach was used in oat to identify random amplified polymorphic DNA (RAPD) marker linkages to genes for oat crown and stem rust resistance (Penner et al. 1993a, and b) and daylength insensitivity (Wight et al. 1994). O'Donoughue et al. (1996) recently reported using BSA and NILs to identify molecular marker associations to additional crown and stem rust resistance genes, enabling the positioning of these genes in the oat linkage map.

The objectives of the study reported here were to (1) map the three dwarfing loci available in oat using RFLP markers and thereby identify regions of the genome which contribute to plant height, and (2) verify the utility of BSA and the hexaploid oat RFLP map for mapping dwarfing genes in cultivated oat.

Materials and methods

Plant materials

Four dwarf lines were selected to represent three distinct dwarfing loci previously identified in hexaploid oat. The OT207 semidwarf line, with the *Dw6* dominant gene, was derived from the tall cultivated oat (*A. sativa* L.) line OT184 by gamma irradiation (Brown et al. 1980). The NC2469-3 (CI 8447) line, with the *Dw7* dominant gene, was selected as a spontaneous mutant from its tall parental line NC2469 (Marshall and Murphy 1981). The AV17/3/10 and the AV18/2/4 dwarf lines, with the *Dw8* dominant gene, were isolated in Japan from accessions of the wild oat *A. fatua* L. and introgressed into the cultivar 'Kanota' by four backcrosses (Morikawa 1989). 'Kanota' has been used to develop the molecular linkage map of hexaploid oat (O'Donoughue et al. 1995) and as the recurrent parent for the Japanese lines. For these reasons, 'Kanota' was chosen as the tall parent in developing the mapping populations in this study. Dwarf and tall genotypes were grown in field nurseries at St. Paul, Minnesota, in 1992 and 1993. Mean plant heights ranged from 65 to 85 cm for OT207, 60 to 80 cm for NC2469-3, and 45 to 55 cm for the Japanese lines, compared with 90 to 110 cm for 'Kanota'.

RFLP analysis

Bulked Segregant Analysis (BSA) (Michelmore et al. 1991) was used to screen the RFLP clones and identify markers putatively associated with the dwarfing genes. BSA was carried out by pooling separately the DNA of 29 dwarf and 17 tall plants from population OT207 × 'Kanota', 13 dwarf and 13 tall plants from population NC2469-3 × 'Kanota', 19 dwarf and 12 tall plants from population AV17/3/10 × 'Kanota', and 23 dwarf and 20 tall plants from population AV18/2/4 × 'Kanota'. Because the dwarf trait is dominant, F_3 progeny tests were needed to distinguish dwarf homozygous from dwarf heterozygous F_2 plants. The F_3 progeny test was done by growing a row of progeny (at least 17 plants) from each F_2 plant to check for heterozygosity. The $F_{2:3}$ families were grown in field nurseries in 1992 or 1993.

Screening blots to identify polymorphisms were made with DNA extracts from the tall and dwarf parents and bulked F_2 samples, each digested with three restriction enzymes. Confirmation of linkage between RFLP markers and dwarfing genes was done through the analysis of mutant × 'Kanota' F_2 populations. The size of the F_2 mapping populations was 94 individuals for OT207 × 'Kanota', 79 for NC2469-3 × 'Kanota', 99 for AV17/3/10 × 'Kanota', and 87 for AV18/2/4 × 'Kanota'.

Tissue samples for the DNA extraction of all parents were from plants grown in a greenhouse or growth chamber. Individual F_2 plants from the mapping populations grown in the field were labeled for later identification, and one or two of their secondary tillers were harvested for DNA extraction. Seeds harvested from these F_2 plants were used for F_3 progeny tests. Tissue from all sources was freeze-dried, mechanically ground, and stored at $-20°C$. The modified CTAB procedure of Saghai-Maroof et al. (1984) was used for DNA extractions. Genomic DNA was digested with *Eco*RI, *Eco*RV, and *Dra*I restriction enzymes (GIBCO BRL) using standard restriction digest procedures recommended by the manufacturer. Twenty-microgram samples of restricted DNA were loaded into 0.8% agarose gels, electrophoresed overnight, and transferred onto Immobilon-N DNA membranes (Millipore) according to the manufacturer's instructions. Probes were labeled with [^{32}P] via random oligonucleotide primer labeling (Feinberg and Vogelstein 1984). Hybridizations were done as described by Rooney et al. (1994a).

The probes for RFLP analysis were from four different libraries. A set of oat *Pst*I genomic probes (denoted with a OG prefix), wheat *Pst*I genomic probes (denoted with a WG prefix), and cDNA probes from an etiolated oat leaf cDNA library (denoted with a CDO prefix) were kindly provided by Dr. M. Sorrells of Cornell University. An oat endosperm cDNA library (denoted with a UMN prefix) characterized at the University of Minnesota was the fourth source of RFLP probes. A core set of probes was chosen to cover the oat hexaploid RFLP map at about 20-cM intervals.

After an evaluation of the RFLP probes on the screening blots, probes for markers that showed putative linkages were hybridized to DNA of the F_2 mapping populations digested with the appropriate enzyme. Putatively linked markers were defined as those for which the bulked dwarf sample had the same RFLP pattern as the dwarf parent and the bulked tall the same pattern as the tall parent. LINKAGE-1 software (Suiter et al. 1983) was used to calculate the linkage values between RFLP markers and dwarfing genes from F_2 data. Recombination values were converted to centimorgan units (cM) using the Haldane mapping function described by Crow and Dove (1990).

Image densitometry analysis

'Kanota' oat monosomic stocks K12 and K14, both lacking chromosome 19, the longest satellited chromosome, and the nullisomic stock K21 lacking chromosome 18, the smallest oat chromosome (Jellen et al. 1993b), were used to confirm the assignment of *Dw7* and *Dw6*, respectively, to those physical chromosomes. A blot with DNA from the K12 and K14 stocks, 'Kanota', NC2469-3, and bulked samples was hybridized with CDO1437. The X-ray film obtained was scanned using a 300 dpi Apple Scanner with the AppleScan software and quantified using densitometry analysis. To detect the bands where the signal intensity was reduced, we measured band intensity using the software Image 1.43 available through the National Institutes of Health, Bethesda, Md., with the procedure described by Rooney et al. (1994a).

Results and discussion

DNA hybridization blots were made to compare RFLP patterns of the various dwarf oat lines, the tall 'Kanota' parent, and bulked samples of F_2 dwarf and tall segregants from crosses of the dwarf lines with 'Kanota' (Table 1). The number of probes screened on the survey blots was 84 for AV17/3/10, 118 for AV18/2/4, 151 for NC2469-3, and 173 for OT207. The number of probes screened with each dwarfing locus reflects in general the number tested before linkage associations were revealed. The highest level of polymorphism was identified between the facultative winter-type 'Kanota' and the spring type OT207, where 61% of the probes detected a polymorphism with at least one restriction enzyme. The level of polymorphism was 44% between 'Kanota' and the winter-type NC2469-3. The levels of polymorphism of 17% and 13% that were detected between 'Kanota' and the Japanese lines, AV17/3/10 and AV18/2/4, respectively, were higher than expected for these BC_4F_6 lines, which had been developed using 'Kanota' as the recurrent parent. The original 'Kanota' was heterogeneous and was reselected at the University of Minnesota to provide a more uniform line for genetic studies. It is likely that this reselected 'Kanota' line differs from the 'Kanota' parent of the Japanese backcross-derived lines.

Of the three restriction enzymes used for screening, *Dra*I identified the highest frequency of polymorphisms for OT207 and NC2469-3, followed by *Eco*RI and *Eco*RV. Rooney (1992) also found that *Eco*RI revealed more RFLPs than *Eco*RV. RFLP levels were similar among the three enzymes for AV17/3/10 and AV18/2/4.

The probes used on the OT207 screening blots represented 42% of the 532 loci that had been mapped on the oat RFLP hexaploid map (O'Donoughue et al. 1995). Thirty-six percent of the mapped loci were represented in the screening of NC2469-3, 23% in the screening of AV17/3/10, and 30% in the screening of AV18/2/4. Of the 38 linkage groups that compose the current oat hexaploid RFLP map, 35 were surveyed for at least one locus for OT207, 34 for NC2469-3, 25 for AV17/3/10 and 31 for AV18/2/4 during the screening process. The linkage groups not surveyed were usually represented by only a few loci. Twenty-seven unlinked

Table 1 Segregation ratios for plant height of $F_{2:3}$ families from crosses of dwarf lines with 'Kanota'

Population	Number of $F_{2:3}$ families			χ^2 (1:2:1)	*P* value
	Dwarf	Heterozygous	Tall		
OT207 (*Dw6*) × Kanota	21	46	31	2.41	0.30
NC2469-3 (*Dw7*) × Kanota	24	50	16	2.53	0.28
AV17/3/10 (*Dw8*) × Kanota	17	22	12	1.94	0.38
AV18/2/4 (*Dw8*) × Kanota	23	18	20	10.73	< 0.01

RFLP loci were also tested for linkage with the dwarfing loci.

The BSA method is effective for rapidly mapping major loci and has been used for mapping quantitative (Chalmers et al. 1993) as well as qualitative traits (Michelmore et al. 1991). The application of this method proved effective in identifying putative linkages between RFLP markers and the oat dwarfing genes. For unlinked polymorphic markers, the RFLP pattern of the bulked samples was similar to that expected from an F_1 individual. The markers putatively linked to the dwarfing genes were those for which the RFLP pattern of the bulked dwarf F_2 progeny was similar to that of the dwarf parent and the pattern of the bulked tall F_2 progeny was similar to that of the tall parent (Figs. 1–3).

One of the factors contributing to the effectiveness of BSA in this study was the use of F_3 progeny tests to distinguish F_2 dwarf homozygous from heterozygous plants for making the bulked samples for probe screening. These tests confirmed that most of the populations were segregating for one major dwarfing locus (Table 1). The AV18/2/4 × 'Kanota' population deviated from the 1:2:1 expected $F_{2:3}$ segregation in having fewer than expected heterozygous dwarf individuals. This deviation could be because not all of the $F_{2:3}$ families for this population were analyzed since some of the F_2 plants set seed poorly due to late maturity. On the other hand, the AV18/2/4 × 'Kanota' F_2 population did segregate at the expected ratio of 3 dwarf: 1 tall.

All of the F_2 mapping populations segregated as expected for both plant height and RFLP loci associated with the dwarfing genes (Table 2). There were no distorted ratios to influence the mapping of the dwarfing genes. For OT207 and NC2469-3, F_3 progeny tests were used to classify the F_2 plants into the three genotypic classes for plant height. F_2 plant height data were used for AV17/3/10 and AV18/2/4 because F_3 data could not be obtained for all F_2 individuals (Table 2).

The marker locus *Xumn145B* was identified as putatively linked to *Dw6* using BSA (Fig. 1). Because sequences recognized by UMN145 had not been placed on the oat RFLP linkage map, this association between marker and gene took the longest to find. For this reason, more probes had to be tested on the OT207 screening blots than were needed for the other dwarfs. After trying all the probes available in our core set of mapped markers, we screened available unmapped markers.

The F_2 linkage data confirmed that the unmapped *Xumn145B* locus is closely linked to the *Dw6* gene. The genetic distance between the locus for the *Dra*I middle band detected by probe UMN145 and the *Dw6* gene was estimated at 3.3 cM with a standard error of 1.3 cM (Table 3). Rooney et al. (1994b) have determined

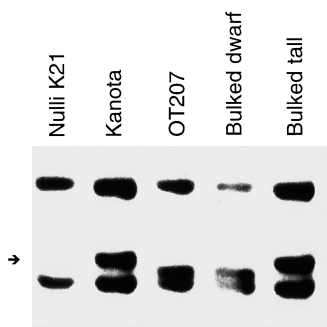

Fig. 1 Autoradiogram of the screening blot identifying a polymorphism between 'Kanota' and OT207 for the *Xumn145* locus that reveals an apparent association with the dwarfing gene in OT207. The absence of the polymorphic sequence in nullisomic K21 is evidence that the sequence (*arrow*) is located on the chromosome pair missing in K21. DNA samples were digested with *Dra*I restriction enzyme

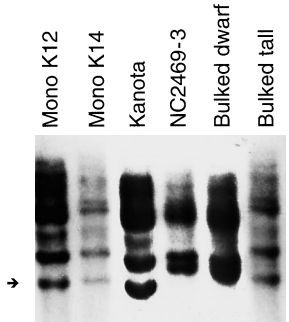

Fig. 2 Autoradiogram of the screening blot identifying a polymorphism between 'Kanota' and NC2469-3 lines for the *Xcdo 1437* locus that reveals an apparent association with the dwarfing gene in NC2469-3. The band associated with *Dw7* (*arrow*) is less intense relative to other sample bands in the monosomics K12 and K14 than it is in 'Kanota', indicating that the sequence is located on the chromosome missing in K12 and K14. DNA samples were digested with *Eco*RV restriction enzyme

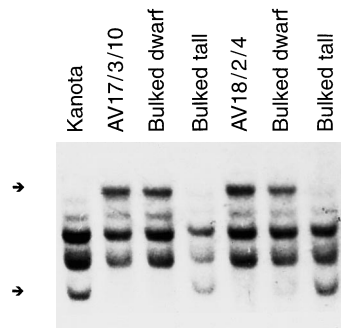

Fig. 3 Autoradiogram of the screening blot identifying a polymorphism between 'Kanota' and the AV17/3/10 and AV18/2/4 lines for the *Xcdo 1319* locus that reveals an association with the dwarfing gene in the AV lines. DNA samples were digested with *Eco*RV restriction enzyme

Table 2 Segregation ratios of plant height and RFLP loci for the mapping populations

Population	Locus	Genotypic classes			χ^2	*P* value
		AA	AB	BB		
OT207 (*Dw6*) × Kanota	Height[a]	22	43	29	1.72[c]	0.42
	Xumn 145B	20	43	31	3.25[c]	0.19
NC2469-3 (*Dw7*) × Kanota	Height	21	43	15	1.53[c]	0.46
	Xcdo1437B	63[b]		16	0.94[d]	0.33
	Xcdo708B	19	44	16	1.25[c]	0.53
AV17/3/10 (*Dw8*) × Kanota	Height	82[b]		17	3.23[d]	0.07
	Xcdo1319A	30	50	19	2.45[c]	0.29
AV18/2/4 (*Dw8*) × Kanota	Height	65[b]		22	0.00[d]	1.0
	Xcdo1319A	27	42	18	1.96[c]	0.37

[a] For height, AA = dwarf phenotype, AB = segregating in $F_{2:3}$ families, and BB = tall
[b] $F_{2:3}$ families not analyzed to distinguish between homozygotes and heterozygotes
[c] Expected ratio is 1:2:1
[d] Expected ratio is 3:1

Table 3 F_2 linkage data for the RFLP markers associated with the *Dw6*, *Dw7*, and *Dw8* dwarfing genes

RFLP locus	Height locus	Dwarf line	Linkage value[a]	Linkage group[b]	Aneuploid location
Xumn 145B[c]	*Dw6*	OT207	3.3 ± 1.3	Undetermined	chr. 18
Xcdo 1437B[c]	*Dw7*	NC2469-3	4.3 ± 2.3	22	chr. 19
Xcdo 708B[c]	*Dw7*	NC2469-3	33.0 ± 4.1	22	chr. 19
Xcdo 1319A[d]	*Dw8*	AV17/3/10	4.9 ± 2.2	3	Undetermined
Xcdo 1319A[d]	*Dw8*	AV18/2/4	6.6 ± 2.6	3	Undetermined

[a] Linkage values are in centiMorgans
[b] Linkage group designation as given in O'Donoughue et al. (1995)
[c] Polymorphism identified with *Dra*I restriction enzyme
[d] Polymorphism identified with *Eco*RV restriction enzyme

that the locus for the *Eco*RI 6.0-kb band detected by probe UMN145 and located on the missing chromosome in the 'Kanota' nullisomic stock K21 is associated with *Pc 91*. To determine if *Dw6* was also on this chromosome and associated with the same *Xumn145* locus as *Pc 91*, we hybridized UMN145 to DNA of K21 digested with *Dra*I (Fig. 1). The K21 nullisomic indeed lacked the DNA fragment for the marker associated with *Dw6*. This finding locates *Dw6* on the smallest oat chromosome and indicates that *Dw6* and *Pc 91* are possibly linked to the same RFLP locus and may be linked to each other. According to Rooney et al. (1994b), this locus is located on the arm present in Sun II ditelosomic stock XIII. This line is missing one arm of the same chromosome that is missing in the K21 nullisomic. The chromosome is designated as chromosome 18 in the generic karyotype of Jellen et al. (1993a).

An association between dwarfing and disease resistance genes has been reported in other small grain cereals. Knott (1989) found that the dominant dwarfing gene present in the wheat cultivar 'Webster' is closely linked to the *Sr30* stem rust resistance gene located on chromosome 5D. Resistance to *Septoria tritici* Blotch in winter wheat is associated with the presence of the dwarfing gene Rht_2 (Baltazar et al. 1990). *Xumn145B* appears to be linked to an important region of the oat genome that contains several important and interesting genes, such as *Pc 91*, which is a complex locus of various rust resistance genes (B.-C. Wu, personal communication). The implication of these results for oat breeding is that the dwarfing gene might be used as a morphological marker to select lines for rust resistance. However, a three-point genetic analysis still needs to be carried out to determine the genetic order and the distance among *Xumn145B*, *Dw6*, and *Pc 91*.

CDO708 was the first probe to reveal a polymorphism putatively linked to *Dw7* using BSA. The F_2 linkage analysis determined that a *Dra*I polymorphic locus was 33 ± 4.1 cM from *Dw7*. To find a closer linkage to *Dw7*, we used the hexaploid oat RFLP map to look for probes that detect loci mapped around *Xcdo708* loci. There are two *Xcdo708* loci on the map; these are located on linkage groups 6 and 22, respectively (O'Donoughue et al. 1995). To determine which *Xcdo708* locus was associated with *Dw7*, we tested the 'Kanota' × NC2469-3 F_2 mapping population with

probes that detected mapped polymorphisms linked to either *Xcdo708* locus. The probes tested detected a *Xumn162* locus located 3.1 cM from *Xcdo708A* on linkage group 6 and locus *Xcdo1437B* located 17.6 cM from *Xcdo708B* on linkage group 22. The expected outcome was that *Dw7* would be linked to *Xumn162* if the locus associated with the dwarfing gene was *Xcdo708A*, or it would be linked to *Xcdo1437B* if the locus being mapped was *Xcdo708B*. The F_2 linkage analysis revealed that *Dw7* is not linked to *Xumn162* and that the dwarfing gene is 4.3 ± 2.3 cM from *Xcdo1437B* (Table 3). This result places *Dw7* on linkage group 22 near *Xcdo1437B*. The linkage distance between *Xcdo708B* and *Xcdo1437B* obtained with the NC2469-3 × 'Kanota' F_2 population was 45 ± 6.4 cM compared to 17.6 cM obtained with the 'Kanota' × 'Ogle' F_6 recombinant inbred lines (O'Donoughue et al. 1995). Even though the mapping distances differed across populations, the use of the oat hexaploid RFLP linkage map was shown to be of value in locating genes of agronomic importance on the oat genetic map.

Linkage group 22 has been assigned to the longest satellited chromosome, which is the chromosome missing in 'Kanota' monosomic stocks 12 (K12) and 14 (K14) (Phillips et al. 1995). To confirm the location of *Dw7* on that physical chromosome, we used CDO1437 to hybridize blots with DNA of K12 and K14 monosomics digested with *Eco*RV (Fig. 2). Image densitometry analysis was used to compare the intensity of the lowest (smallest molecular weight) two bands within each lane. Since the lowest band is the one associated with the *Dw7* gene and its intensity is decreased in the monosomic stocks and not in the tall or Kanota disomic stocks, it was concluded that the placement of this dwarfing gene on linkage group 22 and its assignment to the longest oat satellited chromosome is correct.

The location of *Dw7* on linkage group 22 of the hexaploid oat RFLP map coincides with a major QTL for plant height detected in the 'Kanota' × 'Ogle' mapping population (Siripoonwiwat et al. 1996). Similar results have been found in maize where QTLs have been found in close proximity to mapped qualitative loci for plant height (Beavis et al. 1991; Edwards et al. 1992; Veldboom et al. 1994), date of anthesis, and resistance to first-brood European corn borer (Veldboom et al. 1994). The locations of major dwarfing loci on the oat RFLP linkage map appear to be a starting point for the identification of genomic regions which control plant height. Also, as was observed for *Dw6*, *Dw7* on the hexaploid oat map is located near a disease resistance gene–*Pg13*, a stem rust resistance gene located in linkage group 22 by O'Donoughue et al. (1996).

The BSA approach showed the *Eco*RV polymorphism detected by the probe CDO1319 to be associated with *Dw8* of AV17/3/10 and AV18/2/4 (Fig. 3). F_2 linkage analysis determined that the distance between the *Dw8* gene and *Xcdo1319A* is 4.9 ± 2.2 cM for the AV17/3/10 × 'Kanota' population and 6.6 ± 2.6 cM for the AV18/2/4 × 'Kanota' population (Table 3). The linkage values obtained with the two populations are similar, and their confidence intervals overlap, indicating that the same dwarfing locus is likely being mapped in both populations. The CDO1319 probe also detects two loci on the oat RFLP map, in linkage groups 3 and 5 (O'Donoughue et al. 1995). To identify which locus detected by CDO1319 is associated with *Dw8*, we mapped the *Eco*RV polymorphism detected by CDO1319 with the original 'Kanota' × 'Ogle' mapping population. This was possible because the *Eco*RV polymorphism between 'Kanota' and 'Ogle' for CDO1319 is the same as the one between 'Kanota' and the Japanese lines. The *Xcdo1319* loci presently located on the RFLP map had been mapped with the *Eco*RI restriction enzyme. When the *Eco*RV polymorphism was mapped on the 'Kanota' × 'Ogle' population, the results revealed that the locus associated with *Dw8* is *Xcdo1319A*, located on linkage group 3 (data not shown), thus placing *Dw8* on linkage group 3. The chromosomal location of *Dw8* has not been identified because the assignment of linkage group 3 markers to a physical chromosome still needs to be determined.

Through the molecular genetic mapping of dwarfing genes in oat, we have identified three regions of the hexaploid oat genome that play a role in controlling plant height. The dwarfing mutants mapped in these three regions are distinct from one another in their phenotypic effects, but each is inherited in a dominant monogenic fashion (Milach et al. 1997). There is currently no evidence for homoeology among the chromosome regions carrying the three dominant dwarfing genes nor of orthology of these regions with dwarfing genes in wheat and rye (Van Deynze et al. 1995a; Borner et al. 1996), but homoeology is difficult to determine in hexaploid oat as the species is characterized by numerous chromosomal rearrangements including translocations, duplications, and inversions (Rajhathy and Thomas 1974; Rooney et al. 1994a; O'Donoughue et al. 1995; Van Deynze et al. 1995b; Leggett and Markhand 1995; Kianian et al. 1997). However, the information presented here does give a better understanding of how the dwarf trait in hexaploid oat is genetically determined and may allow more effective manipulation of these genomic regions affecting plant height in cultivated, hexaploid oat.

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References

Baltazar BM, Scharen AL, Kronstad WE (1990) Association between dwarfing genes 'Rht1' and 'Rht2' and resistance to *Septoria tritici* Blotch in winter wheat (*Triticum aestivum* L. em Thell). Theor Appl Genet 79 : 422–426

Barua UM, Chalmers KJ, Thomas WTB, Hackett CA, Lea V, Jack P, Forster BP, Waugh R, Powell W (1993) Molecular mapping of genes determining height, time to heading, and growth habit in barley (*Hordeum vulgare*). Genome 36 : 1080–1087

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor Appl Genet 83 : 141–145

Borner A, Plaschke J, Korzun V, Worland AJ (1996) The relationships between the dwarfing genes of wheat and rye. Euphytica 89 : 69–75

Brown PD, McKenzie RIH, Mikaelsen K (1980) Agronomic, genetic and cytologic evaluation of a vigorous new semidwarf oat. Crop Sci 20 : 303–306

Bush AL, Wise RP, Rayapati PJ, Lee M (1994) Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome 37 : 823–831

Chalmers KJ, Barua UM, Hackett CA, Thomas WTB, Waugh R, Powell W (1993) Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theor Appl Genet 87 : 314–320

Cho YG, Eun MY, McCouch SR, Chae YA (1994) The semidwarf gene, *sd-1*, of rice (*Oryza sativa* L.). II. Molecular mapping and marker-assisted selection. Theor Appl Genet 89 : 54–59

Crow JF, Dove WF (1990) Anecdotal, historical and critical commentaries on genetics: mapping functions. Genetics 125 : 669–671

Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor Appl Genet 83 : 765–774

Farnham MW, Stuthman DD, Pomeranke GJ (1990) Inheritance of and selection for panicle exertion in semidwarf oat. Crop Sci 30 : 328–334

Federizzi LC, Qualset CO (1989) Genetics of plant height reduction and panicle type in oat. Crop Sci 29 : 551–557

Feinberg AP, Vogelstein BL (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137 : 266–267

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In: Russell GE (ed) Progress in plant breeding. Butterworth, London, pp 1–35

Jellen EN, Phillips RL, Rines HW (1993a) C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome 36 : 1129–1137

Jellen EN, Rooney WL, Phillips RL, Rines HW (1993b) Characterization of the hexaploid oat *Avena byzantina* cv 'Kanota' monosomic series using C-banding and RFLPs. Genome 36 : 962–970

Kianian SF, Wu B-C, Fox SH, Rines HW, Phillips RL (1997) Aneuploid marker assignment in hexaploid oat with the C genome as a reference for determining remnant homoeology. Genome 40 : 386–396

Knott DR (1989) The mode of inheritance of a type of dwarfism in common wheat. Genome 32 : 932–933

Leggett JM, Markhand GS (1995) The genomic identification of some monosomics of *Avena sativa* L. cv. 'Sun II' using genomic in situ hybridization. Genome 38 : 747–751

Marshall HG, Murphy CF (1981) Inheritance of dwarfness in three oat crosses and relationship of height to panicle and culm length. Crop Sci 21 : 335–338

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88 : 9828–9832

Milach SCK, Rines HW, Phillips RL, Stuthman DD, Morikawa T (1997) Inheritance of a new dwarfing gene in oat. Crop Sci (in press)

Morikawa T (1989) New genes for dwarfness transferred from wild oats *Avena fatua* into cultivated oat. In: Mattsson B, Lyhagen R (eds) Proc 3rd Int Oat Conf. Svalof AB, Lund, Sweden, pp 41–46

O'Donoughue LS, Wang Z, Röder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP linkage map of oats based on a cross between two diploid taxa (*Avena atlantica* × *A. hirtula*). Genome 35 : 765–771

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38 : 368–380

O'Donoughue LS, Chong J, Wight CP, Fedak G, Molnar SJ (1996) Localization of stem rust resistance genes and associated molecular markers in cultivated oat. Phytopathology 86 : 719–727

Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF, Pinson SRM, Lin S-C, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269 : 1714–1718

Penner GA, Chong J, Levesque-Lemay M, Molnar SJ, Fedak G (1993a) Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet 85 : 702–705

Penner GA, Chong J, Wight CP, Molnar SJ, Fedak G (1993b) Identification of a RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome 36 : 818–820

Phillips RL, Rines HW, Jellen EN, Rooney WL, Wu BC, Kianian S, Riera-Lizarazu O (1995) Oat genome analysis via molecular markers and oat × corn crosses. In: Raupp WJ, Gill BS (eds) Classical and molecular cytogenetic analysis. Proc of a U.S.–Japan Symp. Kansas Agriculture Experiment Station Report 95-352-D, Manhattan, Kan., pp 49–57

Rajhathy T, Thomas H (1974) Cytogenetics of oats (*Avena* L.) Misc Publ Genet Soc Canada No 2

Rayapati PJ, Gregory JW, Lee M, Wise RP (1994) A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. '*avenae*'. Theor Appl Genet 89 : 831–837

Robertson DS (1985) A possible technique for isolating genomic DNA for quantitative traits in plants. J Theor Biol 117 : 1–10

Rooney WL (1992) Identification and characterization of RFLP markers linked to crown rust resistance in oat (*Avena sp.*). PhD thesis. University of Minnesota, St. Paul. Diss Abstr DA9308749

Rooney WL, Jellen EN, Phillips RL, Rines HW, Kianian SF (1994a) Identification of homoeologous chromosomes in hexaploid oat (*A. byzantina* cv 'Kanota') using monosomics and RFLP analysis. Theor Appl Genet 89 : 329–335

Rooney WL, Rines HW, Phillips RL (1994b) Identification of RFLP markers linked to crown rust resistance genes *Pc91* and *Pc92* in oat. Crop Sci 34 : 940–944

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley : Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81 : 8014–8018

Saghai Maroof MA, Yang GP, Biyashev RM, Maughan PJ, Zhang Q (1996) Analysis of barley and rice genomes by comparative RFLP linkage mapping. Theor Appl Genet 92 : 541–551

Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. J Quant Trait Loci 2, Article 3. http://probe.nalusda.gov:8000/otherdocs/jqtl/jqtl1996-03

Suiter KA, Wendel JF, Case JS (1983) LINKAGE 1: a pascal computer program for the detection and analysis of genetic linkage. J Hered 74 : 203–204

Van Deynze AE, Nelson JC, Harrington SN, Yglesias ES, Braga D, McCouch SR, Sorrells ME (1995a) Comparative mapping in grasses. Wheat relationships. Mol Gen Genet 248 : 744–754

Van Deynze AE, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SN, Yglesias ES, Braga DP, McCouch SR, Sorrells ME (1995b) Comparative mapping in grasses. Oat relationships. Mol Gen Genet 249 : 349–356

Veldboom LR, Lee M, Woodman WL (1994) Molecular marker-facilitated studies in an elite maize population : I. Linkage analysis and determination of QTL for morphological traits. Theor Appl Genet 88 : 7–16

Wight CP, Penner GA, O'Donoughue LS, Burrows VD, Molnar SJ, Fedak G (1994) The identification of random amplified polymorphic DNA (RAPD) markers for daylength insensitivity in oat. Genome 37 : 910–914