Theor Appl Genet (2012) 124:1115–1125
DOI 10.1007/s00122-011-1773-7

ORIGINAL PAPER

# Comparative mapping of the oat *Dw6/dw6* dwarfing locus using NILs and association with vacuolar proton ATPase subunit H

**Stephen J. Molnar · Julie T. Chapados · Sivakala Satheeskumar · Charlene P. Wight · Bonnie Bancroft · Winson Orr · Doris E. Luckert · Solomon Kibite**

Received: 2 August 2011 / Accepted: 15 December 2011 / Published online: 4 February 2012


**Abstract** Seven pairs of oat near-isogenic lines (NILs) (Kibite in Crop Sci 41:277–278, 2001) contrasting for the *Dw6* dwarfing gene were used to test for correlation between tall/dwarf phenotype and polymorphic genotype using restriction fragment length polymorphism (RFLP) and other molecular markers selected from the Kanota × Ogle (K×O) (Wight et al. in Genome 46:28–47, 2003) and Terra × Marion (De Koeyer et al. in Theor Appl Genet 108:1285–1298, 2004) recombination maps. This strategy located the *Dw6/dw6* locus to a small chromosomal region on K×O linkage group (LG) KO33, near or at a putative RFLP locus aco245z. Aco245z and other tightly linked flanking markers have potential for use in marker-assisted selection (MAS), and PCR-based markers were developed from several of these. RFLP genotyping of the *Dw6* NILs indicated that 13 of the 14 individual lines were homogeneously maternal or paternal for a large genomic region near *Dw6/dw6*, an unexpected result for NILs. The cDNA clone aco245 codes for a vacuolar proton ATPase subunit H, a potential candidate gene for *Dw6*. Vacuolar proton ATPase enzymes have a central role in plant growth and development and a mutation in subunit C is responsible for the *det3* dwarfing mutation in *Arabidopsis thaliana* (Schumacher et al. in Genes Dev 13:3259–3270, 1999). Aco245 affords the potential of designing highly precise diagnostic markers for MAS for *Dw6*. The *Dw6* NILs have potential utility to investigate the role of vacuolar proton ATPases in growth and development in plants.

Communicated by J. Snape.

S. Kibite: Deceased.

S. J. Molnar (✉) · J. T. Chapados · S. Satheeskumar · C. P. Wight · B. Bancroft · W. Orr · D. E. Luckert
Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Central Experimental Farm, 960 Carling Ave., Ottawa, ON K1A 0C6, Canada
e-mail: steve.molnar@agr.gc.ca

S. Kibite
Lacombe Research Centre, Agriculture and Agri-Food Canada, 6000 C and E Trail, Lacombe, AB T4L 1W1, Canada

## Introduction

Dwarfing genes played a major role in the development of the semi-dwarf wheat varieties at the centre of the "Green Revolution" spear-headed by Dr. Norman Borlaug of CIMMYT in the 1950s (Milach and Federizzi 2001). The dwarfing genes both increased yield and reduced stature, the latter also increasing lodging resistance. Most dwarfing genes are involved in the biochemical pathways producing gibberellins (GAs) in plants and the mutants themselves fall into two classes, GA sensitive and insensitive. As a consequence, dwarfing genes often have many pleiotrophic effects. Although eight dwarfing genes have been reported in oat, only *Dw6*, *Dw7*, and *Dw8* are readily available at present. *Dw6* and *Dw8* are dominant mutants and *Dw7* is partially dominant. All three produce GA-sensitive phenotypes and reduce yield or have other negative attributes. Despite these challenges, there has been considerable interest in deploying *Dw6* in oat breeding programs (Milach and Federizzi 2001; Tanhuanpaa et al. 2006). The specific phenotype observed with *Dw6* is a normal internode number with a reduction in the length of the highest three internodes (Milach et al. 2002).

Through comparative mapping, *Dw7/dw7* has been placed on LG KO22 of the K×O hexaploid oat reference map, which corresponds to chromosome 19, and *Dw8/dw8* has been placed on KO3 (Milach et al. 1997). *Dw7/dw7*

maps to the same location as a K×O height quantitative trait locus (QTL) reported by Siripoonwiwat et al. (1996). *Dw6/dw6* is reported to be linked (at 3.3 cM) to the RFLP molecular marker Xumn145B (or umn145b), based on a *DraI* polymorphism (Milach et al. 1997). Aneuploid analysis suggested that *Dw6/dw6* is on chromosome 18, although the corresponding KO LG could not be determined (Milach and Federizzi 2001). Recently, a single nucleotide polymorphism (SNP) marker derived from a retrotransposon microsatellite amplified polymorphism (REMAP) marker and a SNP marker derived from a random amplified polymorphic DNA (RAPD) marker were developed and linked to *Dw6/dw6*. They were located 5.2 and 12.6 cM from *Dw6/dw6* in the $F_2$ segregating population Aslak × Kontant (Tanhuanpaa et al. 2006), but the map locations of the three loci were not reported. The present study reports the use of comparative mapping and NILs to locate *Dw6/dw6* to a narrow interval on LG KOD_33 and identifies a candidate gene. A preliminary partial report was presented as a conference poster (Chapados et al. 2006).

## Materials and methods

Seven pairs of NILs contrasting for *Dw6* were developed by the late Dr. Solomon Kibite, using OT257 as the common donor of *Dw6* (Table 1). In each case, the tall and short lines constituting a NIL pair consisted of bulked seed from a single $F_{8:10}$ family, selected from a single heterozygous $F_8$ individual, derived by single seed descent from a single $F_2$ individual (Kibite 2001).

Molecular marker techniques used standard RAPD and RFLP protocols (Wight et al. 2003). The protocol of Locatelli et al. (2006) was used to identify amplified fragment length polymorphism markers (AFLP). Markers were selected for this study based on the Terra × Marion (T×M) (De Koeyer et al. 2004) and Kanota × Ogle (K×O) (Wight et al. 2003) recombination maps. The original K×O map of O'Donoughue et al. (1995) guided earlier aneuploid studies cited in this manuscript. The most recent DArT marker-enhanced K×O map of Tinker et al. (2009) was used for final comparative mapping and for data interpretation. The correspondence between LGs in the Wight et al. (2003) map, for example KO11_41+20, KO33, and KO36, and the corresponding LGs in the Tinker et al. (2009) map, for example KOD_11_41+20+45, KOD_33, and KOD_36, is shown in the latter publication. The KO and KOD LGs are collinear for most markers, and both LG nomenclatures are used in the present manuscript for consistency with the literature.

In the RFLP studies, gels included the *Dw6* parents, the *Dw6* NILs, and control lanes of Kanota, Ogle, Terra and Marion, the parents of the K×O and T×M mapping populations. This permitted the accurate comparison of band sizes within each gel to identify alleles shared by the *Dw6* lines and the mapping parents. Banding patterns were then compared with digital images of the autoradiograms used in the original K×O (O'Donoughue et al. 1995) and T×M (De Koeyer et al. 2004) mapping studies, upon which the polymorphic bands had been previously numbered and their assignment to loci recorded. Images of the Kanota and Ogle parental polymorphism autoradiograms for 13 probe/enzyme combinations relevant to the current study (bcd421 *Dra*I; bcd1407 *Dra*I; bcd1414 *Dra*I; bcd1716 *Dra*I; cdo780 *Dra*I, *Eco*RI and *Eco*RV; cdo1255 *Eco*RV; cdo1321 *Dra*I; cdo1380 *Eco*RI; cdo1428 *Dra*I, *Eco*RI and *Eco*RV) and showing the estimated molecular weight of polymorphic bands have been deposited in the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml, accessed September 21, 2011) by Dr. Mark Sorrells of Cornell University. Digital images of other probe/enzyme combinations for both K×O and T×M are unpublished.

A pair of NILs and their two parents were genotyped with DArT markers by Diversity Arrays Pty. Ltd., Australia (http://www.diversityarrays.com) and the data were assessed by calculating quality parameters that were defined and discussed previously (Tinker et al. 2009).

**Table 1** Parentage of *Dw6* NILs derived from OT257 (*Dw6* donor)

| | OT257 × Jasper | Jasper × OT257 | | | OT526 × OT257 | | OT257 × N326-7 | |
|---|---|---|---|---|---|---|---|---|
| Tall NIL | 524-T-02[a] | 525-T-01 | | 525-T-03 | 521-T-02 | 521-T-03 | 517-T-01 | 525-T-02[b] |
| Short NIL | 524-S-02 | 525-S-01 | 525-S-02[c] | 525-S-03 | 521-S-02 | 521-S-03 | 517-S-01 | |
| NIL set | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 |

[a] The full name of each line includes the prefix LAO- (for example LAO-524-T-02)

[b] Tall line 525-T-02 was reported to be derived from Jasper × OT257 (Kibite 2001); however, the current study suggests it was derived from OT257 × N326-7. Tall line 525-T-02 and tall parent N326-7 (and its derived tall line 517-T-01) share diagnostic RFLP patterns for clones aco187, aco227, aco245, cdo421, cdo780, cdo1255, cdo1321, cdo1428 and umn128

[c] Short line 525-S-02 was reported to be derived from Jasper × OT257 (Kibite 2001), and the current study is consistent with this. For two clones (cdo1255 and cdo1321), short line 525-S-02 displayed the tall allele and in both cases it was the tall allele of tall parent Jasper and not that of tall parent N326-7

Given that only four lines were genotyped, analysis was limited to the 990 DArT markers which gave identical scores on three independent technical replications. From these, 550 DArT markers were selected as meeting data quality standards ($P$ value not less than 85%; average call rate not less than 90%) which are more stringent than recommended for general genome mapping.

The standard techniques for the development and mapping of sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers and the specific details regarding CAPS marker ubc360s and SCAR marker ubc264kas are described in Orr and Molnar (2007, 2008). In addition, from RAPD marker acor333, SCAR marker ubc333ks (forward primer GTT TGA TAG TCA GAC TCA TAT AGC, reverse primer GAA TGC GAC GAC GTG TTT CTC, annealing temperature $T = 64$°C, yielding a 1.4 kb product in Kanota which is missing in Ogle) was developed and mapped to KO33 (Table 2). Similarly, from RAPD marker acor165c, SCAR marker ubc165ms (forward primer ACT GTT ACA CCT GTT AGC TCA G, reverse primer GAA GGC ACT GAT TAG ATA AGA ATA, $T = 62$°C, yielding a 0.55 kb product in Marion which is missing in Terra) was developed and mapped to TM1.

DNA sequencing of clone aco245 was done using a LI-COR genetic analyzer and standard forward and reverse primers. The consensus sequence was 1,151 bp in length and has been deposited in GenBank as accession JF913493. Orthologous sequences were identified in the NCBI GenBank database using the BLAST program (http://www.ncbi.nlm.nih.gov/). Syntenic relationships were explored using the Marker View tool available at Gramene (http://www.gramene.org), a resource for comparative genomics in grasses.

## Results

### Parentage of *Dw6* NILs

Seven pairs of near-isogenic lines (NILs) contrasting for *Dw6* were developed by the late Dr. Solomon Kibite using OT257 as the common donor of *Dw6* (Kibite 2001). Their phenotypes had been determined by growth in six environments (3 locations × 2 years) (Kibite 2001). There was a considerable difference between the tall and short members of each NIL pair (mean 36.2 cm, range 32.0–40.3 cm), and for each NIL pair the difference was significant at $P = 0.01$. OT257 is derived from OT207, the *Dw6* donor studied by Milach et al. (1997). The NILs are derived from four crosses but group into three sets based on common parentage (Table 1). At least nine RFLP probes used in the present study suggested that tall line (525-T-02) is misidentified in the earlier publication of Kibite (2001) and is in fact derived from the set three cross (Table 1). Based on two probes, we conclude that short NIL 525-S-02, the alleged partner of tall NIL 525-T-02, is correctly identified as a member of set one (Table 1). Within the present study, to capture the information from the resulting two unpaired lines as well as from the remaining six NIL pairs, the 14 lines were scored individually for correlation with expected genotype.

### Searching for *Dw6/dw6* by mapping umn145 and linked markers

Milach et al. (1997) linked *Dw6/dw6* with the RFLP locus umn145b in a population derived from the cross OT207 (carries *Dw6*) × Kanota. $F_2$ linkage analysis estimated linkage between the umn145b and *Dw6/dw6* loci as 3.3 ± 1.3 cM. As neither Kanota nor Ogle carries *Dw6* and umn145 is not polymorphic in the K×O cross, Milach et al. (1997) were unable to map either locus in the K×O reference population. Establishing the location of *Dw6/dw6* on a LG or chromosome would facilitate the identification of additional linked markers useful not only for marker-assisted selection (MAS) but also for a variety of genetic and genomic studies.

In the Terra × Marion (T×M) mapping population, a *Dra*I polymorphic umn145 locus was mapped to LG TM25, near a plant height QTL (De Koeyer et al. 2004). However, as the band sizes in Terra and Marion differ from those shared by OT207 and Kanota, it is not clear if the umn145 locus on TM25 is homologous or homoeologous to the umn145 locus linked to *Dw6/dw6*.

For the 14 *Dw6* NILs, the same umn145 *Dra*I polymorphism which had been mapped to TM25 correlated with a short/tall phenotype in only 7 of 14 lines (Table 2), which is no better than random chance. A second co-dominant polymorphism correlated in 13 of 14 lines and is likely linked to the *Dw6/dw6* locus; however, this locus could not be mapped as Kanota, Ogle, Terra, and Marion all had the same allele. A fifth *Dra*I band was monomorphic in the NILs. *Eco*RI banding patterns consisted of two monomorphic bands and a pair of co-dominant bands. The latter corresponded exactly with the *Dw6* related *Dra*I bands, and correlated with height in 13 of 14 NILs, but were monomorphic between the mapping parents. Results with both enzymes are consistent with the hypothesis that there are three umn145 loci in hexaploid oat, likely on homoeologous chromosomes, one of which is closely linked to the *Dw6/dw6* locus. Results with *Dra*I suggest that the *Dw6/dw6* locus is located on one of the two homoeologs of TM25. Studies of the detailed K×O reference map have identified KOD_36 as homologous to TM25, its homoeolog KO_33 as homologous to TM1, and a third homoeolog KOD_11_41+20+45 as homologous to an

**Table 2** Alignment of six LG maps, the resultant proposed consensus map of the *Dw6/dw6* region on KOD_33, and the correlation (Cor) between marker genotype and tall/short phenotype across the *Dw6* NILs

| TM1 (KO33) | | | KOD_33 | | | | KOD_11_41+20+45 | | TM25 (KO36) | | | KOD_36 | | | OM15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cM | Map | Cor | cM | Map | Proposed consensus map[a] | Cor | cM | Map | cM | Map | Cor | cM | Map | Cor | cM | Map |
| | * | | | * | bcd1407z | 12/13 | | * | | * | | | bcd1407 | M | | * |
| | * | | 0 | bcd269 | bcd269 | | | * | | * | | | * | | | * |
| | * | | | umn202 | umn202 | | | * | | * | | | * | | | * |
| | * | | 5 | opt-7387 | opt-7387 | | | * | | * | | | * | | | * |
| | * | | | * | umn498z | | | * | | * | | | umn498a | 7/13 | | * |
| | * | | | * | bcd1882z | | | * | | * | | | bcd1882b | 7/14 | | * |
| | * | | | acor360 | acor360 | | | * | | * | | | * | | | * |
| | * | | | * | isu1543z | | 66 | isu1543a | | * | | | * | | | * |
| | ubc165ms | M | | * | ubc165msz | | | * | | * | | | * | | | * |
| | aco187p | 3/3 | | * | aco187z | | | * | | * | | | * | | | * |
| | cdo780x | 3/3 | | * | cdo780z | | | * | | * | | | * | | | * |
| 5 | cdo1321b | 3/3 | | cdo1321b | cdo1321b | 3/3 | | * | 16 | cdo1321a | 7/14 | 14 | cdo1321a | 7/14 | | * |
| | acor165c | M | | * | acor165z | | | * | | * | | | * | | | * |
| | acor264 | M | | * | acor264z | | | * | | * | | | * | | | * |
| | umn128z | 3/3 | | * | umn128z | 13/14 | | * | | * | | | * | | | * |
| | aco227xp | 3/3 | | aco227di | aco227di | 13/14 | | * | | * | | | * | | | * |
| | * | | | * | bcd1860z | | | bcd1860 | | * | | | * | | | * |
| 10 | cdo497x | | | * | cdo497z | | | * | | * | | | * | | | * |
| | * | | | cdo1428b | cdo1428b | 13/14 | | cdo1428a | | * | | | * | | | * |
| | * | | | bcd421b | bcd421b | 13/14 | | bcd421a | | * | | | * | | | * |
| | * | | 6 | opt-2121 | opt-2121 | | | * | | * | | | * | | | * |
| | * | | | * | umn23z | 13/14 | | * | | * | | | umn23 | 7/14 | 5 | umn23b |
| | * | | | * | **aco245z** | **14/14** | | aco245b | 29 | aco245a | | | aco245a | | | * |
| | * | | | * | umn145z | 13/14 | | * | 29 | umn145 | 7/14 | | * | | 5 | umn145 |
| | * | | | * | bcd1716a | 12/13 | | bcd1716b | | * | | 9 | bcd1716a | M | 0 | bcd1716 |
| | * | | 11 | opt-11714 | opt-11714 | | | * | | * | | | * | | | * |
| | * | | | * | cdo1380z | | | * | | * | | 3 | cdo1380 | M | | * |
| | * | | | ubc333ks | ubc333ks | M | | * | | * | | | * | | | * |
| | * | | | * | cdo1090z | | 61 | cdo1090c | | * | | | * | | | * |
| 25 | cdo1255 | 2/3 | | cdo1255 | cdo1255 | 2/3 | | * | | * | | | * | | | * |
| | bcd1414a | M | | bcd1414a | bcd1414a | M | | * | | * | | | * | | | * |
| | * | | | * | og19z | | 43 | og19 | | * | | | * | | | * |
| | * | | 13 | opt-14516 | opt-14516 | | | * | | * | | | * | | | * |
| | * | | | bcd880b | bcd880 | | | bcd880a | | * | | | * | | | * |
| 31 | cdo836b | 2/3 | | cdo836brv | cdo836brv | 2/3 | 30 | cdo836arv | | * | | | * | | | * |
| | * | | 14 | opt-13366 | opt-13366 | | | * | | * | | | * | | | * |
| | * | | | * | bcd1270z | | | bcd1270 | | * | | | * | | | * |
| | * | | | * | cdo1502z | | | cdo1502 | | * | | | * | | | * |
| | * | | | acor333 | acor333 | M | | * | | * | | | * | | | * |
| | * | | 33 | opt-3486 | opt-3486 | | | * | | * | | | * | | | * |

*M* monosomic

Bold values indicate the perfect correlation for inferred locus aco245z

[a] Z after a locus name indicates that the locus on KOD_33 is inferred by comparative mapping

unknown TM LG (De Koeyer et al. 2004) as illustrated in Table 2 and Fig. 1. A umn145 locus was also mapped to LG OM15 in the Ogle × MAM17-5 RIL population and OM15 and KO36 are believed to be homoeologs (Zhu and Kaeppler 2003) (Fig. 1).

The umn145 locus on TM25 co-maps with RFLP locus aco245a and is 13 cM from RFLP locus cdo1321a (Fig. 1) (De Koeyer et al. 2004). Strikingly, on OM15 the umn145 and umn23b loci are coincident, and on KO36 the aco245a and umn23 loci are coincident. RFLP analysis for umn23

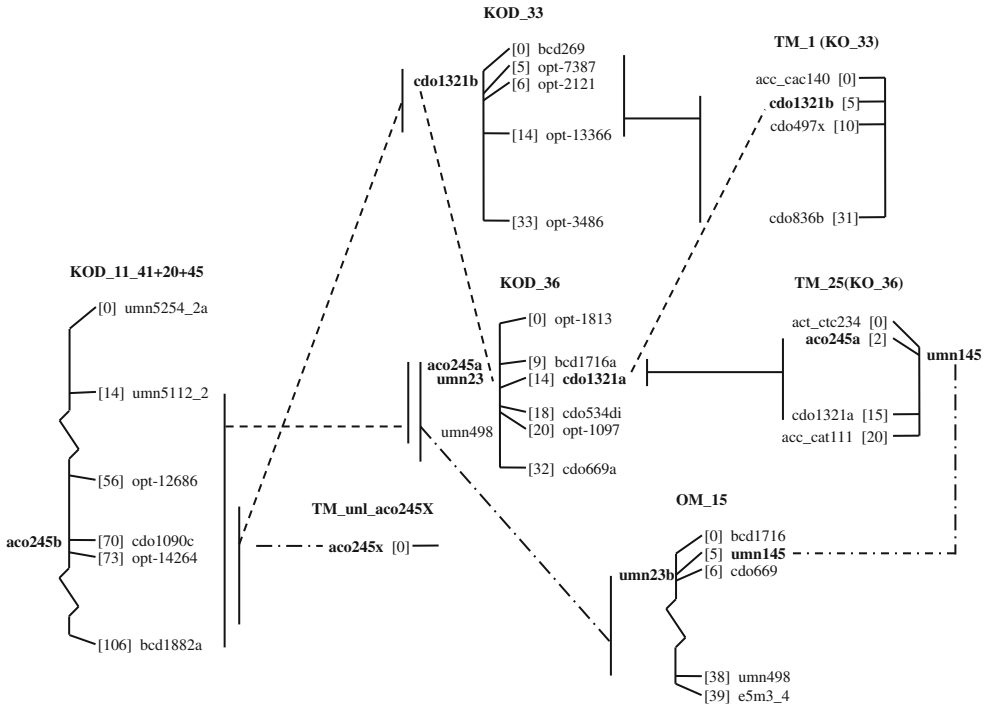


**Fig. 1** Comparative mapping of the *Dw6/dw6* region and its homologs and homoeologs in three hexaploid oat mapping populations (*KOD* Kanota × Ogle DArT map of Tinker et al. 2009, *TM* Terra × Marion map of De Koeyer et al. 2004, *OM* Ogle × MAM17-5 map of Zhu and Kaeppler 2003). Centimorgan distances are shown in *square brackets* for framework markers defining each LG map. Framework markers and selected markers mapped to intervals are shown on opposite sides of the *vertical line* depicting a LG segment. Markers in *bold font* are significant for the current comparative mapping. The KOD_11_41+20+45 LG map is drawn with the 14–56 cM and 73–106 cM regions condensed, and the OM_15 map with the 6–38 cM region condensed. *Vertical lines* indicate the genomic regions being compared as homologous (*solid connecting line*) or homoeologous (*dashed line*) or putative homoeologous (*line* of *mixed dots* and *dashes*)

and *Dra*I showed that the 14 *Dw6* NILs exhibited the same polymorphism as was mapped to KOD_36; however, the correlation with tall/short was only 7 in 14 (Table 2). A second polymorphism correlated in 13 of 14 lines; however, the four mapping parents had identical alleles. This result with umn23 is strong confirmation of the results obtained with umn145. With the second probe, aco245, a strong polymorphism revealed by *Eco*RI correlated perfectly with height in all 14 lines; however, this polymorphism did not occur in either the K×O or T×M mapping populations. This result with aco245 suggests that the *Dw6/dw6* locus is very tightly linked to one of two aco245 loci which are homoeologous to the aco245a locus on KOD_36, and, therefore, is probably on KOD_33 or KOD_11_41+20+45. With the third probe, cdo1321, the *Dra*I polymorphism that was mapped to the cdo1321a locus on both KOD_36 and TM25 was evident across all the *Dw6* NILs; however, the correlation with height was only 7 in 14. In contrast, the *Dra*I polymorphism that was mapped to the cdo1321b locus on both KOD_33 and TM1 was evident only on the third set of NILs but had a perfect correlation of 3 of 3 lines over this limited sample. Results with cdo1321 are consistent with the *Dw6/dw6* locus being on KOD_33.

## Searching for *Dw6/dw6* on LG KOD_33

The 14 NILs were characterized by RFLP analysis using probes bcd421, cdo1428, and aco227, selected because each has been mapped to a locus in the region of interest on KOD_33 (Table 2). For each of the three probes, the same polymorphism that had been mapped to KOD_33 was evident across all the NILs and, in 13 of 14 lines, the mapped polymorphism correlated with height (Table 2). In addition, the aco227 polymorphism that had been mapped to TM1, the homolog of KOD_33, was evident in the third set of NILs and correlated with height in all 3 lines. These results strongly support the hypothesis that the *Dw6/dw6* locus is located on KOD_33.

Table 2 shows a more detailed alignment of the LGs of interest. All markers discussed in this manuscript are included, as well as framework markers used to establish cM distances; however, it should be noted that not all of the markers within the constituent published maps have been included in Table 2. The orders of the markers shown on the maps in Table 2 contain only a few minor changes relative to the published orders in order to facilitate merging of the maps into a consensus order of loci on

KOD_33. The exact order of loci within each bin remains to be determined. A umn145 locus has also been mapped in diploid *Avena strigosa* × *A. weistii* (Kremer et al. 2001); however, neither this map nor that for diploid *A. atlantica* × *A. hirtula* (O'Donoughue et al. 1992) has been included because while they are consistent with the consensus map, they do not enhance it.

Comparative mapping was used to refine the location of the *Dw6/dw6* locus on KOD_33. Available RFLP and PCR-based markers were selected from KOD_33, and its homolog TM1, and their homoeologs (Table 2), and used to genotype the *Dw6* NILs to test for correlation with height. When allele sizes matched that of a published mapped marker, the correlation could be attributed to the locus which had been mapped to that specific LG and is shown as such in Table 2. If the allele sizes observed in the NILs did not match the allele sizes mapped in K×O or T×M; however, the correlation was very high, it was presumed that the correlation could be attributed to the locus near *Dw6/dw6* and therefore on KOD_33. Aco245 was the only probe studied for which 14 of 14 NILs correlated perfectly with tall versus short. At the aco245 locus tall NIL LAO 521-T-03 exhibited the tall allele while at all other tested loci this tall NIL exhibited the short allele. The aco245 locus on KOD_33 was flanked by two genomic regions containing five and two markers, respectively, in which only one NIL, LAO 521-T-03, did not correlate. More proximal are three markers (cdo1321, cdo780, and aco187) which are only polymorphic in NIL set 3 and correlated in 3 of 3 NILs. More distal are two markers (cdo836 and cdo1255) which were polymorphic only in NIL set 3 and correlated for 2 of 3 NILs in both K×O and T×M. Several of the tested markers were monomorphic. Thus, the K×O *Dw6/dw6* locus is quite precisely located on LG KOD_33, tightly linked to the putative aco245z locus, and flanked by multiple RFLP marker loci mapped on KOD_33.

## Potential for detailed mapping of the *Dw6/dw6* region

The NILs were genotyped with high-throughput markers in an attempt to identify additional markers tightly linked to the *Dw6/dw6* locus that could be used for detailed mapping of this genomic region.

Firstly, the *Dw6* NILs were genotyped using eight AFLP primer pairs. The parents of the three *Dw6* NIL sets exhibited 95, 113, and 108 AFLP polymorphisms, respectively, while the six individual NIL pairs exhibited 5, 4, and 6 of 95, 4 and 3 of 113, and 15 of 108 parental polymorphisms. Of the 33 unique AFLP polymorphisms, 25 were in phase with the short/tall phenotype, including three between LAO-521-T-03 and LAO-521-S-03. However, none could be clearly associated with *Dw6* over the limited number of NIL pairs per set, despite the RFLP genotyping which had suggested that much of the KOD_33 LG remained homogeneously maternal or paternal in these *Dw6* NILs.

Secondly, DArT markers were used to genotype the lines from the most informative NIL pair, LAO 521-T-03 and LAO 521-S-03, which exhibit contrasting RFLP alleles only at the aco245 locus, and their parents. The 550 highest quality DArT markers were selected for analysis. Only two were polymorphic on the NIL pair and in phase for the tall/short phenotype: oPt-794288 and oPt-794736. Unfortunately, neither was polymorphic on an oat mapping population (derived from Dal × Exeter) which was genotyped simultaneously with the four *Dw6* related lines, nor have the two DArT markers been mapped in other published studies. They are candidates for being tightly linked to the *Dw6/dw6* locus but confirmation awaits their mapping in another population.

In combination, genotyping the NILs with RFLP, AFLP, and DArT markers sampled approximately 63 (21 probes × 3 loci), 105, and 550 widely distributed loci, respectively. It is known that there is some redundancy among both DArT and AFLP markers and also that both AFLP and DArT markers can exhibit clustering in the genome. Despite these caveats, it is clear that the recovery of only one confirmed and two potential markers tightly linked to *Dw6/dw6* suggests that the introgressed region in NIL LAO 521-S-03 is very small.

## Height-related loci associated with the aco245 loci

Since an aco245 locus has not been mapped directly on either KOD_33 or TM1, there are no reported associations of these putative loci with any traits. However, the nearby bcd269 locus on KO33, the bcd1407 locus on its homoeolog KO36, and the adjacent bcd1860, cdo1428a and bcd421a loci on the third homoeolog KO11_41+20 (Table 2) have all been associated with QTLs for plant height (Table 3). The aco245a locus on TM25 and the co-mapping umn145 locus are peak markers for a QTL for plant height in the T×M population. Thus, the genomic regions containing aco245 and linked loci are rich in height QTLs on all three homoeologs. Markers linked to this genomic region are also associated with QTLs for height in barley and American wild rice, a QTL for coleoptile length in wheat and a QTL for root volume in rice (Table 3). Potential orthologs of the *Dw6/dw6* locus include the maize height mutant sticky chromosome 1 (*st1*) and the barley slender dwarf 4 (*sld4*) mutant (Table 3); however, neither mutant allele has been sequenced. In the future, genomic sequencing will allow for the direct testing of any putative orthologs.

## Development of PCR-based markers for *Dw6/dw6*

PCR-based markers are the markers of choice for MAS. The only existing PCR-based marker within the *Dw6/dw6*

**Table 3** Traits associated with markers near the *Dw6/dw6* locus

| Marker | LG (KO homolog) | Germplasm | Associated locus | References |
|---|---|---|---|---|
| bcd1407 | KO36 | K×O RIL oat pop | Plant height QTL | Siripoonwiwat et al. (1996) and Holland et al. (1997) |
| bcd269 | KO33 | K×O RIL oat pop | Plant height QTL | Siripoonwiwat et al. (1996) and Holland et al. (1997) |
| cdo772 to cdo1380 | | Wheat | Coleoptile length QTL | Rebetzke et al. (2007) |
| bcd1860 | KO11_41+20 | 64 oat accessions | Plant height QTL | Beer et al. (1997) |
| cdo497 | | Rice | Root volume QTL | Hemamalini et al. (2000) |
| cdo497 | | Barley | Plant height QTL | Teulat et al. (2001) |
| cdo1428 and bcd421a | KO11_41+20 | K×O RIL oat pop | Plant height QTL | Siripoonwiwat et al. (1996) and Holland et al. (1997) |
| bcd421 | | Barley | Slender dwarf 4 locus *sld4* | Rossini et al. (2006) |
| aco245a and umn145 | TM25 (KO36) | T×M RIL oat pop | Plant height QTL | De Koeyer et al. (2004) |
| cdo1380 | | Maize | Plant height locus sticky chromosome 1 (*st1*) | Lin et al. (1995) |
| cdo1380 | | American wild rice | Plant height QTL | Kennard et al. (2002) |

region on KOD_33 is the RAPD marker acor360; however, it was monomorphic on the NILs (Table 2). We developed a more robust CAPS marker (ubc360s) from this RAPD marker; however, it mapped to KO2 (Orr and Molnar 2008). RAPD marker acor333 and our derived SCAR marker ubc333ks (details in "Materials and methods") both map to KOD_33, with the SCAR being less than 7 cM from the aco245z locus (Table 2). However, both acor333 and ubc333ks were monomorphic on the NILs. On the homologous TM1 region there are two RAPD markers, acor264 and acor165c, both of which were monomorphic on the NILs. From RAPD marker acor264 we developed the SCAR marker ubc264kas, which mapped to KO17 (Orr and Molnar 2008). From RAPD marker acor165 we developed the SCAR marker ubc165ms (details in "Materials and methods"), which maps 1–6 cM from aco245z but was monomorphic on the NILs (Table 2). The SCAR markers ubc333ks and ubc165ms have potential for MAS for *Dw6* in other germplasm where they are polymorphic. In a polyploid species such as oat, RAPD markers are a good initial choice from which to develop PCR-based SCAR and CAPS markers for MAS because RAPD markers tend to be more genome specific than RFLP markers and RAPD markers are more numerous in oat than microsatellite markers. The most efficient markers are those derived from the correct candidate gene, once it has been identified.

## Potential candidate gene for *Dw6*

The aco245 clone was sequenced (GenBank accession JF913493) and found to have high homology to un-annotated *A. barbata* ESTs (e.g. GR332475.1, GR347136.1, GR359504.1) and many wheat (e.g. AK332556.1, DQ681104.1), barley (e.g. AK250428.1, AK353739.1), and other plant sequences annotated as vacuolar proton ATPase subunit H proteins. The multimeric V-ATPase enzymes are highly conserved proton pumps that are known to play a central role in regulation of tonoplast pH, cell elongation and plant growth (Obroucheva 2008; Sharma et al. 2009). Positional cloning and functional complementation studies of the Arabidopsis *det3* dwarf mutant have shown that the *det3* gene encodes subunit C of the V-ATPase (Schumacher et al. 1999). Thus, the gene on KOD_33 corresponding to the cDNA clone aco245 and coding for vacuolar proton ATPase subunit H is a promising candidate gene for the *Dw6/dw6* locus.

## Syntenic regions

RFLP markers on the published KOD_33 LG (Tinker et al. 2009) and on the proposed consensus map of KOD_33 (Table 2) were used to explore syntenic regions in other grass species. Fifteen of the RFLP clones identified orthologous sequence(s) in at least one of the species *Sorghum bicolor* (sorghum), *Oryza sativa* (*Indica* and/or *Japonica*) (rice) and *Brachypodium distachyon* (Brachypodium) using the View Marker tool on Gramene, a resource for comparative genomics of grasses (http://www.gramene.org). The DNA sequences of 13 of the clones were on deposit with both GenBank and Gramene and these were used directly. The sequences for two clones were on deposit with GenBank but not Gramene. For aco245, our clone of interest, its closest orthologs from wheat (GenBank accession DQ681104), barley, rice, meadow ryegrass, and *Avena barbata* (Barbed oat) were used as proxies, and for clone umn23 a highly

**Table 4** Synteny between oat LG KOD_33 (proposed consensus map of Table 2) and physical maps of grass species showing chromosomal locations (Mb) of orthologs of clones mapped to KOD_33

| *Sorghum bicolor* | | | | *Oryza sativa Indica* and *Japonica* | | | | | *Brachypodium distachyon* | | | | *Avena sativa* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr. 4[a] | | Chr. 2[a] | | Chr. 2[a] | | | Chr. 7[a] | | Chr. 3[a] | | Chr. 1[a] | | KOD_33[a] |
| Clone | Loc. | Clone | Loc. | Clone | Loc. *Ind.* | Loc. *Jap.* | Clone | Loc. *Ind.* | Clone | Loc. | Clone | Loc. | Clone |
| End | 0.0 | End | 0.0 | End | 0.0 | 0.0 | End | 0.0 | End | 0.0 | End | 74.8 | |
| bcd1882 | 4.2 | * | | bcd1882 | 4.2 | 2.0 | * | | bcd1882 | 3.4 | * | | bcd1407 |
| * | | * | | * | | | * | | bcd421 | 12.49 | * | | umn202 |
| cdo497 | 54.3 | * | | * | | | cdo497 | 23.1 | **bcd1428** | 12.5 | * | | bcd1882 |
| cdo1380 | 54.8 | * | | cdo1380 | 24.9 | 23.2 | * | | **cdo1380** | 35.6 | cdo1380 | 53.6 | umn128 |
| * | | * | | * | | | * | | ***cdo1090*** | 41.8 | * | | bcd1860 |
| cdo1428 | 55.0 | * | | cdo1428 | 25.2 | 23.5 | * | | **cdo497** | 49.1 | * | | cdo497 |
| bcd1407 | 55.3 | * | | bcd1407 | 25.6 | 23.9 | * | | * | | * | | cdo1428 |
| umn23 | 55.5 | * | | umn23 | 25.7 | 24.0 | * | | umn23 | 49.9 | * | | bcd421 |
| * | | * | | umn145 | | 24.1 | * | | umn145 | 50.0 | * | | umn23 |
| bcd1716 | 55.8 | * | | bcd1716 | 26.1 | 24.4 | * | | bcd1716 | 50.2 | * | | aco245 |
| umn128 | 56.9 | * | | umn128 | 27.2 | | * | | umn128 | 50.9 | * | | umn145 |
| * | | * | | bcd1860 | 30.9 | 29.0 | * | | bcd1860 | 53.5 | * | | bcd1716 |
| umn202 | 59.7 | umn202 | 63.2 | umn202 | 31.9 | 29.9 | * | | **cdo836** | 55.4 | * | | cdo1380 |
| * | | *cdo1090* | 63.3 | * | | | * | | * | | * | | cdo1090 |
| cdo836 | 66.6 | * | | cdo836 | 36.9 | 34.8 | * | | **umn202** | 56.5 | * | | cdo836 |
| aco245 | 67.1 | aco245 | 70.3 | * | | | aco245 | 25.1 | * | | aco245 | 20.5 | |
| End | 68.0 | End | 77.9 | End | 38.1 | 35.9 | End | 28.0 | End | 59.9 | End | 0.0 | |

[a] Maps of genomic regions orthologous to clones linked to clone aco245 (double underline). Bold and bold italic entries highlight two inversions in the *Brachypodium distachyon* chromosome 3 physical map relative to both the *Sorghum bicolor* chromosome 4 and the *Oryza sativa* chromosome 2 physical maps. Clone cdo1090 (italics) maps to different locations on the *Brachypodium distachyon* chromosome 3 and the *Sorghum bicolor* chromosome 2 physical maps

homologous oat EST (CN819034) was used as a proxy. Most clones had orthologous loci on both sorghum chromosome 4 and rice chromosome 2, known to be highly orthologous chromosomes (The International Brachypodium Initiative 2010), and no conflict in the order of loci was observed (Table 4). A few clones also had orthologous loci on sorghum chromosome 2 and a few other clones on rice chromosome 7, also known to share regions of orthology (The International Brachypodium Initiative 2010). The loci orthologous to clone aco245 are near the ends of sorghum chromosomes 4 and 2 and of rice chromosome 7. In Brachypodium, most clones have orthologs on chromosome 3, consistent with known orthology between these three species. Locus order mirrors that on sorghum chromosome 4 and rice chromosome 2 with the exception of two relatively small inversions (Table 4). As in rice, no aco245 orthologous region was detected near the end of Brachypodium chromosome 3, but a less terminal locus was detected on chromosome 1. Also shown in Table 4 is the order of the loci on the proposed consensus map for oat LG KOD_33. While many pairings of loci are conserved between the KOD_33 map and the other maps, there are major differences in overall organization.

## Discussion

In the current study, RFLP analysis of NILs with clones selected through comparative mapping has tightly linked the oat *Dw6/dw6* dwarfing locus to an aco245z locus on LG KOD_33 of the Kanota × Ogle hexaploid oat reference map. Earlier inheritance studies tightly linked (3.3 ± 1.3 cM) a *Dra*I umn145 polymorphism with *Dw6/dw6* in an OT207 × Kanota mapping population and located this polymorphism to Kanota nullisomic line K21 (Milach et al. 1997). In a similar study, the crown rust resistance locus *Pc91/pc91* was shown to be tightly linked (4.5 cM) to an *Eco*RI umn145 polymorphism in the $F_2$ mapping populations Amagalon × Ogle and Amagalon × Starter, and an identically sized *Eco*RI umn145 polymorphic band was also missing in K21 (Rooney et al. 1994). K21 is missing chromosome 18, the smallest oat chromosome (Rooney et al. 1994). The arm of chromosome 18 purported to carry *Dw6/dw6, Pc91/pc91*, and the umn145b locus also carries an og19 RFLP locus (Rooney et al. 1994) and an og19 locus has been mapped to KO11_41+20 (Wight et al. 2003) (Table 2). Aneuploid analysis also demonstrated that homoeologous loci for both umn145 and og19 occur on

chromosome 16 (Jellen et al. 1993). Fox et al. (2001) concluded that chromosome 18 corresponds to LG KO33 based on their evidence with RFLP clone umn202, and cite supporting evidence from Kianian et al. (1997) that RFLP locus cdo1428b is also located on chromosome 18 by aneuploid analysis and on KO33 by mapping. Generally, caution is advisable when drawing conclusions based on chromosome or chromosome arm cross-comparisons of many different genotypes, since hexaploid oat is known to carry many translocations, leading to only segmental homology and homoeology (Kianian et al. 1997). However, the *Dw6/dw6*-related genomic region appears to be relatively stable and aneuploid analysis is consistent with our independent conclusion from RFLP analysis of the NILs that umn145 homoeologous regions can be expected on KOD_36, KOD_33, and KOD_11_41+20+45, and that the *Dw6/dw6* locus maps to KOD_33. In contrast, in the case of *Pc91/pc91*, while aneuploid analysis suggested that *Pc91/pc91* was linked to *Dw6/dw6*, recently it was mapped in the CDC Sol-Fi × HiFi RIL population to the homolog of KO 1_3_38 near a major translocation (McCartney et al. 2011). The authors hypothesize that the discrepancy regarding the location of *Pc91/pc91* arose because the earlier linkage and aneuploidy analyses employed rather unrelated germplasm. In the case of *Dw6/dw6*, the earlier aneuploid analysis and the current report have studied much more closely related germplasm.

Locating *Dw6/dw6* so precisely was possible because of the somewhat surprising observation that the tall line LAO 521-T-03 exhibited alleles from the short parent for the tested RFLP markers throughout the KOD_33 LG with the single exception of the aco245 locus. In contrast, the other 13 lines were completely either maternal or paternal for a relatively large genomic region flanking *Dw6/dw6*. This differs from common expectation that each NIL pair contrasts for only a small genomic region flanking the target locus. If the aco245 and *Dw6/dw6* loci were actually terminal loci, which all comparative mapping data argues against, then a single recombination event could explain the genotypic patterns observed. It seems more likely that, with respect to the specific parents of the NILs, recombination in this region is deleterious and so only very small exchanges, arising from infrequent double recombination events, are tolerated. Since the NILs derive from the same *Dw6* parent (OT257) crossed to three other lines, it would then be likely that it is OT257 which is somehow anomalous. The original source of *Dw6* was the line OT207, which began as an $M_4$ line having a stable semi-dwarf phenotype selected from OT184 seed mutagenized with 1,150 rads of fast neutron radiation (Brown et al. 1980). Irradiation is known to create chromosomal rearrangements and an inversion may have placed *Dw6/dw6* in a terminal position in OT207. However, chromosome pairing in root-tip squashes from $F_1$ plants derived from crosses between OT207 and six normal height lines showed 21 bivalents in three crosses and 19 bivalents plus 1 quadrivalent in the other three crosses, suggesting to Brown et al. (1980) that OT207 has relatively normal cytology and could be used as a donor of *Dw6* for the development of other semi-dwarf lines.

MAS for *Dw6/dw6* benefits from having numerous PCR-based markers available since only a subset will be polymorphic in any given cross. In the present study, two SCAR markers, ubc333ks and ubc165ms, were developed from RAPD markers and linked to *Dw6/dw6* at less than 7 and at 1–6 cM, respectively. The successful mapping of the *Dw6/dw6* locus to KOD_33 also identifies a wealth of markers mapped to the same region in different cultivated oat populations. Previously, Tanhuanpaa et al. (2006) identified both a REMAP marker and a RAPD marker linked to *Dw6/dw6* in an $F_2$ population derived from the cross Aslak × Kontant. SNP markers developed from them are located 5.2 and 12.6 cM away from the *Dw6/dw6* locus, respectively. Tanhuanpaa et al. (2006) did not map any of the four markers, nor *Dw6/dw6,* to a LG that could be related by comparative mapping to the K×O map. In the future, PCR-based markers developed from the aco245 sequence determined in the present study should be almost perfectly linked to the *Dw6/dw6* locus and, therefore, highly diagnostic for MAS.

The cDNA clone aco245 was sequenced and found to code for vacuolar proton ATPase subunit H protein, a potential candidate gene for the *Dw6/dw6* locus. The multimeric V-ATPase enzymes are highly conserved proton pumps that are ubiquitous in eukaryotes and known to play a central role in regulation of tonoplast pH and cell elongation (Obroucheva 2008; Sharma et al. 2009). V-ATPases influence plant growth and development and response to abiotic stresses. The V-ATPase enzyme has been purified from oat roots (Ward et al. 1992) and oat seedlings (Li et al. 1998).

Studies of the Arabidopsis *det3* dwarf mutant have shown that the dwarfing can be largely ascribed to reduced cell expansion, especially for hypocotyls, petioles, and inflorescence stems (Schumacher et al. 1999). Positional cloning and functional complementation studies revealed that the *det3* gene encodes subunit C of the V-ATPase. Subunit C, like subunit H, is part of the stalk of the enzyme, separating the ball-like head from the membrane intrinsic region. The dramatic phenotypic changes are caused by a relatively minor mutation that resulted in only a twofold reduction in the amount of transcript, which still produces functional enzyme. Expression levels were highest in tissues about to enter into a phase of rapid cell elongation. The latter observation, and the strong effect of *det3* on inflorescence stems, is consistent with the fact that

the oat aco245 clone was derived from an oat immature inflorescence cDNA library (Wight et al. 2003). Schumacher et al. (1999) also report the unpublished results of Li and Chory that regulatory subunit H of the V-ATPase interacts with and is phosphorylated in vitro by the putative brassinosteroids receptor BRI1. Brassinosteroids, gibberellins, and auxin stimulate hypocotyl elongation, whereas ethylene, abscisic acid, and cytokinins inhibit it. In other plant studies, anti-sense constructs to V-ATPase subunit A, a major component of the ball-like head, reduced cell expansion in transgenic carrot root cell lines (Gogarten et al. 1992) and fruit-specific constructs reduced fruit size in transgenic tomato (Amemiya et al. 2006).

Sequenced markers linked to the *Dw6/dw6* genomic region were found to have orthologs within the sequenced genomes of sorghum, rice and Brachypodium. The physical map locations of these orthologs were highly conserved between sorghum chromosome 4 and rice chromosome 2, and with two inversions, also with Brachypodium chromosome 3, consistent with known orthologies (The International Brachypodium Initiative 2010). A second set of orthologous loci suggested a similar relationship between sorghum chromosome 2, rice chromosome 7, and Brachypodium chromosome 1. Loci orthologous to aco245 were located near the ends of sorghum chromosomes 4 and 2 and rice chromosome 7. The absence of an aco245 orthologous locus at the end of rice chromosome 2 may indicate that a nearly terminal location, as is the case for the aco245 orthologous region on sorghum chromosome 4, predisposes such a locus to being lost. In Brachypodium, as in rice, no aco245 orthologous region was detected near the end of Brachypodium chromosome 3, but a less terminal locus was detected on chromosome 1. Comparing the maps also showed that the location of cdo1090 orthologous sequence on Brachypodium chromosome 3 differs from that on sorghum chromosome 2. Likewise, the locations of cdo497 orthologs on Brachypodium chromosome 3 and sorghum chromosome 4 differ from that observed on rice chromosome 7. Since cdo1090 and cdo497 are closely linked markers, these two observations may represent the same phenomenon and suggest that the two chromosomes identified within each species have differences in gene order. In oat, the order of the loci on the proposed consensus map for LG KOD_33 is very different than that on the physical maps of sorghum, rice and Brachypodium. It is not possible to propose what genetic mechanism(s) are responsible; however, genome plasticity due to polyploidy is likely a factor. In Brachypodium, rice and sorghum, the aco245 orthologous sequences are found in regions of high gene density. The real possibility of additional potential candidate genes other than aco245 for the *Dw6/dw6* locus needs careful future investigation.

In conclusion, this study mapped the *Dw6/dw6* locus to the well-characterized KOD_33 oat LG, making many flanking molecular markers available for MAS, several of which were used to develop PCR-based SCAR markers. Vacuolar proton ATPase subunit H was identified as a potential candidate gene for the locus, opening the possibility of designing highly precise diagnostic markers for *Dw6*. In addition, this study identified the potential use of this set of NILs to investigate the role of vacuolar proton ATPases in growth and development in plants, particularly oat.

**Acknowledgments** The research was made possible by generous funding from Quaker Oats (a division of Pepsico), by QTG Canada, and by the Agriculture and Agri-Food Canada Matching Investment Initiative. We thank Drs. Andrzej Kilian and Eric Huttner of Diversity Arrays Pty. Ltd. for DArT genotyping. We appreciate the encouragement of Drs. Burrows, McElroy, and Tinker, and our oat colleagues at AAFC. We also wish to thank the anonymous journal reviewers for their insightful suggestions.

## References

Amemiya T, Kanayama Y, Yamaki S, Yamada K, Shiratake K (2006) Fruit-specific V-ATPase suppression in antisense-transgenic tomato reduces fruit growth and seed formation. Planta 223: 1272–1280

Beer SC, Siripoonwiwat W, O'Donoughue LS, Souza E, Matthews D, Sorrells ME (1997) Associations between molecular markers and quantitative traits in an oat germplasm pool: can we infer linkages? J Agric Genomics 3. http://wheat.pw.usda.gov/jag/papers97/paper197/indexp197.html (published by permission of CAB International)

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

Chapados J, Kibite S, Bancroft B, Molnar SJ (2006) Molecular mapping of the *Dw6* dwarfing locus. Poster abstract, 2006 American Oat Workers' Conference, Fargo, North Dakota, July 23–26. http://wheat.pw.usda.gov/ggpages/oatnewsletter/v50/AOWC/

De Koeyer DL, Tinker NA, Wight CP, Deyl J, Burrows VD, O'Donoughue LS, Lybaert A, Molnar SJ, Armstrong KC, Fedak G, Wesenberg DM, Rossnagel BG, McElroy AR (2004) A molecular linkage map with associated QTLs from a hulless × covered spring oat population. Theor Appl Genet 108:1285–1298

Fox SL, Jellen EN, Kianian SF, Rines HW, Phillips RL (2001) Assignment of RFLP linkage groups to chromosomes using monosomic $F_1$ analysis in hexaploid oat. Theor Appl Genet 102:320–326

Gogarten JP, Fichmann J, Braun Y, Morgan L, Styles P, Taiz SL, DeLapp K, Taiz L (1992) The use of antisense mRNA to inhibit the tonoplast $H^+$ ATPase in carrot. Plant Cell 4:851–864

Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica 112:69–78

Holland JB, Moser HS, O'Donoughue LS, Lee M (1997) QTLs and epistasis associated with vernalization responses in oat. Crop Sci 37:1306–1316

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

Kennard WC, Phillips RL, Porter RA (2002) Genetic dissection of seed shattering, agronomic, and color traits in American wildrice (*Zizania palustris* var. interior L.) with a comparative map. Theor Appl Genet 105:1075–1086

Kianian SF, Wu B-C, Fox SL, 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

Kibite S (2001) Registration of seven pairs of oat near-isogenic lines, dwarf vs tall. Crop Sci 41:277–278

Kremer CA, Lee M, Holland JB (2001) A restriction fragment length polymorphism based linkage map of a diploid *Avena* recombinant inbred line population. Genome 44:192–204

Li X, Su RTC, Hsu H-T, Sze H (1998) The molecular chaperone calnexin associates with the vacuolar $H^+$-ATPase from oat seedlings. Plant Cell 10:119–130

Lin Y-R, Schertz KF, Patterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141:391–411

Locatelli AB, Federizzi LC, Milach SCK, Wight CP, Molnar SJ, Chapados JT, Tinker NA (2006) Loci affecting flowering time in oat under short-day conditions. Genome 49:1528–1538

McCartney CA, Stonehouse RG, Rossnagel BG, Eckstein PE, Scoles GJ, Zatorski T, Beattie AD, Chong J (2011) Mapping of the oat crown rust resistance gene *Pc91*. Theor Appl Genet 122:317–325

Milach SCK, Federizzi LC (2001) Dwarfing genes in plant improvement. Adv Agron 73:35–63

Milach SCK, Rines HW, Phillips RL (1997) Molecular genetic mapping of dwarfing genes in oat. Theor Appl Genet 95: 783–790

Milach SCK, Rines HW, Phillips RL (2002) Plant height components and gibberellic acid response of oat dwarf lines. Crop Sci 42:1147–1154

O'Donoughue LS, Wang Z, Roder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP-based 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

Obroucheva NV (2008) Cell elongation as an inseparable component of growth in terrestrial plants. Russ J Dev Biol 39:13–24

Orr W, Molnar SJ (2007) Development and mapping of PCR-based SCAR and CAPS markers linked to oil QTLs in oat. Crop Sci 47:848–852

Orr W, Molnar SJ (2008) Development of PCR-based SCAR and CAPS markers linked to $\beta$-glucan and protein content QTL regions in oat. Genome 51:421–425

Rebetzke GJ, Ellis MH, Bonnett DG, Richards RA (2007) Molecular mapping of genes for Coleoptile growth in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:1173–1183

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

Rossini L, Vecchietti A, Nicoloso L, Stein N, Franzago S, Salamini F, Pozzi C (2006) Candidate genes for barley mutants involved in plant architecture: an in silico approach. Theor Appl Genet 112:1073–1085

Schumacher K, Vafeados D, McCarthy M, Sze H, Wilkins T, Chory J (1999) The *Arabidopsis det3* mutant reveals a central role for the vacuolar $H^+$-ATPase in plant growth and development. Genes Dev 13:3259–3270

Sharma V, Kumari N, Tripathi BN (2009) V-ATPase in plants: an overview V-ATPase: structure and role in plants. Int J Biotechnol Biochem 5:93–106

Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. J Agric Genomics 2. http://wheat.pw.usda.gov/jag/papers96/paper396/indexp396.html (published by permission of CAB International)

Tanhuanpaa P, Kalendar R, Laurila J, Schulman AH, Manninen O, Kiviharju E (2006) Generation of SNP markers for short straw in oat (*Avena sativa* L.). Genome 49:282–287

Teulat B, Merah O, Souyris I, This D (2001) QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. Theor Appl Genet 103:774–787

The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768

Tinker NA, Kilian A, Wight CP, Heller-Uszynska K, Wenzl P, Rines HW, Bjornstad A, Howarth CJ, Jannink J-L, Anderson JM, Rossnagel BG, Stuthman DD, Sorrells ME, Jackson EW, Tuvesson S, Kolb FL, Olsson O, Federizzi LC, Carson ML, Ohm HW, Molnar SJ, Scoles GJ, Eckstein PE, Bonman JM, Ceplitis A, Langdon T (2009) New DArT markers for oat provide enhanced map coverage and global germplasm characterization. BMC Genomics 10:39

Ward JM, Reinders A, Hsu H-T, Sze H (1992) Dissociation and reassembly of the vacuolar $H^+$-ATPase complex from oat roots. Plant Physiol 99:161–169

Wight CP, Tinker NA, Kianian SF, Sorrells ME, O'Donoughue LS, Hoffman DL, Groh S, Scoles GJ, Li CD, Webster FH, Phillips RL, Rines HW, Livingston SM, Armstrong KC, Fedak G, Molnar SJ (2003) A molecular marker map in 'Kanota' × 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46:28–47

Zhu S, Kaeppler HF (2003) A genetic linkage map for hexaploid, cultivated oat (*Avena sativa* L.) based on an intraspecific cross 'Ogle/MAM17–5'. Theor Appl Genet 107:26–35