Theor Appl Genet (2006) 113:429–437
DOI 10.1007/s00122-006-0308-0

ORIGINAL PAPER

Inheritance and mapping of a powdery mildew resistance gene introgressed from *Avena macrostachya* in cultivated oat

J. Yu · M. Herrmann

Received: 1 November 2005 / Accepted: 6 May 2006 / Published online: 27 June 2006

Abstract The powdery mildew resistance from *Avena macrostachya* was successfully introgressed into hexaploid oat (*A. sativa*). Genetic analysis of F_1, F_2, F_3 and BC_1 populations from two powdery-mildew resistant introgression lines revealed that the resistance is controlled by a dominant gene, tentatively designated *Eg-5*. Molecular marker analysis was conducted using bulked-segregant analysis in two segregating F_3 populations. One codominant simple sequence repeats (SSR) marker AM102 and four AFLP-derived PCR-based markers were successfully developed. The SSR marker AM102 and the STS marker ASE41M56 were linked to the gene *Eg-5*, with genetic distances of 2 and 0.4 cM, respectively, in both mapping populations. Three STS markers (ASE45M56, ASE41M61, ASE36M55) co-segregated with *Eg-5* in one population while two (ASE45M56, ASE36M55) of them linked to *Eg-5* with a genetic distance of 1 cM in another population. The gene was further mapped to be in a region corresponding to linkage group 22_44+18 in the Kanota × Ogle (KO) hexaploid oat map by comparative mapping. To our knowledge, this is the first report of mapping powdery-mildew resistance in hexaploid oat. The new resistance source of *A. macrostachya*, together with the tightly linked markers identified here, could be beneficial in oat breeding programmes.

Communicated by B. Friebe

J. Yu · M. Herrmann (✉)
Institute of Agricultural Crops, Federal Centre for Breeding Research on Cultivated Plants (BAZ), Rudolf-Schick-Platz 3a, 18190 Gross Lüsewitz, Germany
e-mail: m.herrmann@bafz.de

Introduction

Powdery mildew, caused by *Blumeria graminis* D.C. (Speer) f. sp. *avenae* Em. Marchal, is an important disease of oat (*Avena sativa* L.). For economical and environmental reasons, the breeding of resistant varieties would be the most feasible means of controlling this disease and reducing yield losses. Several sources with resistance to powdery mildew, including common oats (Jones 1983; Hsam et al. 1997; Hsam and Zeller 1998), wild oat species such as *A. barbata* (Aung et al. 1977; Thomas et al. 1980), *A. strigosa*, *A. occidentalis* (Herrmann and Roderick 1996), *A. pilosa* (Hoppe and Kummer 1991) and *A. sterilis* (Hayes and Jones 1966), have been reported. Four major powdery-mildew resistance genes were designated as *Eg-1*, *Eg-2*, *Eg-3* and *Eg-4* by Simons et al. (1978). However, because single-gene resistance may become ineffective due to pathogen changes (Hayes and Jones 1966) and a deficiency of major resistance genes, breeding has to deploy "adult plant" resistance, new stable sources of resistance or combinations of available genes (Roderick et al. 2000).

Avena macrostachya, an autotetraploid perennial wild species, possesses several traits with high relevance for oat breeding. Besides winter hardiness (Baum and Rajhathy 1976), resistances to Barley yellow dwarf virus (BYDV), *Erysiphe graminis* (Hoppe and Pohler 1988) and *Rhopalosiphum padi* (Weibull 1986) have also been reported in this wild species. A few crosses of *A. macrostachya* with wild and cultivated oat species were realised to study the genome

constitution and to transfer valuable traits into *A. sativa* (Pohler and Hoppe 1991; Leggett 1985; Santos et al. 2002).

DNA-based molecular markers have greatly increased the capacity for more precise prediction of a genotype in plant breeding. Currently the major molecular markers used for oat mapping are RFLPs and AFLPs (Wight et al. 2004). For high-throughout genotyping, simple PCR-based markers are highly desired by plant breeders. As PCR-based markers, SSR markers are particularly helpful because they are codominant, locus-specific and a dense coverage of the entire genome with SSR markers can be reached. In oat only a very limited number of SSR sequences have been reported by Li et al. (2000), Holland et al. (2001) and Pal et al. (2002). Because of the limited information on oat SSR markers, conversion of AFLP markers into sequence-tagged-site (STS) PCR markers would be beneficial for oat Marker assisted selection (MAS) breeding.

For oat powdery-mildew resistances, no linked molecular markers have previously been reported. In this study, we identified a codominant simple sequence repeat (SSR) marker and developed four AFLP-derived STS markers tightly linked to an incompletely dominant powdery-mildew resistance gene that was derived from *A. macrostachya*. The resistance gene was further mapped to a region corresponding to linkage group 22_44+18 of the 'Kanota' × 'Ogle' mapping population (Wight et al. 2003) by comparative mapping.

Materials and methods

Plant materials

Interspecific crosses between resistant *A. macrostachya* and susceptible *A. magna* and *A. sativa*, respectively, were developed by Pohler and Hoppe (1991). Progenies of these crosses were handed over to one of the authors of the present study in 1992 and were used for the development of stable introgression lines with powdery-mildew resistance from *A. macrostachya*. The 32 BC_1F_1 plants of ((*A. magnaVIR144* × *A. macrostachya*) × AS93) × AS93 displayed a very low seedset of four caryopses per plant. In 86 BC_1F_2 plants striking segregations for several traits including seed and plant morphology, fertility and powdery-mildew resistance were observed. Six BC_1F_2 plants displayed resistance to powdery mildew. Two of the selected resistant plants, referred to as Am428/2 and Am327/1, were used for further backcrosses with susceptible *A. sativa* lines. After selfing the BC_2F_1 and selection of resistant BC_2F_2 plants, most BC_2F_3 progenies displayed segregations for powdery-mildew resistance, a first hint that the powdery-mildew resistance is controlled by a dominant gene.

The cytological examinations of BC_2F_3 and BC_2F_4 displayed a chromosome number of 42 in 41 of the 46 plants tested and only five plants showed aneuploid root-tip cells. Following several more selfing and selection steps, stable hexaploid introgression lines with resistance to powdery mildew were obtained.

To investigate the inheritance of powdery-mildew resistance introgressed from *A. macrostachya*, resistant BC_2F_7 line Am27 and BC_2F_6 line Am28 were crossed with susceptible cultivars 'Neklan' and 'Flämingsprofi' for the development of segregating populations. F_2, F_3 and BC_1 populations of all crosses were tested for segregation analysis of powdery-mildew resistance using detached-leaf segment tests as described below.

For linkage analysis, 180 randomly selected F_3 families from the cross of resistant line Am28 with susceptible cultivar 'Flämingsprofi' and 144 selected F_3 families from the cross of resistant line Am27 with susceptible cultivar 'Neklan' were used.

For comparative mapping, 71 F_{10} recombinant inbred lines of the 'Kanota' × 'Ogle' (KO) mapping population (Wight et al. 2003), kindly provided by Dr. Nicholas A. Tinker (Agriculture and Agri-Food Canada, Ottawa), were used.

Resistance testing

Leaf segments about 2 cm in length from seedlings grown in a glasshouse were placed on the surface of benzimidazole agar (0.6% agar, 30 ppm benzimidazole) in clear rectangular polystyrene boxes. Inoculation was performed using an infection tower with an interchangeable, five-bar air-pressured plexiglass device. A mixture of powdery-mildew collections which had been propagated on leaf segments of a susceptible oat line was used for inoculation. Freshly harvested spores were dispersed over the leaf segments with 200–800 spores per cm^2. For a reliable classification of individuals to the resistant, intermediate or susceptible groups, the resistant and susceptible parental genotypes were also included in each test box. Following 8–10 days incubation in a growth chamber at 18–20°C, 16-h daylength with 4 kLx fluorescent lighting (Philips TLD 58W/25), reaction of leaf segments to powdery mildew was scored using a 0–5 scale (Table 1). For segregation analysis, leaf segments with score 0–2 were grouped as resistant and 3–5 were summarised as susceptible. In F_2 and BC_1 examinations the primary leaves and the first- and second-true leaves were used in subsequent tests,

Table 1 Description of symptoms and scoring of oat powdery-mildew resistance in leaf-segment tests

Score	Symptoms
0	No symptoms of infection
1	Sparse mycelium, no sporulation
2	Weak sporulation, mycelium cover less than 10% of segment surface area
3	Moderate sporulation, mycelium cover about 30% of segment surface area
4	Abundant sporulation, mycelium cover 30–80% of segment surface area
5	Abundant sporulation, mycelium cover more than 80% of segment surface area

resulting in some intermediate scores of 2.5 as a mean of the three tests. For the F_3 generation 15 plants per family were examined.

DNA extraction and bulked-segregant analysis (Michelmore et al. (1991)

Total genomic DNA was extracted from samples of approximately 1 g of young leaf tissue using DNeasy Plant Maxi Kits (QIAGENE GmbH, Hilden, Germany), following the manufacturer's instructions.

For SSR bulked-segregant analysis, two DNA bulks were established by using equal amounts of DNA from twelve homozygous resistant and twelve homozygous susceptible F_3 families, respectively. For AFLP bulked-segregant analysis, two bulks were made by using equal amounts of pre-amplified DNA obtained from twelve homozygous resistant and twelve homozygous susceptible F_3 families, respectively.

SSR analysis

One hundred and fourteen oat SSR primer pairs, of which 61 were from Li et al. (2000), 44 from Pal et al. (2002) and nine from Holland et al. (2001), were used for screening of polymorphisms between the two parents of the mapping population and the two bulks. Each forward primer was labelled with an infrared fluorescent dye (IRD 700, MWG Biotech AG). The PCR amplifications were performed in 10 µl reaction mixes containing 20 ng of template DNA; 5 pmol of each labelled and unlabelled primer; 0.25 U of HotStar Taq DNA polymerase and 1× HotStar Taq PCR buffer (QIAGENE GmbH, Hilden, Germany); and 0.2 mM of dNTPs. The PCR reaction was carried out on a Peltier Thermal Cycler PTC-200 (MJ Research, Waltham MA, USA) using a "Touchdown" PCR profile: started with an initial denaturation at 95°C for 15 min; followed by 20 cycles of 1 min at 94°C, 1 min at 65°C, 1 min at 72°C. Annealing temperatures were progressively decreased by 0.5°C each cycle to 55°C. PCR continued for 23 additional cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C with a final elongation step of 72°C for 10 min. PCR products were separated on an automated laser fluorescence (ALF) sequencer (Pharmacia Biotech) using a KB^{Plus} 6.5% gel matrix (LI-COR GmbH, Bad Homburg, Germany) at 1,500 V, 60 mA, 30 W and 50°C. Fragment sizes were calculated using the Fragment Analyser version 1.00 program (Pharmacia Biotech) by comparison with the external size standards.

AFLP analysis

AFLP analysis was conducted as described by Vos et al. (1995) with modifications. A total of 500 ng genomic DNA was digested with *Eco*RI and *Mse*I. *Eco*RI and *Mse*I adapters were ligated to the restriction fragments. The ligation mixture was 1:10 diluted. Preselective amplification was performed using primers with one additional nucleotide (*Eco*RI + A, *Mse*I + C). Selective amplification was performed using primers with three additional nucleotides. Each of the *Eco*RI selective primers was labelled with an infrared fluorescent dye (IRD 700 or IRD 800, MWG Biotech AG). A total of 256 primer pair combinations were employed for screening. The selective amplification products were detected on a LI-COR automated sequencer (LI-COR, Lincoln, Nebraska) using a KB^{Plus} 6.5% gel matrix (LI-COR GmbH, Bad Homburg, Germany) at 1,500 V, 40 mA, 25 W and 45°C.

Cloning and conversion of AFLP markers

In order to isolate linked AFLP fragments, unlabelled AFLP selective primers were used instead of labelled primers. PCR products were separated on 6% polyacrylamide denaturing sequencing gels. Gels were run at 50 W constant power, silver stained and dried. Appropriate AFLP fragments were excised from the dried gel. The bands were eluted from the gel by incubation in 50 µl of TE buffer at 4°C overnight. Two microliter of TE buffer containing eluted bands were used for re-amplification using the same AFLP selective primers with the same PCR protocols. Amplified products with correct size were recovered from agarose gel using the Qiaquick gel extraction kit (QIAGENE

GmbH, Hilden, Germany). Recovered DNA fragments were cloned using the pGEM-T vector system (Promega GmbH, Mannheim, Germany), following the manufacturer's instructions.

For dominant AFLP markers, the band representing the dominant allele was cloned. For codominant AFLP markers, the bands for both alleles were cloned. For each recovered DNA band, two clones which contain the target fragments confirmed by PCR with corresponding AFLP selective primer pairs were selected for sequencing. New primer pairs were designed based on sequence information of clones. The new primer pairs that revealed polymorphism between the two parents were further used to screen the mapping population.

Linkage analysis

Linkage analysis was performed with the JoinMap 3.0 software (Van Ooijen and Voorrips 2001). A LOD score of 3.0 was established to consider significant linkage and the Kosambi mapping function was used to convert recombination fractions into centiMorgans (cM).

Marker data for the KO map analysis were optained from the Graingenes web site http://www.wheat.pw.usda.gov/GG2/index.shtml .

Results

Inheritance of the powdery-mildew resistance

The two lines, Am27 and Am28 used as parents for the third backcross, showed a nearly complete resistance (score 1) (Table 2) to all powdery-mildew isolates in leaf-segment tests and field experiments at different years and locations. The infection of F_1, and the segregation patterns of F_2, F_3 and BC_1 populations are shown in Table 2. In the cross of Am27 × 'Neklan', the observed segregation pattern in the F_2 population with 277 resistant and 105 susceptible plants fit a 3:1 ratio, supporting a hypothesis that the powdery-mildew resistance in Am27 is controlled by a single dominant gene. This was confirmed by the segregation pattern of 300 F_3 families with 84 nonsegregating resistant families, 136 segregating families and 80 nonsegregating susceptible families, which fit a 1:2:1 F_2 genotypic ratio. The single-dominant gene hypothesis was further supported in analysis of a BC_1 population, in which a 1R:1S segregation was observed and by the segregation analysis of F_2 and F_3 populations of Am28 × 'Flämingsprofi'/'Neklan' (Table 2). Comparing the nearly complete resistant parents Am27 and Am28 with the F_1, F_2 and F_3, several individuals of the segregating populations showed scores of 2, indicating an incomplete gene action of this dominant gene. This dominant powdery-mildew resistance gene derived from *A. macrostachya* was tentatively designated *Eg5* according to the nomenclature of oat genes (Simons et al. 1978).

The phenotypic grouping of score 0–2 and 3–5 as resistant and susceptible reaction patterns, respectively, was validated genotypically by testing F_3 offspring. Among a total of 480 F_2-plants tested, all F_2 plants with reactions scored 0–2 either gave nonsegregating resistant or segregating F_3 progeny, while F_2 plants displaying reactions scored 3 or higher invariantly led to susceptible F_3 progeny. Thus, the grouping

Table 2 Reaction in leaf-segment tests of parental, F_1, F_2, BC_1 plants and $F_{2:3}$ families from crosses of powdery-mildew resistant lines Am27 and Am28 with susceptible cultivars

Generation	Number of plants						Ratio fit	χ^2 value
	Resistant		Segregating	Susceptible				
Score	0+1	2		3	4	5		
'Neklan'					6	5		
'Flämingsprofi'				1	9	1		
Am27/Am28	15							
Am27 × 'Neklan'								
F_1	7	1						
F_2	241	36		21	84		3:1	1.26
BC_1	67	38			97	13	1:1	0.12
F_3	84		136	80			1:2:1	2.72
Am28 × 'Neklan'								
F_1	4	14						
F_2	101	125		0	31	28	3:1	2.81
Am28 × 'Flämingsprofi'								
F_1	7	11						
F_2	202	24		1	60	9	3:1	0.29
F_3	49		83	48			1:2:1	1.10

of scores reflected the underlying resistance genotypes, i.e. *Eg5Eg5* or *Eg5eg5* versus *eg5eg5*.

SSR polymorphisms

A total of 114 SSR primers were screened to identify polymorphisms between resistant parent Am28 and susceptible parent 'Flämingsprofi'. Sixteen primers (14%) gave distinguishable polymorphic patterns and were used for further bulk analysis. Only one SSR primer, AM102, generated polymorphic fragments between the resistant and the susceptible bulks. Fragments amplified in the resistant parent and the susceptible parent using primers AM102F (5′ TGGTCAGCA AGCATCACAAT 3′) and AM102R (5′ TGTGCATG CATCTGTGCTTA 3′) were 201 bp and 213 bp in size, respectively. This SSR-primer pair was then used to genotype 180 F_3 families.

AFLP polymorphisms

A total of 256 AFLP selective primer combinations were screened to identify polymorphisms between resistant parent Am28 and susceptible parent 'Flämingsprofi' and between the two bulks. Among the AFLP bands polymorphic between the two parents, 21 were polymorphic between the two bulks. Eight AFLP markers which gave clear strong polymorphic patterns, including one codominant marker, were chosen for STS-marker development.

Cloning and conversion of AFLP markers

For the eight selected AFLP markers, a total of nine bands were recovered and cloned. Sequence comparison of the two alleles of the codominant AFLP marker E41M56 revealed polymorphisms including five SNPs and one indel (21 bp) (Fig. 1). For all dominant AFLP markers, alignments of sequences of the two clones revealed 100% identity except one marker E37M47 which showed different sequences indicating different bands shared the same size and the same selective bases for this marker. All primer sets derived from AFLP markers were used to check polymorphism between five resistant and five susceptible individuals, and the two parents for both populations. Four primer sets (named as ASE41M56, ASE45M56, ASE41M61 and ASE36M55) revealed the same polymorphism patterns as corresponding AFLP markers in the population of Am28 × 'Flämingsprofi' (Fig. 2) and three primer sets (ASE41M56, ASE45M56 and ASE36M55) revealed the same polymorphism patterns as corresponding AFLP markers in the population of Am27 × 'Neklan'. These primer sets (Table 3) were then used to screen the whole populations for linkage analysis, respectively.

Linkage analysis

Within the Am27 × 'Neklan' population, the SSR marker AM102 and the AFLP-derived codominant STS markers ASE41M56 revealed the same segregating patterns, with a genetic distance of 2 cM to the powdery-mildew resistance gene. Two dominant STS markers (ASE45M56, ASE36M55) revealed a segregation pattern identical to one another detecting loci linked to the powdery-mildew resistance gene with a genetic distance of 1 cM (Fig. 3a).

Upon screening the 180 F_3 Am28 × 'Flämingsprofi' families, a genetic distance of 0.4 cM between AM102 and the powdery-mildew resistance was obtained. The codominant STS markers ASE41M56 co-segregated with SSR marker AM102. Three dominant STS markers (ASE45M56, ASE41M61, ASE36M55) co-segregated with the powdery-mildew resistance gene (Fig. 3b).

Comparative mapping

The SSR marker AM102, the four STS markers and the remaining polymorphic AFLP markers that were not used for STS-marker development were used for

```
1  GAATTCAGGCATAAAACTTGTCGAGACGAATTGGAAGATGCATTTTCCGGGAGGGCCGCA
   |||||||||||||||||||||||   |                     ||  |||||||
1  GAATTCAGGCATAAAACTTGTCGATGCC.....................GGGAGGCCGCA

61 GATACGTGTTATCACACGGTAGATCAGATCAGATAAGGACGGCGTTAA
   |||||||||||||||||||| ||||||||||||||||||||||||||
40 GATACGTGTTATCACACGGTATATCAGATCAGATAAGGACGGCGTTAA
```

Fig. 1 Nucleotide sequences of both the resistant and the susceptible alleles of the converted codominant AFLP marker E41M56. *Underlined* nucleotides are the sequences used for designing AFLP-derived STS marker ASE41M56-87 primers

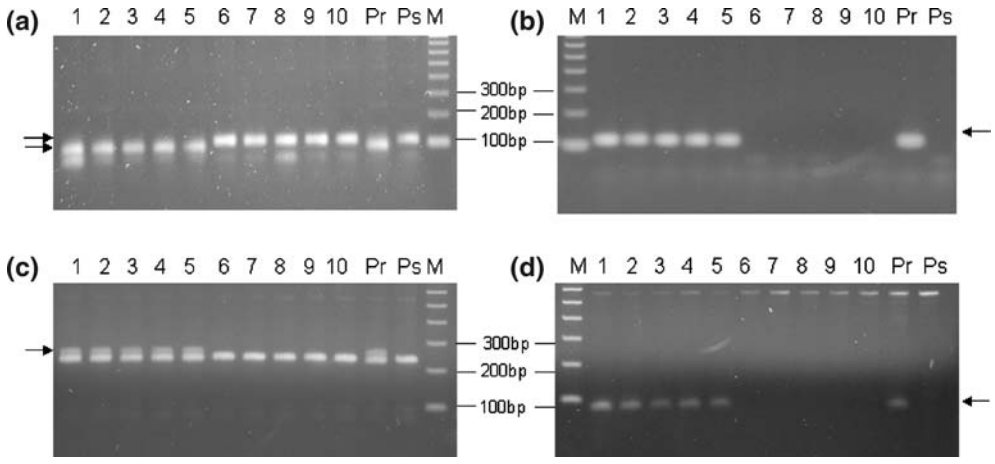

Fig. 2 The PCR products amplified by the STS primers ASE41M56 (**a**), ASE45M56 (**b**), ASE41M61 (**c**) and ASE36M55 (**d**) among the two parents and the F_3 families from Am28 × 'Flämingsprofi'. *Pr* resistant parent Am28; *Ps* susceptible parent 'Flämingsprofi'. *Lines: 1–5* resistant F_3 families; *6–10* susceptible F_3 families; *M* molecular weight marker. The PCR products were separated on 1.2% agrose gel

Table 3 AFLP-derived STS marker primer sequences, amplified fragment length and PCR melting temperatures

STS marker	Primer pair sequence	Product size (bp)	Tm°C
ASE41M56	5′ GAATTCAGGCATAAAACTTGTCG 3′ 5′ AACGCCGTCCTTATCTGATCT 3′	85	58
ASE45M56	5′ TTCATGTATAAGTCCTTAGTTTTATCG 3′ 5′ CATAGCAACACGCACGAAGT 3′	122	56
ASE41M61	5′ TTCAGGTGGGCTAATCTGGT 3′ 5′ ACCACTCACAACCCTTGCTT 3′	245	55
ASE36M55	5′ CCATCAGATTAGTCAAGGTCACA 3′ 5′ CAAAGTATAGCTAAACCGTTGAAA 3′	95	55

screening of the 71 F_{10}-derived RI lines of KO population. The SSR marker AM102 and the AFLP marker E37M48-55 revealed the same polymorphisms in the KO population as in the two bulks. However, the AFLP marker E37M48-55 segregated only in the Am28 × 'Flämingsprofi' population but not in the Am27 × 'Neklan' population (Fig. 3). Linkage analysis revealed that both markers were located on linkage group 22_44+18 in the KO mapping population (Fig. 3c).

Discussion

Wild species have been widely used as important gene resources for introgression of interesting traits into various crops. Until now there are only three reports (Pohler and Hoppe 1991; Leggett 1985; Santos et al. 2002) of successful crosses between *A. sativa* and *A. macrostachya*, but with no specifications of introgressions. In the present study, genes of *A. macrostachya* were introgressed into cultivated oat, using a bridge cross with *A. magna* and an *A. sativa* line with good crossability. After selection in BC_1F_2 for fertility and resistance to powdery mildew and a second backcross with susceptible cultivated oats followed by selfing and selection of valuable plants, stable hexaploid introgression lines with good agronomic value (data not shown) and resistance to powdery mildew were obtained. Additionally, there was no powdery-mildew infection in any of the *A. macrostachya* plants, whereas the *A. magna* and *A. sativa* accessions used in the crossings with *A. macrostachya* showed susceptibility to powdery mildew (results not shown), confirming the resistance gene was derived from *A. macrostachya*.

To investigate the inheritance of the powdery-mildew resistance, we developed several segregating populations by crossing two resistant lines with susceptible cultivated oat cultivars. The segregation patterns of all populations confirmed our hypothesis that the resistance is controlled by a single, incompletely dominant gene, tentatively designated *Eg-5*. The monofactorial dominant gene nature of the resistance derived from *A. macrostachya* makes it useful for powdery-mildew resistance breeding in oat.

In the present study, we identified a tightly linked SSR marker, AM102, to a powdery-mildew resistance gene introgressed in oat. The short genetic distance and the codominant property make AM102 a reliable

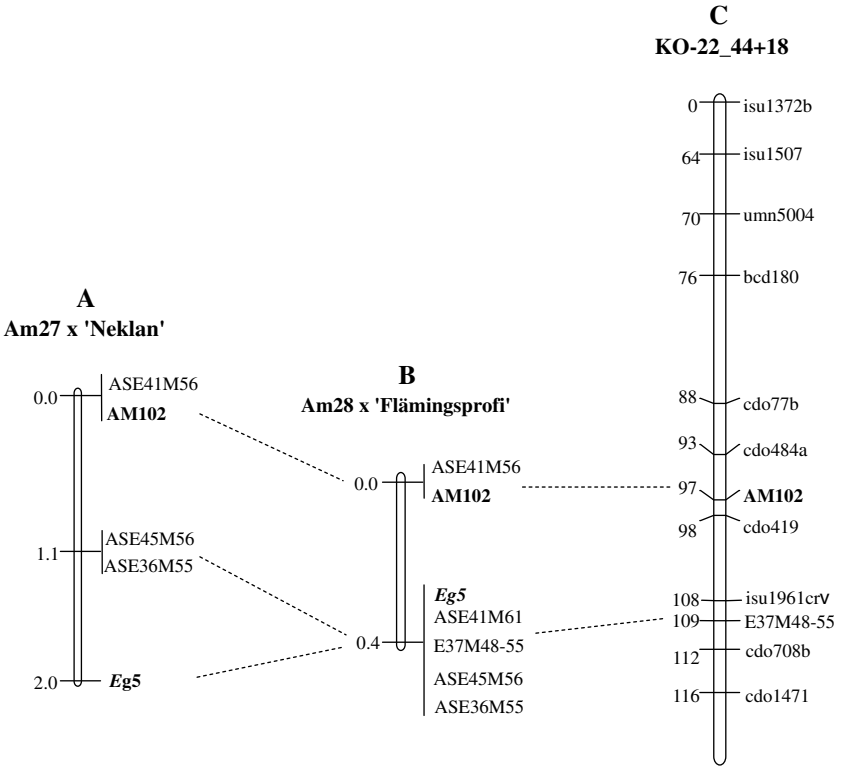

Fig. 3 Genetic mapping of the dominant resistance gene *Eg5* derived from *Avena macrostachya*. **a** Linkage map around *Eg5* from Am27 × 'Neklan'; **b** Linkage map around *Eg5* from Am28 × 'Flämingsprofi'; **c** Linkage map around SSR marker AM102 and AFLP marker E37M48_55 from KO-22_44+18 group

and easily detected marker for backcross selection and gene pyramiding to improve powdery mildew resistance in common oats.

Many studies have been conducted on conversion of AFLP markers into high-throughput; easily handling PCR based markers (Negi et al. 2000; Dussle et al. 2002; Sardesai et al. 2002; Shirasawa et al. 2004). In the current study, nine AFLP bands which gave strong polymorphic patterns were selected for STS marker development. The nine bands corresponded to eight AFLP markers, including one codominant marker and seven dominant markers. Finally four STS markers were successfully developed from eight AFLP markers, including one codominant marker and three dominant markers. The failure of conversion of the remained dominant AFLP markers into STS markers may be due to the difficulty to confirm the correct clones of the polymorphic AFLP bands for some AFLP fragments sharing the same size with polymorphic bands but quite different sequences.

Except ASE41M61 and E37M48-55, the markers found linked to *Eg-5* in the Am28 × 'Flämingsprofi' population were also found linked to the gene in Am27 ×'Neklan' population, with similar recombinations (Fig. 3). Since both populations contained *Eg-5* derived from the same original cross, it is possible they carried similar introgressed segments from *A. macrostachya*. The distances between the two common markers AM102 and E37M48-55 in Am28 ×'Flämingsprofi' and KO population were 0.4 and 12 cM, respectively. This is not surprising due to the small size (71) of KO mapping population used here and the different genetic background between Kanota (*A. byzantina*) and Ogle (*A. sativa*). Another cause for the different genetic distances of both markers may be a reduced recombination frequency within the genomes with introgressions from *A. macrostachya* and *A. magna*. Such lower recombination frequencies leading to a reduced map distances have been found in alien introgression lines in different plant species as potato (Gebhardt et al. 1991) and barley (Ruge et al. 2003).

When compared to the KO reference map (Wight et al. 2003), the *Eg-5* region corresponded to a region on linkage group KO22_44+18 based on the two common markers, AM102 and E37M48-55 (Fig. 3). Several QTLs for resistance to crown rust and BYD have been located on KO22_44+18 group (Zhu et al. 2003) and recently, a resistance gene analog (RGA) marker was also positioned on this linkage group (Irigoyen et al. 2004), giving this group special interests for resistance-gene investigation. However, the linkage between the gene *Eg-5* and the four reported genes *Eg-1*, *Eg-2*, *Eg-3* and *Eg-4* is not clear due to the limited mapping information of oat powdery-mildew resistances. Development of further closely linked markers to oat powdery-mildew resistance genes would be necessary to give a clear picture of the relationships between these genes and would be helpful to deploy combinations of

available resistance genes which will provide more comprehensive and durable protection against powdery mildew in oat breeding.

Acknowledgements We thank Dr. P. Wehling, Dr. B. Hackauf and Dr. B. Ruge for their valuable suggestions and helpful advice.

References

Aung T, Thomas H, Jones IT (1977) The transfer of the gene for mildew resistance from *Avena barbata* (4x) into the cultivated oat *A. sativa* by an induced translocation. Euphytica 26:623–632

Baum BR, Rajhathy T (1976) A study of *Avena macrostachya*. Can J Bot 54:2434–2439

Brugmans B, van der Hulst RGM, Visser RGF, Lindhout P, van Eck HJ (2003) A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Res 31:e55

Dussle CM, Quint M, Xu ML, Melchinger AE, Lübberstedt T (2002) Conversion of AFLP fragments tightly linked to SCMV resistance genes Scmv1 and Scmv2 into simple PCR-based markers. Theor Appl Genet 105:1190–1195

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal WM, Tanksley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Hayes JD, Jones IT (1966) Variation in the pathogenicity of Erysiphe graminis D.C. f.sp. avenae, and its relation to the developement of mildew-resistant oat cultivars. Euphytica 15:80–86

Herrmann M, Roderick HW (1996) Characterisation of new oat germplasm for resistance to powdery mildew. Euphytica 89:405–410

Holland JB, Helland SJ, Sharopova N, Rhyne DC (2001) Polymorphism of PCR-based markers targeting exons, introns, promoter regions, and SSRs in maize and introns and repeat sequences in oat. Genome 44:1065–1076

Holland JB, Portyanko VA, Hoffman DL, Lee M (2002) Genomic regions controlling vernalization and photoperiod responses in oat. Theor Appl Genet 105:113–126

Hoppe HD, Kummer M (1991) New productive hexaploid derivatives after introgression from *A. pilosa* features. Vortr Pflanzenzüchtg 20:56–61

Hoppe HD, Pohler W (1988) Successful hybridisation between *Avena prostrata* and *A. macrostachya*. Cereal Res Commun 16:231–235

Hsam SLK, Peters N, Paderina EV, Felsenstein F, Oppitz K, Zeller FJ (1997) Genetic studies of powdery-mildew resistance in common oat (*Avena sativa* L.) I. Cultivars and breeding lines grown in Western Europe and North America. Euphytica 96:421–427

Hsam SLK, Zeller FJ (1998) Chromosomal location of genes for resistance to powdery mildew in cultivated oat (*Avena sativa* L.). 1. Gene *Eg-3* in the cultivar 'Mostyn'. Plant Breed 117:177–178

Irigoyen ML, Loarce Y, Fominaya A, Ferrer E (2004) Isolation and mapping of resistance gene analogs from the *Avena strigosa* genome. Theor Appl Genet 109:713–724

Jones IT (1983) Transgressive segregation for enhanced level of adult plant resistance to mildew in the oat cross Mostyn × Maldwyn. Euphytica 32:499–503

Leggett JM (1985) Interspecific hybrids involving the perennial oat species *Avena macrostachya*. Can J Genet Cytol 27:29–32

Li CD, Rossnagel BG, Scoles GJ (2000) The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet 101:1259–1268

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 U S A 88:9828–9832

Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection. Theor Appl Genet 101:146–152

Pal N, Sandhu JS, Domier LL, Kolb FL (2002) Development and characterization of microsatellite and RFLP-derived PCR markers in oat. Crop Sci 42:912–918

Pohler W, Hoppe HD (1991) *Avena macrostachya*—a potential gene source for oat breeding. Vortr Pflanzenzüchtg 20:66–71

Roderick HW, Jones ERL, Sebesta J (2000) Resistance to oat powdery mildew in Britain and Europe: a review. Ann Appl Biol 136:85–91

Ruge B, Linz A, Pickering R, Proeseler G, Greif P, Wehling P (2003) Mapping of Rym14Hb, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107:965–971

Santos A, Livingston DP, Murphy JP (2002) Agronomic, cytological and RAPD evaluations of *Avena sativa* × *A. macrostachya* populations. In: Proceedings of Eastern wheat workers and southern small grain workers

Sardesai N, Kumar A, Rajyashri R, Nair S, Mohan M (2002) Identification and mapping of an AFLP marker linked to *Gm7*, a gall midge resistance gene and its conversion to a SCAR marker for its utility in marker aided selection in rice. Theor Appl Genet 105:691–698

Shirasawa K, Kishitani S, Nishio T (2004) Conversion of AFLP markers to sequence-specific markers for closely related lines in rice by use of the rice genome sequence. Mol Breed 14:283–292

Simons MD, Martens JW, McKenzie RIH, Nishiyama I, Sadanaga K, Sebesta J, Thomas H (1978) Oats: a standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters. U.S. Department of Agriculture, Agriculture Handbook 509, Washington DC

Thomas H, Powell W, Aung T (1980) Interfering with regular meiotic behaviour in *Avena sativa* as a method of incorporating the gene for mildew resistance from *A. barbata*. Euphytica 29:635–640

Van Ooijen JW, Voorrips RE (2001) JoinMap version 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen, http://www.kyazma.nl/index.php/mc.JoinMap

Vos P, Hogers R, Bleeker M, Reijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kupier M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Weibull J (1986) Screening for resistance against *Rhopalosiphum padi* (L.). I. *Avena* species and breeding lines. Euphytica 35:993–999

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 48:28–47

Wight CP, O'Donoughue LS, Chong J, Tinker NA, Molnar SJ (2004) Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.). Mol Breed 14:349–361

Zhu S, Kolb FL, Kaeppler HF (2003) Molecular mapping of genomic regions underlying barley yellow dwarf tolerance in cultivated oat (*Avena sativa* L.). Theor Appl Genet 106:1300–1306