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ORIGINAL PAPER

Molecular mapping of the novel powdery mildew resistance gene *Pm36* introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat

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Abstract Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, is one of the most important wheat diseases in many regions of the world. *Triticum turgidum var. dicoccoides* (2n = 4x = AABB), the progenitor of cultivated wheats, shows particular promises as a donor of useful genetic variation for several traits, including disease resistances. The wild emmer accession MG29896, resistant to powdery mildew, was backcrossed to the susceptible durum wheat cultivar Latino, and a set of backcross inbred lines (BC_5F_5) was produced. Genetic analysis of F_3 populations from two resistant introgression lines (5BIL-29 × Latino and 5BIL-42 × Latino) indicated that the powdery mildew resistance is controlled by a single dominant gene. Molecular markers and the bulked segregant analysis were used to characterize and map the powdery mildew resistance. Five AFLP markers (*XP43M32*$_{(250)}$, *XP46M31*$_{(410)}$, *XP41M37*$_{(100)}$, *XP41M39*$_{(250)}$, *XP39M32*$_{(120)}$), three genomic SSR markers (*Xcfd07*, *Xwmc75, Xgwm408*) and one EST-derived SSR marker (BJ261635) were found to be linked to the resistance gene in 5BIL-29 and only the BJ261635 marker in 5BIL-42. By means of Chinese Spring nullisomic–tetrasomic, ditelosomic and deletion lines, the polymorphic markers and the resistance gene were assigned to chromosome bin 5BL6-0.29-0.76. These results indicated that the two lines had the same resistance gene and that the introgressed *dicoccoides* chromosome segment was longer (35.5 cM) in 5BIL-29 than that introgressed in 5BIL-42 (less than 1.5 cM). As no powdery mildew resistance gene has been reported on chromosome arm 5BL, the novel resistance gene derived from var. *dicoccoides* was designated *Pm36*. The 244 bp allele of BJ261635 in 5BIL-42 can be used for marker-assisted selection during the wheat resistance breeding process for facilitating gene pyramiding.

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Introduction

Powdery mildew, caused by *Blumeria graminis* (DC) Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the most important wheat diseases in many regions of the world. The use of resistant cultivars has proven to be an effective and environmentally safe strategy for controlling wheat pathogens and eliminating the use of fungicides. However, since several wheat resistance genes tend to become ineffective within a short period due to frequent changes in the pathogen population, it is necessary to search for new sources of resistance and to use available genes in combinations that will provide effective and more durable resistance. Diversification of sources of resistance has been suggested as a remedy of the problem, provided that an adequate number of resistance genes are available. So far, 35 genes for resistance to wheat powdery mildew (*Pm1–Pm35*) have been identified and assigned to specific chromosomes or chromosome arms (see catalogue of gene by McIntosh et al. 2003; review by Huang and Röder 2004; Zhu et al. 2005; Miranda et al. 2006, 2007). Some of the genes were transferred from wild relatives of wheat, such as *T. turgidum* var. *dicoccoides* and var. *dicoccum*, *T. timopheevii*, *T. monococcum*, *Ae. squarrosa*, *Ae. speltoides*,

Ae. longissima, *Ae. ovata*, or from more distant species, like *Secale cereale* and *Dasypyrum villosum* (see review by Huang and Röder 2004). The tetraploid wheat *T. turgidum* var. *dicoccoides* (2n = 4x = 28, genome AABB), the wild progenitor of tetraploid and hexaploid wheats, shows particular promise as a donor of useful genetic variation for several traits, including disease resistances, drought tolerance, yield components, protein quality and quantity (Feldman and Millet 1993). Populations of emmer wheat was found to be polymorphic for resistance to several pathogens, including powdery mildew, stripe rust, leaf rust and stem rust (Dinoor et al. 1991). The powdery mildew resistance genes *Pm16*, *Pm26*, *Pm30* and *Pm31*, located on chromosomes 4A, 2B, 5B, 6A, respectively, were transferred from var. *dicoccoides* to cultivated wheats (Reader and Miller 1991; Rong et al. 2000; Liu et al. 2002; Xie et al. 2003).

Molecular markers tightly linked to genes of interest can be used in breeding programs to facilitate selection and as starting point for the map-based cloning of such genes. Marker-assisted selection (MAS) would be particularly effective to develop stable resistance to powdery mildew in wheat, where simultaneous or even sequential screening of plants with several pathogen isolates is difficult or impractical. Molecular markers, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites (or simple sequence repeats, SSRs), have been widely used to identify and map powdery mildew resistance genes in wheat. Single nucleotide polymorphisms (SNPs) have not yet been applied to the identification of resistance genes, because only the *Pm3b* gene were recently isolated (Yahiaoui et al. 2004). Currently, the major molecular markers used for wheat mapping are AFLPs and genomic SSRs (gSSRs). AFLPs, developed by Vos et al. (1995), are universal marker techniques enabling the analysis of a large number of marker loci in a single polymerase chain reaction (PCR). However, AFLPs are dominant, laborious and costly, and that limits their use for routine molecular analysis of agronomic traits in breeding practice. Microsatellites represent a valid alternative marker system because of their abundance in plant genomes, easy detection as PCR-based molecular markers on polyacrylamide or high-resolution agarose gel. The advantages of SSR markers include high information content, codominant inheritance, locus specificity and reproducibility. This class of molecular markers is being applied widely in plants for the construction of high-density genetic maps and for identifying specific genes (Gupta et al. 1999). A new type of genetic marker is represented by SSRs derived from ESTs (expressed sequence tags, EST–SSRs). Although EST–SSRs were shown to be less polymorphic than gSSRs (Eujayil et al. 2002), the use of markers from genic regions may be functionally more informative than gSSRs from unexpressed chromosome regions.

The present paper reports the characterization and molecular mapping of a powdery mildew resistance gene introgressed from *Triticum turgidum var. dicoccoides* into durum wheat. This novel powdery mildew resistance gene has been designated *Pm36* (McIntosh, personal communication 2007). The specific objectives of the present study were to (a) determine the inheritance of *Pm36* under controlled conditions, (b) develop a precise map of the *Pm36* gene region by using AFLP, gSSR and EST–SSR markers.

Materials and methods

Plant materials and resistance assays

A population of 94 backcross inbred lines (BILs) was developed using a procedure similar to that of Wehrhahn and Allard (1965) with the main goal to analyse agronomically important quantitative traits according the advanced backcrossed QTL analysis (Tanksley and Nelson 1996). The semi-dwarf and high-yielding cultivar Latino of durum wheat was use as a recurrent parent, and the accession MG29896 of *T. turgidum* L. var. *dicoccoides* was used as donor parent because of its high grain protein content, acceptable seed size, and powdery mildew resistance. Five successive backcrosses were made to Latino followed by five generations of self-pollination. The complete series of BILs and the recurrent parent Latino were evaluated for powdery mildew resistance in replicated trials at Bari, Italy, in 2003 and 2004. Five BILs displayed significant resistance to field natural infection of powdery mildew. To investigate the inheritance of powdery mildew resistance introgressed from var. *dicoccoides*, two of the selected resistant lines, referred to as 5BIL-29 and 5BIL-42, were backcrossed to the susceptible recurrent parent cv. Latino. Adult plant resistance assays were performed on field conditions for parental lines, F_1 and F_2 generations. F_3 progenies from 120 randomly chosen F_2 plants from the cross 5BIL-29 × Latino and 144 F_2 plants from the cross 5BIL-42 × Latino were tested for segregation analysis of powdery mildew resistance under greenhouse conditions. A randomized complete block design with three replications and plots consisting of 1-m rows, with 20 plants at 5-cm spacing, 30 cm apart, was used for each segregating population. For a reliable classification of individuals to the resistant, intermediate or susceptible groups, the resistant and susceptible parental lines, and ten F_1 plants were also included in each replication. The seedling were grown at a diurnal temperature cycle of 16–24°C. A mixture of powdery mildew races, collected in the experimental fields of Bari and propagated on young plantlets of a highly suscep-

tible common wheat cultivar (Fortunato) was used for inoculation seedlings at two-leaf stage. Disease reactions were recorded 12–18 days after inoculation following the rating scale of Saari and Prescott (1975) for appraising the foliar intensity of disease. The level of infection reflects the percentage of plant surface infected (0 = 0–9%; 1 = 10–19%; 2 = 20–29%; …; 9 = 90–100%). Individual plants were considered resistant if no symptoms or less than 20% infection was observed. Progenies for which all plants had a reaction similar to 5BIL-29 and 5BIL-42 were classified as homozygous resistant, and as homozygous susceptible if all reactions were similar to Latino. Progenies that had resistant and susceptible plants were included in the segregating class.

DNA extraction and molecular marker analysis

Total DNA was extracted following the method described by Sharp et al. (1988) from healthy leaves of parents, resistant and susceptible plants. In order to reconstitute the F_2 genotypes, DNA was extracted as pools from leaves of at least 15 plants for each corresponding F_3 family. On the basis of the F_3 trial data, DNA samples from eight non-segregating resistant and eight susceptible F_3 progenies were selected for making two separate DNA bulks with extreme phenotypes to be screened with AFLP and SSR markers according to the Bulked Segregant Analysis (BSA, Michelmore et al. 1991). Primers detecting polymorphisms between the bulks were then used to test each progeny of the two segregating populations.

Amplified fragment length polymorphism assays were performed essentially as described by Vos et al. (1995) with minor modifications. Genomic DNA was digested with restriction endonucleases *Pst*I and *Mse*I. Amplification fragments were separated on 5% denaturing polyacrylamide gels. The polymorphic primer combinations P39M32, P41M37, P41M39, P43M32, P46M31 between the bulks (*Pst*I primer selective bases: P39 AGA, P41 AGG, P43 ATA, P46 ATT; *Mse* primer selective bases: M31 AAA, M32 AAC, M37 ACG, M39 AGA) were tested on each progeny of the segregating populations.

Genomic SSR (gSSR) markers mapped on the chromosomes of the A and B genome of hexaploid wheat were chosen for analysis. The development of the SSR markers, primer sequences, chromosome location, and annealing temperature were reported by Röder et al. (1998) for GWM, Gupta et al. (2002) for WMC, Somers et al. (2004) for BARC, Sourdille et al. (2003) and in the web site http://www.graingenes.gov for CFA and CFD. New EST-derived SSR markers (EST–SSRs), developed by Mauricio La Rota and reported on the public database http://wheat.pw.usda.gov were also used. The primer pair F(5′ TAGCC TGGTACCATTCTGCC) and R(5′ CATTACACCAGAA GGCCTAG) for the BJ261635 EST–SSR marker, tightly linked to powdery mildew resistance gene *Pm36*, was designated based on sequence information reported on http://wheat.pw.usda.gov with the aim to amplify reproducible and heavily bands on agarose gel.

DNA amplification was carried out as described by Blanco et al. (2006). The amplification products were resolved on 6% polyacrylamide gels or 2.5% agarose gels and stained with ethidium bromide. DNA fragments amplified by the BJ261635 marker were also separated by capillary electrophoresis in an automated DNA sequencer (ABI-PRISM 3100 Avant, Applera) according to the instructions of the manufacturer.

Chromosome assignment and linkage analysis

Nulli-tetrasomic lines (NTs) of Chinese Spring (Sears 1954, 1966) and the set of durum wheat cv. Langdon D genome disomic-substitutions lines (Joppa and Williams 1988) were used to assign molecular markers to individual chromosomes. Chinese Spring ditelosomic lines (Sears and Sears 1978) were used for chromosome arm localization. Physical location on 5BL chromosome arm was obtained using a set of wheat deletion lines characterized by terminal deletions (Endo and Gill 1996).

Chi-square (χ^2) tests for goodness-of-fit were used to test for deviation of observed data from theoretically expected segregation. Linkage between DNA markers and the resistance gene was established with JOIMAP 3.0 software (Van Ooijen and Voorrips 2001) with a LOD threshold > 3.0. Genetic map distances were calculated by means of the Kosambi mapping function (Kosambi 1944).

Results

Inheritance of the powdery mildew resistance

The resistant parents and F_1 hybrids of the cross 5BIL-29 × Latino and 5BIL-42 × Latino showed a near-complete adult plant resistance (score = 0–1), while the recurrent cultivar Latino showed medium to high susceptibility (score = 4–7) to natural infection of powdery mildew in field experiments. Although the segregation patterns in both F_2 populations suggested that resistance was controlled by a single dominant gene, the observed number of resistant and susceptible plants (102 and 18 in 5BIL-29 × Latino; 121 and 23 in 5BIL42 × Latino, respectively) did not fit the expected 3:1 ratio because of the excessive number of resistant plants likely due to the escape from the pathogen infection. Favorable temperature and moisture conditions were provided in the greenhouse experiment to minimize environmental effects on powdery mildew initiation and

development, and for accurate assessment of the resistance potential of plant genotypes. The infection of the resistant parents and F_1 plants (scores = 0–1), of the cv. Latino (score = 7–8), and the segregation patterns of F3 populations tested in the greenhouse experiment (Table 1) supported the hypothesis that the powdery mildew resistance in 5BIL-29 and 5BIL-42 is controlled by a single dominant gene. Segregation analysis in F_3 progenies of the cross 5BIL-29 × Latino (27 resistant nonsegregating progenies, 70 segregating progenies and 23 susceptible progenies) and in F_3 progenies of the cross 5BIL-42 × Latino (35 nonsegregating resistant progenies, 70 segregating progenies and 39 susceptible progenies) fit a 1:2:1 genotypic ratio. The gene *Pm36* should be both seedling and adult plant-resistant gene; in fact, there was an almost precise correlation (except for escape) between the disease responses of each F_2 adult plant and the relative F_3 progenies.

Molecular markers analysis

Amplified fragment length polymorphism markers were at first analyzed in the 5BIL-29 × Latino segregating population. A total of 86 AFLP primer combinations (*Pst*/*Mse*) were screened to identify polymorphisms between the parental lines, and between the two DNA bulks obtained by pooling eight powdery-mildew nonsegregating resistant and eight susceptible progenies. The primer combinations produced 9,196 discrete DNA fragments ranging in size from 80 to 900 Kb, with an average of 107 amplified fragments per primer combination. A total of 160 markers resulted polymorphic between resistant and susceptible parents, of which 20 were also polymorphic between the resistant and susceptible bulks. The respective primer combinations were at first assayed on each F_3 progeny component the bulks. Only five AFLPs ($XP43M32_{(250)}$, $XP46M31_{(410)}$, $XP41M37_{(100)}$, $XP41M39_{(250)}$, $XP39M32_{(120)}$) indicated a putative association with the resistance locus and were then used to screen the remaining 104 F_3 progenies. Linkage analysis confirmed the genetic association of the five AFLP markers and the resistance locus, with $XP43M32_{(250)}$ distant 1.1 cM, from *Pm36* (Fig. 1).

The Langdon durum disomic-substitution lines (Joppa and Williams 1988) were used for chromosomal localization of the five AFLP markers. Only two AFLPs,

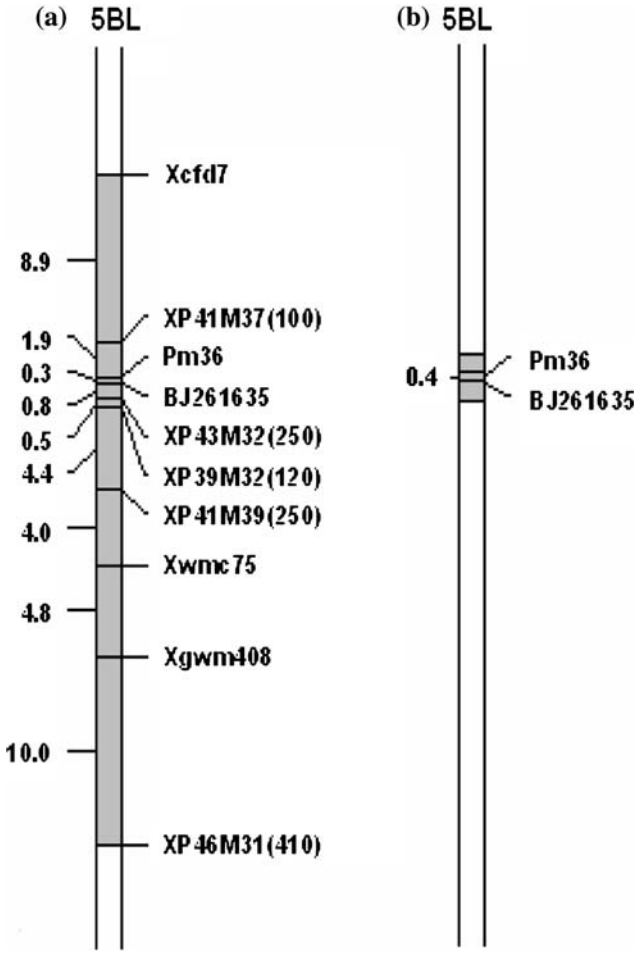

Fig. 1 Genetic linkage maps of the powdery mildew resistance gene *Pm36* on the introgressed 5BL *dicoccoides* segments in the 5BIL-29 (**a**) and 5BIL-42 (**b**) lines of durum wheat. Locus names are indicated on the *right side* of the maps. Kosambi map distances (cM) are shown on the *left side*

$XP43M32_{(250)}$ and $XP39M32_{(120)}$, amplified on cv. Langdon. The first marker was present in all tested lines except LDN5D(5B), in which chromosomes 5B were substituted by the Chinese Spring homoeologous chromosomes 5D, thus indicating that $XP43M32_{(250)}$ was located on 5B chromosome. The second marker, $XP39M32_{(120)}$, gave the same result, but it was also absent in LDN4D(4A). Langdon substitution lines were obtained by interspecific crosses

Table 1 Segregation analysis of the powdery mildew resistance in F_3 progenies of the 5BIL-29 × Latino and 5BIL-42 × Latino mapping populations

Segregating population	Number of F_3 progenies			Expected ratio	χ^2 value	Probability
	Resistant	Segregating	Susceptible			
5BIL-29 × Latino	27	70	23	1:2:1	2.85	$0.30 > P > 0.20$
5BIL-42 × Latino	35	70	39	1:2:1	0.30	$0.90 > P > 0.80$

(*T. turgidum* × *T. aestivum*) that could cause genomic deletions or rearrangements, as reported by Feldman et al. (1997) and Ozkan et al. (2001). The line LDN4D(4A) could contain a deletion of the 5B chromosome region including the $XP39M32_{(120)}$ locus or it did not amplify the $XP39M32_{(120)}$ for an unknown reason.

The parents and the bulks of the 5BIL-29 × Latino segregating population were then analyzed with 42 gSSR markers mapped on 5B chromosome to add more suitable markers for breeding purposes in the *Pm36* region. Three polymorphic gSSRs (*Xcfd7*, *Xwmc75* and *Xgwm408*) were considered potentially linked to the resistance locus and then screened on the complete population of 120 F_3 progenies. Linkage analysis confirmed the genetic association of the three gSSR markers with the powdery mildew resistance gene and with the five AFLP markers (Fig. 1). The nearest gSSR marker, *Xwmc75*, was 10.0 cM distant from *Pm36*. Chinese Spring ditelosomic lines of homoeologous group 5 were used to locate *Xcfd7*, *Xgwm75* and *Xgwm408* on a specific chromosome arm. The markers were absent only in the 5BS ditelosomic, indicating their localization on 5BL chromosome arm. The deletion lines 5BL-6, 5BL-9 and 5BL-16, missing 71, 24 and 21% terminal segments of the 5BL arm, respectively, were then subjected to the three gSSR marker analysis. The fragments detected by the markers were missing in 5BL-6 but were present in 5BL-9 and 5BL-16. Thus, the markers were mapped to the bin 5BL6-0.29-0.76. Because of the strict linkage between the powdery mildew resistance and the gSSR markers, we assumed that the *Pm36* gene is located in the bin 5BL6-0.29-0.76.

To investigate whether 5BIL-29 and 5BIL-42 had the same *Pm* gene, parents and DNA bulks of the 5BIL-42 × Latino segregating population were analyzed with the above three gSSR and five AFLP markers. The absence of any polymorphism suggested that 5BIL-29 and 5BIL-42 could have a different powdery mildew resistance gene.

With the aims of mapping the 5BIL-42 resistance gene and detecting all introgressed *dicoccoides* segments in 5BIL-29 and 5BIL-42 lines, 530 gSSRs mapped on the A and B genomes were screened in the cv. Latino and the accession MG29896 of the var. *dicoccoides* originally used as donor of the powdery mildew resistance (Table 2). One hundred and ninety-eight markers (37.4%) were polymorphic and then tested on Latino, 5BIL-29 and 5BIL-42. Ten markers polymorphic between 5BIL-29 and Latino pointed out that 5BIL-29, besides the 5BL segment with the *Pm36* gene, carried five introgressed *dicoccoides* segments on 1A, 1B, 2A, 5A and 7A chromosomes. The five markers polymorphic between 5BIL-42 and Latino, detecting two *dicoccoides* segments on chromosomes 2A and 3B, were analyzed on the resistant and susceptible DNA bulks. The absence of polymorphism indicated that the 2A and 3B introgressed segments on 5BIL-42 did not carry any *Pm* gene. The screening was then continued with EST–SSR markers. Out of a total of 551 tested markers, 140 (25.4%) were polymorphic between Latino and MG29896 (Table 2). Among them, ten were polymorphic between 5BIL-29 and Latino and detected further four *dicoccoides* segments on chromosomes 2B, 5B, 6B and 7B. Nine polymorphic markers between Latino and 5BIL-42 detected further five *dicoccoides* segments on chromosomes 1A, 2B, 5B, 6B and 7B. Interestingly, the BJ261635 EST–SSR marker amplified two co-migrating bands on 5BIL-29 and 5BIL-42 as well as on DNA resistant bulks, and a single band on Latino and susceptible bulks on agarose gels (Fig. 2). When DNA fragments were separated by capillary electrophoresis, BJ261635 behaved as a co-dominant marker: besides, the 236-bp fragment present in both parents, the susceptible cv. Latino had a 237-bp fragment and the resistant line a 244-bp fragment (Fig. 3). The analysis of each progeny of the mapping populations showed the near complete co-segregation of BJ261635 with the powdery mildew resistance, thus suggesting that 5BIL-29 and 5BIL-42 have indeed the same resistance gene. Analysis of Chinese Spring nulli-tetrasomic, ditelosomic and deletion lines confirmed the BJ261635 location on bin 5BL6 on which were located the

Table 2 Polymorphisms detected by means of gSSR and EST–SSR markers between the cv. Latino of durum wheat and MG29896, the accession of the var. *dicoccoides* donor of the powdery mildew resistance, between Latino and the derived resistant lines 5BIL-29 and 5BIL-42, and between the corresponding susceptible and resistant bulks BS-BR(5BIL-29) and BS-BR (5BIL-42)

Parents or bulks	gSSR markers			EST–SSR markers		
	Tested	Polymorphic		Tested	Polymorphic	
	N	*N*	%	*N*	*N*	%
Latino–MG29896	530	198	37.4	551	140	25.4
Latino–5BIL-29	198	10		140	10	
BS-BR (5BIL-29)	10	3		10	1	
Latino–5BIL-42	198	5		140	20	
BS-BR (5BIL-42)	5	0		20	1	

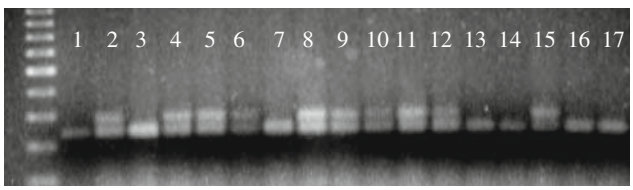

Fig. 2 Electrophoretic profiles of the BJ261635 EST–SSR marker amplified on (*1*) the powdery mildew susceptible durum wheat cv. Latino; (*2*) the resistant parental line 5BIL-42; and on (*3–17*) plants of the segregating population Latino × 5BIL-42. The PCR products were separated on 2.5% agarose gel

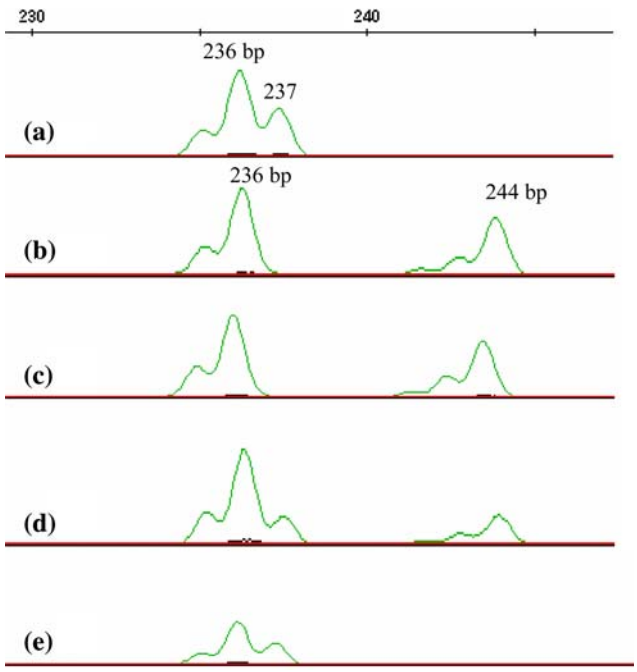

Fig. 3 Electropherograms of the BJ261635 EST–SSR marker amplified on (*a*) the powdery mildew susceptible durum wheat cv. Latino; (*b*) the resistant parental line 5BIL-42; and on (*c*) homozygous resistant, (*d*) heterozygous and (*e*) homozygous susceptible plants of the segregating population Latino × 5BIL-42. The DNA fragments of 236 and 237 bp were localized on 5AL and 5BL chromosome arms, respectively, by Chinese Spring aneuploids. The PCR products were separated by an automated DNA sequencer (ABI-PRISM 3100)

eight AFLP and gSSR markers associated with the resistance locus. Linkage analysis of all segregation data indicated that the introgressed *dicoccoides* segment carrying the *Pm36* gene is about 35.5 cM long in 5BIL-29 and less than 1.5 cM in 5BIL-42 line (Fig. 1).

The BJ261635 sequence was subjected to BLAST analysis at NCBI (http://www.ncbi.nlm.nih.gov/) to find a putative function. The search found a BAC clone (AC137592.2) on chromosome 9 of rice with 86.2% of similarity and unknown function. It is worth noting that the rice region was found to be homoeologous to bin 5BL6 of wheat based on comparative sequence analysis (Sorrels et al. 2003).

Discussion

Wild species have been widely used as important gene resources for introgressing useful traits into various crops. In the present study, a novel resistance powdery mildew gene was transferred from *T. turgidum* var. *dicoccoides* into durum wheat by backcrossing. The backcrossing method has been extensively used in self-pollinating crops to transfer simply inherited characteristics to cultivars which are deficient only in the characteristics being transferred. The development of molecular markers and genetic linkage maps, and the strategies for molecular breeding, have anyway shown that the backcrossing method has also considerable potential for transferring QTLs from unadapted germplasm to elite breeding lines (Tanksley and Nelson 1996; Blanco et al. 2006) and for genetic analyses of quantitative traits.

In order to investigate the inheritance of the powdery mildew resistance derived from var. *dicoccoides*, two segregating populations were developed by crossing two resistant backcross inbred lines (5BIL-29 and 5BIL-42) with the recurrent susceptible cv. Latino of durum wheat. The segregation pattern supported the hypothesis that the resistance is controlled by a single, dominant gene. Five AFLP markers and three gSSR markers (*Xcfd07*, *Xwmc75* and *Xgwm408*), physically mapped on the bin 5BL6-0.29-0.76, were found to be linked to the *Pm36* gene in 5BIL-29. The EST–SSR marker BJ261635 was found to be co-segregating with the *Pm36* gene in both 5BIL-29 × Latino and 5BIL-42 × Latino populations. These results indicated that the two lines had the same resistance gene and that the introgressed *dicoccoides* chromosome segment was longer (35.5 cM) in 5BIL-29 than that introgressed in 5BIL-42 (less than 1.5 cM). As no powdery mildew resistance gene has been reported on chromosome arm 5BL, the emmer gene was designated *Pm36*.

The homoeologous chromosome group 5 seems to play a key role in powdery mildew resistance since several major genes have been found located on these chromosomes, such as *Pm23* on chromosome 5A (McIntosh et al. 2003), *Pm30* on chromosome 5BS (Liu et al. 2002), *Pm36* on chromosome arm 5BL (present study), *Pm2* on chromosome arm 5DS (McIntosh and Baker 1970; Lutz et al. 1995), *Pm34* and *Pm35* on chromosome arm 5DL (Miranda et al. 2006, 2007). Major QTLs for adult plant resistance to powdery mildew were also mapped on 5A and 5B (Keller et al. 1999) and 5D (Chantret et al. 2000; Mingeot et al. 2002). Moreover, the physical mapping of 121 candidate resistance gene sequences by 339 deletion lines localized the highest number (67) of loci on group 5 chromosomes (Dilbirligi et al. 2004). The resistance genes *Pm35* and *Pm36* were located on homoeologous regions of chromosome arms 5DL (Miranda et al. 2007) and 5BL (present study), respectively, as indicated by the flanked homoeologous loci *Xcfd7-5B* and *Xcfd7-5D*, and by the consensus microsatellite map developed by Somers et al. (2004). The existence of homoeo-allelism within the genomes of wheat and within the tribe *Triticeae* have been clearly elucidated by cytogenetic methods and by molecular markers (Sears 1968; Devos et al. 1993), and it has been supposed that these relationships also exist among the resistance loci in the *Triticeae*. Development of further closely linked markers to resistance genes would be necessary to understand

the relationships between these genes, and would be helpful to increase combinations of available resistance which will provide more comprehensive and durable protection against powdery mildew in wheat breeding.

Wheat has an extremely large genome with more than 80% repetitive DNA sequences and that make cloning of agronomically important genes very difficult. However, gene-rich regions contain less repetitive DNA sequences and undergo recombination much more frequently than gene-poor regions. *Pm36* is located in a gene rich-regions on 5BL chromosom arm (Gill et al. 1996) and may be amenable to molecular manipulations as are the small genomes of plants such as tomato and rice (Faris et al. 2000). An important application of closely linked molecular markers to resistance genes is to provide a powerful tool for breeding programs as they can help to detect the resistance genes without the need to perform disease tests, and to shorten the breeding time in marker-assisted breeding compared to classical methodologies. The monofactorial nature of the resistance derived from var. *dicoccoides* makes it useful for powdery mildew resistance breeding in wheat. An EST–SSR marker (BJ261635) tightly linked to *Pm36* was identified. The co-segregation and the co-dominant characteristic make BJ261635 a reliable and easily detectable marker for MAS programs and gene pyramiding in wheat.

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