Theor Appl Genet (2005) 111: 360–369
DOI 10.1007/s00122-005-2029-1

ORIGINAL PAPER

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A new aluminum tolerance gene located on rye chromosome arm 7RS

Received: 3 March 2005 / Accepted: 5 April 2005 / Published online: 19 May 2005

Abstract Rye has one of the most efficient groups of genes for aluminum tolerance (*Alt*) among cultivated species of *Triticeae*. This tolerance is controlled by, at least, three independent and dominant loci (*Alt1*, *Alt2*, and *Alt3*) located on chromosome arms 6RS, 3RS, and 4RL, respectively. The segregation of *Alt* genes and several random amplified polymorphic DNA (RAPD), *Secale cereale* inter-microsatellite (SCIM), and *Secale cereale* microsatellite (SCM) markers in three F_2 between a tolerant cultivar (Ailés) and a non-tolerant inbred line (Riodeva) were studied. The segregation ratio obtained for aluminum tolerance in the three F_2 populations analyzed was 3:1 (tolerant:non-tolerant), indicating that tolerance is controlled by one dominant locus. $SCIM811_{1376}$ was linked to an *Alt* gene in the three F_2 populations studied, and three different SCIMs and one RAPD ($SCIM811_{1376}$, $SCIM812_{626}$, $SCIM812_{1138}$, and $OPQ4_{725}$) were linked to the *Alt* gene in two F_2 populations. This result indicated that the same *Alt* gene was segregating in the three crosses. $SCIM819_{1434}$ and $OPQ4_{578}$ linked to the tolerance gene in one F_2 population were located using wheat–rye ditelosomic addition lines on the 7RS chromosome arm. The *Alt* locus is mapped between $SCIM819_{1434}$ and the $OPQ4_{578}$ markers. Two microsatellite loci (*SCM-40* and *SCM-86*), previously located on chromosome 7R, were also linked to the *Alt* gene. Therefore, the *Alt* gene segregating in these F_2 populations is new and probably could be orthologous to the *Alt* genes located on wheat chromosome arm 4DL, on barley chromosome arm 4HL, on rye chromosome arm 4RL, and rice chromosome 3. This new *Alt* gene located on rye chromosome arm 7RS was named *Alt4*. A map of rye chromosome 7R with the *Alt4* gene, 16 SCIM and RAPD, markers and two SCM markers was obtained.

Communicated by C. Möllers

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Introduction

Aluminum (Al) is a light metal that makes up 7% of the Earth's crust and is the third most abundant element after oxygen and silicon. Al toxicity limits crop growth and productivity on acids soils. According to Van Wambeke (1976), acids soils occupy 1,445 million ha (11%) of the world's land, whereas Haug (1983) estimated that 30–40% of the world's arable soils and up to 70% of potentially arable land are acidic. Plant roots are, therefore, almost always exposed to Al in some form; fortunately, most of this Al occurs as harmless oxides and aluminosilicates. When soils become acid as a result of natural processes or human activities, the predominant form of Al found in acid soils is the toxic trivalent cation Al^{3+}. This ion is highly toxic to plants roots (Kinraide 1991), resulting in a poorly developed root system and thus in a susceptibility to drought stress and nutrient deficiencies (Foy 1988; Kochian 1995). Whereas liming of soil can ameliorate some Al toxicity, it is expensive, ineffective in the subsoil and, in some cases, heavy lime application may have a deleterious effect on soil structure (Rao et al. 1993). Several external Al tolerance mechanisms have been proposed, the exudation of organic acids being one of the most important and studied. The organic acid secretion in the rye roots is delayed for several hours after exposure to Al^{3+}, and the roots exudates malate and citrate (Hede et al. 2001; Ma et al. 2001a). Recently, a wheat gene encoding an Al-activated malate transporter has been detected

(Sasaki et al. 2004). Another strategy for improving crop productivity on Al-toxic acidic soils is to select for cultivars with increased Al tolerance. Cereal crops exhibit variation in Al tolerance, and rye (*Secale cereale* L.) is the most tolerant cereal (Mugwira et al. 1978; Little 1988; Aniol and Madej 1996), whereas barley is the most Al-sensitive (Foy 1983). Rye is an interesting source of resistance genes for wheat (*Triticum* ssp.) through wheat–rye introgression and is useful as a component of triticale (× *Triticosecale* Wittmack). Therefore, the knowledge of the mechanism and the genes that control Al rye tolerance is an important goal and will provide fundamental information than can be used to increase Al tolerance in other cereals. Slootmaker (1974) found that wheat D genome was the most important, followed by the A and B genomes. Aniol and Gustafson (1984) identified Al tolerance genes on wheat chromosome arms 6AL, 7AS, 4BL, 2DL, 3DL, 4DL, and 7D. Aniol (1990) found genes controlling wheat Al tolerance on 2DL, 4DL, and 5AS. Aniol and Gustafson (1984), using wheat–rye addition lines, described the existence of, at least, three different Al tolerance genes in rye: *Alt1* located on rye chromosome arm 6RS, *Alt2* situated on rye chromosome 3R, and *Alt3* that reside on chromosome arm 4RL. Manyowa et al. (1988) found that Al tolerance in rye is controlled by factors on more than one chromosome, though predominantly on chromosome 5R. Ma et al. (2000) indicated the existence of an Al tolerance gene located on the short arm of chromosome 3R, probably involved in the organic acid release in triticale. Aniol (2004) indicated that the major locus responsible for Al tolerance in rye is located on the short arm of chromosome 3R. Al tolerance is genetically controlled (Campbell and Lefever 1981), and several genes with additive effects appear to be involved in wheat (Aniol 1983). The genetics of Al tolerance has been extensively studied in cereal crops. Al resistance in some wheat cultivars is multigenic (Aniol and Gustafson 1984; Aniol 1991) but is controlled by a single dominant gene in other wheat cultivars (Kerridge and Kronstad 1968; Aniol and Gustafson 1984; Fisher and Scott 1987; Larkin 1987). Al tolerance, assessed on the basis of root elongation, segregated as a single dominant locus (3:1) in F_2 populations of wheat (Delhaize et al. 1993; Somers and Gustafson 1995; Riede and Anderson 1996; Basu et al. 1997). A single locus, *AltSB*, was found to control Al tolerance in two highly Al-tolerant sorghum cultivars (Magalhaes et al. 2004). As has been found in other species, Al tolerance in rye seems to be a dominant character (Aniol and Madej 1996). Gallego and Benito (1997) reported the existence of, at least, two dominant and independent loci controlling the rye Al tolerance, and they also detected isozyme loci (*Aco1* and *Ndh2*) located on chromosome 6R that were linked to *Alt1* gene. Gallego et al. (1998) identified two random amplified polymorphic DNA (RAPD) markers, which have been converted to sequence-characterized amplified region (SCAR) markers (*ScR01600* and *ScB15790*) that flanked the gene located on the short arm of chromosome 6R, 2.1 cM and 5.5 cM from the *Alt1* gene. Two RFLPs (*Xbcd1230* and *Xcdo1395*) linked to the wheat *AltBH* gene were located on 4DL chromosome arm (Riede and Anderson 1996). Gallego (1997) detected one RAPD marker (*OPS14715*) linked to rye *Alt3* gene at a distance of 23.5 cM. Rodriguez-Milla and Gustafson (2001) reported a physical characterization of chromosome arm 4DL in wheat, and they mapped the *AltBH* gene to the distal region of the chromosome, between deletion breakpoints 0.7 and 0.86. Miftahudin et al. (2002) reported three AFLP markers tightly linked to the *Alt3* gene, two of these AFLP markers (AMAL4 and AMAL5) flanked the *Alt3* locus at a distance of 0.4 cM and 0.7 cM. Chromosomal localization using cloned AFLP (AMAL4) and a RFLP (*Xbcd1230*) marker indicated that the *Alt3* gene was on 4RL chromosome arm. Moreover, the RFLP marker (*Xbcd1230*) co-segregated with the *Alt3* gene.

Miftahudin et al. (2004) developed two PCR-based codominant markers (B1 and B4) flanking the *Alt3* gene in rye, based on the DNA of a rice (*Oryza sativa* L.) bacterial artificial chromosome (BAC) clone suspected to be syntenic to the *Alt3* gene region. Four additional RFLP markers developed from rice genes/putative genes, spanning 10 kb of a 160-kb rice BAC, were mapped to the *Alt3* region. Two rice markers flanked the *Alt3* locus at a distance of 0.05 cM, whereas two others co-segregated with it (Miftahudin et al. 2005). A major QTL was identified on chromosome 4R through the application of molecular markers to a specific cross of rye; this QTL accounted for 48% of total phenotypic variation and was linked to an RFLP marker at a distance of 2 cM (Hede et al. 2001). The *AltBS* locus mapped near the end of sorghum (*Sorghum vulgare* L.) chromosome 3, and the intertribe map comparisons suggest that the major Al tolerance QTL on rice chromosome 1 is probably orthologous to *AltBS* (Magalhaes et al. 2004). The aims of the present work were to study the genetic control of rye Al tolerance in three different F_2 populations between a tolerant cultivar (Ailés) and a non-tolerant inbred line (Riodeva), to obtain molecular markers linked to the Al tolerance genes and to locate these genes.

Materials and methods

Plant material

To map the *Alt* loci in rye, three different F_2 populations were used: AR1-6 with 118 plants, AR1-25 with 113 plants, and AR6-5 with 150 plants. These F_2 populations were obtained selfing three different F_1 plants originated from different crosses between a tolerant plant from the rye cultivar Ailés (allogamous) and an individual from the non-tolerant inbred line Riodeva. These three F_2 populations were obtained by Gallego and Benito (1997) and are described in Fig. 1.

Hexaploid wheat (*Triticum aestivum* L.) cultivar Chinese Spring (CS), rye cultivar Ailés (A) and the

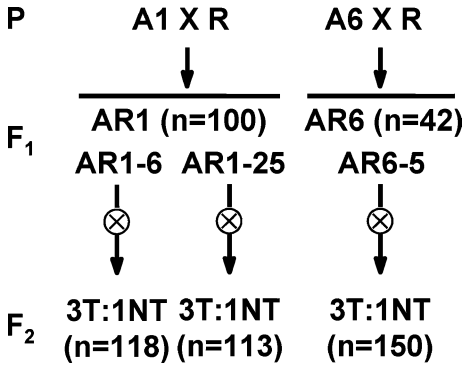

Fig. 1 Scheme of the crosses to analyze the genetic control of aluminum (Al) tolerance in rye. The observed segregation ratios for Al tolerance (tolerant:non-tolerant) in the F_2 populations are shown at the bottom of the figure. *A* Cultivar Ailés, *R* non-tolerant inbred line Riodeva. *Between brackets* are indicated the numbers of plants obtained in the different crosses

inbred line Riodeva (R), the seven disomic wheat–rye Chinese Spring–Imperial (CS-I) addition lines (1R–7R) and the two ditelosomic wheat–rye addition lines 7RS and 7RL, kindly supplied by Dr. A.J. Lukaszewski, were used to assign loci to rye chromosomes. Each disomic and ditelosomic wheat–rye addition line has 42 chromosomes of *T. aestivum* L. cultivar CS and a different pair of homologous chromosomes or chromosome arms of rye *S. cereale* L. cultivar I.

Al tolerance screening test

The Al tolerance tests were carried out using the method of Aniol (1984) at Al concentration of 150 μM (Gallego and Benito 1997). All the plants from the same F_2 population were tested simultaneously, and the R inbred line was used each time as a contemporary control. The plants of the three F_2 populations were classified as tolerant when the mean length of root re-growth was longer than the maximum re-growth (3 mm) found in the inbred line R. The plants of the F_2 populations were classified as non-tolerant when the mean length of root re-growth was shorter than the maximum re-growth (3 mm) found in the inbred line R.

DNA extraction

The young leaves from F_2 plants were frozen in liquid nitrogen and stored at −80°C. The extraction was carried out using a small-scale DNA isolation method (Dneasy Plant Mini Kit, Qiagen).

RAPD markers

RAPD reactions similar to those described by Williams et al. (1990) were performed with minor modifications. Four hundred 10-mer primers (kits A–T from Operon Technologies) were used in this study. The PCR reactions were carried out in a 25-μl volume containing 0.4 U Dynazyme polymerase (F501L), 20 ng genomic DNA template, 10 pmol of primer, 2.5 mM each dNTP, and 2.5 μl 10X Dynazyme reaction buffer. Amplifications were done in a DNA Thermal Cycler PTC (MJ Research) using the following thermocycling program: an initial step of 5 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C, and a final 6-min extension at 72°C. Samples of 20-μl PCR products were analyzed on 1.5% agarose gels running at 90 V for 2 h. The gels were stained using ethidium bromide. RAPD experiments were repeated, at least three times, and only repeatable bands were scored.

Secale cereale inter-microsatellites

One hundred primers (named from 801 to 900) based on di-, tri-, tetra- or pentanucleotide repeats obtained from UBC primer set 100/9 (University of British Columbia) were used. We have adapted the protocol of Zietkiewicz et al. (1994). In this case, each amplification reaction (final volume of 20 μl) was constituted by 1 μl total genomic DNA (5 ng/μl), 1 μl of the corresponding primer (5 μM), 10 μl *Taq*-PCR master mix (Qiagen) and 8 μl ultra-pure distilled water (Qiagen). DNA amplifications were performed in a PTC-100 thermocycler (MJ Research) with an initial step of 5 min at 94°C, followed by 45 cycles of 30 s at 94°C, 45 s at 52°C and 2 min at 72°C, and a final 6-min extension at 72°C. Samples of 20-μl PCR products were analyzed on 1.5% agarose gels running at 90 V for 2 h. The gels were stained using ethidium bromide. SCIM experiments were repeated at least three times, and only repeatable bands were scored.

Secale cereale microsatellites

The 25 pairs of primers used in this work to amplify *Secale cereale* microsatellite (SCM) were previously described by Saal and Wricke (1999). These authors located and mapped several SCM markers, using wheat–rye addition lines and crosses. In this case, each amplification reaction (final volume of 10 μl) was constituted by 1 μl total genomic DNA (30 ng/μl), 0.5 μl each primer (50 μM), 5 μl *Taq*-PCR master mix (Qiagen) and 3 μl ultra-pure distilled water (Qiagen). DNA amplifications were performed in a PTC-100 thermocycler (MJ Research) with an initial step of 5 min at 95°C, followed by 40 cycles of 60 s at 95°C, 60 s at 50–65°C and 30 s at 72°C, and a final 10-min extension at 72°C. Samples of 10-μl PCR products were analyzed on 8% acrylamide gels running at 150 V for 5 h. The gels were stained using silver nitrate.

Bulked segregant analysis

To identify RAPD and SCIM markers linked to the *Alt* loci, we used bulked segregant analysis (Michelmore et al. 1991). Fragments in the *Alt* region were identified by screening two different pairs of pools generated for each F_2 population analyzed. The bulks consisted of 14 Al-tolerant plants and 12 Al-non-tolerant plants from AR1-6, 15 Al-tolerant plants and 11 Al-non-tolerant plants from AR1-25, and 12 Al-tolerant plants and 12 Al-non-tolerant plants from AR6-5. Moreover, F_1 plants, A parentals and the R inbred line were simultaneously analyzed. Primers showing differences within each pair of bulks were applied to all the individual plants of the respective F_2 population to determine their inheritance and the linkage values.

Cloning and sequencing of RAPD and SCIM products

The rye amplification products of interest were linked and transformed using the pCR 2.1 vector system (Invitrogen). The sequences of the cloned fragments were obtained using the automatic DNA ABI-PRISM (Applied Biosystems).

Genetic mapping

Linkage analyses were performed on F_2 segregation data using the MAPMAKER, version 3.0, computer program (Lander et al. 1987). Two loci were considered linked if the LOD score *Z* was greater than 3 and if the two-point distance was less than 45 cM. Genetic distances were calculated using the Kosambi function (Kosambi 1944).

Results and discussion

Al tolerance

The mean length of root re-growth of Al-tolerant (A) and of Al-sensitive (R inbred line) parents at 150 μM of Al were 1.1 mm and 14.6 mm, respectively (Gallego and Benito 1997). Because this concentration of Al clearly distinguished the two parents, we decided to screen the three F_2 populations at 150 μM in order to determine the segregation pattern of the gene(s) controlling Al tolerance. The maximum root re-growth observed in the R inbred line was 3 mm. On this basis, the plants of the F_2 populations analyzed were classified in two different groups, tolerant plants with a length of root re-growth higher than 3 mm and non-tolerant plants with a length of root re-growth between 0 mm and 3 mm (the maximum re-growth found in R). The segregation ratio obtained for aluminum tolerance (*Alt*) in the three F_2 populations analyzed was 3:1 (tolerant:non-tolerant). This result indicated that in these populations the tolerance was controlled by one dominant locus (Table 1).

Moreover, this result indicated that the three F_1 plants used to obtain the three different F_2 analyzed were heterozygous for an *Alt* locus. The χ^2 heterogeneity test was not significant, indicating that the data from the three F_2 populations were homogeneous for *Alt* locus (Table 1). The *SCIM8111376* marker segregated 3:1 (presence:absence) in the three F_2 populations analyzed, and this marker was linked in the three F_2 populations to the *Alt* locus. The χ^2 heterogeneity test for *SCIM8111376* was not significant indicating that the data from the three F_2 populations were homogeneous for this marker (Table 2). Therefore, the same *Alt* locus was segregating in the three different F_2 populations.

Taking into account that the same *Alt* locus was segregating in the three F_2 populations used and that the data from the three F_2 were homogeneous, we have elaborated a graphic with the distribution of root length re-growth in individuals of the three F_2 populations (Fig. 2).

Bulked segregant analysis

Bulked segregant analysis is an efficient procedure to detect dominant markers linked to target loci in F_2 populations. On the basis of Al tolerance, the homozygous dominant and heterozygous genotypes were indistinguishable and were bulked together in the F_2 generations. Therefore, only the RAPD and SCIM fragments associated in *cis* (coupling phase) with the dominant Al-tolerance allele could be detected (Gallego et al. 1998). Fragments in the *Alt* region were identified by screening two different pairs of bulks generated for each F_2 population analyzed. Several ISSR and RAPD

Table 1 Segregation of the *Alt* locus in the three F_2 analyzed. *T* Tolerant, *NT* non-tolerant

F_2 analyzed	Total plants	T plants	NT plants	χ^2 3:1
AR1-6	118	87	31	0.102
AR1-25	113	90	23	1.301
AR6-5	150	112	38	0.009
Total	381	289	92	0.148

Sum of χ^2 = 1.412, χ^2 heterogeneity = 1.264

Table 2 Segregation of the $SCIM811_{1376}$ locus in the three F_2 analyzed

F_2 analyzed	Total plants	T plants	NT plants	χ^2 3:1
AR1-6	117	88	29	0.003
AR1-25	113	91	22	1.844
AR6-5	142	106	36	0.009
Total	372	285	87	0.516

Sum of χ^2 = 1.856, χ^2 heterogeneity = 1.340

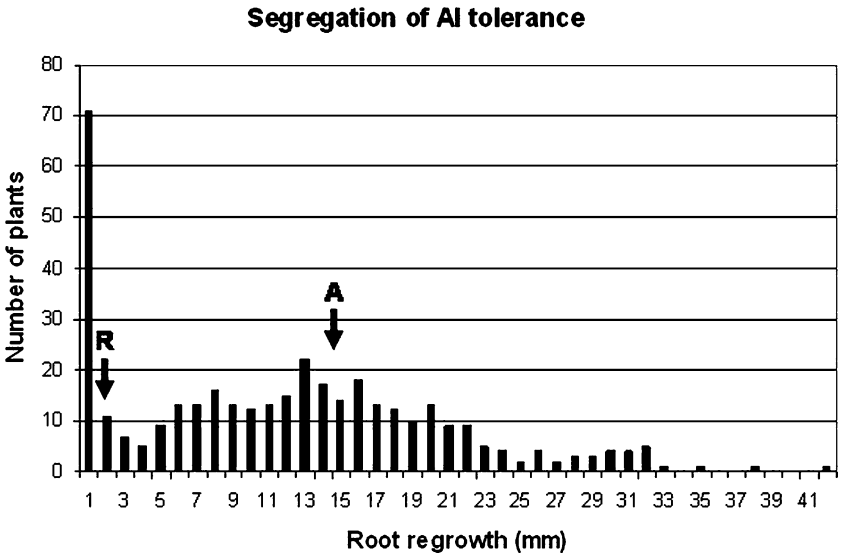

Fig. 2 Distribution of root length re-growth in individuals of the F_2 populations analyzed, after treatment with Al (150 μM). The three F_2 populations AR1-6, AR1-25, and AR6-5 segregated 3:1 (tolerant:non-tolerant). The offspring of the three F_2 were pooled. *Arrows* show the means of root re-growth of the parental cultivars Ailés (*A*) and Riodeva (*R*)

primers that produced a different amplification pattern in the bulk of Al-tolerant (T) and in the bulk of-non-tolerant (NT) plants were detected. The ISSR primers 811, 812, 819, and 881 generated different amplification patterns between T and NT bulks in at least one of the F_2 populations analyzed. The following RAPD primers produced different amplification patterns between T and NT bulks in at least one of the F_2 populations analyzed: OPD1, OPF20, OPJ3, OPJ17, OPN1, OPQ4, OPO2, and OPO7. These ISSR and RAPD primers produced, at least, one DNA fragment present in the T bulk and absent in the NT bulk. Therefore, only the RAPD and SCIM fragments associated in *cis* (coupling phase) with the dominant Al tolerance allele were detected. All the ISSR and RAPD primers previously referred were used to perform individual amplifications in the corresponding F_2 populations. The results obtained for the ISSR primer 811 are indicated in Fig. 3.

Segregation analysis

The tolerant A cultivar is allogamous and presents variability. There are markers that segregate in the three F_2 populations analyzed, for example, the *Alt* locus and the $SCIM811_{1376}$ marker. Moreover, other molecular markers segregate only in two F_2 populations (AR1-6 and AR6-5), like $SCIM812_{626}$, $SCIM812_{1138}$, and $OPQ4_{725}$. At last, there are molecular markers that only segregate in one F_2 population, such as $SCIM881_{1422}$ in AR1-25; $SCIM819_{1434}$, $OPO2_{633}$, $OPO7_{666}$ and $OPQ4_{578}$ in AR6-5; and $OPF20_{709}$, $OPF20_{579}$, $OPJ3_{857}$,

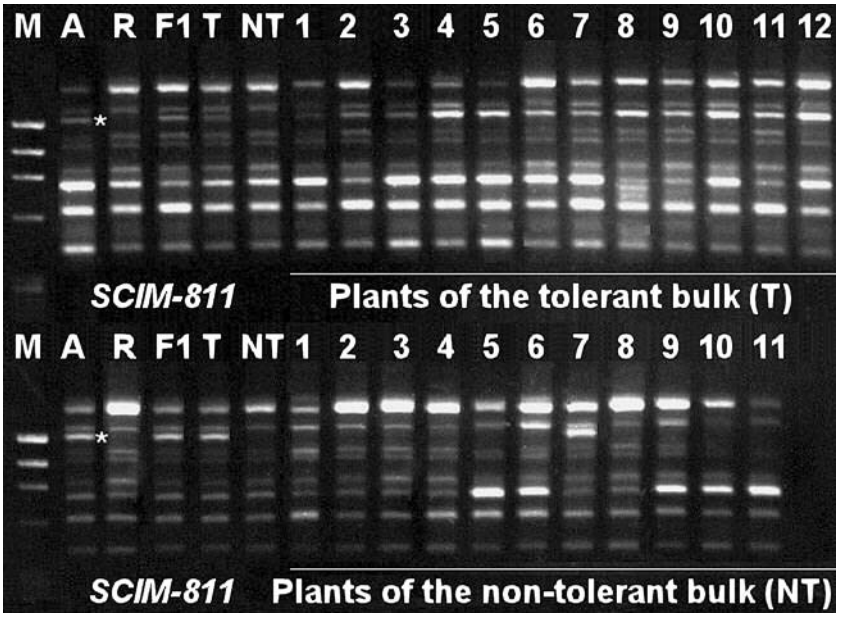

Fig. 3 Amplification patterns obtained with the ISSR primer 811 in bulk of tolerant (*T*) and non-tolerant (*NT*) plants of the AR1-6. The A parental and the F_1 showed the same pattern as the T bulk. The R inbred line showed the same pattern as the NT bulk. The inter-microsatellite $SCIM811_{1376}$ (*) is present in T bulk and in all the plants of this bulk and absent in NT bulk and in all plants of NT bulk except in one plant (number *7*). The plant number 7 of the non-tolerant bulk came from at least one recombinant gamete

OPJ17$_{1245}$, *SCM-86*, *SCM-40*, *OPD1*$_{858}$, *SCIM823*$_{2826}$, and *SCIM823*$_{853}$ in AR1-6. The *Alt* locus, three different SCIMs, and one RAPD (*SCIM811*$_{1376}$, *SCIM812*$_{626}$, *SCIM812*$_{1138}$, and *OPQ4*$_{725}$) segregate in two F_2 populations (AR1-6 and AR6-5). A χ^2 heterogeneity test was performed using the five common loci (*SCIM811*$_{1376}$, *SCIM812*$_{626}$, *SCIM812*$_{1138}$, *OPQ4*$_{725}$, and *Alt*) that segregated in the AR1-6 and AR6-5 in order to pool the data. The χ^2 heterogeneity test was not significant, indicating that data from both F_2 populations were homogeneous.

The SCM markers developed by Saal and Wricke (1999) that segregated in our F_2 populations and their chromosomal location are indicated in Table 3. The other SCM markers obtained by Saal and Wricke (1999) do not segregate in our populations. *SCM40* has been mapped on 7RS chromosome arm by Korzun et al. (2001). All the markers analyzed (SCIM, RAPD, and SCM) in the three F_2 populations showed a dominant inheritance and their segregation ratios fit a 3:1 (presence:absence), except for *SCIM823*$_{853}$ in the F_2 population AR1-6. The segregations of the *SCM-40* and *SCM-86* microsatellites are shown in Fig. 4.

Linkage analysis and map of 7R chromosome

The *SCIM811*$_{1376}$ marker was linked to the *Alt* gene in the three F_2 populations studied and four different markers (*SCIM811*$_{1376}$, *SCIM812*$_{626}$, *SCIM812*$_{1138}$, and *OPQ4*$_{725}$) were linked to the *Alt* gene in two F_2 populations (AR1-6 and AR6-5). The order of the common molecular markers segregating in the F_2 populations AR1-6 and AR6-5 was the same. As we have pointed previously in the study of Al tolerance, these results indicate that the same *Alt* dominant gene was segregating in the three crosses. Taking into account that the χ^2 heterogeneity test for the five common markers was not significant, we analyzed the data of the AR1-6 and AR6-5 populations together in order to obtain a more accurate estimation of the recombinant frequencies. The five common loci (in the following order) of the F_2 populations AR1-6 and AR6-5 were obtained: *SCIM812*$_{626}$, *OPQ4*$_{725}$, *SCIM811*$_{1376}$, *Alt*, and *SCIM812*$_{1138}$. After we fixed this order, we mapped the remaining segregating markers in each F_2 population. The data of the F_2 population AR1-25 were not included in this map, because it only showed two common markers (*Alt4* and *SCIM811*$_{1376}$) with the other F_2 populations analyzed (AR1-6 and AR6-5). The *SCIM819*$_{1434}$ and *OPQ4*$_{578}$ markers linked to the *Alt* tolerance gene in the AR6-5 F_2 population were located using disomic and ditelosomic wheat–rye addition lines on chromosome arm 7RS (Fig. 5).

The *SCM-40* and *SCM-86* markers located on chromosome 7R by Saal and Wricke (1999) that segregated in the AR1-6 population were in the same linkage group as the *Alt* locus. *SCM-40* has been placed on the short arm of chromosome *7R* by Korzun et al. (2001). The other SCM markers located on the 1R, 2R, 3R, 5R and 6R chromosomes behaved independently of the *Alt* locus, but they were linked to other molecular markers segregating in these crosses located in different linkage groups (data not shown). The SCAR *ScR01*$_{600}$ linked at 2.1 cM to *Alt1* locus on the 6RS chromosome arm by Gallego et al. (1998) was also independent of the *Alt* locus segregating in our cross. Therefore, the *Alt* locus that is segregating in our crosses is not *Alt1*. The results obtained indicate that the rye *Alt* locus segregating in our crosses is a new Al tolerance locus located on chromosome 7R. This new locus has been named *Alt4*. The map of the 7R chromosome obtained using the data from the F_2 population AR1-6 and AR6-5 situated the *Alt4* locus between the *OPO2*$_{633}$ and the *OPQ4*$_{578}$ RAPD markers (Fig. 6). The *Alt4* tolerance gene is situated between the *SCIM819*$_{1434}$ and the *OPQ4*$_{578}$ markers, which have been previously located on the 7RS chromosome using wheat–rye addition lines. Therefore,

Table 3 Chromosomal location of the *Secale cereale* microsatellite (*SCM*) markers segregating in the AR1-6 F_2 population has been previously obtained by Saal and Wricke (1999)

Microsatellite	Chromosome or chromosome arm	Microsatellite	Chromosome or chromosome arm
SCM-39	*1RL*[a]	*SCM-109*	5RL
SCM-43	*2RS*[a]	*SCM-2*	6RL
SCM-75	*2RL*	*SCM-28*	6RL
SCM-5	*3RL*	*SCM-304*	6RS
WMS-6	*5RL*	*SCM-40*	7RS[a]
SCM-138	*5RS*	*SCM-86*	7R
SCM-268	*5RS*[a]		

[a]Chromosome arm location obtained by Korzun et al. (2001)

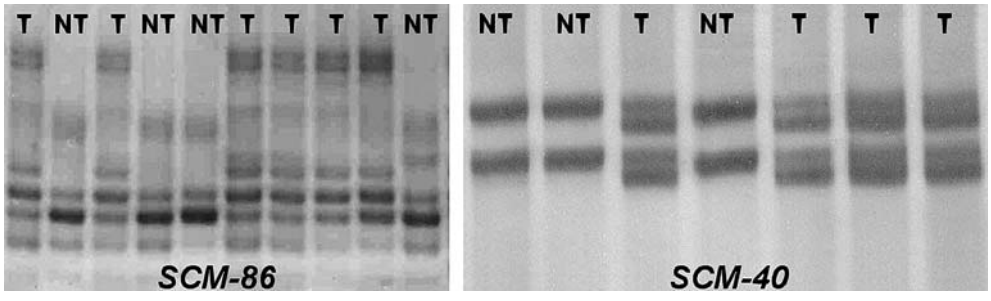

Fig. 4 Segregations of the *SCM-40* and *SCM-86* microsatellites in the F_2 population AR1-6

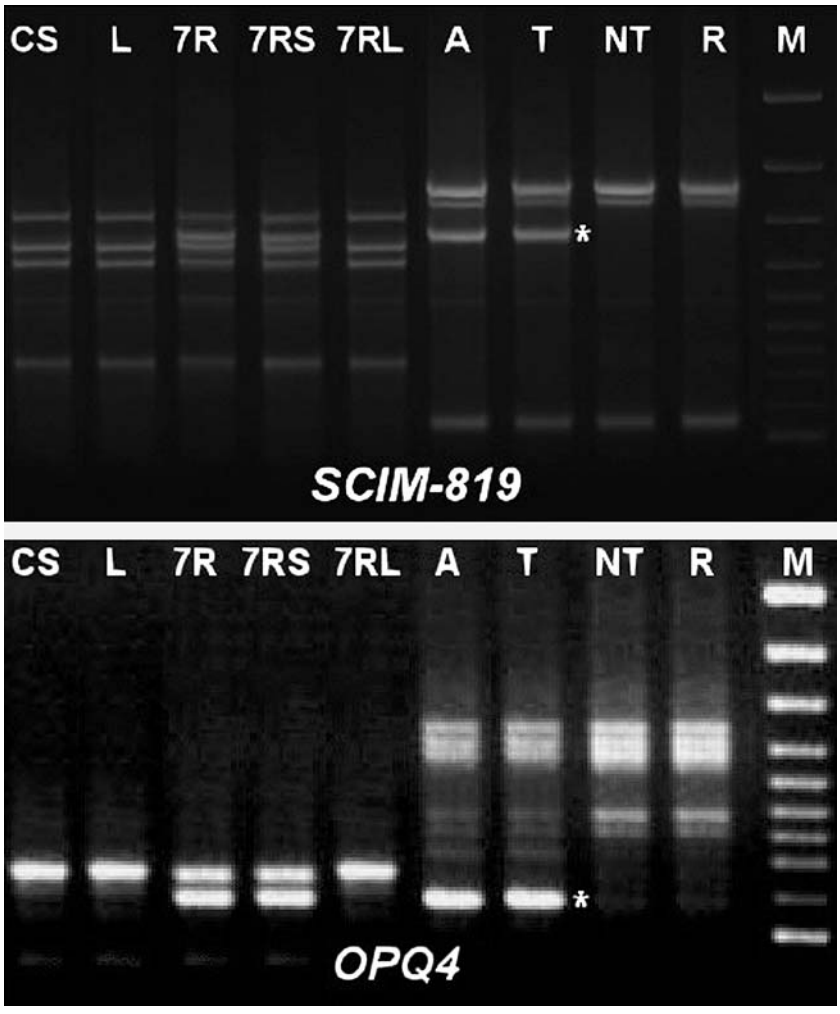

Fig. 5 Chromosomal location of the inter-microsatellite $SCIM819_{1434}$ (*) and the random amplified polymorphic DNA $OPQ4_{578}$ (*) on chromosome arm *7RS*. *CS* Cultivar Chinese Spring wheat, *L* wheat–rye disomic addition lines 1R– 6R, *7R* wheat–rye disomic addition line 7R, *7RS* ditelosomic wheat–rye addition line 7RS, *7RL* ditelosomic wheat–rye addition line 7RL, *T* a tolerant plant of the F_2 AR6-5, *NT* a non-tolerant plant of the AR6-5, *M* molecular size marker. L is not a bulk; the wheat–rye addition lines 1R–6R showed the same amplifications patterns with these primers

the new gene *Alt4* is located on the short arm of 7R chromosome. Moreover, *SCM-40* has been previously located on 7RS chromosome arm by Korzun et al. (2001) and, consequently, all the markers mapped between $SCIM819_{1434}$ and the *SCM-40* must be located on 7RS chromosome arm (Fig. 6).

This map has 18 markers (six SCIMs, ten RAPDs, and two SCMs) and the *Alt4* locus. Most of these DNA markers have been cloned, and their sequences have been sent to the National Center for Biotechnology Information data bank (Table 4).

The map of chromosome 7R obtained with the F_2 population AR1-25 has only three markers, and only two ($SCIM811_{1376}$ and *Alt4*) are common with the markers segregating in the F_2 population AR1-6. The other marker linked to *Alt4* locus in population AR1-25 is the $SCIM881_{1422}$ (Fig. 6). The following markers were only segregating in the F_2 population AR1-6: *SCM-40*, *SCM-86*, $SCIM823_{2826}$, $SCIM823_{853}$, $OPD1_{858}$, $OPF20_{709}$, $OPF20_{579}$, $OPJ3_{857}$, $OPJ17_{1245}$, and $OPN1_{667}$. The following markers were segregating only in the F_2 population AR6-5: $OPQ4_{578}$, $OPO2_{633}$, $OPO7_{666}$, and $SCIM819_{1434}$. The marker $SCIM881_{1422}$ was only segregating in the F_2 population AR1-25.

Comparative mapping of the *Alt4* gene in rye and other triticeae species

Two RFLPs (*Xbcd1230* and *Xcdo1395*) linked to the wheat *AltBH* gene, located on the 4DL chromosome arm, have been reported by Riede and Anderson (1996). Rodriguez-Milla and Gustafson (2001) reported a physical characterization of chromosome arm *4DL* in wheat and they mapped the *AltBH* gene to the distal region of the chromosome, between deletion breakpoints 0.7 and 0.86. Reid et al. (1971) found a single dominant gene (*Alp*) that control Al tolerance in barley. Stølen and Andersen (1978) identified a single dominant gene (*Pht*) on barley chromosome 4H that controls tolerance to high soil acidity. Minella and Sorrells (1992) located the *Alp* gene on barley 4H chromosome (distally from the centromere). Tang et al. (2000) have identified RFLP markers (*Xwg464*, *Xcdo1395*, and *Xbcd1117*) linked to barley aluminum tolerance gene *Alp* and reported that *Alp* could be orthologous of the wheat *AltBH* gene. The *Xcdo1395* marker was located 2.1 cM from *Alp*, whereas in wheat this marker is 11.3 cM from the Al tolerance gene *AltBH*, which resides in the long arm of chromosome 4D (Riede and Anderson 1996). In contrast, Tang

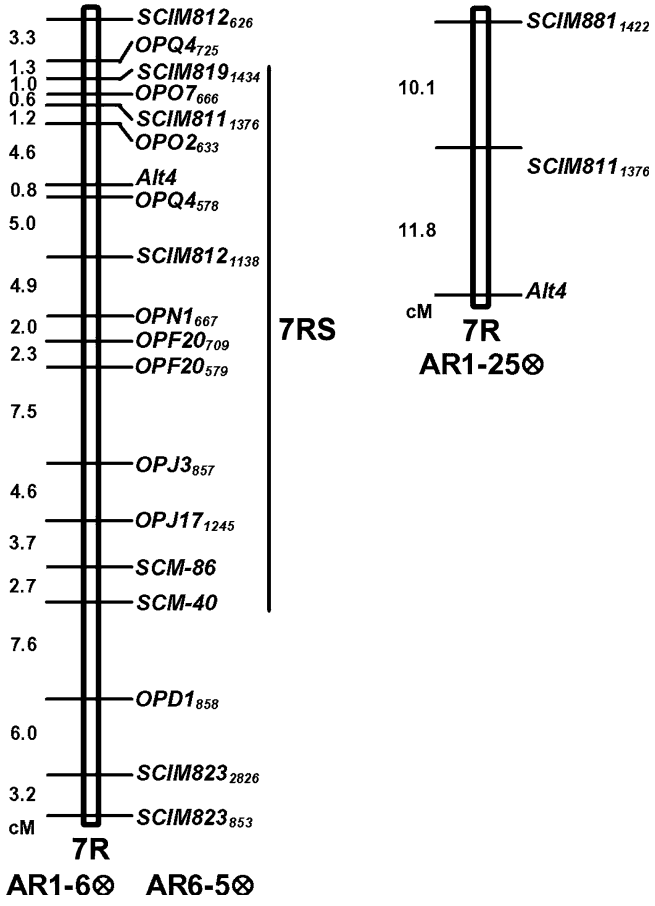

Fig. 6 Mapping of *Alt4* locus on rye chromosome arm 7RS. Data from the AR1-6 and AR6-5 (*left*) and data from the AR1-25 (*right*)

et al. (2000) found that *Xbcd1230* was only weakly linked to Alp (33 cM), whereas in wheat it is 1.1 cM from *AltBH*. Recently, Raman et al. (2002) have identified several AFLP and four microsatellite markers tightly linked to the *Alt* gene on barley 4H chromosome. Nguyen et al. (2003) have reported nine rice QTLs, one for root length under non-stress conditions, three for root length under Al stress (SRL), and five for relative root length (RRL). A major QTL for RRL, which explained 24.9% of the phenotypic variation, was found on chromosome 3 of rice. This QTL for RRL mapped in the same position of a QTL for SRL, which explained 18.7% of the phenotypic variation. A major QTL for SRL, which explained 26.4% of the phenotypic variation, was found on rice chromosome 2. Another major QTL for RRL that explained 20.8% of the phenotypic variation was found on chromosome 8 of rice. This QTL for RRL mapped in the same position of a QTL for SRL that explained 20.8% of the phenotypic variation. Gallego (1997) detected a RAPD $OPS14_{715}$ marker linked to the rye *Alt3* gene at a distance of 23.5 cM. Miftahudin et al. (2002) reported three AFLP markers tightly linked to the rye *Alt3* gene; two of these AFLP markers (AMAL4 and AMAL5) flanked the *Alt3* locus at distances of 0.4 cM and 0.7 cM. The AFLP AMAL4 was cloned and three different types of clones (I, II, and III) were obtained, one of these (type I) was located using wheat–rye addition lines on rye chromosome 4R (Miftahudin et al. 2002). However, these authors were not able to remap the clone in the population analyzed either using PCR or RFLP analysis due to lack of polymorphism between the two parents. Moreover, the RFLP marker (BCD1230) co-segregated with the *Alt3* gene (Miftahudin et al. 2002), but this RFLP has not been located in rye using wheat–rye addition lines. The BCD1230 has been mapped on chromosome arm 4RL using linkage analysis in F_2 populations of rye, but 72.8% of the segregating markers of these crosses showed distorted segregations ($P < 0.01$) including BCD1230 (Ma et al. 2001b). A major QTL was identified on rye chromosome 4R through the application of molecular markers to a specific cross. It accounted for 48% of total phenotypic variation and was linked to an RFLP marker, with a distance of 2 cM (Hede et al. 2001).

The long arm of rye chromosome 4R is partially homoeologous to the short arm of wheat chromosome 7 and partially homoeologous to the short arm of wheat chromosome 6 (Naranjo and Fernández-Rueda 1991; Devos et al. 1993; Naranjo et al. 1997). Therefore, the majority of the 4RL chromosome arm is not homoeologous to the 4DL and 4HL chromosomes. Previous studies (Naranjo and Fernández-Rueda 1991; Rognli et al. 1992, Devos et al. 1993; Naranjo et al. 1997) have stated that the long arm of wheat chromosome 4D is partially homoeologous to the proximal portion of the short arm of rye chromosome 7R and partially homoeologous to the distal portion of the long arm of rye chromosome 5R. Barley chromosome arm 4HL is homoeologous to the wheat chromosome arm 4DL (Tang et al. 2000; Raman et al. 2002). Moreover, Gale and Devos (1998) have obtained a consensus grass comparative map and have pointed out that rice chromosome 3 is homoeologous to wheat chromosome 4. Therefore, the major QTL for RRL located on rice chromosome 3, the wheat *AltBH* gene located on the 4DL chromosome

Table 4 GenBank accession numbers of the markers located on 7R chromosome

Marker	Accession	Marker	Accession	Marker	Accession
$SCIM812_{626}$	AY587498	$OPD1_{858}$	AY587503	$OPN1_{667}$	AY587508
$SCIM811_{1376}$	AY587499	$OPF20_{579}$	AY587504	$OPO2_{633}$	AY587509
$SCIM812_{1138}$	AY587500	$OPF20_{709}$	AY587505	$OPO7_{666}$	AY587510
$SCIM819_{1434}$	AY587501	$OPJ3_{857}$	AY587506	$OPQ4_{578}$	AY587511
$SCIM881_{1422}$	AY587502	$OPJ17_{1245}$	AY587507	$OPQ4_{725}$	AY587512

arm, and the aluminum tolerance gene *Alp* located on 4HL are probably orthologous genes. Taking into account that the rye *Alt3* gene co-segregated with the RFLP *Xbcd1230* Miftahudin et al. (2002, 2004) indicated that this gene could be orthologous to the wheat *AltBH* and barley *Alp* genes and rice QTL for RRL located on rice chromosome 3. Therefore, these authors suggest that the proximal region of the rye 4RL containing the *Alt3* gene is homoeologous to the wheat 4DL and barley 4HL chromosome arms. Following the homoeologous relationships established by Naranjo and Fernández-Rueda (1991), Rognli et al. (1992), Devos et al. (1993) and Naranjo et al. (1997), the new rye *Alt4* gene located on 7RS chromosome in this work is also a candidate to be an orthologous gene. Further analyses should be needed in order to confirm this hypothesis. These results suggest the existence of common and conserved mechanism of aluminum tolerance in the *Triticeae* species.

Acknowledgements This work was supported by the research grants BOS 2000-0561 and AGL 200306470 from the Ministry of Education and Science of Spain (DGICYT) and Praxis XXII/15874/98 from the Ministry of Science and Technology (MCT) of Portugal.

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