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Genetic control of aluminium tolerance in rye (*Secale cereale* L.)

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Abstract Aluminium (Al) tolerance in roots of two cultivars ("Ailés" and "JNK") and two inbred lines ("Riodeva" and "Pool") of rye was studied using intact roots immersed in a nutrient solution at a controlled pH and temperature. Both the cultivars and the inbred lines analysed showed high Al tolerance, this character being under multigenic control. The inbred line "Riodeva" was sensitive (non-telerant) at a concentration of 150 μM, whereas the "Ailes" cultivar showed the highest level of Al tolerance at this concentration. The segregation of aluminium-tolerance genes and several isozyme loci in different F_1s, F_2s and backcrosses between plants of "Ailés" and "Riodeva" were also studied. The segregation ratios obtained for aluminium tolerance in the F_2s analysed were 3 : 1 and 15 : 1 (tolerant : non-tolerant) while in backcrosses they were 1 : 1 and 3 : 1. These results indicated that Al tolerance is controlled by, at least, two major dominant and independent loci in rye (*Alt1* and *Alt3*). Linkage analyses carried out between Al-tolerance genes and several isozyme loci revealed that the *Alt1* locus was linked to the aconitase-1 (*Aco1*), nicotinamide adenine dinucleotide dehydrogenase-2 (*Ndh2*), esterase-6 (*Est6*) and esterase-8 (*Est8*) loci, located on chromosome arm 6RL. The order obtained was *Alt1-Aco1-Ndh2-Est6-Est8*. The *Alt3* locus was not linked to the *Lap1, Aco1* and *Ndh2* loci, located on chromosome arms, 6RS, 6RL and 6RL respectively. Therefore, the *Alt3* locus is probably on a different chromosome.

Key words Aluminium · Inbred lines · Tolerance · Genetic control · Rye

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Introduction

Aluminium (Al) is the most abundant metal in the earth's crust, comprising approximately 7.5% by weight (Haug 1984). It is found in soils primarily in the form of insoluble alumino-silicates or oxides. When solubilized in acid soils, Al (primarily in the form of Al^{3+}) is toxic to many crop plants (Foy et al. 1978; Rao et al. 1993; Kochian 1995). Low pH values (below 5) occur naturally in many volcanic and tropical soils. In addition, the amount of acidity introduced by fertilisers and "acid rain" has outstripped the buffering capacities of soils in many areas, leading to toxic levels of Al (van Breemen 1985).

The primary effect of Al is to inhibit root growth in Al-sensitive genotypes with subsequent effects on nutrient and water uptake (Foy 1983). Root elongation is affected within hours of Al exposure (Wallace et al. 1982) and, as in many plant species, the primary site of Al toxicity in wheat (*Triticum aestivum* L) appears to be the root apex (Bennet and Breen 1991). Ryan et al. (1993) have shown that, in wheat and maize, root elongation is inhibited only when apices are exposed to Al, whereas selectively exposing the remaineder of the root does not inhibit elongation.

Cereals differ in response to Al, rye (*Secale cereale* L) being one of the most tolerant and wheat (*Triticum* ssp.) generally being less tolerant Slootmaker 1974; Aniol and Gustafson 1984; Manyova et al. 1988). Aluminium tolerance is genetically controlled (Campbell and Lefever 1981) and several genes with additive effects appear to be involved in wheat (Aniol 1983). Major genes controlling wheat tolerance to Al were located on chromosome arms 5AS, 2DL and 4DL of hexaploid wheat (Aniol and Gustafson 1984; Aniol 1990). Many triticales have some degree of Al tolerance, but not as much as rye itself. Using wheat-rye addition lines, major genes for Al tolerance in rye were located

on chromosomes 3R, 4R and 6RS (Aniol and Gustafson 1984).

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). Aluminium 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; Sommers and Gustafson 1995; Riede and Anderson 1996). Several Al-sensitive mutants isolated in *Arabidopsis thaliana* segregated 3 : 1 (wild-type : sensitive), as expected for a single recessive mutation (Larsen et al. 1996).

The main objectives of the present work were to study the genetic control of Al tolerance in different cultivars and inbred lines of rye (*Secale cereale* L.) and to obtain some isozyme markers linked to the Al-tolerance genes.

Materials and methods

Plant material

The plant material consisted of two rye cultivars ("Ailés from Spain and "JNK" from Japan) of *S. cereale* L. and two inbred lines of rye ("Riodeva" and "Pool") with more than 30 generations of selfing. Five different F_1 offspring were obtained crossing the five most-tolerant plants of the "Ailés" cultivar (A1, A2, A3, A4 and A6) by the "Riodeva" inbred line (R). These F_1 offspring were named AR1–AR6, respectively. Four different backcrosses (BC) were obtained crossing four F_1 plants (AR1-5, AR1-7, AR1-11 and AR2-6) with the "Riodeva" inbred line. These four backcrosses were named AR1-5 × R, AR1-7 × R, AR1-11 × R and AR2-6 × R). Six different F_2 offspring were also obtained by selfing six F_1 plants (AR1-13, AR1-15, AR2-14, AR3-14, AR4-18 and AR6-17). These six F_2 families were named AR1-13 ⊗, AR1-15 ⊗, AR2-14 ⊗, AR3-14 ⊗, AR4-18 ⊗ and AR6-17 ⊗ (Fig. 1).

Aluminium-tolerance screening test

The Al-tolerance test was carried out using the nutrient-culture, modified-pulse method (Aniol 1984). Seeds were sterilised for 10 min with $HgCl_2$ (0.1%), well rinsed with water, and germinated overnight on filter paper in Petri dishes. Sprouted seeds were sown the next day on a polyethylene net fixed in Lucite frames. Styrofoam blocks were attached to the frames with rubber bands and floated on the surface of the vigorously aerated nutrient solution. Containers with the nutrient solution were placed in a water bath at 25°C under 16-h-per-day illumination. The nutrient solution consisted of: 0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2 \cdot 6H_2O$, 0.01 mM $(NH_4)SO_4$ and 0.04 mM NH_4NO_3. Four-day-old seedlings were incubated for 24 h with aluminium in the form of $AlKSO_4 \cdot 12H_2O$ at the concentration indicated in the experiment. After each exposure to Al, seedlings were removed from the Al-containing solution, thoroughly washed for 2–3 min in running tap water and transferred to Al-free medium for 48 h. Additional root growth after Al-shock was easily assessed by staining the root with a 0.1% aqueous solution of Eriochrome cyanine R for 10 min. After staining, the excess dye was removed by washing under tap water. In seedlings where the aluminium treatment did not destroy the root apical meristem, the root segment growing after Al-treatment was white (unstained), contrasting with the heavily stained root part exposed to aluminium. When the apical meristem was damaged, root tips did not show any re-growth after 48 h in Al-free medium, remaining intensively stained. During all stages of growth, and particularly during Al-treatment, the nutrient solution was maintained at pH 4.0.

Electrophoresis

Electrophoresis was performed in horizontal 12% starch gels, using the buffers and staining methods described by Brewer and Sing (1970). Chenicek and Hart (1987) and Figueiras et al. (1989). The following isozyme systems were used simultaneously over 12-day-old leaf extracts: esterase (EST), leucine, aminopeptidase (LAP), aconitase (ACO), acid phosphatase (ACPH), nicotinamide adenine dinucleotide dehydrogenase (NADH), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI) and peroxidase (PER).

Genetic mapping

Linkage analysis was performed on F_2 segregation data using the MAPMAKER 3.0 computer program (Lander et al. 1987). Genetic distances were calculated using the Kosambi function.

Results

The effect of Al on root growth in different cultivars and inbred lines of rye

The effect of Al on root growth at three different concentrations (50, 100 and 150 μM) in two rye cultivars

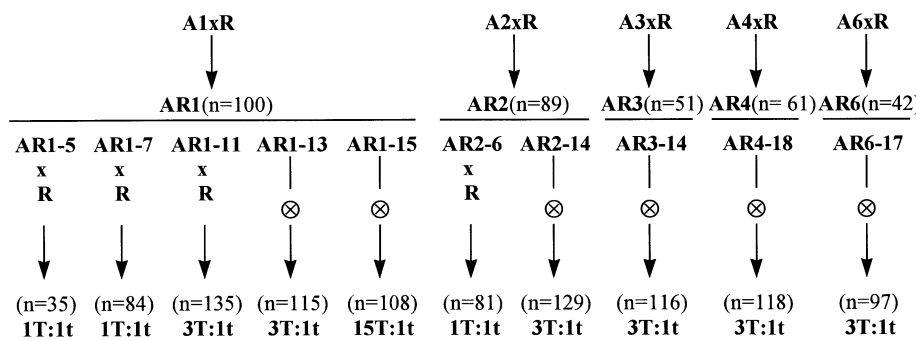

Fig. 1 Scheme of the crosses to analyse the genetic control of Al tolerance in rye. The observed segregation ratios for Al tolerance (tolerant : non-tolerant) in the F_2s and BCs is shown at the bottom of the figure. **A**: "Ailés. *R*: "Riodeva"

Table 1 Mean lengths of root re-growth (mm) at different Al concentrations. The number of plants of each cultivar analysed is indicated in brackets

Source	Al concentration							
	0 μM		50 μM		100 μM		150 μM	
	Mean (*n*)	%t[a]	Mean (*n*)	%t	Mean (*n*)	%t	Mean	(*n*) %t
Ailés	22.6 ± 8 (105)	0	20.8 ± 7.2 (86)	0	16.8 ± 8.2 (126)	0	14.6 ± 8.3 (108)	1.8
JNK	24 ± 7.5 (97)	0	14.9 ± 6.9 (90)	0	11.6 ± 6.9 (104)	0	6.8 ± 6.7 (105)	6.6
Pool	22 ± 7.9 (115)	0	17 ± 8 (100)	0	10.8 ± 7.6 (94)	3.1	7.6 ± 6.9 (103)	4.8
Riodev	19.3 ± 8.2 (98)	0	10.5 ± 6.7 (98)	2.2	6 ± 5.4 (109)	5	1.1 ± 3.1 (114)	28

[a] %t = percentage of plants without root re-growth

("Ailés" and "JNK") and two inbred lines of rye ("Riodeva" and "Pool") was studied. The main effect of Al in rye is to inhibit root growth in all the cultivars and inbred lines analysed. The comparative reduction of the mean length of root re-growth after exposure of the cultivars and inbred lines of rye to different Al concentrations indicates that both the cultivars ("Ailés" and "JNK") and the inbred lines ("Riodeva" and "Pool") have a high degree of Al tolerance, although, "Riodeva" is more sensitive to increasing Al concentrations and "Ailés" showed the highest resistance to Al (Table 1 and Fig. 2).

The Al concentration showing the highest discrimination power among the different rye cultivars analysed was 150 μM, because the mean length of root re-growth in the inbred line "Riodeva" at 150 μM was 1.1 mm and the maximum root re-growth observed in this line was 3 mm, whereas the mean length of root re-growth in "Ailés" at 150 μM was 14.6 mm. Therefore, at this concentration "Riodeva" is non-tolerant and "Ailés" is the most tolerant line.

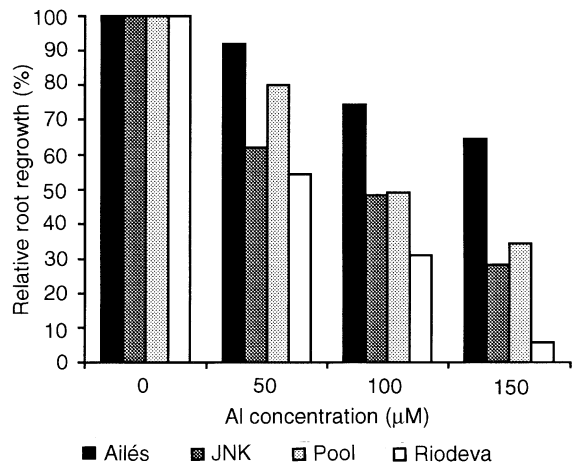

Fig. 2 Relative root re-growth of the four rye populations and at the different Al concentrations (50, 100, 150 μM) analysed, expressed as the percentage of root growth in the absence of aluminium

The genetic control of Al tolerance

The genetic control of Al tolerance was always carried out using a 150-μM Al concentration. It was studied in different F_1s, F_2s and BCs between plants of "Ailés" and "Riodeva".

The mean length of root re-growth of the five F_1s studied ranged between 21 and 17.6 mm (x = 19.4 mm) (Table 2), showing Al-tolerance levels similar to "Ailés", and in so case did individuals appear with a root re-growth less than 7 mm. On this basis, the plants of the F_2s and the BCs analysed 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 and 3 mm (the maximum re-growth found in "Riodeva").

Figure 3 shows the distribution of root re-growth length in two different F_2s and two BCs. The number of tolerant and non-tolerant plants and the segregation observed in the F_2s and BCs analysed are summarised in Table 2.

Linkage analyses between the Al-tolerance gene and several isozyme loci

In order to locate the Al-tolerance genes (*Alt*) and to obtain some isozyme markers linked to these genes, two different F_2s (AR1-13 ⊗ and AR6-17 ⊗) and two BCs (AR1-5 × R and AR1-7 × R) were studied. These crosses were selected because they segregated simultaneously for several isozyme loci and for the *Alt* genes. The remaining crosses did not segregate for the isozyme loci studied and therefore could not be used in linkage studies.

Table 2 Segregations of the aluminium-tolerance loci in the different F_1s, F_2s and BCs analysed (150 μM)

Cross		Tolerant ($\bar{x}_T$)[a]	Non-tolerant ($\bar{x}_t$)[b]	Segregation	χ^2
Ailés	A	108 (14.6)	–	–	–
Riodeva	R	114 (1.1)	–	–	–
AR1	F_1	100 (20)	–	–	–
AR2	F_1	89 (19.5)	–	–	–
AR3	F_1	51 (17.6)	–	–	–
AR4	F_1	61 (21)	–	–	–
AR6	F_1	42 (19.1)	–	–	–
AR1-11 × R	BC	103 (17.7)	32 (0.5)	3:1	0.12
AR1-13 ⊗	F_2	75 (18.4)	35 (1.3)	3:1	2.72
AR1-15 ⊗	F_2	98 (24.4)	10 (0.7)	15:1	1.66
AR1-5 × R	BC	15 (16.0)	20 (1.2)	1:1	0.71
AR1-7 × R	BC	51 (17.5)	32 (1.1)	1:1	4.34*
				9:7	0.91
AR2-14 ⊗	F_2	101 (18.7)	28 (1.2)	3:1	0.66
AR2-6 × R	BC	43 (14.5)	38 (0.7)	1:1	0.3
AR3-14 ⊗	F_2	88 (19.3)	28 (0.7)	3:1	0.04
AR4-6 × R	BC	63 (17.6)	2 (0)	–	–
AR4-18 ⊗	F_2	93 (18.1)	25 (0.8)	3:1	0.91
AR6-17 ⊗	F_2	73 (15.6)	24 (1.3)	3:1	0.006

[a] $\bar{x}_T$: mean length of root re-growth (mm) of tolerant plants (> 3 mm)
[b] $\bar{x}_t$: mean length of root re-growth (mm) of non-tolerant plants (⩽ 3 mm)
* P

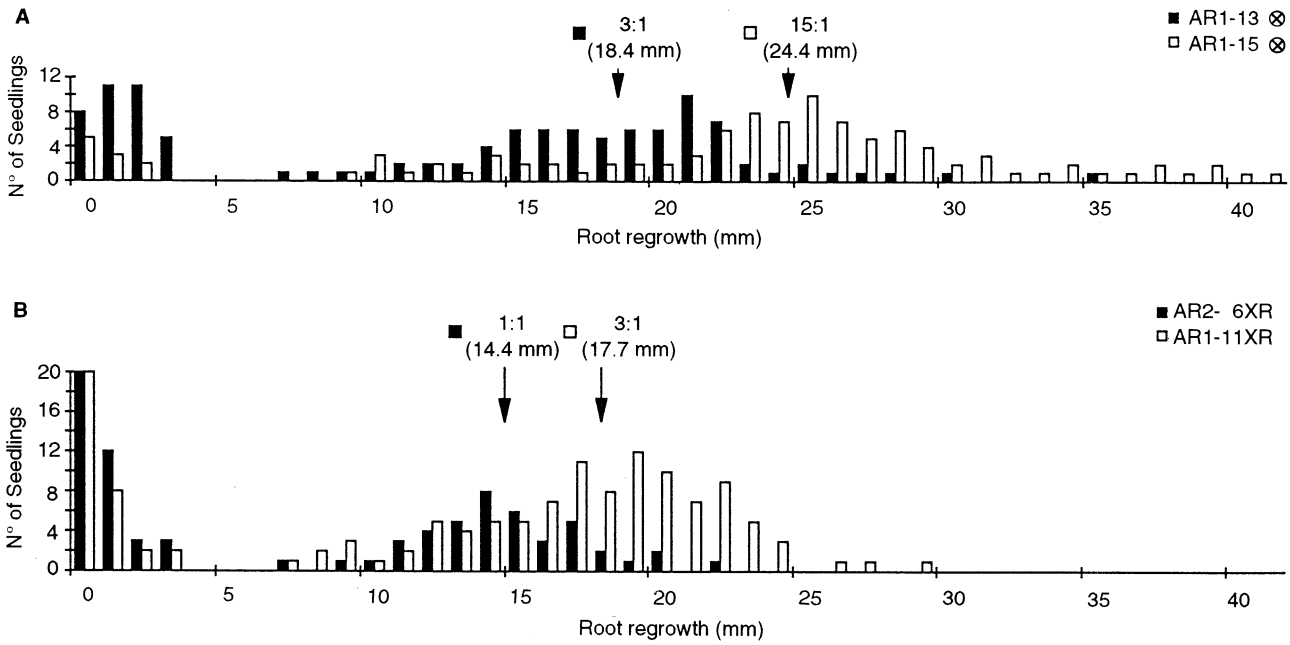

Fig. 3A, B Distribution of root length re-growth in individuals of four progenies, after treatment with Al (150 μM). **A** Two F_2s: AR1-13 ⊗ (3 tolerant : non-tolerant) and AR1-15 ⊗ (15T : 1t); **B** Two backcrosses: AR2-6 × R (1T : 1t) and AR1-11 × R (3T : 1t). *Arrows* show the means of root re-growth inside the groups of tolerant plants

The chromosomal location of the isozyme loci that segregated in each of the crosses studied is shown in Table 3. The existence of linkage between some isozyme loci and *Alt* genes was only detected in the AR6-17 ⊗ F_2. The two-point linkage studies and the genetic distances between *Alt* genes and the isozyme loci were obtained using MAPMAKER 3.0 (Table 4). The order deduced for the loci located on this chromosome is *Alt-Aco1-Ndh2-Est6-Est8* (Fig. 4).

Discussion

Rye has been described as the most aluminium-tolerant cereal crop (Aniol 1990). Our data are in agreement with previous reports based on hydroponic culture tests at similar aluminium concentrations. Aniol et al. (1980) classified different inbred lines into four groups:

Table 3 Chromosomal location of isozyme structural genes that segregated in the different F_2 and backcrosses analysed

Isozyme locus	Chromosome arm	References	Cross
Got3	3RL	Tang and Hart 1975	AR6-17 ⊗, AR1-5 × R and AR1-7 × R
Mdh2	3RL	Salinas and Benito 1985a	AR1-13 ⊗
Pgm1	4RS	Salinas and Benito 1985c	AR6-17 ⊗
Aco2	5RL	Chenicek and Hart 1987	AR1-13 ⊗, AR1-5 × R and AR1-7 × R
Aco1	6RL	Chenicek and Hart 1987	AR1-13 ⊗, AR6-17 × R and AR1-5 × R
Ndh2	6RL	Wehling 1991	AR6-17 ⊗
Est6	6RL	Salinas and Benito 1985b	AR6-17 ⊗
Est8	6RL	Salinas and Benito 1985b	AR6-17 ⊗
Lap1	6RS	Tang and Hart 1975	AR1-13 ⊗, AR1-5 × R and AR1-7 × R

Table 4 Two-point linkage analyses among the isozyme loci and the *Alt* genes considered in this study. The *Aco1* locus presents two active and co-dominant alleles (1 and 2); (11) and (22) are homozygous plants and (12) are heterozygous plants. The remaining loci present one active allele and one null allele; therefore is not possible to distinguish homozygote for the active allele from $^+$ heterozygotes

Loci	Distribution of progeny (phenotype)[a]						χ^2 Lig	Distance (cM)
	T, 11	t, 11	T, 12	t, 12	T, 22	t, 22		
Alt1, *Aco1*	8	16	43	6	22	2	31.17***	24.6
	+, +	+, −	−, +	−, −				
Alt1, *Ndh2*	58	15	11	13			9.94	46.4
Alt1, *Est6*	58	15	21	3			0.75	–
Alt1, *Est8*	53	20	15	9			2.19	–
	11, +	11, −	12, +	12, −	22, +	22, −		
Aco1, *Ndh2*	5	19	40	9	24	0	43.29***	16.8
Aco1, *Est6*	24	0	39	10	16	8	9.33**	42.4
Aco1, *Est8*	8	16	35	14	22	2	19.92***	42.5
	+, +	+, −	−, +	−, −				
Ndh2, *Est6*	52	17	27	1			5.83	26.1
Ndh2, *Est8*	57	12	8	20			26.29***	26.7
Est6, *Est8*	47	32	18	0			10.86***	0

[a] T = Al tolerant plants, t = Al non-tolerant plants

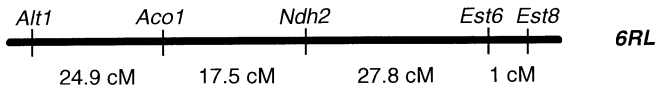

Fig. 4 Linkage map of chromosome 6R containing the *Alt1* locus. Map distances are in Morgans (cM)

(1) sensitive to 30 ppm of aluminium, (2) sensitive to 50 ppm, (3) sensitive to 70 ppm, and (4) tolerant to 70 ppm. Therefore, the inbred line "Pool" and the cultivars "JNK" and "Ailés" could be included in group IV (highly tolerant), whereas the inbred line "Riodeva" could be included in group III (medium tolerant).

The different F_1s analysed indicated that tolerance is dominant to non-tolerance. The observed segregation in F_2s and BCs confirmed the existence of at least two independent loci controlling Al tolerance in rye.

The 3 : 1 and 1 : 1 (tolerant : non-tolerant) segregations observed suggest that there is, at least, one dominant locus controlling Al tolerance in rye (Table 1). Moreover, we found 15 : 1 and 3 : 1 (tolerant : non-tolerant) segregations in one F_2 and one BC, respectively, that can be explained by the presence of two independent and dominant segregating loci. It should be noted that the mean length of root re-growth in these progenies with two Al-tolerance genes segregating simultaneously (AR1-11 × R and AR1-15 ⊗) has the highest values, suggesting an additive effect on this trait (Fig. 3).

Our results indicate that "Ailés" and "Riodeva" differ in two *Alt* genes, but, "Riodeva" showed a high degree of Al tolerance, because at 50- and 100-μM Al concentrations "Riodeva" showed a mean length of root re-growth of 10.5 and 6 mm, respectively. This strongly suggests the existence of additional *Alt* controlling Al tolerance in rye (Aniol et al. 1980; Aniol 1990).

Three Al-tolerance genes (*Alt1, Alt2* and *Alt3*) have been previously located, using wheat-rye addition lines, on chromosomes 6RS, 3R and 4R, respectively (Aniol and Gustafson 1984). Aniol and Madej (1996) studied the Al tolerance in different F_1 crosses, generated from Al-tolerant and non-tolerant inbred lines, showing segregations of 1 : 1 and 3 : 1. Their data indicate that these inbred lines are not homozygous for the *Alt* genes and support the hypothesis of the existence of at least two Al-tolerance loci in rye, which is in agreement with our results.

The 3 : 1 (tolerant : non-tolerant) segregation was previously observed in different F_2s between tolerant and

sensitive wheat cultivars (Delahize et al. 1993; Somers and Gustafson 1995; Riede and Anderson 1996). Larsen et al. (1996) described several Al-sensitive mutants in *A. thaliana*, belonging to eight different complementation groups, that also showed a 3 : 1 segregation in F_2 populations. These results indicate that in these species too there are several genes involved in Al tolerance.

The 9 : 7 segregation ratio observed in one backcross (AR1-7 × R) might be attributed to the presence of a gene that suppress Al tolerance in rye, in the same way as that located on chromosome arm 6BS in wheat (Aniol 1990). Nevertheless, this could also be explained by a significant statistical deviation from a 1 : 1 segregation due to unknown factors. More F_2s showing this segregation are needed to assess the existence of this suppressor gene.

Molecular markers linked to *Alt* genes have not been described in rye to-date. In the present study we have obtained two isozyme loci linked to the rye *Alt1* gene on chromosome 6R. Two RFLPs linked to the wheat Alt_{BH} gene located on chromosome arm 4DL (Riede and Anderson 1996), and two microsatellite marekrs linked to the *als1-1* and *als4* genes (Al-sensitive mutants) located on chromosome 5 of *A. thaliana* (Larsen et al. 1996), have also been reported.

The existence of linkage between isozyme loci and *Alt* genes was only detected in the AR6-17 ⊗ F_2. In this cross the loci *Aco1* and *Ndh2* were linked to the *Alt* locus. Both isozyme loci were previously located and mapped on the long arm chromosome 6R (Benito et al. 1991; Wehling 1991). The *Est6* and *Est8* loci are also located in the same chromosome arm (6RL) but they behave independent of the *Alt* locus, because they are well separated from it on the same chromosome. These results indicate that the *Alt* locus segregating in this cross is located on chromosome 6R. This gene is probably the same as that located by Aniol and Gustafson (1984), using wheat-rye addition lines, on chromosome arm 6RS (*Alt1*); for this reason we have called this locus *Alt1*. The genetic map shown in Fig. 4 was obtained using the multipoint analyses of MAPMAKER 3.0; for this reason the genetic distances indicated in the map are slightly different from the two-point genetic distances presented in Table 4.

The *Lap1* locus was located on chromosome arm 6RS, but it does not segregate in this cross (AR6-17 ⊗). However, *Lap1* does segregate in the AR1-13 ⊗, AR1-5 × R and AR1-7 × R crosses, showing independent behaviour with the *Alt* loci segregating in these crosses. The comparison between the map obtained in this work for chromosome 6R and the maps previously obtained using the same isozyme markers (Table 5) suggests that *Alt1* locus is probably located near to the *Lap1* locus. Therefore, the *Alt* loci that are segregating in the AR1-13 ⊗, AR1-5 × R and AR1-7 × R crosses are not located on chromosome 6R. Moreover, in the AR1-13 ⊗ and AR1-5 × R crosses, the *Aco1* locus is also segregating and showed an independent behaviour to the *Alt* loci. Therefore, these loci are not located on chromosome 6R. Probably, these *Alt* loci could be located on chromosomes 3R and 4R, because the presence of *Alt* genes on these chromosomes have been reported using wheat-rye addition lines (Aniol and Gustafson 1984). The isozyme loci *Got3* and *Mdh2* located on chromosome arm 3RL and the *Pgm1* locus situated on chromosome arm 4RS were independent of the *Alt* loci studied in this work, or else were not segregating simultaneously with them. We have preliminary unpublished data (AR1-13 ⊗ cross) suggesting that the *Alt* locus segregating in this progeny is located on chromosome 4R. This gene could probably be the same as that located by Aniol and Gustafson (1984), using wheat-rye addition lines, on chromosome arm 4R (*Alt3*); for this reason we have named this locus *Alt3*. *Alt* genes were located in the long arm of wheat chromosomes 4A and 4D (Polle et al. 1978; Takagi et al. 1983; Aniol and Gustafson 1984; Riede and Anderson 1996) and in the barley chromosome 4H (Reid 1970; Stolen and Anderson 1978). The existence of *Alt* genes located on the chromosomes of homoeologous group 4 suggests the occurrence of a common mechanism of tolerance in Triticeae species.

The expression of genes from rye controlling tolerance to aluminium tends to be reduced when they are present in a wheat background (Aniol and Gustafson 1984; Aniol 1986). The highly tolerant rye populations we have analysed also have a certain number of Al-sensitive individuals. Therefore, some of the existing triticales could have been obtained using Al-sensitive rye plants. For these reasons, the identification of genetic markers linked to the *Alt1* and *Alt3* rye genes would be of great interest in breeding programs, both in respect of the selection of plants with a high number of Al-tolerance alleles (i.e. homozygous plants in F_2s with

Table 5 Comparison between the genetic distances (cM) obtained using isozyme markers located on chromosome 6R in this work (*) and another previously described

Loci	Distance (cM)	References
Aco1-Ndh2	16.8	*
	16 ± 3.8	Wehling 1991
	16	Philipp et al. 1994
Aco1-Est6	42.4	*
	30.4 ± 4.1	Benito et al. 1991
Aco1-Est8	42.5	*
	29.6 + 4.1	Benito et al. 1991
	33 ± 3.7	Wehling 1991
Ndh2-Est6	26.1	*
Ndh2-Est8	26.7	*
	22 ± 1.6	Wehling 1991
	22 ± 1.6	Wricke 1991
	22	Philipp et al. 1994
Est6-Est8	0	*
	0.5 ± 0.5	Benito et al. 1991
Lap1-Aco1	33.66 ± 3.7	Benito et al. 1991
	41.6 ± 5.6	Benito et al. 1996

segregations 15 : 1) and their maintenance during the breeding processes.

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