ALUMINUM TOLERANCE IN BARLEY: MOLECULAR MAPPING ANALYSES

TOLERÂNCIA AO ALUMÍNIO EM CEVADA: ANÁLISES DE MAPEAMENTO MOLECULAR

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ABSTRACT

Southern Brazil is characterized by acid soils associated to aluminum (Al), and this condition represents a significant restriction to crop production (ANIOL, 1990; BENNET & BREEN, 1991). The initial and most dramatic symptom of Al toxicity is inhibition of root growth, and the reduction in its growth is detectable within minutes after Al addition (JONES & KOCHIAN, 1995; DEGENHARDT et al., 1998). The damage caused in the root system may lead to both nutrient deficiencies and water stress.

The literature contains frequent reports about the basis of Al tolerance in plants and some hypothesis have been proposed. For example, some species accumulate high concentrations of Al and must possess effective mechanisms for detoxifying the Al internally. This can be related to Al complexation by organic ligands, pH effects to reduce the concentration of Al³⁺, compartmentation into vacuole, exudation of various compounds, and development of Al-resistant proteins (TAYLOR, 1991). Another Al tolerance mechanism involves exclusion of Al from the root apex, which may be attained either by the release of Al-chelating ligands or root-induced increases in rhizosphere pH (PELLET et al., 1994; RYAN et al., 1995; TAYLOR, 1988).

Conventional liming or other soil management practices are frequently inefficient for avoiding Al phytotoxicity (RAO et al., 1993). The lime application to the soil is ineffective in the subsoil and deep lime incorporation is technically difficult and expensive. For these reasons, development of cultivars adapted to acid soil is a promising alternative and has been the most effective strategy for crop production in this kind of soil.

The genetics of Al tolerance has been studied in several important crop plants. According to the species, Al tolerance can be monogenic or multigenic. In wheat (Triticum aestivum L. Thell), ANIOL (1990) found that Al tolerance is a complex character, controlled by several major genes, minor modifying genes, and probably by genes controlling suppression of Al tolerance genes; although DELHAIZE et al. (1993) have showed that it is controlled by a single locus. In rye (Secale cereale L.) and maize (Zea mays L.), two major dominant and independent loci controlling the Al tolerance were found (GALLEGO & BENITO, 1997; SIBOV et al., 1999). In rice (Oryza sativa L.), Al tolerance is a multigenic trait (KATIWADA et al., 1996), and in soybean (Glycine max (L.) Merril), it was found to be associated with quantitative trait loci (BIANCHI-HALL et al., 2000). In barley (Hordeum vulgare ssp. vulgare L.), MINELLA & SORRELLS (1992) indicated that the Al tolerance is monogenic with expression of tolerance dependent on Al concentration and allele dose. ECHART & CAVALLI-MOLINA (2002) and RAMAN et al. (2002) had also observed monogenic inheritance although REID (1971) had suggested, in addition to the major gene, the presence of minor gene effects interfering in the Al tolerance.

Advances in DNA marker technology have added a new dimension to the study of genetic traits since the past decade, offering hope that marker technology can clarify the genetics of Al tolerance and aid practical breeding (BIANCHI-HALL et al., 2000). The breeder can use markers for marker-assisted selection (MAS). In addition, genetic information and molecular markers obtained for a trait in one species may be exploited in related species, giving more precise information about a trait through comparative mapping. Although Al tolerance is important for growing barley in many parts of the world, the...
gene localization of this trait was not investigated until recently. TANG et al. (2000) reported three AFLP markers tightly linked to the Alp gene (Al tolerance gene) in the cultivar ‘Dayton’. Xbcd1117, Xwg464, and Xcdo1395, and RAMAN et al. (2002) described four microsatellite markers (Bmac310, Bmag353, HVM68, and HVMCABG) tightly linked to this gene in the cultivar ‘Yambla’.

The objective of the present work was to obtain data about the location of major Al tolerance genes in barley analyzing a F2 population obtained from a cross involving a Brazilian Al tolerant (‘FM-404’) cultivar.

MATERIAL AND METHODS

Genetic material: Cultivars ‘FM-404’ and ‘Harrington’ were crossed to obtain a segregating F2 population. ‘FM-404’ is obtained from individual F2 plants selfed, were also analyzed. University, Ithaca, NY, USA, in 2000.

Evaluation in the parents and F2 generation. To confirm the gene localization of this trait was not investigated until recently. TANG et al. (2000) reported three AFLP markers tightly linked to this gene in the cultivar ‘Dayton’. Xbcd1117, Xwg464, and Xcdo1395, and RAMAN et al. (2002) described four microsatellite markers (Bmac310, Bmag353, HVM68, and HVMCABG) tightly linked to this gene in the cultivar ‘Yambla’.

The objective of the present work was to obtain data about the location of major Al tolerance genes in barley analyzing a F2 population obtained from a cross involving a Brazilian Al tolerant (‘FM-404’) cultivar.

Aluminum tolerance screening: Al response was evaluated in the parents and F2 generation. To confirm the genotype of F2 plants, 12 individuals from each F2 progeny, obtained from individual F2 plants selfed, were also analyzed. Al tolerance analysis was assayed by the hematoxylin staining technique as previously described by POLLE et al. (1978) with some modifications. Disinfected seeds were germinated in Petri dishes and 24 hours later, the seedlings were placed in hydroponic solution following the protocol described by TANG et al. (2000) with 30 µM AICl3 that best distinguished the elite Al tolerant varieties. Disinfected seeds were germinated in Petri dishes and 24 hours later, the seedlings were placed in hydroponic solution following the protocol described by TANG et al. (2000) with 30 µM AICl3 that best distinguished the elite Al tolerant varieties. Disinfected seeds were germinated in Petri dishes and 24 hours later, the seedlings were placed in hydroponic solution following the protocol described by TANG et al. (2000) with 30 µM AICl3 that best distinguished the elite Al tolerant varieties. Disinfected seeds were germinated in Petri dishes and 24 hours later, the seedlings were placed in hydroponic solution following the protocol described by TANG et al. (2000) with 30 µM AICl3 that best distinguished the elite Al tolerant varieties. Disinfected seeds were germinated in Petri dishes and 24 hours later, the seedlings were placed in hydroponic solution following the protocol described by TANG et al. (2000) with 30 µM AICl3 that best distinguished the elite Al tolerant varieties.

DNA extraction: DNA was extracted from leaf tissue of parents and F2 progeny according to the method described by REIDE & ANDERSON (1996).

Microsatellites (SSRs) analysis: Thirty-seven primer pairs (LIU et al., 1996) were used to analyze microsatellite sequences placed in the seven chromosomes of barley (Table 1). Polymerase chain reaction (PCR) amplifications were performed in a thermal cycler (Perkin-Elmer Corporation, model 480, Norwalk, CT, USA). The reaction mixture was that described by SAGHAI-MAROOF et al. (1994) except that the mixture contained 200 µM each deoxynucleotide without radiolabel. Depending on the primer pair used, amplification of the SSRs was performed using different conditions: the conditions 1, 2, and 3 were as described by LIU et al. (1996), while the conditions 4 and 5 were as described by BECKER & HEUN (1995) and RÖDER et al. (1995), respectively (Table 1). PCR products were denatured, separated by 6% denaturing polyacrylamide (sequencing) gels containing 8% urea at 60°C constant current and revealed using silver staining.

AFLP analysis: The AFLP Analysis System I Kit (GIBCO BRL) was used for AFLP analysis following the manufacturer’s recommendations. EcoRI and MseI endonucleases were used to generate the restriction fragments. The pre-amplification was performed with two AFLP primers, each having one selective nucleotide, and the selective amplification with two AFLP primers, each containing three selective nucleotides. The EcoRI primers were end-labelled with 32P using T4 polynucleotide kinase. One hundred and forty four combinations (8 EcoRI x 18 MseI primers) were used for the analysis. Products from selective amplification were separated on 6% denaturing polyacrylamide (sequencing) gel. After drying, gels were exposed to X-ray film.

RFLP analysis: Ten restriction enzymes (BamHI, Drai, EcoRI, EcoRV, HaeIII, HindIII, PstI, SstI, Xbal, and Xhol) were used to digest DNA samples and survey filters were hybridized with 24 wheat, barley and oat (Avena sativa L.) genomic and cDNA clones (Xbcd1117, Xcdo1395, Xzr517, Xwg464, Xabc321, Xbcd1092, Xagb394, Xcdo795, Xcdo541, Xabc464, Xcdo586, Xwgg114, Xcdo669, Xcdo465, Xbcd15, Xwg181, Xcdo1312, Xcdo38, Xmwg542, Xbcd1230, Xagb394, Xmwg634, Xcdo650, and Xbcd1092) that have been mapped to barley chromosome 4 (GRANER et al., 1991; HEUN et al., 1991; KLEINHOF’S et al., 1993, TANG et al., 2000). This chromosome was more intensively investigate because there was previous indication of presence of at least one barley Al tolerance gene. The filters were sequentially washed with procedures previously described by TANG et al. (2000) and then were placed against X-ray film to obtain autoradiographs.

Molecular polymorphism analysis: The polymorphism screening was performed in the parental cultivars for RFLP and SSR markers. For the AFLP technique, the BSA method (MICHELMORE et al., 1991), with two bulks constructed from extreme phenotypes of the F2 population was used, aiming to reduce false positive polymorphisms between parents. Eighty-four plants of F2 generation were analyzed individually with the polymorphic AFLP, SSR, and AFLP markers.

Linkage analysis: Linkage analysis was conducted with the MAPMAKER version 3.0 (LANDER et al., 1987), using Kosambi mapping function, a LOD score of 5.0 and a recombination fraction of 0.30.
Table 1 - Primer sequences, PCR conditions, chromosomal location of barley SSRs, and polymorphism occurrence.

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*PCR conditions: 1, 2, and 3 (LIU et al., 1996); 4 (BECKER & HEUN, 1995); 5 (RÖDER et al., 1995)

RESULTS AND DISCUSSION

To identify the Al tolerant and sensitive genotypes, the hematoxylin staining procedure was used. The technique developed by POLLE et al. (1978) consists of a visual estimation of the stain ability of the root tips of young plants by hematoxylin following growth in the presence of Al. According to the authors, the test was developed based on the evidence that Al enters primarily at near the root tip. Tolerant genotypes tend to accumulate less Al in their roots than sensitive ones, and hematoxylin produces permanent staining upon reaction with Al in the root cells (WRIGHT & DONAHUE, 1953; VOSE & RANDALL, 1962; FOY et al., 1967; HENNING, 1975). The method has given results consistent with those based on root growth measurements (CARVER et al., 1988; POLLE et al., 1978), and has been used in genetic studies, breeding programs, and mapping studies in cereals (TAKAGI et al., 1983; SCOTT & FISHER, 1986; LARKIN, 1987; CARVER et al., 1988; MINELLA & SORRELLS, 1992, 1997; SIBOV et al., 1999; TANG et al., 2000; ECHART & CAVALLI-MOLINA, 2002). This method was chosen because it is simple, accurate and non-destructive and the seedlings can grow to maturity.

The hematoxylin stain procedure allowed classifying the barley plants analyzed into three groups in relation to Al tolerance, according to previous nomenclature described by MINELLA & SORRELLS (1992): Al sensitive plants as C (completely stained), equal to ‘Harrington’; Al tolerant plant as N (non stained), such as ‘FM-404’; and those with an intermediate phenotype similar to ‘FM-404’ but with a fainter staining in the root apical region as P (partially stained). This classification was confirmed by analysis of the descendants from each F$_2$ plant, since no segregation was observed in progenies from F$_2$ plants classified as homozygotes for tolerance (non stained) or sensitivity (completely stained), while 1:2:1 (N:P:C) segregation was observed in plants previously considered heterozygotes (partially stained). This classification was confirmed by analysis of the descendants from each F$_2$ plant, since no segregation was observed in progenies from F$_2$ plants classified as homozygotes for tolerance (non stained) or sensitivity (completely stained), while 1:2:1 (N:P:C) segregation was observed in plants previously considered heterozygotes (partially stained). Among 84 F$_2$ plants analyzed, 24 could be identified as N, 18 as C, and 42 plants were classified as P. The segregation 1:2:1 (N:P:C) observed in F$_2$ ($\chi^2 = 0.43$, $P= 0.513$) indicated the presence of a single gene for Al tolerance segregating in this population. Monogenic inheritance for Al tolerance has already been reported by MINELLA & SORRELLS (1992), TANG et al.
Polymorphism, where only five primer combinations were between the parents. The AFLP analysis also detected a low polymorphism, where only five primer combinations were suitable for segregation analysis in the mapping population (E-AAC/M-CCA, E-AGC/M-CCT, E-AAG/M-CGT, E-ACC/M-CAG, and E-ACA/M-ACT).

Many factors are extremely important in map construction. The genetic distance between parental cultivars has direct effects on the ability to detect polymorphic markers which differentiate them. Although many studies have shown that molecular markers, including microsatellites (BECKER & HEUN, 1995; BIANCHI-HALL et al., 2000), AFLPs (YIN et al., 1999; PIERRE et al., 2000), and RFLPs (GRANER & BAUER, 1993; LEISTER et al., 1999), are efficient for mapping genes, the cultivars ‘Harrington’ and ‘FM-404’ exhibited a low level of inter-cultivar molecular differentiation. The low level of polymorphism observed made more difficult to find molecular markers linked to barley Al tolerance gene. However, a common genetic origin of these cultivars cannot be the explanation for this similarity. ‘FM-404’ is a Brazilian cultivar described as originated from selection from ‘Alpha’, which is derived from the cross ‘Manchuria’/‘Champion of Vermont’ (ARIAS et al., 1983), while ‘Harrington’ is a Canadian cultivar originated from crosses of ‘Klages’/3/Gazelle/’Betzes’/4/Centennial’. Relatively low differentiation between barley lines has been previously reported (GRANER et al., 1991; HEUN et al., 1991; SAGHAI-MAROOF et al., 1994) and has made the development of barley maps fairly laborious.

The 20 polymorphic loci data set evaluated did not allow the construction of a linkage map. Only one short linkage group belonging to chromosome 4 was detected (Figure 1). The co-dominant RFLP marker Xwg464, located on the long arm of chromosome 4H (KLEINHOFS et al., 1993), showed linkage to Al tolerance gene with 21.6 centimorgans (cM) of distance (LOD score 5.0). The microsatellite HVM68, mapped in the same chromosome 4 of barley (LIU et al., 1996), was approximately 21.9 cM from marker Xwg464; in other words, more than 40 cM from Al tolerance gene. Those two markers segregated in 1:2:1 ratio in the F2 population which is in agreement with the segregation of co-dominant loci (Xwg464 - \( \chi^2 = 0.43, P = 0.513 \); HVM68 - \( \chi^2 = 0.79, P = 0.375 \)). No other markers showed linkage to each other or to Al tolerance gene.

Al tolerance was found to be controlled by a single gene and mapped on the long arm of the chromosome 4H, located 21.6 centimorgans from the RFLP marker Xwg464 in the Brazilian barley cultivar ‘FM-404’. The marker Xwg464 had already been found linked to Al tolerance gene in the cultivar ‘Dayton’, but distant 2.1 cM from that. It is possible to presume that the Al tolerance gene in ‘FM-404’ is the gene Alp of ‘Dayton’ based on MINELLA & SORRELLS (1992) results, which showed no segregation for this trait in a cross between ‘FM-404’ and ‘Dayton’ (both Al tolerant cultivars). Differences in the map positions of low and multitypopy probes obtained in other mapping populations have occasionally been reported (BEAVIS & GRANT, 1991; SHERMAN et al., 1995). SCHÖNFELD et al. (1996), mapping RFLPs for resistance genes to powdery mildew in barley, found seven low-copy clones mapped in positions that differed from previously published barley RFLP maps. They suggested that the low-copy characteristic of these probes together with the differences in linkage between them and the target gene are indicating that these RFLP loci may have originated from independent duplication events. Nevertheless, duplication of the RFLP Xwg464 marker cannot be the explanation for the difference observed for the distance between the locus and the Al tolerance loci in the cultivars ‘Dayton’ and ‘FM-404’, because this RFLP marker is a single copy clone.

Other facts that may explain the different distances found in the present analysis and in the research of TANG et al. (2000) would be the occurrence of simple or double crossing-over in different frequencies or the occurrence of chromosomal rearrangements. The occurrence of double crossing-over between the two target genes would underestimate the distance observed between these genes because it would reduce the number of recombinant individuals. However, due to the large difference observed is difficult to affirm that double crossing-over can explain this result. Otherwise, the deletion of a chromosomal segment between these two genes would shorten the distance between them, and the addition of a segment would enlarge the distance, which could explain the difference observed between the distance of the barley Al tolerance gene and the molecular marker.

Due to the distance between the Al tolerance gene and the molecular marker Xwg464, this marker is not appropriated to be used in breeding programs that aim the selection of Al tolerance. Therefore, other crosses with Brazilian cultivars and other molecular markers must be investigated to find a marker tightly linked to Al tolerance gene in Brazilian barley genotypes.

CONCLUSIONS

Al tolerance was found to be controlled by a single gene and mapped on the long arm of the chromosome 4H, located 21.6 centimorgans from the RFLP marker Xwg464 in the Brazilian barley cultivar ‘FM-404’.

REFERENCES


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