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"Molecular mapping of quantitative trait loci (QTL) controlling aluminium tolerance in wheat and barley"

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Abbreviations

AC lines	alfredo chaves lines
AFLPs	amplified fragment length polymorphisms
Al	aluminium
ALF	automated laser fluorescence express DNA sequencer
ALMT1	aluminium activated malate transporter1
Alt	aluminium tolerance
AtALMT1	*Arabidopsis thaliana* ALMT1
BCC	barley core collection
BLAST	basic local alignment search tool
CAPS	cleaved amplified polymorphic sequence
CIAT	International Center for Tropical Agriculture
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo (International maize and wheat improvement center)
cM	centimorgan
CNPMS	Centro Nacional de Pesquisa de Milho e Sorgho (Brazilian national maize and sorghum research center)
CR-EST	crop EST database
CRIS	Current research information system
CSREES	Co-operative state research, education, and extension service
DH	doubled haploid
DNA	deoxyribo nucleic acid
dNTPs	deoxynucleotide triphosphates
EDTA	ethylene diamine tetraacetic acid
ESTs	expressed sequence tags
F_2	second filial generation
Indels	insertion-deletions
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung
ITMI	International Triticeae Mapping Initiative
MAS	marker assisted selection
µl	microlitre

mM	millimolar
Mn	manganese
mW	milliwatt
$NaIO_3$	sodium iodate
NaOCl	sodium hypochlorite
NCBI	National center for biotechnology information
NILs	near isogenic lines
NT	nulli-tetrasomic
OWB	oregon wolfe barley
PCR	polymerase chain reaction
PIC	polymorphic information content
PV	phenotypic variance
QTL	quantitative trait locus
RAPDs	random amplified polymorphic DNA
RFLPs	restriction fragment length polymorphisms
RILs	recombinant inbred lines
RLA	root length in aluminium
RLC	root length in control
RNA	ribo nucleic acid
RTI	root tolerance index
sec	second(s)
SIM	simple interval mapping
SM	single marker analysis
SNPs	single nucleotide polymorphisms
SSRs	simple sequence repeats
STOP1	sensitive to proton rhizotoxicity 1
STSs	sequence tagged sites
USDA	United States Department of Agriculture
USD	US Dollars
V	volt
W	Watt

1 Introduction

Abiotic stress encompasses a wide range of threats to plant health including nutrient deficiencies, nutrient and non-nutrient toxicities, drought, temperature and salinity stresses (http://www.ees.adelaide.edu.au/research/enviro/physiology/abiotic). Physical factors and their interactions are important in determining performance and distribution of plants. In agricultural systems, even if conditions tend to be optimized, the effect of abiotic stresses deriving from changes in the physico-chemical environment is evident at the quantitative and qualitative level. Measures applied to improve plant production and mitigate the consequences of abiotic stresses on production imply chemical and energetical inputs which can have a detrimental effect on the environment (amendants, fertilizers, pesiticides, and irrigation). Among the diverse abiotic stresses, metal toxicity, especially aluminium toxicity takes a major share which affects crop production on a large scale.

Aluminium (Al) is the third most abundant element after oxygen and silicon and the most abundant metal on the earth's crust. Most Al exists as oxides and alumino-silicates and these forms of Al are harmless to plants. However, on acid soil, when the soil pH is below 5.5, Al is solubilised into the soil solution and exists in the form of trivalent cations, Al^{3+}. The chemistry of Al in solution is complicated because Al hydrolyses in a pH dependent manner to form various complexes with hydroxyl groups. Although the toxicity of these soluble Al species varies considerably, Al^{3+} is regarded as the greatest stress to plants. At micromolar concentrations, Al^{3+} can inhibit root growth within minutes or hours. The subsequent effect on water and nutrient uptake results in poor growth and productivity (Ma and Hiradate 2000). Al injured roots become stubby and frequently acquires a brownish colouration. Fine branching and development of root hairs are greatly reduced and the root system often takes on a "coralloid" appearance (Ciamporova 2002).

Most of the cereal crops are sensitive to even low concentrations of Al. Although the poor fertility of the acid soils is due to a combination of mineral toxicities (Aluminium

and Manganese) and deficiencies (Phosphorus, Calcium, Magnesium and Molybdenum), Al toxicity is the single most important factor which constraints crop production on 67% of the total acid soil area (Eswaran et al. 1997). Soil acidity is determined by the amount of hydrogen ion (H^+) activity in soil solution and is influenced by edaphic, climatic, and biological factors. Soils that develop from granite parent materials acidify at a faster rate than soils developed from calcareous parent materials. Sandy soils with relatively few clay particles acidify more rapidly due to their smaller reservoir of alkaline cations and higher leaching potential. High rainfall affects the rate of soil acidification depending on the rate of water percolation through the soil profile. The problem is intensified by acid precipitation from polluted air (Ulrich et al. 1980). Soil acidification is often accelerated by cropping practices such as repeated applications of nitrogen in amounts that exceeds crop uptake (Adams 1984).

Approximately 40% of the arable soils of the world are estimated to be acidic and therefore present Al toxicity hazards. It occurs mainly in two global belts: the Northern temperate belt, dominated by Spodosols, Alfisols, Inceptisols and Histosols where as the Southern tropical belt contains predominantly Ultisols and Oxisols. These soil types are different variations of acid soils with different composition depending on the geographical area where it is present. In developed countries, with the onset of modern agriculture, increased application of lime, phosphate and other nutrients helped to raise the soil pH to 6.0 turning acid soils to highly productive lands (Von Uexkull and Mutert 1995). The worst revealing fact is that most of the acidic Al toxic areas are located in developing and underdeveloped countries in South America, South-East Asia and Central Africa (Wood et al. 2000) where food production is critical. In Brazil alone, over 500 million hectares are covered by acidic soils, comprising roughly two-thirds of its total territory. The Cerrado in Brazil, the Llanos of Venezuela and Columbia, the savannas of Africa and the largely anthropic savannas of tropical Asia, encompass large areas of degraded, sparsely covered or even denuded acid soils which are potentially arable. While acid soils in the northern belt are increasingly protected and re-afforested, the destructive exploitation of timber and abusive modern shifting cultivation have contributed to the loss of > 250 million hectars of tropical forest during the second half

of this century leaving vast areas of anthropic savannas on heavily eroded and degraded acid soils (von Uexküll and Mutert 1995).

Figure 1. Global extent of acid soils - Areas of predominance.

Soil acidity is generally a natural occurrence in tropical and subtropical areas which accounts for 60% of acid soils in the world, but in temperate zones, currently, it is an increasing problem and is largely the result of acid rain in the industrial regions of the USA, Canada and Europe. In United States, for instance, high-input farming practices such as extensive use of ammonium based fertilizers are causing further acidification of agricultural soils, creating new acid soils from previously neutral ones (Jackson and Reisenauer 1984). In Australia, about 90 million hectares of agricultural land can be potentially affected and the annual economic loss is estimated as more than 600 million USD. In Poland, over 60% of arable soils are acidic (Boguszewski 1980). Although soil amendments such as lime can ameliorate soil acidity, and hence Al toxicity, this is neither an economic option for poor farmers nor an effective strategy for alleviating subsoil acidity (Rao et al. 1993). Liming is often not practical because of the slow movement of

lime especially in the deeper layers of highly acidic subsoils (Mugwira et al. 1976). Furthermore, heavy application of lime may have adverse effects on some crops in the rotation or cause deficiencies of certain nutrients (Whitten 1997). Developing Al tolerant cultivars is the most fruitful eco-friendly strategy which gains much attention to stabilise the food security in the marginal areas of the world. The vast increase in human population growth creates a pressure to expand crop production into acid Al toxic soils. Opening the Brazilian Cerrados and similar areas in Latin America, Central Africa and Southeast Asia could contribute greatly to raise the world food production in the future.

Several efforts were undertaken all over the world and are in progress to enhance Al tolerance in many of the important food crops. Brazil can claim one of the major successes in wheat breeding research in the 20th century - transforming wheat production in its acid Al toxic soil. In the 1970s, Brazil and CIMMYT (International Maize and Wheat Improvement Centre) initiated a breeding program to combine Brazilian wheats Al tolerance with the yielding ability of CIMMYT's semi-dwarf wheats. Brazil released at least 22 wheat cultivars developed through this program. By 1990, semi-dwarf wheats covered 63% of Brazil wheat area. These varieties yielded 25% more than older cultivars in Rio Grande do Sul, the most acidic region in Southern Brazil. Collaboration between Brazil's Centro Nacional de Pesquisa de Milho e Sorgho (CNPMS) and CIMMYT's maize program focused on germplasm exchange and improvement, especially, work on Al tolerance received much attention (http://www.cimmyt.org).

In the United States, genetic improvement of tolerance to soil acidity has taken on high priority in breeding programs for several major field crops. The USDA-CSREES and USDA-ARS supported research programs in the Current Research Information System (CRIS) specifically identify acid soil tolerance as a breeding objective. The International Center for Tropical Agriculture (CIAT) combines genomic approaches, plant breeding, and physiology to understand and exploit underlying genetic mechanisms of abiotic stress adaptation for crop improvement (Ishitani et al. 2004). Their research on Al tolerance in tropical grasses illustrates the importance of Al toxicity as an important stress factor. The Australian Barley Molecular Genetics Program which is part of Australian Winter Cereal

Molecular Marker Program and for Molecular Plant Breeding aims at implementing and validating marker trait combinations for acid and Al tolerance (http://www.agric.wa.gov).

Because of its obvious importance, understanding the genetic architecture and physiology behind Al tolerance is highly desirable and has been the focus of intense research over the past several decades. The genetics of Al tolerance were studied in several important cereal crop plants. Accordingly, it is monogenic in some species but polygenic in others. Some plant species and even cultivars within certain species have evolved mechanisms that minimise the harmful effects of Al ions (Kochian et al. 2004; Matsumoto 2000) and the nature of these mechanisms has attracted considerable interest. An exclusion mechanism based on root exudation of Al-chelating organic acids such as malate, citrate, or oxalate has been described in both monocots (Delhaize et al. 1993b; Fontecha et al. 2007) and dicots (Hoekenga et al. 2003; Silva et al. 2001). The organic acid is released into the rhizosphere, where it acts as a ligand for Al^{3+} (the primary toxic Al species), forming a non-toxic complex that does not readily enter the root. It has been shown that Al-activated root malate release co-segregates with the Al tolerance locus in wheat, directly linking the genetic and physiological studies (Delhaize et al. 1993a, 1993b). But there are other instances where the plants detoxify Al internally by forming complexes with organic acids (Ma et al. 2001).

The vast array of molecular tools such as different kinds of molecular/DNA markers, linkage maps, mapping populations and statistical tools enable us to understand the genetics of tolerance to abiotic stresses like Al toxicity and facilitate the study of evolutionary aspects of the genes involved in many of our important crop species. Genetic linkage maps are useful for understanding the genome organisation, for Quantitative Trait Locus (QTL) detection, to establish syntenic relationships and as a platform for map-based cloning. Several types of molecular markers such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STSs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphisms (SNPs) and Expressed Sequence Tags (ESTs) have been used in mapping.

PCR-based markers are highly suitable for genetic mapping and breeding purposes because they require a low amount of DNA, can be easily automated and allow high-throughput screening, can be exchanged between laboratories and are highly transferable between populations. SSR markers are known to be abundant, co-dominant, highly polymorphic even between related species, and reliable, of easy application and with successful adaptation to automation. They are widely used in cultivar fingerprinting, genotype identification, genetic diversity assessment, molecular mapping and marker-assisted breeding (Gupta and Varshney 2000; Röder et al. 1998). Molecular markers have the advantage that they are not influenced by the environment and can be scored at all growth stages of the plant.

QTL mapping allows one to statistically identify individual chromosomal regions or QTLs containing genetic factors that contribute to variation in a complex trait so that this information can be utilised in future either for Marker Assisted Selection (MAS) or for the isolation of these QTLs through map-based cloning (Alonso-Blanco and Koornneef 2000). MAS is a powerful tool for indirect selection of traits of interest at the seedling stage, thus speeding up the conventional plant breeding and facilitating the crop improvement programs (Ribaut and Hoisington 1998). It is particularly useful in alien gene transfer via backcrossing. Once markers that are tightly linked to genes or QTLs of interest have been identified, prior to field evaluation of large number of plants, breeders may use specific DNA marker alleles as a diagnostic tool to identify plants carrying the genes or QTLs (Ribaut et al. 1997). QTL information, in turn, provides tools for physiology, developmental genetics and fine structure genetic analysis.

Aim of the research

The main objective of this study was to map the QTLs for Al tolerance using molecular markers in most important food crops - Wheat and Barley.

- A widespread screening of wheat accessions including the hexaploid, tetraploid and wild relative *Aegilops tauschii* were undertaken to assess their response and

to uncover interesting Al tolerant germplasm from the Gatersleben genebank collections. In addition, a few cytogenetic stocks were also investigated.

- Precise genetic stocks, *Triticum aestivum* L. cv. 'Chinese Spring' / *Aegilops tauschii* introgression lines were investigated to map the QTLs associated with Al tolerance and to study the effect of wild segment introgression in hexaploid wheat background.
- A set of 57 single chromosome recombinant Doubled Haploid (DH) lines, derived from 'Chinese Spring' x 'Chinese Spring' (Synthetic 3B) substitution line was used to construct a genetic linkage map of chromosome 3B, followed by QTL analysis to map the associated locus for Al tolerance on 3B.
- SSR markers closely linked to the major QTL in wheat were validated to evaluate their application in MAS.
- QTL mapping for acid (proton) tolerance *perse* was undertaken using the D genome introgression lines to compare the results with Al tolerance QTLs.
- Screening of barley accessions were carried out to study the diversity in barley for Al tolerance.
- The Oregon Wolfe Barley (OWB) mapping population was investigated to study the inheritance of Al tolerance in barley.

The identification and characterisation of Al tolerance genes will not only greatly advance our understanding of Al tolerance mechanisms, but, more importantly, will be the source of new molecular resources that researchers could use to develop improved crops better suited for cultivation on the acid Al toxic soils that comprise a large fraction of the world's lands.

2 Literature review

Since the aim of the research is mainly focused on genetical aspects of Al tolerance, emphasis in this section is given to the same.

2.1 A brief history of aluminium tolerance breeding

Genetic variation allows different plant species, and different cultivars of the same species, to exhibit differing abilities to grow in acid Al toxic soils. The discovery of genetic variation for Al tolerance in wheat dates back nearly 100 years, when it had a prominent role in the earliest breeding efforts in Brazil and was instrumental to the expansion of wheat production in that country. In the early 1900s, wheat breeding efforts were initiated in areas of Southern Brazil that suffered from severe soil acidity and Al toxicity. Significant variation for wheat cultivars for a trait referred to as "crestamento" (partially burned) was well known. At that time, the basis for "crestamento" was not known, although it is now known as a symptom elicited by toxic levels of Al in the soil. Because of the strong association between tolerance to "crestamento" and superior performance, Al tolerance was by default, probably the earliest trait selected for by the southern Brazilian wheat breeding programs (Garvin and Carver 2003).

One particular cultivar 'Polyssu', selected from landraces cultivated by settlers from Northern Italy, exhibited exceptional resistance. 'Polyssu' was crossed to a series of desirable lines from earlier breeding work, collectively referred to as "Alfredo Chaves" (AC) lines. This proves to be extremely important for Brazilian wheat production; most of the important Brazilian wheat cultivars arising over the next several decades (For e.g., 'Frontana', 'Colonias', 'Fronteira', 'BH1146', 'Maringa') could trace their parentage back to these crosses. As wheat breeding efforts on acid soils expanded globally, Brazilian wheats derived from 'Polyssu' x 'AC' crosses became a corner stone source for genes that confer Al tolerance (Garvin and Carver 2003).

2.2 Comparison of genetics of aluminium tolerance in cereals

The genetics of Al tolerance has been extensively studied in cereal crops mainly in wheat, barley, rye and rice. Tolerance to Al toxicity differs greatly among cereal species, and barley is usually considered the most susceptible member of the Poaceae (Garvin and Carver 2003). The Al tolerance order as reported is maize>rye>triticale>wheat>barley (Polle and Konzak 1985), rye>oats>millet>bread wheat>barley>durum wheat (Bona et al. 1993), and rice>maize>pea>barley (Ishikawa et al. 2000). Several investigations provided an insight into the genetics of Al tolerance in wheat. Al tolerance in wheat cultivars has been found to be under the control of a single dominant gene (Luo and Dvorak 1996; Riede and Anderson 1996). However, there is also evidence to suggest that more than one gene might exist in certain wheat cultivars (Aniol and Gustafson 1984; Camargo 1981; Zhou et al. 2007). F_2 populations from a cross between the well known tolerant cultivar 'Atlas 66' and several Al sensitive parents exhibited a 15:1 segregation pattern, suggesting that two dominant genes contribute to Al tolerance in those populations (Camargo 1981; Berzonsky 1992).

Earlier works found that the wheat D genome is the most important for tolerance to Al, followed by A and B genomes (Slootmaker 1974). The first instance of mapping Al tolerance gene was undertaken in the Brazilian wheat cv. 'BH1146' using RFLP markers. A major gene (Alt_{BH}) was mapped to the long arm of chromosome 4D with the help of markers *Xbcd1230* and *Xcdo1395* at a distance of 1.1 and 11.3 cM respectively (Riede and Anderson 1996). Later, a report on physical characterisation of chromosome arm 4DL mapped the same gene to the distal region of the chromosome (Milla and Gustafson 2001). From a recent study, in addition to the major locus on 4DL, a new minor locus was also reported on chromosome 3B using RILs from a cross of 'Atlas 66' / 'Century' (Ma et al. 2005). Although a substantial body of literatures wind up with a common conclusion of a single QTL/gene, they simultaneously expose a paradox that has been long faced by researchers - that Al tolerance is inherited in a simple fashion in designed crosses; yet, when tolerance is evaluated in a range of germplasm, a broad and continuous distribution from highly tolerant to highly sensitive genotypes is observed, indicative of a

complex multigenic system with varying effects on Al tolerance. This needs further investigations using cultivars from wider geographic scale, which may help to find new interesting alleles or even new genes which could be pyramided to attain the expected potential yield.

In wheat, *TaALMT1* gene (supposed to be the major gene), which encodes for an Al-activated malate transporter was isolated (Sasaki et al. 2004). Molecular characterisation of this gene by Raman et al. (2005b) reveals that the coding region of *TaALMT1* has 6 single nucleotide polymorphisms (SNPs) between the parents 'ET8' and 'ES8' (NILs which differ for Al tolerance for one genetic locus) used in their study. This indicates the possibility of at least two alleles (*TaALMT1-1* and *TaALMT1-2*) for this gene. The same polymorphism occurred between the Al tolerant cv. 'Atlas 66' (*TaALMT1-1*) and Al sensitive cv. 'Scout 66' (*TaALMT1-2*). But the moderately tolerant cv. 'Chinese Spring' had the *TaALMT1-2* allele. This result and the studies on ditelosomic lines of 'Chinese Spring' (Papernik et al. 2001) confirms that additional genes similar to *TaALMT1* are also present, presumably located on A and B genomes. From the same study (Raman et al. 2005b), using a single accession of *Ae. tauschii*, it was found to possess *TaALMT1-1* allele. The authors suggest further work to determine whether the *TaALMT1-2* allele is present in other accessions of *Ae. tauschii* and whether this variation could give an explanation for the different levels of Al tolerance. Repetitive indel (insertion-deletion) markers within the *TaALMT1* gene were studied to examine their utility for routine MAS (Raman et al. 2006). In transgenic barley with expression of the *TaALMT1* gene displayed a capacity for Al-activated malate efflux which was not observed in control plants indicating that a higher level of Al tolerance can be achieved by promoting malate efflux in barley (Delhaize et al. 2004). Despite of lot of research, a 'diagnostic' marker has not yet been found which could be beneficial for MAS of Al tolerant wheat cultivars.

Among the cereal crops barley is the most sensitive to Al toxicity. Even though genotypic variation for Al tolerance exists in barley, production of this crop on acid Al toxic soils is limited than its more Al tolerant relatives (wheat, rye) because of a generally high level of Al sensitivity in the species as a whole. 'Dayton' is one of the most Al-tolerant barley

genotypes (Minella and Sorrells 1992) and a single locus (*Alp*) on the long arm of the chromosome 4H conditions Al tolerance in populations derived from 'Dayton' / 'Harlan hybrid' and 'Dayton' / 'F6ant28B48-16' (Raman et al. 2003; Ried 1970; Tang et al 2000). The same locus was also found to condition Al tolerance in other populations (Ma et al. 2004; Raman et al. 2005; Wang et al. 2006). Minor gene effects for Al tolerance have also been suggested (Echart et al. 2002; Raman et al. 2005a; Reid 1970). Minor QTLs on 3H, 4H, 5H, 6H identified in an F_2 population by Raman et al. (2005a) need further validation in different genetic backgrounds. Markers *Xbcd1230* and *Xcdo1395* (also mapped on wheat) were 2.1 cM and 33.5 cM respectively from the Al tolerance locus *Alp* in barley. The different relative position of the same markers and Al tolerance locus suggest that this chromosome segment has been subject to structural changes in these two species (Tang et al. 2000). The genetic variation may be due to mutations in the Al tolerance gene in barley that may lead to variation in Al tolerance levels.

Rye has one of the most efficient groups of genes for Al tolerance among cultivated species of *Triticeae* and can be used as a source of tolerance genes for wheat (*Triticum ssp.*) through wheat-rye introgression and as a component of triticale (x*Triticosecale* Wittmack). At least, four independent and dominant loci, *Alt1*, *Alt2*, *Alt3* and *Alt4*, located on chromosome arms 6RS, 3RS, 4RL and 7RS, have been reported (Aniol and Gustafson 1984; Gallego and Benito 1997; Gallego et al. 1998; Matos et al. 2005; Miftahudin et al. 2002). Recently, using PCR primers designed from wheat *ALMT1* gene, a rye gene *(ScALMT1 - Secale cereale ALMT1)* was amplified, cloned and sequenced. Subsequently this gene in rye was found to be located on 7RS by PCR amplification using wheat-rye addition lines (Fontecha et al. 2007). This suggests that Al tolerance genes in wheat 4DL, barley 4HL and rye 7RS are orthologs, originated from a common ancestor and have the same function of organic acid efflux. Although extensive inter-chromosomal translocations were detected between these species during evolution, colinearity was found to be retained within the translocated chromosome segments (Devos et al. 1993). Several investigations have established the colinearity within *Triticeae* members (Börner et al. 1998), so also between genomes of species belonging to different tribes within the *Poaceae* (Gale and Devos 1998).

Information on the genetic system controlling Al tolerance in Triticale (x *Triticosecale* Wittmack) is limited. Triticale, a hybrid of wheat and rye, has a higher level of tolerance than wheat and this is attributed mainly to the presence of R-genome (in hexaploid triticale, AABBRR). But it is not still clear whether the genes that regulate the expression of Al tolerance in triticale is the same that of in rye. It was reported that incorporation of the D genome chromosomes into triticale background as substitutions for A and B genome chromosomes improved Al tolerance indicating the efficiency of D-genome of wheat (Budzianowski and Wos 2004).

Molecular mapping of genes conferring Al tolerance in rice suggested that Al tolerance is a complex multigenic trait. Many major QTLs were detected by different molecular markers on chromosomes 1 and 12 (Wu et al. 2000), on chromosomes 1, 2 and 6 (Ma et al. 2002), and on chromosomes 1 and 8 (Nguyen et al. 2002). Later, a major QTL was mapped on chromosome 3 of rice which is syntenic to *Triticeae* group 4 (Nguyen et al. 2003). In sorghum, a major Al tolerance locus, *Alt* $_{SB}$, has been mapped on chromosome 3 (Magalhaes et al. 2004), which corresponds to homoeologous region of *Triticeae* chromosome 3, rice chromosome 1 and maize chromosomes 3 and 8. QTLs associated with Al tolerance in maize have also been mapped on these chromosomes (Ninamango-Cardenas et al. 2003). These studies indicate an evolutionary inheritance of Al tolerance genes in different cereals. A summary of Al tolerance genes/QTLs in cereals is listed in Table A8.

2.3 Aluminium tolerance gene conservation in monocots and dicots

In *Arabidopsis thaliana*, the model dicot plant, inheritance of Al tolerance seems to be complex. Studies using RILs derived from a cross between 'Landsberg erecta' and 'Columbia' detected QTLs on chromosome 1 and 4 (Kobayashi and Koyama 2002). Five epistatic loci were also identified in the same population. The QTL on chromosome 1 had a major effect explaining 40% of the phenotypic variation compared to the QTL on chromosome 4 which explained 16% of the variation. In another study, using RILs with 'Landsberg erecta' and 'Cape verde islands' as parents two QTLs were detected, one on

chromosome 1 and the other on chromosome 3. Eleven sets of epistatic interacing loci were also detected which shared the same chromosomal regions (Kobayashi et al. 2005). The impact of the tolerance factor on chromosome 1 was explained as its involvement in malate excretion as a mechanism of Al tolerance in *Arabidopsis* (Hoekenga et al. 2003). This exclusion principle is similar to that suggested in wheat. An *in silico* analysis of the *Arabidopsis* genome indicated that it contains a number of sequences similar to *TaALMT1* (Sasaki et al. 2004).

Recently, a homolog of the wheat *TaALMT1* named *AtALMT1* in *Arabidopsis* was found and is considered to be a good candidate to be involved with Al tolerance (Hoekenga et al. 2006). This work was the first report of Al tolerance gene conservation between monocot and dicot species. The conservation of such a gene over a broad evolutionary spectrum raises the possibility of identifying yet unknown sources of Al tolerance gene diversity and this knowledge can contribute to the enhancement of the current levels of Al tolerance in economically important grass species.

3 Materials and Methods

3.1 Plant Materials

3.1.1 Screening wheat and barley (Genebank collection, IPK, Gatersleben)

A total of 400 accessions, 320 wheat and 80 barley accessions were screened for Al tolerance. In wheat, tolerance was evaluated for different ploidy levels- hexaploid (*T. aestivum* L., Table A5) and tetraploid wheats (*T. durum* Desf., Table A6), and the diploid wild ancestor of bread wheat, *Ae. tauschii* (Table 6). In addition, a set of 17 'CS'/ 'Synthetic 6x' substitution lines (except 2A, 4A, 7A and 6B) were screened to evaluate individual chromosome effect for Al tolerance.

Table 1. Wheat and barley accessions screened.

Wheat		No. of accessions / lines
Hexaploid -*T. aestivum* L.	153 + 4*	157
Tetraploid - *T. durum* Desf.	63	63
Diploid - *Ae. tauschii* Coss.	83	83
Cytogenetic stocks	'CS'/ 'Synthetic 6x' substitution lines except 2A, 4A, 7A and 6B	17
Total		320
Barley		
H. vulgare L.		80
Sum total		**400**

* Accessions provided from CIMMYT, Mexico

3.1.2 Wheat / *Aegilops tauschii* substitution and introgression lines

A set of seven 'CS / Synthetic 6x' single chromosome substitution lines, in which the individual chromosomes of the D genome donor *Ae. tauschii* replaced their homologs in cv. 'CS' (Law and Worland 1973) was analysed. Further, a set of 84 introgression lines each of which contain a specific sub-chromosomal segment of one *Ae. tauschii* chromosome in the homozygous state in cv. 'CS' background developed by backcrossing

the D genome chromosome substitution lines with the recurrent parent 'CS' was investigated to map the quantitative trait loci (QTL) controlling Al tolerance and acid (proton) tolerance in wheat. These lines have been characterized at the genetic level by Pestsova et al. (2001, 2006). Parent 'Synthetic 6x' had been obtained from a cross of tetraploid emmer and wild grass *Aegilops tauschii* (*T. dicoccoides* var. *spontaneovillosum* x *Ae. squarrosa* ssp. *eusquarrosa*) (McFadden and Sears 1947).

3.1.3 'CS' / 'CS (Synthetic 3B)' DH lines

57 single chromosome recombinant DH (Doubled Haploid) lines from a cross of 'CS' and 3B substitution line ('CS' / 'Synthetic 3B') were used to investigate the molecular markers associated with Al tolerance QTLs. The synthetic parent of this population is the same which was used for developing introgression lines (see 3.1.2). The parents differ only for chromosome 3B on a uniform genetic background for all other chromosomes.

3.1.4 Oregon Wolfe Barley (OWB) mapping population

The Oregon Wolfe Barley (OWB) mapping population is a set of 94 spring barley DH (Doubled Haploid) lines developed from the F_1 of a cross between Dr. R. Wolfe's dominant and recessive morphological marker stocks (DOM x REC) using the *Hordeum bulbosum* method (Costa et al. 2001). DOM and REC, the dominant and recessive morphological marker stocks are described by Wolfe and Franckowiak (1991). The DH lines were developed by the Oregon State University Barley Project. In this study, the OWB population was used to study the inheritance of Al tolerance in barley. Stein et al. (2007) constructed an integrated genetic map of barley based on markers developed from 1,032 expressed sequence tags (ESTs). About 643 markers were informative in the OWB population and the map consists of EST-based RFLPs, -SSRs, -SNPs and several anchor markers.The function of ESTs linked to Al tolerance QTL was derived from a BLASTX search against the public non-redundant protein database NRPEP.

3.2 Phenotyping methods

Since inhibition of root growth is the first visible symptom of Al injury, direct reference to this process in selection seems to be a reasonable approach. Nutrient solution screening is the best and most widely used assay because of the possibility of a precise control

composition. Al tolerance studies should be conducted in solutions containing a level of Al approximating the soil composition, ionic strength, and Al activity. Root length measurement is the most suitable approach for genetic and molecular studies in which a precise quantitative response for Al stress is needed. It is also suitable for identifying genotypes with superior alleles for Al tolerance. Staining techniques are quick and are suitable for screening large collection of germplasm.

3.2.1 Root Tolerance Index (RTI) method.

1) Seeds of each accession / line were sterilized in 3% sodium hypochlorite (NaOCl) solution for 5 minutes and rinsed thoroughly in distilled water.
2) They were pre-germinated on moist filter paper for 84 hours at 7°C and left to germinate at 24°C for approximately 36 hours.
3) Seedlings with similar root length were transferred to holed frames floating in aerated nutrient solution containing appropriate pH, and Al added as aluminium chloride ($AlCl_3.H_2O$) (concentration of Al and pH in Table 2). A control without Al at pH 6.5 was run in parallel. [For acid tolerance test, the only difference is that no Al is added but the pH level is adjusted (Table 2). The control is the same with pH 6.5]. In both cases, pH is adjusted by adding HCl or NaOH. The containers were placed in climate chamber with controlled conditions (24°C, 16 hour light / 8 hour dark regime). Solutions were replaced daily to minimise changes in pH and Al concentration.
4) After 4 days two longest roots of each seedling were measured and averaged per line / accession.
5) Tolerance is evaluated by Root Tolerance Index (RTI) parameter.

   Al tolerance

   RLA = Root Length in Al

   RLC = Root Length in Control

   RTI (Al) = RLA / RLC x 100

   Acid tolerance

   RLAc = Root Length in Acid

   RLC = Root Length in Control

   RTI (Ac) = RLAc / RLC x 100

Tolerance assessment scale;

RTI (%) < 40 % - Sensitive

40 – 49 - Moderately sensitive

50 – 65 - Moderately tolerant

> 65 - Tolerant

3.2.2 Hematoxylin staining (HS) method.

Principle

Hematoxylin, a natural dye, cannot directly stain tissue successfully because it needs a mordant, e.g., Al or Iron, to form a dye-mordant (D-M) complex for efficiency. With mild alkaline treatment this complex is converted into a neutral chelate, which renders a purple colour and is attracted to negatively charged sites, showing a particular affinity for phosphates. In the case of Al-Hematoxylin complex, the major tissue-binding site is thought to be phosphoric acid residue in nucleic acid (nuclear DNA / cytoplasmic and nucleic RNA). The method is based on an exposure of roots to a brief Al shock, after which the effect on root re-growth is recorded.

1) Seeds of each accession / line were sterilized in 3% sodium hypochlorite (NaOCl) solution for 5 minutes and rinsed thoroughly in distilled water.
2) They were pre-germinated on moist filter paper for 84 hours at 7°C and left to germinate at 24°C for approximately 36 hours.
3) Seedlings with similar root length were transferred to holed frames floating in aerated nutrient solution with pH 6.5 for 24 hours.
4) Seedlings were transferred to a nutrient solution containing appropriate Al concentration and pH (Table 2) for 24 hours (Al shock).
5) Roots were thoroughly washed with distilled water and stained with 0.2 % hematoxylin aqueous solution for 15 minutes. Excess dye is washed off. (Preparation of hematoxylin aqueous solution: For 1 litre: 2g hematoxylin powder and 0.2g of sodium iodate ($NaIO_3$) dissolved in 1000ml distilled water).
6) Seedlings are returned back to normal nutrient solution without Al for 24 hours.
7) Scoring scheme: Seedlings with all roots showing continued root re-growth were rated as tolerant (T), those showing re-growth on some roots were rated

moderately tolerant (MT) whereas those with no roots showing re-growth are rated sensitive (S).

Figure 2. Scoring scheme based on root re-growth.

Table 2. Aluminium concentration and pH for different experiments.

Experiments	RTI method	HS method
Screening		
Hexaploid - *T. aestivum* L.	40µM Al, pH 4.5	-
Tetraploid - *T. durum* Desf.	10µM Al, pH 4.5	-
Diploid - *Ae. tauschii* Coss.	-	40 & 80µM Al, pH 4.5
Cytogenetic stocks	40µM Al, pH 4.5	-
H. vulgare L.	20µM Al, pH 4.5	-
QTL mapping		
D-genome introgression lines (Al-test)	30µM Al, pH 4.5	80µM Al, pH 4.5
D-genome introgression lines (acid test)	No Al, pH 4.5	-
'CS'/ 'CS(Synthetic 3B)' DH lines	30µM Al, pH 5.0	-
Oregon Wolfe barley DH lines	20µM Al, pH 4.7	45µM Al, pH 4.7
	10µM Al, pH 4.7	

'-' not applied

Screening methods were a slight modification of Hede et al. (2002). Nutrient solution for both methods was prepared according to a protocol of Nawrot et al. (2001). Details of stock solution are given in Table A1.

3.3 Genotyping methods

3.3.1 Genomic DNA isolation

DNA was isolated from the young leaves (8-10 cm long) collected from 10 to 14 day old seedlings of each accession / line in 2 ml round-bottom eppendorf tubes and stored at -80°C. Isolation of DNA was done following a protocol of Doyle and Doyle (1990). Leaf material of each sample were grinded in "Retsch-Schwingmühle MM300" (2x30 sec and frequency of 25/s) and incubated in 700 µl of extraction buffer in a water bath for 30-45 minutes at 65°C. 700 µl of Chloroform:Isoamylalcohol (24:1) was added and mixed well. Samples were centrifuged for 15 minutes at 8,000 rpm and the supernatent was transferred to a new 1.7 ml tube. The latter step is repeated again. DNA was precipitated by adding 85 µl acetate-mix and 500 µl isopropanol. The pellet, recovered by centrifugation, was washed with 1ml 70% ethanol, centrifuged and supernatant was discarded, then air-dried and resuspended in 2 µl RNaseA-solution and 100 µl TE-buffer. Incubation was done at room temperature until DNA is completely dissolved, stored at 4°C (short term) or -20°C (long term).

3.3.2 Gel electrophoresis

Prior to PCR, the isolated DNA should be checked quantitatively and qualitatively. Genomic DNA was run on 1% agarose gel at 100v for 90 minutes in 1x TAE buffer. A molecular ladder (1Kb) was run as standard for comparison. After the run, the gel is stained with ethidium bromide (a fluorescence dye which intercalates within DNA bases) and visualized under UV light in a transilluminator. According to the band width, DNA is diluted for further steps of PCR experiments.

3.3.3 Polymerase Chain Reaction (PCR)

DNA amplification was carried out in a 96 well thermocycler (Applied biosystems, Foster city, USA) each containing 50-100ng template DNA, 250nM of each primer (one primer was labeled with Cy-5), 200µM of each deoxynucleotide, 1.5mM $MgCl_2$, 1X PCR buffer and 1U of *Taq* DNA polymerase. Amplification was performed on a program for

the initial denaturing step with 94°C for 3 minutes, followed by 45 cycles for 1 minute at 94°C, 1 minute at 50, 55 or 60°C (depending on the annealing temperature,Tm, for each primer), 2 minutes at 72°C and a final extension step at 72°C for 10 minutes. Samples were transferred to 4°C for immediate use or to -20°C for long term storage for later use.

3.3.4 Fragment Analysis

The PCR products were separated on an Automated Laser Fluorescence (ALF) express using short gel cassettes. Denaturing gels (0.35 mm thick) with 6% polyacrylamide were prepared using SequaGel XR (Biozym). The gels were run in 1xTBE buffer (0.09 M Tris-borate (pH 8.3) and 2 mM EDTA) with 850V, 50W with 2 mW laser power and a sampling interval of 1 sec. At a time 40 samples could be loaded. The gels were reused four to five times. In each lane, fragments with known sizes were included as standards (internal standards). The first well will be loaded with an external standard. Fragment sizes were calculated using computer program 'Fragment Analyser version 1.02' by comparing the internal standard size (Röder et al. 1998).

Microsatellite markers used for the study were selected from the reference mapping population of ITMI (International Triticeae Mapping Initiative) and are designated as *gwm* for Gatersleben wheat microsatellites and *gdm* for Gatersleben D genome microsatellites. Gatersleben barley RFLP, microsatellites and SNP markers were designated as GBR, GBM and GBS, respectively.

3.4 Statistical tools

Computer program MAPMAKER (Lander et al. 1987) was employed for linkage map construction. Marker alleles were coded as A (allele from 'CS'), B (allele from 'CS (Synthetic 3B') and '-' (for missing data). Markers were divided into linkage groups using the GROUP command in 'Two point analysis'. 'Multipoint analysis' was used to determine the likely orders among markers. Marker position within the chromosome was determined with the TRY command. Marker orders were confirmed using RIPPLE command, and recombination values were converted to map distances (cM) using

Kosambi (1944) mapping function. Chi-square ($\chi 2$) analysis was performed to test the marker segregation pattern against an expected Mendelian ratio.

QTL analysis is based on the principle of detecting an association between phenotype and marker genotype. QTL analysis was performed using QGENE software (Nelson 1997) by applying the single marker regression analysis and simple interval mapping (SIM) approach. Single marker analysis indicates the chromosome location of QTL, probability values, percentage of phenotypic variation explained by the QTL (R^2) and source of allele. SIM makes use of linkage maps and analyses intervals between adjacent pairs of markers along the chromosome simultaneously. The test statistic LOD (Logarithmic of odds) score is used to identify the most likely position for a QTL where the highest LOD value is obtained. The peak should exceed a specific significance threshold for the QTL to be declared 'statistically significant', which in this case is 3.0 but can be assigned by permutation tests. Dunnet t-test was carried out to compare the control with reference groups.

4 Results

4.1 Screening wheat germplasm

A total of 320 wheat accessions / cultivars, including hexaploid and tetraploid wheats, *Ae. tauschii* and cytogenetic stocks were screened for Al tolerance at seedling stage using nutrient solution culture.

***Hexaploid wheat*:** 157 accessions of hexaploid wheat originated from Australia, Brazil, Canada, India, Poland and U.S.A were evaluated for Al tolerance. Seedlings were treated with 40µM Al and pH 4.5 for stress conditions and a control without Al (pH 6.5) runs in parallel. The reference accessions included were 'Atlas 66' and 'CS' as tolerant and 'Scout 66' as sensitive. A diverse range of tolerance was observed within the materials from highly tolerant to highly sensitive. From the accessions tested, 27 (17%) were tolerant, 61 (39%) showed moderate response and 69 (44%) accessions were found to be sensitive (Table 3). Many Brazilian accessions were highly tolerant with well developed roots. Most accessions from Australia, India and Poland showed moderate response. A high percentage of the collections from U.S.A (66%) were sensitive. Same was the case with Canadian accessions. In general, the mean RTI was higher for Brazil when compared to other countries (Fig. 5). When the pedigree of the tolerant accessions was observed, irrespective of the country of origin, most of them had at least one tolerant Brazilian parent in their lineage (Table 4).

Table 3. Aluminium tolerance diversity in hexaploid wheat.

Country	No. of accessions	Tolerant	Moderately tolerant + moderately sensitive	Sensitive
Australia	26	3	7 + 6	10
Brazil	14	11	1 + 0	2
Canada	31	3	4 + 8	16
India	24	3	11 + 5	5
Poland	21	0	5 + 7	9
U.S.A	41	7	7 + 0	27
Total	157	27	35 + 26 = 61	69

Figure 3. Stress response of tolerant hexaploid wheat cultivars originating from Brazil (left) and a sensitive cultivar from Canada (right) under 40µM aluminium. For each cultivar / accession, roots in control (left) and roots in aluminium stress (right).

The Al tolerant cultivars / accessions showed good root growth and fine lateral branching as was the case in most Brazilian cultivars. Sensitive ones showed reduced or no root growth and had brown coloured root tips (Fig. 3). The effect of Al toxicity at the root tips is shown in Fig. 4. This is a microscopic view of the root tip after 4 days in control and in 40µm Al stress condition. Control root tip looks normal and has fine root hairs, on the other hand, the stressed root tip has stubby appearance which is typical of Al stress and no sign of root hairs is visible.

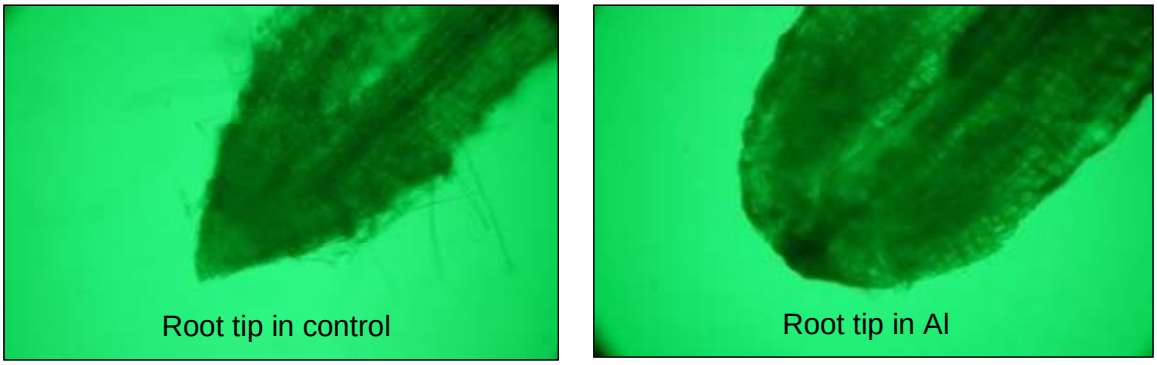

Figure 4. Effect of aluminium toxicity at root tips.

Table 4. Pedigree and origin of aluminium tolerant cultivars. Tolerant Brazilian parents (in bold).

Acc. No	Cultivar name	Pedigree	Origin	RTI (%)
TRI 2959	Frontana 568	Selection from **Frontana**	Brazil	74
TRI 8240	Varanopolis	Trintecino/**Frontana**	Brazil	89
TRI 9553	-	-	Brazil	109
TRI 9691	-	-	Brazil	78
TRI 10431	IAS-20-Iassul	Colonias//**Frontana**/Kenya-58	Brazil	96
TRI 10974	Jesnita	-	Brazil	102
TRI 13776	Carazinho	Colonista/**Frontana**	Brazil	85
* 5919	Frontana	**Fronteira**/Mentana	Brazil	67
* 6071	BH1146	**Fronteira**/**Pontagrossa 1**	Brazil	93
* 26	Pontagrossa 1	Selection from **Polyssu**	Brazil	84
* 1770	Frondoso	**Polyssu**/**Alfredo chavez 6.21**	Brazil	76
TRI 4934	Colotana	Colonicta/**Frontana**	Canada	78
TRI 7360	Atlas 50	**Frondoso**/3/Redhart3/2/ (Noll 28, Hussar/Forward)	U.S.A	67
TRI 6921	Atlas 66	**Frondoso**/3/Redhart3/2/ (Noll 28, Hussar/Forward)	U.S.A	89
TRI 6942	Taylor 49	Trumbull/**Frondoso**	U.S.A	71
TRI 7288	Coker 47-27	**Fronteira**/Hardired	U.S.A	78
TRI 7291	Coastal	**Frondoso**/3/Redhart3/2/ (Noll 28, Hussar/Forward)	U.S.A	82
TRI 7358	Wichita	Early-Blachhull/Tenmarq	U.S.A	89
TRI 7366	Seneca	Portage/Fulcaster	U.S.A	83

* Provided from CIMMYT
'-' not available

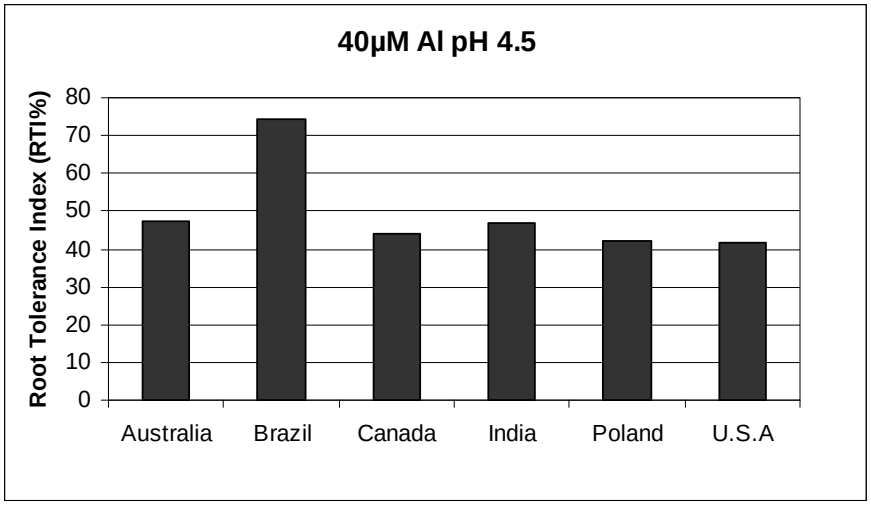

Figure 5. Mean root tolerance index (RTI%) for aluminium tolerance of hexaploid wheat accessions originated from different countries.

***Tetraploid wheat*:** A preliminary screening of tetraploid wheat was carried out under 40 µM Al, pH 4.5 in order to compare the response with that of hexaploid wheat. It was found that this concentration was too high to discriminate the tetraploid wheat accessions since the root tips were brown and swollen, characteristics of high toxicity, for all accessions tested. From previous reports tetraploid wheats are known to be sensitive to Al when compared to hexaploid species. Hence the accessions were screened finally at 10µM Al and pH 4.5. Out of the 63 accessions tested, one was tolerant. Most accessions belonged to the moderately tolerant / moderately sensitive category (46 accessions) and 16 were found to be sensitive (Table 5).

Table 5. Aluminium tolerance diversity in tetraploid wheat.

Country	No. of accessions	Tolerant	Moderately tolerant + moderately sensitive	Sensitive
Australia	3	0	2 + 1	0
Canada	5	0	4 + 0	1
Iran	21	1	11 + 8	1
Poland	2	0	2 + 0	0
Turkey	17	0	8 + 4	5
U.S.A	15	0	4 + 2	9
Total	63	1	31 + 15 = 46	16

***Aegilops tauschii*:** 83 *Ae. tauschii* (genome DD) accessions were evaluated for Al tolerance using the hematoxylin staining method under 45µM Al and pH 4.5. Since *Ae. tauschii* is a wild species it was difficult to obtain seedlings with uniform root length for Al tolerant assessment with RTI method. Hence staining method was used where the seedlings could be selected randomly and evaluated on the basis of root re-growth. Accessions from all countries under study (Table 6) showed moderate re-growth indicating a moderate tolerance at this concentration. At the same pH but at 80µM Al (wheat differentiating medium), root tips were swollen and completely dark stained and showed no signs of re-growth which is an indication of sensitivity. Results show that none of the *Ae. tauschii* accessions tested from all the countries in this study were tolerant.

Table 6. Investigation of genetic diversity in *Ae. tauschii* for aluminium tolerance evaluated by hematoxylin staining method.

Country of origin	No. of accessions	Tolerance score	
		45µm Al	80µm Al
Afghanistan	10	MT	S
Armenia	11	MT	S
Aserbaidshan	16	MT	S
Georgia	7	MT	S
Germany	2	MT	S
Iran	5	MT	S
Kazakhstan	1	MT	S
Kirgistan	3	MT	S
Russia	4	MT	S
Tadshikistan	10	MT	S
Turkmenistan	10	MT	S
Usbekistan	4	MT	S

MT- moderately tolerant
S - sensitive

***Cytogenetic stocks*:** All 'CS x Synthetic 6x' single chromosome substitution lines except 2A, 4A, 7A and 6B were screened for Al tolerance. The lines excluded were shown to be incorrect substitutions (Law and Worland 1973). Few substitution lines had a similar tolerance level like 'CS' but some lines performed better. Lines 3B, 4B and 4D had distinct reduction of tolerance. The variation in tolerance of 3B and 4D is exploited to map QTLs for Al tolerance in this research.

4.2 Mapping QTLs for aluminium tolerance using 'CS' / *Ae. tauschii* substitution and introgression lines

4.2.1 Characterisation of parents, substitution lines and introgression lines

The parents 'CS' and 'Synthetic 6x', the seven D genome substitution lines and the whole set of 84 introgression lines was characterised in 30µM Al at pH 4.5 for stress condition and a pH of 6.5 without Al for control. As per RTI method 'CS' was tolerant (76%) whereas 'Synthetic 6x' was shown to be moderately tolerant (53%) (Fig. 6). All the substitution lines showed a tolerance level below the control parent 'CS'. Except the 4D substitution line which was moderately sensitive, all other lines were moderately tolerant / tolerant. The substitution lines were compared with 'CS' (control) statistically by ANOVA followed by Dunnett's t-test for RTI. The most significant difference ($p<0.05$) was observed between 'CS' and the chromosome 4D substitution line.

Individual characterisation of the introgression lines revealed an overall reduction in tolerance compared to the control parent 'CS'. When the tolerance within the lines was observed for each chromosome separately, a striking difference was seen within chromosome 4D introgression lines (Fig. 7). Except the tolerant lines 4D-1 and 4D-8 all other lines showed significant difference from 'CS' ($p<0.001$). The genetic status of the 4D introgression lines, as determined by SSR genotype allows the delineation of the region of the 'CS' chromosome in which a gene(s) for tolerance is located (Fig. 8). The introgression lines derived from other chromosomes, for instance from 1D, 2D, 3D, 5D, 6D and 7D showed no significant variation from control parent 'CS'. So also the variation observed within the lines for these chromosomes were not significant.

When the same set of lines was evaluated by hematoxylin staining under 80µM Al and pH 4.5, both 'Synthetic 6x' and 'CS' ('Synthetic 4D') were classed as sensitive, but all the other substitution lines and 'CS' recorded significant root re-growth. Root re-growth was also achieved by the 4D-1 and 4D-8 introgression lines, while the remaining 4D introgression lines showed no re-growth. Both RTI and hematoxylin staining methods were used in order to assess the stability of the QTLs under different parameters. The exact position of the putative QTLs and the associated markers were obtained by QTL analysis.

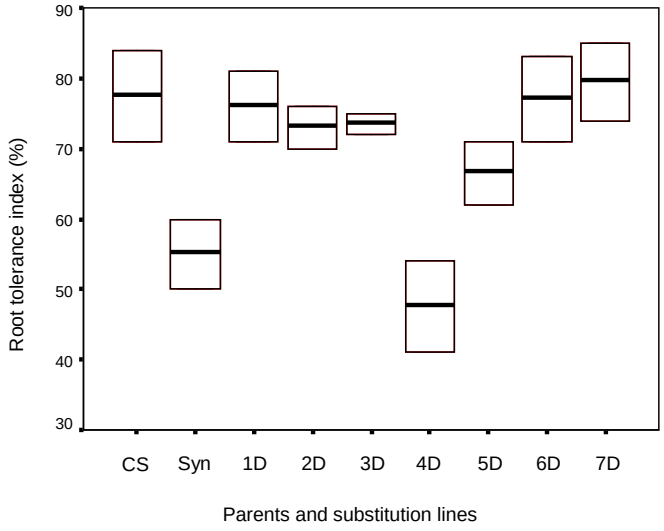

Figure 6. Comparison of RTI of 'CS' with 'Synthetic 6x' and substitution lines under 30µM aluminium, pH 4.5.

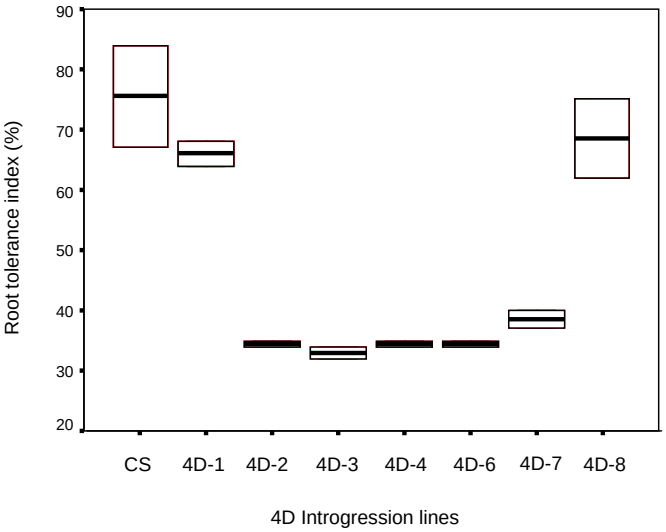

Figure 7. Comparison of RTI of 'CS' with 4D introgression lines under 30µM aluminium, pH 4.5.

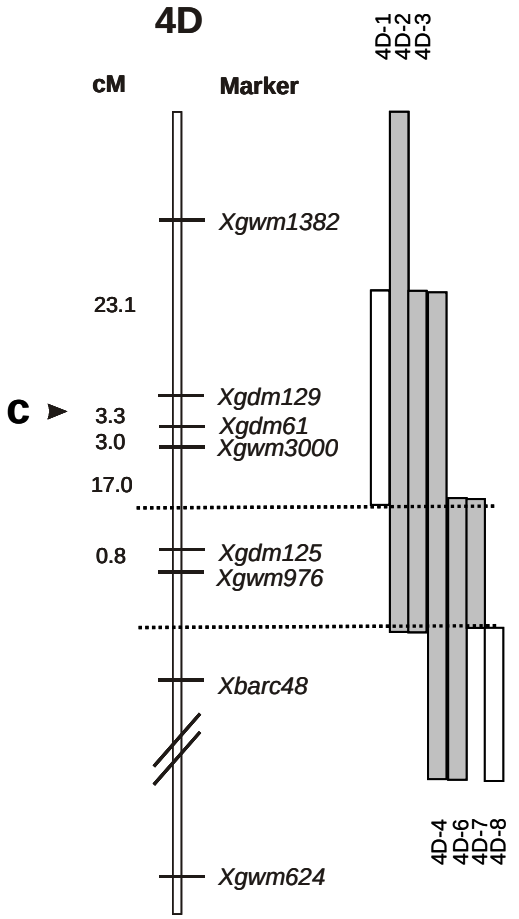

Figure 8. Schematic representation of the set of chromosome 4D introgression lines (Pestsova et al. 2001, 2006). Each bar represents a segment of *Ae. tauschii*. White bars: tolerant lines, grey bars: sensitive lines. C-centromere. The area within the dotted lines indicates the location of positive factors in 'CS' and/or negative factors in *Ae. tauschii*.

4.2.2 Quantitative trait locus (QTL) analysis

QTL analysis was performed with the whole set of 84 introgression lines. The profile of interval analysis indicates that a major QTL for RTI was associated with the SSR loci *Xgdm125* and *Xgwm976*, both mapping to the centromeric region of the long arm of chromosome 4D (Fig. 9, bottom). This QTL was highly significant (p<0.0001) with LOD score 6.69 and explained about 31% of the phenotypic variation (PV) for RTI. The same QTL was observed for RLA (Fig. 9, top) but not for RLC. A root re-growth QTL was located in the same region, although with a higher LOD score (30.54; p<0.0001) and PV (82%) (Fig 9 bottom). Results from single marker analysis for RLA, RTI (Table 7) and

Hematoxylin staining (Table 8) are shown. In all cases the negative allele originated from 'Synthetic 6x'. Hence it could be stated that the tolerance was inherited from the 'CS' parent.

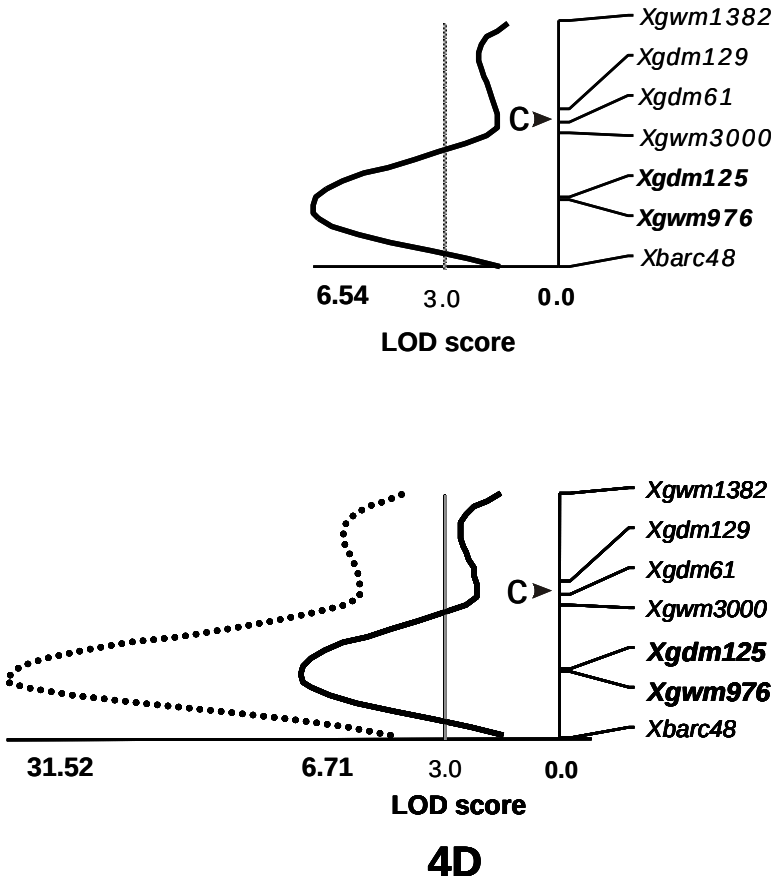

Figure 9. Interval analysis showing the QTL peak associated with aluminium tolerance on chromosome 4D based on RLA (top). QTLs for RTI (bold line) and hematoxylin staining method (dotted line) at the bottom. C-centromere. Closely linked markers are highlighted.

Table 7. Single marker analysis for markers associated with aluminium tolerance. QTL on chromosome 4D evaluated by RTI method - 30µM Al pH 4.5.

Marker	Chrm.	Dist. (cM)	Source	RLA			RTI		
				F-stat	LOD	R^2	F-stat	LOD	R^2
Xgdm125	4D	18	'Synthetic 6x'	35.23	6.50	0.30	36.51	6.69	0.31
Xgwm976	4D	17.2	'Synthetic 6x'	35.23	6.50	0.30	36.51	6.69	0.31

$p < 0.0001$

Table 8. Single marker analysis for markers associated with aluminium tolerance QTL evaluated by hematoxylin staining method - 80μM Al pH 4.5.

Marker	Chrm.	Distance (cM)	Source	F-stat	LOD	R^2
Xgdm125	4D	18	'Synthetic 6x'	364.71	30.54	0.82
Xgwm976	4D	17.2	'Synthetic 6x'	364.71	30.54	0.82

$p < 0.0001$

4.3 Construction of linkage map and QTL analysis using 'CS' x 'CS (Synthetic 3B)' DH lines

4.3.1 Linkage map construction

Parents 'CS (Synthetic 3B)' and 'CS' were screened for SSR marker polymorphism. Of the 21 SSR markers screened, 14 were found to be polymorphic which were subsequently used to screen the population. A linkage map of chromosome 3B was constructed using the marker segregation data of the 57 DH lines. Detailed description of the polymorphic SSR markers are shown in (Table A2). Chi-square (χ^2) analysis showed that the segregation pattern of four of the SSR loci was consistent with the expected 1:1 ratio, while the segregation among the remaining ten was skewed in favour of 'CS'.

4.3.2 Phenotypic characterisation

Screening studies of the cytogenetic stocks of 'CS' / 'Synthetic 6x' substitution lines showed that 'CS (Synthetic 3B)' substitution lines had a lower tolerance (52%) than 'CS' itself (62%). This difference was exploited by recording the response of the set of 57 DH progeny derived from the cross 'CS (Synthetic 3B)' x 'CS'. Using the constructed linkage map of chromosome 3B, QTL analysis was performed to map the position of Al tolerance locus. The parents and the progeny lines were screened under 30μM Al at a pH 5.0. The RTI of the population ranged from 48-70%.

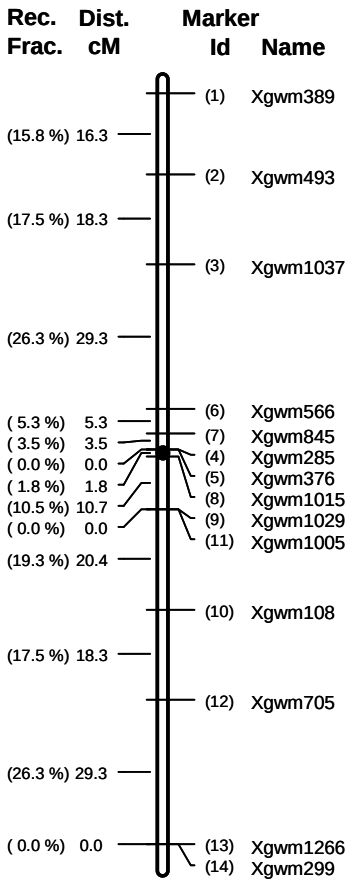

Figure 10. Linkage map constructed for chromosome 3B. Dark circle represents the centromere. Map distance was calculated using Kosambi mapping function (1944).

4.3.3 QTL analysis

QTL analysis was performed with SM and SIM analysis of QGENE software for RLC, RLA and RTI. A highly significant QTL ($p<0.0001$) was associated with the loci *Xgwm1029* and *Xgwm1005* (mapping to the centromeric region of the long arm) for both RLA and RTI (Fig. 11b, 11c). QTL for RLA had a LOD score of 6.79 and the phenotypic variance explained was 42%. The same QTL accounted for 49% of the variation in RTI with a LOD score of 8.36. In both cases, the allele for tolerance originated from 'CS'. Table 9 shows results from single marker analysis. Since no RLC QTL was apparent at this location (Fig 11a), this QTL appears to be specific for the situation where the

seedlings are exposed to Al stress. As this location for an Al tolerance QTL in 'CS' has not been reported in the literature to date, it is therefore designated *Qalt$_{CS}$.ipk-3B*.

Table 9. Single marker analysis of markers associated with aluminium tolerance QTLs on chromosome 3B for RLA and RTI

Marker	Chrm.	Dist. (cM)	Source	RLA			RTI		
				F-stat	LOD	R^2	F-stat	LOD	R^2
Xgwm1029	3B	68	'CS'	40.16	6.79	0.42	53.10	8.36	0.49
Xgwm1005	3B	68	'CS'	40.16	6.79	0.42	53.10	8.36	0.49
Xgwm1015	3B	78.7	'CS'	16.71	3.28	0.23	21.58	4.10	0.28

$p < 0.0001$

Chromosome 3B

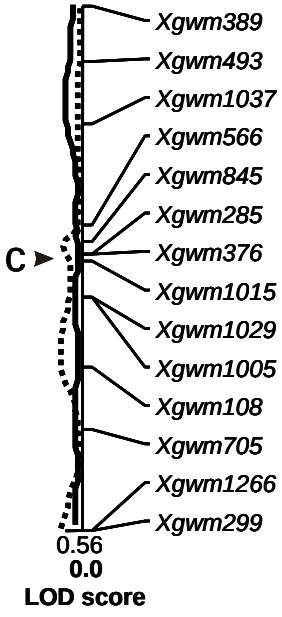

Fig. 11a

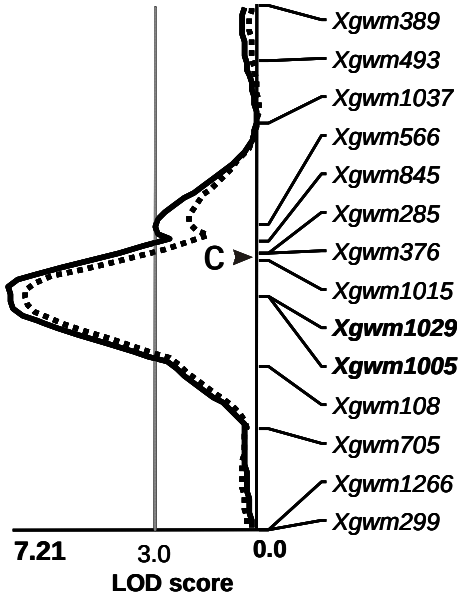

Fig. 11b

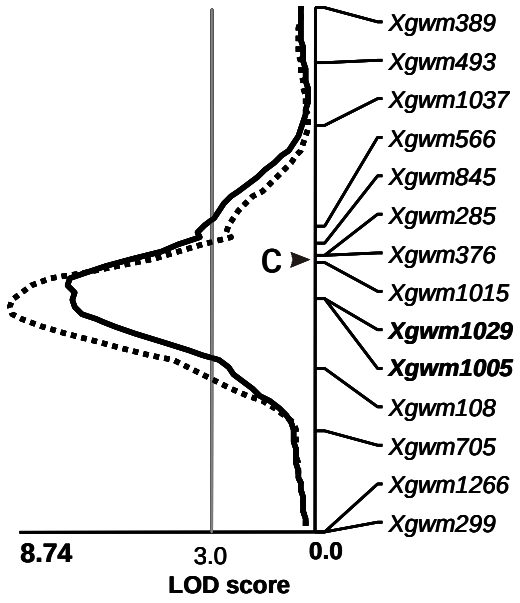

Fig. 11c

Figure 11. QTL interval analysis along chromosome 3B for root length in (a) control medium (RLC) (b) in the presence of aluminium stress (RLA) (c) Root tolerance index (RTI). C-centromere. Regular and dashed lines represent the two separate replicates. Closely linked markers are highlighted.

4.4 Marker validation

Microsatellite markers closely linked to the major Al tolerance QTL on chromosome 4DL in wheat, *Xgwm976* and *Xgdm125*, (Section 4.2.2; Fig. 9) were validated for their association with the trait and utility in MAS. Markers *Xgwm1302* and *Xgwm3156* (Röder et al. unpublished) were also included. The order of the markers from top to bottom along the chromosome is *Xgdm125*, *Xgwm1302*, *Xgwm3156* and *Xgwm976* and the distance between them is 0.0, 1.8 and 0.0 cM respectively. The distance between the markers indicates their close proximity along the chromosome. Marker details are presented in Table A3. Marker validation was performed within a collection of 80 hexaploid wheat accessions from Genebank, IPK, originated from Australia, Brazil and U.S.A., the areas affected with Al stress. A single sensitive accession from Poland and tolerant cv. 'CS' was also included. Phenotypic characterisation was carried out using RTI method. The

accessions were found to be representatives of tolerant, moderately tolerant and sensitive classes. The aim was to find any association between a specific marker allele and Al tolerance.

4.4.1 Allele diversity and marker-trait association

Genomic DNA was isolated and PCR amplification was carried out with primers of *Xgdm125*, *Xgwm1302*, *Xgwm3156* and *Xgwm976*. Amplification products were analyzed using an automated laser fluorescence (ALF) express. 'CS' was used as a control for each run. The output shows that polymorphism exhibited by the markers *Xgwm976*, *Xgdm125* and *Xgwm3156* were not adequate enough to explain the marker-trait association. No specific allele was found to be linked with the trait. For instance, marker *Xgwm3156* had 168 and 170 bp alleles but tolerant and sensitive accessions amplified similar alleles (Fig. 12). But marker *Xgwm1302*, apart from amplifying a locus on chromosome 4D also amplified another locus in the genome (Fig. 13). In order to confirm the genome location of the amplified locus, a nulli-tetrasomic (NT) analysis was carried out. 21 nulli-tetrasomic series of wheat cultivar 'CS' developed by Sears (1966) were amplified with *Xgwm1302*. Since there was no amplification product for NT-4D line for the locus which amplified 182 bp allele in all other lines, it was confirmed that this locus corresponds to chromosome 4D. There was also no product for NT-4B line for the locus which amplified 230 bp allele and hence was concluded that this locus amplified a locus on 4B (Fig. 14). The allele diversity of all validated SSR markers is shown in Table A4.

The 4D locus amplified by marker *Xgwm1302* had 5 different alleles but no association with Al tolerance could be found. On the other hand, the same marker displayed good polymorphism on 4B locus (Fig. 13). The frequencies of alleles of the 4 microsatellite loci were used to calculate the Polymorphic Information Content (PIC) (Table 10). For all the markers on chromosome 4D the PIC values were lower when compared to that locus amplified on 4B by *Xgwm1302*. There were 20 different alleles for the latter locus and the PIC value was as high as 0.91. This was calculated according to the formula $PIC = 1-\sum(Pi)^2$ (Anderson et al. 1993) where Pi is the proportion of the population carrying the i^{th} allele, calculated for each locus.

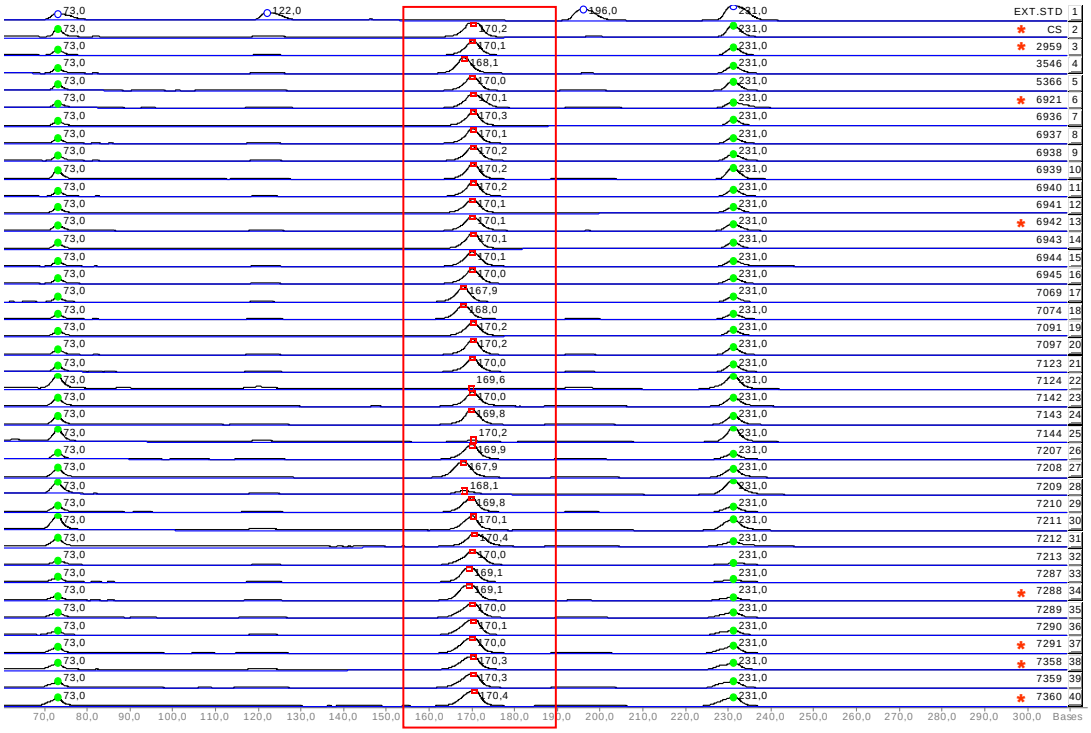

Figure 12. Amplified fragments with *Xgwm3156*. Output of computer software 'Fragment Analyzer'. Topmost peaks are the external standards. Peaks with green colour indicate the internal standards for each lane. Red stars: tolerant accessions.

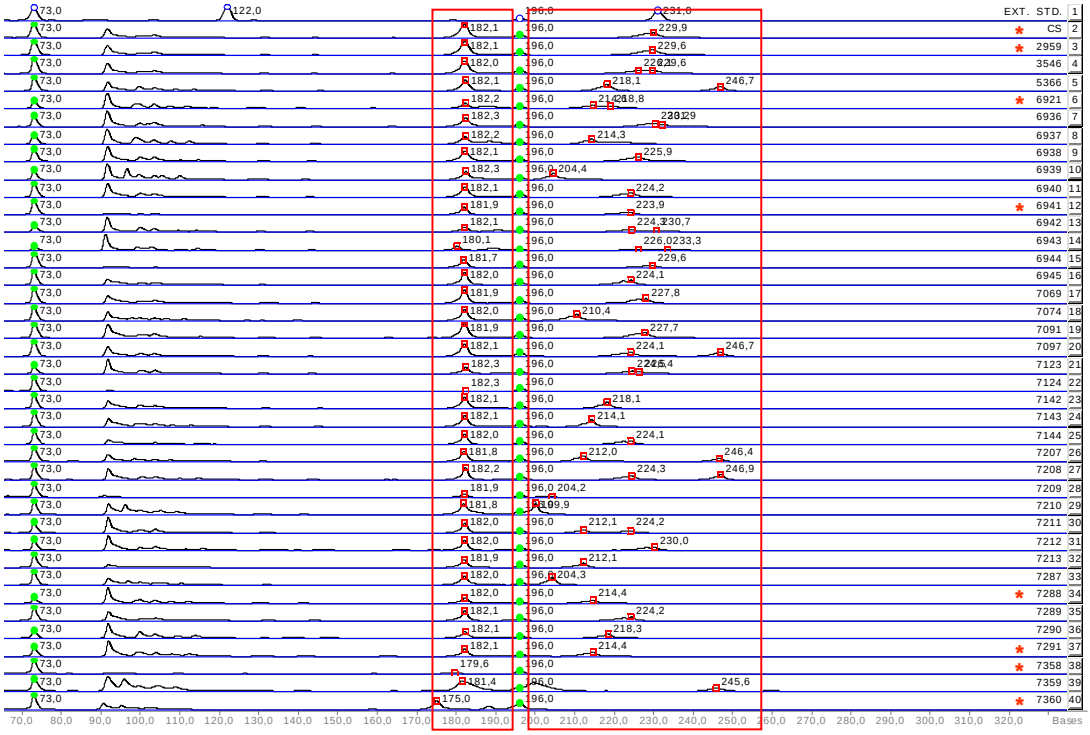

Figure 13. Two loci amplified with marker *Xgwm1302*. Red stars: tolerant accessions.

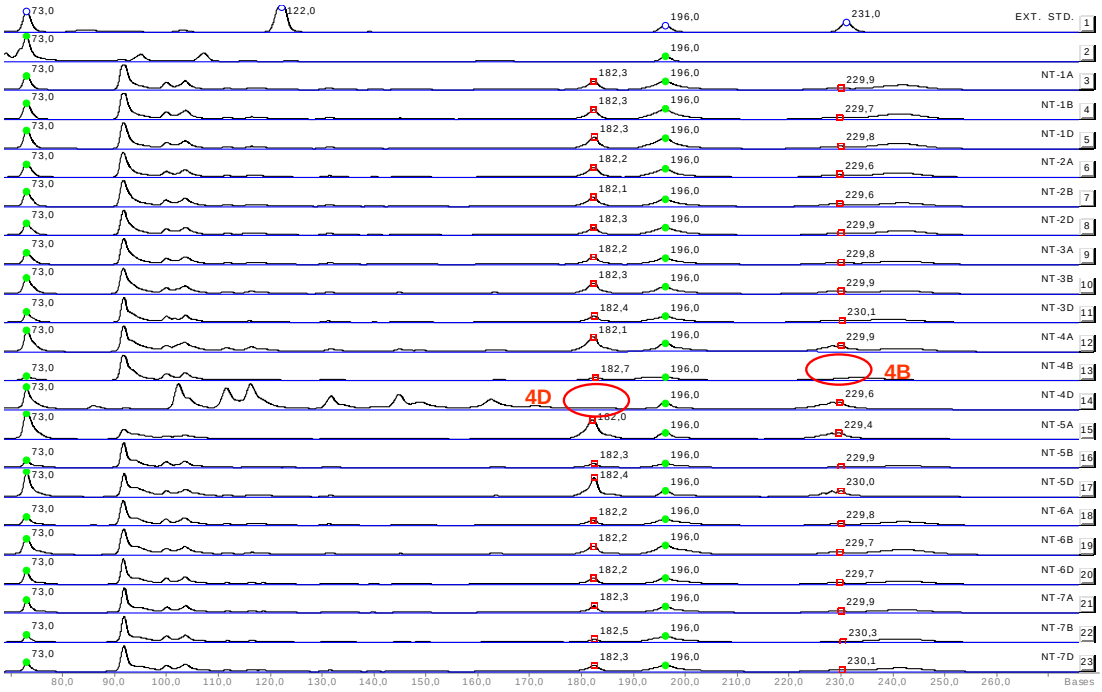

Figure 14. Analysis of nulli-tetrasomic lines of cv. 'Chinese Spring' with *Xgwm1302*.

Table 10. Polymorphic information content (PIC) value and number of alleles amplified per locus by the validated markers.

Marker	Locus amplified	No. of alleles per locus	PIC value
Xgwm976	4D	3	0.38
Xgdm125	4D	3	0.54
Xgwm3156	4D	3	0.44
Xgwm1306	4D	5	0.16
Xgwm1306	4B	20	0.91

Marker *Xgwm1302* amplified 20 different alleles in 80 accessions. It was found that in some tolerant Brazilian accessions an allele of 205 bp was amplified. In order to confirm the specificity of this allele, whether the allele really correlate with Al tolerance, a subset of the original collection, including all tolerant accessions from Brazil, U.S.A and Australia, and some sensitive accessions were evaluated together with a few accessions provided from CIMMYT. Accessions from CIMMYT are the standard Al tolerant cultivars of Brazil used for breeding purposes. Amplification with *Xgwm1302* showed that a 205 bp allele was amplified in most of the tolerant Brazilian accessions/cultivars (Fig. 15, Table 11). Among the materials from CIMMYT, 'Pontagrossa-1' ('PG-1') had the common tolerant allele of 205 bp. 'BH1146', a Brazilian cultivar widely used for genetic studies, had a distinct allele (221 bp) which was not found in any accessions. Another common allele of 218 bp found in three accessions originated from U.S.A and two accessions from Brazil was found to be associated with tolerance trait. One discrepancy was found in an accession from U.S.A where it amplified a 205bp allele but the genotype itself was Al sensitive (RTI - 30%).

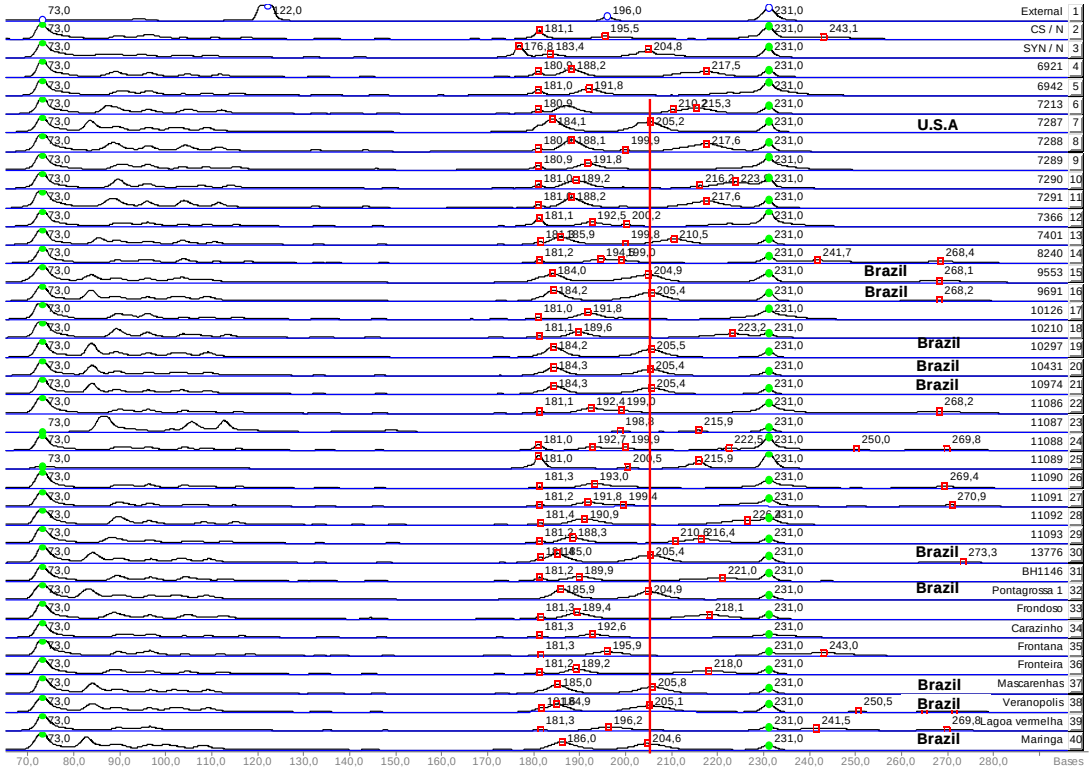

Figure 15. Amplification by *Xgwm1302* on chromosome 4D and 4B. Red line drawn shows the 205 bp alleles specific for most of the tolerant Brazilian accessions.

Table 11. Marker validation of the sub-collection of hexaploid wheat from genebank (IPK) and cultivars from CIMMYT, Mexico.

Serial No.	Accession/ Cultivar	Country	RTI (%)	Score	*Xgwm1302* (4B) - allele size (bp)
1	'CS'	China	55	MT	230
2	TRI 6921	USA	89	T	188, 218
3	TRI 6942	USA	67	T	192
4	TRI 7213	USA	40	MS	215
5	TRI 7287	USA	30	S	205
6	TRI 7288	USA	71	T	218
7	TRI 7289	USA	30	S	192
8	TRI 7290	USA	48	MS	223
9	TRI 7291	USA	83	T	218
10	TRI 7366	USA	71	T	192
11	TRI 7401	USA	21	S	211
12	TRI 8240	Brazil	89	T	194
13	TRI 9553	Brazil	109	T	205
14	TRI 9691	Brazil	78	T	205
15	TRI 10126	Poland	41	MS	192
16	TRI 10210	USA	32	S	190, 223
17	TRI 10297	Brazil	88	T	205
18	TRI 10431	Brazil	96	T	205
19	TRI 10974	Brazil	102	T	205
20	TRI 11086	Australia	60	MT	192
21	TRI 11087	Australia	35	S	216
22	TRI 11088	Australia	58	MT	193, 222
23	TRI 11089	Australia	40	MS	216
24	TRI 11090	Australia	56	MT	193
25	TRI 11091	Australia	37	S	192
26	TRI 11092	Australia	61	T	191, 226
27	TRI 11093	Australia	69	T	216
28	TRI 13776	Brazil	85	T	205
29	*BH1146	Brazil	93	T	221
30	*PG-1	Brazil	84	T	205
31	*Frondoso	Brazil	76	T	218
33	*Frontana	Brazil	67	T	196
34	Fronteira	Brazil	73	T	218
35	Mascarenhas	Brazil	66	T	205
36	Veranopolis	Brazil	89	T	205
37	Lagoa vermelha	Brazil	71	T	196
38	Maringa	Brazil	70	T	205

* Cultivars from CIMMYT

4.5 Mapping QTLs for Acid (Proton) tolerance

The aim of this part of the study was to find QTLs controlling acid / proton tolerance and to compare the results with that of QTLs for Al tolerance. The same set of wheat / *Ae. tauschii* introgression lines used for mapping Al tolerance QTLs was used for this study. As described in the method section (3.2.1), a nutrient solution medium with acid added as HCl is considered as stress condition and a solution with optimal pH for plant growth (6.5) was taken as control. Throughout this investigation, stress was induced by adjusting the pH to 4.5. The seedlings were kept exposed in the respective solutions for 4 days after which the root length was measured and RTI was calculated. 'CS' had a tolerance level of 52% (moderately tolerant) and 'Synthetic 6x' showed 48% (moderately sensitive). RTI of the population ranged between 42-63%.

QTL analysis was carried out. SM and SIM analysis indicated that tolerance to acidity was controlled by minor QTLs (LOD score < 3) (Fig. 16). The QTL for tolerance to acid / proton stress *per se* (from measurement data of root length in acid stress - RLAc) was found to be located on chromosome 6D associated with SSR markers *Xgwm325* and *Xgwm774* (LOD score 2.40, 2.27; $p<0.05$). The QTL originated from 'CS' and accounted for 12% of the phenotypic variation for the trait. For RTI two QTLs were identified, one on chromosome 3D and the other on chromosome 5D. The QTL on chromosome 3D was detected by the markers *Xgwm795* and *Xgdm8* (LOD score 2.55; $p<0.05$) and explained 13% of the phenotypic variation, originated from 'CS'. The QTL on 5D was flanked by *Xgwm174* and *Xgwm182* and had a LOD score of 2.69 explaining 14% of the variance ($p<0.05$). This QTL originated from parent 'Synthetic 6x'.

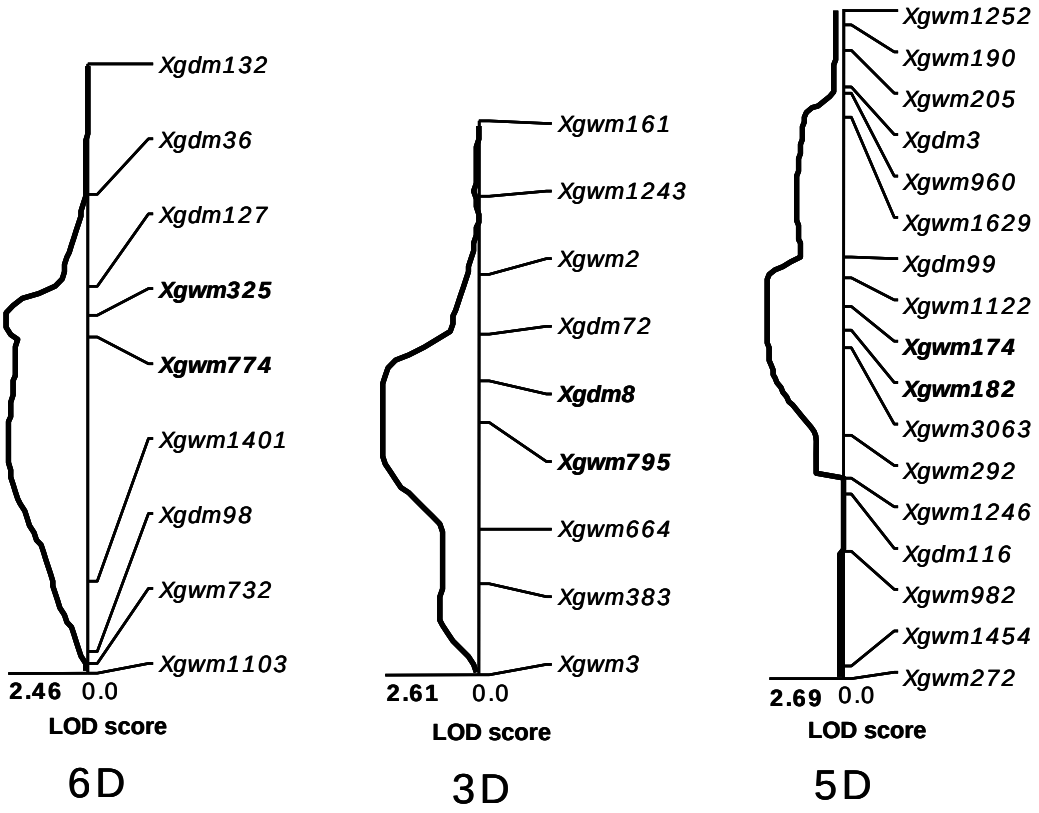

Figure 16. Interval analysis for QTLs for root length in acid stress (RLAc) on chromosome 6D and QTLs for RTI on chromosomes 3D and 5D. Associated markers are highlighted.

4.6 Barley - Screening

80 barley (*Hordeum vulgare* L.) accessions (Genebank, IPK), 28 originated from Australia and 52 from U.S.A were screened in order to assess the genetic variability for Al tolerance. Test was carried out at 20µM Al and pH 4.5. Some accessions are representatives of the Barley Core Collection (BCC). Results show that not a wide variation existed within the barley accessions. RTI ranged from 24-54%, where a high majority of the accessions was sensitive (60%). 31% were classified as moderately sensitive and only 9% of the collection were found to be moderately tolerant. No tolerant accessions were identified. Data are shown in Table A7. 'Dayton', a cultivar from U.S.A generally categorised as tolerant in other studies was shown to be moderately tolerant (RTI - 50%). Cultivars 'Shannon' and 'Grimmett' (Australia), 'James', 'Kansas', 'California Coast' and 'Mo-B-475' (U.S.A) were the other moderately tolerant cultivars identified in this study.

4.7 Mapping aluminium tolerance QTLs in barley

4.7.1 Characterisation

The Oregon Wolfe Barley mapping population consisting of 94 DH lines and parents DOM and REC were investigated to identify the genetic loci controlling Al tolerance in barley. Both RTI and staining method were used to evaluate the tolerance and further for QTL mapping. As the most Al sensitive crop, barley requires a medium with low Al concentration for better differentiation between the parents. As per RTI method under 20µM Al and pH 4.7, parent DOM was tolerant (68%) and performed better than REC which was found to be moderately tolerant (57%). The population showed transgressive segregation stating that some progeny lines performed better than DOM and several lines were worst than REC (Fig. 17). The RTI of the lines ranged from 36-86%.

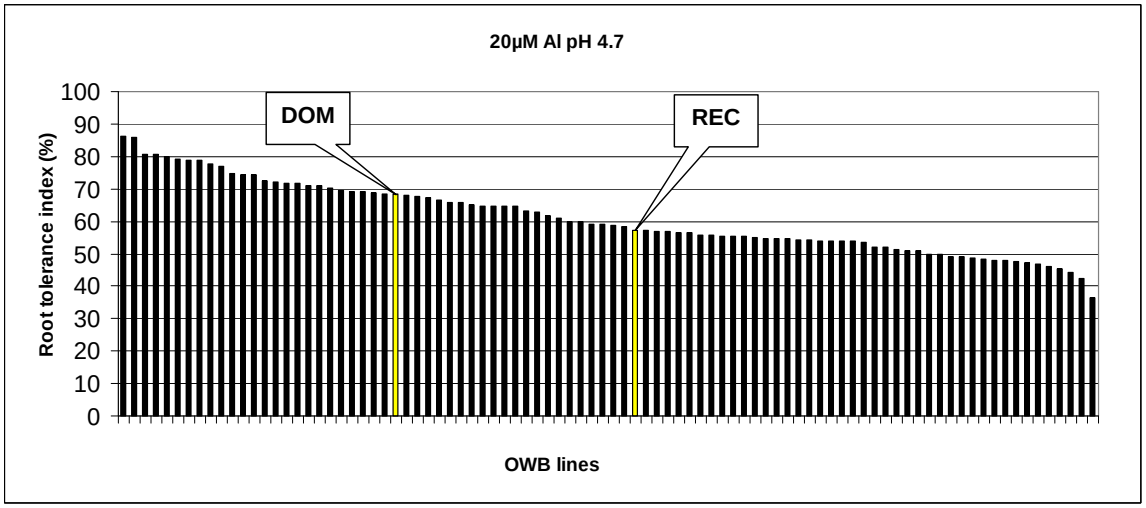

Figure 17. Segregation for aluminium tolerance in Oregon Wolfe Barley (OWB) lines.

4.7.2 QTL analysis

QTL analysis was performed employing a high density transcript map of EST derived SSRs, RFLPs, SNPs and anchor markers. Analysis revealed several minor QTLs on chromosome 3H, 4H and 7H (Fig. 18). Result from single marker analysis is shown in Table 12. QTL on chromosome 4H linked to the marker GBR441 showed the highest LOD score (2.78) and R^2 value (0.16) (p<0.001). Collectively, the QTLs accounted for 36% of the variance in the population. For all loci the positive allele originated from DOM parent. At a lower concentration of 10µM Al and at the same pH of 4.7, two minor

QTLs were identified on chromosome 2 which conditioned Al tolerance. One was associated with a gene *Hot1* which explained 12% of the phenotypic variation with a LOD value 2.50 (p<0.001). The second QTL associated with the EST-RFLP marker GBR1831 accounted for 12% of the variance. Here the QTL source originated from REC parent. For the staining method, the population was treated at a higher stress of 45µM Al, pH 4.7. Analysis of the root re-growth data identified a QTL associated with markers GBM1069 and ABC172 on chromosome 3H as originated from parent REC.

Table 12. QTL mapping in barley - Results from single marker analysis.

Marker	Chromosome	Distance (cM)	Source	R^2 value	LOD score	P-value
RTI - 20µm Al pH 4.7						
GBR441	4H	127	DOM	0.1610	2.78	0.0004
GBR1485	7H	161.3	DOM	0.1093	2.14	0.0020
GBM1043	3H	113.2	DOM	0.1062	2	0.0028
RTI - 10µm Al pH 4.7						
Hot1	2H	190.6	REC	0.1224	2.50	0.0008
GBR1831	2H	169.9	REC	0.1239	2.41	0.0010
GBR0421	2H	47.8	DOM	0.1384	2.17	0.0019
Hematoxylin method - 45µm Al pH 4.7						
GBM1069	3H	21.5	REC	0.1296	2.68	0.0005
ABC172	3H	21.5	REC	0.1235	2.52	0.0008

A summary of the closely linked markers for Al tolerance identified on all chromosomes is shown in Table 13. All markers in close proximity, flanking a distance of 5cM from the QTL, are listed. To explore the putative function of the respective ESTs, a BLASTX (Basic Local Alignment Search Tool) search was performed against the public non-redundant protein database (http://www.ncbi.nlm.nih.gov/sites/gquery). The nucleotide sequences of the barley ESTs are available in v1.5 of the IPK Crop EST database (http://pgrc.ipk-gatersleben.de/cr-est). Only those ESTs whose putative function is relevant to the trait is included in Table 14.

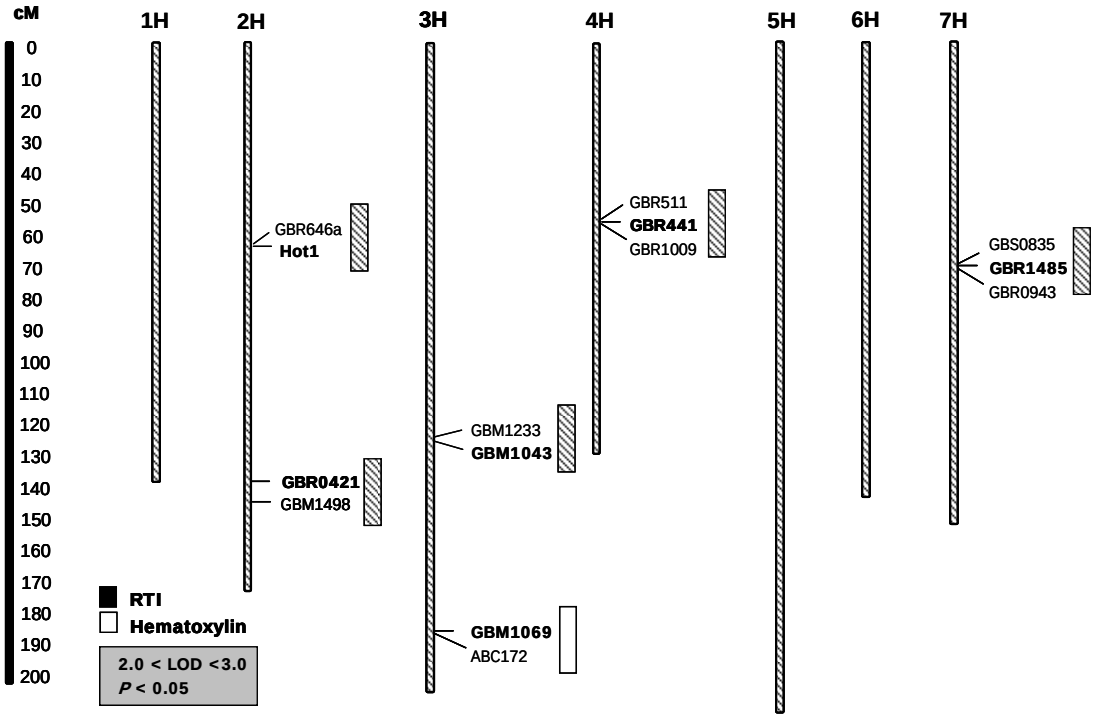

Figure 18. Location of aluminium tolerance QTLs in barley.

Table 13. Markers closely linked to aluminium tolerance locus in barley (proximity of 5cM from the QTL).

Chr.	EST-RFLPs	EST-SSRs	EST-SNPs	Anchor markers
2H	**GBR1831**, GBR1421a, GBR1113, GBR0588, GBR0187, GBR0976, GBR0646a, GBR0879, GBR0516, GBR0337, GBR1074, GBR0095, GBR0060, **GBR0421**	GBM1180, GBM1232, GBM1218, GBM1024, GBM1225, GBM1119, GBM1498	GBS0905, GBS0312, GBS0055, GBS0278, GBS0612, GBS0651,	ABG356 **HOT1**
3H	GBR1657, GBR1425	**GBM1043**, GBM1233, GBM1014, GBS0879, **GBM1069**	GBS0043, GBS0090, GBS0014	ABG499, **ABC172**
4H	**GBR0441**, GBR1009, GBR0511, GBR0064 GBR0026, GBR0016a GBR1149, GBR0304a GBR0884, GBR1053, GBR1422, GBR0031	GBM1452, GBM1236, GBM1067	GBS0506, GBS 0434, GBS 0448, GBS0177 GBS0901, GBS0751 GBS0547,	HVM03
7H	**GBR1485**, GBR0943 GBR0088, GBR0597 GBR1610, GBR0211 GBR1420, GBR0159b	GBM1516, GBM1115 GBM1030	GBS0643, GBS0268 GBS0365, GBS0835 GBS0378	Bmac047b, MWG808

Markers in red- analysed by HS method. Markers in bold- closest markers (see table 12).

Table 14. Putative proteins encoded by ESTs of the closely linked markers for aluminium tolerance

Chr.	EST-markers	Putative function
2H	GBR1831	Putative tRNA isopentenyltransferase [*O. sativa*]
2H	GBR0421	Drought-inducible cysteine proteinase RD21A precursor [*A. thaliana*]
2H	GBM1498	Dehydration responsive element binding protein [*Glycine max*] TINY-like protein [*A. thaliana*]
2H	GBM1024	Harpin-induced protein like [*O. sativa*] NDR1/HIN1-like protein [*A. thaliana*] harpin-induced family protein
2H	GBS0312	PIP2;5 mRNA for plasma membrane intrinsic protein [*H. vulgare*] Plasma membrane intrinsic protein 1 [*T. aestivum*] Plasma membrane integral protein ZmPIP2-1 [*Z. mays*]
2H	GBS0055	TPA: putative cysteine protease Cysteine protease [*T. aestivum*]
3H	GBM1043	Sedoheptulose-1,7-biphosphatase [*T. aestivum*) Sedoheptulose-1,7-biphosphatase precursor [*O. sativa*]
3H	GBR1069	Peroxidase [*A. thaliana*]
3H	GBS0043	Putative nematode-resistance protein [*H. vulgare*, *O. sativa*, *A. thaliana*]
4H	GBR0441	SLT1 – Sodium- and Lithium-tolerant [*A. thaliana*]
4H	GBM1452	Zinc finger (C3HC4-type RING finger) family protein [Arabidopsis thaliana] MYB transcription factor [Arabidopsis thaliana] (EMBL database)
4H	GBS0506	UDP-D-glucuronate decarboxylase [*H. vulgare*, *O. sativa*]
7H	GBR1485	SNF2 domain-containing protein / helicase domain-containing protein / zinc finger protein-related [Arabidopsis thaliana]
7H	GBS0378	Putative ribosomal protein [Oryza sativa] Putative 40S ribosomal protein [Arabidopsis thaliana]
7H	GBS0643	Putative transmembrane protein(TPA regulated locus protein) [Oryza sativa] Transmembrane protein FT27/PFT27-like [Arabidopsis thaliana]
7H	GBS0835	ADP-glucose pyrophosphorylase small subunit a [Hordeum vulgare]. ADP-glucose pyrophosphorylase; glucose-1-phosphate adenylyltransferase [Triticum aestivum]

5 Discussion

5.1 Screening wheat germplasm

In wheat breeding programs, wheat plants are usually evaluated for Al tolerance in acidic soils under field conditions. However, inconsistent phytotoxicity among plots may significantly increase environmental error and decrease accuracy of phenotypic data. In addition, non-stressed treatments are usually applied in a different field with normal soil pH, which may not provide a valid control for proper comparison. An alternative method for evaluating Al tolerance is based on the use of nutrient solution containing a toxic level of Al. Therefore this approach is widely used for screening and genetic studies (Baier et al. 1995; Polle et al. 1978; Samac and Tesfaye 2003).

Genetic diversity is the basis of genetic improvement in plants (Rejesus et al. 1996). Utilisation of new variation for Al tolerance could increase wheat yields in acidic soils without additional inputs. The wheat gene pool consists of diverse biological species, including cultivated, wild and weedy species. But, only a small proportion of the genetic variation for Al tolerance has been studied and utilised in breeding programs (Zhou et al. 2007). This study focus on screening diverse germplasm including hexaploid and tetraploid wheats and wild relative *Aegilops tauschii* in order to evaluate their diversity and potential for Al tolerance.

Hexaploid wheat: 157 hexaploid wheat (*T. aestivum* L.) accessions under study were from selected problematic regions like Australia, Brazil, Canada, India, Poland and U.S.A. Screening parameter was 40µM Al and pH 4.5. Only 27 accessions (17%) were tolerant, 61 (39%) belong to moderately tolerant category and 69 accessions (44%) were found to be sensitive. Only a small percentage of the total wheat accessions screened were tolerant. Nevertheless, genetic variability was observed within the collection as the RTI ranged from 22-108%. Some Brazilian accessions were highly tolerant with well developed roots. The accessions TRI 9553 and TRI 10974 (cv. 'Jesnita') showed exceptional tolerance better than other well known tolerant cultivars like 'BH1146' and

‘Atlas 66’ (tolerant reference). From the Canadian and U.S.A collections sensitive germplasm dominated. The study has revealed that variation for Al tolerance among wheat cultivars was mostly correlated with their origin and the uniform tolerance of the Brazilian accessions could be attributed to the natural occurrence of acidity and Al toxicity in this country.

Some of the well known tolerant cultivars used for breeding purposes are ‘Polyssu’, ‘PG-1’, ‘Fronteira’, ‘Frontana’, ‘Frondoso’, ‘BH1146’ and ‘Carazinho’ all of Brazilian origin, ‘Atlas 66’ from U.S.A and ‘CS’, a land race from China. ‘BH1146’, a spring wheat cultivar (Pontagrosa 1//Fronteira/Mentana) from Brazil and a winter wheat cultivar ‘Atlas 66’ (Frondoso//Redhart3/Noll 28) from the United States have been extensively used as genetic materials to study the inheritance of Al tolerance (Berzonsky 1992; Riede and Anderson 1996). The results from this screening were consistent with the literature (Baier et al. 1995; De Sousa 1998). Hence this Al concentration could be employed for large scale screening and genetic studies. In pedigree analysis, except cv. ‘Wichita’ and ‘Seneca’, all other tolerant cultivars/accessions from U.S.A had their lineage in Brazilian cultivars. The same was the case for one Canadian accession for which the pedigree information was availabe. It is believed that Al tolerant wheat sources may have originated from the Brazilian landraces ‘Polyssu’ and/or ‘AC’ lines which were grown in Rio Grande do Sul where highly acidic soils are very common (Section 2.1; Fig. A1).

De Sousa (1998) screened wheat germplasm for Al tolerance and identified a number of cultivars with high level of tolerance, ‘BH1146’, ‘Embrapa15’, ‘IAC 5-Maringa’, ‘Trigo BR25’, to list a few. In his study tolerance was traced by pedigree analysis to a small number of landraces introduced to Brazil in the early twentieth century. A latest report from molecular screening of diverse germplasm including landraces on the basis of promoter alleles for a gene co-segregating with Al tolerance (Raman et al. 2008) conclude that the alleles associated with Al tolerance in Brazilian cultivars already existed within European landraces and might be carried by Italian and Portuguese immigrants who settled in Brazil. The subsequent strong selection pressure for Al

tolerance on Brazilian soils ensured that these alleles predominated in later cultivar development.

Stodart et al. (2007) evaluated several landraces of bread wheat from 21 different countries and were able to identify some tolerant genotypes which originated from Bulgaria, Croatia, India, Italy, Nepal, Spain, Tunisia, and Turkey. A recent investigation covering a range of wheat accessions including landraces from Asia, and elite breeding lines from Europe and America found some Al tolerant accessions from Asia (Zhou et al. 2007). Their results suggested that some Asian landraces might have different Al tolerance genes than those in American accessions. Because most Asian accessions are not related to American accessions, as their study shows, the Al tolerance in Asian accessions seems not to have originated from Brazilian sources. Probably Al tolerance arose in more than one occasion. This is also clear from the tolerance shown by three Indian accessions and by the cv. 'CS' in this study which has an Asian background.

Screening of germplasm in a wider global scale may unravel different tolerance genes other than from Brazilian sources, so that it might be possible to pyramid the different genes to attain higher yield in Al toxic soils. The reliance on a limited pool of wheat genotypes may lead to a reduction in the genetic variability of commercial cultivars, as well as the possible reduction in the level of Al tolerance due to extensive inbreeding and selection of desirable allele combinations for other traits (Stodart et al. 2007). Since more neutral land areas are getting acidic, it is essential to look for new sources of Al tolerance genes well adapted to these newly developed acidic areas.

***Tetraploid wheat*:** 63 accessions of tetraploid wheat (*T. durum* Desf.) accessions originated from Australia, Canada, Iran, Poland, Turkey and U.S.A were screened under low concentrations of Al (10µM) and pH 4.5. Majority of the accessions (73%) showed moderate response. These include accessions mostly from Iran, Turkey and U.S.A. The single tolerant accession was from Iran. Since there were only few representatives from Australia, Canada and Poland, it was difficult to conclude the real response from this area. While comparatively a large-scale screening of bread wheat genotypes for Al

tolerance has been done in the germplasm pools of many countries, similar data on durum wheat germplasm are lacking. Few investigations classify durum wheat as sensitive (Cosic et al. 1994) but Foy (1996) found some durum wheat from Brazil to be tolerant. Brazilian accessions were not screened in this study due to the lack of material. Probably it would be possible to obtain tolerant germplasm if screened from areas like Brazil where Al toxicity is a natural occurrence.

Nevertheless, this study shows that durum wheat in general is less tolerant than hexaploid wheat and a widespread screening of the materials is essential to find tolerant germplasm better adapted to acid soils around the globe. The comparative higher level of Al tolerance in hexaploid wheat could be due to the presence of additional major genes in the D genome and therefore it is difficult to achieve this level of tolerance in tetraploid wheat.

***Ae. tauschii accessions*:** The diploid grass *Ae. tauschii* Coss. (2n=14) is the D genome progenitor of bread wheat (*T. aestivum* L., 2n=42, AABBDD). The level of genetic variability in the present day species of *Ae. tauschii* is extensive for disease and insect resistance, isozymes and seed storage protein and represents a potential source for the improvement of bread wheat (Lubbers et al. 1991; Spielmeyer et al. 2000; Zhu et al. 2005). The aim of the screening was to study whether genetic diversity existed among the accessions originated from different countries which could be used as a source material for improving Al tolerance in hexaploid wheat via 'Synthetic' wheats. Since *Ae. tauschii* is a wild species, seedlings with uniform root length were difficult to attain. For this reason, RTI method cannot be applied. Staining method serve this purpose since randomly selected roots can be accurately evaluated based on root re-growth of individual plants. To date, there are no reports on Al tolerance assessment in *Ae. tauschii*. In this study, a preliminary screening was undertaken at 45µM Al where all accessions exhibited a moderate tolerance with a slight re-growth of 2-3 millimeter. In fact, this concentration was in general applied for differentiating barley, which is rather sensitive. Wheat differentiating medium require a higher Al concentration, especially for staining method which apply higher stress compared to that of RTI.

At 80µM Al all accessions were found to be very sensitive. No re-growth was observed in any roots and the root tips remained highly stained, an indication of high Al concentration and sensitivity in the root tips. The inability of the species to tolerate Al stress indicate that *Ae. tauschii* lacks the physiological mechanism for tolerance. Probably an extensive screening should be undertaken to find whether tolerant accessions of *Ae. tauschii* exists. In case, if a tolerant accession existed early in the evolution, this might have hybridized with a tetraploid wheat to give rise to a tolerant hexaploid wheat which turned out to be the source of tolerance genes for the later wheat cultivars. In an investigation undertaken to characterise the gene for malate efflux (*TaALMT1- T. aestivum* Al-activated malate transporter1) in wheat which was found to co-segregate with Al tolerance (Raman et al. 2005), a single *Ae. tauschii* accession was also characterised. They found that the tolerant allele *TaALMT1-1* was present in this accession. The allele *TaALMT1-2* was identified in cv. 'Scout 66' which is sensitive. Although this study made an attempt to find a 'diagnostic' marker, they failed to give an explanation for the occurrence of the sensitive allele in 'CS' which is in fact tolerant. It would be interesting to see whether this sensitive allele is present in the *Ae. tauschii* accessions screened in the current study. Characterisation of *Ae. tauschii* in all levels including its physiology and genetics should be undertaken to gain fundamental knowledge which might clarify the existence of a better tolerance in hexaploid wheats compared to that in tetraploids.

***Cytogenetic stocks*:** 'CS'/'Synthetic 6x' single chromosome substitution lines allowed to study the effect of individual chromosomes on Al tolerance. Few substitutions increased the tolerance level of 'CS' indicative of positive factors from 'Synthetic 6x'. Probably the effect of some suppressive factors on 'CS' chromosomes are replaced by the substitution which resulted in an increase in the tolerance level. The substitutions which are significant at this point are 3B and 4D which resulted in considerable decrease of Al tolerance and are important in further QTL mapping experiments in this study.

5.2 QTL mapping in *T. aestivum* L. cv. 'CS' using the D genome introgressions

Wheat arose through spontaneous hybridization of the diploid species *Triticum urartu* (AA genome) and an unknown species (BB genome), to form tetraploid wheat (AABB). Further hybridization with a third ancestral species, *Aegilops tauschii* (DD genome), ~ 10,000 years ago, led to the production of hexaploid bread wheat (2n = 6x = 42; AABBDD) (Devos and Gale, 2000). The diploid D genome progenitor of hexaploid wheat, *Ae. tauschii* (DD), is known to possess rich genetic diversity for resistances to various biotic and abiotic stresses that can contribute to the *T. aestivum* improvement (Assefa and Fehrmann 2004; Börner et al. 2006; Cox et al. 1992; Hussien et al. 1997; Weng et al. 2005; Yang et al. 1998).

The number of loci that control Al tolerance in wheat still remain unresolved. This study is the first of its kind to dissect Al tolerance QTLs using introgressions from wild relative *Ae. tauschii*. The poor performance of 'Synthetic 6x' compared to 'CS' implies two facts: either the accession of *Ae. tauschii* which donated the D genome appears to carry no positive factors for Al tolerance and/or the AB genomes of both parents are likely to be different. Since hexaploid wheat, which represents the outcome of hybridization between tetraploid wheat and *Ae. tauschii*, might have evolved in an environment (*Fertile Crescent*) where Al stress is rare, the wide range of variation and the high tolerance among some of the present day hexaploid wheat cultivars might be due to the selection pressure in acidic and Al toxic environments at later stages. This assumption is supported by Garvin and Carver (2003) who stated the Al tolerance trait in wheat as a derived state rather than an inherent characteristic. Foy et al. (1993) reported similar statement after a case study in sorghum which implies similar evolution in different species of *Graminae*.

The reduction in the tolerance level of the 'CS'/'Synthetic 6x' substitution lines compared to 'CS' implicates that a tolerance gene(s) is present on each of the 'CS' D genome chromosomes and/or that each of the *Ae. tauschii* chromosomes carry a gene(s) promoting sensitivity to Al stress. The largest effect involved chromosome 4D, and the

variation for response among the 4D introgression lines allowed the region responsible for this effect to be defined. Statistically, the introgression lines derived from chromosome 1D, 2D, 3D, 5D, 6D and 7D did not differ significantly from 'CS' even if a small reduction in tolerance was observed when compared to 'CS'. This shows that the main effect QTL might be located on 4D even though some minor factors may be present on other chromosomes. In this experiment two evaluations were made: one is a direct comparison of each introgression line to control parent 'CS' and the other compares each locus of 'CS' with that of *Ae. tauschii* through QTL mapping for associated markers. By QTL analysis, the Al tolerance loci was mapped to the long arm of chromosome 4D near the centromere using SSR markers *Xgdm125* and *Xgwm976*.

Monogenic inheritance for Al tolerance in wheat as controlled by a single dominant gene was repeatedly documented. The importance of this chromosome for the response to Al stress has been known since Riede and Anderson (1996) identified a major gene for Al tolerance on chromosome 4DL in the Brazilian cultivar 'BH1146'. The location of this gene was further defined between *Xgdm125*and *Xpsr914* by Milla and Gustafson (2001), while Ma et al. (2005) identified a QTL linked to *Xgdm125* in cv. 'Atlas 66', *Xgdm125* being one of the markers which tagged the QTL in this study. In a tetraploid wheat background, it was also shown that the introduction of segments of chromosome 4D leads to an improvement in the level of Al tolerance (Luo and Dvorak 1996).

In this investigation, the same QTL on chromosome 4DL was mapped by hematoxylin staining method but with a higher LOD score and R^2 value. This indicates the stability of the QTL over different parameters. The QTL source was shown to be originated from 'Synthetic 6x' which implies that this parent carries the allele with negative effect and the tolerance hence originates from 'CS'. The role of D genome has been confirmed by the earlier results of Gustafson and Ross (1990). All the reports are in consistent with the results from this investigation and shows that the QTL is highly expressed in different genetic backgrounds. This QTL explained 31% of the total phenotypic variation in RTI for the trait. Since the introgression lines cover only the D genome, the rest of the variation which is yet to be explained may be contributed by the A and/or B genomes.

Moreover, the probability of detecting minor QTLs on other chromosomes in the D-genome might not be possible due to the absence of polymorphic markers in those regions.

Several major genes have also been implicated for conditioning the degree of Al tolerance in many wheat varieties. For instance, Al resistance in 'Atlas 66' is determined by a complex genetic mechanism involving more than one gene (Berzonsky 1992). Al resistant NILs that carries only partial resistance from 'Atlas 66' provides indirect evidence to support this assumption (Carver et al. 1993). Tang et al (2002) demonstrated that at least two genetic loci might contribute to Al resistance in 'Atlas 66'. Studies on ditelosomic lines of 'CS' shows that genes located on 2DL, 4DL, 5AS, 6AL and 7AS control Al tolerance (Aniol 1990; Aniol and Gustafson 1984; Ma et al. 2006) suggestive of a complex polygenic inheritance of this trait in wheat.

Synthetic hexaploid wheat provides a convenient instrument for the introduction of desirable genes for several traits from *A. tauschii* to common wheat. The introgression lines used in this study were employed earlier to study the inheritance of genes determining resistance to *Septoria tritici* blotch (Simon et al. 2007), and several morphological and agronomic QTLs (Pestsova et al. 2006). Analysis of RILs of the International Triticeae Mapping Initiative (ITMI) population (Börner et al. 2002) and advanced backcross analysis in wheat (Huang et al. 2003, 2004) demonstrated the ability of genes from *Ae. tauschii* to improve quantitative traits. However, they do not appear to promise a potential source of Al tolerance. A widespread screening of *Ae. tauschii* and a detailed investigation into its Al tolerance genetics and physiology is necessary to come to a conclusion. QTL mapping studies in rice with populations derived from a cross of cultivated (*Oriza sativa* L.) and wild ancestor (*O. rufipogon Griff.*) disclose the contribution of favourable alleles by *O. rufipogon* for Al tolerance (Nguyen et al. 2003). This is not surprising since *O. rufipogon* is a diploid wild species which grows naturally in acidic soils.

In wheat, malate efflux is a well documented tolerance mechanism conditioned by *TaALMT1* (Al activated malate transporter) gene on chromosome 4D which encodes for a membrane protein that facilitates malate release from root tips (Delhaize et al. 1993b; Sasaki et al. 2004). In barley the Al tolerance gene, *Alp*, has been mapped to chromosome arm 4HL, while in rye *ScALMT1* (*Alt4*) is present on 7RS (Fontecha et al. 2007; Matos et al. 2005; Sasaki et al. 2004; Tang et al. 2000) - the latter chromosome arm shares homoeology with the long arm of wheat and barley group 4 chromosomes (Devos et al. 1993). This indicates that Al tolerance through malate efflux is a function of synteny in *Triticeae*.

5.3 QTL detection using 'CS' x 'CS (Synthetic 3B)' DH lines

'CS' x 'CS (Synthetic 3B)' DH population was used to map the QTL as the substitution of chromosome 3B resulted in a reduction of tolerance as is evident from screening studies (section 4.1; *cytogenetic stocks*). A linkage map was developed and QTL analysis was performed. The results from this study reports a major QTL on chromosome 3BL near the centromere (*Qalt$_{CS}$.ipk-3B*) associated with markers *Xgwm1029* and *Xgwm1005*. The existence of a major effect Al tolerance QTL in the centromeric region of chromosome arm 3BL in 'CS' has not been reported in the literature before. Zhou et al. (2007) detected a minor QTL on 3BL in addition to a major QTL on chromosome 4D in a set of progeny derived from 'Atlas 66'. According to the authors, the effect of the QTL was underestimated because of the lack of flanking markers in their study. 'CS' and 'Atlas 66' are unlikely to be related to one another, as the former is a Chinese landrace, and the latter's pedigree ('Frondosa'//'Redhart3'/'Noll28') involves Brazilian cultivars. Thus *Qalt$_{CS}$.ipk-3B* in this study probably represents either a different allele of the 'Atlas 66' minor QTL, or is a distinct locus.

The broad range of genetic variation in wheat, indicative of a complex multigenic inheritance, might be explained by QTLs on chromosome 4DL and 3BL and probably by unmapped QTLs on other genomic regions which would be complicated further by the allelic variants of each locus. According to Macnair (1991) and Macnair et al. (1993) the

complexity of a trait depends on its evolutionary history. The strong selection associated with the evolution of a novel tolerance to a toxic metal ion would be more likely to result in the initial spread of a major gene that gives a reasonable degree of tolerance. Subsequent continued natural selection would lead to more genes spreading that modify and enhance the tolerance making the trait more complex.

The high LOD score and determination coefficient or R^2 value (for both RLA and RTI) for *Qalt$_{CS}$.ipk-3B* indicates that this QTL has a significant role in Al tolerance. Inheritance of Al tolerance in wheat has been extensively demonstrated in literatures. Monogenic control with dominant effect was reported in some wheat cultivars, while multigenic control in others. Berzonsky (1992) proposed that besides a dominant gene in the D genome, genes in A and/or B genomes might also be involved in Al tolerance in wheat cv. 'Atlas 66'.

In addition to the well studied malate efflux (Raman et al. 2005; Ryan et al. 1995, 2001; Tang et al. 2002), phosphate release from the root apex has been proposed as a further means of coping with Al stress in wheat (Pellet et al. 1996, 1997). Investigations with ditelosomic lines of 'CS' by Papernik et al. (2001) reports the reduction in Al tolerance conditioned by genes on chromosome 4DL, 5AS and 7AS which independently act for the same physiological tolerance mechanism through malate release. A comparative study of the Al tolerance and physiological mechanisms in 'Signalgrass' (*Brachiaria decumbens* Stapf cv. Basilik) with that of wheat, *Triticale*, maize and *Arabidopsis* reveals another picture. 'Signalgrass' is one of the most widely sown forage grasses in the tropics with 26.4 million hectares in Brazil alone. Unlike the cereal grasses, it is directly derived from a wild apomictic germplasm that is highly resistance to Al and well adapted to infertile acid soils. The outstanding Al tolerance of 'Signalgrass' compared to the highly tolerant cereal species such as maize and *Triticale* was not associated with the well known mechanism of organic acid release. Instead some strategies based on internal detoxification appear to operate in this species (Wenzl et al. 2001). Whether *Qalt$_{CS}$.ipk-3B* contributes to either malate or phosphate release, or reflects some other tolerance mechanism remains to be investigated.

Taking into account the orthology between homoeologous group 3 of *Triticeae*, sorghum chromosome 3 and rice chromosome 1 (Gale and Devos 1998), *Qalt$_{CS}$.ipk-3B* in this study might corresponds to *Alt$_{SB}$*, a major Al tolerance gene in sorghum (Magalhaes et al. 2004) and to QTL on rice chromosome 1 (Wu etal. 2000; Nguyen et al. 2001, 2002, 2003) and maize chromosome 3 and 8 (Ninamango-Cardenas et al. 2003). Due to the lack of sufficient evidence for QTLs in *Triticeae* group 3, Al tolerance orthology remains limited to *Alt$_{SB}$* and QTLs in rice chromosome 1 so far. The identification of *Qalt$_{CS}$.ipk-3B* might be informative in evolutionary studies through comparative mapping which in turn could provide solutions regarding the physiological mechanisms by which a genotype can tolerate Al toxicity. The association of this QTL with particular SSR markers raises the possibility of improving Al tolerance through marker assisted selection. The potential of SSR markers for MAS is well documented (Gupta and Varshney 2000; Landjeva et al. 2007; Röder et al. 1998).

5.4 Marker Validation

Marker validation involves testing the reliability of the markers to predict the phenotype. This indicates whether or not a marker could be used for routine screening for marker assisted selection (MAS) programs. Once a QTL is tagged with a closely linked marker, the marker should be validated in different genetic backgrounds, in designed and natural populations to ensure its applicability. In this study, marker validation was carried out with the 4 closely linked markers for the major Al tolerance QTL on chromosome 4DL, *Xgwm976, Xgdm125*, *Xgwm3156 and Xgwm1302.* The QTL explained a good proportion of the variance with qualitative (hematoxylin staining - 82%) and quantitative (RTI - 31%) assessment. Hence the closely associated markers could be of great potential for marker assisted introgression or MAS for the development of Al tolerant cultivars in breeding programs.

All the validated markers, in general, were not highly polymorphic within the accessions. The PIC values for *Xgwm976, Xgdm125*, *Xgwm3156 and Xgwm1302* were 0.38, 0.54,

0.44 and 0.16 respectively. This reflects the low level of polymorphism exhibited by the D genome of wheat as is revealed by several authors (Pestsova et al. 2000; Röder et al. 1995; Cadalen et al. 1997). Even though the markers amplified more than two alleles at each locus this could not explain the association of the marker with the trait. On the other hand, *Xgwm1302* amplified a highly polymorphic locus on chromosome 4B, the genome location of which was verified by an NT analysis. NT lines can be used to assign markers to specific chromosomes.

Interestingly, within the original collection, few tolerant Brazilian accessions amplified an allele of 205 bp for *Xgwm1302* on chromosome 4B. A subset of this collection comprising mostly of tolerant accessions / cultivars from the countries under study, together with some standard tolerant breeding cultivars obtained from CIMMYT were investigated. About 30% of the wheat germplasm in CIMMYT includes Brazilian germplasm selected primarily for their Al tolerance and disease resistance as reported by Hettel (1989). The close interaction of breeding programs around the world with CIMMYT is likely to have resulted in the extensive use of few Al tolerant Brazilian cultivars like 'Frontana', 'Fronteira', 'PG-1' and 'BH1146' which were included in this study.

The aim of the subset investigation was to validate whether the same allele of 205 bp will be amplified in the CIMMYT accessions to prove the authenticity of the allele in tolerant germplasm. The results showed that a majority of the tolerant Brazilian accessions (10 out of 16) gave an amplification product of 205 bp (Fig. 15, Table 11). This is an interesting output but, on the other hand, the precise location of amplification is unknown and therefore it was not possible to find out which marker is closely linked to the amplified locus or to which marker does this allele corresponds to. And in fact, no QTL has been reported on chromosome 4B to date. The presence of a different allele of 230 bp in the tolerant cultivar 'CS', of Asian origin, suggest that Al tolerance arose on more than one incident. Appearance of alleles other than 205 bp in the tolerant accessions could be due to recombination which brings together new allelic combinations or due to mutations which create novel alleles. The variability of microsatellites, in general, is due

to an increased rate of mutation which can be explained most frequently due to slippage during DNA replication (Innan et al. 1997).

The occurrence of 218 bp allele in a few tolerant accessions originated from U.S.A and Brazil implies that they share the same Brazilian gene pool. The likely origin of the Al tolerance gene is shown in pedigree chart (Fig. A1). From a historical perspective, this is similar to the transfer of the semi-dwarfing genes (*Rht* genes), the source of which is from Japanese wheat varieties (root source from Norin 10) to the U.S.A and then to the whole world as a result of the wheat improvement programs in U.S.A and CIMMYT, Mexico in the 1960s. Later on, *Rht* genes were mapped on chromosomes 4B and 4D respectively and tagged with potential markers applicable for MAS (Börner et al. 1997; Ellis et al. 2002).

In wheat, *TaALMT1* gene encodes a membrane bound protein (Yamaguchi et al. 2005) which confers Al-activated malate efflux and greater Al tolerance both mapped to the same position on chromosome 4DL. Analysis of the exon sequences of *TaALMT1* has, to date, only two alleles identified neither of which is diagnostic of Al tolerance (Sasaki et al. 2004; Raman et al. 2005). Raman et al. (2006) also reported the utility of CAPS, indels and SSR markers that targeted different regions of the *TaALMT1* gene. They stated that despite of the advantages of the *TaALMT1*-based SSR marker, it cannot be used for MAS because the alleles did not differ sufficiently.

An instance of partial success in MAS was met with the well known dwarfing gene *Rht8* originated from the Japanese landrace *Akakomugi*. The gene was mapped to chromosome 2D in bread wheat by Korzun et al (1998) and was found to be tightly linked to the microsatellite marker *Xgwm261*. This marker has been utilised internationally to screen diverse germplasm, especially the allelic variant of 192bp at this locus has been taken as 'diagnostic' for *Rht8* in many southern and central European wheats (Worland et al. 1998). Later on Ellis et al (2007) found several instances were the 192bp allele of *Xgwm261* was not associated with height reduction and concludes that this allele cannot be always used as 'diagnostic' for *Rht8* gene. This reveals that the possibility of using

even a tightly linked marker for a particular trait could be restricted to a specific gene pool where the cultivars share a common lineage. This was reflected by the current study where the 205bp allele was frequent in many Brazilian accessions/cultivars which might have originated from old landraces possessing this allele. The presence of a different allele in other tolerant Brazilian wheats could be attributed to recombination and/or mutation of the microsatellite marker.

To conclude this part of investigation, it was not possible to find marker-trait correlation for markers on chromosome 4D even though the amplification product on another locus (4B) was found to be interesting. This also demonstrates the difficulty to find 'diagnostic' markers for Al tolerance as is the case with other quantitative traits. In addition, the low level of polymorphism displayed by the microsatellites on chromosome 4D makes the situation more difficult. More polymorphic markers need to be developed which could amplify unique alleles and explain the association with Al tolerance. This can facilitate approaches like MAS and map-based cloning of Al tolerance genes in the future.

5.5 Mapping QTLs for Acid (Proton) tolerance

Growth of crop plants is limited in acid soils due to complex stress factors which consist of a series of toxicities and nutrient deficiencies (Kochian 1995; Salazar et al. 1997). Acid soil syndrome consists of phyto-toxicity to excess ions, such as Al^{3+}, Manganese (Mn^{2+}), and protons (H^{+}), and deficiency of essential nutrients including phosphorous (P), calcium (Ca), and Magnesium (Mg) (Lopez-Bucio et al. 2000; Horst et al. 1999; Rengel and Zhang 2003). Rhizo-toxicity of Al is among the most important of the stress factors that cause severe inhibition of root growth and thus enhancement of drought sensitivity of crop plants (Foy 1988) in acid soils. Much of the research is concentrated on Al^{3+} toxicity in several crop species and also in the model dicot, *Arabidopsis thaliana*. In contrast, little is known of the genes that control acid or H^{+} or proton tolerance, although H^{+} rhizo-toxicity causes severe inhibition of root growth of wheat, *Arabidopsis* and spinach in hydroponic culture (Kinrade 1998, 2003; Koyama et al. 2001; Linkes et al. 1997; Yang et al. 2005). Calcium displacement by protons in the root tip cell membrane is known to be

the part of toxic action (Kinraide et al. 1994). Proton rhizo-toxicity is also observed under certain soil conditions such as organic acid soil (Lumsdon et al. 2005) and acid sulphate soil (Rosicky et al. 2004). Therefore identification of genes that regulate acid (proton or H^+) tolerance is also important for the molecular breeding of crops tolerant to acid soils. Since acidity and Al toxicity are associated together, an interesting point is; whether Al and acid tolerance are regulated by similar physiological mechanisms encoded by the same genetic factors or do they follow distinct patterns?

An investigation was undertaken using the D genome introgression lines which was used for mapping Al tolerance QTLs, in order to have a good comparison. A major QTL for Al tolerance was identified on chromosome 4DL. For acid tolerance, a single QTL for RLA was found on chromosome 6D. But QTLs for RTI were identified on chromosome 3D and chromosome 5D. No QTLs for Al tolerance were mapped on chromosome 6D, 3D or 5D so far. This shows that Al and acid (proton) tolerance are controlled by different genetic loci and hence it is possible that they display different tolerance mechanisms. In contrast to the large amount of literature on the genetics and physiology of Al tolerance, little is known about how plants cope with proton stress. To date, there is no report about acid tolerance studies in wheat. In barley, Stolen and Anderson (1978) found that both pH (acid) and Al tolerance are controlled by a locus on chromosome 4H.

QTL mapping studies in the model plant *A. thaliana* reveals that the major QTLs for acid (proton) tolerance did not overlap with the identified Al tolerance QTLs. This indicates that the major genetic factors controlling Al and proton tolerance are clearly distinct as shown in the *Landsberg erecta* / *Columbia* RIL population used in their study (Ikka et al. 2007). This outcome assumes importance in light of the discovery of Al tolerance gene conservation in monocots and dicots. A homolog of the wheat *TaALMT1* named *AtALMT1* was identified in *Arabidopsis* (Hoekenga et al. 2006). This makes it easier to make a general statement that, in wheat like in *Arabidopsis*, acid (proton) tolerance might be controlled by different genetic loci as is shown in our study. Further research in wheat using specific populations for acid tolerance is necessary to come to a precise conclusion. A recent research reported that zinc finger protein (STOP1- *sensitive to proton*

rhizotoxicity) is essential for proton tolerance in *Arabidopsis* but it is found to co-regulate a key gene in Al tolerance. The *stop1 Arabidopsis* mutant lacked the induction of the *AtALMT1* gene which encodes a malate transporter, which is associated with Al-induced malate exudation. It is not known whether *stop1* is involved in the mechanism of phenotypic variations of the proton and Al tolerance of *Arabidopsis* (Iuchi et al. 2007).

Generally, pH expresses a logarithmic function and hence even small changes in pH values may drastically influence the availability or excess of elements in the soil or solution cultures. And hence acid or Al studies should be conducted with utmost care given to pH adjustments during the solution preparation. Furthermore, the solutions should be changed daily to control the pH.

An interesting research in spinach reveals the significance of acid tolerance in plants. Spinach is a vegetable with high oxalate concentration in its tissues and its efflux is stimulated by Al treatment. Oxalate efflux from the roots plays a major role in the Al tolerance of buckwheat (*Fagopyrum esculentum*), which is considered to be one of the most Al tolerant species. Despite of the presence of high oxalate in spinach, they are relatively sensitive to Al stress. The influence of pH itself on root growth may have masked the potential for oxalate to protect the plants from Al toxicity. Hence the research concludes that tolerance to both acid and Al toxicity is necessary for a plant to survive in acidic, Al toxic soils (Yang et al. 2005).

5.6 Screening barley

The response of barley genotypes originated from Australia and U.S.A under 20µM Al, pH 4.5 showed a narrow range of genetic variation. Most of the accessions were sensitive where the RTI ranged between 24 and 39%. Since no tolerant accessions were identified, it is apparent, at least from this study, that the potential to improve the tolerance in barley is remote. In fact, Australia and U.S.A has large acidic areas where there is a possibility to obtain tolerant cultivars. Perhaps the collection under study which was chosen randomly did not include tolerant accessions except the standard accession HOR3111

(cv. 'Dayton'). 'Dayton' is one of the tolerant barley cultivars used for genetic studies which was categorised as moderately tolerant in this study. Probably the screening pH should be raised to 4.7 at the same Al concentration in order to get a better differentiation among the moderately tolerant accessions. Variability for this trait in *H. spontaneum* (C. Koch) and other wild species originating in acid soils should be evaluated. Another potential approach would be to transfer tolerant genes from wheat (*T. aestivum* L.) or rye (*S. cereale* L.) into barley. Al toxicity is affected by many factors such as pH, concentration of Al, temperature and concentrations of salts in the solution. The concentration of calcium greatly influences the degree of Al toxicity (Rengel 1992). Therefore Al tolerance evaluation may change in different solutions making the comparison between laboratories difficult even at the same pH and Al concentration.

5.7 QTL mapping for aluminium tolerance in barley

Barley (*Hordeum vulgare* L.) is an excellent system for genome mapping and map-based analysis. Main advantages of barley for genetic studies are its diploid nature (2n=14) with seven cytologically distinct chromosomes (Bennett and Smith, 1976), and they are homoeologous to those of common wheat (Moore et al. 1995), which allows barley to serve as a model system for the more complex polyploid cereals. However, Al tolerance studies was difficult in barley because on one hand barley is sensitive when compared to other crops and on the other hand the property of Al ions to hydrolyse in a pH dependent manner makes it difficult to control the Al concentration (different species of Al ions exist at different pH but Al^{3+} is the most phytotoxic) while adjusting the pH of the nutrient solution.

The aim of this investigation is to map Al tolerance QTLs in barley using the Oregon Wolfe Barley DH population. The saturated transcript map of barley serves as a valuable resource for the analysis of gene-trait associations and for comparative genome analysis where the functional orthologs in other crop species can be compared and exploited for the improvement of different traits (Stein et al. 2007). Analysis from the present study revealed several minor QTLs affecting Al tolerance in barley. QTLs were found on

chromosome 3H, 4H and 7H all originated from DOM parent at 20µM Al, evaluated on the basis of RTI method. Minor gene effects for Al tolerance have also been suggested from other researches (Echart et al. 2002; Raman et al. 2005a; Reid 1970). The minor QTLs found on chromosome 3H and 4H in this study were consistent with the reports of Raman et al. (2005a) who identified several QTLs for root elongation under Al stress on 3H, 4H, 5H and 6H in an F_2 population from 'Ohichi' / 'F6ant28b48-16'. In many cases where monogenic inheritance for Al tolerance was reported, a major effect QTL was found on chromosome 4H. Minella and Sorrells (1997) mapped the Al tolerance gene *Alp* on 4H in cv. 'Dayton', one of the most Al tolerant barley cultivars, and Tang et al. (2000) confirmed this location. The occurrence of a single major gene on chromosome 4H was also reported by Raman et al. (2002) and Wang et al. (2006).

In this study, both parents might have contributed favourable alleles for Al tolerance as was clear from the transgressive segregation of the progeny lines. The presence of different major and/or minor genes with additive effects resulted in recombinant genotypes with greater tolerance than the better parent which could be used as breeding source for developing Al tolerant barley cultivars. At a lower concentration of 10µM Al, two minor QTLs were detected as originated from REC. One QTL is putatively linked with the gene *Hot1* and the other with EST-RFLP marker GBR0421 on chromosome 2H. A report from Hong and Vierling (2000) states that *Hot1* locus encodes the *Hsp101* gene (heat shock protein), necessary for acquired thermo-tolerance in plants, after a study in *A. thaliana*. Interestingly, Richards et al. (1998) found among several genes previously reported to be regulated by Al, one gene encoding a heat shock protein to be down-regulated in a study in rye which probably might be the same gene associated with Al tolerance in barley. BLASTX search performed through the CR-EST (IPK) and NCBI database provided information about the potential biological function of the ESTs. The ESTs on chromosome 2H coded proteins related with stress response. For instance, the putative homolog of GBR0421 in *A. thaliana* encoded a drought-inducible cysteine proteinase. Similarly GBM1498 was associated with dehydration responsive element binding protein and GBM1024 with plant defence related harpin-induced family proteins

respectively. The potential role of NDR1/HIN1-like protein coded by GBM1024 in plant defense was demonstrated by Varet et al. (2002).

The identification of QTLs on chromosome 3H by both methods confirm the fact that some stable genetic factors for Al tolerance are located on this chromosome. Different QTLs appeared under different stress conditions which imply that the expression of tolerance was dependent upon Al concentration. Similar case was reported by Minella and Sorrells (1992). Investigations which reported only a single major gene on 4H were derived from tolerant x sensitive crosses. In the current study, irrespective of the Al concentrations used, both parents were moderately tolerant even if one was superior to the other. It could be possible that DOM and REC had similar QTL alleles for several loci (monomorphic) and hence could not be mapped in this study. Oram (1983) demonstrated the appearance of Al tolerant genotypes in an F_4 population of a cross between two most susceptible barley cultivars (C17115/Weeah) and suggested that transgressive segregation observed might be due to more than one locus which determines Al tolerance. The current study reveals that Al tolerance in barley might be controlled by factors residing in different genomic regions rather than a single gene model, even though the individual contribution is small.

The EST associated with GBM1043 (3H) was found to encode an enzyme of Calvin cycle, Sedoheptulose-1,7-biphosphatase (SBPase), in *O. sativa* and *T. aestivum*. The increased production of SBPase was reported to enhance photosynthesis and growth in early plant developmental stages (Lefebvre et al. 2005). GBR1069 and GBS0043 on chromosome 3H coded peroxidase enzyme (*A. thaliana*) and a putative nematode-resistance protein respectively (*A. thaliana*, *O. sativa* and *H. vulgare*). In general, the peroxidase activity is stimulated by different biotic and abiotic stresses.

The EST associated with the closely linked marker GBR0441 for the 4H QTL corresponds to a putative Sodium- and Lithium-tolerant (*SLT1*) gene locus in *Arabidopsis thaliana*. Whether this gene play the role of a general metal stress tolerance in different species is not known. An EST in close proximity, GBM1452, coded for Zinc finger

family protein and MYB transcription factor in *A. thaliana*. A recent research reported that Zinc finger protein (STOP1) is essential for acid tolerance and is also found to co-regulate a key gene in Al tolerance in *A. thaliana* (Iuchi et al. 2007). Probably the same protein family might play a similar role for regulating Al tolerance in barley. MYB proteins are a superfamily of transcription factors that play regulatory roles in developmental processes and defense responses in plants (Yanhui et al. 2006). The findings from Ma et al. (2004) indicate that the gene controlling Al tolerance on chromosome 4H is identical to that for Al-activated citrate secretion in barley since both are tightly linked to SSR marker Bmag353. Secretion of citrate is one of the Al tolerance mechanisms in barley. The ESTs associated with the 7H QTL in this study mainly coded for ribosomal and transmembrane proteins together with proteins related to the Zinc finger family. Perhaps the membrane bound proteins are keys to regulate Al tolerance through organic acid secretion which is a well documented mechanism for Al tolerance in many cereal crops. In conclusion, many ESTs associated with Al tolerance might be Al stress regulated genes. Probably the combined effect of all the genes which results in the production of different stress regulating proteins through a network of signal transduction and metabolic pathways, might contribute the tolerance to Al in barley as is evident from the identification of different minor QTLs in this study.

Genetic analysis of Al tolerance in highly diverse barley genotypes (Minella and Sorrells 1992; Tang et al. 2000; Raman et al. 2002, 2003, 2005a; Ma et al. 2004; Wang et al. 2006) indicates a narrow genetic base for Al tolerance in barley. The present available genetic variation could be due to mutations in genes that may lead to variation in tolerance levels. Minella and Sorrells (1992) reported a little chance for improving Al tolerance in barley. In this case, genetic manipulation could be an alternative way to improve the tolerance by exploiting the genes from related species. In barley, no Al tolerance genes has been isolated and characterised so far. Since Al could have diverse effects and act differently in different species (Delhaize and Ryan 1995), the Al induced gene expression change of barley deserves further study.

The QTLs on chromosome 3 and 4 receives attention because of its syntenic relationship within *Triticeae* so also in other members of *Poaceae* (detailed discussion in section 5.8). Barley, wheat and rye detoxify Al by secreting organic acid anions from the roots (Ma et al. 2001) but they may differ in the process leading to the secretion. Therefore, although the gene for Al-activated secretion of organic acid anions might be located on similar chromosomal regions in barley, wheat and rye, the structure and expression of the gene that regulates the secretion process may differ, resulting in different secretion patterns, different compounds secreted (barley-citrate; wheat-malate; rye-citrate and oxalate) and different secretion amounts so also the number and effect of genes involved may differ which is reflective of their tolerance levels (rye>wheat>barley).

5.8 Conservation of aluminium tolerance QTLs / Genes in Grasses

The grass family *Poaceae* is highly diverse and contains ~ 10,000 species (Kellogg 2001) many of which are our most important staple crops. The extremely broad adaptation of the grasses to diverse environments (Kellogg 1998), including adaptation to the widespread Al toxic acid soils, raises the question whether adaptation to Al toxicity in different grass species is associated with mutations in a limited number of genes or whether a far more diverse range of genes contributes to Al tolerance. Studies indicated that the Al tolerance gene(s) are conserved in grass species.

Comparative mapping using RFLP markers not only revealed the remarkable similarity among the genomes of the three diploid ancestors of wheat (*T. urartu*, *A. squarrosa* and the still unrevealed donor of the B genome) but also allowed the extension of the comparisons to genomes of other *Triticeae* members - barley and rye. Although the rye genome was found to be re-arranged relative to that of wheat by a few major translocations most RFLP probes revealed that the same loci in wheat, barley and rye were arranged in exactly the same order along large stretches of their chromosomes (Fig. 19; Devos et al. 1993). In *Triticeae* tribe, the major gene conferring Al tolerance in wheat, barley and rye seems to be due to homoeologous loci on the long arm of the group 4 chromosomes. Several investigations including this study mapped Al tolerance QTL on

chromosome 4DL in wheat (Luo and Dvorak 1996; Ma et al. 2005; Riede and Anderson 1996). In barley a QTL with major effect was mapped by Tang et al. (2000) on 4HL. In rye which is the most Al tolerant species of *Triticeae* and one of the most tolerant in cereal species, several major Al tolerance QTLs were reported. But the one on chromosome 7RS receives special attention because the short arm of chromosome 7 of rye shares homoeology with wheat and barley group 4 chromosomes (Devos et al. 1993).

Recently, using PCR primers designed from wheat *ALMT1* gene, a rye gene *(ScALMT1 - Secale cerealeALMT1)* was amplified, cloned and sequenced. Subsequently, this gene in rye was found to be located on 7RS by PCR amplification using wheat-rye addition lines (Fontecha et al. 2007). This suggests that Al tolerance genes in wheat 4DL, barley 4HL and rye 7RS are orthologs, originated from a common ancestor. Several investigations have established the colinearity within *Triticeae* members (Börner et al. 1998, 1999a, 1999b), so also between genomes of species belonging to different tribes within the *poaceae* (Gale and Devos, 1998). One of the mechanisms for Al tolerance in the *Triticeae* is Al exclusion from the root tips (Kochian 1995). This mechanism is mediated by Al-activated release of organic acids from roots (malate, citrate and oxalate), which chelate Al^{3+} in the rhizosphere and prevents its entry into the root apex. This physiological evidence is strongly supported by the orthologous loci controlling Al tolerance in the *Triticeae*.

The rice genome is one of the smallest among the grasses and has become pivotal to grass genetic studies. The relationship between the three major cereals wheat, maize and rice based on the genetic maps revealed that the conservation of gene order was restricted to a small number of rice linkage segments despite these species having been isolated for up to approximately 60 million years. This made possible to describe the genes not only on the chromosome maps of individual maize and wheat but also of other crop species (sorghum, sugarcane and foxtail millet) in terms of these segments (Fig. 20; Moore et al. 1995). Barley / rice colinearity has been well demonstrated by Stein et al. (2007). In their study among the 1,032 genetically mapped barley ESTs, 46% were assigned to syntenic linkage groups of rice. Individual chromosomal pairs such as barley 3H / rice Os01

showed a colinear organization over almost their entire length with few exceptions. In this context, the Al tolerance QTLs on chromosome 3H in barley may find its possible orthologs in rice chromosome 1, the information of which would be helpful to get an indepth knowledge of barley Al tolerance genetics which might aid in the improvement of the trait.

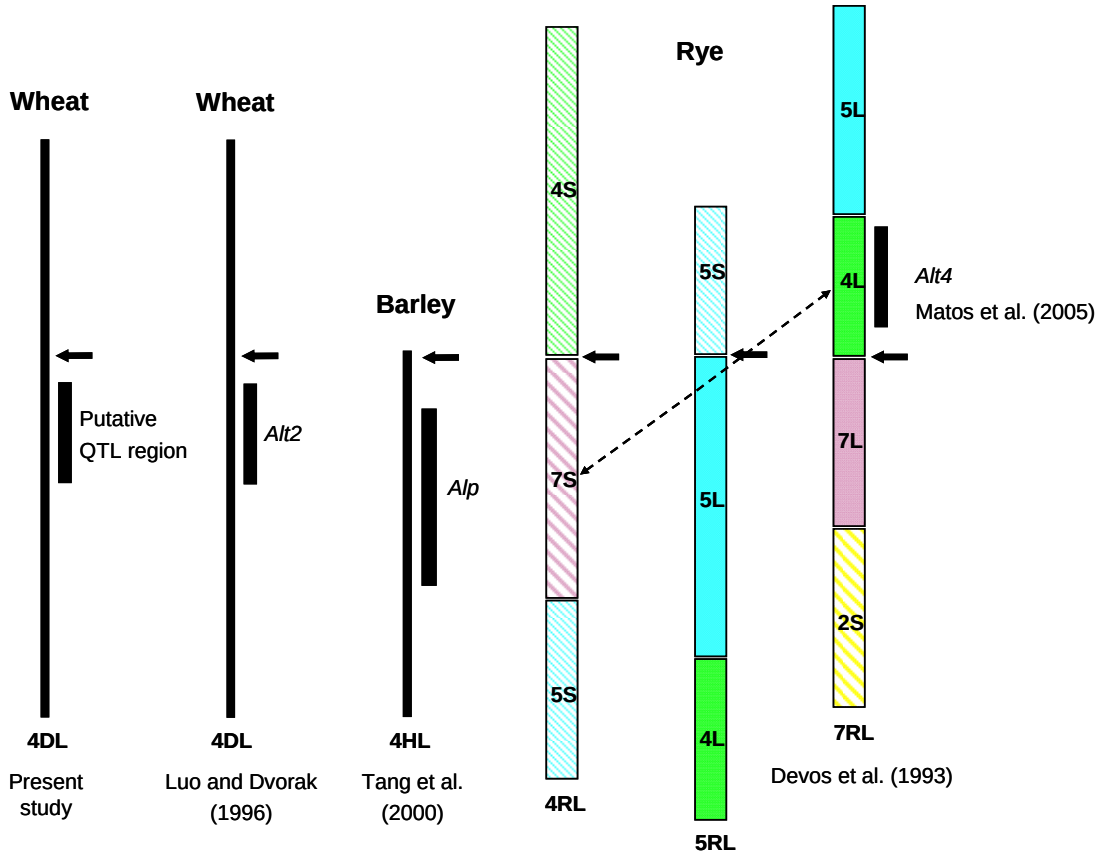

Figure 19. Synteny for aluminium tolerance locus in *Triticeae* (wheat, barley and rye). Bold arrows shows position of centromere. Rectangular black bars represent QTL position. Dotted arrow in rye shows the evolutionary translocations.

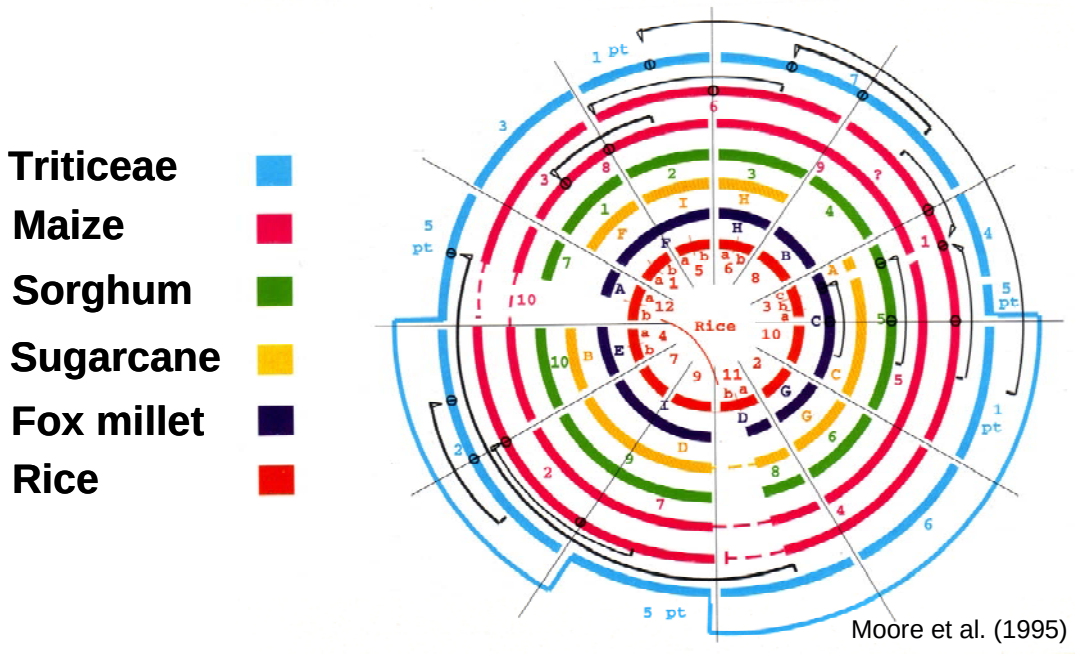

Figure 20. The circular model of grass genome.

Note: Alignment of the genomes of six major grass crop species with rice linkage segments (LS), whose order reflects the circularized ancestral grass genome. The LS are defined by radiating lines and formed into chromosomes (broken colour-coded and numbered lines). Thin dashed lines corresponds to the duplicated segments. Inversions within LS are not shown. LS forming parts (pt) of *Triticeae* chromosome 5 are shown as a series of segments connected by coloured lines. Alignment is based on the genetic map of D genome of wheat. Red line indicates the duplicated segments shown as blocks11b and 12b. Insertion of one segment into another is shown by black lines with arrow indicating the direction and point of insertion. The points of chromosome breakage involved with insertion events are indicated by black bisected circles.

Genetic analysis for Al tolerance in sorghum, a member of the tribe Andropogoneae, revealed that this trait was encoded by a single major locus, *Alt* $_{SB}$ (Magalhaes et al. 2004) on chromosome 3 which is not a syntenic region of group 4 chromosomes of wheat, barley and rye. Instead it maps to a homoeologous region of *Triticeae* chromosome 3, rice chromosome 1 and maize chromosomes 3 and 8. QTLs associated with Al tolerance in maize and rice has also been mapped on these chromosomes (Ninamango-Cardenas et al., 2003; Nguyen et al., 2001). *Qalt*$_{CS}$*.ipk-3B* in wheat and the QTL on chromosome 3HL

in barley from this study might correspond to the above mentioned colinearity of *Triticeae* group 3.

The comparative frame work of molecular markers can be used for map-based prediction of the location of QTLs / genes that determines important traits. For instance, in diploid oats, molecular markers for Al tolerance QTLs have been identified through comparative mapping and QTL analysis. RFLP markers were tested in regions were comparative mapping indicated the potential for orthologous QTLs for Al tolerance in other grass species. Four QTLs were identified of which the major QTL is a possible ortholog of group 4 chromosomes of *Triticeae* (Wight et al., 2006). Likewise, the sorghum major QTL (Alt_{SB}) was also identified through synteny-based comparative mapping (Magalhaes et al. 2004). Recently, a homolog of the wheat *TaALMT1* named *AtALMT1* in *Arabidopsis* was found and is considered to be a good candidate to be involved with Al tolerance (Hoekenga et al. 2006).

The use of comparative mapping to integrate information from genomes of a range of plant species to reference genomes such as that of rice or *Arabidopsis* has become pivotal to modern plant genomics. Chromosomal re-arrangements that disrupt colinearity may reduce the likelihood of finding an ortholog of a gene/QTL of interest in the expected syntenic position of a single given reference genome. This issue may be mitigated through broader evolutionary comparisons among different members of grasses, the knowledge of which could be used to improve the Al tolerance of economically important grass species.

6 Summary

The aim of this investigation was to map the quantitative trait loci (QTLs) controlling aluminium (Al) tolerance in wheat and barley with the aid of a nutrient solution culture approach. To gain a comprehensive understanding of the Al tolerance genetics a widespread screening of wheat and barley was undertaken. Closely linked markers for Al tolerance in wheat were validated to assess the potential of the markers for marker assisted selection of tolerant cultivars.

In total 320 wheat accessions consisting of 157 hexaploid (*T. aestivum* L.), 63 tetraploid (*T. durum* Desf.) and 83 *Ae. tauschii* Coss. accessions, and 17 cytogenetic stocks were screened for Al tolerance evaluation. Hexaploid wheats showed a wide range of variation from highly tolerant to highly sensitive which shows obviously the potential to improve Al tolerance in wheat. Exceptional tolerance was shown by some Brazilian accessions and the pedigree of most of the tolerant accessions originated from other countries had their lineage in Brazilian accessions. The high Root Tolerance Index (RTI) shown by the Brazilian cultivars could be attributed to the natural occurrence of highly acidic Al toxic soils in this country. In contrast, the tetraploid wheats showed comparatively less tolerance than hexaploids even under low stress conditions. *Ae. tauschii*, the D genome progenitor of hexaploid wheat, was also found to be sensitive. From the cytogenetic stocks, 'Chinese Spring' ('CS') x 'Synthetic 6x' single chromosome substitution lines were investigated. The distinct reduction in tolerance of the 3B and 4D substitution lines were exploited to map the QTL(s) on the respective chromosomes in this study.

QTLs for Al tolerance in wheat were mapped using a set of 84 'CS' / *Ae. tauschii* Coss. substitution and introgression lines. A major QTL with LOD score 6.69 ($p < 0.0001$) was found to be associated with the SSR loci *Xgdm125* and *Xgwm976*, both mapping to the centromeric region of the long arm of chromosome 4D. The QTL accounted for 31% of the phenotypic variation for RTI. The stability of the QTL was confirmed using the hematoxylin staining method with which the same QTL was identified for root re-growth by the same SSR loci but with a much higher LOD score (30.54) and phenotypic

variation explained (82%). In both cases the negative QTL originated from 'Synthetic 6x' indicating that tolerance was derived from 'CS'. The study showed that wild relative *Ae. tauschii* harbours negative alleles for Al tolerance.

Another set of 57 doubled haploids (DH) lines derived from a cross of 'CS' x 'CS (Synthetic 3B)' was exploited to develop a linkage map of SSR markers and further for mapping the Al tolerance QTL. Analysis identified a significant QTL ($p<0.0001$) associated with markers *Xgwm1029* and *Xgwm1005* for both Root Length in Aluminium (RLA) and RTI, mapping to the centromeric region of the long arm of chromosome 3B. QTL for RLA had a LOD score of 6.79 and explained 42% of the phenotypic variance. RTI QTL had a LOD score of 8.36 and accounted for 49% of the variance in this population. Since an Al tolerance QTL at this location in 'CS' has not been reported in the literature to date, it is therefore designated $Qalt_{CS}.ipk\text{-}3B$.

SSR markers closely linked to the major Al tolerance QTL on chromosome 4DL, *Xgdm125* and *Xgwm976*, were validated for their association with the trait within a collection of 80 hexaploid wheat accessions. Markers *Xgwm1302* and *Xgwm3156* (Röder et al. unpublished) in close proximity with the QTL were also included. The polymorphism exhibited by the markers was not sufficient enough to explain a marker-trait correlation. But a second locus amplified by the marker *Xgwm1302* on chromosome 4B was polymorphic and a specific allele of 205bp correlated with the Al tolerance in many tolerant Brazilian accessions. The specificity of the allele was tested in a few standard tolerant Brazilian cultivars provided from CIMMYT. The 205bp allele was found to be present among some other unique alleles. Nevertheless, this is an interesting result where the 4B locus should be studied and exploited.

Since Al toxicity is always associated with acidity, an investigation was carried out to find out whether both the traits are controlled by the same genetic loci or they exhibit a different pattern. Hence the D genome introgression lines used to map Al tolerance QTL was evaluated to map acid (proton / H^+) tolerance in wheat so that the results can be compared. This study shows that acid tolerance is conditioned by QTL on chromosome

6D for RLA and by chromosomes 3D and 5D for RTI. None of the QTLs overlapped with Al tolerance QTLs (4DL) indicating that both traits are controlled by different loci.

Screening of 80 accessions of barley (*H. vulgare* L.) showed a narrow range of genetic variation for Al tolerance. RTI ranged from 24-54% where a majority of the accessions were found to be sensitive (60%). No tolerant accessions were identified. QTL mapping in barley was performed in Oregon Wolfe Barley mapping population consisting of 94 DH lines derived from parents DOM and REC. Marker analysis was performed by employing the recently published integrated barley transcript map which has coverage of 1,032 Expressed Sequence Tags (EST)-based markers (EST-SSRs, -RFLPs and -SNPs). Several minor QTLs were identified which were located on chromosomes 2H, 3H, 4H and 7H under different stress conditions using RTI method. With hematoxylin staining method a root re-growth QTL was found to be identified on chromosome 3H. The putative function of the EST-markers associated with Al tolerance was referred through a BLASTX search performed with the CR-EST database (The IPK Crop EST Database) combined with the information from the NCBI (National Center for Biotechnology Information) database in certain cases. Many ESTs coded for stress regulatory proteins but some seems to have a putative role in Al and/or acid stress.

In conclusion, this research establishes a polygenic inheritance for Al tolerance trait in wheat and barley and also presents the possibility to improve Al tolerance in wheat using SSR markers linked to the major QTL *Qalt$_{CS}$.ipk-3B*. The 4DL QTL in wheat demonstrates the well established colinearity of gene order in *Triticeae* and extended to other species in the grass family, the information of which could be utilised for the improvement Al tolerance in economically important cereal crops.

7 Zusammenfassung

Ziel der Untersuchungen war es, quantitativ vererbte Loci (Quantitative Trait Loci, QTL) für Aluminiumtoleranz in Weizen und Gerste mittels Hydrokultur genetisch zu kartieren. Parallel zu einem umfangreichen Screening auf Al Toleranz sollte beim Weizen geprüft werden, ob zum Merkmal eng gekoppelte Marker genutzt werden können um Al tolerante Sorten markergestützt zu selektieren.

Insgesamt wurden 320 Weizengenotypen bestehend aus 157 hexaploiden (*T. aestivum* L.), 63 tetraploiden (*T. durum* Desf.) und 83 *Ae. tauschii* Coss. Akzessionen sowie 17 cytogenetischen Tester-Linien unter Aluminiumstress getestet. Die hexaploiden Weizen zeichneten sich durch eine große Variabilität von hoch tolerant bis hoch anfällig aus und können somit potentiell für die Verbesserung der Al-Toleranz im Weizen genutzt werden. Außergewöhnlich hohe Toleranz zeigten brasilianische Akzessionen und solche von anderen geographischen Regionen, die in ihrem Stammbaum brasilianisches Ausgangsmaterial hatten. Die hohen RTI (Root Tolerance Index) Werte der brasilianischen Herkünfte sind auf das natürliche Vorkommen von sehr sauren Al toxischen Böden in diesem Land zurückzuführen. Im Gegensatz zu den hexaploiden Weizen zeigten die tetraploiden Formen eine vergleichsweise niedrige Toleranz und das nur bei bei geringer Stressbehandlung. *Ae. tauschii*, der D-Genom-Spender des hexaploiden Weizens war ebenfalls relativ anfällig. Von den cytogenetischen Tester-Linien wurden die ‚Chinese Spring' (‚CS') x ‚Synthetic 6x' (‚Syn') Einzelchromosom-Substitutionslinien analysiert. Dabei zeigten die Chromosomen 3B und 4D Substitutionslinien ein verändertes Toleranzverhalten. Dieses Material wurde weiterführend genutzt, um QTLs zu identifizieren.

Zunächst wurde eine Population von 84 ‚CS'/*Ae. tauschii* Coss. Introgressionslinien getestet, um QTLs für Al-Toleranz zu kartieren. Dabei wurde mittels RTI-Methode ein Haupt-QTL mit einem LOD-Signifikanzwert von 6,69 ($p<0,001$) identifiziert, gekoppelt zu den SSR Markern *Xgdm125* und *Xgwm976*, beide kartiert in der Zentromerregion auf dem langen Arm von Chromosom 4D. Dieser QTL bestimmte

31% der phänotypischen Variabilität. Die Stabilität des QTLs wurde mittels der Hematoxylin-Färbungsmethode bestätigt. Hierbei hatte der QTL, gekoppelt zu den Markern *Xgdm125* und *Xgwm976*, einen deutlich höheren LOD Wert (30,5) und eine phänotypische Variabilität von 82%. In beiden Fällen war der negative Effekt auf die Toleranz durch ‚Synthetic 6x' bedingt. Das bedeutet, dass *Ae. tauschii* negative Allele für Al-Toleranz trägt.

Eine weitere Population von 57 doppelt haploiden (DH) Linien, entwickelt aus einer Kreuzung von ‚CS' x ‚CS(Syn 3B)' wurde genutzt, um vorerst eine genetisch Kopplungskarte unter Verwendung von SSR Markern für Chromosom 3B zu entwickeln. Anschließend wurde die Population für die Kartierung von Al-Toleranz QTLs verwendet. Dabei konnte ein signifikanter QTL ($p<0,001$), gekoppelt zu den Markern *Xgwm1029* und *Xgwm1005* sowohl mittels RTI-Methode (LOD Wert 8,36; 49% phänotypische Variabilität) als auch unter Verwendung der Meßwerte der absoluten Wurzellängen unter Al-Stress (LOD Wert 6,79; 42% phänotypische Variabilität) identifiziert werden. Beide Marker befinden sich in der Zentromerregion auf dem langen Arm von Chromosom 4B. Diese QTL war bisher noch nicht in der Literatur beschrieben und erhielt die Bezeichnung $Qalt_{CS}.ipk\text{-}3B$.

SSR Marker *Xgdm125* und *Xgwm976*, eng gekoppelt zu dem Haupt-QTL auf Chromosom 4DL wurden genutzt, um ihre Kopplung zur Al Toleranz unter Verwendung 80 hexaploiden Weizenakzessionen zu validieren. Zusätzlich wurden die Marker *Xgwm1302* und *Xgwm3156* (Röder et al. unveröffentlicht), ebenfalls eng gekoppelt zum QTL, verwendet. Eine Korrelation zwischen Marker und Merkmal (Al-Toleranz) konnte nicht gefunden werden. Indes war ein zweiter Locus, amplifiziert durch den Marker *Xgwm1302* auf Chromosom 4B polymorh und ein spezifisches Allel von 205 bp korrelierte mit der Al-Toleranz zahlreicher brasilianischer Akzessionen. Die Spezifität des Allels wurde unter der Einbeziehung weiterer toleranter brasilianischer Standardsorten, bereitgestellt vom CIMMYT Mexiko, geprüft. Auch hier war das 205 bp Allel neben einigen anderen nachweisbar. Dieser potentielle 4B Locus sollte weiter analysiert und künftig genutzt werden.

Da die Al Toxizität immer an eine Versauerung der Böden gekoppelt ist, wurde weiterführend untersucht, ob die Reaktion gegenüber beider Merkmale durch identische oder verschiedenen Genloci kontrolliert wird. Aus diesem Grunde wurden die D-Genom Introgressionslinien, die bereits für die Kartierung von Al-Toleranz QTLs genutzt wurden, unter sauren Bedingungen aber ohne Zusatz von Al evaluiert. Diese Experimente haben gezeigt, dass die Säure-(Protonen, H^+) Toleranz durch QTLs auf den Chromosomen 6D (Wurzellänge unter Stress) sowie 3D und 5D (RTI-Methode) determiniert wird, nicht jedoch aud Chromosom 4D (Al-Toleranz). Demzufolge unterliegen beide Merkmale unterschiedlichen genetischen Mechanismen.

Ein Screening von 80 Gerstenakzessionen (*H. vulgare* L.) hat gezeigt, dass es hier eine sehr eingeengte genetische Variabilität für das Merkmal Al-Toleranz gibt. Die RTI-Werte schwankten zwischen 24-54%. Die Mehrzahl der Akzessionen (60%) war sensitiv. Die bereits vorhandene Oregon-Wolfe-Barley Kartierungspopulation, bestehend aus 94 DH Linien und entwickelt aus einer Kreuzung zwischen DOM und REC wurde genutzt, um auch in Gerste eine QTL-Kartierung durchzuführen. Verwendet wurde dazu die jüngst veröffentlichte Gersten-Transkriptom-Karte, bestehend aus 1.032 EST(Expressed Sequence Tags)-basierten Markern (EST-SSRs, -RFLPs and -SNPs). Unter Verwendung der RTI-Methode wurden auf den Chromosomen 2H, 3H, 4H und 7H Minor-QTLs detektiert. Mit der Hematoxylin-Färbungsmethode konnte der QTL auf Chromosom 3H bestätigt werden. Die mögliche Funktion der ESTs gekoppelt mit der Al-Toleranz wurde mittels BLASTX Analyse unter Verwendung der CR-EST Datenbank (The IPK Crop EST Database) in Kombination mit der NCBI Datenbank ermittelt. Zahlreiche ESTs kodieren Stress-Regulations-Proteine; einige spielen eine mögliche Rolle für Al und/oder Säure-Stress.

Schlussfolgernd hat die Arbeit gezeigt, dass das Merkmal Al-Toleranz in Weizen und Gerste polygen vererbt wird. Insbesondere im Weizen besteht jedoch die Möglichkeit, die Toleranz unter Verwendung von SSR Markern, gekoppelt zu dem Haupt-QTL

Qalt$_{CS}$*.ipk-3B*, zu verbessern. Der QTL auf Chromosom 4DL bestätigt die existierende Ko-Linearität der Gene innehalb der Triticeae aber darüber hinaus auch in anderen Arten der Familie der Gräser. Diese kann genutzt werden, um die Al-Toleranz von ökonomisch bedeutenden Getreidearten zu verbessern.

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Table A 1. Nutrient solution composition

Components	Final concentration	Stock solution 200 * (g/l)	Molecular Weight (g)
Macro-nutrients	**mM**		
$Ca(NO_3)_2.\ 4H_2O$	0.4	18.89	236.15
$MgSO_4.7H_2O$	0.2	9.86	246.48
KNO_3	0.4	8.09	101.1
$(NH_4)_2SO_4$	0.0435	1.16	132
Micro-nutrients	**µM**		
$MnSO_4.H_2O$	0.2	0.0068	169.01
$CuSO_4.5H_2O$	0.03	0.0016	249.68
$ZnSO_4.7H_2O$	0.08	0.0046	287.55
NaCl	3.0	0.0350	58.44
$Na_2MoO_4.2H_2O$	0.01	0.00046	241.95
H_3BO_3	1.0	0.0124	61.83
Iron source	**µM**		
$FeNa_2EDTA$	1.0	0.073	367.05

Iron should be added fresh. Phosphate is omitted from the solution to avoid precipitation of aluminium phosphate which ameliorates Al toxicity. Al is added fresh as $AlCl_3.6H_2O$ (Mol.wt. 241.43g/mol).

Table A 2. Descriptive features of polymorphic markers on chromosome 3B used for mapping aluminium tolerance QTLs

Markers	Annealing temperature (Tm)	Fragment size (bp)	Motifs & Repeats	CS allelle (bp)	Synthetic 3B allele (bp)
Xgwm389	60	130	(CT)14,(GT)16	130	142
Xgwm1037	55	140	(GA)35	184	189
Xgwm285	60	243	(GA)27	238	225
Xgwm376	60	147	(CA)16, (GA)22imp	141	135
Xgwm566	60	130	(CA)21, (GA)2, (TA)8	130	124
Xgwm845	60	196	(GA)23imp	191	204
Xgwm1015	50	149	(GT)20	153	177
Xgwm1029	60	217	(CT)15?	214	209
Xgwm108	60	132	(GT)29imp	137	143
Xgwm1005	60	152	(CA)13	145	165
Xgwm705	50	97	(GA)?	96	99
Xgwm655	60	177	(CA)37	172	169
Xgwm1266	60	157	CT	165	173
Xgwm299	55	208	(GA)31, (TAG)4	192	182

Table A 3. Descriptive features of markers linked to aluminium tolerance QTL on chromosome 4D

Markers	Annealing temperature (Tm)	Fragment size (bp)	Motifs & Repeats
Xgdm125	60°C	149	(CA)29
Xgwm0976	60°C	244	(GT)13
Xgwm1302	60°C	228	(GA)36
Xgwm3156	60°C	176	-

'-' missing data

Table A 4. Allele diversity of the SSR markers validated for aluminium tolerance

Serial. No.	Accession Number	Country	RTI (%)	*Xgwm976* (4D)	*Xgdm125* (4D)	*Xgwm3156* (4D)	*Xgwm1302* (4D)	*Xgwm1302* (4B)
1	CS	China	55	248	144	170	182	230
2	TRI 2959	Brazil	74	248	144	170	182	230
3	TRI 3546	Brazil	47	246	144	168	182	226, 230
4	TRI 5366	Brazil	26	248	144	170	182	218, 246
5	TRI 6921	USA	89	248	144	170	182	188, 218
6	TRI 6936	USA	37	248	144	170	182	230, 232
7	TRI 6937	USA	29	248	144	170	182	214
8	TRI 6938	USA	32	248	144	170	182	226
9	TRI 6939	USA	42	248	146	170	182	204
10	TRI 6940	USA	32	248	146	170	182	224
11	TRI 6941	USA	34	248	146	170	182	224
12	TRI 6942	USA	67	248	146	170	182	192
13	TRI 6943	USA	36	248	146	170	180	226
14	TRI 6944	USA	33	248	146	170	182	230
15	TRI 6945	USA	33	248	146	170	182	224
16	TRI 7069	USA	38	246	144	168	182	228
17	TRI 7074	USA	-	244	146	168	182	210
18	TRI 7091	USA	31	248	146	170	182	238
19	TRI 7097	USA	32	248	146	170	182	224, 246
20	TRI 7123	USA	30	248	146	170	182	224, 226
21	TRI 7124	USA	38	248	144	170	182	-
22	TRI 7142	USA	32	248	144	170	182	218
23	TRI 7143	USA	32	248	146	170	182	214
24	TRI 7144	USA	22	248	144	170	182	224
25	TRI 7207	USA	36	248	146	170	182	212, 246
26	TRI 7208	USA	38	246	144	168	182	224, 246

table A4 contd.

27	TRI 7209	USA	35	246	146	168	182	204
28	TRI 7210	USA	32	248	146	170	182	200
29	TRI 7211	USA	23	248	146	170	182	212, 224
30	TRI 7212	USA	34	248	144	170	182	230
31	TRI 7213	USA	40	248	146	170	182	215
32	TRI 7287	USA	30	248	144	170	182	205
33	TRI 7288	USA	71	248	144	170	182	218
34	TRI 7289	USA	30	248	144	170	182	192
35	TRI 7290	USA	48	248	146	170	182	223
36	TRI 7291	USA	83	248	144	170	182	218
37	TRI 7358	USA	78	248	142	170	180	-
38	TRI 7359	USA	36	248	144	170	182	246
39	TRI 7360	USA	82	248	142	170	-	-
40	TRI 7362	USA	41	248	144	170	182,188	218
41	TRI 7366	USA	71	248	144	170	182	192
42	TRI 7401	USA	27	248	144	170	182	211
43	TRI 8240	Brazil	89	248	144	170	182	194
44	TRI 9553	Brazil	109	248	144	170	182	205
45	TRI 9691	Brazil	78	248	144	170	182,184	205
46	TRI 10126	Poland	41	248	144	170	182,190	196
47	TRI 10210	USA	32	248	146	170	182	190, 223
48	TRI 10297	Brazil	88	-	146	170	182	205
49	TRI 10431	Brazil	96	248	146	170	182	205
50	TRI 10974	Brazil	102	248	146	170	182	205
51	TRI 11086	Australia	60	246	146	168	182	192
52	TRI 11087	Australia	35	246	146	168	182	216
53	TRI 11088	Australia	58	246	146	168	182	193, 222
54	TRI 11089	Australia	40	248	144	170	182	216

table A4 contd.

55	TRI 11090	Australia	56	246	146	168	182	193
56	TRI 11091	Australia	37	248	146	170	182	220, 246
57	TRI 11092	Australia	61	246	146	168	182	192
58	TRI 11093	Australia	69	246	146	168	182	216
59	TRI 11094	Australia	43	246,248	144	168	182,184	228
60	TRI 11095	Australia	48	246,248	144	168	182	230
61	TRI 11096	Australia	30	248	146	168	182	212
62	TRI 11097	Australia	55	246	144	168	182	210
63	TRI 11098	Australia	39	248	146	170	182	210
64	TRI 11101	Australia	47	246	144	168	182	212
65	TRI 11102	Australia	38	248	144	170	182	220
66	TRI 11107	Australia	42	246	146	168	182	230
67	TRI 13776	Brazil	85	248	146	170	182	205
68	TRI 17622	Australia	39	246	144	172	-	202, 226
69	TRI 17715	Australia	39	248	146	170	182	210
70	TRI 19350	Australia	35	246,248	144	168	182	212
71	TRI 19840	Australia	42	248	144	170	182	212
72	TRI 20129	Australia	45	246	144	168	182	210, 224, 228
73	TRI 21021	Australia	68	248	144	168	184	208
74	TRI 21172	Australia	82	248	144	168	182	-
75	TRI 22704	Australia	34	248	142	168	-	-
76	TRI 22916	Australia	34	246	144	168	182	218
77	TRI 22918	Australia	51	246	142	168	182	212

'-' Indicates missing data

Table A 5. Characterisation of hexaploid wheat for aluminium tolerance

Serial No.	Accession No.	Cultivar name	Country of Origin	RTI (%)	Score
1	TRI 1112	'Wysokolitewka'	Poland	38	S
2	TRI 1140	'Polnischer Land'	Poland	32	S
3	TRI 1188	'Kadolzer Winterweizen Nr. 5'	Poland	36	S
4	TRI 1298	'Lozinka Mikulicka'	Poland	35	S
5	TRI 1299	'L. Mudka Goetka'	Poland	56	MT
6	TRI 1304	'Graniatka Janasza'	Poland	45	MS
7	TRI 1367	'Dankowska Zachodnia'	Poland	35	S
8	TRI 2219	-	Canada	56	MT
9	TRI 2959	'Frontana Nr. 568'	Brazil	74	T
10	TRI 3074	'Waratah'	Canada	60	MT
11	TRI 3189	'Kanada IV'	Canada	51	MT
12	TRI 3241	'Canadischer Weizen'	Canada	59	MT
13	TRI 3493	'Pelissier'	Canada	53	MT
14	TRI 3546	-	Brazil	47	MS
15	TRI 3631	'Renown'	Canada	55	MT
16	TRI 3969	-	India	80	T
17	TRI 3992	-	India	72	T
18	TRI 4041	'Huron Ottawa 3'	Canada	43	MS
19	TRI 4058	'Manitoba 329'	Canada	35	S
20	TRI 4060	'Manitoba I'	Canada	33	S
21	TRI 4287	'Dawsons Golden Chaff'	Canada	71	T
22	TRI 4934	'Colotana'	Canada	78	T
23	TRI 4939	'Hope'	Canada	36	S
24	TRI 4941	'Chul'	Canada	35	S
25	TRI 5366	'Frontana'	Brazil	26	S
26	TRI 6921	'Atlas 66'	U.S.A	89	T
27	TRI 6936	'Knox'	U.S.A	37	S
28	TRI 6937	'Orfed'	U.S.A	29	S
29	TRI 6938	'Quanah'	U.S.A	32	S
30	TRI 6939	'Sanford'	U.S.A	42	MS
31	TRI 6940	'Saunders'	U.S.A	32	S
32	TRI 6941	'Taylor'	U.S.A	34	S
33	TRI 6942	'Taylor 49'	U.S.A	67	T
34	TRI 6943	'Triumph'	U.S.A	36	S
35	TRI 6944	'Vermillion'	U.S.A	33	S
36	TRI 6945	'Westar'	U.S.A	33	S
37	TRI 7069	'Washington Hybrid'	U.S.A	38	S
38	TRI 7074	'White Odessa'	U.S.A	-	-
39	TRI 7091	-	U.S.A	31	S
40	TRI 7097	'Ohio 14656 Hultio Mediterri'	U.S.A	32	S

table A5 contd.

41	TRI 7123	'Norka'	U.S.A	30	S
42	TRI 7124	'Sonora'	U.S.A	38	S
43	TRI 7142	'Marfed'	U.S.A	32	S
44	TRI 7143	'Lemhi'	U.S.A	32	S
45	TRI 7144	'Russel'	U.S.A	22	S
46	TRI 7207	'Baarf 38'	U.S.A	36	S
47	TRI 7208	'Big Club 43'	U.S.A	38	S
48	TRI 7209	'Exchange'	U.S.A	35	S
49	TRI 7210	'Frontana-Thatcher'	U.S.A	32	S
50	TRI 7211	'Hope x Timstein Minn 2776'	U.S.A	23	S
51	TRI 7212	'Kentana'	U.S.A	34	S
52	TRI 7213	'Onas 53'	U.S.A	40	MS
53	TRI 7287	-	U.S.A	30	S
54	TRI 7288	'Coker 47-27'	U.S.A	71	T
55	TRI 7289	'Centana'	U.S.A	30	S
56	TRI 7290	'Henry'	U.S.A	48	MS
57	TRI 7291	'Coastal'	U.S.A	83	T
58	TRI 7358	'Wichita'	U.S.A	78	T
59	TRI 7359	'Austin'	U.S.A	36	S
60	TRI 7360	'Atlas 50'	U.S.A	82	T
61	TRI 7362	'Lee'	U.S.A	41	MS
62	TRI 7366	'Seneca'	U.S.A	71	T
63	TRI 7401	'Brevor'	U.S.A	27	S
64	TRI 7494	'Pembina'	Canada	15	S
65	TRI 7603	'Talbot'	Canada	34	S
66	TRI 7734	'Winalta'	Canada	29	S
67	TRI 7735	'Westmont'	Canada	31	S
68	TRI 7736	'Kent'	Canada	32	S
69	TRI 7740	'Kenhi'	Canada	34	S
70	TRI 7741	'Lake'	Canada	35	S
71	TRI 7742	'Selkirk'	Canada	36	S
72	TRI 7748	-	Canada	35	S
73	TRI 8240	'Varanopolis'	Brazil	89	T
74	TRI 8316	'NP 825'	India	53	MT
75	TRI 8317	'NP 829'	India	54	MT
76	TRI 8318	'NP 832'	India	45	MS
77	TRI 8347	-	India	65	MT
78	TRI 8414	'C 286'	India	50	MT
79	TRI 8415	'C 303'	India	35	S
80	TRI 8432	'NP 891'	India	49	MS
81	TRI 9553	-	Brazil	109	T
82	TRI 9556	'Chris'	Canada	49	MS
83	TRI 9560	'NP 860'	India	47	MS

table A5 contd.

84	TRI 9561	'NP 875'	India	33	S
85	TRI 9582	'C 322/63'	Poland	58	MT
86	TRI 9588	'C 457/66'	Poland	41	MS
87	TRI 9589	'M 866/64'	Poland	42	MS
88	TRI 9590	'M 941/64'	Poland	54	MT
89	TRI 9617	'M 866/64'	Poland	35	S
90	TRI 9618	'M 941/64'	Poland	53	MT
91	TRI 9621	'Kb-5'	Poland	33	S
92	TRI 9628	'Unmedpur Mummy'	India	41	MS
93	TRI 9637	'NP 200'	India	41	MS
94	TRI 9668	'R 322-63'	Poland	32	S
95	TRI 9691	'Carazinho'	Brazil	78	T
96	TRI 9692	'NP 852'	India	41	MS
97	TRI 9707	'Selkirk'	Canada	38	S
98	TRI 9723	'Manitou'	Canada	36	S
99	TRI 9743	'CB 163-1'	Canada	70	T
100	TRI 9935	'Chinook'	Canada	58	MT
101	TRI 9936	'Hercules'	Canada	54	MT
102	TRI 9941	'Super X'	Canada	43	MS
103	TRI 10125	'Balta'	Poland	51	MT
104	TRI 10126	'Grana'	Poland	41	MS
105	TRI 10210	'Scout 66'	U.S.A	32	S
106	TRI 10297	-	Brazil	88	T
107	TRI 10375	'C 145/65'	Poland	24	S
108	TRI 10378	-	India	45	MS
109	TRI 10381	'M 1/66'	Poland	40	MS
110	TRI 10431	'IAS 20 Iassul'	Brazil	96	T
111	TRI 10974	'Jesnita'	Brazil	102	T
112	TRI 11045	'Bastion'	Poland	57	MT
113	TRI 11086	'Darkan'	Australia	60	MT
114	TRI 11087	'Duramba'	Australia	35	S
115	TRI 11088	'Eagle'	Australia	58	MT
116	TRI 11089	'Egret'	Australia	40	MS
117	TRI 11090	'Falcon'	Australia	56	MT
118	TRI 11091	'Festignay'	Australia	37	S
119	TRI 11092	'Gambee'	Australia	61	MT
120	TRI 11093	'Gamenya'	Australia	69	T
121	TRI 11094	'Gamut'	Australia	43	MS
122	TRI 11095	'Gatcher'	Australia	48	MS
123	TRI 11096	'Glaive'	Australia	30	S
124	TRI 11097	'Halbred'	Australia	55	MT
125	TRI 11098	'Heron'	Australia	39	S
126	TRI 11101	'Olympic'	Australia	47	MS

table A5 contd.

127	TRI11102	'Pinnacle'	Australia	38	S
128	TRI 11107	'Timgalen'	Australia	42	MS
129	TRI 11767	-	Poland	43	MS
130	TRI 11769	-	Poland	53	MT
131	TRI 12348	-	India	33	S
132	TRI 12350	-	India	61	MT
133	TRI 12351	-	India	43	MS
134	TRI 12352	-	India	42	MS
135	TRI 12353	-	India	36	S
136	TRI 12624	-	India	50	MT
137	TRI 12626	-	India	35	S
138	TRI 12627	-	India	45	MS
139	TRI 12630	-	India	41	MS
140	TRI 12922	'Chinese Spring'	China	55	MT
141	TRI 13776	'Carazinho'	Brazil	85	T
142	TRI 17591	-	Canada	42	MS
143	TRI 17592	-	Canada	33	S
144	TRI 17622	-	Australia	39	S
145	TRI 17715	-	Australia	39	S
146	TRI 19350	-	Australia	35	S
147	TRI 19840	-	Australia	42	MS
148	TRI 20129	-	Australia	45	MS
149	TRI 21021	-	Australia	68	T
150	TRI 21172	-	Australia	82	T
151	TRI 22704	-	Australia	34	S
152	TRI 22916	-	Australia	34	S
153	TRI 22918	-	Australia	51	MT
154	* 26	'PG-1'	Brazil	84	T
155	* 1770	'Frondoso'	Brazil	76	T
156	* 5919	'Frontana'	Brazil	67	T
157	* 6071	'BH1146'	Brazil	93	T

* CIMMYT accessions

'-' missing data; T-tolerant; MT-moderately tolerant; MS-moderately sensitive; S-sensitive

Table A 6. Characterisation of tetraploid wheat for aluminium tolerance

Serial No.	Accession No.	Cultivar name	Country of Origin	RTI (%)	Score
1	TRI 438	'Velvet Don'	U.S.A	58	MT
2	TRI 440	'Braunspelziger Arnautka'	U.S.A	49	MS
3	TRI 441	'Amber'	U.S.A	40	MS
4	TRI 698	-	Turkey	47	MS
5	TRI 707	'Kaza-Kus'	Turkey	45	MS
6	TRI 709	-	Turkey	47	MS
7	TRI 710	-	Turkey	49	MS
8	TRI 749	-	Turkey	37	S
9	TRI 754	-	Turkey	50	MT
10	TRI 1516	-	Turkey	39	S
11	TRI 1691	-	Turkey	54	MT
12	TRI 1964	-	Turkey	55	MT
13	TRI 2154	-	Turkey	39	S
14	TRI 2163	-	Turkey	46	MS
15	TRI 2167	-	Turkey	44	MS
16	TRI 2169	-	Turkey	37	S
17	TRI 2177	-	Turkey	44	MS
18	TRI 2182	-	Turkey	52	MT
19	TRI 2186	-	Turkey	48	MS
20	TRI 3493	'Pelissier'	Canada	42	MS
21	TRI 3653	'Mindum'	U.S.A	37	S
22	TRI 4059	'Arnautka'	U.S.A	48	MS
23	TRI 4164	-	Turkey	37	S
24	TRI 5291	'Akrona'	U.S.A	53	MT
25	TRI 5294	'Arnautka'	U.S.A	38	S
26	TRI 5326	'Kubanka'	U.S.A	39	S
27	TRI 5330	'Mindum'	U.S.A	39	S
28	TRI 5336	'Pulawska'	Poland	48	MS
29	TRI 5515	-	Iran	53	MT
30	TRI 5549	-	Iran	42	MS
31	TRI 5552	-	Iran	37	S
32	TRI 5593	-	Iran	43	MS
33	TRI 5862	-	Iran	49	MS
34	TRI 5867	-	Iran	49	MS
35	TRI 5908	-	Iran	45	MS
36	TRI 5923	-	Iran	47	MS
37	TRI 5932	-	Iran	47	MS
38	TRI 6023	-	Iran	50	MT
39	TRI 6056	-	Iran	44	MS
40	TRI 6071	-	Iran	65	T
41	TRI 6145	-	Iran	45	MS

table A6 contd.

42	TRI 6183	-	Iran	53	MT
43	TRI 6241	-	Iran	47	MS
44	TRI 6248	-	Iran	57	MT
45	TRI 6263	-	Iran	52	MT
46	TRI 6264	-	Iran	60	MT
47	TRI 6437	-	Iran	44	MS
48	TRI 6526	-	Iran	53	MT
49	TRI 6697	-	Iran	50	MT
50	TRI 7217	'Vernum L.D. 153'	U.S.A	34	S
51	TRI 7282	'Langdon'	U.S.A	34	S
52	TRI 7283	'Ramsey'	U.S.A	37	S
53	TRI 7737	'Sentry'	Canada	44	MS
54	TRI 9936	'Hercules'	Canada	44	MS
55	TRI 11047	'Wakooma'	U.S.A	38	S
56	TRI 11048	'Wascana'	U.S.A	28	S
57	TRI 11087	'Duramba'	Australia	41	MS
58	TRI 17582	-	Poland	48	MS
59	TRI 17590	-	U.S.A	44	MS
60	TRI 17591	-	Canada	47	MS
61	TRI 17592	-	Canada	36	S
62	TRI 17622	-	Australia	49	MS
63	TRI 26330	-	Australia	54	MT

T-tolerant; MT-moderately tolerant; MS-moderately sensitive; S-sensitive

Table A 7. Characterisation of barley accessions for aluminium tolerance

Serial No.	Accession No.	Cultivar name	Country of origin	RTI (%)	Score
1	HOR 35	-	Australia	36	S
2	HOR 36	'Trabut barley'	Australia	32	S
3	HOR 37	'Reka'	Australia	30	S
4	HOR 259	'Kuan'	U.S.A	33	S
5	HOR 276	'Hero'	U.S.A	32	S
6	HOR 295	'Horn'	U.S.A	41	MS
7	HOR 312	'Spartan'	U.S.A	34	S
8	BCC 878	'Horsford'	U.S.A	36	S
9	BCC 884	'Jackson'	U.S.A	35	S
10	BCC 885	'Kamiak'	U.S.A	35	S
11	BCC 923	'Ridawn'	U.S.A	37	S
12	BCC 931	'Shonkin'	U.S.A	37	S
13	BCC 944	'Weal'	U.S.A	40	MS
14	HOR 945	'G.H. 1 Nr. 22'	Australia	36	S
15	HOR 946	'G.H. 1 Nr. 22'	Australia	38	S
16	HOR 1242	'Australische Frühe'	Australia	40	MS
17	HOR 1264	'Australische Frühe'	Australia	39	S
18	HOR 1274	'Vaughn'	U.S.A	37	S
19	HOR 1443	'New Cross'	U.S.A	44	MS
20	BCC 1701	'Forrest'	Australia	42	MS
21	BCC 1702	'Galleon'	Australia	45	MS
22	BCC 1703	'Stirling'	Australia	40	MS
23	BCC 1704	'Grimmett'	Australia	53	MT
24	BCC 1705	'Clipper'	Australia	40	MS
25	BCC 1706	'Lara'	Australia	49	MS
26	BCC 1707	'Weeah'	Australia	36	S
27	BCC 1708	'Brindabella'	Australia	35	S
28	BCC 1709	'Yerong'	Australia	38	S
29	BCC 1711	'Kaputar'	Australia	36	S
30	HOR 2256	'Pryor 472'	Australia	36	S
31	HOR 2257	'Pryor'	Australia	38	S
32	HOR 2592	'Chevron'	U.S.A	37	S
33	HOR 2633	'Australie'	Australia	35	S
34	HOR 2747	'Bonneville'	U.S.A	39	S
35	HOR 2748	'Cascade'	U.S.A	42	MS
36	HOR 2751	'Feebar'	U.S.A	49	MS
37	HOR 2960	'Australische'	Australia	26	S
38	HOR 2971	'Frühe Australische Zweizeilige'	Australia	32	S
39	HOR 2983	'Blackhull'	U.S.A	34	S
40	HOR 2994	'Juliaca'	U.S.A	47	MS
41	HOR 3093	'Dutchess'	U.S.A	36	S

table A7 contd.

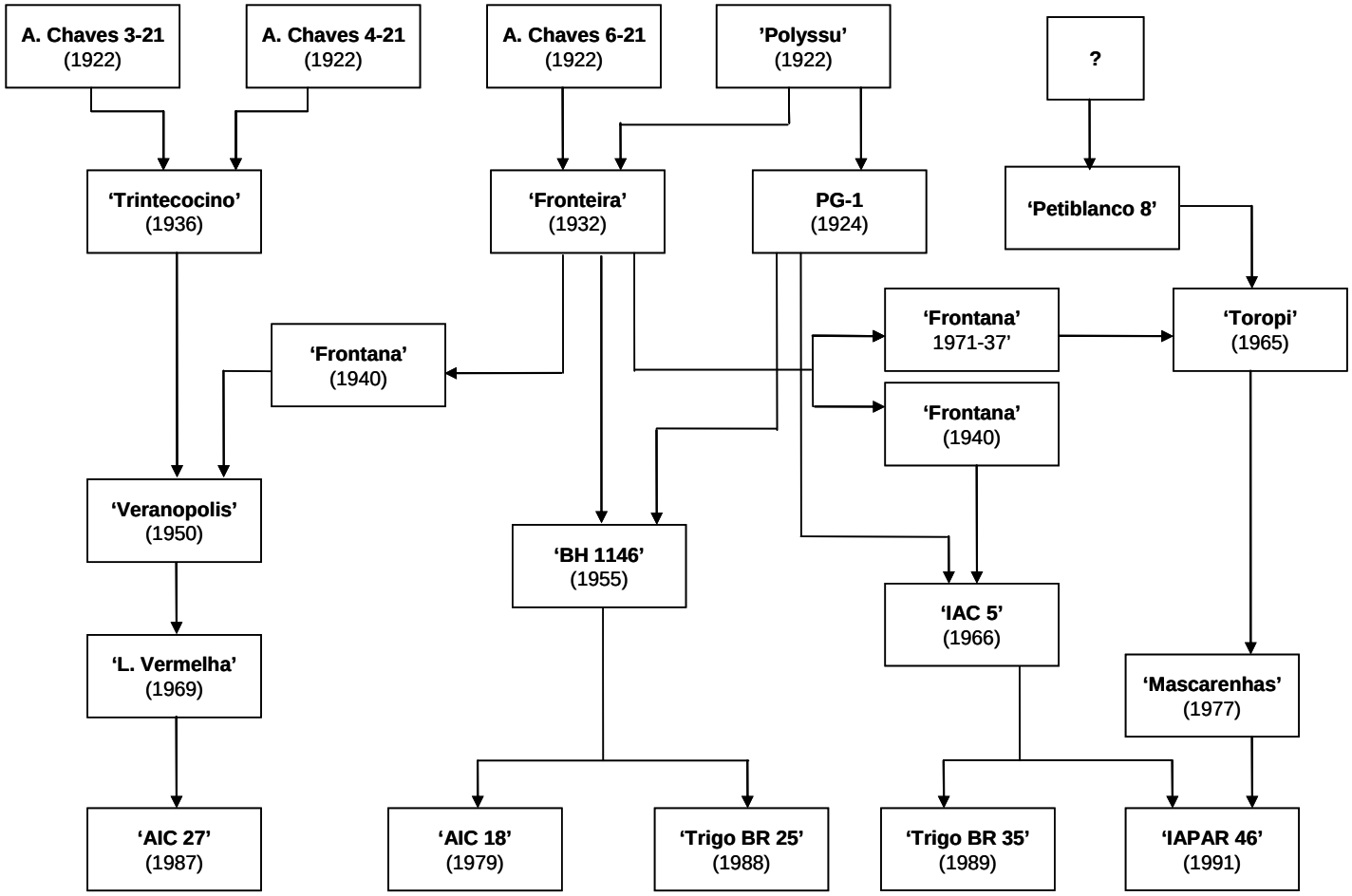
42	HOR 3094	'Kenbar'	U.S.A	38	S
43	HOR 3106	'Brier'	U.S.A	44	MS
44	HOR 3111	'Dayton'	U.S.A	50	MT
45	HOR 3119	'James'	U.S.A	52	MT
46	HOR 3120	'Kansas'	U.S.A	52	MT
47	HOR 3121	'Kearney'	U.S.A	45	MS
48	HOR 3123	'Kenbar'	U.S.A	37	S
49	HOR 3125	'Khayyam'	U.S.A	36	S
50	HOR 3131	'Mercer'	U.S.A	42	MS
51	HOR 3134	'Mo-B-475'	U.S.A	54	MT
52	HOR 3135	'Nebraska 53417'	U.S.A	46	MS
53	HOR 3160	'Wong'	U.S.A	45	MS
54	HOR 3303	'Wade'	U.S.A	40	MS
55	HOR 3632	'Liberty'	U.S.A	35	S
56	HOR 3876	'Atlas 46'	U.S.A	37	S
57	HOR 3926	'Barbless'	U.S.A	45	MS
58	HOR 4018	'Alagon'	U.S.A	24	S
59	HOR 4021	'Chinerme'	U.S.A	30	S
60	HOR 4075	'Orge d'Australie'	Australia	31	S
61	HOR 4080	'Anabee'	Australia	33	S
62	HOR 4084	'Research'	Australia	32	S
63	HOR 4085	'Resibee'	Australia	35	S
64	HOR 4112	'California Coast'	U.S.A	52	MT
65	HOR 4206	'Cape'	Australia	33	S
66	HOR 4609	'Clayton'	U.S.A	39	S
67	HOR 4692	'Kerr'	U.S.A	31	S
68	HOR 4695	'Miller'	U.S.A	36	S
69	HOR 4906	'Keowee'	U.S.A	39	S
70	HOR 4908	'Milton'	U.S.A	36	S
71	HOR 4998	'Abate'	U.S.A	33	S
72	HOR 6935	'Durani'	U.S.A	40	MS
73	HOR 6961	'Bay'	U.S.A	46	MS
74	HOR 9514	'Dickson 628'	U.S.A	42	MS
75	HOR 9526	'Suwon 31'	U.S.A	36	S
76	HOR 9553	-	U.S.A	32	S
77	HOR 9584	'Pendleton 372'	U.S.A	41	MS
78	HOR 9588	'Briggs'	U.S.A	42	MS
79	HOR 10030	'Shannon'	Australia	51	MT
80	HOR 19232	'Davidson'	U.S.A	45	MS

BCC- Barley core collection
MT-moderately tolerant; MS-moderately sensitive; S-sensitive

Table A 8. Aluminium tolerance Genes / QTLs in cereals

Species	Crosses	Designation and location	Flanking markers	Reference
H. vulgare	Yambla/WB229	*Alt* (4H)	Bmag353, HVM68/HVM3	Raman et al. (2002)
H. vulgare	Dayton/Harlon	*Alp* (4HL)	Xbcd1117/ Xwg464, Xcdo1395	Tang et al. (2000)
H. vulgare	Dayton/Harlon	*Alp* (4HL)	HVM68/Bmag353	Raman et al. (2003)
T. aestivum	BH1146/Anahuac	Alt_{BH} (4DL)	Xbcd1230/Xcdo1395	Riede and Anderson (1996)
T. aestivum	BH1146/Anahuac	Alt_{BH} (4DL)	Xgdm125/Xpsr914	Milla and Gustafson (2001)
T. aestivum	Atlas 66/Century	QTL (4DL)	Xgdm125/Xwmc331	Ma et al. (2005)
S. cereale	Ailes/Riodeva	*Alt1* (6RS)	ScR01600/ScB15790	Gallego et al. (1998a)
S. cereale	M39A-1-6/ M77A1	*Alt3* (4RL)	AMAL5/AMAL5	Miftahudin et al. (2002)
O. sativa	IR64/ *O. rufipogon*	*QAlRr1.1* (1) *QAlRr3.1* (3) *QAlRr7.1* (7) *QAlRr8.1* (8) *QAlRr9.1* (9)	RFLP, SSR	Nguyen et al. (2003)
O. sativa	CT9993/IR62266	*qALRR-1-1* *qALRR-1-2* *qALRR-2* *qALRR-3* *qALRR-4* *qALRR-7* *qALRR-8* *qALRR-9* *qALRR-10* *qALRR-12*	RFLP, AFLP, SSR	Nguyen et al. (2002)
O. sativa	Koshihikari/Kasalath	QTL (1) QTL (2) QTL (6)	C86, R2460, G200	Ma et al. (2002)
Z. mays	Cat-100-6/S5187	*Alm1* (10S) *Alm2* (6S)	CSU70, UMC130	Sibov et al. (1999)
Z. mays	L53/L1327	QTL1 (2) QTL2 (6) QTL3 (6) QTL4 (8) QTL5 (8)	RFLP, SSR	Ninamango-Cardenas et al. (2003)
A. strigosa	Clav 9011/Clav2921	Linkage group F	RFLP-comparative mapping	Wight et al. (2006)
S. bicolor	SC283/BR007	Alt_{SB} (3)	AFLP- comparative mapping	Magalhaes et al. (2004)

Figure A 1. Probable origin of main aluminium tolerance genes in some selected Brazilian wheat cultivars and year of release (De Sousa 1998).



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Publications

Peer reviewed

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Erklärung

Hiermit erkläre ich, dass mit dieser wissenschaftlichen Arbeit noch keine vergeblichen Promotionsversuche unternommen wurden.

Die eingereichte Dissertation mit dem Thema: “Molecular mapping of quantitative trait loci (QTL) controlling aluminium tolerance in wheat and barley” habe ich selbständig und nur unter Verwendung der angegeben Literatur und Hilfsmittel angefertigt.

Des weiteren erkläre ich, dass keine Strafverfahren gegen mich anhängig sind.

Gatersleben, den 21.05.2008

Sheeba Navakode Gangadharan