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Review:

Aluminium tolerance in barley (*Hordeum vulgare* L.): physiological mechanisms, genetics and screening methods*

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Abstract: Aluminium (Al) toxicity is one of the major limiting factors for barley production on acid soils. It inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poor plant growth and yield. Plants tolerate Al either through external resistance mechanisms, by which Al is excluded from plant tissues or internal tolerance mechanisms, conferring the ability of plants to tolerate Al ion in the plant symplasm where Al that has permeated the plasmalemma is sequestered or converted into an innocuous form. Barley is considered to be most sensitive to Al toxicity among cereal species. Al tolerance in barley has been assessed by several methods, such as nutrient solution culture, soil bioassay and field screening. Genetic and molecular mapping research has shown that Al tolerance in barley is controlled by a single locus which is located on chromosome 4H. Molecular markers linked with Al tolerance loci have been identified and validated in a range of diverse populations. This paper reviews the (1) screening methods for evaluating Al tolerance, (2) genetics and (3) mechanisms underlying Al tolerance in barley.

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INTRODUCTION

Aluminium (Al) toxicity is one of the major constraints on crop productivity on acid soils, which occur on up to 40% of the arable lands of the world (Kochian, 1995). Al is the third most abundant element in the earth's crust and is toxic to plants when solubilised into soil solution at acidic pH values (Kochian, 1995). A total of 3950 million hectares of land is classed as having acidic soil, of which 15% is used for planting of annual and perennial crops (von Uexküll and Mutert, 1995). Soils are becoming more acidic by certain farming practices, for example the application of ammonium-based fertilizers (Kochian *et al*., 2002), and accumulation of organic matter (Williams, 1980). In Australia, about 90 million hectares of agricultural land can be potentially affected and annually economic loss is estimated at more than USD 600 million (http://www.science.org.au/nova/071/071box01.htm). At low pH, Al is solubilised as phytotoxic Al^{3+} ions from non-toxic Al silicates and oxides (Hoekenga *et al*., 2003).

Although crop production on acid soils can be sustained by application of lime, runoff pollution is an undesirable effect (de la Fuente *et al*., 1997). Liming is often not economic or practical because of the slow movement of lime especially in the deeper layers of subsoils (Foy *et al*., 1965; 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). Thus, developing cultivars with improved tolerance

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to acid soil stress is a solution to address this problem (Scott and Fisher, 1989). In recent years, extensive research has been focused on this area, including evaluation of germplasm for Al tolerance, the biochemistry and physiology of Al toxicity and tolerance, and the genetics of Al tolerance. Barley is the fourth most important cereal crop globally. It is considered to be more sensitive to acidic soils than rye, oat, rice, and wheat (Bona *et al*., 1993; Ishikawa *et al*., 2000). This paper reviews advances in screening methods, mechanisms and genetics underlying Al tolerance especially in barley and related cereals.

EFFECT OF Al TOXICITY ON GROWTH, DEVELOPMENT AND YIELD OF CROP PLANTS

Symptom of Al toxicity

Al toxicity is considered to be a complex of nutritional disorders of growth and development of plants, which may be manifested as a deficiency of essential nutrients like calcium, magnesium, iron or molybdenum; decreased availability of phosphorus or as toxicity of Mn and H^+ (Alam and Adams, 1980; Foy, 1984; 1988; 1992; Kamprath and Foy, 1985; IRRI, 1974; Clark *et al*., 1981; Furlani and Clark, 1981; Foy and Fleming, 1982). The primary response to Al stress in plants occurs in roots, as reduced elongation at the tip, followed by swelling and distortion of differentiated cells, as well as root discolouration (Foy *et al*., 1978; Bergmann, 1992; Hossain *et al*., 2005). Within meristematic and root cap cells, Al toxicity is associated with an increased vacuolation and turnover of starch grains (de Lima and Copeland, 1994), as well as disruption of dictyosomes and their secretory function (Bennet *et al*., 1985; Puthota *et al*., 1991). The foliar symptoms in some plants resemble those of phosphorus deficiency manifested by overall stunting, small, dark green leaves, late maturity, purpling of stems, leaves and leaf veins, and yellowing and death of leaf tips (Foy, 1992). Al stress decreases total chlorophyll concentration and photosynthetic rate, but the decline in transpiration rate is most severe (Ohki, 1986). Al toxicity also appears as an induced calcium deficiency or as reduced Ca^{2+} transport within plants, causing curling or rolling of young leaves, inhibited growth of lateral branches, or a collapse of growing points or petioles (Foy, 1992).

Effect of Al toxicity on plant growth and development

Al toxicity inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poorer plant growth and yield (Alam, 1981; Clarkson, 1966; Foy, 1983; Foy *et al*., 1967; Gauthier, 1953; Reid *et al*., 1969; 1971). Relative shoot and root dry weights in tolerant barley cultivars were two-fold and three-fold respectively compared to susceptible cultivars (Foy, 1996). Al toxicity also limits both rooting depth and degree of root branching (Foy, 1992). Parker (1995) demonstrated that there are two responses to Al: an initial acute inhibition of growth that is followed by a later chronic Al effect on root growth. Al toxicity decreases drought tolerance and the use of subsoil nutrients (Carver and Ownby, 1995).

Gallardo *et al*.(1999) reported 50% and 30% reduction of grain yield, respectively for sensitive and tolerant cultivars of barley when they were grown in naturally acidic soil (pH 4.9) with a large amount of extractable Al compared to that grown in non acidic soil (pH 5.8). Oram (1983) reported that Australian barley cultivars are generally tolerant to high soil manganese levels but sensitive to high Al levels. Australian cultivars did, however, outperform most tolerant lines introduced from Denmark in terms of grain and straw yield in a field trial experiment on soil with lime added (Oram, 1983).

Factors affecting Al toxicity

Al toxicity is affected by many factors such as pH, concentration of Al, temperature, and concentrations of cations and anions in culture solution. A pH 5.0 or above will reduce Al solubility (Reid *et al*., 1971) thus reducing Al toxicity. Root elongation depended critically on the concentration of Ca^{2+}, whether in the presence or absence of Al, with at least 0.2 mmol/L Ca^{2+} being essential for optimum growth (Kinraide *et al*., 1985). The concentration of Ca^{2+} greatly influences the Al toxicity at a given pH and Al concentration. As the Ca^{2+} concentration approached 1 mmol/L the inhibition by 1 μmol/L Al was nearly eliminated (Kinraide *et al*., 1985). Increased concentrations of basic cations in solution of the root

rhizosphere, particularly calcium, have been shown to ameliorate Al toxicity (Brady *et al*., 1993). Mg^{2+} at concentration of 0.5 mmol/L can also alleviate Al toxicity as did Ca^{2+} (Kinraide *et al*., 1985). Application of NH_4Cl to a soil with a high exchangeable Al significantly reduced barley seedling emergence, shoot and root weights, spike numbers/m^2 and grain numbers/spike whereas $NaNO_3$ significantly increased all these parameters. At harvesting, soil analysis showed that NH_4Cl significantly reduced soil pH and increased soil Al and Mn contents and this was confirmed by tissue analysis of shoots and roots (Stange *et al*., 1995). NH_4^+-N induced release of H^+ from the roots particularly whilst NO_3^--N significantly increased pH by release of OH^- (Borie *et al*., 1994). Adding excess P in nutrient solution will precipitate and detoxify Al (Kinraide *et al*., 1985; Reid *et al*., 1971). Field experiments identified that phosphate fertilizer allowed barley to withstand larger concentrations of soluble and exchangeable Al (Bache and Ross, 1991). Silicon (as $Na_2SiO_3{\cdot}5H_2O$) significantly ameliorated the toxic effects of Al on root and shoot growth and decreased the Al concentration in the roots of barley seedlings under 25 and 50 μmol/L Al treatment (Hammond *et al*., 1995), but exaggerated it at a higher concentration of Al (above 75 μmol/L) in barley (Liang *et al*., 2001). In an Al-tolerant cultivar, Al-exposed plants pre-treated with Si exuded up to 15 times more phenolics than those plants not pre-treated with Si (Kidd *et al*., 2001). However, Cocker *et al*.(1998) reported that Si does not reduce Al phytotoxicity as a result of Al/Si interactions in the external media, and that the mechanism of amelioration has an *in planta* component.

SCREENING OF PLANTS FOR Al TOLERANCE

Genetic improvement of crops for acid soil tolerance has been accelerated by the availability of screening criteria for detecting Al tolerance. Laboratory- and greenhouse-based techniques are widely employed which are usually non-destructive, and can be applied in early developmental stages from seedlings only a few days old to flowering stage of the plants. Field-based screening techniques are more laborious, time consuming and expensive. Choice of a particular screening test is influenced by the kind of material under selection, i.e., germplasm collections for identifying suitable parents, segregating populations, or advanced breeding lines under consideration for release.

Nutrient solution culture

Nutrient solution culture is the most common screening medium for Al tolerance, which provides easy access to root systems, tight control over nutrient availability and pH, and non-destructive measurement of tolerance (Carver and Ownby, 1995). The experimental conditions including culture media, pH, Al concentration, and time of treatment used for screening barley germplasm are summarised in Table 1. Because Al toxicity is affected by these factors, the experimental conditions need to be optimized.

There are two major criteria for evaluation of Al tolerance in nutrient solution culture. First, 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 (Hede *et al*., 2002). Second, root staining is quicker and more efficient. It is suitable for screening a large segregating population derived from improved germplasm (Hede *et al*., 2002). Haematoxylin (Polle *et al*., 1978; Minella and Sorrells, 1992; Bona *et al*., 1998; Tang *et al*., 2000) and eriochrome cyanine R (Fig.1) (Aniol, 1995; Ma *et al*., 1997; Wang *et al*., 2006) stains have been used for evaluation of barley germplasm. Other parameters such as ratio of root to shoot fresh weight (Ma *et al*., 2004), ultra-weak luminescence (UL) analysis (Hu *et al*., 2002) and nitro blue tetrazolium (NBT) reduction (Maltais and Houde, 2002) have also been used for assessment of germplasm for Al tolerance.

Variation in temperature in the growth chamber and minor fluctuation of pH of the nutrient solution, as it affects effective Al concentration, can reduce repeatability of the results (Moore *et al*., 1977). The concentration of Al and duration of exposure are varied inversely. A long exposure to Al for 3~4 weeks requires much lower (about one third) concentrations than brief exposure of 24 h. Shuman *et al*.(1993) found effective screening of wheat cultivars using

Table 1 Experimental conditions used for Al tolerance screening in barley by hydroponic culture

Source	pH	Al (μmol/L)	Temperature (°C)	Treatment time	Light/ dark (h)	Culture media	Material
Ma *et al*.(1997)	4.5	0, 5, 10, 20, 40	25/20	24 h	14/10	1 mmol/L $CaCl_2$	604 barley lines
Zhao *et al*.(2003)	5.0	10	25	24 h		Same as above	21 varieties
Gallardo *et al*.(1999)	4.8	0, 50, 100, 200	25	15 d	14/10	Ca 1270, K 750, NO_3 3710, NH_4 310, PO_4 100, SO_4 120, Fe 17.9, B 6.6, Mn 2.4, Zn 0.6, Cu 0.2, Mo 0.1 (μmol/L)	3 cultivars
Raman *et al*.(2002)	4.0, 4.3	100, 50, 10	24/18	Pulse treatment 1 d or 4 d, recovery 3 d	16/8	Ca 1000, Mg 400, K 1000, NO_3 3400, NH_4 600, PO_4 100, SO_4 401.1, Cl 78, Na 40.2, Fe 20, B 23, Mn 9, Zn 0.8, Cu 0.3, Mo 0.1 (μmol/L)	F_2 and F_3
Raman *et al*.(2003)	4.3	50, 25	24/18	Pulse treatment 1 d and recovery 3 d	16/8	Same as above	F_3
Minella and Sorrells (1992)	4.0	30, 60, 90	25	17 h	17/0	$CaCl_2$ 4, KNO_3 6.5, $MgCl_2$ 2.5, $(NH_4)_2SO_4$ 0.1, NH_4NO_3 0.4 (mmol/L)	37 barley cultivars and F_2s
Maxim and Duta (1996)	3.9~4.1	30, 60, 90	22	17 h	17/0	Same as above	24 barley genoytpes
	4.1, 4.2	74, 148		5 d for relative root elongation			
Tang *et al*.(2000)	4.0	50		24 h		Same as above	F_2, F_3
Reid *et al*.(1971)	4.8	4×10^{-6}	18~21	18 d	12/12	Ca 50.8, Mg 6.6, N 56, S 3.8, K 29.4, P 3, Na 0.01, Cl 0.34, Mn 0.13, Fe 1, B 0.07, Zn 0.04, Cu 0.0.1, Mo 0.005 ($\times10^{-6}$)	30 varieties
Kinraide *et al*.(1985)	4.5	0, 0.25, 0.5, 0.75, 1.0		48 h		0.2 mmol/L $CaSO_4$	Dayton, Kearney
Lisitsyn (2000)	4.3	1~2 mmol/L	21~23	5~7 d		Distilled water	75 barley cultivars
Hossain *et al*.(2005)	4.5	50~100	22	24 h		$CaCl_2$ 4, KNO_3 6.5, $MgCl_2$ 2.5, $(NH_4)_2SO_4$ 0.1, NH_4NO_3 0.4 (mmol/L)	6 barley varieties

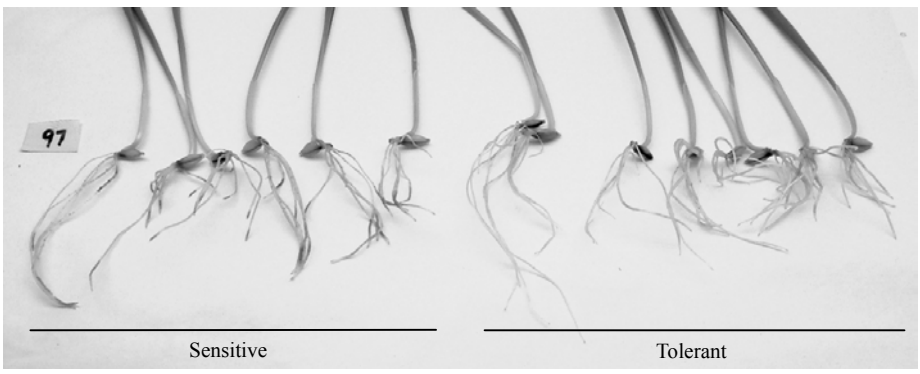

Fig.1 Eriochrome Cyanine R staining of F_3 family segregation for Al tolerance

very low Al levels in solution to minimize Al precipitation and to more closely represent actual environmental stresses compared to traditional short-term exposure with higher Al concentrations. The longer exposure makes solution culture technically more difficult, requiring constant adjustments of pH, water loss and nutrient loss.

Other variables to consider in solution-based screening are nutrient composition and standards for measuring tolerance. Changes in nutrient composition can change the intensity of Al stress at a given concentration (Foy *et al*., 1988; Little, 1988; Scott and Fisher, 1989). Higher concentration of phosphorus may lead to Al-phosphate precipitates in Al solution and protect plants against Al toxicity. Hence, phosphorus is often avoided in nutrient solution, particularly in short-term Al exposures when phosphorus needs are satisfied by seed reserves. Tamas *et al*.(2006), however, indicated several disadvantages of hydroponics. For example, the transfer of young germinated barley seedlings to hydroponics can cause significant stress and requires a long period for the onset of several mechanisms to adapt to hydroponics, especially in plants sensitive to hypoxia. They have developed a filter-paper-based system for cultivating germinating barley seeds for Al tolerance analysis (Tamas *et al*., 2006). In their method, millimolar Al concentrations were used to cause similar Al toxicity symptoms on roots as micromolar Al concentrations in hydroponics due to the high affinity of filter paper to Al monomeric forms.

Measurements of shoot and root growth as total dry weight for long-term culture also provide good separation of Al-tolerant and sensitive genotypes. The hematoxylin staining method which was originally described by Polle *et al*.(1978) with applications to genotypic classification (Takagi *et al*., 1981; Ma *et al*., 1997; Carver *et al*., 1988), genetic characterization (Ruiz-Torres and Carver, 1992), and selection (Fisher and Scott, 1987; Carver *et al*., 1993) has been widely used in wheat and other species with higher levels of Al tolerance. Polle *et al*.(1978) recommended a qualitative scale to rate genotypes as completely, partially, or unstained for 3 different concentrations of Al in solution. These rating parameters are applicable when the seminal roots have a consistent stain pattern, but some genotypes show a differential staining pattern (none vs complete) among roots on the same plants, or between plants of a single genotype, making genotypic/individual plant classification confusing and difficult (Ruiz-Torres and Carver, 1992; Hossain *et al*., 2005). A modified method was developed by Hossain *et al*.(2005) as follows: pre-germinated seedlings (2 d at 22 °C) were cultured for 3 d in nutrient solution (Al free) followed by 24 h growing in a solution with 50 or 100 μmol/L Al, and then 48 h regrowth in Al free nutrient solution. A particular feature of this method involves the culture of individual plants in small tubes rather than in bulk tanks, to avoid cross contamination. Following this method, seminal root regrowth length (SRRL) and relative seminal root regrowth length (RSRRL) showed significant differences between tolerant and sensitive cultivars.

Screening with soil bioassay

The use of soil media has received less attention than solution media because of the complications of creating a soil environment with a specific type and amount of phytotoxicity (Foy, 1976). Mitigating effects of other nutrients (e.g., Ca, P, or Mg) or organic matter must be considered as well as other factors like variability at the soil collection site, time of collection, and soil storage condition (Scott and Fisher, 1989). Results from soil bioassays may lack consistency when the same soil is used repeatedly due to the effects of continuous wetting and drying on soil chemistry.

There has been some attention to the development of rapid soil bioassays in screening for acid soil tolerance. Fundamental to such an assay is that reduction in length of the primary root is the first visual indicator of Al sensitivity and that reduction in primary root length only two days after germination under dark conditions is equally effective in discriminating genotypes as root lengths measured later under light conditions (Aitken *et al*., 1990). Early root growth under dark conditions is primarily supported by the seed reserves without possible confounding effects of nutrient uptake. Screening in soils representative of the targeted production area where soil acidity is a yield limiting factor provides a critical intermediate step in selection of tolerant genotypes after preliminary screening in nutrient solution but before more lengthy and costly screening under natural field conditions (Carver and Ownby, 1995).

Field screening

The most direct screening for acid soil tolerance is the measurement of yield of both grain and total biomass under field conditions. The result is an integrated measurement of tolerance expressed throughout development. The procedure is to conduct tests in unamended and lime added blocks to allow a direct measurement of tolerance and to ensure that acid soil tolerance is not related to low yield potential in the absence of stress. Soil management practices are otherwise equal between blocks. The data from the field experiments are often reported as the ratio of grain yield in the unamended block to that in the lime-amended block to adjust for differences in yield potential without acid soil stress. However, Scott and Fisher (1989) showed that equally responsive genotypes may have equal absolute tolerances (yield differential between blocks) but appear quite different in their yield ratios. Thus, the ratios should be interpreted with caution, or at least not reported alone.

The major obstacle for field screening is the inherent spatial variability for pH or plant nutrients (e.g., P) in soil, which influences Al stress severity. Spatial variability can greatly bias the interpretation of the field screening results (Ball *et al*., 1993) and, in turn, lead to an inefficient selection response if not considered in the experimental design or in the statistical analysis by use of a covariate or nearest-neighbour method. Adjustments must also be made in field data, either statistically or intuitively, for a variable response to other environmental factors, such as disease or insect pressures and water supply.

MECHANISMS OF Al TOLERANCE IN PLANTS

During the past two decades there have been many hypotheses to explain the mechanisms to cope with Al stress among plants. In general, strategies that various plants use to tolerate Al fall into two categories: (1) external resistance mechanisms, by which Al is excluded from plant tissues, especially the symplastic portion of the root meristem; and (2) internal tolerance mechanisms, conferring the ability of plants to tolerate Al ion in the plant symplasm where Al that has permeated the plasmalemma is sequestered or converted into an innocuous form (Kochian, 1995).

The possible external resistance or exclusion mechanisms of Al tolerance are: immobilisation of Al at the cell wall or low cell wall cation exchange capacity, selective permeability of the plasma membrane, formation of a plant induced pH barrier in the rhizosphere or root apoplasm, exudation of chelate ligands, exudation of phosphate, and Al efflux (Kochian, 1995; Taylor, 1991), Al^{3+}-induced changes in the membrane protein, and ATPase activity of the microsomal membrane function (Matsumoto *et al*., 1992; Wagatsuma *et al*., 1995). A metabolism-dependent exclusion of Al from root apical meristem has been described, which involves inhibition of Al accumulation in root tips (Rincon and Gonzales, 1992). Foy (1996) also reported that when barley plants were grown at pH 4.4, the accumulation of Al and phosphorus in shoots of susceptible cultivars were three times and two times higher, respectively, than in that of the tolerant cultivars. Accumulation of such high concentrations of Al and P in the aerial parts of the plants are considered to be toxic to growth and development of the plants.

The internal resistance mechanisms are those which operate within the symplasm and are mediated at the cellular level either by detoxification or immobilisation of Al ions that have penetrated into plant cells (Taylor, 1995). The possible mechanisms for internal resistance are: chelation of Al in the cytosol, compartmentation in the vacuole, evolution of Al-tolerance enzymes and elevated tolerance of enzyme activity.

Exudation of organic acids

The mechanism(s) of Al tolerance in plants have not been fully elucidated, but the suggestion that the release of various di- and tricarboxylic acids can form strong complexes with Al has lead to various studies attempting to show that plants use this as a defence mechanism against Al toxicity (Ishikawa *et al*., 2000; Kochian *et al*., 2005). Exudation of organic acids, mainly citric and malic acids appears to be one of the main mechanisms for Al tolerance (Carver and Ownby, 1995; Gallardo *et al*., 1999; Ishikawa *et al*., 2000). Ma *et al*.(2001) proposed two patterns of Al-stimulated efflux of organic acids: (1) the pattern where no discernible delay is observed between the addition of Al and the onset of organic acid efflux (Delhaize *et al*., 2001; Ryan *et al*., 1995; Zheng *et al*., 1998) and (2) the pattern where the efflux of organic

acids is delayed for several hours (Li *et al*., 2000; Ma *et al*., 2001; Yang *et al*., 2006). At sufficient concentrations, these organic acids can form complexes with Al ions, prevent the Al ions from binding to the fixed negative sites of the cell wall and plasma membrane, and confer Al tolerance to plants to maintain the normal functions of the cell wall and plasma membrane (Ishikawa *et al*., 2000). Wagatsuma and Yamasaku (1985) reported that Al tolerant barley cultivars exclude Al actively outside the plasmalemma of root cells, and excluded Al may be polymerised and/or react with phosphorus to form Al precipitates, resulting in low Al content in the root cell protoplast. Zhu *et al*.(2003) reported an Al-exclusion mechanism in Al-tolerant mutant cell lines of barley by exudation of malate and citrate. A negative correlation between accumulation of Al in cells and release of citrate and malate in the medium was observed (Zhu *et al*., 2003), however, the authors did not find any positive correlation between synthesis of citrate and malate in the cells and the amount of these organic acids released in the medium. Citrate secretion from the root apices of barley plays an important role in excluding Al and thereby detoxifying Al based on a positive correlation between citrate secretion and Al resistance in 21 barley varieties (Zhao *et al*., 2003). Miyasaka *et al*.(1991) observed that Al-tolerant snapbean cultivars when grown under sterile conditions for relatively long periods (8 d) exuded citric acid to a level that reached 26% of initial Al (mol/mol). This response was not seen in an Al-sensitive cultivar and the authors concluded that exudation of citric acid into the medium provides Al tolerance, either by chelating external Al and thus preventing its entry into the roots or by mobilizing phosphate that had been precipitated with Al in the root apoplast (Millard *et al*., 1990).

It has been reported that the accumulation of Al phosphate on the root surface of the tolerant barley cultivar Dayton was about two times higher than that on the susceptible cultivar Kearney as revealed by X-ray photoelectron spectroscopy, and that the tolerant cultivars create a barrier to the transport of Al to the interior of the roots.

Binding Al in the cell wall

The interaction of Al with cell wall constituents remains a relatively unexplored aspect of Al phytotoxicity. Binding of Al to charge sites on the cell wall surface is a prerequisite for uptake and toxicity. Plants with a higher root cation exchange capacity are generally more sensitive to Al than similar lines with lower cation exchange capacity (Blamey *et al*., 1990). In terms of a specific interaction with Al, Blamey *et al*.(1993) have provided evidence that Al displaces Ca^{2+} from pectic acid in the cell wall, which reduces the movement of water and mineral nutrients through the cell wall interstices. In the presence of Al, an increase in Ca^{2+} concentration in the root environment reduced the porosity of the root cell wall, which is regulated by the arrangement of Ca^{2+} pectate, thereby regulating the movement of solutes and limiting the uptake of Al in the root cell (Blamey and Dowling, 1995; Carpita and Gibeaut, 1993). However, Kinraide *et al*.(1992) have concluded that negative charges on the cell wall pectins as well as the charge sites on membrane lipids and proteins, do not play a significant role in differential Al tolerance.

An association between Al toxicity and accumulation of Al phosphate precipitates in the apoplasm was reported (Clarkson, 1967). It is still unclear, however, whether Al-tolerant plants actively release phosphate to immobilize Al in the apoplast. Evidence for active efflux of cell phosphate in Al-tolerant sugar beet was reported (Lindberg, 1990). However, it should be noted that cellular phosphate often leaks into the cell wall region as part of the Al stress effect (Ownby, 1993). Various studies have suggested that tolerance of low phosphate, and high efficiency in uptake and distribution of phosphate, may be characteristics of Al-tolerant cultivars (Foy *et al*., 1978). There is evidence that Al-tolerant wheat cultivars are able to absorb and translocate phosphate to shoots in the presence of Al.

Production of root-cap mucilage

The mucilage exudates from root tips, predominantly comprising pectin and D-polygalacturonic acid were found to protect roots from toxic Al, and removal of root cap mucilage caused an increase in Al uptake and phytotoxicity (Horst *et al*., 1982). Henderson and Ownby (1991) noted a strong correlation between root mucilage volume and Al tolerance in winter wheat cultivars. The mechanism of protection by mucilage is not clear yet. It has been assumed that a mucilage droplet would create a boundary layer in which diffusion of Al to the root surface is slowed and

where the organic acid/Al ratio would likely be much more favourable than in the rhizosphere as a whole (Henderson and Ownby, 1991).

Exclusion of Al in the plasmalemma

There is circumstantial evidence that the constituents of the cell wall and plasma membrane are directly involved in an exclusion mechanism, however, details are yet to be explored. The plasmalemma has an essential role in cell metabolism and growth, and is the primary target site of selective Al toxicity. The effect of Al on membrane integrity and function includes binding of Al to membrane lipids (Haug and Shi, 1991), as well as inhibition of ATPase activity (Matsumoto and Yamaya, 1986), NADH-linked electron transfer (Loper *et al*., 1993) and ion channel functions (Rengel and Elliott, 1992). Al toxicity was associated with an increase in the ratio of phosphatidylcholine to phosphatidylethanolamine, which could increase membrane permeability (Lindberg and Griffiths, 1993). Caldwell (1989) observed that a root membrane isolated from an Al-sensitive cultivar appeared to bind more Al than did a tolerant cultivar and inferred that Al could displace Ca^{2+} from membrane protein-binding sites.

GENETICS OF Al TOLERANCE

Genotypic difference in Al tolerance

Tolerance to Al toxicity or acidic soils 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).

Al tolerance has been evaluated by different methods around the world (Foy *et al*., 1965; Maxim and Duta, 1996; Ma *et al*., 1997; Minella and Sorrells, 1992; Foy, 1996; Xu *et al*., 1991; Read and Scott, 1983; Read and Oram, 1995; Hossain *et al*., 2005). Some barley varieties with high Al tolerance were identified, although most of them were sensitive (Foy *et al*., 1965; Minella and Sorrells, 1992; Maxim and Duta, 1996). In general, 6-row cultivars were more tolerant than 2-row and 4-row types, husked more tolerant than naked, and winter cultivars more tolerant than spring ones (Xu *et al*., 1991). All eight tolerant barley cultivars ranked by Minella and Sorrells (1992) were six-row cultivars. Two row barleys having Al tolerance have also been reported (Raman *et al*., 2002).

A wide range of genetic variation for Al tolerance exists in barley (Reid *et al*., 1969) and has been exploited to develop varieties with increased Al tolerance (Foy *et al*., 1965). In Australia, some breeding lines outperformed significantly better on acid soils than Al sensitive lines (Oram, 1983) and have been released for commercial cultivation on acid soils, for example Brindabella, Yambla, and Tulla.

Inheritance of Al tolerance in barley

Al tolerance in barley has been assessed by root staining using hematoxylin (Minella and Sorrells, 1992; Tang *et al*., 2000) and eriochrome cyanine (Ma *et al*., 1997; Wang *et al*., 2006). These qualitative variations have been assessed as stained, unstained and partially stained to represent Al-sensitive, tolerant and intermediate genotypes respectively. Al tolerance on the basis of relative root regrowth in barley has also been assessed as a quantitative trait (Raman *et al*., 2005a). In some cases continuous quantitative phenotypic variations could be grouped into discrete classes. Raman *et al*.(2002) classified tolerant, sensitive and intermediate genotypes in an F_2 segregating population for Al tolerance, evaluated on the basis of root growth rate. Similarly, Ma *et al*.(2004) classified Al tolerant and Al sensitive genotypes by root/shoot ratio, with ratios>0.24 being classified as tolerant, and <0.24 being classified as sensitive. Previous work carried out by Tang *et al*.(2000) and Raman *et al*.(2002) highlighted the need to use F_3 populations for Al tolerance evaluations. Both the studies reported phenotypic misclassifications at F_2 stage, thus it was recommended to perform F_3 family testing to confirm the genetic constitution of an individual F_2.

Research to date has shown that the Al tolerance in barley is under a single locus control. The earliest work was with the barley cultivar Dayton, which was reported to have Al tolerance conferred by a single dominant gene, designated as *Alp* (Reid, 1970). Stϕlen and Anderson (1978) reported a dominant allele *Pht* at one locus which controls high tolerance to acidic soil conditions. The single locus model was

confirmed by Minella and Sorrells (1992), based on extensive crosses. The results further indicated that the expression of the tolerance (dominant or recessive) was dependent on the Al concentration. Al tolerance segregation in F_2 populations from crosses between Dayton (Al tolerant)/Harlan Hybrid (Al sensitive), Harrington (Al sensitive)/Brindabella (Al tolerant), Yambla (moderate Al tolerant)/WB229 (Al tolerant), and F6ant28B48-16 (Al sensitive)/Honen were in a monogenic fashion (Raman *et al*., 2001; 2002; Tang *et al*., 2000; Wang *et al*., 2006). Oram (1983), however, obtained Al tolerant genotypes in an F_4 population of a cross between two of the most susceptible cultivars (CI7115/Weeah) of barley and suggested that transgressive segregation might be due to more than one locus which determines Al tolerance. Minella and Sorrells (1992) did not find any transgressive segregation in segregating populations derived from crosses between tolerant/susceptible and moderately tolerant/tolerant using 37 barley genotypes of diverse genetic and geographical origin. Duke (1982) suggested additive genetic effects in a composite cross of winter barley varieties from all over the world grown on Al-toxic soils resulted in considerable increase in tolerance within one generation.

Location of the gene conferring Al tolerance

The locus *Pht* conferring tolerance to low soil pH was located on chromosome 4H (Stϕlen and Anderson, 1978). The locus *Alp* conferring Al tolerance of Dayton was located on the long arm of chromosome 4H by crossing Dayton with trisomic Shin Ebisu 16 (Minella and Sorrells, 1997). This result was later on confirmed by restriction fragment length polymorphism (RFLP) mapping analysis (Tang *et al*., 2000) and simple sequence repeat (SSR) marker linkage analysis (Raman *et al*., 2003). The same chromosomal location (4H) of other proposed Al tolerance loci was confirmed including *Alt* from WB229 by amplified fragment length polymorphism (AFLP), SSR and analysis of wheat-barley chromosome addition lines (Raman *et al*., 2002) and *Alp3* from Brindabella (Raman *et al*., 2001). However, it is not known whether *Alp*, *Pht*, *Alt*, and *Alp3* are allelic. Recently, Raman *et al*.(2005a) identified several quantitative trait loci (QTLs) for root elongation under Al stress on 3H, 4H, 5H and 6H in an F_2 population from Ohichi/F6ant28B48-16. These additional indicative QTLs require further validation in different genetic backgrounds.

Identification of molecular markers linked with the Al tolerance gene(s)

Molecular markers are highly regarded as an efficient selection tool to indirectly select traits linked to Al tolerance loci (Raman *et al*., 2002). In recent years, a significant effort has been made in developing molecular markers for agronomic traits for efficient marker-assisted selection (MAS) in breeding programs. Table 2 shows the results from studies of molecular markers linked with the Al tolerance gene(s) in barley, and closely related cereals such as wheat and rye.

Different molecular marker systems such as RFLP, AFLP, SSR, sequence tagged site (STS), and diversity array platform have been used to identify Al tolerance alleles in barley (Fig.2). Tang *et al*.(2000) reported three RFLP markers flanking the Al tolerance gene *Alp*, which was 2.1 cM proximal to the marker BCD1117 and 2.1 cM distal to the markers WG464 and CDO1395. RFLP-based marker-assisted selection (MAS) of the *Alp* is both time-consuming and labour intensive and generally involves radioactive isotopes and hence is not suitable for high throughput analysis in breeding programs (Raman *et al*., 2003). Therefore, SSR markers (e.g. Bmag353, Bmac186 and Bmac310) were identified that showed tight linkage with the *Alp* locus (Raman *et al*., 2003).

In an independent study, markers Bmac310, Bmag353, HVM68 and HVRCABG were found to be tightly linked to a major Al tolerance locus *Alt* in a F_2 population from Yambla (moderately tolerant of Al)/WB229 (tolerant of Al) (Raman *et al*., 2002). The *Alt* gene was 1.6 cM proximal to marker Bmag353 and HVM68 and 1.6 cM distal to HVM3. Two SSR markers (Bmac310 and HVRCABG) and six AFLP markers co-segregated with *Alt* (Raman *et al*., 2002). These SSR markers were used to distinguish other source for Al tolerance in different populations derived from Harrington (Al sensitive)/Brindabella (Raman *et al*., 2001), Ohichi/F6ant28B48-16 (Raman *et al*., 2005a) and F6ant28B48-16/Honen (Wang *et al*., 2006).

These markers have enabled fast tracking Al tolerance alleles in different breeding programs in

Table 2 Molecular mapping of Al tolerance in cereals

Species	Crosses	Ranking Al tolerance		Gene/ QTLs	Designation and chromo-some location	Contribution	Mapping population	Marker species	Flanking markers	Distance (cM)	Reference
		Al stress	Criteria								
Hordeum vulgare	Yambla/ WB229	100 μmol/L 4 d+50 μmol/L 5 d	Root regrowth	Gene	*Alt* (4H)	Single major	Wheat-barley chromosome addition lines 67 F_2	AFLP and SSR	Bmag353, HVM68/ HVM3	1.6/1.6	Raman *et al*.(2002)
Hordeum vulgare	Dayton/ Harlan	50 μmol/L 24 h	Hematoxy-lin stain	Gene	*Alp* (4HL)	Single	48 F_2	RFLP	Xbcd1117/ Xwg464, Xcdo1395	2.1/2.1	Tang *et al*.(2000)
Hordeum vulgare	Dayton/ Harlan	50 μmol/L 24 h	Haema-toxylin stain	Gene	*Alp* (4HL)	Single	48 F_2	SSR	HVM68/ Bmag353	5.3/3.1	Raman *et al*.(2003)
Triticum aestivum	BH 1146/ Anahuac	1.7 mmol/L 17 h+0 mmol/L 24 h	Root regrowth	Gene	Alt_{BH} (4DL)	85%	101 F_5 RILs	RFLP	Xbcd1230/ Xcdo1395	1.1/11.3	Riede and Anderson (1996)
Triticum aestivum	BH 1146/ Anahuac	37 μmol/L 72 h	Root tolerance index	Gene	Alt_{BH} (4DL)	Single dominant	91 F_5 RILs	RFLP SSR AFLP	Xgdm125/ Xpsr914	4.8/1.1	Milla and Gustafson (2001)
Secale cereale	Ailes/ Riodeva	150 μmol/L 24 h	Root regrowth	Gene	*Alt1* (6RS)	Dominant	F_2	RAPD & SCARs	ScR01600/ ScB15790	2.1/5.5	Gallego *et al*.(1998a)
Secale cereale	M39A-1-6/ M77A1	2×10^{-6} 72 h	Root tolerance index	Gene	*Alt3* (4RL)	Single	F_6 RILs	AFLP	AMAL5/ AMAL5	0.4/0.7	Miftahudin *et al*.(2002)
Oryza sativa	IR64/*Oryza rufipogon*	1.48 mmol/L 14 d	Relative root length	QTLs	*QAlRr1.1* (1) *QAlRr3.1* (3) *QAlRr7.1* (7) *QAlRr8.1* (8) *QAlRr9.1* (9)	9.0% 24.9% 22.5% 20.8% 9.9%	171 F_6 RILs	RFLP SSR			Nguyen *et al*.(2003)
Oryza sativa	CT9993/ IR62266	30×10^{-6} 10 d	Relative root length	QTLs	*qALRR-1*-1 *qALRR-1*-2 *qALRR*-2 *qALRR*-3 *qALRR*-4 *qALRR*-7 *qALRR*-8 *qALRR*-9 *qALRR*-10 *qALRR*-12	24.1% 18.5% 13.4% 12.8% 20.1% 10.3% 28.7% 19.3% 17.7% 19.7%	146 DH lines	RFLP AFLP SSR			Nguyen *et al*.(2002)
Oryza sativa	IR1552/ Azucena	1 mmol/L 2 and 4 weeks	Relative root length	QTLs	QTLs 2 weeks (1) (3) (12) QTLs 4 weeks (1) (9) (12)	 19% 9% 10% 15% 9% 20%	150 F_9 RILs	AFLP RFLP			Wu *et al*.(2000)
Oryza sativa	Koshihikari/ Kasalath	50 μmol/L 24 h	Relative root elongation	QTLs	QTL (1) QTL (2) QTL (6)	11.1% 7.3% 8.7%	183 backcross inbred lines	RFLP	C86 R2460 G200		Ma *et al*.(2002)
Zea mays	Cat-100-6/ S1587	31×10^{-6} 7 d	Net root length	Genes	*Alm1* (10S) *Alm2* (6S)	24.2 7.67 (*F*-test value)	56 inbred lines	RFLP	CSU70 UMC130		Sibov *et al*.(1999)
Zea mays	L53/ L1327	222 μmol/L 7 d	Net root length	QTLs	QTL1 (2) QTL2 (6) QTL3 (6) QTL4 (8) QTL5 (8)	10.9% 5.3% 15.6% 7.4% 8.6%	168 $F_{3:4}$	RFLP SSR			Ninamango-Cárdenas *et al*.(2003)

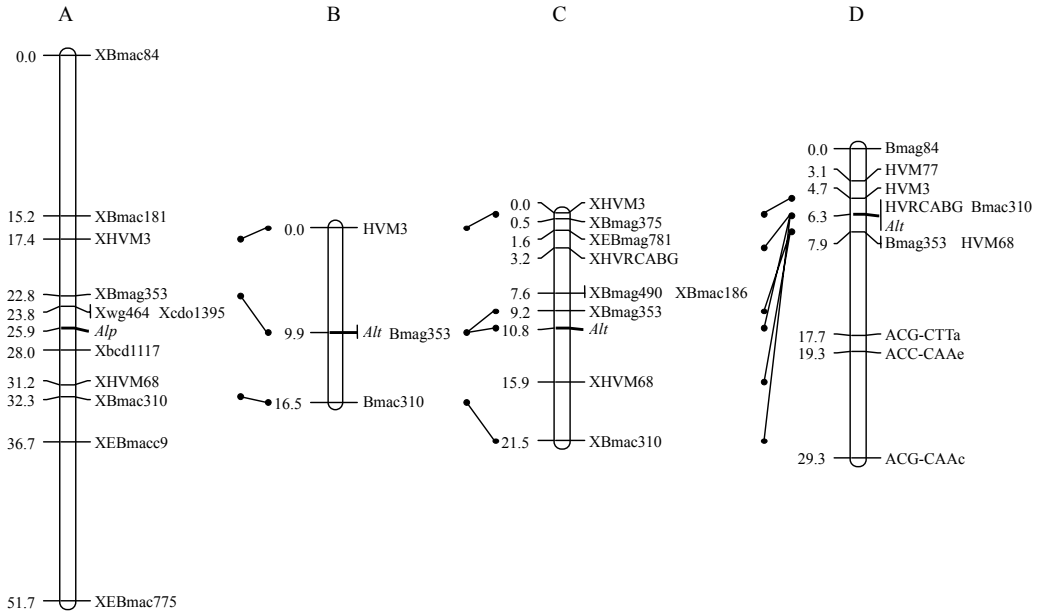

Fig.2 Comparison map of aluminium tolerance locus in barley
A: Dayton/Harlan hybrid (Tang *et al*., 2000; Raman *et al*., 2003); B: Murasakimochi/Morex (Ma *et al*., 2004); C: F6ant28B48-16/Honen (Wang *et al*., 2006); D: Yambla/WB229 (Raman *et al*., 2002)

Australia. Molecular mapping especially with the SSR markers has allowed validating the 'hypothesised' mechanism for Al tolerance in barley (Zhao *et al*., 2003). For example, Ma *et al*.(2004) reported that tightly linked marker Bmag353 explained 51.3% of phenotypic variance for citrate excretion in an intercross population from Murasakimochi/Morex.

Most of the mapping studies employed smaller population comprising 48~100 individuals. It is clear that improved mapping resolution is needed. Firstly, the Al tolerant cultivar Dayton possesses blue aleurone colour which is not desirable for malting barley varieties. The marker Bmag353, which is linked with Al tolerance, could also explain about 54% of phenotypic variation for blue aleurone colour (Li *et al*., 2003; Read *et al*., 2003), indicating the possible tight linkage between these loci. Secondly, barley has a large genome of about 4800 Mb (Arumuganathan and Earle, 1991) and the genetic map covers about 1800 cM (Becker and Heun, 1995), so the mapping interval of 1 cM should correspond to a DNA segment of several megabases. Higher resolution mapping is not only very important to reduce genetic drag during the marker-assisted introgression process but also useful in positional cloning of the Al tolerance gene and may lead to genetic engineering of crops. This strategy has been used to isolate the *Mlo* gene for powdery mildew resistance in barley (Büschges *et al*., 1997; Simons *et al*., 1997).

Comparison of genetics of Al tolerance in cereals

The major gene conferring Al tolerance in barley, wheat and rye seems to be due to orthologous loci on the long arm of the group 4 chromosome. Al tolerance in-wheat cultivars has been found to be under the control of a single dominant gene (Delhaize *et al*., 1993; Riede and Anderson, 1996; Luo and Dvorak, 1996). However, there was also evidence to suggest that more than one Al tolerance gene might exist in certain wheat cultivars (Aniol and Gustafson, 1984; Camargo, 1981; Aniol, 1997). Riede and Anderson (1996) used RFLP markers to map the gene Alt_{BH} conferring Al tolerance in Brazilian wheat cv BH1146 on the long arm of chromosome 4D (4DL). RFLP markers BCD1230 and CDO1395 were 1.1 and 11.3 cM from the Alt_{BH} locus respectively. Milla and Gustafson (2001) used AFLP and SSR markers and mapped this locus to a 5.9 cM interval between markers GDM125 and PSR914, while marker BCD1230 co-segregated with Alt_{BH}. However, CDO1395 and BCD1230 were 2.1 cM and 33.5 cM respectively from the Al tolerance locus *Alp* in barley

(Tang *et al*., 2000). The different relative position of the same markers and Al tolerance locus suggested that this chromosome segment has been subject to structural changes in these two species (Tang *et al*., 2000).

In rye, Al tolerance was reported to be controlled by a single gene on chromosome 4R (Miftahudin *et al*., 2002), 7R (Matos *et al*., 2005), three major genes on 3RL, 4RL, and 6RS (Aniol and Gustafson, 1984; Gallego *et al*., 1998b), and the major locus on 3RS (Aniol, 2004). Gallego *et al*.(1998a) mapped a gene conferring Al tolerance, designated as *Alt1*, on the short arm of chromosome 6R by RAPD (random amplified polymorphic DNA) and SCAR (sequence characterized amplified regions) markers. Miftahudin *et al*.(2002) mapped *Alt3* on 4RL with AFLP and RFLP markers using an F_6 rye recombinant inbred line (RIL) population. They found that the RFLP marker BCD1230 co-segregated with *Alt3*. This marker had previously showed co-segregation with Alt_{BH} locus in wheat (Milla and Gustafson, 2001) or was tightly linked with this gene (1.1 cM) (Riede and Anderson, 1996) in wheat.

Molecular mapping of genes conferring Al tolerance in rice suggested that Al tolerance was a complex multigenic trait. The 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), or on chromosomes 1 and 8 (Nguyen *et al*., 2002). The common chromosome 1 did not seem to correspond to most of the genes that had been mapped for Al tolerance in other species (Nguyen *et al*., 2001) because homeologous chromosome 4 of the *Triticeae* generally corresponds to chromosome 3 in rice. However, in later work a major QTL explaining 24.9% of the phenotype variation was found on chromosome 3 of rice, which is conserved across cereal species (Nguyen *et al*., 2003). In sorghum, a major Al tolerance locus has been mapped on chromosome 3, which is not a syntenic region of group 4 chromosomes of wheat, barley and rye. Instead, it maps to a homeologous region of *Triticeae* chromosome 3, rice chromosome 1 and maize chromosomes 3 and 8. QTLs associated with Al tolerance in maize and rice have also been mapped on these chromosomes (Ninamango-Cárdenas *et al*., 2003). These studies indicate evolutional inheritance of Al tolerance genes in different cereals.

Genes or proteins regulated by Al stress

Studies on genes regulated by Al stress have indicated the complexity of Al toxicity. In rye, Al-regulated genes were found belonging to 13 major functional categories involved in cell elongation and division, oxidative stress, iron metabolism and other cellular mechanisms. The transcripts of two different genes coding for tonoplast intrinsic proteins (TIPs) decreased under Al stress, which would suggest generation of a lower turgor pressure in the cell elongation zone resulting in reduced root growth (Milla *et al*., 2002). Watt (2003) indicated that 14 of the 50 cDNAs up-regulated by Al stress in sugarcane were involved in signalling events and the regulation of gene expression. Mao *et al*.(2003) identified several Al-regulated genes related to the metabolism of cell wall components in rice.

The expression of Al-induced genes in a transgenic *Arabidopsis* plant could ameliorate Al stress and/or oxidative stress (Ezaki *et al*., 2000). The four transgenic lines (*AtBCB-Arabidopsis* blue copper-binding protein, *parB*-tobacco GST, *NtPox*-tobacco peroxidase, *NtGDI1*-tobacco GDP dissociation inhibitor) ameliorate Al toxicity through different mechanisms. *AtBCB* may suppress Al absorption, *NtGDI1* promotes a release of Al in the root tip region, whereas *parB* and *NtPox* enhance the enzyme activities which diminish oxidative damage caused by Al. More resistant transgenic plants could be produced by combination of these four genes (Ezaki *et al*., 2001). Cruz-Ortega *et al*.(1997) suggested that synthesis of 1,3-β-glucanase during Al stress in wheat could be as a protective response against pathogen attack. *TaMDR1* encoding multidrug resistance-like protein was induced in both Al sensitive and Al-tolerant wheat cultivars but the concentration for the induction was lower in the Al sensitive cultivar than in the Al-tolerant one (Sasaki *et al*., 2002). A clone *OsAR28* coding for an unknown protein could be a candidate gene for Al tolerance in rice (Mao *et al*., 2003).

Isolation and cloning of genes for Al tolerance

More than 50 genes with expression induced by Al stress have been isolated from a range of plant species. However, most of these genes are general stress-inducible genes, whose expressions are turned on by oxidative stress (Ezaki *et al*., 1996; Richards *et al*., 1998; Watt, 2003), pathogen infection (Hamel *et*

al., 1998; Cruz-Ortega *et al*., 1997; Watt, 2003), other metal ions (Snowden *et al*., 1995), and water-stress and cold stress (Watt, 2003). They are correlated with Al toxicity rather than tolerance to Al stress.

Most recently, an *ALMT1* gene, which encoded for an Al-activated malate transporter was isolated and characterised (Sasaki *et al*., 2004; Raman *et al*., 2005b). This gene co-segregated with Al tolerance in wheat and increased the tolerance of tobacco cells (Sasaki *et al*., 2004). This discovery gave an explanation of a physiological mechanism of Al exclusion in wheat. The gene was induced in rice and was expressed in the transgenic lines. However, the Al tolerance of these transgenic plants was not increased (Sasaki *et al*., 2004). Transgenic barley with expression of the *ALMT1* gene displayed a capacity for Al-activated malate efflux which was not observed in control plants. Plants expressing *ALMT1* were also more tolerant to Al stress based on root growth in both hydroponic culture and acid soil experiments. This indicated that a higher level of Al tolerance can be achieved by promoting malate efflux in barley (Delhaize *et al*., 2004; Ryan *et al*., 2004). A homolog of the wheat *ALMT1* named *AtALMT1* in *Arabidopsis* was the best candidate from the 14 member *AtALMT* family to be involved with Al tolerance (Hoekenga *et al*., 2006).

Genetic analysis of Al tolerance in highly diverse barley genotypes (Minella and Sorrells, 1992; Tang *et al*., 2000; Raman *et al*., 2001; 2002; 2003; 2005a; Ma *et al*., 2004; Wang *et al*., 2006) indicates that the genetic base for Al tolerance is very narrow. The genetic variation in Al tolerance may be due to mutations in the Al tolerance gene in barley, that may lead to variation in Al tolerance levels (e.g. highly/moderately tolerant). Minella and Sorrells (1992) reported that there is a little chance for barley improvement for Al tolerance. More research is required to screen barley germplasm, in order to identify better sources for Al tolerance. Alternatively, genetic manipulation can be used to improve the Al tolerance levels in barley. So far, there is no gene conditioning Al tolerance which has been isolated and characterised in barley. 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. Furthermore, the mechanisms of Al tolerance include Al exclusion and tissue tolerance (Taylor, 1988; Kochian, 1995). It is still unknown whether any gene relates to a tissue tolerance mechanism, or if it exists.

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