CHAPTER 15

Acid Soils and Aluminum Toxicity

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Acid soils limit crop production on 30-40% of the world's arable land and up to 70% of the world's potentially arable land (Haug, 1983). Although the poor fertility of acid soils is due to a combination of mineral toxicities (aluminum and manganese) and deficiencies (phosphorus, calcium, magnesium, and molybdenum), Al toxicity is the single most important factor, being a major constraint for crop production on 67% of the total acid soil area (Eswaran et al., 1997). Therefore, this chapter will focus on Al toxicity.

Although aluminum toxicity can be ameliorated by surface application of lime, this is often not economically or physically feasible. Hence, combining the use of Al tolerant cultivars with liming is often the most effective strategy for improving crop production on acid soils. To breed genotypes with improved Al tolerance, reliable, efficient screening methods must be available to the researcher. Several screening methods have been employed for this purpose, from genotype screening in the laboratory to soil bioassays and field evaluations.

Plant species differ in their Al tolerance; some are inherently more tolerant than others—for example, cassava (*Manihot esculenta* Crantz), cowpea (*Vigna unguiculata* L. Walp), groundnut (*Arachis hypogea*), pigeon pea (*Cajanus cajan* L. Millsp.), potato (*Solanum tuberosum*), rice (*Oryza sativa* L.), and rye (*Secale cereale* L.) (Little, 1988). Rye is one of the most stress tolerant species in the Triticeae family. Several studies comparing Al tolerance in rye with that of other cereals have shown that rye has the highest tolerance, followed by triticale (X *Triticosecale* Wittmack), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) (Mugwira et al., 1976, 1978; Aniol and Madej, 1996).

Information on the mechanisms of Al tolerance, and the genetic control and chromosome location of Al tolerance genes in different crop species are not only of interest for those crops, but also for other, more or less closely related species. With the identification of molecular markers closely linked to Al tolerance genes in, for example, rye, marker assisted selection (MAS) strategies can be applied to introgress rye Al tolerance genes into wheat.

Global Expanse of Acid Soils

There are several estimates of the extent of acid soils in the world. According to van Wambeke (1976), acid soils occupy 1,455 million ha (11%) of the world's land, while Haug (1983) estimated that 30-40% of the world's arable soils and up to 70% of potentially arable land are acidic. Von Uexkull and Mutert (1995) estimated the global expanse of acid soils (defined as soils with pH <5.5 in their surface layers) to be 3,950 million ha, or approximately 30% of total ice-free land in the world. This is in accordance with Eswaran et al. (1997), who estimated that globally around 26% of total ice-free land is constrained for crop production by soil acidity. Acid soils occur mainly in two global belts (Figure 1): the northern belt, with cold, humid temperate climate, and the southern tropical belt, with warmer, humid conditions (von Uexkull and Mutert, 1995).

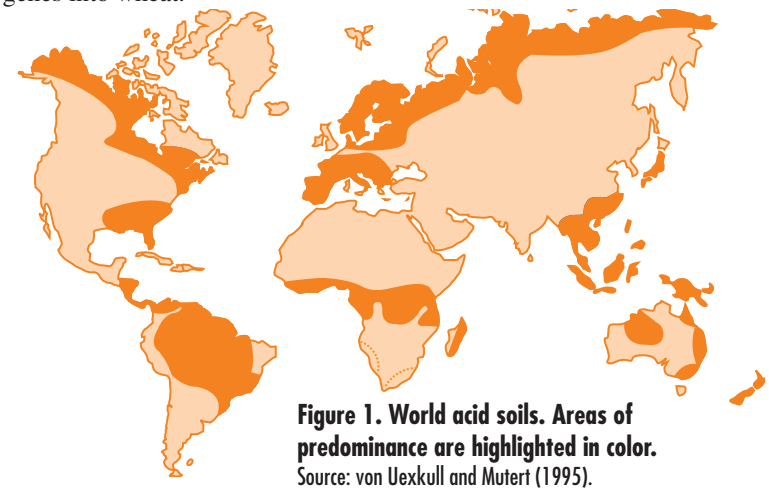

Figure 1. World acid soils. Areas of predominance are highlighted in color.
Source: von Uexkull and Mutert (1995).

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Most acid soils are forests and woodlands (66.3%, or 2,621 million ha), while 17.7% (699 million ha) are covered by savanna, prairie, and steppe vegetation. Only 212 million ha (5.4%) of the world's acid soils are cropped (von Uexkull and Mutert, 1995). The acid soils of the world comprise large areas of potentially arable land, of which the savanna region of Brazil, called Los Cerrados, is a good example. Its total area is approximately 205 million ha, of which about 112 million ha are potentially arable. Much of the remainder could be used for forest plantations and improved pastures for animal production. Similar areas are found in Colombia, Venezuela, Central Africa, and Southeast Asia (Borlaug and Dowswell, 1997).

The Chemistry of Soil Acidity

Development of acid soils

Soil acidity is determined by the amount of hydrogen (H^+) activity in soil solution and 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. Soil acidification is intensified by the removal of cations through the harvesting of crops and by acid precipitation from polluted air (Ulrich et al., 1980). Organic matter decaying to form carbonic acid and other weak acids also contributes to acidification (Carver and Ownby, 1995).

Aluminum is the most abundant metal in the earth's crust, comprising approximately 8% by weight (FitzPatrick, 1986); it is also a major constituent in a wide array of primary and secondary minerals. Under acid soil conditions, these primary and secondary minerals dissolve to a limited extent, releasing Al into the soil solution, where it may be hydrolyzed and contribute to soil acidity. Among the most important soil cations that hydrolyze and contribute significantly to soil acidity are Al^{3+} and Fe^{3+} (Thomas and Hargrove, 1984). Soil acidification is often accelerated by certain cropping practices such as repeated applications of nitrogen in amounts that exceed crop uptake (Adams, 1984). Net H^+ production occurs through natural processes such as nitrification of ammonical nitrogen.

Neutralizing soil acidity

Acidity and Al toxicity in surface soil can be ameliorated through liming. A liming material is defined as a material whose Ca and Mg compounds are capable of neutralizing soil acidity (Barber, 1984). The bulk of agricultural lime comes from ground limestone, and can be calcite ($CaCO_3$), dolomite ($CaCO_3$, $MgCO_3$), or a mixture of the two. Other materials are used to neutralize soil acidity, including marl, slag from iron and steel making, flue dust from cement plants, and refuse from sugar beet factories, paper mills, calcium carbide plants, rock wool plants, and water softening plants (Thomas and Hargrove, 1984). However, total use of these materials is relatively small, and they are generally applied only in areas close to their source.

Lime is usually broadcast on the soil surface and then mixed with the soil during tillage operations. In water, $CaCO_3$ dissolves and hydrolyzes to form OH^- ions that can subsequently react with both H^+ ions formed from hydrolysis of Al^{3+} and exchangeable Al^{3+} (Thomas and Hargrove, 1984). Other compromising factors are NO_3^- uptake and the subsequent release of OH^- (which can neutralize part of the H^+), NO_3^- denitrification, and NH_3 volatilization. Management practices that optimize N-use efficiency and ultimately reduce the amount of NO_3^- lost through leaching could slow the rate of acidification (Carver and Ownby, 1995).

Factors of Acid Soil Infertility

Acid soils are phytotoxic as a result of nutritional disorders, deficiencies, or unavailability of essential nutrients such as calcium, magnesium, molybdenum, and phosphorus, and toxicity of aluminum, manganese, and hydrogen activity (Foy et al., 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara et al., 1998). The solubility of soil compounds and, therefore, nutrient availability to plants is related to soil pH (Figure 2).

Aluminum toxicity

Aluminum toxicity is considered the most important growth-limiting factor for plants in acid soils (Foy et al., 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara et al., 1998). The primary response to aluminum stress occurs in the roots (Foy et al., 1978; Foy, 1984, Taylor, 1988, Jayasundra et al., 1998). Aluminum-injured roots are stubby and brittle. Root tips and lateral roots thicken and turn brown. The root system as a whole is affected, with many stubby lateral roots and no fine branching. Such roots are inefficient in absorbing nutrients and water (Foy et al., 1978).

The main symptom of Al toxicity is rapid inhibition of root growth. A number of mechanisms may cause this, including Al interactions within the cell wall,

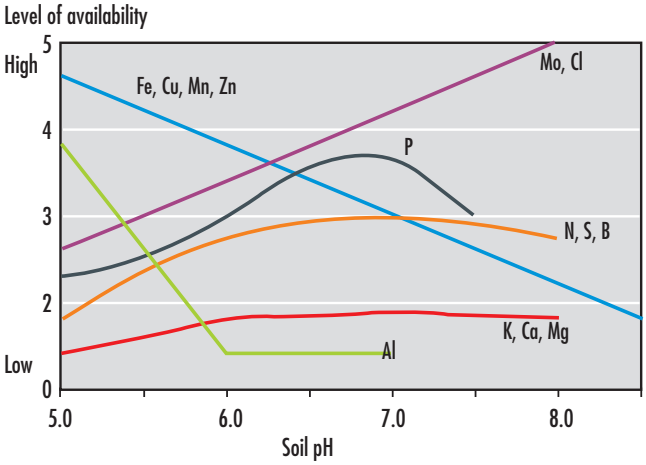

Figure 2. Relationship between level of availability of different elements and soil pH.
Source: Goedert et al. (1997).

the plasma membrane, or the root symplasm (Taylor, 1988; Marschner, 1991; Horst, 1995; Kochian, 1995). According to Ryan et al. (1993), the root apex is the critical site for Al toxicity. They demonstrated in maize (*Zea mays* L.) that for root growth to be inhibited, the terminal 2 to 3 mm of the root (root cap and meristem) must be exposed to Al. Application of Al to the next 3 mm of the root (elongation zone) did not result in significant root growth inhibition.

Ryan et al. (1993) investigated whether the root cap affords protection from the inhibitory effects of Al and found that decapped roots were no more susceptible to Al-induced growth inhibition than intact roots. They concluded that the root cap does not provide protection from Al damage. This is in disagreement with other studies that suggest the root cap provides protection from Al through its involvement in signal perception and hormone distribution (Bennet and Breen, 1991).

Aluminum Tolerance Mechanisms

Aluminum tolerance can be divided into mechanisms that facilitate Al exclusion from the root apex (external tolerance mechanisms) and mechanisms that confer the ability to tolerate Al in the plant symplasm (internal tolerance mechanisms) (Taylor, 1988; Carver and Ownby, 1995; Kochian, 1995). Due to the common assumption that most Al in the root is apoplasmic and that penetration of Al into the symplasm in general is very low, the amount of research addressing internal tolerance mechanisms is limited compared to research on external mechanisms. However, it has been demonstrated that 50-70% of total Al might be present in the symplasm (Tice et al., 1992) and that Al can be present in the symplasm after only 30 minutes' exposure to a solution containing Al (Lazof et al., 1994).

Several external tolerance mechanisms have been suggested, of which the most important are: 1) exudation of organic acids (Hue et al., 1986; Suhayda and Haug, 1986; Miyasaka et al., 1991; Delhaize et al., 1993; Basu et al., 1994b; Ryan et al., 1995; Pellet et al.,1995; de la Fuente et al., 1997); 2) immobilization at the cell wall (Mugwira and Elgawhary, 1979; Blamey et al., 1990; Taylor, 1991; Kochian, 1995); 3) exudation of phosphate (Taylor, 1991; Ryan et al., 1993; Pellet et al., 1997); 4) active Al efflux across the plasma membrane (Zhang and Taylor, 1989; 1991; Taylor, 1991); 5) production of root mucilage (Horst et al., 1982; Henderson and Ownby, 1991); 6) Al exclusion via alterations in rhizosphere pH (Foy et al., 1965; Mugwira et al., 1976; Mugwira and Patel, 1977; Foy, 1988; Taylor, 1988; Taylor, 1991; Kochian, 1995; Pellet et al., 1997), and 7) selective permeability of the plasma membrane (Wagatsuma and Akiba, 1989; Taylor, 1991).

The most important internal tolerance mechanisms are Al-binding proteins, chelation in the cytosol, compartmentation in the vacuole, evolution of Al tolerant enzymes, and elevated enzyme activity (Taylor, 1991). Substantial experimental evidence supports the synthesis of Al-binding proteins (Aniol, 1984b; Picton et al., 1991; Rincon and Gonzalez, 1991; Delhaize et al., 1991; Basu et al., 1994a; Somers and Gustafson, 1995; Somers et al., 1996; Basu et al., 1997).

Genetic Mechanisms of Aluminum Tolerance

Acidity in the surface soil can be corrected by applying agricultural lime. When the subsoil layers are acidic, amelioration of the surface layer will not allow the plant roots to penetrate the acid layer and reach critical water and nutrient supplies below it. Selection and development of genotypes with enhanced tolerance to acid soils and toxic levels of Al is the only reasonable solution to this problem.

The genetics and chromosome localization of aluminum tolerance genes have been extensively studied in cereal crops, with emphasis on wheat. For chromosome manipulation in wheat and triticale breeding, it is important to know which wheat and rye chromosomes carry genes for aluminum tolerance (Aniol and Gustafson, 1984).

Genetic control in wheat

Slootmaker (1974), who first roughly located genes for acid soil tolerance in wheat, found the D genome to be most important, followed by the A genome and the B genome. Aniol and Gustafson (1984) identified Al tolerance genes on chromosome arms 6AL, 7AS, 4BL, 2DL, 3DL, 4DL, and 7D, confirming that Al tolerance genes are mainly located in the A and D genome. Aniol (1990) found genes controlling Al tolerance on 2DL, 4DL, and 5AS.

According to Kerridge and Kronstad (1968), a single dominant gene is responsible for Al tolerance in a cross between the two wheat varieties Druchamp and Brevor, but additional genes are present in Atlas 66. Aniol (1984a) concluded that several genes are responsible for Al tolerance in wheat. This is consistent with Lafever and Campbell (1978) and Campbell and Lafever (1981), who found that Al tolerance in wheat is not simply inherited and that the expression of Al tolerance is additive, with high heritability.

Genetic studies conducted by Camargo (1981) demonstrated that Al tolerance in Atlas 66 is determined by a complex genetic mechanism involving at least two dominant major genes and perhaps some minor genes. Previous studies had identified a gene on chromosome 5D but Berzonsky (1992) concluded that Al tolerance in Atlas 66 is determined not only by dominant genes located on the D genome but also by genes on the A and/or B genomes. Studying different crosses, Rajaram et al. (1991) found the presence of two complementary dominant genes in one parent and one recessive gene in two other parents. Camargo (1984) also reported recessive Al tolerance in wheat.

Other studies have shown Al tolerance to be a simply inherited trait based on a single major dominant gene (Delhaize et al., 1993; Somers and Gustafson, 1995; Somers et al., 1996; Basu et al., 1997). Recently, an RFLP clone on chromosome 4DL has been linked to a gene conferring tolerance to Al in the wheat variety BH1146 from Brazil (Riede and Anderson, 1996).

Genetic control in rye

Rye is one of the most stress tolerant species in the Triticeae family (Little, 1988), and several studies comparing Al tolerance in rye with other cereals demonstrate that on average rye has the highest Al tolerance, followed by triticale, wheat, and barley (Slootmaker, 1974, Mugwira et al., 1976; Mugwira et al., 1978; Manyowa et al., 1988; Aniol and Madej, 1996; Hede et al., 2001a).

Genes for Al tolerance in rye have been located on chromosomes 3R, 4R, and 6RS (Aniol and Gustafson, 1984). Manyowa et al. (1988) found that Al tolerance in rye is controlled by factors on more than one chromosome, though predominantly on chromosome 5R. As has been found in other species, aluminum tolerance in rye seems to be a dominant character (Aniol and Madej, 1996). Gallego and Benito (1997) investigated the segregation of Al tolerance genes and several isozyme loci in a population segregating for Al tolerance and found that Al tolerance in rye is controlled by at least two major dominant and independent loci. Two isozyme loci (*Aco1* and *Ndh2*) were linked to a segregating aluminum tolerance gene on chromosome 6R. Evaluating the segregation ratios in several rye populations, Hede et al. (2001b) found Al tolerance in rye to be controlled by several dominant alleles with different effects at two or three independent loci. A major QTL was identified on chromosome 4R through the application of molecular markers to a specific cross. It accounted for 48% of total phenotypic variation and was linked to an RFLP marker with a distance of 2 cM.

Genetic control in triticale

Many triticales have a high degree of tolerance to Al, but not as much as rye itself (Mugwira et al., 1976; Mugwira et al., 1978; Hede et al., 2001c). Apparently certain wheat genes suppress the expression of Al tolerance genes from rye, yet others allow expression of rye Al tolerance. Aniol and Gustafson (1984) demonstrated that the expression of Al tolerance from 6R depends on which wheat chromosome is substituted. Gustafson and Ross (1990) found suppressors of rye Al tolerance on chromosome arms 4AL, 5AL, 6AL, 7BS, 7BL, and 3DS. Similarly, activators of rye Al tolerance were present on the chromosome arms 2AL, 5AS, 6BS, 1DS, 1DL, 2DL, 4DL, 5DS, 5DL, 6DL, 7DS, and 7DL.

Genetic control in barley

Barley is regarded the most Al sensitive of the small-grain cereals. Genetic analyses have shown that tolerance to acid soil in barley is inherited as a single dominant gene (Stølen and Andersen, 1978) plus multiple alleles (Minella and Sorrells, 1992). Stølen and Andersen (1978) found that tolerance to high soil acidity is controlled by a single dominant gene, designated *Pht*, on chromosome 4. Reid (1971) found Al

tolerance in barley cultivars Dayton and Smooth Awn 86 to be controlled by a single dominant gene, designated *Alp*. Minella and Sorrells (1997) studied the inheritance and chromosome location of *Alp* and found that the *Alp* gene is distally located from the centromere on chromosome 4, suggesting that tolerance to low pH (*Pht*) and aluminum tolerance (*Alp*) are controlled by the same locus.

Genetic Resources to Enhance Al Tolerance

Plant genetic resources are a rich source of valuable traits that could be used to improve crop species. Aluminum tolerance in wheat can be enhanced by incorporating tolerance genes present in the primary, secondary, and tertiary genepools of the Triticeae family. Wheat's primary genepool includes hexaploid, tetraploid, and diploid wheats. It is relatively easy to transfer genes within species of the primary genepool.

Aegilops species and rye belong to the secondary genepool, while triticale is classified between the primary and secondary pools. Most species in the secondary genepool are fairly easy to cross with wheat, although there can be problems with the expression of alien genes in a wheat background. The tertiary genepool consists of annual and perennial forage grasses that are difficult to tap into without the use of specialized techniques.

Significant variation for response to aluminum has been identified in wheat. An efficient way of identifying Al tolerant accessions in a genebank collection is to evaluate those from areas with highly acidic soils and aluminum problems. Some of the most Al tolerant wheats and ryes (such as BH1146 and Blanco) come from Brazil, where vast expanses of land have acid soils.

Hede et al. (1996) investigated whether as a result of natural and human selection, wheat landraces collected from acid-soil regions are more likely to be Al tolerant than those collected from regions with neutral or basic soils. They evaluated the aluminum tolerance of Mexican landraces collected from soils with different acidity levels and found that landraces from acid-soil regions were not more Al tolerant than those from regions with basic or neutral soil; rather, the opposite was true. The most likely explanation is either that the acidity of soils in Mexico is not strong enough to have selected wheats with higher tolerance, or that there was no genetic diversity for Al tolerance in lines brought into Mexico from Europe.

Thus the most appropriate germplasm for increasing aluminum tolerance is likely to be found in areas of extremely low pH (e.g., Brazil). Rye could be used as an indicator; if rye with high Al tolerance is found in an area, wheat with good tolerance will probably also occur there. The wheat landrace Barbela, grown for centuries in certain acid-soil regions of Portugal, was outyielded only by the local rye. Barbela was found to have high levels of Al tolerance; it also has small rye-chromosome segments representing up to 5% of a chromosome (Ribeiro-Carvalho et al., 1997). Barbela may be a good source of Al tolerance genes, especially since it has not yet been utilized in wheat improvement. A similar situation was found in Ecuador, where a highly Al tolerant rye was reported (Baier et al., 1996); it might be worthwhile to screen the wheat landraces from that region for Al tolerance.

Rye has a large amount of variation that could be transferred to wheat. Triticale (a cross between rye and wheat) could serve as a bridging parent to transfer Al tolerance genes from rye to wheat. Slootmaker (1974) studied a number of *Aegilops* species for Al tolerance and found they possess little variation for the trait. He concluded that the reason for this lack of variation is that soils in the center of origin of *Aegilops* (the Fertile Crescent in Asia Minor) are not acid. Nevertheless, *Ae. umbellulata* has been found to have useful levels of aluminum tolerance (Mujeeb-Kazi, pers. comm.).

Relatively little is known about the reaction to acid soil of the species in the tertiary genepool, which are also extremely difficult to utilize in wheat improvement. The preference is thus to utilize species of the primary genepool. However, it may not be possible to achieve greater Al tolerance than found in BH1146 utilizing genetic resources from the primary genepool. The variation found in the secondary and tertiary genepools has great potential for improving the levels of Al tolerance in wheat. The potential payoff in terms of improved Al tolerance may make it worthwhile to use extraordinary means to access the genes conferring such tolerance.

Strategies for Screening for Aluminum Tolerance

Different screening methods have been used to evaluate Al tolerance: cell and tissue culture (Conner and Meredith, 1985), nutrient solution culture (Baier et al., 1995), soil bioassays (Stølen and Andersen, 1978; Ring et al., 1993), and field evaluations (Johnson et al., 1997). Laboratory- and greenhouse-based techniques for screening for Al tolerance are widely used because they are quick, highly accurate, non-destructive, and can be applied at early developmental plant stages. Field-based techniques are more laborious (Carver and Ownby, 1995).

Nutrient solution culture

By far the most common screening medium for Al tolerance is solution culture, which provides easy access to the root system, strict control over nutrient availability and pH, and non-destructive measurements of tolerance (Carver and Ownby, 1995). Different assays have been applied to identify Al tolerant and Al sensitive genotypes, of which the most widely used are hematoxylin staining of root tips and root growth measurement (Baier et al., 1995; Carver and Ownby, 1995). Plant parameters such as root and top dry weight, height, tiller number, and number of spikelets per ear have also been used to evaluate Al tolerance (Mugwira et al., 1976; Mugwira et al., 1978; Manyowa et al., 1988).

Aluminum-induced callose (1,3-b-D-Glucan) synthesis after short Al treatment in nutrient solution has been reported to correlate well with Al tolerance (Zhang et al., 1994; Basu et al., 1997; Horst et al., 1997). Results obtained using the nutrient solution technique have proven to be highly relevant to acidic field conditions. Genotypes classified as Al tolerant based on the nutrient solution evaluation very often show improved agronomic performance under acid soil and Al stress (Carver et al., 1988; Rajaram and Villegas 1990; Ruiz-Torres et al., 1992; Rengel and Jurkic, 1993; Baier et al., 1995).

Hematoxylin staining method

The hematoxylin staining method is an extremely powerful tool for observing tolerance without laborious quantitative measurements. The hematoxylin dye forms complexes with tissue Al that has been immobilized as $AlPO_4$ by phosphate on or immediately below the root surface (Ownby, 1993).

There are several variations of the hematoxylin method. Polle et al. (1978) used the hematoxylin-staining pattern of root tips as an indicator of Al tolerance. As the intensity of staining increases, reflecting a higher level of Al uptake, the level of tolerance decreases. Another procedure using hematoxylin, the modified-pulse method, evaluates Al tolerance based on the ability of Al tolerant seedlings to continue root growth after a short pulse treatment with high Al concentrations (Aniol, 1984a). Aluminum sensitive seedlings do not show root re-growth because their apical meristem has been damaged. This method can be applied to determine Al tolerance through either measuring root regrowth (Aniol and Gustafson, 1984; Gustafson and Ross, 1990; Gallego and Benito, 1997) or evaluating seedlings on a 1 to 3 scale (tolerant, medium tolerant, and susceptible) based on their ability to present root regrowth (Rajaram and Villegas, 1990; Riede and Anderson, 1996).

Laboratory protocol for the hematoxylin method (modified pulse method)

1. Sterilize seeds by placing in a 3% sodium hypoclorite solution for 5 minutes and rinsing thoroughly with water (Figure 3.1).
2. Place seeds on moist filter paper in petri dishes for 84 hours at 7 °C and leave to germinate at room temperature (18-20 °C) for approximately 24 hours (Figure 3.2).
3. Place seedlings with similar root lengths (5-10 mm) and endosperm size on a polyethylene net fixed on lucite frames; attach styrofoam blocks to the frames with rubber bands so they will float (Figure 3.3).
4. Place frames in plastic containers with nutrient solution (4 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2$, 0.1 mM $(NH_4)_2SO_4$, and 0.4 mM NH_4NO_3) maintained at pH 7. Place plastic containers in a water bath kept at 25 °C. Grow seedlings in the nutrient solution for 32 hours. Continuously aerate the nutrient solution during the whole process (Figure 3.4).
5. Transfer frames with seedlings to a nutrient solution containing Al (pH 4.0) and keep in solution for 17 hours (Figure 3.5).
6. Thoroughly wash roots with water and stain with a 0.2 % hematoxylin aqueous solution for 15 minutes. Wash off excess dye.
7. Return seedlings to nutrient solution for 24 hours (Figure 3.4).
8. Remove seedlings from trays; measure root growth or rate seedlings as tolerant (T), susceptible (S), or moderately tolerant (MT). Seedlings with all roots showing continued growth are rated T, whereas seedlings with no roots showing re-growth are rated S. Seedlings showing re-growth on some roots are rated MT (Figure 3.6).

Root growth method

The root growth method considers two Al tolerance parameters: root growth (RG) and a root tolerance index (RTI) (Baier et al., 1995). The RG parameter is measured root growth under Al stress while RTI is root growth under Al stress compared to root growth without Al stress. A low-ionic-strength nutrient solution combined with a low Al concentration is used, as evidence suggests that Al tolerance studies should be conducted using solutions containing ionic strength and Al activity approximating soil composition (Blamey et al., 1991). Assessment of Al tolerance based on root growth and RTI has been used extensively in genetic and molecular studies (Somers and Gustafson, 1995; Baier et al., 1996; Riede and Anderson, 1996; Somers et al., 1996).

Laboratory protocol for the root growth method

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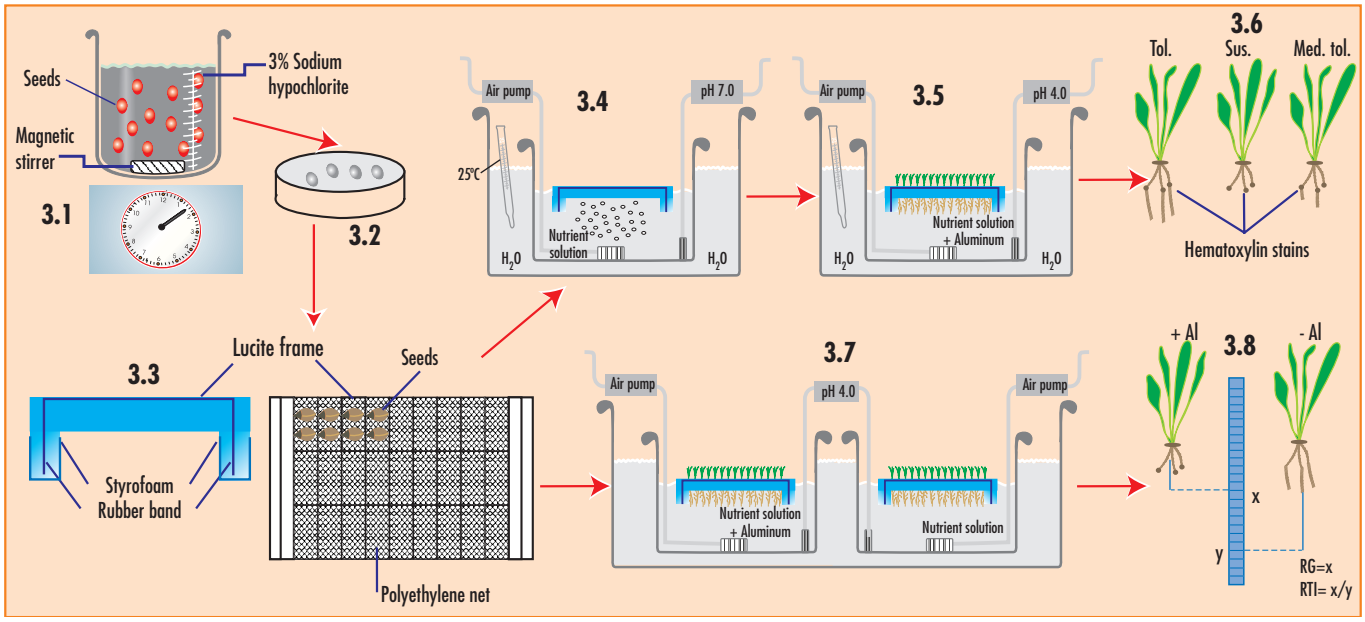

Figure 3. Laboratory procedures for nutrient solution methods.

2. Place seeds on moist filter paper in petri dishes for 84 hours at 7 °C and leave to germinate at room temperature (18-20 °C) for approximately 24 hours (Figure 3.2).
3. Place seedlings with similar root lengths (5-10 mm) and endosperm size on a polyethylene net fixed on lucite frames; attach styrofoam blocks to the frames with rubber bands so they will float (Figure 3.3).
4. Place frames in plastic containers with a low-ionic-strength nutrient solution (400 µM $CaCl_2$, 650 µM KNO_3, 250 µM $MgCl_2$, 10 µM $(NH_4)_2SO_4$, 40 µM (NH_4NO_3) containing Al and maintained at pH 4.0. As a control, a similar experiment but without Al in the medium is conducted at the same time. Replace solutions daily to minimize changes in pH and aluminum concentration. Leave containers for four days (preferentially in a growth chamber maintained at 25 °C, 16-hour day/ 8-hour night, and 70% relative humidity) (Figure 3.7).
5. Remove seedlings from trays; measure the longest primary root of each seedling and average, combining data within each genotype. RG is calculated as the mean root growth of seedlings after four days in a solution containing Al, while RTI is calculated as the ratio between RG and the mean root growth of seedlings after four days' growth in a solution without Al (control) (Figure 3.8).

Comparing the hematoxylin and root growth methods

The optimal Al concentration for screening genotypes depends on the plant species being evaluated. Since rye is more Al tolerant than wheat, the optimum Al concentration for rye is higher than that for wheat. However, optimum Al concentration also depends on the purpose of screening. If it is part of an on-going breeding program and the aim is simply to identify the most Al tolerant plants, higher Al concentrations can be applied. However, if the purpose is to quantitatively characterize the Al tolerance of genotypes, a lower Al concentration has to be applied to better separate the germplasm. Hede et al. (2001a) found that with the hematoxylin method, the most appropriate Al concentration for separating rye populations was 50 mg/l. At higher Al concentrations very few rye plants showed root re-growth. With the root growth method, the best separation of genotypes was achieved using the lowest Al concentration (4 mg Al/l).

Several studies have demonstrated that the primary response to Al stress occurs in the roots. Root growth under Al stress is therefore used as an indirect estimate of Al tolerance in several screening techniques. However, Al tolerance as measured by root growth under Al stress is a combination of Al tolerance (Al tolerance alleles) and root vigor. Thus a relative scale like RTI should be a better indicator of root performance under Al stress, since it can eliminate genotype-specific differences in root growth and standardize comparisons between genotypes (Baier et al., 1995). Since RTI is the relative growth of the genotype in Al solution compared to its potential growth without Al, this parameter is a measure of Al tolerance alone.

To improve precision of the root growth method, Baier et al. (1995) suggest using seedlings with similar vigor, achieved by selecting seedlings with similar sized

endosperm and initial root length. Since seed age is also very important for plant and root vigor, seeds should be regenerated before evaluating for Al tolerance and other traits that may be affected by seed age. This ensures that differences in root growth are not due to differences in vigor caused by seed age (Hede et al., 2001a).

Hede et al. (2001a) compared the hematoxylin and root growth methods to determine whether they identify the same Al tolerant genotypes. Using the root growth method and evaluating genotypes in a solution with and without Al, five different classes of root growth under Al stress were identified, each with a specific combination of root vigor and Al tolerance (Figure 4).

Class 1 and 4 are both very Al tolerant because their RTI is close to, or equal to, 1. However, due to differences in root vigor, classes 1 and 4 will differ in RG values. An experiment considering RG only will never be able to identify class 4 as Al tolerant. Class 2 is a combination of high root vigor and intermediate Al tolerance. This combination results in high RG values but intermediate RTI values. Class 3 has a combination of intermediate root vigor and intermediate Al tolerance, resulting in intermediate RG and RTI values. Class 5 is Al susceptible with a combination of low root vigor and low Al tolerance, resulting in low RG and RTI values.

Measuring RG only will select genotypes with good root growth under Al stress. However, they are not necessarily the most Al tolerant genotypes, defined as those with the most favorable Al tolerance alleles. For example, the RG parameter will characterize classes 3 and 4 as equally Al tolerant, although the RTI reveals that class 4 is actually more tolerant (Figure 4).

Hede et al. (2001a) concluded that the only way to separate the effects of root vigor and Al tolerance is to include a non-stressed control in the experiment. However, due to its quick, inexpensive, and easy screening protocol, the hematoxylin method is still a very efficient way to evaluate large numbers of seedlings from segregating populations derived from elite germplasm. The root growth method (including the RTI parameter), in which the root length of every single plant has to be measured before and after Al treatment, is more laborious than the hematoxylin method. The extra cost of the root growth method is justified when screening new or exotic germplasm, such as genebank accessions. The RTI parameter will identify genotypes that may have superior alleles for Al tolerance, even though their genetic background may lack desirable agronomic characters such as plant and root vigor (e.g., class 4 genotypes).

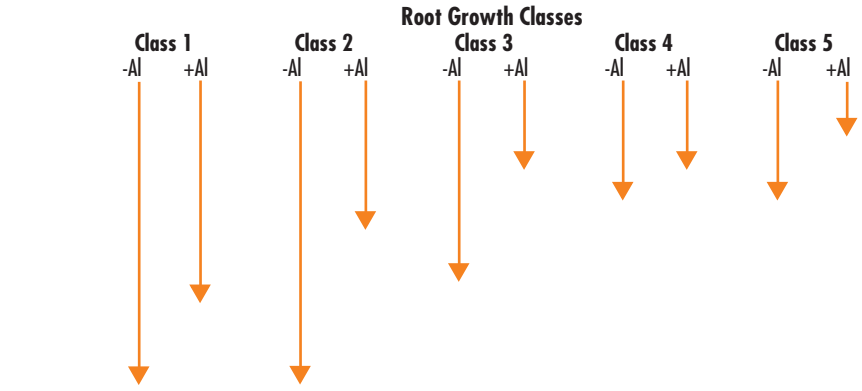

Figure 4. Five classes of root growth without Al stress (-Al) and with Al stress (+Al), and how the specific combination of root vigor and Al tolerance of each type translates into the Al tolerance parameters root growth and root tolerance index.
Source: Hede et al. (2001a).

Cell and tissue culture

The application of cell and tissue culture may offer a way of screening for Al tolerance, given that Al resistance can be expressed at the cellular level (Taylor, 1995). However, this methodology has not yet been substantially explored in wheat, possibly due to the technical difficulty of culturing cells in a low pH, Al-toxic medium (Carver and Ownby, 1995) and there are only a few examples in the literature in which cell and tissue culture was applied (Conner and Meredith, 1985; Parrot and Bouton, 1990).

Soil bioassays

Soil bioassays are not necessarily done on soils from the targeted production area, but screening in a soil representative of the targeted area could be a critical intermediate step, after screening in nutrient solution but before more tedious and expensive field evaluations (Carver and Ownby, 1995). Soil bioassays have a distinct advantage over nutrient solution culture when Al tolerance may be influenced by soil dependent external factors (Ring et al., 1993). The use of soil media has received

less attention than solution media for Al tolerance evaluation, and relatively few examples of its use can be found in the literature (Slootmaker, 1974; Stølen and Andersen, 1978).

Field evaluation

The ultimate and most direct method of evaluating for Al tolerance is by measuring economic yield (forage or grain) under field conditions. Field evaluation is normally conducted in two duplicate tests: one in an unamended and naturally acid plot, and the other in a lime-amended plot. The data are reported as the ratio of grain yield in the unamended plot to that in the lime-amended plot to adjust for differences in yield potential without acid soil stress (Carver and Ownby, 1995; Johnson et al., 1997).

The two most important problems observed when evaluating for Al tolerance in the field are the presence of fungal pathogens such as take-all (incited by *Gaeumannomyces graminis* var. *tritici*), in which infection is often favored by the application of lime to low pH soils (Johnson et al., 1997), or spatial variability of pH in the surface and sub-surface soil layers (Carver and Ownby, 1995). There are several examples of evaluating for Al tolerance in the field, but they are more expensive and laborious (Stølen and Andersen, 1978; Ruiz-Torres et al., 1992; Baier et al., 1995; Johnson et al., 1997).

Conclusions

The global demand for wheat and other crop species is increasing as a consequence of rapid population growth. Projections by the International Food Policy Research Institute (IFPRI) indicate that the world demand for wheat will rise from 552 million tons in 1993 to 775 millions tons by 2020 (Rosegrant et al., 1997). This is a total increase of about 40%, or 2% annually. To meet this future demand, productivity in both favorable and marginal environments needs to increase. Most opportunities for opening new agricultural land have already been exploited. However, opening the Brazilian Cerrados and similar areas in Latin America, Central Africa, and Southeast Asia could contribute greatly to raising world food production in the future.

To crop these large areas, a program aimed at developing Al tolerant cultivars and sound cropping practices must be implemented. The first step would be to identify Al tolerant genotypes through an efficient screening and evaluation process. Field evaluation, the ultimate and final test, is rather laborious, since the presence of acid soils in the field often is not very homogeneous. A number of quick and highly efficient laboratory techniques have thus been developed. As discussed earlier, which screening technique to use is strongly determined by the type of germplasm to be evaluated and the screening objective.

Due to its low cost and simple nature, the hematoxylin method is very efficient when working with large populations derived from well-adapted germplasm. However, when evaluating germplasm with superior Al tolerance alleles, but a poor agronomic background, and inferior plant and root vigor, the root growth method with the RTI parameter is the preferred screening method. The frequency of genotypes with superior Al tolerance alleles but poor root vigor is often high in exotic germplasm. Once identified, the Al tolerance alleles may be introgressed into a genotype possessing desirable agronomic characteristics via a backcrossing program using the inexpensive and less laborious hematoxylin method. The root growth method, including the RTI parameter, is also the most suitable approach for genetic and molecular studies requiring a precise quantitative response for Al tolerance.

References

Adams, F. 1984. Crop response to lime in the southern United States. In: *Soil acidity and liming*. Adams, F. (ed.). American Society of Agronomy, Inc., Madison, WI. pp. 211-265.

Aniol, A. 1984a. Introduction of aluminum tolerance into aluminum sensitive wheat cultivars. Z. Pflanzenzuchtg. 93:331-339.

Aniol, A. 1984b. Induction of aluminum tolerance in wheat seedlings by low doses of aluminum in the nutrient solution. Plant Physiol. 75:551-555.

Aniol, A. 1990. Genetics of tolerance to aluminium in wheat (*Triticum aestivum* L. Thell). Plant and Soil 123:223:227.

Aniol, A., and J.P. Gustafson. 1984. Chromosome location of genes controlling aluminum tolerance in wheat, rye, and triticale. Can. J. Genet. Cytol. 26:701-705.

Aniol, A., and L. Madej. 1996. Genetic variation for aluminum tolerance in rye. Vortr. Pflanzenz, chtg. 35:201-211.

Baier, A.C., D.J. Somers, and J.P. Gustafson. 1995. Aluminum tolerance in wheat: Correlating hydroponic evaluation with field and soil performances. Plant Breed. 114:291-296.

Baier, A.C., D.J. Somers, and J.P. Gustafson. 1996. Aluminum tolerance in triticale, wheat and rye. In: Triticale Today and Tomorrow, Guedes-Pinto, H. et al. (eds.). Kluwer Academic Publishers. pp. 437-444.

Barber, S.A. 1984. Liming materials and practices. In: *Soil acidity and liming.* Adams, F. (ed.). American Society of Agronomy, Inc., Madison, WI. pp. 171-209.

Basu, A., U. Basu, and G.J. Taylor. 1994a. Induction of microsomal membrane proteins in roots of an aluminum-resistant cultivar of *Triticum aestivum* under conditions of aluminum stress. Plant Physiol. 104:1007-1013.

Basu, U., D. Godbold, and G.J. Taylor. 1994b. Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. J. Plant Physiol. 144:747-753.

Basu, U., J.L. McDonald, D.J. Archambault, A.G. Good, K.G. Briggs, T. Aung, and G.J. Taylor. 1997. Genetic and physiological analysis of doubled-haploid, aluminium-resistant lines of wheat provide evidence for the involvement of a 23 kD, root exudate polypeptide in mediating resistance. Plant and Soil 196:283-288.

Bennet, R.J., and C.M. Breen. 1991. The aluminum signal: New dimensions to mechanisms of aluminum tolerance. Plant and Soil 134:153-166.

Berzonsky, W.A. 1992. The genomic inheritance of aluminum tolerance in 'Atlas 66' wheat. Genome 35:689-693.

Blamey, F.P.C., D.C. Edmeades, and D.M. Wheeler. 1990. Role of root cation-exchange capacity in differential aluminum tolerance of Lotus species. J. Plant Nutr. 13:729-744.

Blamey, F.P.C., D.C. Edmeades, C.J. Asher, D.G. Edwards, and D.M Wheeler. 1991. Evaluation of solution culture techniques for studying aluminum toxicity in plants. In: *Plant-Soil Interactions at Low pH.* Wright, R.J. et al. (eds.). Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 905-912.

Borlaug, N.E., and C.R. Dowswell. 1997. The acid lands: One of agriculture's last frontiers. In: *Plant-Soil Interactions at Low pH.* Moniz, A.C. et al. (eds.). Brazilian Soil Science Society. pp. 5-15.

Camargo, C.E.O. 1981. Wheat improvement. I. The heritability of tolerance to aluminum toxicity. Bragantia 40:33-45 (in Portuguese).

Camargo, C.E.O. 1984. Wheat improvement. VI. Heritability studies on aluminum tolerance using three concentrations of aluminum in nutrient solutions. Bragantia 44:49-64 (in Portuguese).

Campbell, L.G., and H.N. Lafever. 1981. Heritability of aluminum tolerance in wheat. Cereal Res. Common. 9:281-287.

Carver, B.F., and J.D. Ownby. 1995. Acid Soil Tolerance in Wheat. Advances in Agronomy 54:117-173.

Carver, B.F., W.P. Inskeep, N.P. Wilson, and R.L. Westerman. 1988. Seedling tolerance to aluminum toxicity in hard red winter wheat germplasm. Crop Sci. 28:463-467.

Conner, A.J., and C.P. Meredith. 1985. Large scale selection of aluminum-resistant mutants from plant cell culture expression and inheritance in seedlings. Theor. Appl. Genet. 71:159-165.

De la Fuente, J.M., V. Ramirez-Rodriguez, J.L. Cabrera-Ponce, and L. Herrera-Estrella. 1997. Aluminum Tolerance in Transgenic Plants by Alteration of Citrate Synthesis. Science 276:1566-1568.

Delhaize, E., T.J.V. Higgins, and P.J. Randall. 1991. Aluminum tolerance in wheat: Analysis of polypeptides in the root apices of tolerant and sensitive genotypes. In: *Plant-Soil Interactions at Low pH.* Wright, R.J. et al. (eds.). Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 1071-1079.

Delhaize, E., P.R. Ryan, and P.J. Randall. 1993. Aluminum Tolerance in Wheat (*Triticum aestivum* L.). II: Aluminum-Stimulated Excretion of Malic Acid from Root Apices. Plant Physiol. 103:695-702.

Eswaran, H., P. Reich, and F. Beinroth. 1997. Global distribution of soils with acidity. In: *Plant-Soil Interactions at Low pH.* Moniz, A.C. et al. (eds.). Brazilian Soil Science Society. pp. 159-164.

FitzPatrick, E.A. 1986. An introduction to soil science. Longman Scientific & Technical. pp. 2-55.

Foy, C.D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. In: *Soil Acidity and Liming.* Adams, F. (ed.). American Society of Agronomy, Inc., Madison, WI. pp. 57-97.

Foy, C. D. 1988. Plant adaptation to acid, aluminum-toxic soils. Commun. Soil Sci. Plant Anal. 19:959-987.

Foy, C.D. W.H. Armiger, L.W. Briggle, and D.A. Reid. 1965. Differential aluminum tolerance of wheat and barley varieties in acid soils. Agro. J. 57:413-417.

Foy, C.D., R.L. Chaney, and M.C. White. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29:511-566.

Gallego, F.G., and Benito, C. 1997. Genetic control of aluminum tolerance in rye (*Secale cereale* L.). Theor. Appl. Genet. 95:393-399.

Goedert, W.J., E. Lobato, and S. Lourenco. 1997. Nutrient use efficiency in Brazilian acid soils: Nutrient management and plant efficiency. In: *Plant-Soil Interactions at Low pH.* Moniz, A.C. et al. (eds.). Brazilian Soil Science Society. pp. 97-104.

Gustafson, J.P., and K. Ross. 1990. Control of alien gene expression for aluminum tolerance in wheat. Genome 33:9-12.

Haug, A. 1983. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant. Sci. 1:345-373.

Hede, A.R., B. Skovmand, and O. Stølen. 1996. Evaluation of Mexican wheat landraces for tolerance to aluminum. In: Abstracts, 5th International Wheat Conference. Ankara, Turkey: CIMMYT. p. 184.

Hede, A.R., B. Skovmand, J.-M. Ribaut, D. González-de-León, and O. Stølen. 2001a. Evaluation of aluminum tolerance in a spring rye collection using two hydroponic screening techniques. Submitted to Plant Breeding.

Hede, A.R., J.-M. Ribaut, B. Skovmand, D. González-de-León, and O. Stølen. 2001b. Genetic dissection and molecular mapping of aluminum tolerance in rye. In preparation.

Hede, A.R., B. Skovmand, N. Bohorova, J.-M. Ribaut, D. González-de-León, and O. Stølen. 2001c. Expression of rye aluminum tolerance in primary triticale. In preparation.

Henderson, M., and J.D. Ownby. 1991. The role of root cap mucilage secretion in aluminum tolerance in wheat. Current Topics in Plant Biochemistry and Physiology 10:134-141.

Horst, W.J. 1995. The role of the apoplast in aluminum toxicity and resistance of higher plants: A review. Z. Pflanzenernahr. Bodenk. 158:419-428.

Horst, W.J., A. Wagner, and H. Marshner. 1982. Mucilage protects root meristems from aluminium injury. Z. Pflanzenphysiol. Bd. 105:435-444.

Horst. W.J., A.K. Pschel, and N. Schmohl. 1997. Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. Plant and Soil 192:23-30.

Hue, N.Y., G.R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminium toxicity in subsoils. Soil Sci. Society of America J. 50:28-34.

Jayasundara, H.P.S., B.D. Thomson, and C. Tang. 1998. Responses of cool season grain legumes to soil abiotic stresses. Advances in Agronomy 63:77-151.

Johnson, J.P., B.F. Carver, and V.C. Baligar. 1997. Productivity in Great Plains acid soils of wheat genotypes selected for aluminium tolerance. Plant and Soil 188:101-106.

Kerridge, P.C., and W.E. Kronstad. 1968. Evidence of genetic resistance to aluminum toxicity in wheat (*Triticum aestivum* Vill., Host.). Agron. J. 60:710-711.

Kochian, L.V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46:237-260.

Lafever, H.N., and L.G. Campbell. 1978. Inheritance of aluminum tolerance in wheat. Can. J. Gen. Cytol. 20:355-364.

Lazof, D.B., J.G. Goldsmidth, T.W. Rufty, and R.W. Linton. 1994. Rapid uptake of aluminum into cells of intact soybean root tips. Plant Physiol. 106:1107-1114.

Little, R. 1988. Plant soil interactions at low pH. In: *Problem Solving – The Genetic Approach*. Commun. Soil Sci. Plant Anal. 19:1239-1257.

Manyowa, N.M., T.E. Miller, and B.P. Forster. 1988. Alien species as sources for aluminium tolerance genes for wheat, *Triticum aestivum*. Proc. 7th Int. Wheat Genet. Symp. pp. 851-857.

Marschner, H. 1991. Mechanisms of adaptation of plants to acid soils. Plant Soil 134:1-24.

Minella, E., and M.E. Sorrells. 1992. Aluminum tolerance in barley: Genetic relationships among genotypes of diverse origin. Crop Sci. 32:593-598.

Minella, E., and M.E Sorrels. 1997. Inheritance and chromosome location of *Alp*. A gene controlling aluminum tolerance in "Dayton" barley. Plant Breeding 116:465-469.

Miyasaka, S.C., J.G. Buta, R.K. Howell, and C.D. Foy. 1991. Mechanism of aluminum tolerance in snapbeans. Root exudation of citric acid. Plant Physiol. 96:737-43.

Mugwira, L.M., and S.U. Patel. 1977. Root zone pH changes and ion uptake imbalances by triticale, wheat, and rye. Agronomy Journal 69:719-722.

Mugwira, L.M., and S.M. Elgawhary. 1979. Aluminum accumulation and tolerance of triticale and wheat in relation to root cation echange capacity. Soil Sci. Soc. Am. J. 43:736-740.

Mugwira, L.M., S.M. Elgawhary, and K.I. Patel. 1976. Differential tolerances of triticale, wheat, rye, and barley to alumium in nutrient solution. Agronomy Journal 68:782-787.

Mugwira, L.M., S.M. Elgawhary, and S.U. Patel. 1978. Aluminium tolerance in triticale, wheat and rye as measured by root growth characteristics and aluminium concentrations. Plant and Soil 50:681-690.

Ownby, J.D. 1993. Mechanisms of reaction of hematoxylin with aluminium treated wheat roots. Physiol. Plant. 87:371-380.

Parrot, W.A., and J.H. Bouton. 1990. Aluminum tolerance in alfalfa as expressed in tissue culture. Crop Sci. 30:387-389.

Pellet, D.M., D.L. Grunes, and L.V. Kochian. 1995. Organic acid exudation as an aluminium tolerance mechanism in maize (*Zea mays* L.). Planta 196:788-795.

Pellet, D.M., L.A. Papernik, D.L. Jones, P.R. Darrah, D.L. Grunes, and L.V. Kochian. 1997. Involvement of multiple aluminium exclusion mechanisms in aluminium. Plant and Soil 192:63-68.

Picton, S.J., K.D. Richards, and R.C. Gardner. 1991. Protein profiles in root tips of two wheat (*Triticum aestivum* L.) cultivars with differential tolerance to aluminum. In: *Plant soil interactions at low pH.* Wright, R.J. et al. (eds.). Dordecht, The Netherlands: Kluwer Academic Publishers. pp. 1063-1070.

Polle, E., C.F. Konzak, and J.A. Kittrick. 1978. Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. Crop Sci. 18:823-827.

Rajaram, S., and E. Villegas. 1990. Breeding wheat (*Triticum aestivum*) for aluminium toxicity tolerance at CIMMYT. In: *Genetic aspects of plant mineral nutrition.* El Bassam, N. et al. (eds.). Dordecht, The Netherlands: Kluwer Academic Publishers. pp. 489-495.

Rajaram, S., M.M. Kohli, and J. Lopez-Cesati. 1991. Breeding for tolerance to aluminum toxicity in wheat. In: *Plant-Soil Interactions at Low pH*. Wright, R.J. et al. (eds.). Dordecht, The Netherlands: Kluwer Academic Publishers. pp. 1019-1028.

Reid, D.A. 1971. Genetic control of reaction to aluminum in winter barley. In: Barley Genetics II. Proc. 2nd. Int. Barley Genet. Symp. Nilan, R.A. (ed.). Pullman, WA: Washington State University Press. pp. 409-413.

Rengel, Z., and V. Jurkic. 1993. Evaluation of *Triticum aestivum* germplasm from Croatia and Yugoslavia for aluminum tolerance. Euphytica 66:111-116.

Ribeiro-Carvalho, C., H. Guedes-Pinto, G. Harrison, and J. S. Heslop-Harrison. 1997. Wheat-rye chromosome translocations involving small terminal and intercalary rye chromosome segments in the Portuguese wheat landrace Barbela. Heredity 78(5):539-546.

Riede, C.R., and J.A. Anderson. 1996. Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci. 36:905-909.

Rincon, M., and R.A. Gonzalez. 1991. Induction of protein synthesis by aluminum in wheat (*Triticum aestivum* L.) root tips. In: *Plant-Soil Interactions at Low pH.* Wright, R.J. et al. (eds.). Dordecht, The Netherlands: Kluwer Academic Publishers. pp. 851-858.

Ring, S.M., R.P. Fisher, G.J. Poile, K.R. Helyar, M.K. Konyers, and S.G. Morris. 1993. Screening species and cultivars for their tolerance to acidic soil conditions. Plant Soil 155/156:521-524.

Rosegrant, M.W., Sombilla, M.A., Gerpacio, R.V., and Ringler, C. 1997. Global food markets and US exports in the twenty-first century. Paper prepared for the Illinois World Food and Sustainable Agriculture Program Conference "Meeting the Demand for Food in the 21st Century: Challenges and Opportunities for Illinois Agriculture", May 27, 1997.

Ruiz-Torres, N.A., B.F. Carver, and R.L. Westerman. 1992. Agronomic performance in acid soils of wheat lines selected for hematoxylin staining pattern. Crop Sci. 32:104-107.

Ryan, P.R., J.M. Ditomaso, and L.V. Kochian. 1993. Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44:437-446.

Ryan, P.R., E. Delhaize, and P.J. Randall. 1995. Malate efflux from root apices and tolerance to aluminium are highly correlated in wheat. Aust. J. Plant Physiol. 22:531-536.

Slootmaker, A.L.J. 1974. Tolerance to high soil acidity in wheat related species, rye and triticale. Euphytica 23:505-513.

Somers, D.J., and J.P. Gustafson. 1995. The expression of aluminum stress induced polypeptides in a population segregation for aluminum tolerance in wheat (*Triticum aestivum* L.). Genome 38:1213-1220.

Somers, D.J., K.G. Briggs, and J.P. Gustafson. 1996. Aluminum stress and protein synthesis in near isogenic lines of *Triticum aestivum* differing in aluminum tolerance. Physiol. Plant. 97:694-700.

Stølen, O., and S. Andersen. 1978. Inheritance of tolerance to low soil pH in barley. Hereditas 88:101-105.

Suhayda, C.G., and A. Haug. 1986. Organic acids reduce aluminum toxicity in maize root membranes. Physiol. Plant. 68:189-95.

Taylor, G.J. 1988. The physiology of aluminum phytotoxicity. In: Metal ions in biological systems: Aluminum and its rule in biology, Sigel, H., and A. Sigel (eds.). Vol. 24:123-163, Marcel Dekker, New York.

Taylor, G.J. 1991. Current Views of the aluminum stress response: The physiological basis of tolerance. Current Topics of Plant Biochemistry and Physiology 10:57-93.

Taylor, G.J. 1995. Overcoming barriers to understanding the cellular basis of aluminum resistance. Plant Soil 171:89-103.

Thomas, G.W., and W.L. Hargrove. 1984. The chemistry of soil acidity. In: *Soil Acidity and Liming.* Adams, F. (ed.). American Society of Agronomy, Inc., Madison, WI. pp. 3-56.

Tice, K.R., D.R. Parker, and D.A. McMason. 1992. Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. Plant Physiol. 100:309-318.

Ulrich, B., R. Mayer, and P.K. Khanna. 1980. Chemical changes due to acid precipitation in a loess-derived soil in Central Europe. Soil Sci. 130:193-199.

Van Wambeke, A. 1976. Formation, distribution and consequences of acid soils in agricultural development. In: Proceedings of Workshop on Plant Adaptation to Mineral Stress in Problem Soils. Wright, M.J. and S.A. Ferrari (eds.). Spec. Publ. Cornell Univ., Agric. Exp. Stn., Ithaca, NY. pp. 15-24.

Von Uexkull, H.R., and E. Mutert. 1995. Global extent, development and economic impact of acid soils. Plant and Soil 171:1-15.

Wagatsuma, T., and R. Akiba. 1989. Low surface negativity of root protoplasts from aluminum-tolerant plant species. Soil Sci. Plant Nutr. 35:443-452.

Zhang, G., and G.J. Taylor. 1989. Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. Plant Physiol. 91:1094-1099.

Zhang, G., and G.J. Taylor. 1991. Effects of biological inhibitors on the kinetics of aluminum uptake by excised roots and purified cell wall material of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. J. Plant Physiol. 138:533-539.

Zhang, G., J. Hoddinott, and G.J. Taylor. 1994. Characterization of 1,3-*b*-D-Glucan (callose) synthesis in roots of *Triticum aestivum* in response to aluminum toxicity. Plant Physiol. 144:229-234.