Chapter 9

Effects of Soil pH and Aluminum on Plant Respiration[1]

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Summary

Interactions among external (soil) pH, cellular pH, and their effects on respiratory metabolism are complex. While the effects of changes in the apoplastic pH on the cytosolic pH are not clearly understood, pH directly affects enzymatic reactions in the cell, and pH-regulated ion uptake has profound indirect effects on cellular respiratory metabolism. A major consequence of soil acidification is the release of aluminum in solubilized forms from its insoluble forms, which, in turn, adversely affects the uptake of cations, causes organic acid secretion, and inhibits cell division and growth in the roots. Consequently, the respiratory metabolism is redirected to meet the needs of organic acid efflux from the roots. The effects of changes in external pH on cellular pH and consequent effects of this change on respiratory metabolism, particularly through effects on soil aluminum are summarized.

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I. Introduction

Soil pH is affected by the biogeochemistry of the soil and by changes in ionic strength attributable to inputs from the environment in the form of acidic deposition and/or fertilizer addition. Acidic deposition from the environment (NO_X and SO_X) contributes to a lowering of the soil pH (Van Breemen, 1985), which causes leaching of nutrients as well as solubilization of aluminum (Al^2) (Marschner, 1995). Solubilized Al competes with cation uptake, further compounding the effects of low pH on nutrient uptake (Lawerence et al., 1995). Combined actions of changes in pH, perturbation of nutrient uptake, and solubilization of Al have profound effects on growth, development, and metabolism, including respiratory metabolism in plants.

In this chapter we review the complex relationships among pH, Al solubilization, and respiratory metabolism in plants. Since in nature most pH effects occur in soil and soil solution, we focus primarily on interactions in the root; where relevant, studies with in vitro cell cultures and effects on the above-ground plant parts are also discussed. A critical limiting factor in this discussion is that we lack an understanding of the regulation of cellular pH and its response to changes in external or apoplastic pH. And because soil pH has both direct and indirect effects on plant metabolism, it is not possible to study these effects independently of one another. Finally, solubilization of Al, which is one of the primary effects of lowering of the soil pH, has numerous direct and indirect effects on respiratory metabolism.

II. Relationship Between External (Soil and Apoplast) and Internal (Symplast) pH

The regulation of intracellular pH is a fundamental physiological process of immense importance to nearly every metabolic activity in the plant cell, particularly the transport of nutrients and plant hormones, as well as to numerous enzymatic reactions (Raven and Smith, 1974; Smith and Raven, 1976; Minocha, 1987; Kurkdjian and Guern, 1989). Thus, it is not surprising that cells allocate a significant amount of energy to the regulation of cytosolic and organellar pH. The metabolic production and consumption of large quantities of H^+ and OH^- ions within the cytosol, together with transport of H^+ across cellular membrane systems, constantly challenge the homeostatic mechanisms of pH regulation in the cell (Sanders and Bethke, 2000). Cytosolic pH is tightly regulated within a relatively narrow range of 7.0–7.5, even under a wide range of pH (5.0–8.0) of the bathing solution, though local variations in the pH of cellular compartments and organelles are common (Minocha, 1987; Marschner, 1995; Sanders and Bethke, 2000). However, Smith (1984) and Gehl and Colman (1985) argued that changes in the cytosolic pH are much more pronounced in response to external pH than commonly believed, particularly when cells are challenged by an external pH outside the range of 4.5 to 8.0. Other physical and chemical factors that influence cytosolic pH include light, temperature, hypoxia, and metabolic poisons (Smith and Raven, 1979; Kurkdjian and Guern, 1989; Roberts et al., 1992). The effects of hypoxia on cytoplasmic pH, however, vary with the time of treatment (Roberts et al., 1992). Vacuolar pH generally is much lower (5.0–6.5, but as low as 2.0–4.0), and is not regulated as tightly as the cytosolic pH (Torimitsu et al., 1984; Sanders and Bethke, 2000). Chloroplast pH ranges from 6.5 to 8.0, and is affected by the rate of photosynthesis (Heldt et al., 1973; Davis, 1974).

Most animal cells are constantly bathed in a pH at or near the cytosolic pH. For animal cell culture media also, the pH is controlled experimentally by the incorporation of buffers or by rapid replacement of the medium. By contrast, most plant cell culture media are poorly buffered, and traditionally adjusted to a pH of 5.5 + 0.3 initially; the operating pH of the medium can fluctuate, and reach values that are much lower than the starting pH, especially when ammonium is the source of N (Minocha, 1987; Goodchild and Givan, 1990). In nature, except for plants growing in calcareous soils, plant roots often are surrounded by an acidic pH (Marschner, 1995). Since both pH gradients and differences in potential across the plasmalemma and organellar membranes play essential roles in transport processes and energy relationships in cells (Malkin and Niyogi, 2000), external (apoplastic and ambient environment) pH could have significant effects on respiratory metabolism and consequently on the growth of plant cells

Abbreivations: NO_X – nitrous oxides; OAA – oxalo acetic acid; PEP – phosphoenolpyruvate; ROS – reactive oxygen species; SO_X – sulfur oxides; TCA – tricarboxyloic acid

[2] All ionic forms of aluminum are represented by Al in this chapter; although the toxicity of different forms varies considerably; the most toxic form Al^{3+} occurs only at pH ≤4.5.

in vitro and that of plants in nature.

A major difficulty in interpreting experimental data on the effects of external pH on metabolic reactions in the cell is measuring cytosolic and organellar pH accurately. All direct (microelectrodes) and indirect (partitioning of weakly-acidic dyes or shift in ^{31}P NMR spectrum) methods used to date have provided, at best, a crude estimate of the steady-state pH of a given cellular compartment (Heldt et al., 1973; Moon and Richards, 1973; Walker and Smith, 1975; Boron and Roos, 1976; Burt et al., 1979; Roberts et al., 1992; Bligny and Douce, 2001; Hesse et al., 2002).

Several mechanisms of cytosolic pH regulation were discussed by Smith and Raven (1979) and later by Marschner (1995). While metabolic control of pH through enzymatic reactions was proposed by Davies (1973a,b), Raven and Smith (1974, 1978) and Smith (1984) argued that cytosolic pH might be regulated more by a biophysical rather than by a biochemical pH-stat. They suggested that H^+ transport across the plasmalemma, and probably the tonoplast, plays a major role in the regulation of cytosolic pH. This argument is supported by the existence of proton pumps in both the plasma membrane and the tonoplast. It is now believed that both biophysical and biochemical mechanisms contribute to the regulation of cytosolic pH (Marschner, 1995; Edwards et al., 1998). Smith (1984) suggested that the fine control of cytosolic pH begins to break down when cells are challenged with an external pH below 4.5, and that significant net influx of H^+ causes a downward shift in the cytosolic pH (Rent et al., 1972; Lane and Burris, 1981; Torimitsu et al., 1984; Gehl and Colman, 1985). Similarly, a decrease in the cytosolic pH occurs when anions are taken up in excess.

Finally, plant roots not only respond to pH changes in the soil, but also contribute to the modulation of ambient soil pH by a variety of mechanisms including: differential uptake of NO_3^- and NH_4^+, release of H^+ and/or OH^-, changes in the uptake and release of CO_2, and release of various organic anions and cations (Minocha, 1987; Sorensen et al., 1989; Hoffland et al., 1992; Yan et al., 1992). The modulation of apoplastic and rhizosphere pH by the roots is species specific and heavily dependent on the buffering capacity of the soil. This feature of roots further complicates the interpretation of results on the effects of changes in soil pH on physiological processes in plants. A similar modulation of the nutrient solution's pH also occurs in cell culture systems (Minocha, 1987).

III. Soil pH and Respiration

Our current understanding of the effects of external pH on the overall metabolism of cells, particularly respiratory metabolism, is rather limited and highly speculative (Minocha, 1987; Marschner, 1995; Lambers et al., 1998). In terrestrial environments, increased acidification is more pronounced than alkalinization. The effects of pH changes on plant roots may be short term (rapid) or long term, direct and acute or indirect and slow. It is generally believed that short-term effects of low pH contribute more to root physiology than long-term effects (Kochian, 1995). Lowering the pH of the bathing solution (hence the apoplastic pH) can influence plant cell metabolism in several ways. Whereas some of these effects are due to direct interactions of increased $[H^+]$ with the cell wall and plasmalemma, others are mediated through effects on nutrient availability, uptake and assimilation, and the release and influx of cytotoxic cations. It is difficult to estimate the extent of various forms of respiration (e.g., dark respiration, photo-respiration, including non-phosphorylating respiration and alternative respiration, etc.) precisely, or to understand their metabolic significance and regulation in the plant (Lambers et al., 1998; Siedow and Day, 2000). The dozens of individual reactions of the respiratory metabolism, that are localized in various cellular compartments, are affected directly by cytosolic and/or organelle pH. The effects of pH on individual enzymes often studied in vitro are subject to misinterpretation because of the complexity of these reactions and their sites of localization in the cells. In the following section we discuss the effects of pH on respiratory metabolism in plant cells; examples are drawn from the literature on cell cultures, crop plants, and forest trees.

A. Direct Effects of Soil pH on Respiration

Root respiration constitutes a major proportion of the total respiratory carbon metabolism in plants; under certain conditions, it can account for half or more of the carbon fixed in a day (Van der Werf et al., 1992; Lambers et al., 1998). Root respiration depends on the rate of growth and nutrient supply to the root, both of which are affected directly by changes in soil pH (Van der Werf et al., 1992). The soil pH varies with soil depth resulting in strong pH gradients (up to 2 pH units) within the rhizosphere and the bulk of the soil (Marschner et al., 1991; Godbold and Jentschke,

1998). Over long periods at low pH, a reduced growth rate due to reduced ion absorption and transport, will adversely affect root respiration.

Rapid direct effects of low pH on root elongation presumably do not involve synthesis of new proteins or hormones, nor do they affect root dry weight (Peiter et al., 2001). Perhaps the primary cause is via interactions with auxin-induced H^+-driven cell elongation, which is accompanied by increased respiration (Rayle and Cleland, 1992). This pH effect would be largely apoplastic via buffering of the H^+ ions that are secreted (high pH effect), or by preventing H^+ secretion from the membrane (low pH effect) or by inhibiting cellulose microfibril sliding or interfering with cellulosic bonds by stabilizing them (freezing the cell wall) and preventing elongation of the cell wall.

Soil CO_2 concentration can increase quickly in response to flooding and acidity, and high CO_2 could adversely affect root respiration (Thomas et al., 1973; Nobel and Palta, 1989; Palta and Nobel, 1989; Qi et al., 1994; Lambers et al., 1996, 1998; Wlodarczyk et al. 2002). Suggested mechanisms of CO_2 effects on root respiration include its direct inhibition of cytochrome oxidase, malic enzymes, and other mitochondrial enzymes (Thomas et al., 1973; Gonzales-Meler et al., 1996; Lambers et al., 1998; Drake et al., 1999).

The presence and the extent of plant symbionts, pathogens, and mycorrhizal associations in the root directly affect its respiratory metabolism, and these associations also are subject to adverse effects of low pH (Marschner, 1995; Qian, 1998). Nitrogen-fixing microbes generally promote respiration due to a combination of increased nutrient uptake and increased growth rate. Mycorrhizae have a similar positive effect on root respiration and ion uptake.

The overall extent and the rates of respiration (and its regulation) in the above-ground parts of the plant have been studied extensively (Lambers et al., 1998; Siedow and Day, 2000), yet little is known about the direct effects of leaf exposure to acid mist/clouds on leaf respiration. Leaf respiration depends on three factors: maintenance of biomass, growth, and transport of nutrients (Lambers et al., 1998). It is the last one that is affected the most by changes in soil pH. Major factors that govern respiration in green tissues are the process of photosynthetic carbon fixation and transport of photosynthetic products from the leaf (Siedow and Day, 2000). In C_3 plants, the extent of photorespiration may be variable but it responds more to CO_2 levels than to pH. Also, indirect effects of low pH on leaf respiration via its effects on stomata closure can be important under acid mist conditions (Sanders and Bethke, 2000). The major effects of ambient cloud pH on respiration would occur at night when there is increased vapor condensation due to lower temperature, though there is little direct evidence for such an effect.

B. Soil pH, Ion Uptake and Respiration

The most significant indirect effects of changes in soil pH on respiratory metabolism in plants probably result from alterations in nutrient supply and uptake. A large proportion of cellular respiratory metabolism is devoted to N absorption and assimilation in plants (Lambers et al., 1996). While a part of this is perhaps related to the energy dependence of N uptake and assimilation, equally important is the needed carbon skeleton for N assimilation that is provided by the TCA cycle (Forde and Clarkson, 1999, and references therein). The interactions among NO_3^-, NH_4^+ and soil pH play a critical role in N uptake by the roots (Britto et al., 2001b; Kronzucker et al., 2001 and references therein).

Both the relative amounts of NH_4^+ and NO_3^- in the soil and their absorption by roots are affected by pH in complex ways. For example: (a) at relatively high soil pH (7.0–9.5), passive influx of free ammonia (NH_3) increases total N uptake; (b) a low vacuolar pH acts as a facilitator of NH_3 transport and trapping (in the form of NH_4^+); and (c) high-affinity transport systems for NH_4^+ apparently are unaffected by pH changes over a wide range (4.5–9.0), though this transport is presumably H^+-coupled (Wang et al., 1993a,b; Britto et al., 2001a). It should be noted that preferential uptake of NH_4^+ by roots (and also by cells in culture) results in lowering the soil (or medium) pH, while the opposite is true when NO_3^- is taken up (Forde and Clarkson, 1999; Britto and Kronzucker, 2002).

Vanhala (2002) summarized the seasonal variation in soil respiration in three forest sites that differed in N, water availability, and pH. Although seasonal variation in soil chemical properties was small, soil respiration rates decreased from spring to summer, with minimum values at the end of August. The soil respiration rates increased again in autumn, but values were not close to those in the spring. At a constant temperature (14 °C), the respiration rate was determined by moisture and pH, while at a constant moisture content, it depended heavily on

the amount of organic matter (N and C-org) and pH. When the respiration rate was calculated on the basis of the N concentration, the variation was explained primarily by pH.

Inorganic phosphate (Pi) is a key nutrient that is crucial for the biosynthesis of macromolecules and for the whole gene-expression machinery. Phosphate also plays a predominant role in plant respiratory metabolism because of its involvement in energy transfer reactions (Siedow and Day, 2000). Cellular Pi levels are tightly associated with its metabolic demand, and regulated by its supply, which is significantly affected by pH of the nutrient solution. In plants, several high-affinity Pi transporters have been cloned that regulate the balance of cellular Pi and its organellar compartmentation in various tissues. Several of these are PO_4^{3-}/H^+ transporters whose activity responds to external pH. A reduction in nutritional availability of PO_4^{3-} results in a decrease in cellular Pi, ATP, and ADP, which affects numerous metabolic pathways that use these phosphorylated moieties. Apart from direct effects of Pi deficiency on the respiratory pathway (Gauthier and Turpin, 1994), significant indirect effects include altered N metabolism (Johnson et al., 1996b), an increase in amino acids and NADH (Johnson et al., 1996a; Juszczuk and Rychter, 1997), and an increase in alcohol dehydrogenase activity (Neumann et al., 2000). Some of these effects are related to the increased production of organic acids (see discussion below) that involves changes in the activities of certain respiratory enzymes, e.g., PEP carboxylase, malate dehydrogenase, PEP phosphatase, citrate synthase, phosphofructophosphatase, and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (Duff et al., 1989b; Theodorou et al., 1992; Delhaize et al., 1993a,b; Johnson et al., 1994, 1996a,b; Watt and Evans, 1999b).

An example of how pH and Al-induced reduction in Ca^{2+} uptake, Al-induced efflux of citrate (see discussion below), and availability of cellular Pi could modulate at least one key step in respiration is their effect on the properties of the vacuolar PEP phosphatase, which presumably converts PEP into pyruvate (reviewed in Givan, 1999). K^+, Mg^+, and ADP are positive effectors of this enzyme, while ATP, citrate, and Ca^{2+} are negative effectors (Duff et al., 1989a, 1991). As described later, Al causes a reduction in cellular [Ca] (which also is a direct effect of low pH), and also induces a significant efflux of citrate; both of which, combined with reduced ATP and Pi, tend to promote the conversion of PEP to pyruvate, which could then serve as a substrate for synthesis of citrate and other organic acids (Fig. 1). A reduction in the concentrations of citrate and ATP also enhances the activity of key glycolytic reactions that involve phosphofructokinase. Levi and Gibbs (1976) and Steup et al. (1976) demonstrated that high amounts (2–5 mM) of PPi promoted a breakdown of starch in the chloroplast by plastid glycolysis.

In addition to effects on the uptake of N and P, pH profoundly affects the availability as well as the uptake of many other nutrients, including Ca, Mg, Mn, Fe, and K. In turn, these nutrients play significant but complex roles in respiration (Marschner, 1995; Siedow and Day, 2000).

IV. Interactions Among Soil pH, Aluminum and Respiration

Bound as oxides and complex aluminosilicates, Al is the most abundant metal in the Earth's crust. Until recently, surface-water concentrations of Al ions (particularly Al^{3+}) have remained minimal due to the insolubility of Al hydroxide complexes at neutral pH (Macdonald and Martin, 1988). The lowering of soil pH due to acidic deposition and excess fertilization leads to the solubilization of otherwise insoluble Al in the mineral soil horizon. On the soil particles, Al competes with Mg and Ca for ion-exchange sites, leading to leaching of these nutrients into mineral soil and eventually into surface water (Lawrence et al., 1995). Al profoundly affects plant growth and development through inhibition of cell division (via inhibition of DNA, RNA and protein synthesis), reduction of cell enlargement, and cytotoxicity by direct interactions with the plasmalemma (Delhaize and Ryan, 1995; Sparling and Lowe, 1996; Ma et al., 2001; Kochian et al., 2002). The direct and indirect effects of Al on cation uptake, membrane properties, and organic acid efflux, all are tied to cellular respiration The discussion here is limited mostly to the effects of Al on membrane properties and metabolic processes that are related (Fig. 1) and presumably are responsible for effects on plant respiration.

The degree to which Al effects are seen in plants depends on environmental factors that include the amount and species of Al ions, amounts of other ions present, amount and form of organic matter present, and plant genotype (Karr et al., 1984; Kochian et al., 2002). While most plants exclude this metal or absorb it only in small quantities, several widely spread taxa

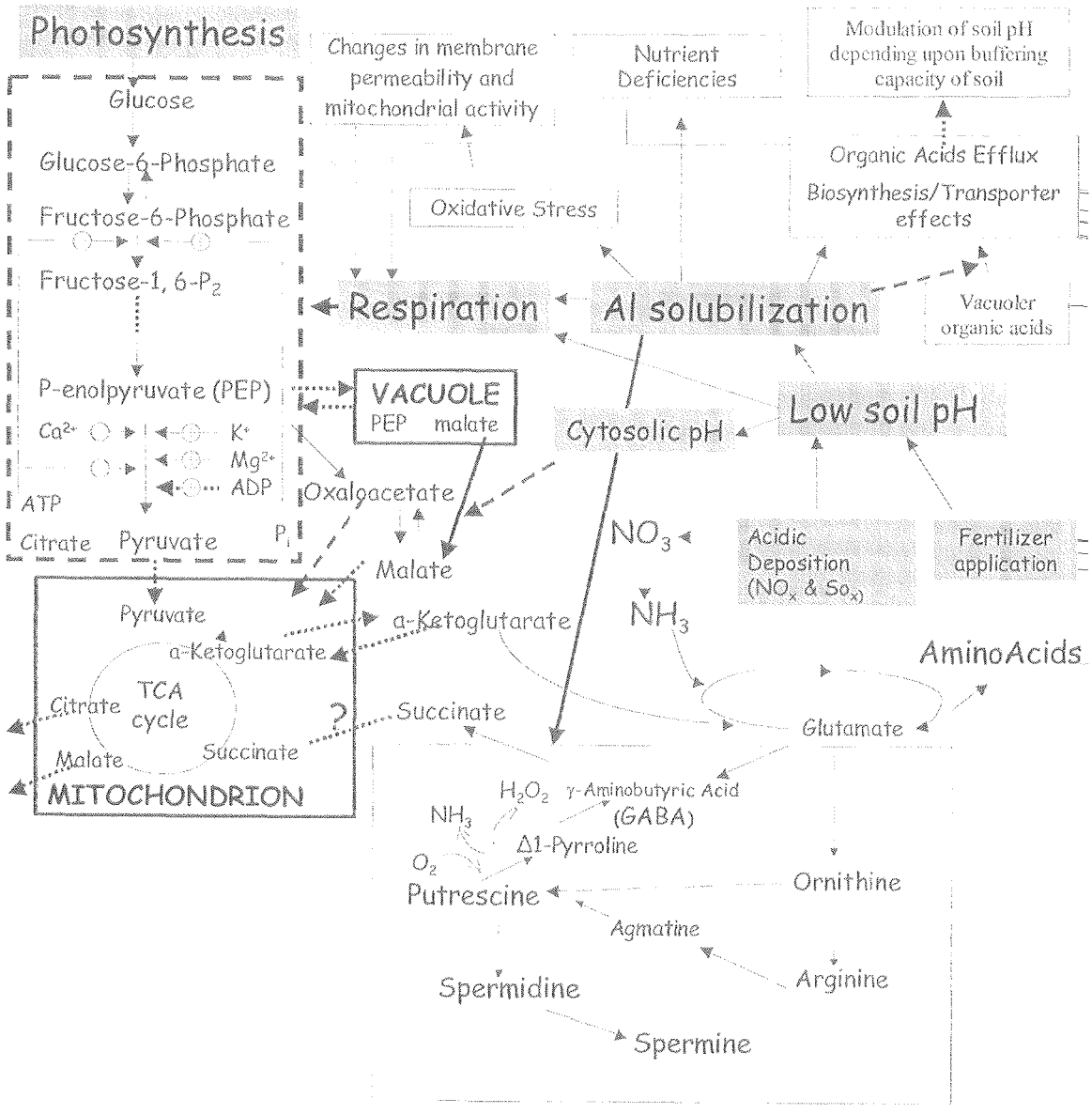

Fig. 1. Interactions among low pH, Al solubilization and the respiratory pathway in plants. Only selected parts of the pathways that are relevant to the discussion in this chapter are shown here.

are known to be hyperaccumulators of Al (Jansen et al., 2002). Soil pH dictates the total amount of Al that is solubilized, and thus available for physiological effects, and also affects the inorganic forms of Al that determine its toxicity. The Al^{3+}, $Al^{2+}(OH)$, and $Al^{+}(OH)_2$ monomers generally are regarded as the toxic forms of Al in aqueous systems; of these, Al^{3+} is many times more toxic than the hydroxyl forms, although a pH ≤4.5 is required for its formation (Delhaize and Ryan, 1995). Tolerance/resistance mechanisms have evolved in plants to cope with an acidic rhizosphere environment where Al concentrations are relatively high (Piñeros and Kochian, 2001).

The complexity of interactions among the effects of Al and pH on respiration is evident from: (a) multiple effects of soil pH on Al availability, Al speciation, and uptake, and (b) variable pH of the cellular compartments and resulting effects on Al speciation

and compartmentation in the symplast (Kochian et al., 2002). The results of a study by Godbold and Jentschke (1998) who attempted to experimentally analyze the interaction between Al and pH in a nutrient solution further illustrate the complexity of this interaction. The uptake of ions by the plant is heavily dependent on the ion exchange capacity of the root apoplast, which is subject to major influence of pH (Meychik and Yermakov, 2001). Al, whose solubilization and specific forms depend on pH (the lower the soil pH, the higher the overall Al solubilization and the availability of Al^{3+}), readily displaces Ca and Mg in the apoplast (Godbold et al., 1988; Schröder et al., 1988). While the soil and the nutrient solution chemistry suggest that maximum Al toxicity should coincide with maximum Al solubility, lowering of pH to around 3.2 actually reduces Al toxicity (Grauer and Horst, 1990; Kinraide, 1993). In the root cortical cell walls of Norway spruce (*Picea abies*) seedlings, the cation-exchange capacity (for Ca, Mg, etc.) was the highest at pH 4.0, within the range of 3.2–5.0 that was tested (Godbold and Jentschke, 1998; see also, Godbold et al., 1988; Schröder et al., 1988; Keltjens, 1995). In the presence of Al, the highest cation-exchange capacity was at pH 3.2; it decreased as the pH of the solution increased, as did the amount of Ca and Mg present in the root apoplast. Higher Al in the soil or the nutrient solution was associated with reduced foliar Ca and Mg in some plants, but there was no effect on foliar Mg levels in others (Godbold et al., 1988; Schröder et al., 1988; Moustakas et al., 1995; Oleksyn et al., 1996; Lee et al., 1999; Minocha et al., 2000). The effect of Al on both shoot and root growth was maximum at pH 5.0 and minimum at pH 3.2.

A. Aluminum in the Apoplast and Symplast

A major controversy concerning the physiological effects of Al is whether its effects are an apoplastic or a symplastic phenomenon (Kochian et al., 2002). There is a large body of evidence attesting to the apoplastic effects of Al. However, under acidic conditions, Al^{+3} can enter the cell by transporters, by adsorption endocytosis, or by forming complexes with Fe^{+3}, amino acids and organic acids (Kochian, 1995), and thus show symplastic effects. Possible apoplastic interactions of Al include immobilization of cell walls, chelation with organic acids, and stimulated efflux of phosphate. Examples of the symplastic interactions are chelation by organic acids, proteins, and other organic ligands in the cytosol; compartmentation in vacuoles; and superinduction of Al-sensitive enzymes.

The plasma membrane is known to be an effective barrier to Al entry into the cytoplasm (Cuenca et al., 1991; Marienfeld and Stelzer, 1993; Marienfeld et al., 1995). Other researchers have reported that Al enters the symplast in the root cells, where it can accumulate and/or be transported to the shoots (Matsumoto et al., 1976; Asp et al., 1988; Zhang and Taylor, 1989; Lazof et al., 1994, 1997). For the primary effects of short-term exposure to Al, its entry into the symplast is not considered necessary (Olivetti and Etherton, 1991; Huang et al., 1992; Rengel, 1992). There is also evidence that Al can directly alter plasma-membrane properties by interacting with certain phospholipids and altering their peroxidation levels (Akeson and Munns, 1989; Rengel, 1996; Yamamoto et al., 1997; Zhang et al., 1997) and ion-channel proteins (Rengel, 1992). One of the few early studies of Al effects on membrane functions showed accelerated leakage of K^+ from the plasma membrane (Woolhouse, 1969). Later studies showed the activation of anion channels similar to the S-type Cl^- channel in the membrane in response to Al (Ryan et al., 1997; Zhang et al., 2001). Its ability to chelate extracellular organic acids, to affect membrane properties, and to stimulate efflux of phosphate all will have significant effects on cellular respiration rates.

Cytosolic effects of Al are generally believed to be less important, because at cytosolic pH of about 7, Al exists mostly as $Al(OH)_4^-$ which has little toxicity (Martin, 1988; Kinraide and Parker, 1990; Kochian, 2000). According to Martin (1988), most of the Al in cytosol is tied up, and its available concentration is low, about 10^{-10} M. However, it could still compete for Mg binding sites even at extremely low concentrations (Kinraide and Parker, 1990; Martin, 1992). For example, binding of Al to ATP is 7 orders of magnitude greater than that of Mg, and the $Al\text{-}ATP^{4-}$ complex binds more strongly to hexokinase than Mg-ATP complex. This would result in a negative impact of Al on respiration. It has not yet been possible to make credible assertions about intracellular localization of Al because of the differences in instruments, sample preparation methods, operating conditions, and lengths of Al exposure used in various studies (Rengel, 1996).

B. Direct Effects of Aluminum on Respiration

There have been several reports on the effects of Al treatment on the rates of cellular respiration in a variety of plants. Upon exposure to Al, the respiration rates of carrot (*Daucus carota*) suspension cultures decreased significantly (Honda et al., 1997), as did the respiration rates of white spruce (*Picea glauca*) seedlings (Nosko et al., 1988). In carrot, Al exposure also caused a reduction in cellular ATP level. Al inhibited respiration in cut rose leaves (Son et al., 1994), inhibited mitochondrial respiration and oxidative phosphorylation in the roots of rice (*Oryza sativa*) seedlings (Hao and Liu, 1989), as well as red spruce (*Picea rubens*) and tobacco (*Nicotiana tabacum*) cultures (Ikegawa et al., 1998; Minocha et al., 2001; Yamamoto et al., 2002). Schaberg et al. (2000) observed reduced respiration in elongating shoots of red spruce following Al exposure. Dark respiration was inhibited in the seedlings of Japanese red pine (*Pinus densiflora*) when Al was added to the culture medium (Lee et al., 1999, 2001). Yamamoto et al. (2002) reported an increase in reactive oxygen species, inhibition of respiration, depletion of ATP, and loss of growth in tobacco and pea plants 12 h after Al exposure. These results were used to hypothesize that mitochondrial dysfunction may lead to the production of reactive oxygen species, which may be a key factor in growth inhibition by Al. Other cases where Al caused an increase in respiration are red spruce seedlings and wheat (*Triticum aestivum*) roots (McLaughlin et al., 1990, 1991; Collier et al., 1993).

In most of the above-mentioned cases, the target site for Al effects on respiration was not identified. Three kinds of effects of Al on plant respiratory metabolism can be postulated: (a) directly by interactions with the plasmalemma and organellar membranes, (b) those related to the uptake of ions, and (c) those related to Al-induced efflux of organic acids.

The effects of Al on the activities of enzymes involved in ATP generation and utilization via effects on ATP synthase and ATPases also have been reported. Increases in the activities of vacuolar ATPase and mitochondrial ATP synthase concomitant with a reduction in the plasma membrane ATPase activity were observed in an Al-resistant wheat variety (Hamilton et al., 2001). Changes in the level of transcript for vacuolar ATPase paralleled changes in its enzyme activity, indicating that this response occurs at the level of gene expression. However, no change in the transcript levels of mitochondrial ATP synthase was observed. The induction of vacuolar ATPase activity is thought to be a homeostatic mechanism required to provide energy for Na^+/H^+ antiport that delivers Na^+ to vacuoles. However, upon exposure to Al, an increase in vacuolar ATPase may be needed to maintain the cytosolic pH neutrality or to sequester Al in vacuoles via an Al^+/H^+ antiport similar to the Na^+/H^+ antiport. Currently, there is no direct evidence for such Al^+/H^+ co-transporters in plants (Hamilton et al., 2001 and references therein).

C. Aluminum, Organic Acid Metabolism, and Respiration

As discussed earlier, solubilized Al interferes with the uptake of many essential cations, particularly Ca, Mg, Fe, and Mn (Delhaize and Ryan, 1995; Ryan et al., 2001; Ma et al., 2001). The importance of these ions in respiration was also discussed in relation to the effects of pH. Al has specific interaction with the uptake of Ca and P, with the former it competes for carriers; with the latter, it forms insoluble $Al_3(PO_4)$ both in the soil and in the apoplast. Pellet et al. (1995) showed that PO_4^{3-} secretion and organic acid secretion might be regulated independently.

One of the best documented phenomena in relation to Al effects in plants is its role in inducing the efflux of low-molecular-weight organic acids from intact roots as well as from cells in culture (reviewed in Delhaize et al., 1993a,b; Delhaize and Ryan, 1995; Ma, 2000; Ma et al., 2001; Ryan et al., 2001; Kochian et al., 2002; Minocha and Long, 2004). Acids that are commonly released include: citrate, malate, succinate, and oxalate. Often, a particular acid is secreted in a species-specific manner, and more than one acid can be secreted simultaneously. Secretion of organic acids is greater in Al-resistant varieties, is Al induced, and is dependent on Al concentration. This secretion is several folds greater in the apex than in the mature parts of the root, suggesting localized detoxification and injury (Ryan et al., 1995). While the induction of organic acid secretion by Al is well established, the mechanisms of signal perception and transduction that cause the efflux, the signals that cause increased production of these acids, and the specific enzyme(s) involved in their biosynthesis are not known.

Several organic acids are present in all living organisms as intermediates of the TCA cycle, the main respiratory pathway that oxidizes malate and pyruvate to release energy (Bray et al., 2000; López-Bucio et

al., 2000; Siedow and Day, 2000). Some of these are present both in the cytosol and in the mitochondria, and can accumulate in large quantities in vacuoles. Organic acids also are linked with other metabolic processes, e.g., amino-acid biosynthesis, regulation of cytosolic pH and osmotic potential, chelation of cations, and charge balance during excessive carbon uptake (Edwards et al., 1998). Questions remain with respect to Al-induced organic acid secretion; for example: since the raw materials for the synthesis of most organic acids that are secreted are in the mitochondria, what triggers their release from this organelle, or from the vacuole, into the cytosol for its eventual efflux? Is it simply a matter of equilibrium between the two compartments, or active mitochondrial and vacuolar membrane transporters are involved? If the latter is the case, then how are these transporters regulated by Al? Does Al enter mitochondria? It is believed that Al interactions could occur at any or all of these levels to cause efflux of a particular acid.

The mechanisms that regulate the secretion of organic acids as well as their biosynthesis to support increased secretion in response to Al treatment have been widely debated. Ma (2000) discussed two patterns for Al-induced organic acid secretion. In the first, there is no lag phase between Al exposure and organic acid secretion (rapid, short-term responses); activation of anion channels is implicated in this approach. The second entails a marked lag phase between Al exposure and acid secretion (slow and long-term effects); gene activation may play a part in affecting organic acid metabolism in this pattern. Furthermore, it is still not known whether Al functions from the outside or the inside of the membrane to induce rapid changes in organic acid efflux. In addition to increased organic acid efflux in response to Al, there are examples of an increase in biosynthesis and accumulation of organic acids in cells without increase in efflux (Keerthisinghe et al., 1998; Watt and Evans, 1999a; Neumann and Römheld, 2000). The lack of a positive correlation between the cellular content of an acid and its efflux may largely be due to compartmentation of the acid (Ryan et al., 2001).

The secretion of organic acids from roots in the presence of solubilized Al at low pH is an excellent example of the complex interaction among pH, Al, and respiration. In addition to solubilization of Al, a decrease in external pH causes an increase in the membrane potential (make it more negative), thus promoting the passive efflux of organic acids from the cytosol. In turn, this can enhance the release of acids from the vacuole into the cytosol. There are two additional consequences of low pH-induced organic acid efflux from the root: (a) the efflux of organic acids from the cells is accompanied by the release of K^+, and (b) the release of organic acids into the rhizosphere modulates the rhizosphere pH and soil microbe populations (Delhaize et al., 1993b; Marschner, 1995). If the efflux of both the organic acids and a cation such as K^+ continues, it will result in adverse physiological effects on osmotic potential of the root cells as well as in the carbon balance in the cells. The latter will have serious consequences for the respiratory metabolism of the root. Few experimental data are available on the prolonged effects of low pH and high Al levels on these rhizosphere interactions.

For Al-induced secretion of organic acids over extended periods, it can be argued that both the effects on anion channels and altered carbohydrate metabolism (respiration) must be involved. The extent of organic acid secretion is in the same range as the H^+/K^+ fluxes (Kochian, 2000), and the total amount of carbon secreted as organic acids can be significant; e.g., as high as 23% of the net photosynthate was secreted as organic acids by *Lupinus albus* within 13 weeks of growth (Dinkelaker et al., 1989). The role of Al in stimulating the release of organic acids from mitochondria and vacuoles is not clear. Whereas involvement of anion channels in organic acid efflux from the cell has been demonstrated (Kochian, 2000; Ryan et al., 2001), their nature and distribution in the plasmalemma, the mitochondrial membranes, and the tonoplast, and the regulation of their function by Al and other factors is poorly understood. Direct or indirect effects of Al on anion channels may include induction of more channels, and induction of second messengers to activate ion channels. It is speculated that the Al effect is not through direct interaction with the anion channels, but occurs through cascades of signal transduction steps, possibly involving Ca, kinases, phosphatases, or other regulatory proteins (Ryan et al., 2001). Al may cause transient Ca release by interfering with G protein and IP_3-mediated signal transduction pathways (Coté and Crain, 1993; Haug et al., 1994); it also may interact directly with calmodulin, a Ca-binding protein involved in signal transduction pathways. Al binds to calmodulin 10-times more strongly than Ca, though the results have been contradictory (You and Nelson, 1991). Despite recent advances in gene cloning and the availability

of the entire genome sequence of several plants, little is known about the genes encoding specific anion channels involved in organic acid efflux.

The source of the organic acids that are secreted in response to Al is equally controversial. Since increased activities of enzymes of the TCA cycle are primarily responsible for their biosynthesis, increases in both enzyme activities (involving biosynthesis and post-translational modulation) and substrate availability are essential. Delhaize et al. (1993b) showed that the cellular content of malate was not affected by a higher rate of its efflux, indicating a homeostatic regulation in a constitutive manner. Basu et al. (1994) also observed increased production of malate concomitant with its increased efflux. Although in wheat no immediate effect of Al on organic acid biosynthesis via PEP carboxylase and NAD-malate dehydrogenase was observed, increased PEP carboxylase activity was associated with Al-induced organic acid secretion in maize (Gaume et al., 2001). Ryan et al. (1995) observed that both Al-sensitive and Al-resistant wheat showed similar activity of PEP carboxylase and malate dehydrogenase, key enzymes involved in malate synthesis. However, it should be noted that both of these enzymes have multiple forms (Givan, 1999; Siedow and Day, 2000). Li et al. (2000) showed that species differ in their response to Al in terms of organic acid efflux. For example, efflux of acids was rapid in wheat and slow in rye (6–10 h); isocitrate dehydrogenase, PEP carboxylase, and malate dehydrogenase were not affected; citrate synthase increased in rye but not in wheat; low temperature had no effect on malate exudation in rye but citrate exudation was lower; and inhibition of the citrate carrier (between mitochondria and cytosol) inhibited citrate secretion into the medium in both species. It was concluded that while both metabolism and carriers were affected by Al in rye, only carriers were affected in wheat.

Mugai et al. (2000) characterized citrate secretion in two bean (*Phaseolus vulgaris*) varieties that differed in Al tolerance. They showed that the resistant variety secreted more citrate than the sensitive variety. Also, the activities of citrate synthase and $NADPH^+$-isocitrate dehydrogenase were higher in the former, but those of PEP carboxylase were not significantly different under Al stress. A dual effect of Al on both organic acid production and secretion can be possible through its effects on NADP-dependent isocitrate dehydrogenase. The inhibition of this enzyme by Al could increase the cellular levels of citrate (Chiba, 1999). This might have a negative effect on the TCA cycle and respiration, causing a reduction in ATP production, followed by a passive secretion of acids due to the semipermeability of the plasma membrane being compromised.

The complexity of interactions among key metabolites involved in organic acid synthesis is illustrated by the various routes of utilization of PEP in the respiratory metabolism (Fig. 1). There are at least three known metabolic fates of PEP in the cell: (a) its conversion into pyruvate by cytosolic or plastidic PEP kinase, (b) its conversion into pyruvate by vacuolar PEP phosphatase, and (c) its carboxylation in the cytosol by PEP carboxylase to OAA (Givan, 1999; Siedow and Day, 2000). Pyruvate is then transferred into mitochondria for entry into the TCA cycle. Oxaloacetate can also enter the TCA cycle in mitochondria or it can be reduced in the cytosol to malate, which then enters the mitochondria and the joins the TCA cycle. The cytosolic malate could serve as a direct source for its efflux in response to Al. If this were to occur, its impact on TCA cycle reactions could be significant; however, little information on this is currently available.

The secretion of organic acids in response to Al has been implicated as a mechanism for Al resistance in plants (Delhaize and Ryan, 1995; Jones, 1998; Ma et al., 2001). Al resistance by organic acids secretion could operate through several mechanisms, for example, by detoxification of the extracellular Al by binding to organic acids (Delhaize and Ryan, 1995; Jones, 1998; Ma et al., 2001) or by increasing the availability of essential nutrients for uptake by roots (Jones, 1998). However, Ishikawa et al. (2000) did not observe a correlation between Al resistance and citric and malic acid exudation among various species or even between two cultivars of the same species. They suggested that there might be more effective alternative Al resistance mechanisms that operate in addition to or in place of the exudation of malic and citric acids in these species.

Several laboratories are attempting to exploit the relationship between the accumulation and/or secretion of organic acids and the resistance of plants to Al by transgenic manipulation of organic acid metabolism in order to produce Al-resistant plants (de la Fuente et al., 1997; Koyama et al., 1999; Tesfaye et al., 2001). For example, tolerance to Al was conferred to transgenic tobacco and carrot plants by overexpression of a citrate synthase gene and to alfalfa (*Medicago sativa*) by overexpression of a malate dehydrogenase

gene. However, Delhaize et al. (2003) failed to see any effect of either an overexpression of a mitochondrial citrate synthase or a reduction in cytosolic isocitrate dehydrogenase on accumulation or efflux of citrate in transgenic tobacco. Nevertheless, these studies provide evidence for the potential role of Al in the respiratory metabolism of plants.

In summary, the inhibition of Ca and Mg uptake and the induction of phosphate extrusion by Al would together have a potential negative effect on respiration in plants. However, its effects on the induction of organic acid secretion should enhance certain reactions of the respiratory metabolism in plant cells.

D. Aluminum and Non-Phosphorylating Respiration

It is believed that overall flux of carbon through the TCA cycle is governed by reoxidation of NADH via the regenerative electron-transport chain and the cellular utilization of ATP (Simons and Lambers, 1998; Siedow and Day, 2000; Vanlerberghe and Ordog, 2002). The overall rate of oxygen consumption is regulated by ADP and Pi levels in the cell. Other controlling factors are availability and turnover of the substrate, with some modulation provided by the non-phosphorylating oxidation reactions. This might yield less ATP per cycle of electron flow, allowing the TCA cycle to continue. This mechanism also would allow continued production of organic acids for efflux in response to Al. The extent of non-phosphorylating or cyanide-resistant alternative oxidation reactions is subject to factors such as the amount of α-keto acids in the cell as well as a variety of environmental stresses (Simons and Lambers, 1998; Vanlerberghe and Ordog, 2002; Millenaar and Lambers, 2003). Conditions that reduce the flow of electrons through the cytochrome-based electron transport chain enhance alternative respiration. It is thought that the mechanism in part involves stimulation of the alternative oxidase by increased pyruvate in response to its reduced utilization as NADH builds up in the cells. Once it is activated, a high rate of alternative respiration may continue, allowing the sustained production of precursors and intermediates of the TCA cycle. Unfortunately, there is no direct evidence on the effects of Al on alternative oxidase. However, it can be argued that in the presence of Al, overall ATP utilization may be reduced, and enhanced alternative oxidase may serve a useful purpose in allowing a continued supply of organic acids for efflux. This situation might be similar to the situation with rotenone-insensitive, NADH dehydrogenase-driven electron flow, which bypasses Complex I (Siedow and Day, 2000). This non-phosphorylating electron flow also allows higher levels of respiration to continue under conditions of low ATP demand and utilization. The alternative oxidase also prevents the accumulation of superoxide and other hydroxyl radicals, which usually are formed under conditions of a highly reduced state of the electron transport chain. A possible role of these alternative oxidation reactions under adverse environmental conditions (chilling, drought, osmotic stress, etc.) has been discussed (Simons and Lambers, 1998; Lambers et al., 1998; Siedow and Day, 2000; Millenaar and Lambers, 2003).

E. Aluminum and Photorespiration

Photosynthesis, photorespiration, and dark respiration have strong direct and indirect metabolic interactions in regulating carbon and energy flow within the cell. Although occurring in different organelles, these three pathways share several intermediates as well as products, and affect the energy status of the cell in both competitive and complementary ways. Cytosolic and organellar pH, cytosolic content of Ca, Fe, Mg, P, and other ions, enzyme activities (all of which are affected by ambient pH), and the presence of solubilized Al in the environment have significant effects on photosynthesis, photorespiration and dark respiration (Moustakas et al., 1993; Simons et al., 1994; Marschner et al., 1995). Unfortunately, there are no data on the concurrent effects of Al on photosynthesis and photorespiration.

F. Aluminum, Oxidative Stress, and Respiration

Oxidative stress results from conditions that promote the formation of reactive oxygen species (ROS) that damage or kill cells. Among the numerous environmental factors that cause oxidative stress is Al (Bray et al., 2000). Generally, ROS are formed during certain redox reactions and during reduction of oxygen or oxidation of water by mitochondrial or chloroplast electron-transfer chains. A possible sequence of events leading to an increase in ROS during stress was discussed by Møller (2001). As much as 1–5% of the consumed oxygen could go into ROS production depending on the plant species and the overall rate of respiration (Møller, 2001). To prevent oxidative damage, plant cells are equipped with a scavenging

system consisting of antioxidants of low molecular weight (e.g., ascorbate, glutathione, and vitamin E), and several protective antioxidant enzymes (Asada and Takahashi, 1987; Kuzniak, 2002). Polyamines and flavanoids also may provide some protection against free radicals (Bray et al., 2000). When these defenses are overwhelmed, as occurs during biotic and abiotic stress, the mitochondria are damaged by oxidative stress (Møller, 2001).

There is evidence that Al causes oxidative stress in plants, and there might be an overlap between protective mechanisms for Al stress and oxidative stress (Ezaki et al., 1998, 2000; Richards et al., 1998). Basu et al. (2001) showed that Al caused an induction of mitochondrial superoxide dismutase in oil seed rape (*Brassica napus*). Sakihama and Yamasaki (2002) demonstrated that Al stimulated the phenolic-dependent lipid peroxidation in barley (*Hordeum vulgare*) roots. A strong link between lipid peroxidation (oxidative stress) and cell viability under Al exposure also was reported by Ingo and Wolfgang (2000). Ogawa et al. (2000) found that Al treatment of roots activated several of the antioxidant enzymes in the needles of Hinoki cypress (*Chamaecyparis obtusa*). Lidon et al. (1999) reported that ethylene production, acyl peroxidation, and activities of anti-oxygenic enzymes in maize shoots were affected by Al. For example, activities of copper/zinc-superoxide dismutase, catalase and glutathione reductase were inhibited while those of ascorbate peroxidase and dehydroascorbate reductase were increased in response to Al. An Al-resistant wheat cultivar had higher levels of superoxide dismutase, and reduced glutathione and malondialdehyde, suggesting a lower level of oxidative stress compared to an Al-sensitive cultivar (Dong et al., 2002). In our studies with red spruce suspension cultures, Al caused a dose-dependent decrease in several antioxidant enzymes, e.g., glutathione reductase, monodehydroascorbate reductase, and ascorbate peroxidase (R. Minocha, S. Minocha, S. Long and L. Jahnke, unpublished). Genes for mitochondrial superoxide dismutase, glutathionine S-transferase, peroxidase, and blue copper-binding protein respond to Al and oxidative stress in various plant species. These studies suggest that there may be common protective mechanisms among several types of stress, including pathogen stress, oxidative stress, heat shock stress, and Al stress.

G. Aluminum, Polyamines, and Respiration

The aliphatic polyamines (putrescine, spermidine, and spermine) play an important role in the growth and development of all living organisms (Cohen, 1998). The polyamine metabolism interacts with the respiratory metabolism in two ways (Fig. 1): (a) their biosynthesis depends on a continuous supply of glutamate (derived from α-keto glutarate) for the production of ornithine and arginine, the primary substrates for polyamine biosynthesis; and (b) their oxidative catabolism yields succinate that could contribute to the cytoplasmic pool of this acid that can be secreted. Polyamines carry a net positive charge at cellular pH and also play a role in modulating cellular pH (Cohen, 1998). In addition, polyamines can affect several mitochondrial functions via electrostatic interactions (Votyakova et al., 1999).

Abiotic stress conditions including low pH, high SO_2, nutrient deficiency or oversupply, and high Al cause increased cellular concentrations of polyamines, particularly putrescine (Dohmen et al., 1990; Santerre et al., 1990; Flores, 1991; Minocha et al., 1992, 1996, 1997, 2000; Wargo et al., 2002). There often is an inverse relationship between the cellular concentrations of putrescine and those of Ca, Mg, Mn, and K in response to Al treatment (Minocha et al., 1992, 1996; Zhou et al., 1995); these cations also respond to apoplastic pH. A key distinction between the polyamines (organic cations) and the inorganic cations is that the cytoplasmic availability of the latter can change only in response to external stimuli by recompartmentalization, because their cellular levels are derived entirely from uptake and transport, which depend upon their availability in the soil solution. By contrast, polyamines are synthesized within the cell, allowing adjustment of their cellular concentrations to meet physiological needs in situ. For example, substitution of Ca by putrescine at certain sites in the cell could increase Ca availability for key signal transduction pathways at times of Ca deficiency, e.g., in the presence of high concentrations of Al.

On the basis of extensive work from our group using cell cultures and mature conifer and hardwood trees in the field, we have proposed that putrescine could be used as a potential biochemical indicator of Al stress (indirectly Ca deficiency) in visually healthy trees (Minocha et al., 1996, 1997, 2000; Wargo et al., 2002). The availability of early biochemical indicators can aid in assessing the current status of (Al) stress in visually healthy trees, which, in turn, can be use-

ful in planning potential treatments and management strategies designed to alleviate the deleterious effects of stress or remediate the cause(s) of stress.

V. Conclusions and Perspectives

Profound metabolic and, consequently, growth and developmental changes occur when plants are exposed to high acidity in the soil. The factors that regulate soil pH, and the cellular signals that modulate the resulting changes in metabolism, are complex and poorly understood. Oxidative metabolism is only one of a multitude of cellular responses that are affected by changes in pH, directly, and through changes in soil nutrient levels, including a major effect through Al toxicity. The latter (Al toxicity) in itself is a complex phenomenon, an understanding of which is attracting increasing attention. A better understanding of the complexity of interactions between pH and Al will allow us to produce genetically improved plant varieties that can cope with the deleterious consequences of man made climatic changes that contribute to environmental acidity.

Potential resistance to low pH is a multigenic trait. Only a few mutant/resistant varieties of commercially important plants are available that show high yields in acid soils. As a result, breeding for low-pH resistance is a formidable task. In recent years, powerful techniques have become available to analyze global changes in gene activity in response to one or more environmental factors. Techniques such as Serial Analysis of Gene Expression (SAGE), Differential Display Reverse Transcriptase (DDRT) PCR, macroarrays, and microarrays (DNA chips) allow simultaneous analysis of the expression of thousands of genes within short periods of a specific treatment (Kuhn, 2001; Bohnert et al., 2001; Wu et al., 2001; Seki et al., 2002). These techniques have not yet been applied to studies of the effects of pH changes in the environment on molecular changes in the plant cell, particularly those related to respiratory metabolism. Currently there is no EST (Expressed Sequence Tags) database for genes responding to low pH or Al. High-throughput molecular techniques should allow us to distinguish between direct effects of pH changes on gene expression in the presence or absence of indirect consequences due to nutrient uptake and Al toxicity. Likewise, high-throughput analysis of thousands of metabolites in the cells should reveal changes in respiratory and related metabolic changes in the cell. Initial results on the effects of salinity, drought, cold, and pathogen infection on gene expression have been revealing with respect to changes that occur within hours of such treatments; many of the genes identified are related to respiratory metabolism (Bohnert et al., 2001; Seki et al., 2002). It is hoped that similar studies will lead to the identification of the genes whose expression changes in response to alterations in pH and Al, and also to the identification of signal-transduction pathways that are responsible for these changes. These studies should then lead to the cloning of pH-inducible regulatory elements (promoters, enhancers, etc), which would be useful in altering the expression of other genes of interest whose expression may impart tolerance or resistance to such pH changes.

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