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Overcoming barriers to understanding the cellular basis of aluminium resistance

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Abstract

There appears to be an emerging consensus that resistance to aluminium (Al) is mediated at the cellular level. Virtually all current hypotheses which seek to explain the basis of Al resistance have a cellular focus, including those which postulate that external mechanisms limit the rate of Al entry across the membrane and/or protect sensitive extracellular sites, as well as those which postulate that internal mechanisms detoxify Al in the cytoplasm. If Al resistance is a cellular phenomenon, it should be expressed in single cells. Attempts to demonstrate resistance in cell culture systems, however, have not been uniformly satisfying. Considerable uncertainty has arisen from use of experimental conditions which favour formation of insoluble or non-toxic Al species. This problem has plagued research which has attempted to select for Al resistance in cell culture systems, as well as research which has attempted to express existing patterns of differential resistance in cell culture systems. Despite technical problems such as this, work at the cellular level has provided some important contributions. Most importantly, we now know resistance to Al can be expressed at the cellular level. We have discovered also that plant cells accumulate Al much more rapidly in cell culture systems than in intact roots and that isolated cells are more sensitive to Al than complex tissues. While this type of research is still hampered by a number of technical barriers, it would appear that more rapid progress could be achieved if greater emphasis was placed on true "experimental" work. Furthermore, we need to begin evaluating experimental data in the context of an integrated Al stress response if we are to achieve a full understanding of the cellular basis of Al resistance.

Introduction

If recent reviews concerning the physiology of the aluminium (Al) stress response provide an accurate view of current thinking (see for example, Cumming and Taylor, 1990; Haug, 1984; Taylor, 1988a, 1991), there is an emerging consensus that resistance to Al must be mediated at the cellular level. This conclusion has been explicitly stated by several authors (Conner and Meredith, 1985a; Haug, 1984). Furthermore, virtually all current hypotheses which address the physiological basis of Al resistance reflect processes which should operate within single cells. Surprisingly, however, few reports clearly demonstrate direct Al toxicity or differential resistance to Al at the cellular level. In many ways, this reflects difficulties inherent in working with Al. The complex chemistry of Al is certainly a major barrier. This has led to uncertainty about the identity of the primary toxic species. In cell culture systems, incomplete control of the speciation of Al may also preclude expression of Al toxicity and Al resistance. Difficulties also arise from the need to measure small quantities of cytoplasmic Al against an overpowering background of noise (Al in the cell wall). Equally problematic is the paucity of information about the primary toxic effects of Al and the lack of truly isogenic germplasm.

All these barriers have limited our progress in understanding the cellular basis of Al resistance. We should not, however, use the existence of such barriers to justify complacency about our conceptual approach to identifying resistance mechanisms. In a recent review (Taylor, 1991), I suggested that we tend to focus our efforts on searching for a single resistance mechanism which can provide an explanation for plant growth on Al-toxic soils. This approach is not likely to be well suited for identifying a suite of physiological adaptations which act in a coordinated fashion to provide protection against Al stress. Thus, our conceptual approach to research may itself be a barrier. In many

ways, conceptual barriers such as this may be more significant than technical barriers.

In this review, I will attempt to justify concerns about recent efforts to demonstrate Al resistance at the cellular level. In highlighting successes that have been achieved, I will illustrate how technical barriers have been overcome to provide substantive insight into the physiology of Al toxicity and resistance. This paper is not an exhaustive review of the literature, but offers the reader a critical view of current research and ideas, providing a framework from which to consider ways of overcoming conceptual barriers to understanding the cellular basis of Al resistance.

Current hypotheses emphasise the cellular basis of resistance

The often-cited distinction between external (exclusion) and internal resistance mechanisms continues to serve as a useful conceptual tool with which to categorise hypotheses about Al resistance. Basing the distinction between external and internal mechanisms on the site of metal detoxification or immobilisation (Taylor, 1988a, 1991), either in the apoplasm or symplasm, also helps to highlight the fundamental cellular basis of most current hypotheses. This is not to suggest that whole plant parameters cannot be involved in determining resistance. In fact, the importance of whole plant parameters was recently emphasised in split pot experiments with *Mucuna pruriens* (Hairiah et al., 1992). Several external mechanisms proposed in the literature can also take on whole plant characteristics. Ultimately, however, resistance must be mediated by adaptations and processes occurring within single cells.

External resistance mechanisms or exclusion mechanisms include those which serve to limit the rate of entry of Al into the cytosol. In this sense, exclusion is a more descriptive term than external resistance. However, the latter term more accurately reflects the fact that external resistance mechanisms can also protect sensitive extracellular sites from Al-induced injury (Taylor, 1991; Tice et al., 1992). Extracellular lesions may be important in Al toxicity. In fact, Rengel (1992) has argued that many of the toxic effects of Al might be mediated by extracytosolic lesions such as disruption of normal functioning of the plasma membrane. If external resistance mechanisms play a role in limiting the rate of Al transport across the plasma membrane or the extent of extracytosolic injury, resistant plants must possess specific adaptive features at the plasma membrane or cell wall. These adaptations may only be expressed in cells of the root periphery. However, the potential for unregulated apoplastic flow in immature regions of the root or at sites where emergence of lateral roots disrupts the functional apoplastic barrier at the endodermal or exodermal Casparian band (Peterson, 1988) suggests that external resistance mechanisms may be required by all cells of the plant. External resistance mechanisms might take a variety of forms, including (1) immobilisation of Al at the cell wall or low cell wall CEC, (2) selective permeability of the plasma membrane, (3) formation of a plant-induced pH barrier in the rhizosphere or root apoplasm, (4) exudation of chelator ligands, (5) exudation of phosphate, and (6) Al efflux (Taylor, 1988b, 1991). Each of these mechanisms must be mediated by cellular events and should (at least to some extent) be expressed in single cells.

If internal resistance mechanisms are defined as those which operate within the symplasm, these mechanisms must also be mediated at the cellular level. This is not to say that they will operate in every living cell of the plant, although this might be the case if a resistance mechanism is constitutively expressed. If an internal resistance mechanism is inductively expressed, Al must be present in the immediate environment of a cell and external resistance must be incomplete or ineffective before induction will occur. Thus, we might expect differences in the extent to which these mechanisms are expressed within the individual cells which comprise an intact functioning plant. We would not, however, expect such mechanisms to operate differently in whole plant and cell systems. All current hypotheses regarding internal resistance mechanisms (Taylor, 1988b, 1991) can be viewed in this way, including (1) chelation in the cytosol, (2) compartmentation in the vacuole, (3) evolution of Al-tolerant enzymes, or (4) elevated enzyme activity.

Demonstrating resistance to aluminium at the cellular level

Does Al toxicity occur in cell culture systems, or is it strictly a whole plant phenomenon? These important questions were posed more than 15 years ago by Carole Meredith (1978a). In the years since Meredith's pioneering work, a number of papers have concluded that Al toxicity and resistance are both expressed at the cellular level. However, recent progress in under-

standing the physical chemistry of Al provides a new tool with which to evaluate these early studies. Current knowledge about the speciation of Al in dilute media and the relative toxicity of various Al complexes leads me to question whether many of these conclusions are still justified. Most of the uncertainty in this field arises from the use of experimental conditions which favour the formation of insoluble or non-toxic Al species. This problem has plagued research which has attempted to select for Al resistance in cell culture systems and research which has attempted to express existing patterns of differential resistance in cell culture systems.

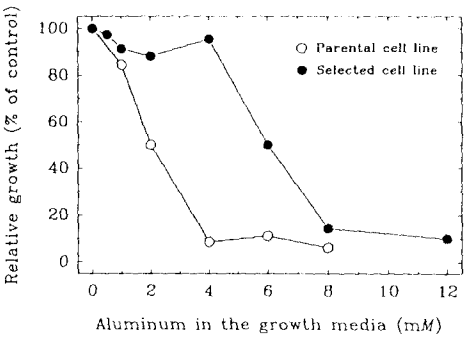

Fig. 1. Effect of $AlCl_3$ on growth of a parental cell line of *Daucus carota* and a cell line selected for growth in the presence of 4 m*M* $AlCl_3$ (see text). Growth of cell suspensions are expressed as percent relative to control. Adapted from Ojima and Ohira (1983).

Selecting for resistance to aluminium

In her pioneering work, Meredith (1978a) demonstrated that treatment of callus cultures of *Lycopersicon esculentum* with 200 and 400 μM Al reduced relative growth rates of two independent lines. Furthermore, prolonged exposure to 200 μM Al permitted the selection of variants which maintained rapid rates of growth in the presence of Al (Meredith, 1978b). In these experiments, modified CS5 and MS media (pH 5.9–6.0) were used and Al was supplied as an EDTA complex. At the time, use of Al-EDTA appeared to be a plausible means of keeping Al in solution under conditions of high pH (Meredith, 1978a). We now know, however, that the activity of Al^{3+} under these conditions would be limited to at least 1.0×10^{-10} *M* by formation of the Al-EDTA complex. Moreover, formation of micronutrient-EDTA complexes would reduce concentrations of copper, iron, manganese and zinc to between 5.4×10^{-8} and 4.8×10^{-20} *M*. These calculations do not take into account the exchange properties of agar, which could further reduce the activities of free cations. With this in mind, one might predict that the growth effects observed by Meredith (1978a) reflected an EDTA-induced micronutrient deficiency, rather than direct Al toxicity.

Similar concerns might be raised with respect to several other recent reports of Al resistance in cell culture systems. For example, Smith et al., (1983) selected what they believed to be Al resistant callus cultures of *Sorghum bicolor* on a modified MS medium containing Al-EDTA. Unfortunately, these authors used the same experimental conditions as Meredith (1978a, b), thus their conclusions should also be reconsidered. It is the work of Kunihiko Ojima and his colleagues, however, which provides the most well known and openly acknowledged example of cell culture work which used experimental conditions conducive to formation of solid phase Al (Ojima and Ohira, 1983, 1988; Ojima et al., 1984). Ojima's group developed what they believed to be Al-resistant cell suspensions of *Daucus carota* by repeatedly subculturing an Al-sensitive cell line on an R2 medium containing 4 m*M* $AlCl_3$ (pH 4.5 to 4.7). Cell lines selected in this way showed better growth than a parental line over a range of Al treatments in the R2 medium (Fig. 1; Ojima and Ohira, 1983). At first sight, these data appear consistent with selection for resistance to Al. The concentration of added Al required to inhibit growth of the selected cell line by 50% was three fold higher (6 m*M*) than that required for 50% inhibition of the non-selected cell line (2 m*M*). Furthermore, in subsequent papers, Ojima's group demonstrated that the selected line also exuded more citrate into the growth medium (Ojima and Ohira, 1988; Ojima et al., 1984). Thus, exudation of citrate provided a plausible mechanistic explanation for resistance (Ojima and Ohira, 1985). Like most cell culture media, however, the R2 medium contains high concentrations of phosphate (2 m*M*). Thus, formation of solid phase Al would be expected. Indeed, Ojima and Ohira (1983) reported that most of the Al present at the beginning of their experiments was in the form of insoluble gels composed of Al-hydroxide polymers and Al-phosphate. Speciation calculations show that the activity of Al^{3+} in these solutions was probably less than 10 μM, being limited primarily by formation of solid phase complexes with sulfate and phosphate. Over the period of the experiment, these gels became solubilised as Al-resistant cells exuded citrate into the medium, however, the non-toxic nature of Al-citrate complexes has been clearly demonstrated (Conner and Meredith, 1985a).

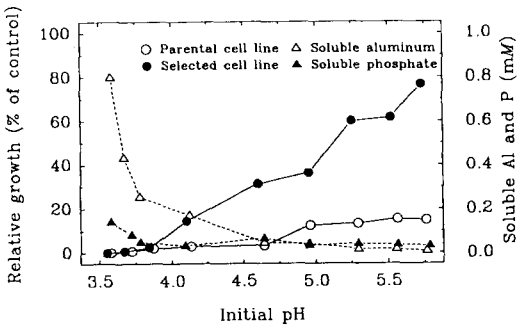

Fig. 2. Relative growth (percent of Al free control at pH 5.6) of parental and selected cell lines of *Daucus carota* as a function of increasing pH in the presence of 30 m*M* MES, 4.0 m*M* $AlCl_3$, and 2 m*M* NaH_2PO_4. Concentrations of soluble Al and soluble phosphate (dotted lines) are also indicated. Adapted from Koyama et al. (1988).

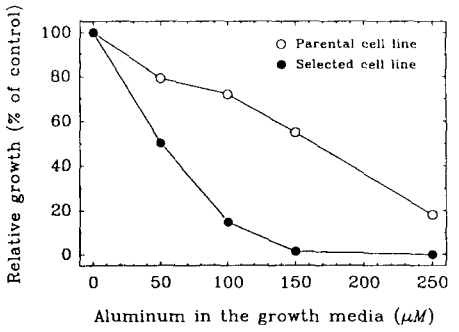

Fig. 3. Relative growth (percent of control) of parental and selected cell lines of *Daucus carota* as a function of increasing concentrations of $AlCl_3$ at pH 4.0. Phosphate was initially supplied as 0.1 m*M* NaH_2PO_4. Additional aliquots of NaH_2PO_4 were delivered two (0.2 m*M*) and four (0.3 m*M*) days after inoculation. Adapted from Koyama et al. (1988).

In two important papers, Koyama et al. (1988, 1990) effectively retracted the early conclusions of Ojima's group. They recognised that when Al is added to culture media containing inorganic phosphate, "it is difficult to distinguish between the toxic effect of the Al ion itself and interference with phosphate availability" (Koyama et al., 1988). To deal with this problem, they grew their cell lines over a pH range from 3.5 to 5.8 in the presence of 4 m*M* $AlCl_3$ and 2.0 m*M* NaH_2PO_4. Neither cell line showed any growth below pH 4.0 where, they argued, soluble Al was the primary toxic factor. As pH increased to 5.8 where little soluble Al was detected, the selected cell line outperformed that of the parental line (Fig. 2; Koyama et al., 1988). Furthermore, in contrast to parental cells, selected cells were capable of utilising phosphate from an insoluble $FePO_4$ colloid in media where this was the sole source of phosphate. The authors concluded that the primary selection factor imposed in their earlier studies was lack of soluble phosphate rather than direct Al toxicity. This selection pressure facilitated the development of a phosphate-efficient cell line in which efficiency was mediated by a more rapid (high Vmax) phosphate transport system (Koyama et al., 1992) and by release of citric acid into the medium (Koyama et al., 1988, 1990). The importance of phosphate efficiency in these selection experiments was emphasised by the fact that parental cells actually outperformed selected cells in experiments designed to minimise precipitation of solid phase Al-phosphate (Fig. 3; 0-250 μM $AlCl_3$, 0.1 m*M* NaH_2PO_4, pH 4.0). Speciation calculations show that soluble phosphate levels would be largely unaffected by treatment with Al under these conditions, but solid phase Al-sulfate and soluble Al-sulfate and Al-phosphate complexes would still limit the activity of Al to less than 15 μM.

While these data provide a convincing argument for phosphate efficiency, one note of caution should be expressed. The experiments designed by Koyama et al. (1988) did not allow a clear distinction of phosphate deficiency from direct Al toxicity (see Fig. 2). In their high Al, high phosphate medium, a gradual change from Al toxicity to phosphate deficiency stress occurred as pH increased. Furthermore, the importance of soluble Al-phosphate complexes in mediating phosphate deficiency or ameliorating Al toxicity and the possible role of direct H^+ toxicity cannot be discounted. Thus, the major stress factor operating in their experiments cannot be defined unambiguously. In this context, it is interesting to note that Ojima and Ohira (1988) reported that whole plants regenerated from their selected cell line showed greater resistance to Al than parental lines when grown in dilute nutrient solutions lacking phosphate (pH 4.0). Patterns of hematoxylin staining in regenerated plants were also consistent with enhanced resistance to Al. While the superior performance of regenerated plants could perhaps be attributed to phosphate efficiency, this explanation does not appear to account for the observed pattern of hematoxylin staining. Is it possible that Ojima and Ohira's (1988) selected cell line was phosphate-efficient and Al-resistant? It is difficult to be sure.

Ojima and Ohira's (1983) work provided at least one additional observation that warrants repeating. They found that their parental and selected cell lines were equally sensitive to Al-EDTA over a range of concentrations from 0 to 800 μM (Fig. 4). One can infer from these data that growth in an R2 medium containing 4 m*M* $AlCl_3$ (Ojima and Ohira, 1983) provided

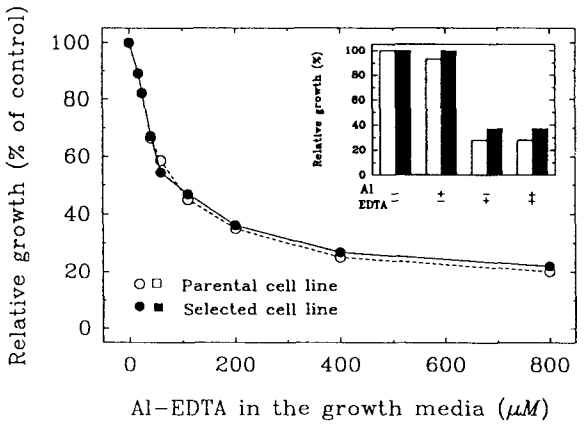

Fig. 4. Effects of Al, EDTA, and Al-EDTA on the relative growth (expressed as percent of control) of a parental and a selected cell line of *Daucus carota* Data presented in the inset suggest that the apparent toxicity of Al-EDTA was due to the presence of excess EDTA, not excess Al. Adapted from Ojima and Ohira (1983).

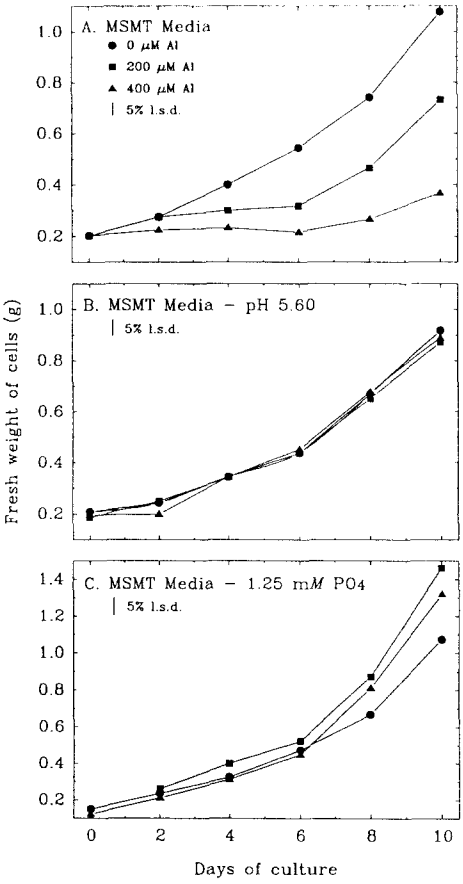

Fig. 5. The effects of Al toxicity (0, 200 400 μ*M*) on growth of plated cell suspensions of *Nicotiana plumbaginifolia*. **a** Growth of cells on a modified medium (MSMT) with low Ca (0.05 m*M*), phosphate (10 μ*M*), and pH (4.0) in the absence of EDTA. **b** Growth of cells on MSMT media with pH set to 5.6. **c** Growth of cells on MSMT with 1.25 m*M* phosphate. Adapted from Conner and Meredith (1985a).

a very different selection pressure than growth in the presence of Al-EDTA (Meredith, 1978a, b). In fact, in experiments varying the supply of Al and EDTA, Ojima and Ohira (1983) were able to demonstrate that the toxicity of Al-EDTA was due to excess EDTA, not excess Al (see inset, Fig. 4). This observation is consistent with my earlier suggestion that Meredith's (1978a, b) experiments with *Lycopersicon esculentum* actually selected for micronutrient efficiency rather than Al resistance. Interestingly, Ojima and Ohira (1983) did not question Meredith's earliest (1978a) claim of Al resistance in *Lycopersicon esculentum*. In preparing a revised MS culture medium, however, Conner and Meredith (1985a) clearly demonstrated the non-toxicity of both Al-citrate and Al-EDTA and cautioned against the use of chelating agents in cell culture experiments. Implicit in this statement is the conclusion that early reports of Al-resistant variants in *Lycopersicon esculentum* (Meredith, 1978a) and *Sorghum bicolor* (Smith et al., 1983) are no longer consistent with current views.

Conner and Meredith's (1985a-c) work provided what appears to be the first convincing demonstration of Al toxicity in a cell culture system. They developed a modified MS medium (MSMT; Murashige and Skoog medium modified for metal toxicity) which they argued allowed for expression of toxicity (Conner and Meredith, 1985a). Recognising that high phosphate concentrations and high pH favour the formation of solid phase Al, these authors lowered phosphate concentrations from 1250 μM to 10 μ*M* and pH from 5.8 to 4.0. In addition, calcium was supplied at 0.50 (or 0.1 m*M*), and EDTA was eliminated. Under these conditions, growth of plated cell suspensions was severely limited, presumably by phosphate deficiency (speciation calculations show that the activity of phosphate, primarily H_2PO_4, in their medium was reduced to 0.44 μ*M*). Thus, Conner and Meredith (1985a) had to inoculate experiments with phosphate loaded cells and replace their culture media every two days. With Al (200, 400 μ*M*) supplied as $Al_2(SO_4)_3.18H_2O$, the predicted activity of Al^{3+} in the MSMT media would be less than 20 μ*M*, but Al-induced changes in the activities of other nutrient elements would not be expected. Thus, inhibition of the growth of plated cell suspension of *Nicotiana plumbaginifolia* observed by Conner and Meredith (1985a) likely represented direct Al

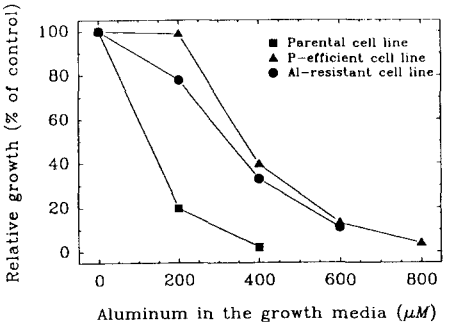

Fig. 6. Relative growth (percent of control) of parental cell line and putative phosphate efficient and Al-resistant cell lines of *Daucus carota* as a function of increasing concentration of $AlCl_3$ at pH 4.0. Phosphate was initially supplied as 0.1 m*M* NaH_2PO_4. Additional aliquots of NaH_2PO_4 were delivered at two (0.2 m*M*) and four (0.3 m*M*) days after inoculation. Adapted from Ojima et al. (1989).

toxicity (Fig. 5a). Restoration of pH or phosphate concentrations to standard MS levels (pH 5.6, 1.25 m*M* phosphate) alleviated expression of toxicity (Figs. 5b, c), confirming the importance of maintaining low pH and low phosphate concentrations in experiments with Al. Using their MSMT medium, Conner and Meredith (1985b, c) were able to isolate Al-resistant variants from wild type cell cultures of *Nicotiana plumbaginifolia* and regenerate fertile, Al-resistant plants which produced progeny segregating for resistance. Segregation ratios were consistent with a single dominant mutation (Conner and Meredith, 1985c).

More recently, Ojima's group also appears to have been successful in selecting for resistance to Al in both *Nicotiana tabacum* and *Daucus carota*. In responding to concerns about formation of solid phase Al in their media, Ojima et al., (1989) selected *Nicotiana tabacum* for growth in an R2 medium under conditions that favour formation of solid phase Al (4 m*M* $AlCl_3$, 2 m*M* phosphate, pH 5.0) as well as conditions designed to minimise precipitation of Al-phosphate (2.5 m*M* $AlCl_3$, 0.1 m*M* phosphate, pH 4.0). They argued these conditions provided selection for phosphate efficiency and Al resistance respectively. Growth of the parental cell line and the putative phosphate-efficient and Al-resistant cell lines were then evaluated over a range of AlC_3 concentrations in R2 medium (pH 4.0) containing 0.1 m*M* phosphate (additional phosphate was also added after two (0.2 m*M*) and four (0.3 m*M*) days of growth). Both selected cell lines outperformed the parental cell line over a range of added Al (Fig. 6). While the activity of Al^{3+} in their low phosphate, R2 medium was likely limited to less than 15 μM by formation of soluble complexes with sulfate and phosphate and solid phase Al-sulfate, it would appear that soluble P levels were unaffected by treatment with Al. Furthermore, Arihara et al. (1991) demonstrated elevated resistance to Al in plants of *Daucus carota* which were regenerated from callus selected for resistance on this medium. These data suggest that Ojima and his coworkers were successful in selecting for resistance to Al in both *Nicotiana tabacum* (Ojima et al., 1989) and *Daucus carota* (Arihara et al., 1991).

Expressing existing patterns of aluminium resistance in cell culture systems

Although success in selecting for resistance to Al in cell culture systems has been limited, Conner and Meredith's work with *Nicotiana plumbaginifolia* and Ojima's work with *Nicotiana tabacum* and *Daucus carota* suggest that resistance is indeed a trait which can be expressed at the cellular level. This important finding raises another interesting question. Can preexisting differences in resistance also be expressed at the cellular level? Unfortunately, published literature addressing this question is meager. In a recent paper, Parrot and Bouton (1990) attempted to demonstrate differences in the response of callus cultures of *Medicago sativa* to Al. Callus was derived from explants from an Al-sensitive and an Al-resistant line developed as part of a divergent selection procedure at the whole plant level (Hartel and Bouton, 1989). Callus from the Al-resistant line outperformed callus from the Al-sensitive line under conditions of Al stress (400 μM) in a Blaydes medium which had been modified according to the specifications of Conner and Meredith (1985a). Unfortunately, Parrot and Bouton (1990) did not provide full dose response information for their whole plant and callus culture systems. Thus, it is not possible to evaluate whether resistance was fully expressed, in this case at the tissue level. There is also reason to believe relatively complex tissues like callus may respond differently to Al than cell suspensions. Nonetheless, these results are encouraging in as much as pre-existing genotypic differences were reproduced in a cell culture system.

It is with this backdrop of experimental work that I began working with cell suspension cultures of *Phaseolus vulgaris*. Our approach was similar to Parrot and Boutin's (1990) work in that we attempted to generate Al-resistant and Al-sensitive cell suspensions using callus derived from plants differing in resistance to Al. Unfortunately, our early experiments clearly demon-

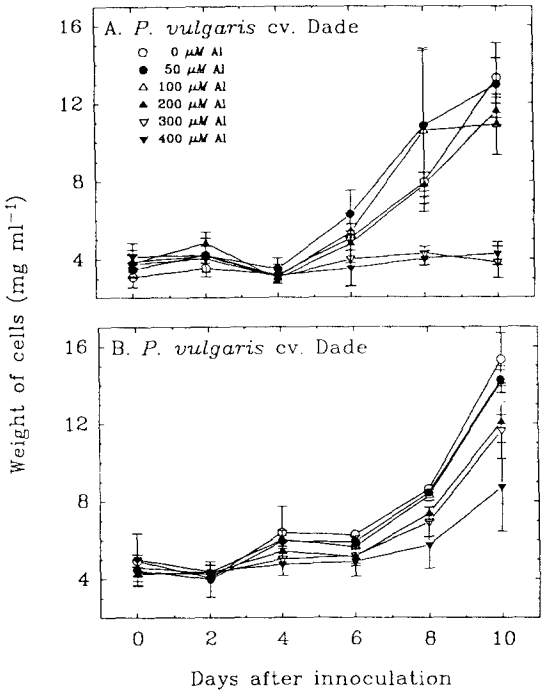

Fig. 7. Effect of Al on growth of cell suspensions derived from an Al-resistant cultivar (Dade) of *Phaseolus vulgaris*. Cells were grown on a modified MS medium containing 0.5 m*M* Ca, 0.68 m*M* total phosphate, and an initial pH of 4.5. Phosphate was added to cell suspensions on a daily basis in exponentially increasing quantities (0.15 d^{-1} RAR). Panels **A** and **B** represent two repeat experiments with identical experimental designs. The lack of reproducibility between experiments appears to reflect plant-induced pH changes in the culture media.

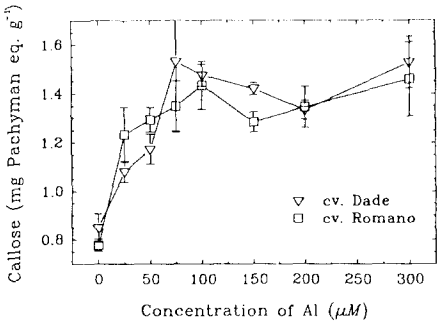

Fig. 8. Effect of Al on synthesis of callose in cell suspensions derived from an Al-resistant (Dade) and an Al-sensitive (Romano) cultivar of *Phaseolus vulgaris*. Cells were exposed to Al for 2 h in 1.0 m*M* $CaCl_2$ at pH 4.5. Callose was quantified as mg Pachyman equivalents $gram^{-1}$ dry weight.

strated that use of Conner and Meredith's (1985a) MSMT medium would not be appropriate for use with *Phaseolus*. Our cells were sensitive to low pH (4.0) and did not grow well when the culture media were changed every two days to provide adequate phosphate. Thus, in our experiments, phosphate was added to a modified MS medium (0.5 m*M* Ca, 0.68 m*M* total phosphate, initial pH 4.5) on a daily basis in exponentially increasing quantities (0.15 day^{-1} relative addition rate). Under these conditions, the absolute concentration of phosphate was maintained at low levels, and an Al-induced inhibition of growth was observed at concentrations above 200 μM Al (Fig. 7A). Unfortunately, our results were not consistent. In several experiments where cells raised the pH of the culture medium, the response to increasing Al supply was not as dramatic (Fig. 7B), emphasising the importance of careful control of pH. In subsequent experiments, we found that control of pH could be achieved in the absence of Al stress by using a pthalate buffer system (pH 4.5), but the combined stresses of low Ca, low phosphate, low pH, mildly toxic concentrations of pthalate, and Al resulted in poor growth in long-term (10 day) experiments.

To overcome this problem, we moved to short-term experiments (2h) in which sensitivity to Al was evaluated using Al-induced synthesis of callose as a short term marker for injury (Wissemeier et al., 1992). Shaeffer and Walton (1990) previously demonstrated that treatment with Al (100-1000 μM) induced synthesis of callose in protoplasts isolated from *Avena sativa*, *Triticum aestivum* and *Hordeum vulgare*. Furthermore, in *Avena sativa* and *Triticum aestivum*, synthesis of callose in protoplasts was correlated with the extent of Al injury observed in whole plants. Unfortunately, however, the high pH (6.0) of Shaeffer and Walton's (1990) experimental medium raises questions about the solubility and speciation of Al. In fact, when pH was buffered at pH 4.0 with *N*-hydroxyethyl piperazine-*N'*-propanesulfonic acid (Hepps), Al had no effect on callose synthesis. In our experiments, cells were exposed to Al in simple solutions containing 1 m*M* $CaCl_2$ and 0–300 μM $AlCl_3$ (pH 4.5) where the calculated activity of Al^{3+} ranged from 0 to 20 μM. Aluminium-induced injury was clearly reflected by increased callose synthesis in both the Al-resistant cv. Dade and the Al-sensitive cv. Romano. Once again, however, differences in callose production between cultivars were not consistently expressed (Fig. 8).

These results are encouraging in that they clearly demonstrate Al toxicity in a cell culture system, but they are disappointing in that they also appear to suggest that differential resistance to Al is not expressed reliably at the cellular level. Several explanations are possible. In *Phaseolus vulgaris*, resistance

to Al appears to be an inducible trait (Cumming et al., 1992). If a period of stress is required before a resistance mechanism is "switched on" one might predict that short-term injury would be observed in both Al-resistant and Al-sensitive cell suspensions. Differences in callose production would only be expected to occur after a period of acclimation. It is also possible that resistance to Al is only expressed at the whole plant level, or, at least at a higher level of structural complexity (such as callus). Mechanisms such as exudation of chelator ligands, exudation of phosphate, or the plant-induced pH barrier require that root cells modify the micro-environment of the root soil interface (Taylor, 1991). Isolated cells may not be capable of altering the micro-environment at the cell surface sufficiently to express resistance. In this context, it is interesting to note that Miyasaka et al. (1991) reported a 70-fold increase in exudation of citrate from roots of the Al-resistant cultivar Dade (*Phaseolus vulgaris*) under conditions of Al stress, while exudation from the Al-sensitive Romano was only slightly affected. This is the kind of mechanism which may rely on the formation of a micro-environment at the cell surface. Perhaps *Phaseolus vulgaris* provides an ideal experimental system for testing the importance of structural complexity and induced responses in resistance to Al.

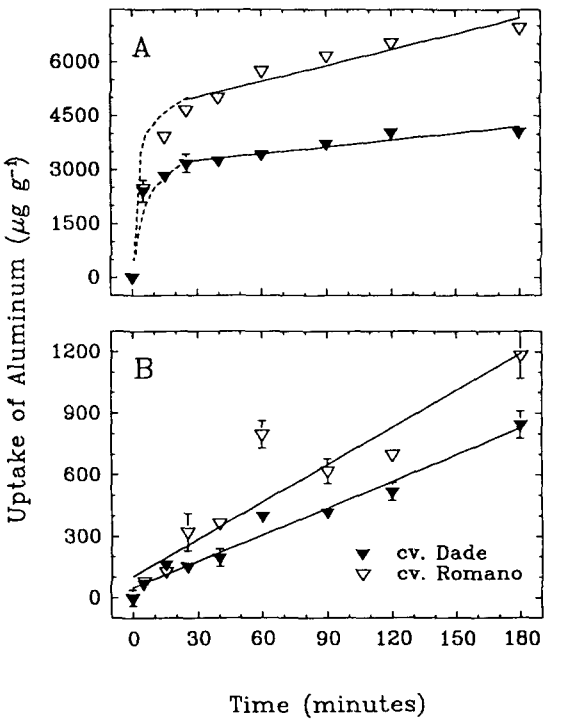

Fig. 9. Short-term kinetics of Al uptake by cell suspensions derived from an Al-resistant (Dade) and an Al-sensitive (Romano) cultivar of *Phaseolus vulgaris*. Cells were exposed to 75 μM $AlCl_3$ and 1.0 mM $CaCl_2$ at pH 4.5. Uptake of Al was measured directly after the 0-180 min absorption period (**a**) or after desorption in 9 mM citrate (**b**). Adapted from McDonald and Taylor (1994).

Important contributions of cell culture work

While the extent of literature which clearly demonstrates Al toxicity and differential resistance in cell culture systems is still limited, some important contributions to our understanding of the physiology of Al stress have been made. Observation of Al toxicity and differential resistance to Al in cell culture systems provides at least a preliminary confirmation of the cellular basis of toxicity and resistance. This in itself is a major step forward. Another important contribution has been to highlight the extreme sensitivity of isolated cells to Al. Conner and Meredith (1985b) documented the enhanced sensitivity of cell suspensions of *Nicotiana plumbaginifolia* to Al when compared with plated cells, callus cultures, and in vitro propagated shoots and seedlings. Two hundred μM Al was sufficient to abolish growth in cell suspensions, while growth of more complex tissues was reduced by less than 40% (Table 1). This enhanced sensitivity to Al was attributed to the intimate contact between cell suspensions and their bathing medium. This could reduce the possibility of maintaining micro-environments in the immediate vicinity of the cells, which Conner and Meredith (1985b) argued should facilitate entry of Al into cells. Experiments in my laboratory have clearly demonstrated the propensity of cell suspensions of *Phaseolus vulgaris* to accumulate Al. Short-term experiments with cell suspensions derived from the Al-resistant cultivar Dade and the Al-sensitive cultivar Romano have shown that patterns of Al uptake by cell suspensions are qualitatively similar to those observed in excised roots. Uptake is biphasic with a rapid, saturable phase superimposed over a linear phase of uptake with time (Fig. 9a). However, in cell suspensions, uptake of Al during the rapid, saturable phase is an order of magnitude greater than in excised roots (Mc Donald and Taylor, 1994). This loosely bound Al can be effectively desorbed with citrate, leaving a linear phase of uptake (Fig. 9b) in which the rate of Al uptake is approximately three times more rapid in cell suspensions of *Phaseolus vulgaris* (Mc Donald and Taylor, 1994) than in excised roots of *Phaseolus vulgaris* (unpublished data) or *Triticum aestivum* (Zhang and Taylor, 1989, 1991).

Table 1. Influence of aluminium on performance of *Nicotiana plumbaginifolia* under varying developmental conditions

Developmental condition	Growth parameter	Total aluminium concentration (μM)					
		0	200	400	600	800	1000
Cell suspension	Relative growth[a]	432	0	0	0	0	0
Plated cells	Relative growth[a]	371	242	160	20	8	-2
Smeared callus	Relative growth[a]	312	194	178	132	126	130
In vitro shoots	Percent rooting	80	60	60	40	30	40
Seedlings	Percent survival	97	89	65	2	1	0

[a] Relative growth is expressed as growth relative to control without Al. Data from Conner and Meredith (1985a).

An alternative explanation for the enhanced sensitivity of isolated cells to Al is their high metabolic activity. Cell cultures are in a state of active growth and division, a state which is only paralleled in intact roots at meristematic sites. Few studies have compared the effects of Al on mature, differentiated regions of the root with the younger root tip, where cell division and growth is rapid. Where this focus has been adopted, results have been dramatic. Rincon and Gonzales (1992) reported that uptake of Al in root tips of an Al-sensitive cultivar of *Triticum aestivum* was 9 to 13 times greater than in an Al-tolerant cultivar. In the Al-tolerant cultivar, Al uptake was stimulated by CCCP and cycloheximide, suggesting that some form of metabolism-dependent exclusion might account for reduced uptake. In more mature regions of the root, differences between Al-tolerant and Al-sensitive cultivars were not as dramatic and less affected by CCCP and cycloheximide. Similarly, Huang et al. (1993) reported that exposure of root tips to Al inhibited translocation of Ca from roots to shoots more dramatically than when Al was applied to mature regions of the root. Equally dramatic are the results of Ryan et al. (1993) who demonstrated that exposure of the terminal 2 to 3 mm of the root tip is required for expression of Al toxicity. Application of Al to other portions of the root had little or no effect on growth and caused minimal damage to root tissues. These results suggest that studies on whole roots may not provide an accurate picture of the response of actively dividing cells (in root tips or cell culture systems) to Al. With this in mind, it is perhaps not surprising that the response of individual cells to Al stress is different from whole plant responses. In fact, it is tempting to speculate that the response of cell culture systems to Al may more accurately reflect processes occurring at the sensitive root tip than responses of whole roots. Unfortunately, data to support this speculation are not yet available.

In highlighting successes that have been achieved with the use of cell culture systems, a note of concern must be expressed. Mc Donald and Taylor's (1994) work with *Phaseolus vulgaris* demonstrated that accumulation of Al by cell suspensions is extensive, and patterns of uptake are sensitive to experimental conditions. Uptake of Al from high volume, low concentration solutions showed a biphasic pattern similar to that observed in studies with intact or excised roots (Fig. 9). In contrast, uptake from low volume, high concentration solutions saturated within 20 minutes (Mc Donald and Taylor, 1994). One can infer from these results that the toxicity of Al will also be dramatically affected by growth conditions. Growth experiments with cell culture systems frequently lead to high cell densities with a tremendous potential for accumulation of Al. If relatively constant levels of Al stress are to be maintained over time, Mc Donald and Taylor's (1994) results suggest that experiments must be conducted using higher volumes and lower concentrations than is currently the practice. While expression of Al toxicity and differential resistance appear to have been achieved, failure to maintain constant levels of stress over time continues to be a problem.

Overcoming barriers to understanding

Problems encountered in adapting standard cell culture media and techniques for use in studies with Al illustrate how technical barriers have hampered our progress in understanding the cellular basis of Al resistance. It is not unrealistic to suggest that the multiplicity of technical and conceptual barriers which have faced scientists working with Al can account for the

slow rate of progress we have seen, not only in cellular studies, but throughout the discipline. Fortunately, some of the technical barriers are beginning to fall as new techniques are developed and new experimental approaches are implemented. Others, however, remain largely intractable at this time. In as much as conceptual barriers are solely of our own creation, they should be relatively easy to overcome. Nonetheless, conceptual barriers continue to have as much impact on our science as technical barriers. Perhaps the first step in overcoming these barriers is to acknowledge their presence.

Technical barriers

The complex physical chemistry of aluminium

One of the most significant technical barriers limiting our progress is the lack of information about the relative toxicity of various Al species. There is little doubt that we have made progress on this topic, especially in recent years. Monumental efforts on the part of scientists like Tom Kinraide, Dave Parker, Paul Bertsch, and Pax Blamey have heightened our awareness of the complex physical chemistry of Al and the importance of taking these complexities into account in designing experiments. Each of these scientists has repeatedly called for experimental designs which provide control of the species of Al which are present under a given set of environmental conditions (see for example, Blamey et al., 1983; Kinraide, 1991; Kinraide and Parker, 1989; Shann and Bertsch, 1993). In describing the difficulties which have been encountered in cell culture studies, I have already illustrated the potential impact of ignoring their pleas. Many, however, have had difficulty in responding to these concerns. This point was effectively communicated to me when Dave Parker visited my lab early in 1993. He described how dilution of a 100 m*M* stock solution of $AlCl_3$ with distilled water actually favours loss of monomeric Al through precipitation. In the process of diluting the stock solution from 100 m*M* to 1 μM, pH rises from 3.4 to 5.6. This drives the ratio of $(Al^{3+}) / (H^+)^3$ above $10^{8.8}$, at which point precipitation is predicted to occur. This problem can be easily rectified by acidifying stock solutions. Given my background, this rather simple observation is something that would never have occurred to me and yet has the potential to affect my ability to draw accurate conclusions from experimental data.

In venturing into a field where most of us are unwilling or unable to go, scientists such as the ones cited earlier have provided important new information about the relative toxicity of the various Al species present in solution (see reviews by Kinraide, 1990, 1991; Kinraide and Parker, 1990; Kinraide and Ryan, 1991). They speak with confidence about the extreme toxicity of the Al_{13} polymer and describe circumstantial evidence that Al^{3+} itself is toxic. They also provide evidence for the non-toxicity of $Al(OH)^{4-}$ and various Al complexes with fluoride, sulfate, and chelator ligands such as citrate and EDTA. On the other hand, uncertainty about the relative toxicity of the various hydroxy-Al species continues to be a problem. Perhaps most distressing are the lucid arguments as to why clear assignment of toxic lesions to a particular Al species may not be possible. It will be difficult to distinguish sensitivity to mononuclear hydroxy Al from pH-dependent Al^{3+} toxicity because independent control of hydroxy Al and pH are not possible. In addition, alkalanisation of the rhizosphere near root apices may favour the formation of what appears to be the highly toxic Al_{13} species, giving a false impression of the relative toxicity of Al^{3+} and the monomeric hydroxy-Al species (Kinraide, 1991; Kinraide and Parker, 1989, 1990; Kinraide and Ryan, 1991; Parker and Bertsch, 1992). An understanding of the relative toxicity of the wide variety of Al species which are present under natural conditions would certainly be an important asset in our efforts to understand the physiological basis of resistance. Such information will not likely be available in the near future.

Incomplete information about the toxic effects of aluminium

If our understanding about the relative toxicity of various Al species is incomplete, it is not surprising that we have yet to develop a clear understanding of specific toxic lesions which are induced by exposure to Al. While complete information about the toxic effects of Al need not be considered a prerequisite to understanding the physiological basis of resistance, such information would be an important asset. One key piece of information which is still lacking is the extent to which trans-membrane transport of Al is required for expression of Al toxicity. A number of authors have argued that the toxic effects of Al could be explained by an apoplastic lesion (see for example, Rengel, 1992). If we knew this was true, then we could legitimately conclude that internal resistance mechanisms are of little significance in protecting plants from Al-induced injury. This would permit us to focus our efforts on

external resistance mechanisms. In some respects, our efforts to identify potential resistance mechanisms are somewhat akin to searching for the needle in the haystack. If we knew in which part of the haystack to start searching, our chances of success would be markedly improved.

Measuring cytosolic aluminium

An equally important technical barrier to understanding the cellular basis of Al resistance is the lack of information about the extent of trans-membrane transport of Al. We do not know which species of Al are capable of crossing the plasma membrane, what rates of transport might be realised, or the extent to which Al is accumulated in the cytoplasm. Much of the problem in plant systems can be attributed directly to the cell wall, which may represent a significant sink for Al, perhaps accounting for 70 to 90% of total uptake (Clarkson, 1967; Huett and Menary, 1979). If these numbers are correct, any effort to quantify transport of Al across the plasma membrane will be complicated by the problem of measuring a small cytosolic Al signal in the midst of overpowering noise from Al in the cell wall.

Despite these difficulties, some important nuances are beginning to appear. One of the more important is the possibility that uptake in the cell wall may not be as substantive as previously indicated (Clarkson, 1967; Huett and Menary, 1979). In short-term kinetic studies (3 h) with *Triticum aestivum*, Zhang and Taylor (1989) demonstrated that uptake of Al was biphasic, with a rapid saturable phase of uptake (saturating in 30 min) superimposed over a linear phase of uptake with time. The saturable phase of uptake, which was readily desorbed with citrate, appeared to represent exchange in the cell wall. In a subsequent paper, Zhang and Taylor (1990) suggested that the linear phase of uptake included uptake across the plasma membrane and metabolism-dependent accumulation in the cell wall (possibly precipitation or polymerisation of Al in the apoplast). Because of the complex nature of the linear phase of uptake, it was not possible to isolate uptake across the plasma membrane. Nonetheless, it can be estimated that the cell wall accounted for 57 to 72% of total Al uptake during a 3 h exposure (Zhang and Taylor, 1991). While these estimates of Al uptake in the apoplasm are considerably lower than those from early studies, it is still possible that they may be too high. Using confocal laser scanning microscopy and the fluorophore morin, Tice et al. (1992) localised Al in roots tips of an Al-resistant and an Al-sensitive cultivar of *Triticum aestivum* and concluded that uptake of Al across the plasma membrane dominated (56–70%) total uptake in short-term (48h) experiments.

In attempting to account for their unique results, Tice et al. (1992) suggested that Zhang and Taylor's (1990) metabolism-dependent linear phase of uptake in the cell wall may have reflected experimental conditions (75 μM AlK $(SO_4)_2$, 1 mM $CaSO_4$, pH 4.5) which were conducive to precipitation or polymerisation of Al. Recent work in my laboratory (Archambault and Taylor, unpubl.) has confirmed these concerns. We repeated Zhang and Taylor's experiments using uptake conditions (3 h exposure to 50 μM $AlCl_3$ in 1 mM $CaCl_2$, pH 4.5) more similar to those used by Tice et al. (1992). Under these modified conditions, Al in the apoplasm was almost completely desorbed with citrate and did not make a significant contribution to the linear phase of uptake. We also repeated the experiments of Tice et al. (1992) using graphite furnace AAS to measure directly Al in the cell wall and discovered that $CaCl_2$ is not an effective agent for desorption of tightly bound Al in the apoplasm. Purification of cell wall material from roots which had undergone Tice et al.'s (1992) lengthy desorption protocol demonstrated that 30 to 50% of the Al in the residual fraction was localised in the apoplasm. Thus, it would appear that Tice et al.'s (1992) use of the fluorophore morin may not have been capable of detecting Al which was tightly bound to the cell wall.

The important point arising from these experiments is that use of experimental conditions which minimise precipitation or polymerisation of Al in the cell wall (Tice et al., 1992) and use of desorption agents which permit near complete desorption of Al from the apoplasm (Zhang and Taylor, 1990) may provide a means of isolating uptake of Al across the plasma membrane. Using these techniques, we have estimated that uptake of Al in the apoplasm accounts for as little as 33% of total Al uptake in short-term experiments with excised roots, and this apoplastic Al is almost completely removed by desorption with citrate (Archambault and Taylor, unpubl. data). This suggests that the problem of measuring a small cytosolic Al signal in the midst of overpowering background noise may not be insurmountable. This conclusion relies upon Shi and Haug's (1990) assertion that desorption of Al using citrate is also effective in removing Al from the external surface of the plasma membrane. Several of our preliminary experiments have indicated that this assumption is valid. If trans-membrane transport of Al

can be isolated in plant systems, it should be possible to determine whether cationic species of Al are capable of crossing the plasma membrane. Experiments designed to answer this question have yet to be performed.

The lack of isogenic germplasm

In many ways, the lack of isogenic germplasm may well be the single most important factor which limits our progress in understanding the cellular basis of resistance. With a truly isogenic system, the task we face becomes much simplified. All we have to do is identify differences between genotypes. By definition, these differences would be directly related to the presence or absence of a single gene. We would still face the task of separating cause and effect. This task is not likely to be trivial. Nonetheless, the experimental effort required would be considerably less demanding.

While a number of research groups are working towards development of near-isogenic germplasm, the extent of current literature which has made use of such germplasm is still limited. Delhaize et al. (1993a) analysed uptake and distribution of Al in root apices of near-isogenic lines of *Triticum aestivum* developed through a backcrossing program using the Al-resistant cultivar, Carazinho, and the Al-sensitive cultivar, Egret. Aluminium-sensitive backcross isolines accumulated more Al in root apices than Al-resistant isolines, but the experimental design did not allow for clear separation of cause and effect. In another paper, Delhaize et al. (1993b) demonstrated that roots of their Al-resistant isoline exuded 5 to 10 times more malate into the apoplasm/rhizosphere than their Al-sensitive line. Furthermore, enhanced exudation of malate was a consistent characteristic of Al-resistant plants in populations segregating for Al resistance. To my knowledge, the only other paper reporting results of experiments using near-isogenic, Al-resistant plant material is that of Ryan and Kochian (1993) which investigated the effect of Al on Ca uptake in root apices of several Al-resistant and Al-sensitive backcross isolines of *Triticum aestivum*. They found that Al inhibited Ca influx in Al-sensitive isolines, but had relatively little effect on Ca influx in Al-resistant isolines. Again, while these studies indicated a correlation between inhibition of Ca influx and sensitivity to Al, the question of cause and effect was not resolved. Nonetheless, these papers represent an important first step towards use of near-isogenic plant material. Unfortunately, similar studies using cell systems have not yet appeared.

Conceptual barriers

Separating cause from effect

In identifying the lack of isogenic germplasm as a technical barrier, I expressed concern about separating cause from effect. Even in experiments which make use of isogenic systems, an important question still arises. Do observed differences between genotypes play a role in mediating resistance, or are they an indirect result of a resistance mechanism providing protection from Al-induced injury in one genotype but not in another? To be fair, this question is not so much a conceptual barrier as it is an issue that must be addressed in formulating a rigorous experimental design. While the importance of proper experimental design is widely accepted, our published work does not always reflect attention to this issue. Much of the current literature dealing with mechanisms of Al toxicity and resistance is largely descriptive in nature. This kind of research can support correlative conclusions, but often falls short of providing the kind of experimental data we need to reject alternative hypotheses. Of course, an experimental approach is not a panacea to our problems. For experimental research to be successful, it must be coupled with awareness of, and attention to, possible alternative hypotheses. We need only to look as far as Carole Meredith's and Kunihiko Ojima's early work on cell cultures of *Nicotiana plumbaginifolia* and *Daucus carota* to illustrate the dangers of an incomplete consideration of alternative hypotheses (Meredith, 1978a; Ojima and Ohira, 1983, 1988 ; Ojima et al., 1984).

An integrated response to aluminium stress

At the risk of downplaying the importance of the barriers discussed above, I have become increasingly convinced that our reductionist approach to science may present the most substantive barrier to understanding the cellular basis of Al resistance. In closing my 1991 review on mechanisms of Al resistance (Taylor, 1991), I suggested that we tend to focus our efforts on searching for a single resistance mechanism which can provide an explanation for what must be a complex multigenic system. A search for a single mechanism which accounts for the full range of resistance to Al observed in a number of crop species may be futile. If resistance is mediated by a suite of physiological adaptations which act in a coordinated fashion to provide protection against Al stress, a single adaptive trait will only have a minor impact on plant performance. This view of the Al stress response has several important

repercussions. Perhaps most importantly, it may be unwise to reject hypotheses on the basis of research with genetically diverse material. Even if experiments are designed with a rigorous experimental approach, data which are inconsistent with a given hypothesis could arise if the hypothesis is invalid or if other adaptations play an important role in resistance.

This point can be illustrated by several recent reports in the literature. For example, I conducted a series of experiments which demonstrated a positive correlation between rates of nitrate (NO_3^-) and ammonium (NH_4^+) depletion from solutions, plant-induced pH of growth solutions, and cultivar resistance to Al (Taylor and Foy, 1985a, b, c). These results were consistent with the hypothesis that Al-resistant genotypes avoid the toxic effects of Al by maintaining a more rapid rate of NO_3^- uptake and a lower rate of NH_4^+ uptake than Al-sensitive genotypes. The resulting differences in cation/anion balance would lead to differences in plant-induced pH and resistance to Al. However, when the relative supply of NO_3^- and NH_4^+ in growth solutions was varied to provide experimental control over plant-induced pH, differences in plant-induced pH had a relatively minor impact on performance under conditions of Al stress (Taylor, 1988b). I concluded that resistance could not be explained solely by the plant's ability to maintain a high solution pH in mixed nitrogen solutions (Taylor, 1988b). I still stand by this conclusion, but I would be reluctant to go a step further and conclude that these data suggest that the plant-induced pH barrier does not play a role in resistance. The lack of a major effect arising from experimental manipulation of plant-induced pH could also reflect the operation of a variety of other resistance mechanisms which worked in concert to mediate Al resistance. A second illustration of this point is provided by recent work by Ryan et al. (1993). Short-term measurements of root elongation in intact and decapped roots of *Zea mays* indicated that removal of the root cap had little effect on subsequent growth of root tips in the presence and absence of Al. The authors concluded that the root cap does not provide significant protection from Al injury in this species (Ryan et al., 1993). While this carefully worded conclusion appears justified, I would be reluctant to go one step further and suggest that the root cap does not play a role in an integrated response to Al stress.

The possibility that resistance is mediated by an integrated suite of adaptive responses provides a powerful incentive for use of isogenic plant material. It is only when we are working with a truly isogenic system that we can be confident that experimental manipulation of a resistance mechanism should lead to dramatic changes in performance under conditions of stress. Even with an isogenic system, however, caution must be exercised. It is possible that the single gene of interest might function to provide a message to initiate, or perhaps regulate an integrated response. If this is true, then identifying a specific adaptive response does not rule out the possibility that other adaptations might also contribute to resistance. I would argue that this is a realistic scenario. We are beginning to see reports of a variety of short-term responses to Al stress which could reflect induction of an integrated suite of resistance mechanisms. This is nicely illustrated with recent work on Al-induced proteins in *Triticum aestivum*. Rincon and Gonzales (1991) reported Al-induced changes in the profiles of proteins extracted from Al-resistant and Al-sensitive cultivars. After 24 h of exposure, three newly synthesised proteins were observed in both the resistant and sensitive cultivar, and one additional cultivar-specific protein was synthesised in each of the resistant and sensitive cultivars. Short-term changes in expression of cytoplasmic proteins have also been reported by Ownby and Hruschka (1991) and Picton et al. (1991). Such changes in protein expression are not limited to the cytosol. Basu et al. (1994a, b) observed induction of a membrane-bound protein and several proteins in root exudates of *Triticum aestivum*. In each case, proteins were expressed in a time-and dose-dependent fashion. Unfortunately, direct evidence linking synthesis of this wide array of proteins to induction of a specific resistance mechanism is still lacking. Indeed, in some cases it would appear that these proteins are not involved in resistance (Delhaize et al., 1991). The possibility remains, however, that we are on the verge of identifying a suite of proteins which mediate resistance as part of an integrated response to Al stress.

Summary

While recent efforts to study mechanisms of resistance at the cellular level have been plagued by technical difficulties, techniques for expression of Al toxicity and differential Al resistance in cell culture systems are now available. These techniques provide confirmation that resistance to Al has a fundamental cellular basis. At the same time, differences in the extent of Al uptake by isolated cells and the sensitivity of such cells to Al suggests that the ability of plant cells to alter the

micro-environment within the apoplasm of the root tip affects the way that individual cells respond to Al stress. Cell culture techniques offer a powerful tool to investigate mechanisms of Al resistance, however, a decision to work with cell systems dictates that care must be exercised in selection of genetic material and experimental conditions to ensure that proper control for Al stress has been achieved. While near-isogenic cell lines are not yet available, there does not appear to be any reason why they can not be developed.

While we still have much to learn about the means by which plants are capable of growing in the face of Al stress, the past few years have provided a number of exciting advances. In some cases, these advances have helped to overcome some of the technical barriers which have hindered progress in our field. As we begin to understand the complex physical chemistry of Al, measure the rate of Al transport across the plasma membrane, and make use of near-isogenic Al-resistant genetic material, we can expect our progress towards understanding the cellular basis of Al resistance to improve dramatically. I believe, however, that a complete understanding will ultimately require a greater emphasis on true experimental manipulation of potential resistance mechanisms. Perhaps more importantly, we must begin to interpret the results of these experiments in the context of a suite of physiological adaptations which act in a coordinated fashion to provide protection against Al stress. This is the direction that a number of laboratories throughout the world appear to be heading. I believe this new direction will allow us to continue our progress in overcoming barriers to understanding the cellular basis of Al resistance.

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