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Multiple Aluminum-Resistance Mechanisms in Wheat[1]

Roles of Root Apical Phosphate and Malate Exudation

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Although it is well known that aluminum (Al) resistance in wheat (*Triticum aestivum*) is multigenic, physiological evidence for multiple mechanisms of Al resistance has not yet been documented. The role of root apical phosphate and malate exudation in Al resistance was investigated in two wheat cultivars (Al-resistant Atlas and Al-sensitive Scout) and two near-isogenic lines (Al-resistant ET3 and Al-sensitive ES3). In Atlas Al resistance is multigenic, whereas in ET3 resistance is conditioned by the single *Alt1* locus. Based on root-growth experiments, Atlas was found to be 3-fold more resistant in 20 μM Al than ET3. Root-exudation experiments were conducted under sterile conditions; a large malate efflux localized to the root apex was observed only in Atlas and in ET3 and only in the presence of Al (5 and 20 μM). Furthermore, the more Al-resistant Atlas exhibited a constitutive phosphate release localized to the root apex. As predicted from the formation constants for the Al-malate and Al-phosphate complexes, the addition of either ligand to the root bathing solution alleviated Al inhibition of root growth in Al-sensitive Scout. These results provide physiological evidence that Al resistance in Atlas is conditioned by at least two genes. In addition to the *alt* locus that controls Al-induced malate release from the root apex, other genetic loci appear to control constitutive phosphate release from the apex. We suggest that both exudation processes act in concert to enhance Al exclusion and Al resistance in Atlas.

Al toxicity is one of the major factors that limits the productivity of crop plants in acid soils. A number of crop species and cultivars exhibit significant genetically based variability in their response to the toxic levels of soil Al (Kochian, 1995). This variability has served as the basis for a considerable amount of recent research on the underlying mechanisms that result in crop Al resistance. We are beginning to understand the cellular processes that confer Al resistance in plants. Most of the recent work has focused on Al exclusion from the root apex as a primary mechanism of Al resistance (Kochian, 1995).

Root exudation of organic acids that can chelate Al^{3+} in the rhizosphere and, thus, detoxify Al was first reported in an Al-resistant snapbean genotype (Miyasaka et al., 1991). Several studies have shown that Al resistance is associated with metabolically dependent Al exclusion from the root tip (Zhang and Taylor, 1991; Rincon and Gonzales, 1992). Delhaize and co-workers (1993b) demonstrated, in near-isogenic wheat (*Triticum aestivum*) lines exhibiting differential Al resistance, that Al exclusion was linked to the Al-stimulated exudation of malate. They found that malate was released only from the root apex (the primary site of Al toxicity) (Ryan et al., 1993) of Al-resistant lines when exposed to Al. Exuded malate can chelate and presumably lower the Al^{3+} activity in the rhizosphere, thus reducing Al toxicity. It was shown that malate exudation, and not synthesis, was the rate-limiting step for this Al-resistance mechanism (Delhaize et al., 1993b; Ryan et al., 1995a). Subsequently, several laboratories have confirmed that the association between Al-induced exudation of malate and Al resistance is widespread in wheat (Basu et al., 1994; Ryan et al., 1995b; Huang et al., 1996). In contrast, in Al-resistant and -sensitive maize varieties and lines, Pellet et al. (1995) found that Al rapidly triggered exudation of citrate from the root apex of the Al-resistant maize genotypes.

The work of Delhaize and co-workers (1993a, 1993b) showed that in near-isogenic wheat lines, Al resistance was encoded by the single *Alt1* locus that conditioned Al-induced malate release from the root apex. However, Al resistance can also be multigenic in wheat, controlled by several major and minor genes (see Carver and Ownby, 1995, and refs. therein). In a genetic study of the ditelosomic lines of Chinese Spring wheat, Aniol (1990) found that Al resistance was linked to at least three different chromosome arms: the short arm of chromosome 5A and the long arms of chromosomes 2D and 4D. An earlier report suggested that the major Al-resistance genes were located on the long arms of 2D and 4D in the same ditelosomic lines (Takagi et al., 1983).

Thus, it is possible that wheat has several different genes associated with Al resistance that control the exudation of different Al-chelating compounds. Pi efflux from roots (Elliot et al., 1984; Cogliatti and Santa Maria, 1990) is another potential Al-resistance mechanism in plants. In the presence of Al, Pi efflux could lower the rhizosphere Al^{3+} activity via the formation of Al-Pi complexes either in the apoplasm, on the root surface, or in the rhizosphere (Taylor, 1991; Lüttge and Clarkson, 1992, and refs. therein). In an Al-resistant sugar beet cultivar Lindberg (1990) speculated that a metabolically dependent efflux of Pi was occurring in the presence of Al. Also, Pettersson and Strid

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(1989) suggested that in Pi-sufficient wheat roots, Al-Pi complexes could form due to an efflux of Pi from the symplasm. However, in another study no differences were observed in the amount of Pi bound to Al in the cell walls of Al-resistant or -sensitive wheat varieties (Miranda and Rowell, 1989). In all of these studies Pi efflux was not measured as a function of position along the root. Since the root apex is the primary site for Al toxicity (Ryan et al., 1993), root apical Pi efflux, and not Pi efflux, from the mature root is the important transport process in Al resistance. Therefore, to be an effective Al-resistance mechanism, Pi efflux at the root tip should be greater in Al-resistant than in Al-sensitive varieties. Pellet et al. (1995) observed an Al-associated release of Pi from the root apex of an Al-resistant maize line; however, it was not clear if this efflux was a part of the Al-resistance mechanism in maize.

The objective of the present work was to investigate the role of exudation of different Al-chelating compounds in the mechanisms of Al resistance. Both Al-resistant and -sensitive wheat cultivars, in which Al resistance is controlled by several genes, and near-isogenic lines, in which Al resistance is controlled by the single *Alt1* locus, were studied. Based on the results of this study, we suggest that root apical phosphate exudation is a second mechanism of Al resistance that is conditioned by one or more genes that are different from those controlling Al-induced malate release from the root apex.

MATERIALS AND METHODS

Plant Materials and Seedling Growth

Seeds of winter wheat (*Triticum aestivum* L.) cultivars (Al-resistant Atlas 66 and Al-sensitive Scout 66) were obtained from Dr. J. Peterson (University of Nebraska, Lincoln). Seeds of ET3 and ES3, homozygous Al-resistant and Al-sensitive near-isogenic wheat lines (Fisher and Scott, 1987), were provided by Dr. E. Delhaize (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia).

Seedlings were grown in sterile culture to prevent microbial degradation of organic compounds that were either excreted by seedlings or added to growth solutions. Depending on the experiment seeds were either surface-sterilized for 20 min in 5.25% NaOCl and then rinsed eight times with 40 mL of sterile water, or exposed to Cl_2 gas for 2 h (Huang et al., 1996). Disinfected seeds were germinated aseptically in Petri plates containing 1% agar, 100 μM $CaCl_2$, pH 4.5, in the dark for 24 h at 30°C.

In experiments designed to monitor root exudates from whole plants, two to three germinated seeds were added to 125-mL flasks containing 20 mL of filter-sterilized control solution (100 μM $CaCl_2$, pH 4.5). The flasks were incubated on a shaker (132 rpm) in a growth chamber with a 20°C day (16 h)/15°C night (8 h) cycle for 4 d. Prior to Al treatment, solutions were decanted from the flasks and the seedlings were rinsed twice, first with 20 mL of the sterile control solution and then with the appropriate sterile Al treatment solution (100 μM $CaCl_2$ + 0, 5, or 20 μM $AlCl_3$, pH 4.5). The flasks were then refilled with the same sterile Al treatment solution. Flasks were placed on a shaker in a growth chamber during the daylight period (see above), and the seedlings were exposed to Al treatment for 7 h. Preliminary studies demonstrated that when the whole seedling was in contact with the exudation solution, an accurate measurement of root organic acid and Pi exudation was obtained, since the shoot and seed exuded almost no organic acids or Pi into the bathing solution. At the end of any experiment, the solutions were collected, checked for sterility by streaking onto agar plates, weighed, frozen (−20°C), and lyophilized before being analyzed for organic acids and phosphate.

In experiments designed to study the spatial aspects of phosphate exudation, root segments of 5-d-old seedlings were excised under sterile conditions. Both the apical root segment (either 0–5 or 0–10 ± 2 mm from the apex) and the adjacent subapical segment (either 5–10 ± 2 or 10–20 mm from the apex) were used for Pi exudation studies. Root segments were transferred under sterile conditions into 1.5-mL vials containing 1.0 mL of sterile 100 μM $CaCl_2$ control solution at pH 4.5. Vials with root segments were placed on the shaker in the growth chamber for 2 to 3 h to remove organic acids or phosphate released from cut cells. After two rinses (one with control solution, the second with the corresponding Al treatment solution) root segments were exposed to the appropriate Al treatment for 7 h.

To investigate the ability of malate or phosphate to ameliorate Al toxicity, solutions containing different concentrations of malic acid (100–500 μM) or K_2HPO_4 (40–500 μM) and 100 μM $CaCl_2$ (pH 4.2) were prepared with or without 5 μM $AlCl_3$. KCl was added to maintain the K^+ concentration at 1.5 mM for every solution. Three seedlings of the Al-sensitive variety Scout were grown under sterile conditions in 60 mL of the appropriate solution (in 125-mL flasks) as previously described, and root elongation was measured after 3 d of growth. GEOCHEM-PC (Parker et al., 1995) was used to assess the free Al^{3+} activity in the different amelioration treatments. Stability constants for the formation of Al-malate complexes were added to the database of GEOCHEM-PC after the transformation for zero ionic strength, following the Davies modification of the extended Debye-Hückel equation, according to Lindsay (1979): $Al^{3+} + Mal^{2-} = AlMal^{+}$; log K = 5.54 (Martell and Motekaitis, 1989, after the transformation for zero ionic strength) and $Al^{3+} + 2Mal^{2-} = AlMal_2^{-}$; log K = 11.3 (Nordstrom and May, 1989, after the transformation for zero ionic strength).

For Al-phosphate complex formation, values from Stumm and Morgan (1981; at 25°C and zero ionic strength) were employed in the GEOCHEM-PC database:

$$Al^{3+} + HPO_4^{2-} = AlHPO_4^{+}; \quad \log K = 8.0, \quad \text{and}$$

$$Al^{3+} + H_2PO_4^{-} = AlH_2PO_4^{2+}; \quad \log K = 3.0.$$

To assess the level of Al resistance exhibited by the two wheat cultivars and two near-isogenic lines, sterilized seeds pregerminated (as indicated above) were grown in 125-mL flasks (three seeds per flask) on a shaker in a growth chamber (see conditions above) in 40 mL of 100 μM $CaCl_2$ with 0, 5, or 20 μM $AlCl_3$ at pH 4.5. Total root

elongation was measured after 3 d of growth. Percentage of root growth was expressed in terms of root growth inhibition relative to control plants (grown without Al).

Determination of Organic Acids and Phosphate in Root Exudates

To analyze organic acids and inorganic anions in the root exudates, an ion chromatography system (Dionex 300, Dionex, Sunnyvale, CA) was used (Pellet et al., 1995). The system employed an ion-exchange analytical column (AS11, 4 mm, Dionex) with an eluent gradient of NaOH in 18% high-purity methanol. Concentrations of organic acids and phosphate were determined via measurement of electrical conductivity; for more details, see Pellet et al. (1995).

Seed Phosphorus Content

Seed phosphorus concentrations were determined via inductively coupled plasma emission spectrometry after wet digestion of seeds in a solution of ultrapure nitric and perchloric acids.

RESULTS

Root Growth Inhibition

Differential inhibitory effects of Al on root growth in the four wheat genotypes are depicted in Figure 1. In the Al-resistant cv Atlas a slight root growth inhibition was observed in 5 and 20 μM Al compared with the control treatment. The Al-resistant, near-isogenic line ET3 was almost as Al-resistant as Atlas in 5 μM Al. However, in the presence of 20 μM Al, root growth of ET3 was inhibited by 66%; thus, ET3 was over three times more sensitive to Al than Atlas at this Al concentration. Both Al-sensitive genotypes, ES3 (near-isogenic line) and Scout (cultivar), exhibited a strong reduction of root growth in 5 μM Al (65–70% inhibition) and 20 μM Al (88% root-growth inhibition).

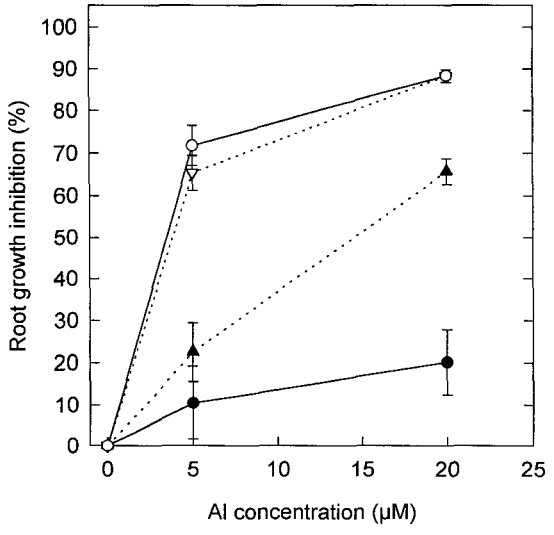

Figure 1. Al-induced root-growth inhibition (% root growth inhibition = [1 − (root length in Al/root length without Al)] × 100) in the four wheat genotypes. Two Al-resistant and two Al-sensitive wheat genotypes were grown in solutions containing the different Al concentrations for 3 d before measurement of root growth. Error bars = SE (n = 5). ●, Atlas; ▲, ET3; ▽, ES3; ○, Scout.

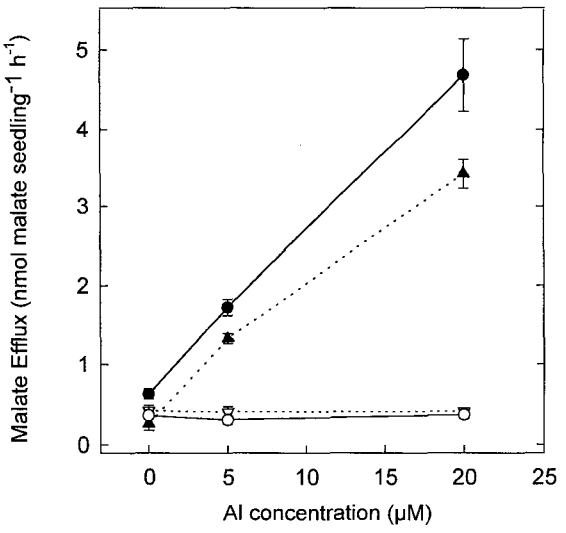

Figure 2. Effect of Al concentration on malate exudation in the two Al-resistant and two Al-sensitive wheat genotypes. Seedlings were exposed to Al for 7 h. Pooled data are of two separate experiments with five to seven replicates. Error bars = SE. ●, Atlas; ▲, ET3; ▽, ES3; ○, Scout.

Malate Exudation

The Al-resistant wheat genotypes Atlas and ET3 both released malate in response to Al exposure. In the presence of 5 and 20 μM Al, Atlas exuded slightly more malate than ET3 (Fig. 2). Both genotypes exhibited a linear increase in malate exudation in response to increasing levels of Al. In contrast, the Al-sensitive wheat genotypes Scout and ES3 exhibited a low rate of malate exudation that was insensitive to Al exposure. In the Al-resistant genotypes Atlas and ET3, the rates of malate exudation in the presence of 20 μM Al were 11- and 8-fold higher, respectively, than in the Al-sensitive wheat lines. Malate exudation was previously shown to be localized primarily to the first 3 to 5 mm of the root apex in wheat (Ryan et al., 1995b; Huang et al., 1996).

Root Phosphate Exudation

The Al-resistant cv Atlas exhibited a constitutive phosphate exudation that was unaffected by Al exposure (Table I). Phosphate exudation in Atlas was much higher than in the other three wheat genotypes (Fig. 3). The Al-resistant, near-isogenic line (ET3) and the two Al-sensitive wheat genotypes (ES3 and Scout) exhibited a similarly low rate of Pi exudation that was approximately 30% of the Pi efflux that was observed in Atlas. In Atlas the rate of Pi exudation was slightly higher than the rate of malate efflux in the presence of 5 μM Al; however, in 20 μM Al, malate efflux was twice that of Pi exudation (compare Figs. 2 and 3).

Pi exudation was localized to the first 5 mm of the root apex in Atlas (Fig. 4). Longer apical root segments (10 mm) did not exude significantly more Pi than the apical 5-mm

Table I. *Effect of Al concentration on Pi exudation in Al-resistant Atlas wheat seedlings*

Seedlings were exposed to Al for 7 h. Pooled data are of two separate experiments.

Al Concentration	Pi Exudation
μM	*nmol plant^{-1}h^{-1}*
0	1.90 (0.18)[a]
5	2.33 (0.28)
20	2.13 (0.47)

[a] SE, n = 13–14.

segments. Subapical root segments (5–10 and 10–20 mm back from the root tip) exhibited a 5- to 8-fold lower Pi exudation than did the apical root segments. It is interesting that the rates of Pi exudation from the root apex (0- to 5-mm segments) were comparable with the values observed for intact root systems of whole plants, assuming three roots per plant for these 5-d-old seedlings (Fig. 3). This result suggests that in whole plants, neither the seed, the shoot, nor the more basal root regions contribute significantly to Pi efflux in Atlas.

Since no P was added to the growth solutions in these exudation experiments, seed P was the only phosphorus source. Thus, seed P concentration and content were determined to see if the higher rate of root Pi exudation in Atlas was the result of greater seed P reserves (Table II). There were no major differences in seed P concentration or content among Atlas, ET3, or ES3. However, all three genotypes had considerably larger seed P reserves than the Al-sensitive cv Scout. Although P content in ET3 and ES3 was larger than in Scout, the three genotypes showed similarly low rates of Pi efflux (Fig. 3). These results indicate that seed P reserves do not play a major role in root Pi efflux or Al resistance.

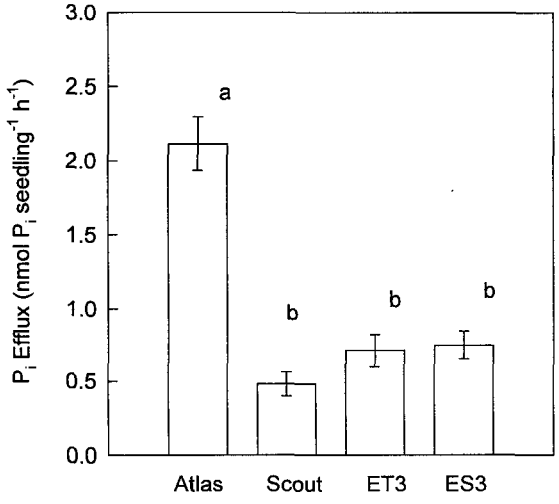

Figure 3. Constitutive phosphate exudation in the two Al-resistant and two Al-sensitive wheat genotypes. Rates are averages over three Al concentrations (0, 5, and 20 μM Al). Seedlings were exposed to Al for 7 h. Pooled data are of two separate experiments with five to seven replicates. Error bars = SE. Lowercase letters represent indices of Tukey's test ($P < 0.05$).

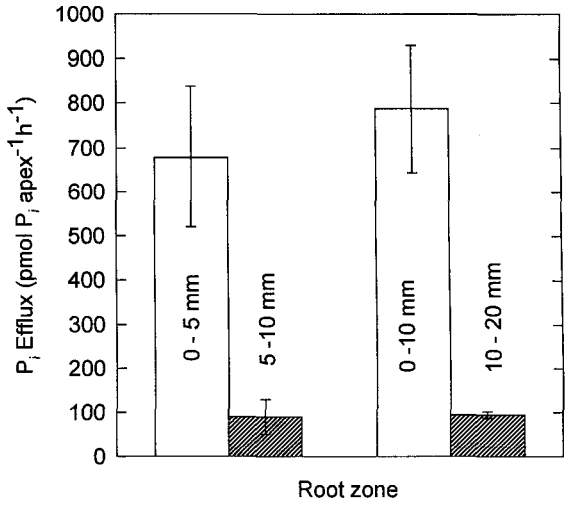

Figure 4. Phosphate exudation in excised root segments of Al-resistant Atlas. Either the terminal 5 or 10 mm of the root or the next subapical 5 or 10 mm was used for phosphate exudation experiments. The exudation period was 7 h. Error bars = SE (n = 5–7). Open bar, Apex; striped bar, basal.

Amelioration of Al Toxicity

The ability of malate and phosphate to protect roots against Al-induced injury was assessed in a bioassay with the Al-sensitive cv Scout (Fig. 5). In the presence of 5 μM Al (pH 4.2) root elongation in Scout seedlings was severely inhibited after 3 d (72% root-growth inhibition). The addition of 40 μM phosphate to the 5-μM Al solution (pH 4.2) significantly ameliorated Al toxicity, as root growth inhibition decreased to 49%, and the addition of 500 μM Pi to the Al-containing growth solution further decreased root growth inhibition (23% inhibition of root growth). Under the sterile conditions imposed for this experiment, the addition of 100 μM malate almost completely restored normal root growth (7% inhibition of root growth) in the presence of 5 μM Al. The predicted effect of the increasing concentrations of malate and phosphate (0–500 μM) on Al^{3+} activity as calculated with GEOCHEM-PC is presented in Figure 5 as the relative Al^{3+} activity, where relative Al^{3+} activity equals the Al^{3+} activity in the presence of phosphate or malate divided by the Al^{3+} activity in the absence of either ligand (expressed as a percent). There was a strong correlation between the experimentally derived bioassay data and the predicted reduction in Al^{3+} by malate or phosphate (correlation coefficient = 0.78 for malate [$P <$

Table II. *Seed weight, total seed P concentration, and seed P content of four wheat genotypes*

Variety	Seed Dry Weight	P Concentration	P Content
	mg	*mg/kg*	*μg P/seed*
Atlas 66	30.9 (0.65)[a]	4528 (82.5)	140 (2.9)
ET3	29.7 (1.11)	4025 (70.1)	121 (4.5)
ES3	28.7 (1.0)	4094 (15.9)	117 (3.9)
Scout 66	26.0 (0.88)	2940 (79.9)	76 (3.4)

[a] SE, n = 5.

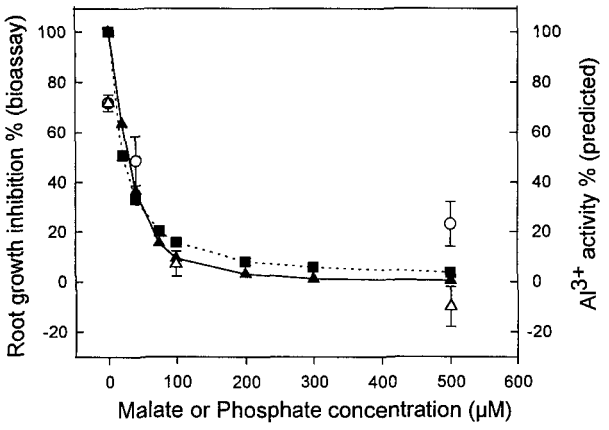

Figure 5. Root growth inhibition for Scout grown in 5 μM $AlCl_3$ (% root growth inhibition = [1 − (root length in Al/root length without Al)] × 100) exposed to increasing levels of malate (0–500 μM) or phosphate (0–500 μM). Error bars denote SE (n = 4). ○, Phosphate; △, malate (bioassays). Effect of increasing malate or phosphate concentrations on relative Al^{3+} activity (% relative Al^{3+} activity = [{Al^{3+}} with ligand/{Al^{3+}} without ligand] × 100) as predicted by GEOCHEM-PC (100% = 2.8 μM Al^{3+} activity, in the absence of any ameliorant; total Al concentration = 5 μM, pH 4.2). Gibbsite formation was not allowed for calculations in GEOCHEM-PC. Correlation coefficient for relationship between root growth inhibition and predicted relative Al^{3+} activity is 0.78 ($P < 0.01$) for phosphate and 0.95 ($P < 0.001$) for malate. ■, Phosphate; ▲, malate (both predicted).

0.01] and 0.95 for phosphate [$P < 0.001$]), providing additional evidence to support the idea that phosphate exuded from the root apex helps to ameliorate Al toxicity.

DISCUSSION

Multiple Mechanisms of Al Resistance

The most significant findings presented in this paper involve the identification and characterization of the multiple physiological mechanisms of Al resistance. It has been well documented, based on genetic studies, that Al resistance in wheat is under multigenic control (see, for example, Takagi, 1983; Aniol, 1990; Carver and Ownby, 1995). To our knowledge, this study provides the first physiological evidence supporting the genetic findings for Al resistance. Our results suggest that differences in malate exudation alone cannot account for the increased Al resistance exhibited by Atlas in comparison with ET3, particularly at the highest Al levels tested here (20 μM Al). As shown in Figure 1, at 5 μM Al there was no significant difference in seedling growth between Atlas and ET3; however, solutions containing 20 μM Al elicited a 20% inhibition of root growth in Atlas, whereas the same treatment had a much more dramatic inhibition on root growth by ET3 (66% inhibition). This large difference in root-growth inhibition was associated with only a modest difference in malate exudation between Atlas and ET3 (Fig. 2).

The results presented here confirm the findings by Ryan and co-workers (1995b) showing that the near-isogenic line ET3 exhibited a moderate Al resistance. ET3 was significantly more Al resistant than Al-sensitive ES3, but was considerably more sensitive (approximately two to three times) than the Al-resistant cv Atlas (Ryan et al., 1995b) (Fig. 1). Some of the data in table I of Ryan et al. (1995b) can be used to support the possibility of multiple resistance mechanisms in wheat, if one considers only the seven most Al-resistant wheat varieties determined for the highest Al concentration used in that study. If root-growth performance for these seven varieties is plotted against rates of malate efflux, no significant correlation is found (r = 0.39, n = 7). However, for the same group of seven varieties, the same type of analysis yields a significant correlation (r = 0.75, $P < 0.05$, n = 7) between root growth performance at a lower Al concentration (3 μM) and malate exudation. This observation suggests that in Al-resistant varieties, malate exudation accounts for only part of the resistance when exposed to higher levels of Al. The results presented in Figures 3 and 4 indicate that constitutive Pi exudation localized to the root apex might be an additional Al-resistance mechanism employed by Atlas (and possibly other Al-resistant cultivars), which increases this genotype's ability to exclude Al.

The presence of malate exudation but not Pi exudation in ET3 suggests that these two Al-resistance mechanisms are not conferred by the same genetic locus. Thus, in addition to the *Alt1* locus, which encodes Al resistance in ET3 and is correlated with malate release (Delhaize et al., 1993a, 1993b), other genetic loci regulating phosphate exudation must be involved in Al resistance in Atlas. These results are supported by previous genetic analyses of Al resistance in wheat, including the work of Campbell and Lafever (1981), which indicated that Al resistance in Atlas was multigenic, and research showing that Al resistance is linked to several different chromosome arms in wheat (Takagi, 1983; Aniol, 1990). We suggest that multiple genes control the release of several Al^{3+}-chelating compounds in Atlas, and the sum of these exudation processes results in increased Al resistance.

The constitutive root apical Pi exudation was significantly higher in Atlas than in any of the other wheat genotypes (Figs. 3 and 4). Furthermore, the addition of relatively low levels of Pi (40 μM) and malate (100 μM) to the bulk solution in the presence of Al alleviated the effects of Al-induced root injury in Al-sensitive Scout. Predictions from the speciation program GEOCHEM-PC (modified with stability constants for Al-phosphate and Al-malate, as indicated in "Materials and Methods") were in good agreement with the data obtained from the root-growth bioassay (Fig. 5). Based on the constants for the formation of the different Al-phosphate complexes, phosphate alleviation of Al toxicity was due primarily to the formation of the Al-HPO_4^+ complex. GEOCHEM predicted that Al-mal_2^- is the predominant Al-malate complex formed in our experiments. Data from our bioassays and calculations using GEOCHEM-PC indicated that malate was more effective than Pi in alleviating Al toxicity, particularly at higher ligand concentrations (≥100 μM, Fig. 5). Indeed, comparisons of the stability constants used for the Al-malate and Al-phosphate complexes indicate that malate has a higher affinity for Al than does Pi. Nonetheless, Pi should contribute an additional ameliorative effect to malate by chelating additional Al^{3+} in the rhizosphere.

Calculations using the measured ligand exudation rates in a computer-simulation model that describes malate and phosphate radial diffusion from the root (Jones and Darrah, 1994a, 1994b) indicated that steady-state phosphate and malate concentrations after 1 h of exudation in the presence of 20 μM Al should be approximately 34 and 75 μM, respectively, at the surface of the root apex in Atlas. In this concentration range phosphate and malate should act in an additive fashion to lower the Al^{3+} activity in the rhizosphere. Under these conditions at pH 4.5, calculations using GEOCHEM-PC indicated that free Al^{3+} activity would increase by 60% (from 0.75 μM to 1.22 μM Al^{3+}) if Al-phosphate complex formation was not considered. This result suggests that relatively low Pi concentrations at the root apex surface may have a moderate effect in lowering rhizosphere Al^{3+} activity, thus contributing an additional ameliorative effect to that conferred by malate exudation.

Because phosphate has a high affinity for protons as well as for Al^{3+}, the apical exudation of phosphate may be involved in another possible Al exclusion mechanism that is associated with the moderate increase in the apical rhizosphere pH measured in Atlas (Pellet et al., 1996). This possibility will be the focus of future work.

Root Apical Pi Exudation

Because most acidic soils are P-deficient, an Al-resistance mechanism that involves the loss of an essential mineral nutrient that is limiting may seem to have questionable adaptive significance. However, because this loss is localized to a small portion of the root, negative effects on the plant are minimized. Also, Pi efflux from plant roots is a natural consequence of P nutrition and P balance in plants (Bielski and Fergusson, 1983). Under conditions of growth-limiting P supply, Pi efflux diminishes in absolute terms but increases relative to Pi influx and becomes a significant component of net uptake (Elliott et al., 1984). On the other hand, plants well supplied with P exhibit increased Pi efflux compared with P-stressed plants (Pettersson and Strid, 1989; Cogliatti and Santa Maria, 1990; Adalsteinsson et al., 1994, and refs. therein). In agreement with this observation, the data presented in Table I indicate that Atlas has the highest seed P content, which correlates with the significant root Pi efflux in this genotype. Similarly, Pellet et al. (1995) showed that in the presence of Al, an Al-resistant corn variety exuded more Pi from the root tip than an Al-sensitive inbred line. Recent analysis of seeds from these maize genotypes indicated that the seed P content of the Al-resistant maize line was 38% higher than in seeds from the Al-sensitive maize line (D.M. Pellet, unpublished data). It has also been shown that the application of Pi fertilizers to surface soil horizons enhanced the ability of plant roots to penetrate the surface soil horizons and to enter acidic subsoils, where Al concentrations limit root growth (Miranda and Rowell, 1987; McLaughlin and James, 1991). According to the authors, Al-phosphate formation was a possible cause for this beneficial response. In line with the present work, this effect could have been due to increased root Pi efflux and rhizosphere Al-Pi complex formation at the root tips of plants well supplied with Pi.

However, a higher P content in seeds of the Al-resistant genotype cannot fully explain the differences in Pi exudation observed here (Fig. 3). Atlas has almost twice the seed P content of Scout, yet its roots exuded up to four times more Pi than Scout (Fig. 3; Table I). Additionally, ET3 and ES3 both had seed P concentrations that were similar to Atlas, and yet they both exuded low levels of Pi (Fig. 3). These results suggest that the increased Pi efflux in Atlas involves an alteration in Pi transport at the root-cell plasma membrane. Presumably, Pi efflux is mediated by a plasma membrane anion channel. Thus, the situation is analogous to the Al-induced malate release observed in ET3 and Atlas. In ET3 it has been shown that this process is regulated at the transport step, and not via malate synthesis in the cytoplasm (Delhaize et al., 1993b; Ryan et al., 1995a). In the study by Ryan and co-workers (1995a) results from experiments using anion channel blockers suggested that Al-induced malate release is mediated via an anion channel. The one significant difference between the malate and phosphate efflux processes is that Pi efflux is constitutive, and is not induced by Al. Because plants growing in acid soils are continuously exposed to toxic levels of Al, the selection of a constitutively expressed Al-resistance mechanism might be expected.

The differential Al resistance observed here in Atlas, Scout, ET3, and ES3, along with the differences in malate and Pi exudation, are not a result of working with low-salt-grown seedlings. We have previously shown that in Atlas and Scout, the responses of root growth to Al exposure and the differential Al resistance were identical whether 5-d-old seedlings were grown in full nutrient solution or low-salt media ($CaCl_2$, pH 4.5) (Miyasaka et al., 1989). Based on visual inspection of the seedlings, as well as inductively coupled plasma emission spectrometry analysis of shoot and root tissue mineral composition, it was clear that the low-salt-grown seedlings were not deficient in any of the essential macronutrients or micronutrients. We have found that in 5-d-old wheat seedlings, the seed mineral nutrient reserves are sufficient to adequately supply the nutritional needs of the seedlings.

In conclusion, this study presents, to our knowledge, the first evidence in support of multiple physiological processes functioning in concert to enhance Al resistance in an important crop plant. We suggest that these two processes, Al-induced malate release and constitutive Pi release, are controlled by different genes and result in the enhanced Al-resistant phenotype expressed in Atlas wheat.

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