**Al^{3+} - Ca^{2+} interactions in aluminum rhizotoxicity**

**II. Evaluating the Ca^{2+}-displacement hypothesis**

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**Abstract.** Several mineral rhizotoxicities, including those induced by Al^{3+}, H^+, and Na^+, can be relieved by elevated Ca^{2+} in the rooting medium. This leads to the hypothesis that the toxic cations displace Ca^{2+} from transport channels or surface ligands that must be occupied by Ca^{2+} in order for root elongation to occur. In this study with wheat (*Triticum aestivum* L.) seedlings, we have determined, in the case of Al^{3+}, that (i) Ca^{2+}, Mg^{2+}, and Sr^{2+} are equally ameliorative, (ii) that root elongation does not increase as Ca^{2+} replaces Mg^{2+} or Sr^{2+} in the rooting media, and (iii) that rhizotoxicity is a function solely of Al^{3+} activity at the root-cell membrane surface as computed by a Gouy-Chapman-Stern model. The rhizotoxicity was indifferent to the computed membrane-surface Ca^{2+} activity. The rhizotoxicity induced by high levels of tris(ethylenediamine)cobaltic ion (TEC^3+), in contrast to Al^{3+}, was specifically relieved by Ca^{2+} at the membrane surface. The rhizotoxicity induced by H^+ exhibited a weak specific response to Ca^{2+} at the membrane surface. We conclude that the Ca^{2+}-displacement hypothesis fails in the case of Al^{3+} rhizotoxicity and that amelioration by cations (including monovalent cations) occurs because of decreased membrane-surface negativity and the consequent decrease in the membrane-surface activity of Al^{3+}. However, TEC^3+, but not Al^{3+}, may be toxic because it inhibits Ca^{2+} uptake. The nature of the specific H^+-Ca^{2+} interaction is uncertain.

**Key words:** Aluminum toxicity – Calcium displacement – Electrical potential – Root – *Triticum*

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**Introduction**

Investigators of mineral rhizotoxicity have long had an interest in the interactions between Ca^{2+} and the rhizotoxic ions. Elevated levels of Ca^{2+} in the rooting media can relieve the toxicity induced by Al^{3+}, H^+, Na^+, and other cations (Foy 1984; Hanson 1984). One hypothesis that has been offered repeatedly to explain the Ca^{2+} amelioration is that the toxic cations displace Ca^{2+} from transport channels or surface ligands that must be occupied by Ca^{2+} in order for root elongation to occur. In this study with wheat (*Triticum aestivum* L.) seedlings, we have determined, in the case of Al^{3+}, that (i) Ca^{2+}, Mg^{2+}, and Sr^{2+} are equally ameliorative, (ii) that root elongation does not increase as Ca^{2+} replaces Mg^{2+} or Sr^{2+} in the rooting media, and (iii) that rhizotoxicity is a function solely of Al^{3+} activity at the root-cell membrane surface as computed by a Gouy-Chapman-Stern model. The rhizotoxicity was indifferent to the computed membrane-surface Ca^{2+} activity. The rhizotoxicity induced by high levels of tris(ethylenediamine)cobaltic ion (TEC^3+), in contrast to Al^{3+}, was specifically relieved by Ca^{2+} at the membrane surface. The rhizotoxicity induced by H^+ exhibited a weak specific response to Ca^{2+} at the membrane surface. We conclude that the Ca^{2+}-displacement hypothesis fails in the case of Al^{3+} rhizotoxicity and that amelioration by cations (including monovalent cations) occurs because of decreased membrane-surface negativity and the consequent decrease in the membrane-surface activity of Al^{3+}. However, TEC^3+, but not Al^{3+}, may be toxic because it inhibits Ca^{2+} uptake. The nature of the specific H^+-Ca^{2+} interaction is uncertain.
virtually halt growth and that additions of ameliorative cations to the toxic Al3+ solutions relieve growth inhibition while simultaneously depressing Ca2+ uptake.

In this article we evaluate the general Ca2+-displacement hypothesis for the rhizotoxicities of three cations — Al3+, H+, and tris(ethylenediamine)cobaltic ion (TEC3+) — by considering whether these toxicities are relieved by increased activities of Ca2+ at the surface of root cell membranes.

Materials and methods

**Seedling culture.** The principal data used to evaluate the hypothesis come from experiments of short-term seedling culture in chemically simple rooting media. The details and the rationale for the methods have been presented in previous articles (Kinraide 1991). Essentially, newly germinated wheat (Triticum aestivum L.) was cultured for 2 d in an aerated, 25°C, basal medium of CaCl2 variously supplemented with AlCl3 and other chloride salts. The Al3+-sensitive cultivar Tyler was used in experiments performed years ago with a seed lot now exhausted. More recent experiments were performed with the Al3+-sensitive cultivar Scout 66. Unless otherwise stated, the rooting media for the experiments contained 0.4 mM CaCl2, and the pH was 4.3 in experiments with Tyler and 4.5 in experiments with Scout 66. Some results are presented as relative root lengths (RRLs) according to the formula RRL = 100 (Lc - Ls)/(Lc Ls), in which Lc is the mean root length in the presence of the toxicant (e.g., Al3+), Ls is the mean root length in the corresponding toxicant-free (or, in the case of H+, low-toxicant) control, and Lc is the mean root length in toxicant-sufficient to saturate growth-inhibitory processes. Additional notes on culture conditions will be presented with the results.

**Ion activities at the membrane surface.** The first step in the estimation of ion activities at the membrane surface is the computation of ion concentrations and activities in the rooting medium. In the case of Al-containing solutions, the hydrolysis products of Al3+ must be considered according to methods described previously (Kinraide 1991). Then a Gouy-Chapman-Stern model may be used to compute the membrane-surface electrical potential (E0), which is a function of the charge density on the membrane surface, the ion concentrations in the bulk medium, and the binding of ions to the membrane surface (Lau et al. 1981). Finally, the ion activities at the membrane surface are computed by the Nerst equation using E0 and the ion activities of the rooting medium. The model parameters used in a previous study (Kinraide et al. 1992) were used here. In that study, the rhizotoxicity of Al3+ and La3+ was assessed in terms of the computed chemical activities of those ions at the cell-membrane surface ([Al3+]o and [La3+]o). For a large body of experimental data, [Al3+]o and [La3+]o were much better correlated with rhizotoxicity than were [Al3+]E and [La3+]E (activities of the ions in the external rooting media).

**Results**

**Rhizotoxicity of Al3+.** The rhizotoxicity of Al3+ is alleviated by Ca2+ and other cations in the following order of effectiveness: C1+ > C2+ > C3+. This amelioration has been attributed to the reduction by cations of the negativity of E0 (Kinraide et al. 1992). The Al3+ cation would be less attracted to the less-negative membrane surface, and the relative root elongation would be a function of [Al3+]o alone, irrespective of the identity of the ameliorating cation. Though it was not explicitly stated in previous publications, it can be inferred that the elongation of Al3+-intoxicated roots is independent of [Ca2+]o above some threshold level that seems to have been exceeded in most published reports. For example, Na+ relieved Al3+-toxicity in a published experiment with Tyler wheat comprising solutions factorial in [Al3+]E and [NaCl]E (Kinraide and Parker 1987b). Figure 1 shows that [Al3+]E was a poor predictor of toxicity, but [Al3+]o (not computed in 1987) correlated highly with RRL. Though [Ca2+]o was constant, both [Ca2+]E and [Ca2+]o varied widely in the experiment but without effect upon RRL (Fig. 1B).

A more stringent test for a specific role for Ca2+ in Al3+ rhizotoxicity is provided by Ca2+-Mg2+ replacement experiments. Two series of solutions were prepared in which ([Ca2+]E + [Mg2+]E) = 1 mM in the first and ([Ca2+]E + [Mg2+]E) = 2 mM in the second. In each series, the ratio [Ca2+]E/([Ca2+]E + [Mg2+]E) ranged from 0.1 to 1.0. Figure 2 illustrates that this ratio had no effect upon root elongation whether in the presence or absence of Al3+. The sum of the divalent cations was important, however; RRL was 45 at 1 mM and 74 at 2 mM. The ratio [Ca2+]E/([Ca2+]E + [Mg2+]E) had little effect upon either [Al3+]E or [Al3+]o, but [Ca2+]E and [Ca2+]o both increased about tenfold. Apparently, neither the control roots nor the Al3+-inhibited roots were limited by cell-surface Ca2+.

The preceding experiments were performed years ago with Tyler wheat, so more recent experiments were done with Scout 66 in order to further compare the effects of several divalent cations. The cations were Ca2+, Mg2+, Sr2+, and hexamethonium (HXM2+). The last is a very