Aluminum exclusion and aluminum tolerance in woody plants

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INTRODUCTION

Aluminum (Al) is a prevalent constituent of most soils and is one of the major stresses to plants in acid soils. Most of the Al in soils is incorporated into aluminosilicates and other precipitated forms, which are harmless to plants. Under acid soil conditions, these minerals solubilize to a limited extent, and the toxic ion Al\(^{3+}\) is released into the soil solution (Kimble, 1997). This form of Al is capable of inhibiting root growth and damaging cells at the root apex, which is the most sensitive part of the root to Al\(^{3+}\) (Ryan et al., 1993; Kochian, 1995). However, the mechanism underlying Al\(^{3+}\) toxicity is not clearly understood. Because Al\(^{3+}\) can interact with a number of extracellular and intracellular structures, many different mechanisms of Al\(^{3+}\) toxicity have been proposed. These mechanisms include modification of the cell wall, disruption of the plasma membrane and transport processes, interruption of signaling pathways, and Al\(^{3+}\) binding to the DNA (Kochian et al., 2005).

Al\(^{3+}\) toxicity is a major research topic, because many crop plants are susceptible in acid soils, and their growth and yield are limited by high Al\(^{3+}\) concentrations. Less attention is paid to native plant communities, which tolerate acidic soil conditions over large areas in different biomes. Acid soils occupy about 30% of the ice-free land area in the world and primarily occur in the humid tropics and the boreal region. Large parts of these soils (about 67%) are covered by forests and woodland (von Uexküll and Mutert, 1995; Figure 1). The biodiversity and high biomass production of both tropical and boreal forests suggest that their plants are not affected by Al\(^{3+}\) toxicity. Al\(^{3+}\) toxicity may occur in forests that are exposed to acid deposition deriving from air pollutants (Cronan and Schofield, 1979; Ulrich et al., 1980; Larssen et al., 2006). On sensitive sites, acid deposition accelerates soil acidification and leads to increased Al\(^{3+}\) concentrations in the soil solution (Blaser et al., 1999; Fossler et al., 1999). Recently, it was found that soils, affected by acid deposition, showed signs of recovery due to the reduction in sulfate deposition (Stoddard et al., 1999; Evans et al., 2001). However, inputs of nitric acid and ammonia continue to alter the chemistry of forest soils and are likely to promote acidification (Cotef Pannatier et al., 2011; Zang et al., 2011). Acid soils characteristically contain high amounts of Al\(^{3+}\) and low amounts of the base cations (BC) Ca\(^{2+}\), Mg\(^{2+}\), and K\(^{+}\), which are important plant nutrients. Since Al\(^{3+}\) and BC interact at the plasma membrane surface, it is not the soil Al\(^{3+}\) concentration alone that determines the plant responses to Al\(^{3+}\) exposure (Vreeland and Westra, 1993; Cronan and Gregal, 1995; Kinraide, 2003). A ratio of Ca\(^{2+}\)/Al\(^{3+}\) or BC/Al\(^{3+}\) in the soil solution lower than 1 is widely used as an ecological indicator for potentially adverse effects of Al\(^{3+}\) stress and nutrient imbalance on tree growth. Alternative indicators are based on the Al and Ca concentrations in fine roots and provide information on the availability of toxic Al\(^{3+}\) in the soil (e.g., Brunner et al., 2004; Richter et al., 2007; Vanguelova et al., 2007).

Al\(^{3+}\) EXCLUSION AND Al\(^{3+}\) TOLERANCE MECHANISMS

The mechanisms conferring resistance to Al\(^{3+}\) have been the focus of intensive research in crop plants and in the model plant Arabidopsis. Many different mechanisms have been suggested, but for most of them, the supporting genetic and physiological evidence is not provided. Therefore, these mechanisms have to remain speculation or hypotheses until supporting data is provided. One exception is the Al\(^{3+}\) -induced efflux of organic acids.
from roots, which has been demonstrated to be a major Al$^{3+}$ resistance mechanism in several plant species (Delhaize et al., 1993, 2007).

Following Ryan and Delhaize (2010) and Horst et al. (2010), the term “Al$^{3+}$ resistance” is used here as a plant property that allows a plant to grow with little or no injury under elevated Al$^{3+}$ conditions. The potential mechanisms conferring resistance to Al$^{3+}$ can be broadly divided into those that exclude Al$^{3+}$ from the root symplast (exclusion mechanisms) and those that enable plants to cope with Al$^{3+}$ safely, once it enters the symplast (internal tolerance mechanisms; e.g., Kochian, 1995). Exclusion mechanisms depend on the release of ligands which chelate and detoxify Al$^{3+}$ externally and limit its uptake in the cytosol. Tolerance mechanisms include those that chelate the Al$^{3+}$ entering

**FIGURE 1** World acid soils and world forests. (A) pH of topsoil (0–30 cm), (B) pH of subsoil (30–100 cm), and (C) forest cover. Soil pH is presented in two classes: pH ≤ 4.5 (strongly acid soils) and pH 4.6–5.5 (moderately acid soils). Data were retrieved from the Harmonized World Soil Data Base (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012). Forest cover is presented in four classes: ≤ 5, 6–25, 26–75, and 76–100%. Data were retrieved from the Food Insecurity, Poverty and Environment Global GIS Database (FGGD; FAO and IIASA, 2007).
the root cells, with subsequent transport and sequestration into less sensitive parts of the plant and subcellular compartments. The physiology, biochemistry, and molecular biology of these mechanisms have been thoroughly discussed in several review articles (e.g., Jones and Ryan, 2003; Kochian et al., 2004; Ma, 2007; Poschenrieder et al., 2008; Horst et al., 2010; Ryan et al., 2013; Delhaize et al., 2012; Inostroza-Blancheteau et al., 2012). The proposed principles of these mechanisms are summarized in Figure 2.

In this paper, we review the literature on proposed Al\(^{3+}\) resistance mechanisms of woody plants. We summarize information obtained from crops and Arabidopsis, and then review relevant results from similar studies in woody plants. In addition, we discuss the occurrence of Al accumulators and Al excluders in different forest biomes of the world.

**Al\(^{3+}\) EXCLUSION**

**RELEASE OF SUBSTANCES THAT CHELATE AND DETOXIFY Al\(^{3+}\)**

The best-documented mechanism of Al\(^{3+}\) exclusion is the Al\(^{3+}\)-activated efflux of organic acids from roots. Typical organic acids released by plants are citrate, malate, and oxalate. These organic acids are deprotonated anions at the pH found in the cytosol, and once transported out of the root, they chelate the toxic Al\(^{3+}\) in the rhizosphere, forming stable and non-toxic complexes. Citrate and malate are present in all plant cells because they are involved in the mitochondrial respiratory cycle. Oxalate is a common cellular constituent involved in Ca\(^{2+}\) regulation, ion balance, and metal detoxification (Franceschi and Nakata, 2005; Rahman and Kawamura, 2011). The three organic acid anions form complexes with Al\(^{3+}\) with the following order of strength: citrate > oxalate > malate (Liberi and Franceschi, 1987; Jones and Ryan, 2003). Physiological and genetic evidence from several plant species shows that the Al\(^{3+}\)-activated efflux of organic acid anions indeed confers resistance to Al\(^{3+}\). The most convincing support comes from genotypes within a species that show contrasting levels of Al\(^{3+}\) resistance. In wheat (Triticum aestivum), for example, a pair of near-isogenic lines that differ in resistance at a single locus was used to show that the Al\(^{3+}\)-activated efflux of malate from roots was greater in the resistant genotype than in the sensitive genotype (Delhaize et al., 1993). The same wheat genotypes were used to clone the Al\(^{3+}\)-activated malate transporter (TaALMT1) gene, the first Al\(^{3+}\) resistance gene isolated from plants (Sasaki

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**Symplast**

| Plasma membrane | Apoplast |
|-----------------|---------|
| **Exclusion mechanisms** |
| OA [OA-\(\text{Al}^{3+}\)] |
| OH\(^+\) [\(\text{OH}^- + \text{Al}^{3+} \rightarrow \text{Al(OH)}^2^-\)] |
| P [\(\text{Al}^{3+} + \text{P} \rightarrow \text{AlP}^-\)] |
| PME [\(\text{Al}^{3+} + \text{P} \rightarrow \text{AlP}^-\)] |

**Internal tolerance mechanisms**

1. Chelation of Al\(^{3+}\) in cytosol [OA-\(\text{Al}^{3+}\)]
2. Sequestration of Al\(^{3+}\) to cell compartments (e.g., vacuoles)
3. Activation of Al\(^{3+}\)-tolerant metabolic pathways

**FIGURE 2** | Mechanisms of plant roots to deal with Al\(^{3+}\) (modified from Jones and Ryan, 2003; Inostroza-Blancheteau et al., 2012). OA, organic acids; P, pectin; PME, pectin methylesterase.
like genes were isolated from several additional plant species, including Arabidopsis and rape (Brassica napus; Hoekenga et al., 2006; Ligaba et al., 2006; see also Delhaize et al., 2007). Citrate efflux, on the other hand, was found to be mediated by members of another protein family, the multidrug and toxic compound extrusion (MATE) family (Furukawa et al., 2007; Magalhaes et al., 2007). The molecular background of oxalate exudation has not yet been identified.

Numerous studies, some of which are described here, have found that woody plants release organic acid anions following Al\(^{3+}\) exposure. In the model tree poplar (Populus), the Al\(^{3+}\)-activated release of organic acid anions has been studied both at the physiological and molecular level. In young rooted cuttings of Populus tremula, Al\(^{3+}\) induces the release of citrate and oxalate (Qin et al., 2007). In a follow-up study with the same poplar clone, Grisel et al. (2010) identified a MATE gene with a 60% amino acid sequence identity to the AtMATE1 gene of Arabidopsis. The poplar gene is induced by Al\(^{3+}\) in both root and stem tissue, but not in the leaves, consistent with a function of this gene in the efflux of citrate from roots. In seedlings of two other poplar species, Populus tremula and Populus trichocarpa, Al\(^{3+}\) induced the exudation of citrate, malate, and oxalate from roots (Naik et al., 2009). In these species, organic acids accounted for 20–64% of the total C released upon Al\(^{3+}\) exposure (Naik et al., 2009). The minimal concentrations of Al\(^{3+}\) required to induce organic acid exudation in Populus tremula are between 50 and 100 \(\mu\text{M}\) Al\(^{3+}\) (Table 1; Qin et al., 2007). Using the same solution culture medium, similar threshold values were found in the two coniferous trees Cryptomeria japonica and Pinus thunbergii (Hirano et al., 2010). Although these studies clearly demonstrate that Al\(^{3+}\) induces the release of organic acid anions from roots, direct physiological and genetic evidence for their role in Al\(^{3+}\) resistance has not been established. For example, it is not known whether the organic acid anions are released primarily from the root tip, which would be indicative for a role in Al\(^{3+}\) resistance.

Woody plant species vary considerably in the organic acid compounds they release in response to Al\(^{3+}\) exposure. Many species exude more than one organic acid anion, with various combinations of malate, oxalate, and succinate. In the broad-leaved deciduous and evergreen trees and shrubs assayed so far, citrate is the most common organic acid anion identified (Table 2 and references therein). Of the five coniferous tree species analyzed, three released oxalate, and the two remaining citrate and succinate, respectively (Table 2).

Little is known about other substances that may be released by roots to chelate Al\(^{3+}\). Proposed compounds include polypeptides, phenolic compounds, cyclic hydroxamates, and rhizodeposition in the form of mucilage (Jones and Ryan, 2003; Poschenrieder et al., 2008). In the tea plant, Camellia sinensis, Morita et al. (2011) observed beside of oxalate an increase of the release of caffeine, a phenolic compound, in response to Al\(^{3+}\) exposure. Phenolic compounds were also exuded in Al\(^{3+}\)-treated Eucalyptus camaldulensis and two Malaleuca species (Nguyen et al., 2003).

### Table 1 | Organic acid release from roots of Cryptomeria japonica (\(\mu\text{mol g}^{-1} \text{day}^{-1} \mu\text{mol g}^{-1} \text{day}^{-1}\)) and Pinus thunbergii (\(\mu\text{mol g}^{-1} \text{day}^{-1}\)) after exposition to Al\(^{3+}\) (according to Qin et al., 2007; Hirano et al., 2010).

| Tree species           | Al\(^{3+}\) concentration (\(\mu\text{M}\)) | Citrate | Malate | Oxalate |
|------------------------|------------------------------------------|---------|--------|---------|
| Cryptomeria japonica   | 0                                        | 0.10a   | 0.14a  | 0.14a   |
|                        | 100                                      | 0.39    | 0.14   | 0.49    |
|                        | 500                                      | 0.41    | 0.14   | 0.75    |
|                        | 1000                                     | 0.38    | 0.13   | 0.70    |
|                        | \(P\) value                             | ns      | ns     | ns      |
| Pinus thunbergii       | 0                                        | 0.03a   | 0.03a  | 0.47    |
|                        | 100                                      | 0.03a   | 0.03a  | 1.08    |
|                        | 500                                      | 0.10    | 0.03a  | 2.93    |
|                        | 1000                                     | 0.11    | 0.03a  | 4.04    |
|                        | \(P\) value                             | *       | ns     | **      |
| Populus tremula        | 0                                        | 0.0a    | 0.0a   | 0.1     |
|                        | 50                                       | 0.0a    | 0.0a   | 4.5     |
|                        | 100                                      | 1.9     | 0.0a   | 3.9     |
|                        | 200                                      | 18.4    | 0.0a   | 5.6     |
|                        | 500                                      | 20.5    | 0.0a   | 25.7    |
|                        | 1000                                     | 20.3    | 0.0a   | 18.8    |
|                        | \(P\) value                             | ***     | ***    | ***     |

\(a\) Below detection limit. *ANOVA: ***\(P < 0.001\), **\(P < 0.01\), *\(P < 0.05\); ns, not significant.

**RAISING THE pH IN THE RHIZOSPHERE**

According to Kochian et al. (2005), only one study to date has unequivocally demonstrated that raising the pH in the rhizosphere can protect plants from Al\(^{3+}\). For two distinct classes of Al\(^{3+}\)-tolerant Arabidopsis mutants, it was shown that Al\(^{3+}\) resistance is mediated by the exclusion of Al\(^{3+}\) from the root either by exudation of malate and citrate (Larsen et al., 1998) or by H\(^+\) influx at the root apex (Degenhardt et al., 1998). The H\(^+\) influx resulted in an increase in the rhizosphere pH, and subsequently in a significant decrease in the Al\(^{3+}\) activity around the root tip. However, there is currently no evidence to support that this mechanism operates in Arabidopsis ecotypes. Whether the roots of woody plants make use of such a mechanism remains elusive.

**MODIFICATION OF Al\(^{3+}\) BINDING SITES IN THE CELL WALL OF ROOT CELLS**

The cell wall of root cells has been suggested to be a site of both Al\(^{3+}\) toxicity and Al\(^{3+}\) exclusion (Horst et al., 2010). It has been determined that up to 90% of the Al\(^{3+}\) absorbed by roots can be localized to the apoplast (Kochian, 1995). The primary site of Al\(^{3+}\) binding is probably the pectin matrix, which is largely composed of homopolymers of galacturononic acid (Mohnen, 2008; Horst et al., 2010). Al\(^{3+}\) is known to bind far more strongly to pectin than Ca\(^{2+}\), whose binding to the cell wall is required for proper cell wall functioning (Franco et al., 2004). It has been proposed that Al\(^{3+}\) binds to the cell wall through a replacement of Ca\(^{2+}\), making
Table 2 | Al\(^{3+}\)-activated release of organic acids from roots of woody plants.

| Plant species          | Al\(^{3+}\)-activated organic acids | Reference |
|------------------------|-------------------------------------|-----------|
| **Non-mycorrhizal roots; coniferous trees** |
| Cryptomeria japonica   | Citrate, oxalate                     | Hirano et al. (2012) |
| Pinus abies            | Oxalate                             | Heim et al. (2000) |
| Pinus sylvestris       | Oxalate                             | Ahonen-Jonnath et al. (2000) |
| Pinus thunbergii       | Citrate, oxalate                     | Hirano et al. (2012) |
| **Non-mycorrhizal roots; broad-leaved trees (deciduous)** |
| Populus tremula        | Citrate, oxalate                     | Qin et al. (2007) |
| Populus tremuloides    | Citrate, malate, oxalate, succinate | Naik et al. (2009) |
| Populus trichocarpa    | Citrate, malate, oxalate, succinate | Naik et al. (2009) |
| **Non-mycorrhizal roots; broad-leaved trees and shrubs (evergreen)** |
| Acacia auriculiformis  | Citrate, oxalate                     | Nguyen et al. (2003) |
| Camellia ancinensis    | Oxalate                             | Morita et al. (2011) |
| Cinnamomum camphora    | Citrate                             | Ishikawa et al. (2000) |
| Citrus grandis         | Citrate, malate                      | Osawa et al. (2011) |
| Citrus junos           | Citrate                             | Yang et al. (2011) |
| Citrus sinensis        | Citrate, malate                      | Yang et al. (2011) |
| Eucalyptus camaldens/cusis (Eucalyptus camaldens/cusis) | Citrate, oxalate                     | Tahara et al. (2008) |
| Eucalyptus camaldens/cusis (Eucalyptus camaldens/cusis) | Citrate, oxalate                     | Tahara et al. (2008) |
| Eucalyptus cloeziana   | Citrate                             | Silva et al. (2004) |
| Eucalyptus dunnii      | Citrate, malate, oxalate            | Silva et al. (2004) |
| Eucalyptus globulus    | Citrate, malate                      | Silva et al. (2004) |
| Eucalyptus grandis     | Citrate                             | Silva et al. (2004) |
| Eucalyptus saligna     | Citrate                             | Silva et al. (2004) |
| Eucalyptus uniphyla    | Citrate, malate, oxalate            | Silva et al. (2004) |
| Melaleuca bracteata    | Citrate                             | Tahara et al. (2008) |
| Melaleuca cajuputi     | Citrate, malate                      | Tahara et al. (2008) |
| Melaleuca cajuputi     | Citrate, oxalate                     | Nguyen et al. (2003) |
| Melaleuca leucadendria | Citrate                             | Nguyen et al. (2003) |
| **Mycorrhizal roots** |
| Picea abies            | Succinate                           | Heim et al. (2000) |
| Picea abies            | Oxalate                             | Eldhuset et al. (2007) |
| Pinus densiflora      | Citrate                             | Tahara et al. (2005) |
| Pinus sylvestris       | Oxalate                             | Ahonen-Jonnath et al. (2000) |

The references are divided into studies dealing either with non-mycorrhizal roots or with mycorrhizal roots.

Several studies have suggested that the pectin content and the degree of pectin methylation are important determinants of the amount of Al\(^{3+}\) that can bind to the cell wall of root cells. In maize (Zea mays), rice (Oryza sativa), and common bean (Phaseolus vulgaris), differences in the pectin content and/or the degree of pectin methylation were linked with Al\(^{3+}\) sensitivity/resistance (Etschka et al., 2005; Yang et al., 2008; Rangel et al., 2009). Al\(^{3+}\)-resistant lines of all three plant species were found to have a higher degree of pectin methylation, and a lower cell wall Al content when compared to Al\(^{3+}\)-sensitive lines, supporting a role for pectin methylation in Al\(^{3+}\) exclusion. A modulating role for the degree of pectin methylation is further supported by the finding that the expression of pectin methylesterase (PME), the enzyme responsible for the demethylation of pectin, was lower in Al\(^{3+}\)-resistant lines than in Al\(^{3+}\)-sensitive lines (Maron et al., 2008; Yang et al., 2008).

Current evidence, based on X-ray microanalyses, indicates that the apoplast is a major site of Al accumulation also in woody plants. In Al\(^{3+}\)-treated seedlings of the conifer Picea abies, Al was found in both epidermal and cortical cells of the root tip (Heim et al., 1999). In both cell types, more than 88% of the total Al localized to the cell wall. In addition, it was observed that the amount of Ca in the cell wall of both cell types was much lower in Al\(^{3+}\)-treated seedlings than in control plants, suggesting that Al\(^{3+}\) replaced Ca\(^{2+}\) at the exchange sites of the cell wall. These findings are further substantiated by results of a study conducted in Picea abies and Populus tremula, cultivated in a model ecosystem for 3 years (Brunner et al., 2008). In Picea abies, Al accumulated continuously over time in the cell wall of root epidermal cells, whereas in Populus tremula, Al accumulated in the cell wall of both root epidermal and cortical cells. In both species, Al did not accumulate intracellularly (Table 3).

In root cells of woody plants, little evidence exists about the relationship between cell wall polysaccharides and Al\(^{3+}\) sensitivity/resistance. In a set of poplar clones, representing several interspecific crosses, it was found that the Al content of the root symplast was higher in Al\(^{3+}\)-resistant clones than in Al\(^{3+}\)-sensitive clones (Smith et al., 2011). The Al content of the root symplast, on the other hand, was lower in Al\(^{3+}\)-resistant clones than in Al\(^{3+}\)-sensitive clones. This pattern of cellular Al distribution suggests that the cell wall of root cells prevented Al\(^{3+}\) from entering the root symplast. Additional parameters investigated were pectin and callose, the latter of which is a widely used indicator of early Al\(^{3+}\) exclusion. A modulating role for the degree of pectin methylation, and the cell wall more rigid, and thus reducing its extensibility which is required for normal cell elongation (Tabuchi and Matsumoto, 2001).
low, but even at these concentrations, \( \text{Al}^{3+} \) remains a hazard. The very high affinity of \( \text{Al}^{3+} \) for oxygen ligands allows it to compete with other ions for metabolically important sites despite a large disparity in their concentrations (Ions and Ryan, 2003).

Indeed, studies of several woody plant species demonstrate that intracellular \( \text{Al}^{3+} \) is chelated by organic acid anions. In the small shrub Melastoma malabathricum, upon entering the root, \( \text{Al}^{3+} \) binds to citrate, and the \( \text{Al} \)-citrate complex itself is transported from the root to the shoot (Watanabe and Osaki, 2001). In the leaves, the \( \text{Al} \)-citrate complex is transformed into \( \text{Al} \)-oxalate 1:1, 1:2, and 1:3 complexes. The former two complexes are potentially toxic to the plant. A similar transformation of \( \text{Al} \)-organic acid complexes is described for the tea plant. Upon entering the root cell, \( \text{Al}^{3+} \) binds to oxalate and then is transported from the root to the shoot in the form of \( \text{Al} \)-citrate and \( \text{Al} \)-malate complexes (Morita et al., 2004, 2008). In a comparison of several Eucalyptus species, the concentration of root tip malate was found to correlate positively with the degree of \( \text{Al}^{3+} \) resistance in the presence of \( \text{Al}^{3+} \) (Silva et al., 2004). In contrast, in the poplar clones of the above-mentioned study, the concentrations of symplastic citrate and formate correlated closely with \( \text{Al}^{3+} \) sensitivity (Smith et al., 2011).

Besides organic acids, there are other complex forming compounds, e.g., phenolic substances, that bind \( \text{Al}^{3+} \) in the cytosol. For example, in the tea plant, \( \text{Al} \)-catechin complexes were described (Nagata et al., 1992). In the sepal of the small shrub Hydrangea macrophylla, \( \text{Al}^{3+} \) is bound to both \( \text{J} \)-catecholquinic acid and delphinidin 3-glucoside, where \( \text{Al}^{3+} \) is thought to play a role in stabilizing the two organic compounds, and thus caus-

For example, in the tea plant, \( \text{Al} \)-catechin complexes were described (Nagata et al., 1992). In the sepal of the small shrub Hydrangea macrophylla, \( \text{Al}^{3+} \) is bound to both \( \text{J} \)-catecholquinic acid and delphinidin 3-glucoside, where \( \text{Al}^{3+} \) is thought to play a role in stabilizing the two organic compounds, and thus caus-

Ofei-Manu et al. (2001) in a series of woody plants upon \( \text{Al}^{3+} \). An increase in root phenolics has been observed by Osawa et al., 2002; Jolzi et al., 2011; Gonzalez-Santana et al., 2012). Interestingly, the chloroplasts of Qualea grandiflora and Callicethone major, two \( \text{Al} \)-hyperaccumulating woody plant species from the Vochysiaceae family, which grow in the Brazilian Cerrado, have been suggested as a primary compartment for \( \text{Al} \) sequestration (de Andrade et al., 2011). \( \text{Al}^{3+} \) can also be sequestered into cells specialized for storage functions, e.g., idioblasts containing \( \text{Ca} \)-oxalate crystals have been considered as a location for \( \text{Al}^{3+} \) detoxification in the leaves of Conchora olitoria (Maren, 2004).

### Table 3: Al accumulation in fine roots of Picca abies and Populus tremula [Al] concentrations of bulk material; Al net counts of compartments using energy-dispersive X-ray spectroscopy (EDX)-analyses after growth in weakly acidic soil (pH 6.5) with different length of exposition time (according to Brunner et al., 2009).

| Tree species | Time (year) | \( \text{Al} \) concentration (mg g\(^{-1}\)) | \( \text{Al} \) counts in epidermal cells | \( \text{Al} \) counts in cortical cells |
|-------------|------------|---------------------------------|-----------------------------------|---------------------------------|
| Picca abies | 0.5        | 2.12                            | 213                               | 83                              | 126                             | 94 |
|             | 1.5        | 8.23                            | 294                               | 73                              | 122                             | 72 |
|             | 2.5        | 9.50                            | 355                               | 101                             | 140                             | 80 |
| Populus tremula | 0.5 | 1.81                            | 168                               | 67                              | 96                              | 65 |
|             | 1.5        | 16.40                           | 359                               | 50                              | 152                             | 51 |
|             | 2.5        | 5.36                            | 338                               | 57                              | 163                             | 63 |

\( P \) value (*): *P* = 0.05, **P** = 0.01, ***P*** = 0.001; ns, not significant.

(No statistical analysis possible because of missing replicates.)
Transport of Al from the root to the shoot is likely to involve complexes of Al with organic acids (see above). Little is known about the transport of Al across the plasma membrane and further sequestration into subcellular compartments. Transport across membranes requires transport proteins. Candidates for such proteins have been identified in the Al3+-sensitive mutants als1 and als3 of Arabidopsis. Both mutants are mutants in genes encoding proteins that belong to the ATP-binding cassette (ABC) transporter superfamily. ALS1 (Al3+-sensitive) is a half type ABC transporter, whereas ALS3 is an ABC transporter-like protein, lacking the ABC domain. The functions and substrates of ALS1 and ALS3 are not known, but the mutant phenotypes, the subcellular localization of the proteins, and tissue-specific gene expression have led to the assumption that the two proteins sequester and transport Al3+ to overcome Al3+ toxicity. ALS1 is likely to be involved in the intracellular transport of Al3+ to vacuoles of root tip cells and cells of the plant vasculature (Larsen et al., 2005). ALS3 is mainly localized to the plasma membrane of root cortex cells and phloem cells throughout the plant, suggesting that it mediates the intercellular redistribution of accumulated Al away from sensitive tissues (Larsen et al., 2005). In Populus tremula, Grisel et al. (2010) identified an ALS3-like gene with a 79% amino acid sequence identity with the Arabidopsis ALS3 gene. The poplar gene was found to be expressed in the root, stem, and leaves, and was strongly induced by Al3+ in the root (44-fold, Figure 3A). In addition, the poplar gene was inducible by Al3+, but not by La3+ (lanthanum; Figure 3B), consistent with the finding that the Arabidopsis mutant als3 is not affected by La3+ (Larsen et al., 1997).

**ACTIVATION OF METABOLIC PATHWAYS TO OVERCOME THE TOXIC EFFECTS OF Al3+**

Moderate Al3+ concentrations are not fatal, and roots may at least partially recover (see also Matsumoto and Motoda, 2012). This is well documented in Populus tremula treated with either no Al3+ or increasing concentrations of Al3+ up to 1,000 μM. Two phases of root growth could be distinguished: a rapid Al3+-induced growth inhibition (within 6 h at Al3+ concentrations > 250 μM) and a subsequent phase of growth recovery (within 2 days at Al3+ concentrations < 500 μM; Grisel et al., 2010). The root growth of plants treated with 1,000 μM Al3+ further decreased. This pattern of root growth recovery may reflect the success of the roots in activating metabolic pathways to overcome the toxic effects of Al3+ and/or Al3+ resistance mechanisms. Matsumoto and Motoda (2013) suggested that the recovery of roots exposed to Al3+ is associated with the reduction of Al3+-induced oxidative stress. In Populus tremula, two genes of the oxidative stress pathway (a peroxidase gene and an alternative oxidase gene) were strongly induced upon Al3+ exposure after 6 h (>8-fold), while their expression decreased to control levels after 2 days (Grisel et al., 2010).

Two additional genes of Populus tremula that may play a role in root growth recovery encode CorA-like Mg2+ transporters (Grisel et al., 2010). These genes were induced up to fivefold by Al3+. The activity of a homologous CorA-like Mg2+ transporter from Arabidopsis was shown to be blocked by micromolar concentrations of Al3+, when expressed in bacteria (Li et al., 2001). In addition, the same CorA-like Mg2+ transporter alleviated Al3+ toxicity when overexpressed in plants (Ding et al., 2006). Mg2+ has been reported to be able to alleviate Al3+ toxicity in a number of crop plants (see reviews of Rose et al., 2011; Chen and Ma, 2013). Various mechanisms have been put forward to explain how Mg2+ can alleviate Al3+ toxicity. These mechanisms include increased ionic strength of the solutions, reduction in Al3+ saturation at the apoplastic exchange sites, and decreased Al3+ activity at the root cell plasma membrane surface (Rose et al., 2011). However, the identified Mg2+ transporters indicate that, besides of electrostatic interactions, biochemical processes may be involved in the rescue of Al3+ toxicity.

**ADAPTATIONS**

The tropical forests and the forests of boreal and temperate regions have evolved on geological timescales under very different conditions. The forests of boreal and temperate regions were repeatedly affected by the Pleistocene glaciations, while the impact of the Pleistocene climatic fluctuations was certainly much less severe in tropical regions. Most tropical forests have not been disturbed for hundreds of thousands of years, and thus typically grow on highly acidic soils and elevated ionic strength of the solutions. Reduction in Al3+ saturation at the apoplastic exchange sites, and decreased Al3+ activity at the root cell plasma membrane surface (Rose et al., 2011). However, the identified Mg2+ transporters indicate that, besides of electrostatic interactions, biochemical processes may be involved in the rescue of Al3+ toxicity.
weathered soils, which are strongly acidic, both in the topsoil and the subsoil (Figures 1A,B).

Accumulating evidence shows that in tropical regions both Al excluders and Al accumulators occur. Examples of woody species that are strong excluders are *Melaleuca quinquenervia*, *Acacia mangium*, and *Leucaena leucocephala* (Osaki et al., 1997), which are all capable of exuding organic acid anions from their roots (see also Table 2). Examples of woody species that store high amounts of Al in leaves are *Melastoma malabathricum* and *H. macrophylla*. Other woody species, such as *Vaccinium macrocarpon*, store high amounts of Al in their roots (Osaki et al., 1997). Most Al-hyperaccumulator plants are shrub-type broad-leaved woody plants.

Phylogenetic analyses indicate that Al hyperaccumulation is a trait that has arisen a number of times, and this trait is scattered over more than 20 orders across about 45 families belonging to magnoliids (e.g., Laurales), eudicots (e.g., Proteales), rosids (e.g., Malpighiales, Myrtales), and asterids (e.g., Gentianales, Ericales; Table 4; Jansen et al., 2002). To date, Al accumulation in tall trees has only been found in a few tree species of the Euphorbiaceae (Ohawa et al., 2013). The predominance of Al accumulators within non-flowering plants suggests that Al accumulation evolved early in the evolution of land-plants and is probably a primitive characteristic associated with survival in ancient Al-rich environments (Jansen et al., 2002).

An analysis of the variation in foliar Al and macronutrient concentrations in a global dataset of plant species in a phylogenetic framework showed that the frequency distribution of foliar Al concentration in tropical regions clearly had two peaks (“bimodal”); whereas in temperate regions it showed only one peak (“unimodal”; Metali et al., 2012). This conclusion supports the hypothesis that Al accumulators and non-Al accumulators exist as distinct, but overlapping, groups of species. The estimated threshold value of foliar Al concentrations that distinguish Al accumulators from non-Al accumulators varies geographically. The foliar threshold of tropical plants is higher (2.3–3.9 mg g\(^{-1}\)) than that of temperate plants (1.1 mg g\(^{-1}\); Metali et al., 2012). Among angiosperm species, there was a significant phylogenetic signal in foliar Al concentrations, substantiating results of previous studies, suggesting a greater prevalence of Al accumulators in some families than in others (e.g., Jansen et al., 2002, 2003). A phylogenetical signal may also arise when related species occupy relatively similar habitats that differentially influence nutrient uptake and accumulation (Thompson et al., 1997).

For example, Schreg et al. (2010) have provided evidence for significant associations between high soil Al and Mn concentrations and the distributions of trees in the Vochysiaceae and Myrtaceae on a 50-ha plot in a semideciduous moist forest in Panama.

Compared to soils of tropical regions, soils of boreal and temperate regions are generally younger, although large areas, such as Siberia and Beringia, were ice-free during the Last Glacial Maximum (26,500–18,000 year before present; Clark et al., 2009).

However, soils of these ice-free areas were severely affected by cold climate and permafrost, strongly limiting soil chemical and soil biological processes. As a consequence, soils in boreal and temperate regions are generally less weathered and thus less acidic than tropical soils, particularly in the subsoil (Figure 1B). In contrast, topsoils of the boreal regions are highly acid due to the strong acidifying effect of the coniferous litter during the incomplete decomposition process and the formation of humic acids (Schachtschabel et al., 1992).

Aluminum concentrations measured in roots from temperate or boreal woody species suggest that the immobilization of Al\(^{3+}\) in the cell wall is most likely an important adaptation. Al concentrations in the fine roots of common temperate and boreal woody species usually exceed the limit for being hyperaccumulators (>1 mg g\(^{-1}\)). For example fine roots of *Abies alba*, *Castanea sativa*, *Fagus sylvatica*, *Pinus abies*, *Pinus cembra*, and *Pinus montana* all have values of 1–10 mg g\(^{-1}\) (Ziyyat et al., 1996; Brunner et al., 2002; Genenger et al., 2003; Hirano et al., 2006; Richter et al., 2011; see also Table 3). All these woody species are ectomycorrhizal, suggesting that ectomycorrhizal structures, such as fungal mantle and Hartig net, further contribute to the accumulation of Al in roots by immobilizing Al in the cell wall of the fungal hyphae (Brunner and Frey, 2000; Heim et al., 2003) or in the fungal vacuoles (Martin et al., 1994). Ectomycorrhizas in temperate and boreal regions have, we assume, the function not only to take up water and nutrients but also to immobilize toxic Al\(^{3+}\). Because ectomycorrhizal trees and ericoid-mycorrhizal Ericales dominate in temperate and boreal regions, we propose that this mechanism to immobilize Al\(^{3+}\) is a major mechanism for excluding Al\(^{3+}\) from the roots of woody plants in these regions, a suggestion first proposed by Jansen et al. (2002). Moreover, mycorrhizal fungi may well contribute significantly to the cycling of Al in forest ecosystem because high concentrations of Al can be found in their fruiting bodies (Smit and Hoffland, 2009).

An additional adaptation of tree roots from temperate or boreal forests to acid soils is the observation that the roots have a shorter lifespan, which means that the turnover rate of roots exposed to elevated Al\(^{3+}\) concentrations is higher (Godbold et al., 2003; Leuschner et al., 2004; Richter et al., 2013). Reasons for a shorter lifespan could be that Al accumulation has reached its saturation point faster compared to roots from non-acid soils, causing the root to die earlier.

Some woody plants in temperate regions have not evolved strategies to deal with high levels of Al\(^{3+}\) and thus are not adapted to acid soils. Examples are the two species *Fraxinus excelsior* and *Panama.*
**Aluminum in woody plants**

Acer pseudoplatanus, which both are not ectomycorrhizal. They grow mainly on forest sites with high pH and high base saturation, and are unlikely to occur on very acid soils (Vézina-Blaschke et al., 2002). In a recent study, Walther et al. (2013) showed that Fraxinus excelsior and Acer pseudoplatanus respond much more sensitively to soil properties than Fagus sylvatica does. The soil properties limiting their distribution are Al in the case of Fraxinus excelsior, and Al together with nutrient availability (C/N ratio) in the case of Acer pseudoplatanus. On the contrary, no soil-induced distribution limits were detectable for Fagus sylvatica (Walther et al., 2013).

Differences in Al$^{3+}$ resistance not only exist among different tree species but also among local populations of the same species. Kidd and Proctor (2000) assessed the level of Al$^{3+}$ resistance in four populations of Betula pendula growing on soils that differ in soil acidity and levels of Al$^{3+}$. Using a solution culture system which simulates natural soil solutions, the authors found that seedlings originating from populations from acid mineral soils (pH 4.3, Al$^{3+}$ concentration 21.1 mg l$^{-1}$), had a significantly higher level of Al$^{3+}$ resistance (measured with the root elongation rate) compared to seedlings from populations from non-acid soils (pH > 4.8, Al$^{3+}$ < 5.3 mg l$^{-1}$). A similar study was performed by Wilkins and Hodson (1989) who analyzed two populations of Picea abies growing on an acid mineral soil and a calcareous soil, respectively. The growth of seedlings from the acid soil was slightly stimulated by the Al$^{3+}$ treatment, whereas the growth of that from the calcareous soil was greatly reduced. The results of these studies suggest that high Al$^{3+}$ soil conditions are a significant force for population divergence due to strong selective pressure associated with adaptation.

Further evidence for population adaptation to acid soil conditions comes from a study of Pinus contorta growing along a steep gradient of soil acidity at the northern coast of California (Eckert et al., 2012). In this area, five well-developed marine terraces exist, whose soils represent a chronosequence ranging from fertile mineral soil and a calcareous soil, respectively. The growth of seedlings from the acid soil was slightly stimulated by the Al$^{3+}$ treatment, whereas the growth of that from the calcareous soil was greatly reduced. The results of these studies suggest that high Al$^{3+}$ soil conditions are a significant force for population divergence due to strong selective pressure associated with adaptation.

**CONCLUSION**

Current evidence indicates that woody plants native to acid soils have evolved various strategies to overcome Al$^{3+}$ stress. These strategies include exclusion of Al$^{3+}$ from the root tip, probably through the release of organic acid anions. The formation of ectomycorrhizal structures, which are capable of accumulating Al in the cell wall of hyphal cells, may also be regarded as an exclusion strategy, reducing the degree of contact of root cells to Al$^{3+}$. Internal strategies rely on the transport and sequestration of Al in aerial parts of the plant, where the Al may be stored in the cell wall of different leaf tissues, or in subcellular compartments, like chloroplasts or possibly vacuoles. Some woody plant species may also store high levels of Al in the cell wall of root cells. The biochemical and molecular mechanisms underlying these strategies, however, remain largely to be determined. The few studies performed so far in woody plants provide hints in which direction research may focus. With the exception of poplar, forest trees are generally not amenable to genetic engineering for testing the impact of candidate genes. Similarly, progenies of controlled crosses, which are valuable for testing segregation of specific traits and genes, exist only for a few tree species. An alternative approach would involve the identification of genes that play a role in adaptation through association of genetic variation with particular soil Al$^{3+}$ conditions. This has already been initiated and has provided insights into the mechanisms of population adaptation to Al$^{3+}$-rich environments.

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