Review Article

Aluminium Toxicity Targets in Plants

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Aluminium (Al) is the third most abundant metallic element in soil but becomes available to plants only when the soil pH drops below 5.5. At those conditions, plants present several signals of Al toxicity. As reported by literature, major consequences of Al exposure are the decrease of plant production and the inhibition of root growth. The root growth inhibition may be directly/indirectly responsible for the loss of plant production. In this paper the most remarkable symptoms of Al toxicity in plants and the latest findings in this area are addressed. Root growth inhibition, ROS production, alterations on root cell wall and plasma membrane, nutrient unbalances, callose accumulation, and disturbance of cytoplasmic Ca^{2+} homeostasis, among other signals of Al toxicity are discussed, and, when possible, the behavior of Al-tolerant versus Al-sensitive genotypes under Al is compared.

1. Introduction

Aluminium (Al) ranks third in abundance among the Earth's crust elements, after oxygen and silicon, and is the most abundant metallic element. A large amount of Al is incorporated into aluminosilicate soil minerals, and very small quantities appear in the soluble form, capable of influencing biological systems [1].

Al bioavailability, and in consequence, toxicity, is mainly restricted to acid environments. Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. The production of staple food crops, in particular grain crops, is negatively influenced by acid soils [2]. Some agricultural practices, as removal of products from the farm, leaching of nitrogen below the plant root zone, inappropriate use of nitrogenous fertilizers, and build-up in organic matter, are causing further acidification of agricultural soils.

When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxy cations and $Al(H2O)_6^{3+}$ (Al^{3+}), that then exchange with other cations. On that conditions, Al^{3+} also forms the mononuclear species $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, and $Al(OH)_4$ [3]. The mononuclear Al^{3+} species and Al_{13} are considered as the most toxic forms [4, 5].

Although some crops (e.g., pineapple, tea) are considered tolerant to high levels of exchangeable Al, for most crops it is a serious constraint. Species and genotypes within species greatly differ in their tolerance to Al. For most crops, fertilization and attempts of soil correction (e.g., liming) may not be enough per se to reduce Al toxicity (e.g., as the soil reaction remains strongly acid), and in most target countries these strategies may also be jeopardized by economical constrains [6]. Therefore, it is imperative to fully understand the mechanisms that are used by the Al-tolerant species to cope Al toxicity, as well which genotypes, within the most resistant/tolerant cereal species, are more suitable to grow in acidic soils in order to increase world cereal production. Furthermore, the development of new cultivars (or the reinvestment in ancient genotypes from Al rich regions) with increased Al-tolerance is fundamental and economic solution to increase world food production.

2. Aluminium Toxicity

2.1. Root Growth. A major consequence of Al toxicity is the inhibition of root growth, and this outcome has been reported during the last century (e.g., [7]) for innumerous species [8–15]. Consequently, root growth inhibition has been widely used to assess Al toxicity.

Root growth is the combination of cell division and elongation. Only during the last decade, researchers started to look at the cell cycle (de)regulation induced by Al, with some works focusing unbalances on mitosis phase and very few on other interphase phases (e.g., [15]). Decrease of mitotic activity was reported as a consequence of Al exposure in root tips of several species as wheat [16, 17], maize [18, 19], barley [20], and bean. [19]. Some authors defended that inhibition of cell elongation was the primary mechanism leading to root growth inhibition [21, 22]. The reason for that is that root growth inhibition could occur within a short time period—30 min in Al-sensitive maize [23]—and that cell division is a slow process (cell cycle takes usually several hours to be completed). However, Doncheva et al. [18] reported inhibition of cell division (decrease of S-phase cells) in the proximal meristem after 5 min Al exposure and inhibition of root cell division in the apical meristem within 10 or 30 minutes. Furthermore, Al can accumulate in the nuclei of cells in the meristematic region of the root tip within 30 minutes [15]. Therefore, whereas inhibition of cell elongation or cell division is the primary mechanism leading to root growth inhibition is still unclear. More recently, Yi et al. [24] reported that Al exposure led to abnormal progress through mitosis and induced micronuclei formation in *Vicia faba* roots, which is in agreement with Al-induced chromosome aberrations found in wheat roots [25] and Al-induced chromosome stickiness and breaks in *Oryza sativa* [26]. From the literature review, it is evident that Al leads to cell cycle unbalances, but many questions still remain to clarify. For example it still remains unclear how and where Al exerts its influence throughout the cell cycle, if these changes are species and region dependent (most studies are performed in root apices), how the putative changes are exerted through time, and/or if they may be reversible after Al removal.

The root growth inhibition and increase in root diameter observed in roots exposed to Al [27] suggested that plant cytoskeleton could be a cellular target of Al phytotoxicity [28]. Blancaflor et al. [28] and Horst et al. [29] studied Al-induced effects on microtubules and actin microfilaments and showed that microtubules and microfilaments are altered, in their stability, organization, and polymerization, when exposed to Al. Also, in *Triticum turgidum* Al treatment led to disorganization of actin filaments and formation of actin deposits [30]. Zhang et al. [31] showed that Al inhibited actin and profilin genes. Profilin, as an actin-binding protein, provides cells with the ability to remodel the cytoskeleton [32]. In *Arabidopsis thaliana* a decrease in profilin expression resulted in an elongation defect [33]. Furthermore, Sivaguru et al. [34] and Čiamporová [21] showed that organization of cytoskeleton is most sensitive in the distal transition zone of the root apex, providing evidence that this zone represents a potential target with respect to Al toxicity.

The most sensitive root zone to Al toxicity is under great attention. Earlier, it was hypothesized that root cap played a major role in the mechanism of Al toxicity/protection [35]. However, Ryan et al. [9] demonstrated that the removal of the root cap had no effect on the Al-induced inhibition of root growth in maize. Furthermore, the same authors

also suggested that the meristem is the primary site of Al toxicity. Later, Sivaguru and Horst [36], applying Al to 1 mm root segments, reported that Al accumulation in the distal transition zone (DTZ: 1-2 mm) led to a rapid inhibition of the root elongation and suggested that this root zone is the primary target of Al in an Al-sensitive maize cultivar.

2.2. Oxidative Stress. Al-induced oxidative stress and changes in cell wall properties have been suggested as the two major factors leading to Al toxicity [22, 37]. Oxidative stress occurs when any condition disrupts the cellular redox homeostasis. The reactive oxygen species (ROS) have the capacity to oxidize cellular components such as lipids, proteins, enzymes, and nucleic acids, leading to cell death. Metals are known to act as catalysts in ROS production and to induce oxidative damage in plants. Al itself is not a transition metal and cannot catalyze redox reactions; however, Al exposure leads to oxidative stress [37–43]. Because aluminium ions form electrostatic bonds preferentially with oxygen donor ligands (e.g., carboxylate or phosphate groups), cell wall pectin and the outer surface of the plasma membrane seem to be major targets of aluminium [37]. Al binding to biomembranes leads to rigidification [44], which seems to facilitate the radical chain reactions by iron (Fe) ions and enhance the peroxidation of lipids [38].

Al induction lipid peroxidation has been reported for some species, including barley [45], sorghum [46], triticale [42], rice [40], greengram [47], and wheat [48]. Yamamoto et al. [37] found that, for *Pisum sativum* seedlings treated with Al in a simple Ca solution, Al accumulation, lipid peroxidation, and callose production had a similar distribution on the root apex surface and were accompanied by root growth inhibition. However, the loss of membrane integrity was only detected at the periphery of the cracks on the surface of the root apex. Furthermore, Yamamoto et al. [38] concluded that the Al enhancement of lipid peroxidation is an early symptom of Al accumulation and appears to cause partly callose production, but not root growth inhibition. Later, however, in maize, Al treatment did not induce lipid peroxidation, indicating that lipids are not the primary cellular target of oxidative stress in maize [39]. So, it seems that cellular target of oxidative stress depends on plant species.

Plant cells are equipped with a defensive system composed by enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (G-POX), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST), and gluthatione reductase (GR) and nonenzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), α-tocopherol, and carotenoids that help to detoxify the ROS. Some works reported ROS production and alterations in the antioxidant system as a consequence of Al exposure. In pea seedlings, ROS production is detected in root apex after two hours of Al exposure and increased with time exposure [38]. In maize roots, Al treatment also led to increase in ROS production rate in all epidermal cells, only within 10 min of Al exposure and continued to increase during Al exposure [41]. APX and

SOD activity increased in roots of both Al-resistant and Al-sensitive triticale cultivars (with higher magnitude in the sensitive one), but changes were detected first in the sensitive cultivar (6 h) and then in the resistant (12 h) [42]. Boscolo et al. [39] reported for maize root tips an increase of SOD and APX activities. Furthermore, these authors found that SOD and APX activity is inversely proportional to root growth rate and, therefore, suggested that the increase of O_2^- and H_2O_2 production is related to Al toxicity. An increase in SOD, APX, and GR activities was reported for greengram seedlings, whereas a decrease in CAT activity and glutathione and ascorbate contents was also found at higher Al concentrations [47]. These authors justified the decrease in CAT activity due to the fact that this enzyme is photosensitive and, therefore, needs constant synthesis and suggested that glutathione and ascorbate may be able to detoxify the ROS directly [47]. Devi et al. [49] found an increase in manganese superoxide dismutase (MnSOD) activity in both sensitive and tolerant cell lines of tobacco and in AsA and GSH contents, mostly in the tolerant line. These data indicated that AsA and GSH seem to be in part responsible for the tolerance mechanisms of the tolerant line to Al. Activities of SOD, CAT, and APX also increased in roots of plants and in cultured tea cells exposed to Al [50]. owever, However, plants of this species provide a complex scenario compared with other models, as aluminium may show a stimulatory effect on plant growth. That increase seemed to result in increased membrane integrity, since lipid peroxidation reduced with Al exposure [50].

These findings reporting increase of antioxidants (enzymatic and nonenzymatic) are accompanied with others that prove gene regulation associated with oxidative stress. For example, Ezaki et al. [51] expressed nine genes derived from *Arabidopsis*, tobacco, wheat, and yeast in *Arabidopsis* ecotype Landsberg. An *Arabidopsis* blue-copper-binding protein gene (*AtBCB*), a tobacco glutathione-S-transferase gene (*parB*), a tobacco peroxidase gene (*NtPox*), and a tobacco GDP-dissociation inhibitor gene (*NtGDI1*) conferred a degree of resistance to Al: significative differences in relative root growth and decrease in Al content and oxidative damages. They also showed that overexpression of three Al-induced genes in plants conferred oxidative stress resistance. Furthermore, overexpression of the *parB* gene simultaneously conferred resistance to both Al and oxidative stresses. Therefore, Ezaki and coworkers concluded that some of the genes induced during Al exposure and oxidative stresses play protective roles against both stresses. Cançado et al. [52] identified a maize Al-inducible cDNA encoding a glutathione-S-transferase (GST). Expression of that gene (GST27.2) was upregulated in response to various Al concentrations in both Al-tolerant and Al-sensitive maize lines. Recently, using Al-sensitive *Medicago truncatula* cultivar Jemalong genotype A17, 324 genes were upregulated and 267 genes were downregulated after Al exposure [53]. Upregulated genes were enriched in transcripts involved in cell-wall modification and abiotic and biotic stress responses, while downregulated genes were enriched in transcripts involved in primary metabolism, secondary metabolism, protein synthesis and processing, and the cell cycle. Known markers of Al-induced

gene expression including genes associated with oxidative stress and cell wall stiffening were differentially regulated in that study [53]. For maize plants, Al exposure led to alteration in gene expression, mostly in the Al-sensitive genotype. Although Al-sensitive genotype showed changes in the expression of more genes, several Al-regulated genes exhibited higher expression in the tolerant genotype [54]. So, it is clear that expression of some genes confers Al resistance and contributes to reduce oxidative stress.

2.3. Cell Wall, Plasma Membrane, and Nutrient Unbalances. Al accumulation is primarily and predominantly in the root apoplast (30–90% of the total absorbed Al) (e.g., [42, 55]) of peripheral cells and is only very slowly translocated to more central tissues [19, 56, 57]. The primary binding of Al^{3+} in the apoplast is probably the pectin matrix, with its negatively charged carboxylic groups [57, 58].

Several works reported increases of pectin levels in Al-sensitive genotypes [29, 43, 57–60], and some also detected increase in Al contents in the same sensitive genotypes [29, 57, 60]. These findings indicated that pectin plays a major role in the binding of Al and suggested that some of the additional Al accumulation in sensitive genotypes bound in the newly formed cell wall pectin [43, 57, 58]. Binding of Al to the pectin matrix and other cell wall constituents could alter cell wall characteristics and functions such as extensibility [61], porosity, and enzyme activities thus leading to inhibition of root growth [57]. Another mechanism for Al toxicity targeted to the apoplast invokes a rapid and irreversible displacement of Ca^{2+} from cell wall components by Al ions [22, 61]. Accumulation of Al occurs predominantly in the root apoplast. Nevertheless, Al accumulates also in the symplast and with a fast rate [19]. Recently, Xia et al. [62] reported a transporter, *Nrat1* (Nramp aluminium transporter 1), specific for Al^{3+} localized at the plasma membrane of all rice root tips cells, except epidermal cells. Those authors referred that the elimination of the *Nrat1* enhanced Al sensitivity, decreased Al uptake, increased Al binding to cell wall and concluded that this transporter is required for prior step of final Al detoxification through sequestration of Al into vacuoles. Furthermore, given its physicochemical properties, Al can interact strongly with the negatively charged plasma membrane. For instance, Al can displace other cations (e.g., Ca^{2+}) that may form bridges between the phospholipid head groups of the membrane bilayer [63]. Furthermore, Al interaction with plasma membrane could lead to depolarization of the transmembranar potential (e.g., [64]) and/or reduction of H^+-ATPase (e.g., [65]) which, in turn, can alter the activities of ions near the plasma membrane surface and impede the formation and maintenance of the transmembrane H^+ gradient [2]. Moreover, Al changes in plasma membrane can modify the uptake of several cations (e.g., Ca^{2+}, Mg^{2+}, K^+, NH_4^+) [8, 66–68]. These changes are related to direct Al^{3+} interactions with plasma membrane ion channels [69] and changes in membrane potential.

Nutritional unbalances induced by Al exposure were reported for several plant species. Eleven families of pteridophytes presented different nutritional unbalances (mostly

in Ca, Mg, P, K) depending on Al accumulation [70], and in maize, Al had negative effects on the uptake of macro- and micronutrients, with Ca and Mg being the macro- and Mn and Zn the micronutrients more affected [68]. Also, the maize Al-tolerant genotypes accumulated higher concentration of Ca, Mg [68], and K [71] than the sensitive genotypes. In wheat, both sensitive and tolerant genotypes presented a decrease in K and Mg contents in roots, whereas Ca, Al, Si contents increased [72]. However, the sensitive wheat genotype showed more nutritional unbalances and Al accumulation than the tolerant one in both roots and shoots [72]. Al exposure led to an increase of Ca accumulation in rye-sensitive genotype, contrarily to the tolerant rye genotype [73]. However, other studies reported different results in Al-induced nutritional imbalances in maize: Lidon et al. [74] referred that all elements in roots, except K, Mn, and Zn, increased in Al-treated roots and that in shoots Ca and Mg had little variation. Reference [67] reported that only the specific absorption rate of B was correlated to the Al-induced root growth inhibition. Al exposure led to decrease in K, Mg, Ca, and P contents and uptake in rice plants, and, as observed in maize, the tolerant cultivar presented less negative effects in nutrient content than the sensitive one [75]. In tomato cultivars, Al exposure decreased the content of Ca, K, Mg, Mn, Fe, and Zn in roots, stems, and leaves [76]. Zobel et al. [27] related changes in fine root diameter with changes in concentration of some nutrients, as N, P, and Al. It seems that the differential tolerance to Al may be due to their differences in uptake, ability to keep adequate concentrations and to use the nutrients efficiently. Differences in nutrient uptake, accumulation, and translocation are evident between plant species and within each species. Furthermore, since each author utilized different Al concentrations, diverse nutritive solutions and time exposures, it is difficult to make a general and accurate model of Al-induced nutritional unbalances.

2.4. Cytoplasmic Ca^{2+}.

Disturbance of cytoplasmic Ca^{2+} homeostasis is believed to be the primary target of Al toxicity [77] and may be involved in the inhibition of the cell division or root elongation by causing potential disruptions of Ca^{2+}-dependent biochemical and physiological processes [34, 77, 78].

In wheat root apices, [44] found that Al inhibits Ca^{2+}-dependent phospholipase C, which acts on the lipid substrate phosphatidylinositol-4,5-biphosphate. The authors hypothesized that phosphoinositide signaling pathway might be the initial target of Al. In accordance, Zhang et al. [31] found Al-induced inhibition of genes related to phosphoinositide signaling pathway and hypothesized that the gene inhibition could result in disruption of this pathway. Also, it was reported that components of the actin-based cytoskeleton interact directly with phospholipase C in oat [79].

Most works reported an increase in cytoplasmic Ca^{2+} when plants were exposed to Al [13, 80, 81]. However, Jones et al. [82] reported a decrease in cytoplasmic Ca^{2+} in tobacco cell cultures in the presence of Al. Furthermore, Zhang and Rengel [80] reported an increase in cytoplasmic Ca^{2+} in two lines with different tolerance to Al and correlated it with the

inhibition of root growth in both lines. Moreover, Ma et al. [13] correlated cytoplasmic Ca^{2+} to root growth response. Moreover, alteration in cytoplasmic Ca^{2+} homeostasis can occur within few minutes (20–30 minutes) in root hair tips of *Arabidopsis thaliana* [82].

It is certain that Al exposure influences cytoplasmic Ca^{2+} homeostasis, but it is still unclear if it is a primary cause of Al-induced inhibition of root growth or a secondary effect. The source of Ca^{2+} for the increase of cytosolic Ca^{2+} activity could be extracellular and/or intracellular but is still insufficiently documented, as well the effects on increased cytosolic Ca^{2+} (for review see [77]).

2.5. Callose.

The induction of callose ($1,3$-β-D-glucan) formation in Al-exposed roots has been reported in many plant species (e.g., [20, 41, 67, 83–86]). Al-induced callose formation in root tips is recognized as an excellent indicator of Al sensibility [81, 86–90], and some works negatively correlated root elongation with callose formation during Al exposure (e.g., [86, 91]). Recently, it was reported that Al induced callose accumulation not only in the root meristematic regions but also in mature zones, in both wheat and rye genotypes [72, 73]. In maize roots, Jones et al. [41] found a close spatial and temporal coordination between Al accumulation and callose production in roots. Also, in wheat, callose accumulation in root tissues was progressive with Al-exposure, and, contrarily to the tolerant genotype, the sensitive one presented callose deposition at inner cell layers [72, 73]. Still, Tahara et al. [86] reported that, in some Myrtaceae species, induction of callose formation was not accompanied by root growth inhibition and suggested that callose formation is a more sensitive indicator to Al than root elongation.

Since Al induces a transient rise of cytosolic Ca^{2+}, an increase of callose accumulation under Al stress is not unexpected. Cytosolic Ca^{2+} is one of the prerequisites for the induction of callose synthesis, but not the only factor modulating increases in callose synthesis and deposition [81]. Callose formation, as response to Al, is described in sensitive and, to a lesser extent, in tolerant roots [85, 87]. In a less extent, callose deposition has been considered as a mechanism to prevent Al from penetrating into the apoplast. Also, this accumulation is reported to inhibit the symplastic transport and cell communication by blocking plasmodesmata, avoiding Al-induced lesions in the symplast [92]. However, callose deposition in sensitive roots has also been shown to lead to uncontrolled rigidity of cell walls [41] leading ultimately to protoplast degradation.

2.6. Others.

Al-induced effects/damages are first detected in the root system [18, 93]. Changes in the root system may affect nutrient uptake, which can lead to nutritional deficiencies in shoots and leaves [94]. Except for Al-accumulator plants, Al accumulates more in roots than in leaves [95]. In some species, Al-induced alterations in leaves were considered indirect, since Al accumulation was not detected in leaves [94]. Nevertheless, alterations in leaves induced by Al exposure were reported for many species. Several works reported leaves biomass reduction [96], thickness [95], lipid

peroxidation [97], nutritional imbalances [98], changes in the photosynthetic performance [99], and changes in chlorophyll contents [96, 97, 99, 100], among others. Reductions in carbon dioxide (CO_2) assimilation rate due to Al toxicity are reported for several species [94, 99–101], and some works indicated that Al exposure induced damage of the photosystem II [97, 102]. Very few works focused on the consequence of Al treatment in the carbohydrate metabolism. The effects of Al exposure on Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) content and activity are still unclear, and the few reports available were performed in citrus [99, 100] and in wild rice [103].

3. Conclusions

Most studies on Al toxicity are performed with different media composition, Al concentration, and period of exposure. Also, there is a large variation between genotypes. This battery of nonharmonized experimental data needs caution during interpretation, mostly concerning generalizations of functional models. So, it would be important to uniform the experimental procedures in order to better comprehend the plant response to Al exposure and the mechanisms of Al tolerance.

Acknowledgments

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