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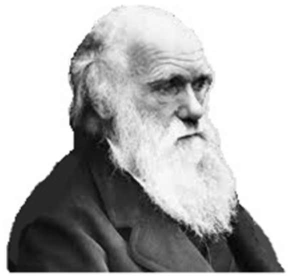

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A study on the toxicity of aluminum in rice, *Oryza sativa* L.

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ABSTRACT

Aluminum trivalent (Al^{3+}), which commonly occurs on 40% of arable land, is highly phytotoxic to crop growth and yield in acidic soils. As one of the major pollutants in the atmosphere, this element reduces chlorophyll activity, CO_2 assimilation, and photosynthesis. Rice is a staple food crop in India and Asian countries. A widely recognized metal toxicity of rice (*Oryza sativa*) includes soluble aluminum. The processes of senescence are known to be characterized by loss of chlorophyll, lipids, total protein, photosynthetic activity, and RNA. The author illustrates the aluminum effect in rice plants (ADT 43 & PA 6129) under different aluminum exposure levels (100 μM, 200 μM, & 300 μM), in regard to photosynthetic activity (total chlorophyll degradation, depleted CO_2 fixation, inhibited stomatal conductance) bioaccumulation, and histological analysis during leaf senescence. Rice varieties PA 6129 and ADT 43 were compared to assess photosynthetic degradation, bioaccumulation, and histological changes associated with aluminum-mediated degradation. Consequently, accelerated leaf senescence was observed after prolonged exposure to variety PA 6129 with increasing aluminum concentration. As an alternative, there is ADT 43, a precision aluminum tolerance mechanism.

***Keywords*:** Aluminum, Phytotoxicity, Leaf senescence, Rice, ADT 43, PA 6129

1. INTRODUCTION

Aluminum (Al) is one of the most abundant elements in earth's crust. In acidic soils, it inhibits crop production.[1] The world's arable land is acidic to a degree of 70%.[2] Hybrid

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Aduthurai 43 variety (ADT 43) one of the most commonly grown varieties of rice in Tamilnadu (Dept. of Agriculture Govt. Tamilnadu, and Tamil Nadu Agricultural University, 2012).[3]

For studies on good irrigated crops in Punjab, a hybrid variety of hybrid Pro Agro 6129 (PA 6129) was selected.[4] The toxicity of Al^{3+} is one of the major limitations of CO_2 assimilation by plants.[5-9] Senescence contribute to decline the productivity important flora include rice economically, A photosynthesis activity is central to the growth and productivity of plant and chlorophyll disappearance has been considered as principal criteria to study the leaf senescence by several investigators since long time.[10-12] Many researchers have considered chlorophyll disappearance as the principle criterion to study leaf senescence for quite a while. Over a long period of time, several investigators have used this approach to study leaf senescence. The onset of senescence is also accompanied by a decrease in photosynthesis and the disappearance of chlorophyll. [13-14]

The turning yellow of leaves during senescence suggests carotenoids more stable than chlorophyll during senescence.[15] On exposure to gaseous pollutants, Posthumus (1983) displayed similar symptoms, including chlorosis and growth depression, in addition to abscission of leaves, flowers, fruits, and damage to cones and seeds.[16] A new wild collection of Cicer was screened for aluminum toxicity tolerance, and accessions were identified that were more resistant to aluminum than current domestic cultivars.[17] It binds to several sites in cells, such as the cell wall, plasma membrane, cytoskeleton, and nucleus, and triggers several cytotoxic reactions.[18-19]

Researchers reviewed the effects of aluminum exposure on different plant leaves, particularly protein metabolism and starch metabolism.[20-21] To date, little is known about the aluminum effect on regulation of leaf senescence in *Oryza sativa* L.

Hence, this study should be of great relevance to developing rice crops in acidic soils since it addresses the biochemistry, accumulation, and cytological mechanisms associated with Al toxicity and tolerance in rice.

2. MATERIALS AND METHODS

Table 1. Materials and methods of various parameters followed by standard methods.

Sl	Categories	Parameters	Methods to estimated samples
1	Photosynthesis	Total chlorophyll content	According to Arnon (1949) method.[22]
		Rate of photosynthesis	By used an Infrared gas analyzer: LCA – 3 as described by Vadell and Medrano (1992) with the formula of C-index = 1 (Ci / Ca) ----II (Ci and Ca are intercellular and ambient CO_2 concentrations).[23]
		Stomatal Conductance	

2	Bio-accumulation	Estimation of aluminum	By atomic absorption spectrophotometry (AAS) with a Graphite Furnase at the wavelength of 309.3 nm as described by Gorsky et al., (1978).[24]
3	Histology	Microscopic study	Used Nikon Labphoto 2 microscopic Unit. Tissue of control and experimental rice plants studied by the method described by Easu (1964).[25]
		Staining methods	O'brien et al. (1964).[26]
4	Statistical analysis	All the data of obtained by each parameter analyzed for the significance	According to the method Duncan's multiple tests (Duncan's 1955).[27] The significance were calculated at 5% level ($p>0.05$). Each value is a mean of eight estimations, Percent decrease / increase over control is given in bars.

The study was conducted using rice plants from two varieties of *Oryza sativa* L. (ADT 43 and PA 6129). Rice plants were grown in natural conditions with pots. Experimental planting was made after 45 days of leaf growth. Using deionized water, leaves were detached from plants and washed for 30 seconds with mercuric chloride solution before being surface sterilized with distilled water.

With distilled water and various aluminum concentrations in petri dishes (100 µM, 200 µM and 300 µM with four replications), Leaf bits were placed in each with four Leaf bits, while distilled water alone was used as the control (Figures 1 and 2). In each Petri dish, there was a light intensity of 150 wm^{-2} and a temperature of 27 ± 3 °C; the solution was changed daily. The samples were measured at 24, 48, 72, and 96 hours after incubation for physiological, biochemical, and histological studies. Each parameter was estimated by standard methods as shown in Table 1.

3. RESULTS

3. 1. Total Chlorophyll estimation

In the present study, the total chlorophyll concentration declined with increasing aluminum exposure time. Over the control, however, aluminum hastened chlorophyll degradation. Also, aluminum accelerated degradation in PA 6129 more rapidly than in ADT 43 (Figure 1).

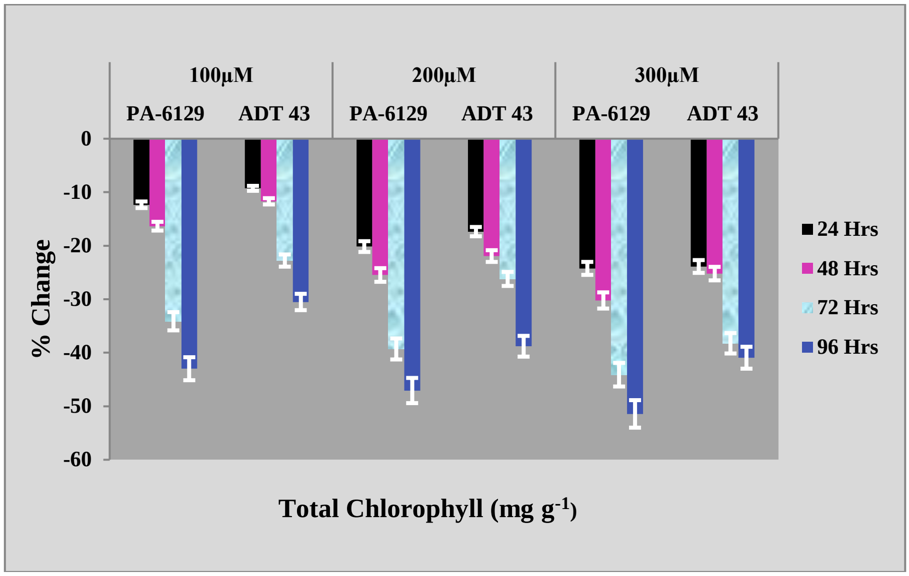

Figure 1. Percent decrease in total chlorophyll (mg g^{-1}) in two rice varieties (PA 6129, ADT 43) when exposed to different aluminum concentrations for 24, 48, 72, and 96 hours over the control

3. 2. Photosynthetic rate

According to ADT 43, aluminum tolerance is evident by the lesser inhibition of CO_2 fixation rate in photosynthetic rate upon exposure to aluminum over time and toxicity intensity. Aluminum toxicity regimes of moderate and severe severity influence stomata behavior in a measurable way, as measured by stomata conductance (Figure 2).

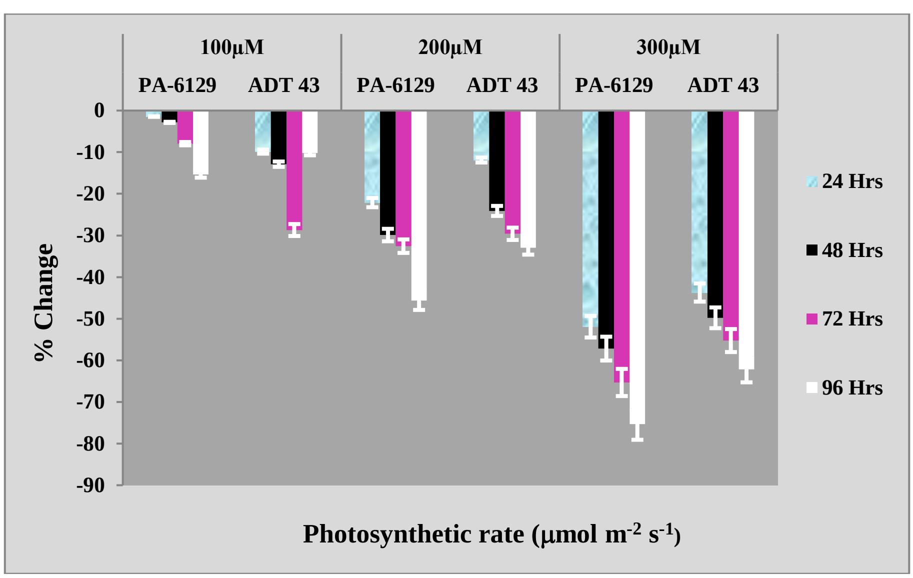

Figure 2. Percent decrease over the control in the photosynthetic rate (µmol m^{-2} s^{-1}) in leaf of two rice varieties (PA-6129, ADT-43) and on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

3. 3. Stomata conductance

Studies on the stomatal conductance exhibited considerable decrease on exposure to aluminum toxicity in both varieties. Further it was noticed that the degree of decrease in stomatal conductance was more in PA 6129 than ADT 43 (Figure 3).

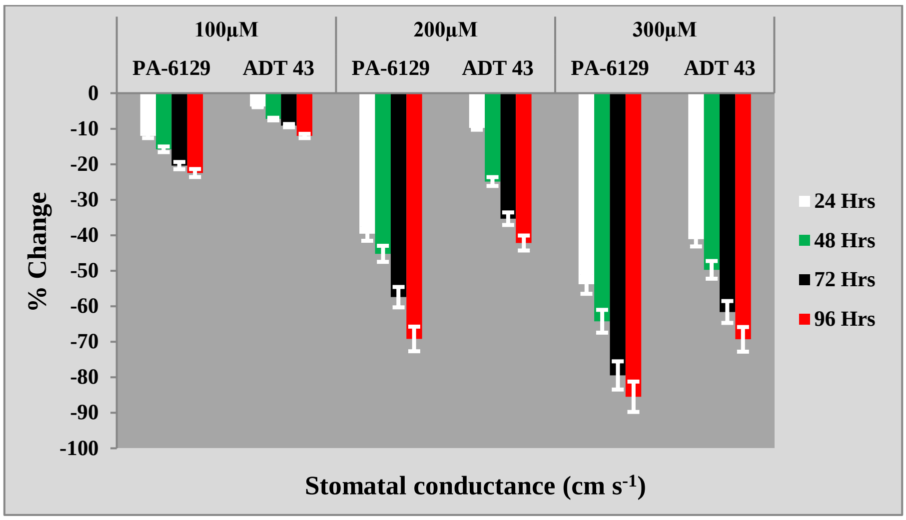

Figure 3. Percentage decrease in leaf stomatal conductance (cm s^{-1}) of two rice cultivars (PA-6129, ADT-43) with different concentrations of aluminum at 24, 48, 72, and 96 hours over control.

3. 4. Bioaccumulation

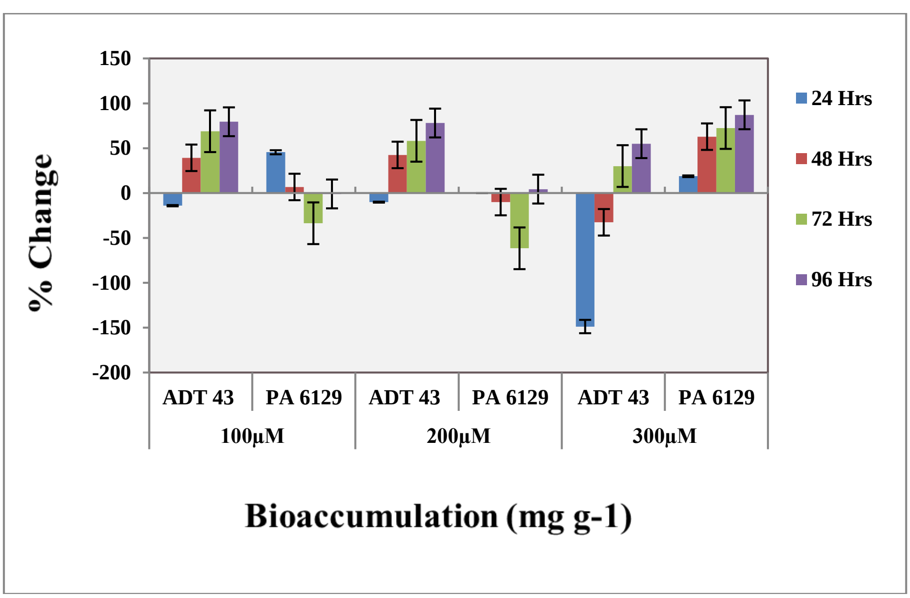

Figure 4. Percent decrease in bioaccumulation (mg g^{-1}) over the control for two rice varieties (PA 6129, ADT 43) exposed to different aluminum concentrations at 24, 48, 72, and 96 hours.

This study found that the bioaccumulation of aluminum increased as the concentration in the 300 µm range increased in both varieties (PA 6129 and ADT 43). While rice leaves of PA 6129 showed a greater rate of bioaccumulation than ADT 43(Figure 4).

3. 5. Histology

Microscopic examination of aluminum-treated rice leaves revealed interesting anatomical changes. A time-dependent and dose-dependent effect was observed in the tissue. As can be seen in Figures 5 and 6, the ADT 43 and PA 6129 varieties exhibit degrading leaf structure. At higher levels of aluminum exposure, PA 6129 showed conspicuous histological changes (A defective midrib, metaxylem, bulliform cells, phloem, damaged epidermis cells, irregular thickening with damaged cell walls, and dead cells and tissue excreted at the vacuolar level). These changes are more apparent with increasing exposure time. In contrast, very mild to insignificant changes were observed in rice leaves exposed to aluminum in ADT 43, indicating tolerance to aluminum.

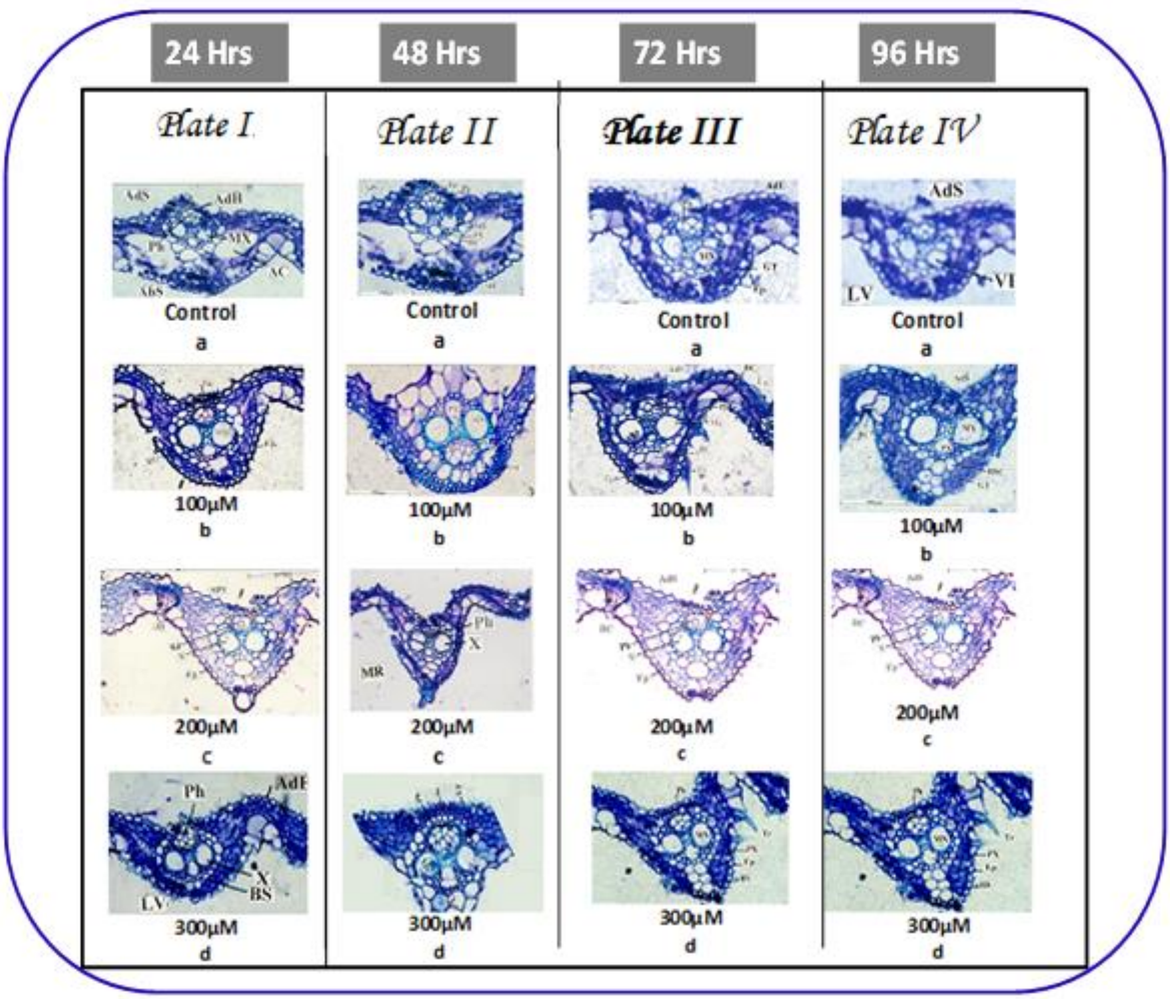

Figure 5. Transverse sections of ADT 43 rice leaves exposed exposed to different concentrations [a - Control; b - 100 µm; c - 200 µm; d - 300 µm] of aluminum at 24, 48, 72 and 96 hours.

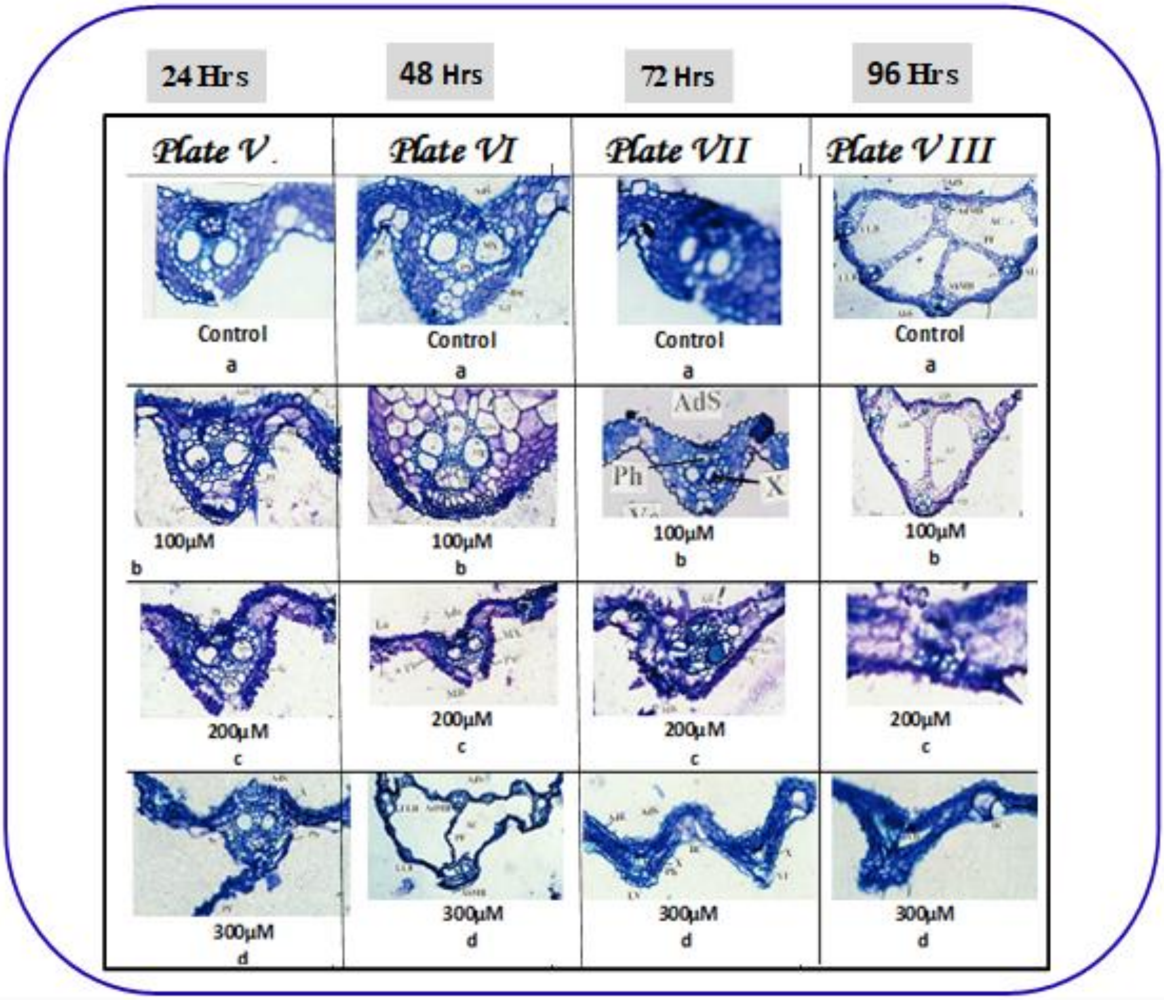

Figure 6. Transverse sections of PA 6129 rice leaves exposed exposed to different concentrations [a - Control; b - 100 µm; c - 200 µm; d – 300 µm] of aluminum at 24, 48, 72 and 96 hours.

4. DISCUSSION

In the photosynthesis process, chlorophyll content and stomata activity control plant growth and productivity. Aluminum may be able to influence leaf senescence in rice leaves through this study. Researchers have long considered the chlorophylls disappearance as a primary criterion to study leaf senescence, as well as photosynthetic decline.[28-38] These results align with those of previous studies examining the effects of exposure to heavy metals and other toxicants. At higher doses, aluminum appears to have a greater impact on total chlorophyll content. Compared to controls, aluminum exposures resulted in greater chlorophyll degradation, suggesting that aluminum accelerates cellular senescence. Further, it is worth noting that chlorophyll degradation was higher when aluminum concentration was higher. On the contrary, Abdel-Basset et al., (1995) found that metals at high concentrations inhibited chlorophyllase activity.[39] Aluminum might have enhanced the enzyme chlorophyllase because the chlorophyll degradation rate increased with higher aluminum doses.

Furthermore, assimilation rates of photosynthetic CO_2 may decline in glycophytes during aluminum stress due to either stomatal (decreased stomatal conductance) or non-stomatal (biochemical dysfunction) responses. As a result, aluminum retarded carbon dioxide assimilation[40-45] Photosynthesis rates are higher in certain species that are tolerant of aluminum, and Sorghum exhibits toxic properties that are influenced by abiotic stress.[46] In their study, Ribeiro et al., (2013) examined the rate of photosynthetic depletion, reduced stomatal conductance, and decreased transpiration rate.[47] In this study, the author found that changes in photosynthetic metabolism could provide insight into where toxic injury occurs and which tissue organelles are involved. In PA 6129, where Al3+ accelerated degradation more than ADT 43, considered a resistant type, the study found. Overall, the author suggests aluminum reduces photosynthetic metabolism.

As the leaf is one of the important large organs for aluminum accumulation.[48] The author found significant amounts of accumulation on rice varieties ADT 43 and PA 6129 after their leaves were exposed to aluminum at 100μM, 200μM and 300μM. Despite this, the bioaccumulation of aluminum in the leaves of ADT 43 was considerably lower than that of PA 6129. Comparing ADT 43 to controls at 300 μM, no significant difference in bioaccumulation of aluminum was observed. Aluminum is probably not reaching the leaf tissues at this concentration using ADT 43 due to a possible formation of an aluminum organic acid complex with the leaf epidermis as a result of aluminum binding to the organic acids of plants. Aluminum has been shown to be a very effective complexing agent for organic acids.[49] Smaller quantities of aluminum that reach the epidermal layer of ADT 43 rice leaves may be within limits by detoxification, but PA 6129 could cause structural and functional damage with influence of aluminum consequence. Previous studies have shown that plants tolerant to aluminum secrete organic acids and increase the level of secretion when exposed to higher levels of aluminum. [50-52]

ADT 43 showed no morphological changes in comparison to control and PA 6128 when aluminum was studied. A concentration of aluminum (300 μm) at which a maximum deformity was observed in the midrib, metaxylem, bulliform cells, and phloem cells of the rice variety PA 6129. An earlier study made similar findings regarding heavy metal exposures, such as cadmium. According to MacFarlanc and Burchett (2001), heavy metals accumulate within the cell wall system. [53] Additionally, it appears that the study also documented aluminum accumulation in cell wall system of senescing leaves, resulting in significant histological changes in PA 6129 rice leaves. While the accumulation of aluminum and translocation into the cell wall were less in ADT 43 than in PA 6129, the degree of accumulation and translocation was still statistically significant. We observed anatomical changes in rice leaves along with a number of adaptive characteristics for survival in heavy metal-laden soils.

5. CONCLUSIONS

In this study, aluminum enhanced cell wall maturation at higher concentrations in leaf parts. Furthermore, these changes at the cell wall level significantly changed PA 6129. Moreover, ADT 43 displays tolerance to aluminum ions and shows no significant anatomical changes in rice leaves. As organic acids have shown to be an effective complexing agent for aluminum, it is most likely that the decrease in aluminum absorption is caused by the binding of aluminum[54-58]. There are most effective organic detoxifiers with hydroxyl (OH) or carboxylic groups (citric acid and tartaric acid) and form stable rings with aluminum and are non-toxic to plants, this may account for ADT 43 tolerability. Overall, this study concluded that aluminum enhanced the onset of photosynthetic, bioaccumulation, and histological alterations similar to those associated with senescence in rice leaves. The study also concludes that the changes occur at a faster rate in variety PA 6129 than in variety ADT 43, and that leaf senescence was accelerated by increasing aluminum concentration over time. In contrast, Aluminum Oxalate Complex may have evolved from an earlier phase of concentration to execute a nontoxic pattern by secreting organic acids in response to aluminum, an evolved mechanism of aluminum tolerance.

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