Review

Exploring Aluminum Tolerance Mechanisms in Crops: A Comprehensive Review of Genetic, Metabolic, and Physiological Adaptations in Acidic Soils

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Article

Exploring Aluminum Tolerance Mechanisms in Crops: A Comprehensive Review of Genetic, Metabolic, and Physiological Adaptations in Acidic Soils

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Abstract: Aluminum (Al), making up a third of the Earth's crust, is a widespread toxic contaminant, particularly in acidic soils. It impacts crops at multiple levels, from the cellular to the whole plant. This review delves into aluminum's reactivity, including its cellular transport, involvement in oxidative redox reactions, development of specific metabolites, and the influence of genes on the production of membrane channels and transporters, as well as its role in triggering apoptosis leading to senescence. It discusses the involvement of channel proteins in calcium influx, vacuolar proton pumping, the suppression of mitochondrial respiration, and the initiation of programmed cell death. At the cellular nucleus level, the effects of Al on gene regulation through alterations in nucleic acid modifications, such as methylation and histone acetylation, are examined. The review in addition outlines the pathways of Al induced metabolic disruption specifically citric acid metabolism, regulation of proton excretion, induction of specific transcription factors, modulation of Al-responsive proteins, changes of citrate and nucleotide glucose transporters and overall metal detoxification pathways in tolerant genotypes. It also considers the expression of phenolic oxidases in response to oxidative stress, their regulatory feedback on mitochondrial cytochrome proteins, and their consequences on root development. Ultimately, the review focuses on the selective metabolic pathways that facilitate Al exclusion and tolerance, emphasizing compartmentalization, antioxidative defense mechanisms, and the control of programmed cell death to manage metal toxicity.

Keywords: toxic metals; environmental pollution; antioxidant defense; organic acid exudation; programmed cell death; vacuolar processing enzymes

1. Introduction

Acidic soils, comprising nearly 40% of the Earth's crust, include about 50% of arable land. These soils can support certain crop species, though they often require altered physiological responses. In acidic soils, aluminum (Al) is the most abundant element, significantly impacting rice cultivation, which occupies 13% of acidic land [1]. Aluminum exists in various forms, including aluminum silicate and sulfate, and can dissolve into active trivalent aluminum ion (Al^{3+}), particularly under low pH conditions in the rhizosphere. Dominant forms like aluminum hydroxide cations [$Al(OH)_2^+$, $[Al(OH)_3^+]$ can cause oxidative stress. Both inorganic and organic residues in the rhizosphere

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contribute to Al release into acidic water bodies [2]. Exchangeable Al^{3+} binds with silicates, clays, and other residues, affecting mineral availability to plants. High Al concentrations, depending on soil pH, can damage root systems.

In acidic soil roots are impaired with membrane permeability, hydraulic conductivity, mineral acquisition, replacement of other nutrients, and those collectively are responsible for plant growth and productivity [3]. Aluminum toxicity involves inducible gene expression and two primary stress mechanisms. Aluminum tolerance in roots through both internal and external detoxification processes includes organic acid-based ligand formation and that helps bio exclusion.

Aluminum accumulation is more sensitized in the root apex, root cap and extended permanent region of root where an altered carbohydrate metabolism is directly related for metal uptake. In Al tolerant genotypes the uptake of metal may not be related to impeded ion uptake for nitrate (NO_3^-) like ions [4]. In cereals, the inhibition of such ion uptake is reversed by ammonium (NH_4^+) treatment which suggests the co-transport of Al with other cations. Still, the interaction of Al with NO_3^- is a possible clue for mineral imbalances under acidic soil. The increase in amino acid under Al treatment is also assumed for an enhanced degradation of proteins in root zone. Likewise, a proportion of amines (asparagines, glutamine) is the product of Al induced protein hydrolysis or oxidation, and thereby accumulation of free amino acids are served as bio-indicator of acidic soil [5]. On the other hand, the uptake of ions in reduced rate due to Al is in competitive manner according to pH of the solution. Thus, the reduction in NO_3^- absorption releases more protons (H^+) into rhizosphere that otherwise favors acidic pH of the soil and more Al absorption. A fall in NO_3^- uptake is also an inducing signal for rhizosphere secretion by Al sensitive species to release some special metabolites. Thus, NO_3^- uptake would be a preliminary symptom of Al toxicity within short period of application of the metal particularly, in tolerant plants.

Toxicity to Al is another path irrespective of tolerant and susceptible species where different chemicals may functions as elicitors for defense mechanism. In relation to acidic soil grown crops species there are exhibition of various mechanisms of Al tolerance [6]. Few predominant chemical species (malate, oxaloacetate, citrate and other a few organic acids) released into rhizosphere and are acted as Al chelators. This is an extracellular strategy of Al avoidance by specific reactions in different tolerant genotypes with variations in sequestration of metal [7]. In intracellular pathway the same strategy for metal chelation is operative where H^+ pump mediated cytoplasmic and vacuolar acidity is accomplished. Thus, Al sensitivity is also established by acidolysis of cytoskeleton and biomolecules and is registered in different degrees in non-tolerant species. A subsequent imprisonment of the metal into non-cellular or apoplastic spaces also sets another tolerance mechanism. Moreover, Al toxicity also converts the change of membrane potential which is often correlated with membrane surface binding capacities of other cations [8]. A change of plasma membrane potential induced by Al mediated depolarization can also regulate calcium ion (Ca^{2+}) uptake influencing growth of the plants. The effect of cytoplasmic calcium ion (Ca^{2+}) concentration is directly regulating many processes of cell metabolism where Al injury is responsible. Aluminum-dependent flux of Ca^{2+} from extracellular spaces regulates the inhibitions of root elongation which may be reversed by acidification of the soil [9]. Therefore, cellular pH of the roots is the determining factor for the availability of some signaling molecules (e.g. Ca^{2+}) for interaction of Al in soil. A pH dependent synthesis of complex polysaccharides (like 1-3-glucan) in the roots influences metabolism of calcium where roles of calmodulin (CaM) like binding proteins are important [10].

Despite a significant literature describing the toxicity of Al in plants, a little is deciphered about the specific ion effects like oxidative stress. It was reported earlier that besides of water relation and other cellular anomalies by Al as a metal in general, the changes of redox is the prime key of Al vulnerability. A leading work for accumulation of reactive oxygen species (ROS) and thereby, the induction of antioxidation machineries are the keys of Al tolerance [11]. A change of cellular redox inducing oxidative stress is the initiation of different chemical reactions like peroxidation of lipids, carbonylation of protein, tautomerization of nucleic acids is the processes of degeneration of plants under Al toxicity. Furthermore, Al induced inhibitions of cellular respiration, adenosine triphosphate (ATP) depletion can compel the plants to bypass the electron flow from electron transport chain (both

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in chloroplast and mitochondria) into molecular oxygen. At cellular level in root the depletion of energy utilizing pathways and the dysfunction of organic acid cycle in the mitochondria set the loss of viability of roots [12]. Thus, oxidative stress is set as the key underlying mechanism of Al toxicity where interference of other redox metals (iron, chromium, zinc) in the tissue are complementary to degenerative process of toxicity. More insights are yet to be known for the ROS production through other pathways linked to cellular reactions as major determining factor for root growth inhibitions under Al contamination.

This review aims to understand thoroughly the Al resistance mechanisms in tolerant genotypes and other species in acidic soils. It emphasizes the need to develop cultivars or identify specific traits to support adaptation to Al-rich acidic soils. Understanding increased Al^{3+} sensitivity is crucial for constraints of food production in specific agro-climatic zones, it is imperative to understand the tolerance mechanism for crop in breeding purpose. Metal detoxification primarily relies on exclusion mechanisms with organic acids forming stable complexes with Al^{3+}

[13]. Genetic studies in rice, a crop highly sensitive to acidic soils, have identified several gene families (ALMT, MATE, ABC) and transcription factors (ART1, ASR5, *Os*WRKY22) crucial for Al exclusion and tolerance [14]. This review also reveals the importance of omics technologies like genomics, proteomics, and metabolomics to understand the complex traits in crops under Al toxicity using intradisciplinary approaches in crop science. It may cover the generations of metabolites under metal stress with their qualitative and quantitative distribution, biosynthesis, catabolism, and the functional network in combination for tolerance. It also finally discusses the development of selective markers based on specific metabolomes, and their roles for metal tolerance in plants through gene overexpression and, chemical elicitation.

2. Aluminum Bioavailability in Different Forms to Sensitize the Plants

High complex effluents from industrial outlets composed of different aluminum (Al) compounds are present in varying quantities and, responsible for toxicity in acidic soil. In soil (pH < 4) increasing crop susceptibility to metal toxicity by a high concentration of Al can cause soil acidification which is more targeted to failure of crop productivity [15]. In spite of this, soil acidification is achieved by other agronomic factors through the accumulation of basic cations (K^+, Na^+, Mg^{2+}, Ca^{2+}) etc. Aluminum is the major component covering (> 7%) of metallic elements in the Earth's crust after silicon, however, showing no significant roles exhibiting in plant growth and development [16]. Aluminum is precipitated in soil from major ores like aluminosilicate that undergoes initial solubilization (at pH < 5.0) resolving as eventual phytotoxic form. Trivalent aluminum ion can also accompany different hydroxides of metal like aluminum hydroxide cation [$AlOH^{2+}$, $Al(OH)_3$, and $Al(OH)_4$] in partial to fully solubilization for plants' absorption. This is based on a gradual depletion of soil pH that corresponds to a better solubility of Al salt with different valences (Figure 1). Notwithstanding all valences of Al, Al^{3+} is the most toxic and stress-producing forms when distributed through apoplastic and symplastic spaces in root system. Plant species irrespective of taxa when subjected to intense and prolonged duration of Al exposure undergo variable levels of modification through intra and intercellular components [17]. Physiologically those cover changes in cell wall components, modification of transport processes across the membrane, disruption of cytoskeletons, altered cell signaling energy-yielding metabolism, and finally mutation of nuclear material [18]. Notably, plants that responded to Al^{3+} toxicity are distinctly categorized into tolerant as well as sensitive species. Thereby, the tolerance mechanism has been in search in acidic soil vulnerable for the plants' growth at physiological and genetic levels. The most predominant line of investigation is based on the absorption of Al through the root system which ultimately cause changes in root physiology, biochemistry and, molecular biology. In the second line of research approaches the relevance of oxidative stress with specific Al^{3+} ions at the cellular level are well established [19]. Different oxidative metabolites, their varying expression through tolerant and, sensitive cultivars and, regulation through biochemical reactions are the major domains of Al-induced oxidative stress in research. This is more intricated with other findings, where partially known or hypothetical ligands are reported to form complex compounds with free Al in the cytosol

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[20]. In this view, the biosynthetic and catabolic genes are responsible for studying in concern to tolerant cultivars. Therefore, the acquired information may be relevant in application of molecular breeding and biotechnological devices for the development of tolerant genotypes against Al toxicity.

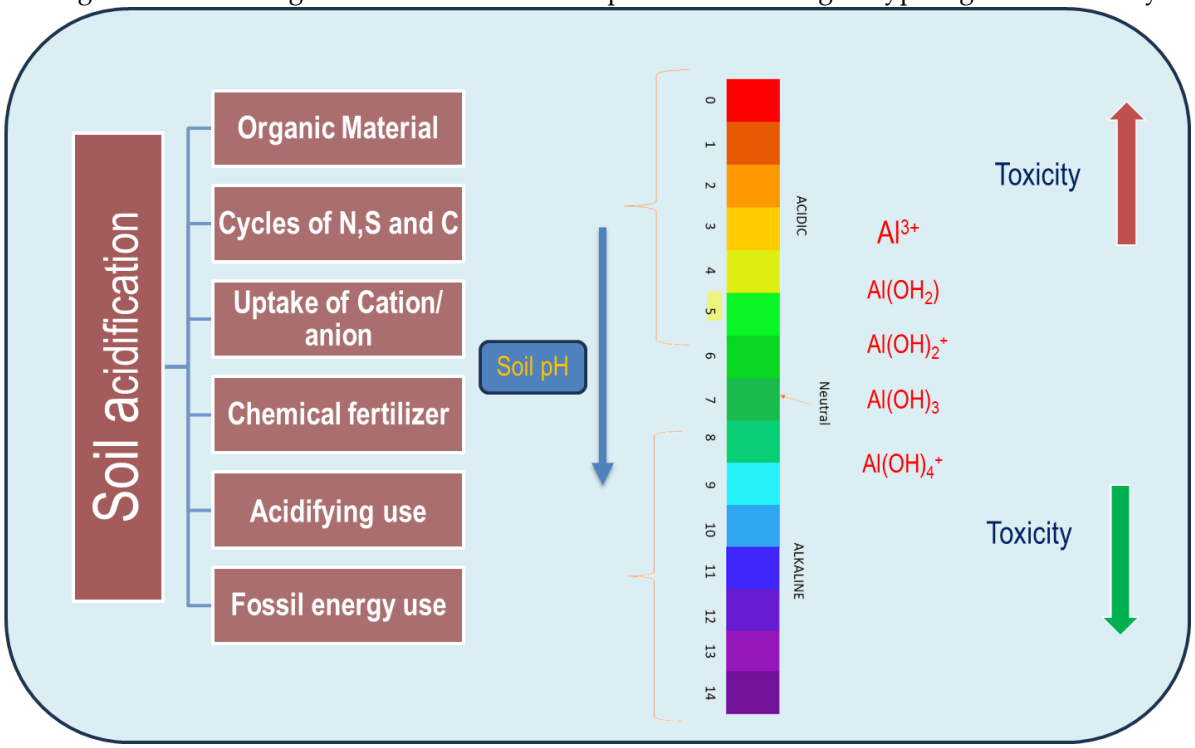

Figure 1. Illustration of soil acidification's impact on aluminum chemistry, highlighting its transformation into various inorganic forms. Aluminum reactivity varies with solubility, heavily influenced by ionic changes. Different aluminum species are formed based on acidic pH levels, with aluminum ion (Al^{3+}) being the most predominant in acidic conditions, forming stable complexes with sulfate ion ($SO4^{2-}$), hydroxide (OH^{-}), phosphate ion ($PO4^{2-}$), and silicon (Si). As pH increases, aluminum toxicity diminishes, leading to diverse complexes like aluminum hydroxide ions [$Al(OH)_3$, $Al(OH)_4^{+}$, $Al(H_2O)_6^{3+}$], and other insoluble hydroxides. Al^{3+} actively enters plant roots in environments with a pH lower than 5. At neutral pH, $Al(OH)_3$ forms, characterized by higher insolubility and non-toxicity. In environments with a pH greater than 7, aluminate $Al(OH)^{4-}$ speciation occurs, often complexing with other molecular species such as $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$.

3. Aluminum-Induced Oxidative Stress and Metabolic Alterations

Industrial processes and human activities are primary contributors to the dispersal of toxic forms of aluminum (Al) in the Earth's crust. These activities release various toxic molecular forms of Al, posing significant threats to both the environment and plant life. Sources of these toxic forms include the processing of ores, the use of fertilizers and herbicides, and the disposal of solid wastes such as sewage sludge and alloy emissions from different industries [21]. A key indicator of Al toxicity in plant cells is the generation of reactive oxygen species (ROS). Particularly, roots are highly sensitive to Al exposure, leading to the production of various ROS. This generation of ROS occurs not only in the cytosol but also within cellular organelles such as chloroplasts, mitochondria, and peroxisomes which are rich in electron transport chains in cell [22]. Even the nucleus is affected, where peroxidation of the nuclear membrane can initiate cascades of free radicals, including hydroperoxides and peroxy radicals those interact with nucleotides, and additional chemical residues. Research has identified several genes activated in the nucleus those act upon tolerance mechanisms through cytoplasmic and organellar compartments. Key genes include *ADP-ribosyltransferase 1*, *ferric reductase defective like 4*, *acetolactate synthase 1*, *Nramp family 1* (*ART1*, *FRDL4*, *ALS1*, *NRAT1*) along with various antioxidants and proteins involved in the ascorbate (AsA)-glutathione (GSH) cycle [23].

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Mitochondria can also be significant sources of ROS, especially where malate and organic acid transporters facilitate the entry of aluminum ion (Al^{3+}). The inter-membrane spaces, housing the electron transport chain, are prone to reactive oxygen species (ROS) generation, particularly hydroxyl radicals resulting from oxidative decarboxylation reactions. The redox status is crucial for understanding the metabolic impact of Al on tissues, with pyridine nucleotides ($NADH/NAD^+$, $NADPH/NADP^+$, $FADH_2/FAD^+$) playing a key role in signaling through ROS [24]. This ROS trigger various harmful reactions including lipid peroxidation, protein carbonylation, and formation of bulky nucleotide adducts. The dual roles of reactive oxygen species (ROS) as a critical signaling residues as well as in inducing oxidative stress is more contextual for signaling against aluminum ion (Al^{3+}). Root tips affected with Al^{3+} are demonstrated with detoxification mechanisms through cell wall modification. Reactive oxygen species and its exposure on outer surface of plasmamembrane induce a complex network in wall modifying enzymes [25]. There are a few noticeable over expressed proteins like expansions, xyloglucan endo-transglucosylase, pectin actyl esterase are responsible for cell wall elasticity as well as cross linking with secondary deposition. Reactive oxygen species, particularly, hydrogen peroxide (H_2O_2) is most important inducer in the reaction where Al^{3+} is chelated with complex carbohydrate residues. Formation of superoxide radicals on apoplastic and membrane bound NAD(P)H oxidase (NOX) and respiratory burst oxidase homolog (RBOH) are most important for their activities in signaling processes. A number of enzymes like superoxide dismutase, xanthine oxidase and finally a few classes III wall bound peroxidases are important for different ROS/free radicals in sensitization for aluminum (Al). In presence of Al^{3+}, cross linking of cell wall residues, mostly xyloglucans increase the simultaneous formation of ROS and overexpression of variants of peroxidases. Reactive oxygen species on the cell wall, apoplastic spaces are protruded with membrane transporters like nod26-like intrinsic protein (NIP), halimione portulacoides plasma membrane amino acid transporter 1(HmPALT1), and nitrate transporter 1 (NRAT1) with simultaneous restriction of Al^{3+} entry [26]. In latter stage with the establishment of metal ion within cytosol, ROS signal membrane transporters are overexpressed for organic acid exudation. A few phenolic residues are also induced to chelate Al^{3+} on rhizosphere that prevent entry of ions through root system. The path of ROS development under Al^{3+} toxicity is quite consistent with other external stimuli. The intricate control mechanism employing RBOH happens to be most useful in metal effected tissues for transmission of signal following its transduction [27]. Additionally, NOX derived ROS is also accompanied by a number of apoplast harboring proteins mostly peroxidases (PRXs), polyamine oxidases (PAO), quinone reductase, and other metal bearing anime oxidases. An oxidative signal, mainly through altered mitochondrial electron pathways also promotes ROS generation [28]. Finally, within nuclear membrane, ROS exposure activates genes encoding for antioxidative cascades for regulation of oxidative stress. Translocation of ROS as signal through cytosol can also induce a number of membranes bound transporters like *Oryza sativa* L. aluminium-activated malate transporter 1 (*Os*ALS1), vacuolar amino acid transporter 1 (VALT1) for conjugations of Al^{3+} as well as direct detoxification with non-thiols residues and phytochelatin [29]. Reactive oxygen species produced by herbicides or pesticides like xenobiotic toxicity functions in reliable biomarking for specific plant species. The metals inserted within the xenobiotics have a distinct path to be detoxificated in the plant system initially by spatio-temporal transitory accumulation of free radicals (e.g. H_2O_2) and that may act in evocation of signal. This is followed by compartmentalization of metal in cytosolic or even apoplastic spaces in conjugation with glutathione like compounds [30]. Elicitors like melatonin are effective in chelation of pesticide like residues following transport into vacuoles by ATP-binding cassette transporters (ABC) transporter as found in detoxification of Al^{3+}.Therefore, ROS sensing and its signal transduction would be dependent on type of stimuli and plants' genotypic potential to minimize its effects either by direct sequestering or / and antioxidation.

Among the plethora of free radicals, hydrogen peroxide (H_2O_2) stands out for its dual role in signaling, potentially leading to either beneficial or detrimental outcomes in the context of Al toxicity. The synthesis of macromolecules such as lignin, which relies on peroxidase activity on H_2O_2, is an example of an immediate response to Al stress. Oxidative stress, indicated by the accumulation of H_2O_2, fuels the peroxidase activity necessary for lignin biosynthesis [31]. Concurrently, plants

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develop a defense system comprising both enzymatic and non-enzymatic antioxidants to cope with H_2O_2 accumulation.

In chloroplasts, Al toxicity triggers ROS production through mechanisms involving the electron transport chain in Photosystems I and II. Various ROS forms are generated, including singlet oxygen, superoxide, hydrogen peroxide and, hydroxyl radicals. The uptake of Al^{3+} induces the expression of certain genes in the chloroplast genomewhich includes membrane-bound ATP-independent proteases that play a significant role in Al tolerance mechanisms [32]. The mechanisms of Al tolerance are complex, involving the synergistic contributions of metal compartmentalization, chloroplast fluorescence, mitochondrial oxidative reactions, and peroxisomal carbon oxidation (Figure 2). This multifaceted approach to Al tolerance underscores the intricate network of responses of plants deployed against oxidative stress induced by metal.

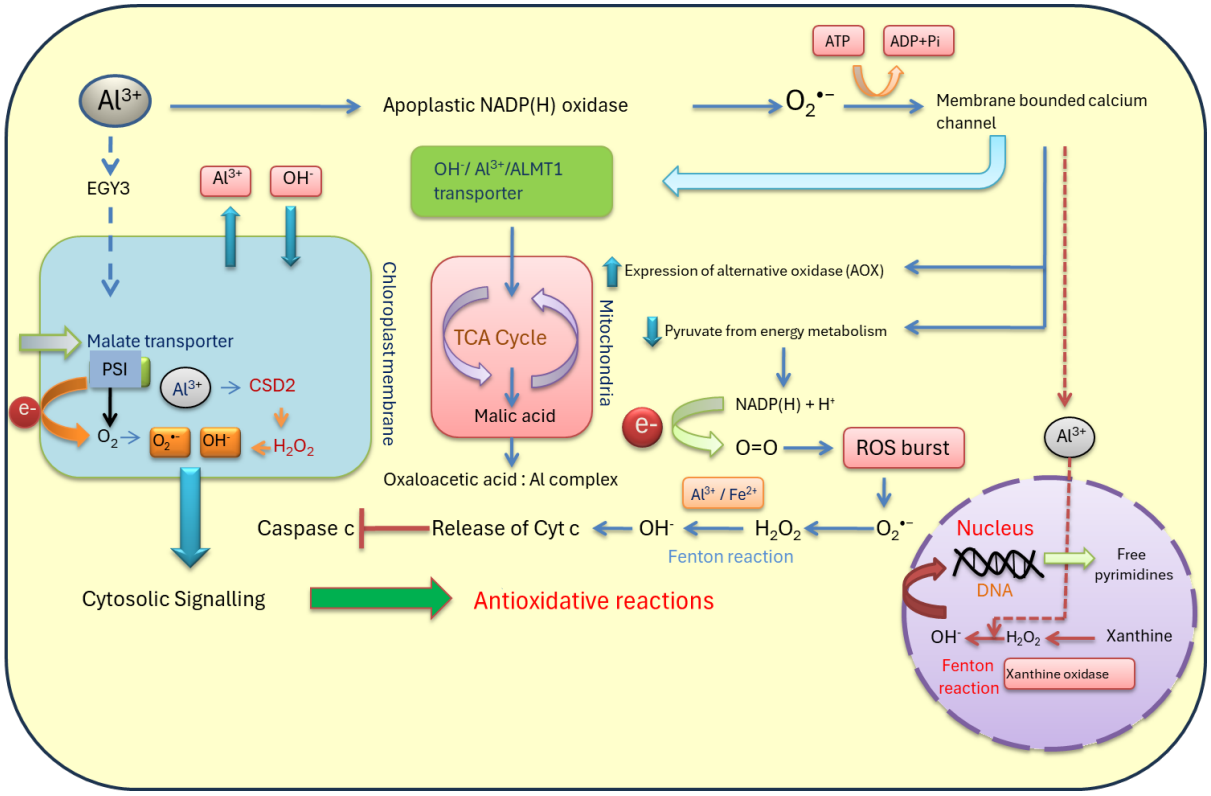

Figure 2. Integrated approach for reactive oxygen species (ROS) mediated cellular tolerance of plants to aluminum (Al) toxicity. Aluminum shares a common path for organic acid transporter (OA:Al^{3+}) where the ions diffuses inside the cytosol and interact the mitochondrial oxidative cycle. Malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) are the major enzymatic sources where nicotinamide adenine dinucleotide [NAD(H) + H^+] contributes electrons to O_2 into superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). In sensitive cultivars H_2O_2 leads the formation of hydroxide ion (OH^-) with Fenton reaction. On the other hand, nucleus is activated on nuclear membrane oxidation which also produces free radical and other genes *angiotensin II receptor type 1, nitrate transporter 1* (*ATR1, NRT1* etc) induction. Aluminum induced release of cytochrome c inhibits caspase like activity forwarding cell apoptosis. Chloroplast's reactions in another form can also induce ROS formation through introduction of ethylene dependent gravitropism deficient and yellow green light 3 (*EGY3*) like metallo proteases that is efficient in production of H_2O_2 which is retrograded in downstream signaling. The *EGY3* can also induce malate transporter where Al^{3+} can invade PSI. The later is induced to develop $O_2^{\bullet -}$/OH^- like free radicals and are engaged in chloroplast membrane oxidation. Aluminum ion (Al^{3+}) can also induce a chain of oxidative reaction where nucleotides are released freely by the action of xanthine oxidase like activities.

Aluminum profoundly impacts root growth and extends its detrimental effects to other plant organs. Many species show signs of toxicity at different organizational levels, from morphological

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changes to nuclear alterations, largely due to their oxidative sensitivity to the metal [33]. The solubilization of Al^{3+} in the rhizosphere, a result of soil acidification, leads to the formation of various chemical species of Al. This complicates agricultural practices in such soils, especially with the co-presence of other metals like ferrous ion (Fe^{2+}) and various Al salt intermediates. In regions like the root meristem, endodermis, and cortical tissues exposed to Al^{3+} causes lipid peroxidation and the accumulation of reactive oxygen species (ROS) as demonstrated in maize cultivars. This results in significant inhibition of tissue elongation, highlighting the root as the primarily affected organ [34]. Aluminum toxicity triggers oxidative stress, altering cellular and membrane integrity and disrupting the redox balance within cells. Different ROS types can initiate peroxidation reactions on macromolecules such as lipids, proteins, and nucleic acids compromising cell viability.

Although Al is not a transition metal and does not directly catalyze redox reactions, it can still contribute to the oxidation of biomolecules, leading to oxidative stress. This often involves Al forming electrostatic bonds with biomolecular groups such as phosphate (PO_4^{3-}) or carboxylate (COO^-) on plasma membrane pectins [35]. The formation of callose within cell walls serves as an adaptive strategy to mitigate free radical damage. Transition metals, in conjunction with Al, contribute to the pool of ROS. Excessive Al on the membrane surface can increase the uptake of redox-active iron (Fe) into the rhizosphere, creating a vicious cycle of ROS production in plant tissues [36]. Crop species, including barley, rice and, gram, suffer from Al toxicity at their root apices that leading to growth inhibition due to lipoxygenase activity. In maize, factors such as early senescence and altered water relations, alongside oxidative stress, amplify the detrimental effects [37].

In acidic soils, aluminum (Al) accumulation can inhibit the function of cellular proton-ATPase pumps (H^+/ATPase), leading to the efflux of potassium and other essential elements from the membrane. The impact of Al-induced oxidative stress varies among crop species, depending on their genetic makeup. Metabolomic responses in susceptible species are critical in acidic conditions, where micromolar concentrations of Al^{3+} have specific toxic effects, impairing water and nutrient uptake. Combating soil acidification, a mounting global concern, involves selecting Al-tolerant genotypes and identifying and cloning specific genes related to Al tolerance [38]. A comprehensive understanding of cellular responses and nuclear regulatory mechanisms is vital for developing Al-tolerant crop lines. Metabolomics, reflecting changes in metabolites expression triggered by various Al species, needed further investigation. The interaction between nitrate and Al in root uptake significantly affects primary metabolomes [39]. Gene expression changes involve transcription factors like suppressor of gamma response 1 (SOG1), and DNA damage signaling proteins such as Ataxia-telangiectasia-mutated, and Rad3-related (ATR). In acidic environments, where Al toxicity is prevalent, there is a higher ratio of ammonium ion to nitrate ion (NH_4^+ to NO_3^-). Plants resistant to Al exhibit a preference for NH_4^+ over NO_3^-, facilitating better solubilization of Al in the rhizosphere (Figure 3).

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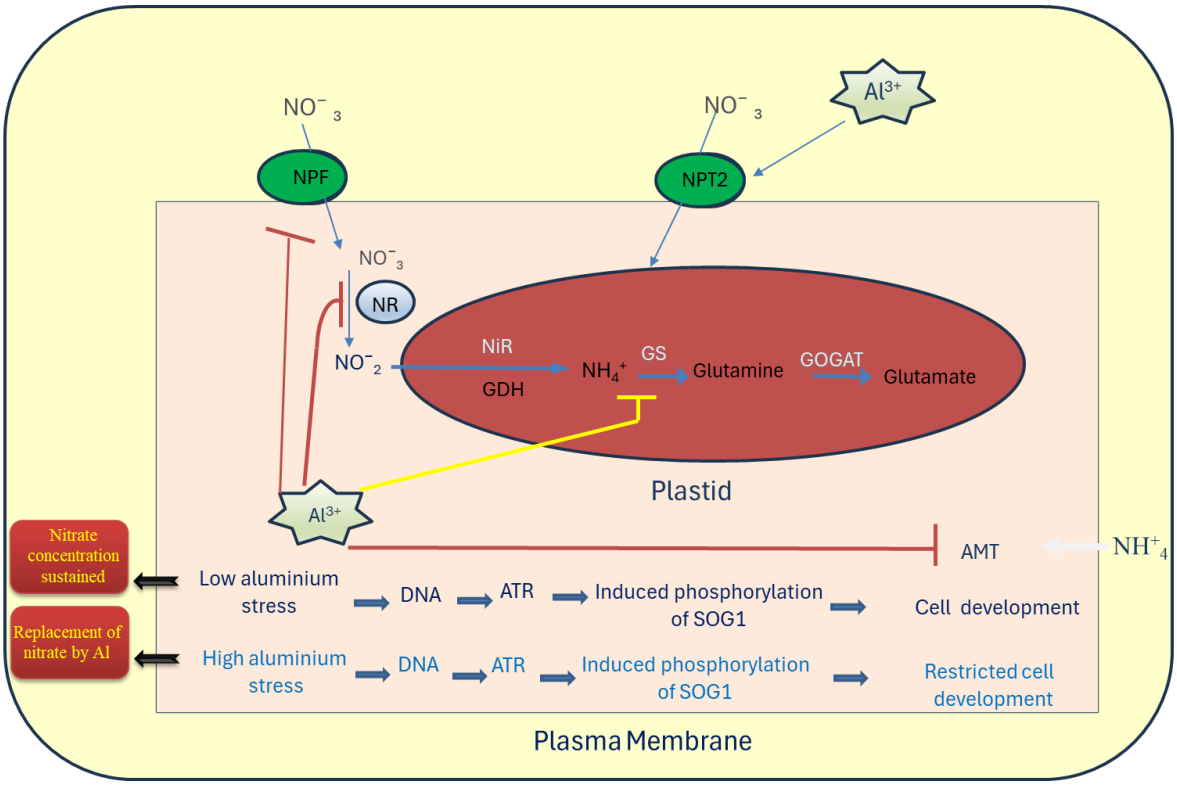

Figure 3. Depiction of ammonium ion (NH_4^+) and nitrate ion (NO_3^-) absorption by aluminum (Al) at root-soil interfaces. Ammonium ion (NH_4^+) lowers the rhizospheric pH, increasing the inhibition of metal uptake in plant roots due to competitive inhibition between aluminum ion (Al^{3+}) and H^+. Conversely, rhizospheric pH becomes more alkaline with NO_3^+ accumulation, aiding in the desorption of metals into the rhizosphere but enhancing their transport into roots. Nitrate addition increases the negative electrical potential on the root surface, facilitating the conversion of NH_4^+ to NO_3^-. Excess NH_4^+/H^+ can displace soluble NO_3^- in roots. In sensitive cultivars, NH_4^+ can influence the binding of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia mutated rad3-related (ATR) with suppressor of gamma response 1 (SOG1) for DNA damage recognition, leading to arrested cell growth in roots under high Al stress. The caption also references key enzymes and transporters involved in nitrogen metabolism: nitrite reductase (NiR), nitrate reductase (NR), glutamine synthase (GS), glutamate dehydrogenase (GDH), glutamine oxoglutarate aminotransferase (GOGAT), nitrate transporter 1/peptide transporter family (NPF), and nitrate transporter 2 family (NRT2).

4. Metabolomes Induction to Aluminum Responses in Plants

The response to aluminum (Al) toxicity in plants involves two primary pathways: exclusion and oxidative tolerance [40]. Exclusion involves several channel proteins on cell membranes and tonoplasts, aided by specific metabolites, including anion transporters like malate/hydroxide ion that facilitate Al sequestration into vacuoles or apoplastic spaces [41]. Solubility of various organic acid anions like citrate, lactate, oxalate, and succinate in acidic environments also plays a role [42]. Carbohydrate biosynthesis, particularly for cell wall components such as pectin, acidic heteropolysaccharides, D-galacturonic acid, and glycoside-containing residues like rhamnose and galactose, is crucial. Pectin, a prevalent residue for aluminum ion (Al^{3+}) chelation, is recycled from primary metabolites. Monosaccharides like galactose and cellobiose bind Al^{3+} on the cell wall, while lignin, among the heteropolysaccharides, is pivotal for covalent Al binding, influencing metal compartmentalization [43]. Al-tolerant plant genotypes exhibit enhanced lignin biosynthesis, supporting anatomical adaptations like vascular lumen thickening under metal stress [44]. Lignin and phenolics like coumarins, derived from the phenylpropanoid or shikimic acid pool, are significant [45]. Key enzymes overexpressed under Al toxicity include phenylalanine ammonia lyase (PAL) and coumarate CoA ligase [46], especially in rice cultivars. In plants a multistep bio-exclusion

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of Al^{3+} from root cells is understood specifically for tolerant species where ions are entered through symplast of tissues crossing the cell wall (Figure 4).

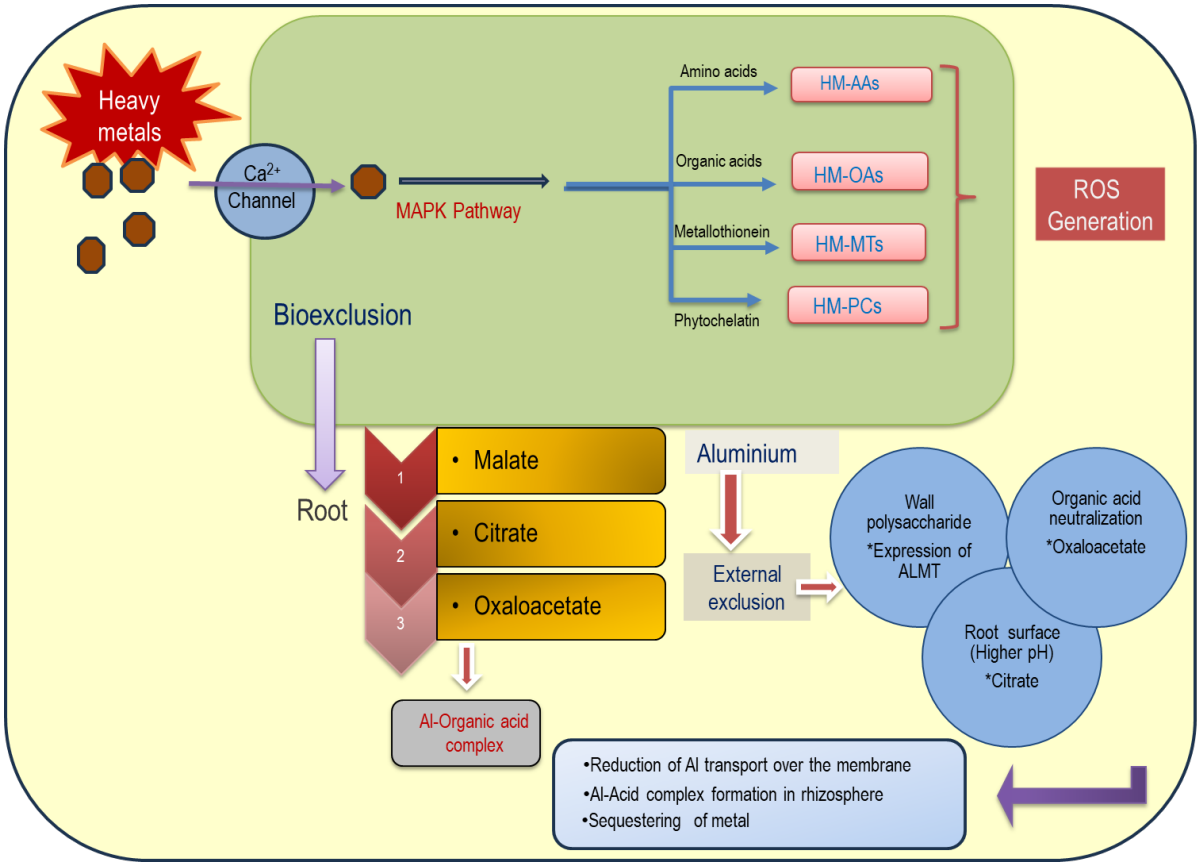

Figure 4. Illustration of aluminum tolerance mechanisms in plants through bio-exclusion during stress. The plant's response to aluminum stress includes formation of aluminum-organic acid complexes leading to increased alkalization of the root surface. This process involves the induction of malate transporters, activation of proton ATPases, and alterations in cell wall polysaccharide composition. These adaptations aim to minimize aluminum accumulation within cells, thereby mitigating oxidative damages through enhanced antioxidation pathways.

Aluminum tolerance also involves malate transporters (AlMTs) in wheat and *Arabidopsis*, essential for Al exclusion [47]. Increased cytoplasmic calcium (Ca^{2+}) is another aspect of Al tolerance, affecting cell division inhibition in roots [48]. In wheat root apices, Al toxicity inhibits phospholipase C (PLC), affecting downstream phosphatidyl inositol 4, 5 bisphosphate (PIP2) signaling. PIP2 and its associated genes, along with actin proteins, play roles in Ca^{2+} signaling under Al stress [49]. The source of Ca^{2+} in Al toxicity is still uncertain, but it influences downstream metabolism and plant sensitivity [50].

Aluminum toxicity impacts secondary metabolite metabolism, particularly in cell walls and phloem tissue [51]. Genes for transporters like glucosyl transferase and antioxidative secondary metabolites like hydroxy ferulic acids have been identified [52]. Hormonal biosynthesis genes and metal chelators are also implicated in various secondary metabolite pathways [53]. Metabolites overexpressed under Al toxicity include those involved in carotenoid and indole alkaloid biosynthesis and pinene degradation, indicating distinct mechanisms for Al exclusion and tolerance. Adenosine triphosphate binding box (ABC) proteins play roles in energy-dependent Al transport and ATP hydrolysis [54]. Aluminum toxicity also correlates with increased lipid and copper transporters, affecting ROS homeostasis and gene expression related to malondialdehyde and protein carbonyl content [55]. Therefore, metabolite fluxes in response to Al are complex, reflecting specific ion effects and oxidative stress metabolism.

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5. Root Phenotypes and Quantitative Trait Loci for Aluminum Toxicity

Soil acidification, particularly from ammonium fertilizer use, has been a major issue in recent decades. This is especially true in areas with inorganic fertilizers and lowland rice fallows [56]. Studies show that excess aluminum ion (Al^{3+}) in soil disrupts root growth, primarily by hindering microtubule formation in root hairs. Therefore, tolerant cultivars should possess several quantitative trait loci (QTLs) those enable sensing of soil acidity, induction of receptor protein on cell membranes, perception of aluminum (Al)-induced redox changes, and amplification of antioxidation pathways [57]. Major QTLs related to Al toxicity might also be linked to water or other metal stresses often involving channel proteins, gateway proteins and other transmembrane domains [58].

Other vital QTLs include those aiding in the development of transcription factors for cell wall modifications, such as pectin methyl esterase genes. These genes, upregulated within six hours, help form stable complexes with Al. Their enzyme activity also relates to the metal's adsorption capacity, suggesting bio-sequestering [59]. Aluminum stress remodels cell wall structures, involving sugar residues and special QTLs with glucosyl transferase on the cell wall, impacting not only glucose but also galactose, glucuronic acid, and xylose modifications [60,61].

Recent developments have introduced strategies to modulate complex interactions of environmental stresses, including the use of bio-stimulants to modify root growth under Al toxicity [62]. For instance, pyroligneous acid, a common bio-stimulant, can reduce Al-induced root growth inhibition, enhancing resilience to metal toxicity and restoring yield [63]. This compound, derived from organic residue carbonation in low-oxygen conditions, contains a mix of bioactive water-soluble moieties, such as sugar and alcohol derivatives esterified with phenolics and organic acids.

In crops like *Triticum*, certain QTLs, like those for anaerobic carbohydrate metabolism (alcohol dehydrogenase, lactate dehydrogenase, pyruvate decarboxylase), have been identified for Al toxicity resistance [64]. Bio-stimulants can also enhance antioxidative enzymes like peroxidases and promote transcription factors (Auxin Response Factor) in seedlings pre-treated for Al toxicity. These QTLs link carbon concentration mechanisms to Al toxicity, focusing on the development of primary metabolites like organic acids and glucosyl residues [65]. Aluminum tolerance also benefits from improved photosynthetic reserve acquisition, particularly in C_4 plants.

6. Metabolite Shifting from Central Carbon Metabolism

Plants have adapted cellular and metabolic strategies to mitigate aluminum (Al) toxicity. Typically, the citric acid pool and other organic acids form complexes with Al, aiding in its exclusion [66]. This mechanism involves a slow Al entry into root cells, followed by acid secretion, acid ionization and the formation of chelate compounds with Al at the center [67]. In the rhizosphere, plants secrete malic acids and siderophores to chelate metals extracellularly [68]. Tolerant cultivars are capable of bio-excluding metal and detoxifying intracellular Al by forming non-toxic aluminum-chlorohydrate (Al-COOH) complexes, a result of an enhanced carbon concentration mechanism.

In both C_4 and C_3 species, stable carboxylated products like 3-phosphoglyceraldehyde are crucial for Al interaction [69]. Central carbon metabolism supplies carbon intermediates for osmolytes, antioxidants, and other secondary metabolites aiding in Al tolerance [70]. Calvin cycle, glycolysis, hexose monophosphate shunt, and the citric acid cycle contribute to the respiratory substrate, growth, and yield under stress, requiring substantial adenosine triphosphate (ATP) consumption [71]. Studies indicate metabolic shifts from primary metabolism to transitory pathways with specialized metabolites, particularly shikimic acid pathway-derived flavonoids in roots [72].

Mitochondrial metabolism, involving oxidative organic acid decarboxylation and oxidative phosphorylation, is essential for ATP biosynthesis in response to Al influx in roots. Glycolytic reactions produce pyruvate, influencing downstream organic acid pathways. These acids, particularly malic and citric, can lower intracellular and rhizospheric pH, reducing Al solubility [73]. Enhanced photosynthetic activity in Al-tolerant cultivars is linked to these downstream residues from the central carbon pool [74].

Identifying specific root metabolic pathways and shifts in metabolic reactions is essential for understanding photosynthesis and respiration in tolerant species. Recent advancements in omics,

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especially metabolomics, offer insights into complex reaction webs under Al stress [75]. Analyzing qualitative and quantitative aspects of metabolomes helps understand signal transduction and genome alteration in response to Al, with rice varieties serving as effective models for studying root-based metabolite changes [76]. Both constitutive (ALMT families) and inductive (MATE families) metabolomes play significant roles in Al tolerance, whether through exclusion or detoxification.

7. Aluminum-Induced Signaling for Reactive Oxygen Species Development

Aluminum toxicity is most severe in its soluble form, particularly as free aluminum ion (Al^{3+}), which is prevalent in acidic environments as aluminum hydroxide ion [$Al(OH)^{+}$ and $Al(OH)^{2+}$] [77]. Aluminum-induced phytotoxicity primarily stems from specific ion effects, leading to reactive oxygen species (ROS) generation and various cellular impacts. This ROS generation often results in programmed cell death (PCD), characterized by caspase-like activation, chromatin condensation, nuclear dehydration, and chromatin fragmentation [78]. Aluminum tolerance in plants involves successful regulation of cellular redox, enabling root growth even with significant metal accumulation [79]. This tolerance is a genotypic trait linked to antiapoptotic processes that balance cellular redox.

In mitochondria, tolerant species regulate mitochondria-dependent PCD, including cytochrome c release and caspase protease activation [80]. Reactive oxygen species generation in plants under aluminum (Al) stress is a key factor in oxidative damage, similar to that observed under biotic and abiotic stress [81]. Aluminum toxicity triggers oxidative bursts in chloroplast and mitochondrial compartments, disrupting normal redox reactions [82]. Although Al is not a transition metal and doesn't catalyze redox reactions like Fenton's reaction, it induces ROS formation in mitochondria through processes like univalent reduction of O_2 [83]. The resultant hydrogen peroxide (H_2O_2), while cytotoxic, also plays a dual role in signaling for acclimation and tolerance at low concentrations. However, higher H_2O_2 concentrations accelerate PCD and senescence.

The relationship between Al toxicity, ROS bursts, and antioxidative enzyme synthesis is crucial for understanding plant survival under oxidative stress [84]. This understanding can help mitigate lipid peroxidation in cellular membranes and compartments. Current research focuses on the correlation between Al-toxicity and plant survival in acidic soils, where Al^{3+} bioaccumulation is a concern. Agronomic measures to neutralize soil acidity, such as the use of amending chemical residues, are essential for managing this issue.

8. Comprehensive Genomics for Aluminum Toxicity

Aluminum (Al) tolerance in different species, including *Oryza sativa* L., which is more tolerant than other cereals like maize, rye, sorghum, and wheat, involves varied gene expressions in roots against the metal [20]. This includes specific Al-responsive gene expressions involving transcription factors (TFs), particularly basic leucine zipper (bZip) and regulatory enzymes like protein kinases [85]. Gene regulation in the context of Al toxicity is understood to involve post-translational modifications such as phosphorylation and adenylation of TFs, which can be characterized at the nuclear level [86].

This mechanism includes repression, activation and co-regulation of genes related to metal tolerance. Aluminum signaling involves different chemical species interactions with root proteomes, leading to changes in gene expression and root activities [87]. Molecular studies in rice have identified and cloned several quantitative trait loci (QTLs) like signal transduction and activation of RNA 1, Nramp family 1, *Oryza sativa* acetolactate synthase 1, *Oryza sativa* magnesium transporter 1 (STAR2, Nrat1, OsAlS1, OsMGT1), which are significant in the context of aluminum ion (Al^{3+}) tolerance [88]. These genes are involved in the generation of superoxides, free radicals, and other reactive oxygen species (ROS) producing genes.

In *Arabidopsis*, salicylic acid-induced nicotinamide adenine dinucleotide phosphate hydrogen [NADP(H)] oxidase reactions are common for specific ROS production, regulating Al toxicity metabolomes [89]. Aluminum ion initially induces nitrogen oxidase (NOX) activity, linked to superoxide production. Two pore segment channel 1 (TPC1) channel protein activation on the

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membrane increases Ca^{2+} influx [90]. This influx triggers salicylic acid synthesis and accumulation, which in turn boosts NADP(H) production in root tissues, affecting cellular redox [91].

Vacuole-mediated cell death, a genome responsive pathway for Al toxicity, is another mechanism, differing from mitochondria-associated cellular death where redox-responsive genes are crucial [92,93]. This process, regulated by proteolytic genes, leads to vacuole collapse and membrane disruption in roots, especially under Al exposure [94]. Protease activity, NADP(H) oxidase, and TPC1 activation are considered key paths for Al resistance [95].

Aluminum phytotoxicity also involves vacuolar membrane lysis, leading to vacuole collapse. In tissues with excessive Al, caspase-like activities trigger vacuolar processing enzymes (VPE) functioning, disrupting cell membranes. VPE functions are also linked to root growth sensitivity, particularly in meristematic regions sensitive to Al toxicity. Numerous genes including VPE1 QTLs are upregulated in response to Al toxicity indicating their involvement in cell death events [96].

9. Special Metabolomic Pathways to Register Aluminum Toxicity

Mitochondrial pathways in response to aluminum (Al) toxicity involve a specific branch point at the ubiquinone (ubQ) site, triggering alternative pathways. The upregulation of genes related to vacuolar processing enzyme 1 (VPE1) quantitative trait loci (QTL) has been linked to cell death events under Al toxicity. Alternative oxidase (AOX) diverts electrons from the main transport chain to reduce molecular oxygen without energy production, thereby reducing reactive oxygen species (ROS) burden under Al toxicity [97]. Excessive Al causes complex II and III to be bypassed, leading to a reduced ubQ pool, indicating oxidative redox activity [98].

Under high aluminum (Al) toxicity, there's a significant increase in alternative oxidase (AOX) transcripts, regulated both transcriptionally and translationally. In non-stressed plants, both transcript abundance and enzyme activity of AOX are low. Aluminum accumulation downregulates root respiration through complexes III and IV, diminishes succinate-dependent electron transfer, and reduces cytochrome oxidase activity [99]. This results in increased ROS and free radicals, consuming energy to reduce ROS through nicotinamide adenine dinucleotide phosphate hydrogen NADP(H), flavin adenine dinucleotide ($FADH_2$), and other reducing equivalents. Plants responding to Al is finally accomplished at the nuclear level where a number of gene expression ensure various intermediate products those singly or in combination influence the cellular phenomena (Figure 5).

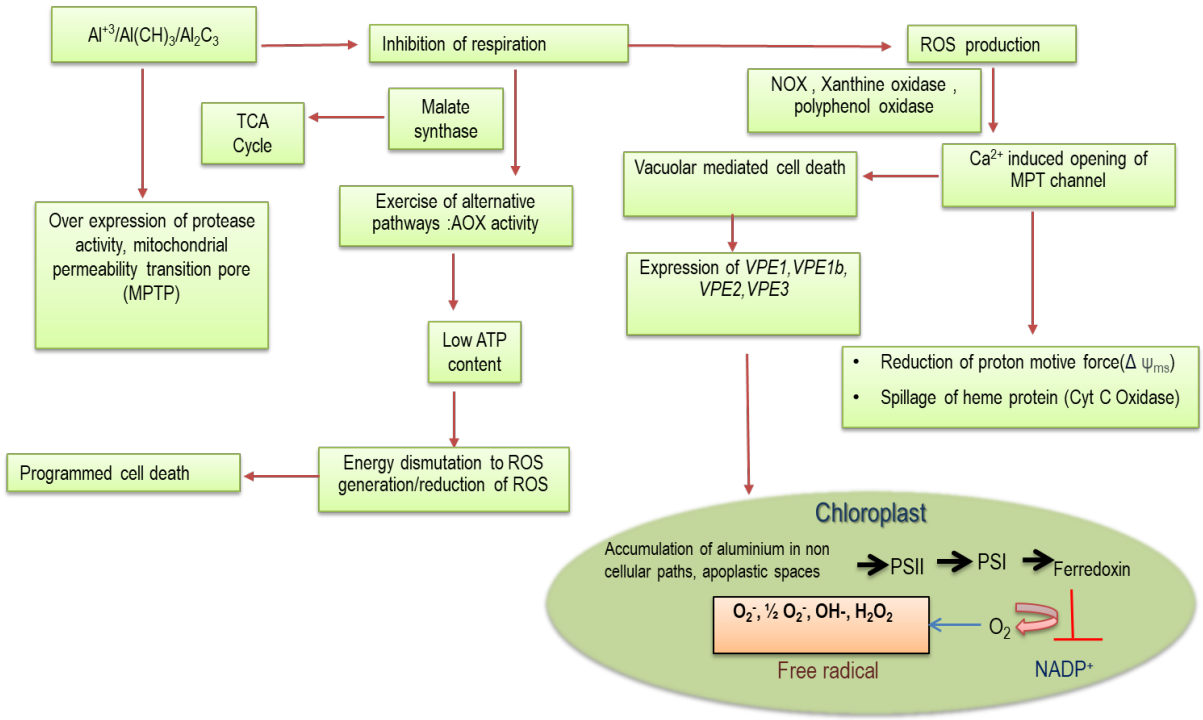

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Figure 5. Depicting the genotoxic effects of aluminum (Al) through various intermediate gene expressions that individually or synergistically influence cellular phenomena. Initially, aluminum ion (Al^{3+}) stimulates ATP production, while simultaneously down regulating the expression of other electron transport carriers, leading to the dismutation of oxygen into free radicals/ reactive oxygen species (ROS). This ROS production triggers the tonoplast membrane, where calcium ion (Ca^{2+}) activates mitochondrial permeability transition (MPT) channels. Concurrently, vacuole-mediated cell death is facilitated by the overexpression of vacuole-localized cysteine protease genes like *vacuolar processing enzyme* genes (*VPE1, VPE1b, VPE2, VPE3*), releasing Al from the vacuole into the cytosol. The over activation of MPT channels disrupts the proton motive force ($\Delta\psi m$) and causes spillage of cytochrome c oxidase, underlying the oxidative damage. The tricarboxylic acid (TCA) cycle is activated, triggering anaplerotic reactions and inducing genes for malate synthase, citrate synthase, and oxaloacetate decarboxylase. Mitochondrial membrane leakage, linked to the overexpression of mitochondrial membrane permeability transition pore (MPTP) proteins and increased protease activity, also contributes to this process. Collectively, these events culminate in programmed cell death (PCD) of the roots, completing the cycle of Al toxicity.

Differential gene expression shows that major tricarboxylic acid (TCA) cycle enzymes like succinate dehydrogenase, alpha-ketoglutarate dehydrogenase, and malate synthase are targeted to generate NADP(H), bypassing complexes III and IV to AOX [100]. Aluminum-treated tobacco roots experience a significant increase in ROS and a consequent decline in adenosine triphosphate (ATP) synthesis [101]. This suggests that Al-induced inhibition of respiration down regulates mitochondrial ATP production, affecting the bioenergetics status of tissues and potentially signaling programmed cell death (PCD) in roots. This effect may extend to shoot tissues, where Al toxicity leads to early senescence and foliage abscission [102].

Furthermore, the mitochondrial genome under Al toxicity overexpresses special proteins on the outer membrane. Excess Al toxicity opens mitochondrial transition pores, leading to the leakage of matrix-based proteins, loss of matrix potential ($\Delta\Psi m$) and impaired ATP generation [103]. Aluminum toxicity stimulates these pores, releasing cytochrome c oxidase proteins, ultimately triggering programmed cell death (PCD) [104]. Oxidative stress, therefore, is linked to mitochondrial metabolomics, potentially driven by elevated calcium ion (Ca^{2+}) levels and increased ROS, leading to further peroxidation reactions. Mitochondrial permeability transition, influenced by Ca^{2+} under Al toxicity, involves specific membrane proteins in the process of cell death.

10. Special Metabolites and Their Contribution to Aluminum Tolerance in Plants

Tobacco cells exposed to aluminum (Al) toxicity accumulate caffeic acid and chlorogenic acids via the phenylpropanoid pathway. A key enzyme in this process is L-phenylalanine ammonia-lyase (PAL), whose activity is linked to phosphate availability in plant tissues. Aluminum toxicity induces phosphate deprivation, increasing PAL activity [105]. The phenolics produced by this pathway act as antioxidants, protecting against lipid and protein peroxidation and macromolecule carbonylation. When exposed to a combination of aluminum ion (Al^{3+}) and ferrous ion (Fe^{2+}), plant cells activate these antioxidants, indicating tolerance against these metals. This suggests that the combined effects of metals are mediated by the induction of phenolic antioxidants like phenylpropanoid residues [106].

Organic acids from the tricarboxylic acid (TCA) cycle are other metabolites reducing rhizotoxicity in Al-contaminated soil. Aluminum ion (Al^{3+}), prevalent in acidic soils, induces proton (H^+) secretion in some sensitive species. The acidic pH, with its high H^+ concentration, enhances solubilization in the soil, mitigating phytotoxic impacts. A lower pH can also reduce the negative charge of biological membranes, decreasing Al binding affinity [107].

In *Arabidopsis*, citrate induction is crucial for the expression of the transcription factor *Arabidopsis thaliana* SENSITIVE TO PROTON RHIXOTOXICITY 1 (AtSTOP1), associated with specific H^+ transporters. Mutations in STOP1 can reduce Al tolerance due to inadequate citrate production, indicating AtSTOP1's role in activating other genes, including those in antioxidative cascades, for co-tolerance against Al^{3+}.

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Other metabolites include methylated residues formed during reactions to Al toxicity, contributing to H^+ de-acidification. A protein similar to glycerophosphodiesterase (GPD) is overexpressed at the transcript level in tobacco soon after Al exposure, necessary for methylation of CCGG islands in the promoter regions of antioxidative genes. This methylation pattern changes with Al exposure, similar to the response to herbicide-induced reactive oxygen species (ROS) [108]. Since ROS is common in metal induction, demethylation may occur through the development of oxygen radicals. Cytosine residue methylation decreases upon Al^{3+} application in roots, indicating metal induction can cause total DNA methylation, potentially leading to genotoxicity.

11. Metabolomes under Regulation of Signal Transduction and Protein Turnover

Several residues are believed to be involved in signaling and perception through the cell membrane during aluminum (Al) transportation. Specific guanosine triphosphate (GTP) binding proteins, isolated from cell membranes, are known to function in ion channel opening, cell volume proliferation and energy-mediated processes [109]. Various species, including cereals and *Arabidopsis*, show mass proteome expression changes in roots exposed to Al, indicating global gene expression alterations related to antioxidation under metal toxicity. Vacuolar hydrogen ion ATPase (H^+/ATPase) activity in roots is suggested to create an electrochemical gradient for aluminum ion (Al^{3+}) activation through a proton (H^+) antiporter system [110]. In Al-tolerant *Triticum* species, a tonoplast ATPase protein is overexpressed, linked to metal absorption, though reports vary on ATPase expression levels across different crop species irrespective of Al sensitivity and tolerance. This suggests that Al compartmentalization is an avoidance strategy, with plants variably responding to H^+/ATPase proteome expression for exclusion [111].

Mitochondrial ATPase activity, isolated from mitochondrial intermembrane space, varies with protein subunit expression. For instance, soybean shows reduced membrane-bound ATPase activity but increased mitochondrial ATPase under Al toxicity. Parallel expression of organic acid-secreting proteins and P-type ATPase suggests dual roles in sequestering Al in vacuoles or apoplastic spaces following esterification. In legumes like soybean, an increase in citrate synthase activity from the tricarboxylic acid (TCA) cycle correlates with co-transport by ATPase-driven activity.

Homeo-domain proteins from the MCM1, AGAMOUS, DEFICIENS and SRF-Box (MADS-Box) family in soybean have distinct DNA binding domains for regulatory factors in Al tolerance genes [112]. Metabolomes contributing anaplerotic reactions with citrate, malate, and oxaloacetate are crucial for chelating metals in apoplastic spaces. In some rice cultivars, tolerance to Al is linked to overexpression of MADS-Box factors, enhancing expression of *vacuolar processing enzyme* (*VPE*) genes and H^+ extrusion [113].

Protein regulation through lysis is also significant in Al toxicity, where root growth retardation is linked to protein lysis [114]. In rice and other crops, the induction of proteasomes and heat shock proteins (HSPs) occurs under varying Al^{3+} concentrations where HSPs are involved in protein folding, transport, stress resistance, and gene expression [115]. This suggests the necessity of protein post-translational modification under Al toxicity. The induction of HSP70 and its derivatives might be a key factor in Al tolerance, with protease activity indicating tolerance through the turnover of degenerative proteins. In tolerant cultivars, protease-mediated lysis of misfolded proteins may alleviate degradation from root membrane lysis and growth inhibition [116].

12. Transcriptional Control of Aluminum Tolerance in Roots Under Acidic Cytosol

Acidic environment is the most favorable keys to aluminum (Al) sensitivity of root physiology where a number of genes activation is directly or indirectly involved through transcription factors. Transcription factors have been in knowledge in rice since last decades with regards to plant responses to different stimuli. The factors like ADP-ribosyltransferase 1 (*ART1*) in rice are most important for Al tolerance as already mentioned earlier [117]. Now it is in quest to identify a few interacting proteins with *ART1* in future. Already nine of the downstream genes induced upon *ART1* factors have been functionally characterized for Al tolerance exclusively in rice [118]. Moreover, tolerant species to Al have also been scanned for a few quantitative trait loci (QTL) and used for

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molecular markers. Still the fine mapping and cloning of those QTLs are yet to be deciphered for breeding. Soil enriching with low pH is a major domain for cultivated crops in which transcriptome-wide association studies combining with genome wise associated studies are most important to identify the direct involvement of gene versus trait association. Besides, well characterized *ART1* regulated gene, the membrane localized polypeptide of 53 amino acids residue is identified [119]. The gene *C-terminal domain phosphatase* (*CTD3*) which is basically metal specific and exhibit no transport ability for Al but directly binds to Al in roots [120]. Moreover, *CTD3* from rice (*OsCTD3*) is specifically required with a strong promoter for its expression on membrane to detoxify the Al. External detoxification in roots for Al also involves a lot of acid secretion in rhizosphere in contrast to internal bio-accumulation of metal. In a number of cases, biomolecules like pectin, hemicelluloses and other polysaccharides on the root symplast as well as endodermal casparian strips are required for sequestration of the metal [121].

The modality of gene regulation with transcription factors through phosphorylation-dephosphorylation, adenylation-deadenylation is important for cell signaling with Al. In acid soil a C_2H_2 zinc-finger protein, homologue (sensitive to proton rhizotoxicity 1) to SENSITIVE TO PROTON RHIXOTOXICITY 1 (STOP1) as found in *Arabidopsis* can regulate malate transporters *Arabidopsis thaliana* multidrug and toxic compound extrusion 1, *Arabidopsis thaliana* aluminum activated malate transporter 1 (*AtMATE1*, *AtALMT1*). These two factors can bind the basic upstream elements [GGN(T/g/a/C)V(C/A/g)S(C/G)] where the downstream genes would be malate, citrate, oxalate and other organic acid [122]. This promoter can control a minimum of 30 genes covering *expansin A10, magnesium transporter 1, signal transduction and activation of RNA 1, ferric reductase defective like 4/2,* (*EXPA10, MGT1, STAR1/2, FRDL4, FRDL2*) expressed on extracellular or intracellular spaces [123]. The specific ATP binding cassettes and membrane binding domain similar to *E.Coli* ABC transporter resemble the two basic transcription factors (STAR1-2) of the *ALMT1* gene are more characterized. The homeodomain complex, STAR1-STAR2 localized at the membrane that transport pyrimidine nucleotide (UDP-glucose) that is required for modification of cell wall and callose [124]. Transcription factors like FRDL4 is required for secretion of organic acids under Al toxicity in roots for internal detoxification. This factors is basically $(cys\text{-}cys)_n$, a homologue peptide can directly bind with Al and reducing Al detoxification, however, externally. On the other hand, magnesium transport and upregulation of *MGT1* can reduce internal Al concentration superseding other metals [125]. For vacuolar sequestering of Al other member of Nramp family 1 (Nrat1) is important specifically for aluminum ion (Al^{3+}). For the same species a tonoplast bound ABC transporter is recognized from rice *Oryza sativa acetolactate synthase 1* (*OsALS1*) which takes part for activations of promoter under low pH. There exists a strong correlation for Al tolerance with expression of *Os*ALS1, *Os*FRDL4 like factors in roots under acidic pH of soil [126]. Along with the normal ability in organic acid transportation, genes encoding expansin like proteins on cell wall elongation in root tips are dependent on activation of transcription factors. From the most sensitive cereals to Al (e.g. rice) another few transcription factors like ART2 are also important to interact with other factors (Figure 6). Likewise, wrinkled transcription factor (WRKY) domains which is characterized with a zinc–finger structure (either Cx4-5Cx22-23HxH or Cx7Cx23HxC) on the upstream sequence containing W-BOX promoter is also applicable for Al sensitivity [127]. For many factors against Al toxicity like STAR1/2 can interact with WRKY in a dimer configuration where the *cis*-element is represented with a consensus sequence of TTGACC/T [128]. The expression of *OsFRDL4* is controlled by *Os*WRKY22 where localization of expression is plasma membrane, nucleus, tonoplast etc. Sometimes there found the co-expression of *Os*WRKY22 and ART1 in dimer configuration within the nucleus for citrate secretion. There are a few factors like abscisic acid stress and ripening (*ASR*) genes are identified in response to wider activation of Al tolerant genes. These ASR factors are mostly acted as chaperons and transcription factors and amenable to *ASR5* gene involving in Al tolerance in rice [129]. *ASR5* is nucleus as well as cytoplasm specific in expression and regulates the co-expression of different gene from Al-induced stress tolerance. In more recent version of genome wise array of activating genes, ASR5 *trans*-factor is shown to bind STAR1 promoter and in downstream can also regulate *Nrat1*. From rice also, *ALS1* encodes an ABC transporter belonging to transporter associated antigen

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processing protein (TAP) can be induced only by Al^{3+} but not with other metals at low pH [130]. These are more advocated with expression of transcription factors for the genes expressed in rice *Oryza sativa pectin methylesterase* (*OsPME*) required for pectin methyl esterase in Al detoxification for apoplastic spaces. Thereby, it would be conclusive that transcriptome for Al tolerance is mostly based on sensitization of transcription factors where proteins either singly or in combination can regulate the expression when molecular mechanism is concern.

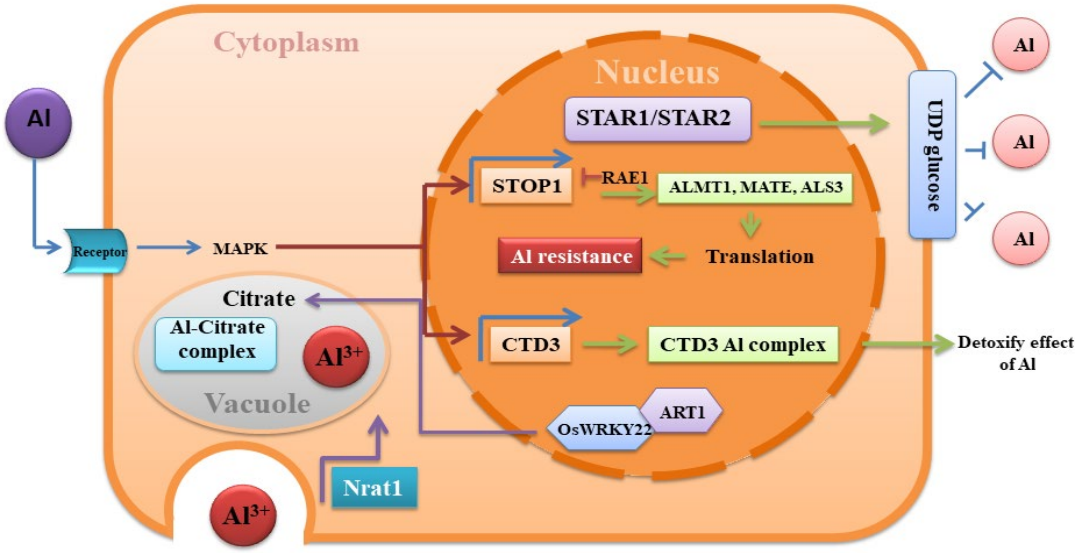

Figure 6. A hypothetical model for control of organic acid (citrate) exudation in root against aluminum ion (Al^{3+}) toxicity. At the nuclear level, activation of SENSITIVE TO PROTON RHIXOTOXICITY 1 (STOP1) initiate the signaling pathway which otherwise activate *multidrug and toxic compound extrusion 1, Arabidopsis thaliana aluminum activated malate transporter 1, amylotrophic lateral sclerosis 3* (*AIMTE, MATE, ALS3*) genes. STOP1 also upregulates the expression of *RNA export 1 (RAE1)* which in feedback regulation reduce STOP1 through degradation and ubiquitination. The *signal transduction and activation of RNA 1 and 2* (*STAR1, STAR2*) otherwise induces UDP glucose like residues which conjugates with aluminum (Al) for extracellular detoxification. Transcription factors like *Oryza sativa* wrinkled transcription factor (*Os*WRKY22) forms dimer with ADP-ribosyltransferase 1 (ART1) and collectively induces citrate biosynthesizing gene. Expressed proteins Nramp family (Nrat1) on cellular membrane engulfs Al^{3+} within the apoplastic space for chelation.

13. Phytohormone Signaling and Functioning for Aluminum Tolerance

Phytohormones are the most active as well as crucial signaling residue in plant development even act under environmental stress to rescue the sustainability. Aluminum undoubtedly invites a metal stress and also is coincided by plant hormones including auxin, gibberellins, ethylene, abscisic acid, jasmonic acid and other secondary elicitors like nitric oxide [131]. External application of plant growth regulators and their synthetic analogues in most of the cases record the similar effects. Rice under acidic soil shows a strong affinity of aluminum ion (Al^{3+}) sensitivity and thereby, induces specific transcription factors like ADP-Ribosyltransferase 1 (ART1) (C_2H_2-zinc finger). This factor is played with specifically regulation of 31 downstream genes under auxin control [132]. The genes include *rice* aluminum transporters like nicotinate riboside transporter 1/*Oryza sativa* nicotinate riboside transporter 4 (*NRAT1*/*OsNRAMP4*) those are characteristically aluminum (Al) tolerance gene where auxin metabolism is involved. Since, inhibition of root growth is the most vulnerable for Al toxicity, changes in auxin synthesis and translocation may be important as recorded in *Brassica* [133]. Auxin signaling with its principle functioning for stress induced root growth inhibition has demonstrated the expression of auxin transporter proteins like *Oryza sativa pin formed 3* (*OsPIN3t*). This transporter activity is also observed with the reduction of adventitious growth in *Arabidopsis*. The toxicity of Al inhibits expression of *pin formed 2* (*PIN2*) gene encoding protein for auxin transport towards root. Downregulation of its expression can reduce endogenous auxin concentration in roots

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and thereby, sensitivity of Al is more marked with impede root growth [134]. Other transporter genes for auxin including (*PIN2*), *ferric reductase defective like 4* (*FRDL4*), *auxin resistant 1* (*AUX1*) are also proposed to correlate with ethylene production. Ethylene happens to be undoubtedly a good signaling residue resulting impeded root growth under Al toxicity [135]. Ethylene has a direct relationship with Al^{3+} stress where respiratory burst oxidase homolog (RBOH) mediated reactive oxygen species (ROS) alteration can induce its synthesis. Ethylene is also played as direct signaling residue for auxin biosynthesis where genes like *Aux1*, *PIN1*, *tryptophan aminotransferase related 1*(*TAA1*) are involved for root growth inhibition under Al stress [136]. On the other hand, abscisic acid (ABA) typically recognized as stress hormone also cloned from rice where few homologues abscisic acid-stress-ripening (*ASR* family) are demonstrated ABA and Al^{3+} stress at a time. The increase of synthesized ABA is directly related to cellular signaling where hydrogen peroxide (H_2O_2) is most important ROS. The signaling cascade under Al stress as perceived through cell membrane bound receptor is also co-induced with *9-cis-epoxycarotinoid dioxygenase* (*NCED*). This gene encodes the protein which splits the carotenoids for ABA biosynthesis. Higher expression of *NCED* accumulates intra cellular ABA in support to osmotic adjustment under Al^{3+} stressed crops [137]. ABA synthesis in Al treated root cell can induce ATP-binding cassette transporter (ABC) and nitrate transporter1/peptidetransporter (NPF) for its translocation in different organs. Subsequently, ABA serves as a stimulus for resistance mechanism against Al toxicity by increasing water use efficiency, decrease transpiration rates [138]. Additionally, ABA accumulation in some root tissues induces the formation of protein complex via receptors like pyrabactin resistance 1/pyrabactin resistance-like (PYR/PYL), and protein phosphatase 2C (PP2C). This complex induces activation of some protein kinases which otherwise activate by phosphorylation of specific anion channels to release malate against Al accumulation [139]. This family encodes specific transcription factors amino-terminal enhancer of split-related 5 (*Os*AsR5) responsible for co-expression of *signal transduction and activation of RNA 1* (*START1*), Nramp family of factors [140]. Another gene *FRDL4* has also been cloned from roots sensitive to Al^{3+} where the tolerance to the metal is manifested by development of ROS. The ROS otherwise would be an inducer for different genes related to Al tolerance most specifically synthesis of organic acids. The organic acids in turn are also induced by some growth regulators like ethylene under Al stress [141] Ethylene in wheat has also been reported with negative control for *Al activated malate transporter* (*ALMT1*) that reduces the root secretion of organic acids. Thereby, mutation of this gene would be sufficient for sensitive to Al toxicity [142]. Ethylene has also been reported for Al tolerance, however, in cross talk with auxin interference. The expression of ethylene biosynthetic genes like *1-aminocyclopropane-1-carboxylic acid synthase* (*ACSs*), *1-aminocyclopropane-1-carboxylic acid oxidase* (*ACOs*) those induce a direct promotion of ethylene biosynthesis under Al^{3+} toxicity in sensitive cultivars [143]. On signaling pathways ethylene activates the transcription factors like ethylene insensitive 3 and 1 (EIN3 and EIN1) expression which otherwise controls the root growth. EIN3 has a good binding ability to the promoters of a few genes *YUCCA 9* (*YUC9*) to interfere the growth of root apex transition zone under Al toxicity [144]. Toxicity of Al in root sensitization finds expression of few genes *TAA1*, *YUCs* [*YUC3/5/7/8/9*]) for transcription factors in regulation of ethylene biosynthesis in contrast to auxin. Therefore, the toxicity of Al in root growth moderation would be a supplementary effect where auxin and ethylene expression in effected tissues is simultaneous. Ethylene is also responsible for regulation of both local auxin biosynthesis as well as auxin transport by induction of genes like *TAA1*/*YUCs* and *PIN2*, *AUX1* respectively [145]. The distinct action of ethylene in reversal of auxin sensitivity may differ through plant species when exposed to Al^{3+}, still, their molecular mechanism is obscure. Gibberellins (GAs) are most implicated for metal tolerance in preservation of carbohydrate metabolism and vegetative growth. In that context, Al^{3+} induced inhibitions of root elongation following sugar translocation to roots are also reported as possible path for metal tolerance. Still, downregulation of Al induced ROS accumulation and changed root cell wall deposition may be apprehended as membrane localized Al sequestering [146]. The function of GAs with multiple attributes may collectively include biosynthesis of bioactive GAs in tomato can improve growth, pigment content, CO_2 fixation rate in soybean under metal stress [147]. Cytokinin is less explored under Al toxicity but is also related to cell wall alkalization, influx

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of H^+ over the membrane and in a few cases alteration of expression for cell wall modifying genes. Cytokinin biosynthesis is directly influenced by auxin mediation where transcription factors like transport inhibitor response 1 (TIR1), auxin signaling f-box (AFB) are responsible for encoding other factors on upstream binding sequences. The factors include most importantly auxin response factor (ARF) 7-19 which otherwise involved the isopentenyl pyrophosphate (IPTs) residue biosynthesis as precursor of cytokinin [148]. Growth substances like jasmonic acids is also played in differential manner against Al toxicity. Jasmonic acid application in Al sensitive root species induces adaptive responses in plants by regulation of ROS metabolism (Figure 7). Two genes, *coronatine insensitive 1* (*COI1*) and (*myeloblastosis viral oncogene homologue 2*) *MYC2* are found to be over expressed in root growth inhibition by altering the ethylene concentration. The phenotypic analysis with mutants insensitive to jasmonic acid biosynthesis and signaling recorded in response to Al^{3+} with direct involvement for ethylene accumulation. The inhibitions of formation and depolymerizations of cortical microtubules in those mutants are responsible for *COI1* gene expression under Al stress [149]. Moreover, malate transporter expression by *aluminum activated malate transporter* (*ALMT*) is also dependent on regulation of jasmonic acid signaling where *COI1* is involved. Therefore, phytohormones being a crucial signaling residue in favor of Al tolerance where pivotal roles are played mostly on metal sequestering, ion complex formation, water balance, and ROS homeostasis.

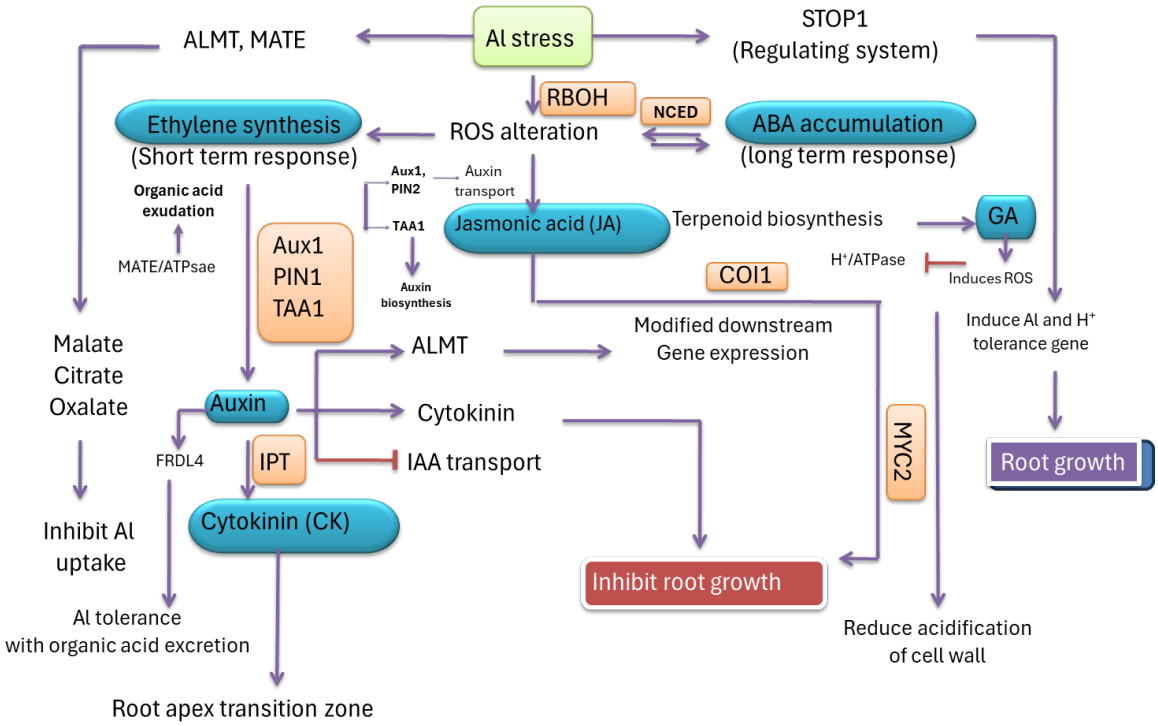

Figure 7. The involvement and crosstalk of plant growth substances against aluminum (Al) sensitivity in plants. Growth hormones like auxin (Aux), gibberellic acids (GA), abscisic acid (ABA), cytokinin (CK), jasmonic acid (JA), ethylene those induce several cellular processes in roots of plants exposed to Al hyper accumulation. Both external and internal detoxification by exclusion, sequestering, chelation reactions are important under hormonal influence. Reduction of H^+ by inhibition of H^+/ATPase activity is important to downregulate the acidification of apoplast of root to reduce the aluminum ion (Al^{3+}). Ethylene induces auxin accumulation as well as auxin transportation by pin formed 2 (PIN2) mediated polar transport that results root growth inhibition. Acidification of cell wall by organic acids (malate, citrate, oxalate) is important for ligand formation with metal. Abscisic acid and ethylene has synergistic actions with a negative regulation where respiratory burst oxidative homologue (RBOH) and short term response respectively are important. Abscisic acid can induce also reactive oxygen species (ROS) biosynthesis directly influence Al tolerance genes. Other substances like JA and CK are distantly related to Al tolerance by over expression of *coronatine insensitive 1, myeloblastosis viral oncogene homologue 2* (*COI1, MYC2*) and direct inhibition of root growth.

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14. Conclusions and Future Perspective

The discussion highlights that aluminum (Al) toxicity impacts plants in two main phases: the establishment of metal-induced specific ion effects and the development of an oxidizing redox environment prone to peroxidation reactions. Initially, physiological and cellular events are affected, including reduced root membrane permeability, increased dehydration, vacuolar disintegration, nutrient release over the tonoplast membrane, cytosolic pH alterations, caspase activity acceleration leading to programmed cell death (PCD) and dissolution of cellular metabolites into soluble products. Conversely, oxidative redox changes can lead to biomolecule degradation, including lipid peroxidation, protein carbonylation, and demethylation of specific coding regions or gene promoters. Altered metabolite fluxes, particularly in organic acid turnover, can enhance Al toxicity tolerance. Another key tolerance mechanism is the pattern change in secondary metabolites, especially the development of complex polysaccharides like callose, preferred in selective cultivars. This development is also linked to alternative oxidase (AOX) expression and ATPase activities, which help prevent electron misfiring from cellular organelles and stimulate energy-producing pathways. Additionally, specialized transcriptional regulation by MCM1, AGAMOUS, DEFICIENS and SRF-Box (MADS Box) proteins and other transcription factors is crucial for activating specific genes under Al toxicity. This process is complemented by protein degradation mechanisms that impede root growth, countered by the expression of heat shock proteins (HSPs) or chaperones, which aid in refolding misfolded proteins. Therefore, Al tolerance is a multifaceted process involving both enzymatic antioxidation cascades and bio-exclusion of the metal through diverse gene expressions. Future prospects include profiling different proteomes in Al-tolerant species to understand and manage various pathways under Al stress. For instance, examining proteomes involved in cysteine and methionine metabolism could shed light on methyl cycling and glutathione metabolism for antioxidation. Recent advances in proteomic sequencing are poised to provide deeper insights into precise protein expression and its role in Al tolerance.

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