UNIVERSIDADE FEDERAL DE VIÇOSA

Integrated omics approaches for understanding and improving aluminum tolerance in popcorn: from mechanistic insights to breeding

Matheus Pereira Ribeiro
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2025

MATHEUS PEREIRA RIBEIRO

Integrated omics approaches for understanding and improving aluminum tolerance in popcorn: from mechanistic insights to breeding

Thesis submitted to the Genetics and Breeding Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Jose Marcelo Soriano Viana

Co-advisers: Humberto J. de O. Ramos
Vítor Batista Pinto

VIÇOSA - MINAS GERAIS
2025

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
R484i
2025

Ribeiro, Matheus Pereira, 1994-
Integrated omics approaches for understanding and improving aluminum tolerance in popcorn: from mechanistic insights to breeding applications / Matheus Pereira Ribeiro. – Viçosa, MG, 2025.
1 dissertação eletrônica (106 f.): il. (algumas color.).

Texto em inglês.
Orientador: José Marcelo Soriano Viana.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Biologia Geral, 2025.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2025.553
Modo de acesso: World Wide Web.

1. Cereais - Efeito do alumínio. 2. Estresse oxidativo. 3. Genômica. 4. Proteômica. I. Viana, José Marcelo Soriano, 1963-. II. Universidade Federal de Viçosa. Departamento de Biologia Geral. Programa de Pós-Graduação em Genética e Melhoramento. III. Título.

CDD 22. ed. 572.52673

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

MATHEUS PEREIRA RIBEIRO

Integrated omics approaches for understanding and improving aluminum tolerance in popcorn: from mechanistic insights to breeding

Thesis submitted to the Genetics and Breeding Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: July 18, 2025.

Assent:

Matheus Pereira Ribeiro
Author

Jose Marcelo Soriano Viana
Adviser

Essa tese foi assinada digitalmente pelo autor em 31/08/2025 às 16:51:04 e pelo orientador em 31/08/2025 às 17:27:59. As assinaturas têm validade legal, conforme o disposto na Medida Provisória 2.200-2/2001 e na Resolução nº 37/2012 do CONARQ. Para conferir a autenticidade, acesse https://siadoc.ufv.br/validar-documento. No campo 'Código de registro', informe o código **NRJS.T576.UFRK** e clique no botão 'Validar documento'.

To my parents, José Antônio and Ana; to my sister, Aline; to my fiancée, Ana Carolina, for their unwavering support, encouragement and love.

ACKNOWLEDGMENTS

To God for guiding my steps.
To my parents José Antônio and Ana, and to my sister Aline, for their support, affection, love and for believing in my potential.
To my grandparents Amélia (in memoriam), Librantino (in memoriam) and Maria for their love and example.
To my fiancée Ana Carolina, for her love, respect and for supporting me in the most difficult moments. To her parents Sérgio and Márcia, for their friendship.
To my uncles and cousins, for their affection and support.
To my all family, for their support.
To Universidade Federal de Viçosa and the Programa de Pós-Graduação em Genética e Melhoramento, for the opportunity to pursue my education.
This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).
To my adviser, Professor José Marcelo Soriano Viana, for his guidance, friendship and teachings.
To my co-advisers, Professor Vitor Batista Pinto and Professor Humberto Josué de Oliveira Ramos, for their guidance, attention and teachings.
To my friends from the Programa Milho-Pipoca, Programa Milho, Laboratório de Genética Molecular, Laboratório de Sementes and Laboratório de Enzimologia, for their teamwork and teachings.
To Grupo de Pesquisa em Biologia do Estresse Vegetal (UENF) and Laboratório Max Feffer Genética de Plantas (ESALQ/USP) for the partnership and collaboration in the development of this work.
To all my friends with whom I have lived since my undergraduate studies, for their friendship, support and for making this journey so joyful.
To everybody that somehow made this dream come true.

ABSTRACT

RIBEIRO, Matheus Pereira, D.Sc., Universidade Federal de Viçosa, July, 2025. **Integrated omics approaches for understanding and improving aluminum tolerance in popcorn: from mechanistic insights to breeding**. Adviser: Jose Marcelo Soriano Viana. Co-advisers: Humberto Josue de Oliveira Ramos and Vítor Batista Pinto.

Aluminum (Al) toxicity is one of the main limiting factors for agricultural productivity in tropical and subtropical regions, directly affecting plant morphology, physiology and metabolism. In acidic soils, soluble Al^{3+} impairs root growth, reduces nutrient uptake and triggers oxidative stress, with serious consequences for food crops such as maize (*Zea mays* L.). The first chapter, an integrative review, discusses advances in the understanding of Al tolerance in cereals, focusing on maize, rice, wheat, barley, sorghum and, especially, popcorn. The review details the two main tolerance mechanisms: (i) exclusion via exudation of organic acids and phenols, and (ii) internal tolerance based on vacuolar sequestration, cell wall modification and activation of the antioxidant system. The analysis addresses the roles of genes such as MATE, ALMT, STOP1, WRKY and ABC transporters, as well as the contribution of omics approaches, such as genomics, transcriptomics, proteomics, metabolomics and epigenomics, for the identification of regulatory pathways, functional targets and molecular markers. The study highlights that multi-omics integration has revealed coordinated responses between tissues at the molecular level, becoming the basis for the application of modern tools such as marker-assisted selection, genomic prediction and gene editing. The second chapter, of an experimental nature, describes an integrated proteomics and metabolomics approach in a popcorn inbred line previously selected for its tolerance to Al. Seedlings were subjected for 72 h to nutrient solutions with and without Al, and root and leaf tissues were analyzed by LC-MS/MS and GC×GC/TOF-MS. A total of 423 differentially accumulated proteins and 68 differentially accumulated metabolites were identified, with most of the changes occurring in the roots. The tolerance response included the induction of enzymes of the citric acid cycle, citrate synthase, biosynthetic pathways of phenolics, malate and glutathione, in addition to the activation of antioxidant systems. In the leaves, adjustments in energy metabolism, selenium pathways and redox signaling were observed. The integrated analysis revealed functional convergence between proteins and metabolites, highlighting key strategies such as exudation of organic acids, detoxification of reactive oxygen species and structural reinforcement. Together, these studies significantly expand the understanding of the molecular mechanisms of Al tolerance in popcorn,

providing promising biomarkers for genetic improvement programs. They demonstrate the power of integrative biology to transform molecular data into practical applications, driving the development of more resilient cultivars adapted to environments with high soil acidity.

Keywords: proteomic; metabolomic; Al tolerance mechanisms; antioxidant metabolism; TCA cycle

RESUMO

RIBEIRO, Matheus Pereira, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Abordagens ômicas integradas para compreender e melhorar a tolerância ao alumínio em milho-pipoca: de insights mecanicistas a aplicações no melhoramento**. Orientador: Jose Marcelo Soriano Viana. Coorientadores: Humberto Josue de Oliveira Ramos e Vítor Batista Pinto.

A toxidez por alumínio (Al) é um dos principais fatores limitantes à produtividade agrícola em regiões tropicais e subtropicais, afetando diretamente a morfologia, fisiologia e metabolismo das plantas. Em solos ácidos, o Al^{3+} solúvel compromete o crescimento radicular, reduz a absorção de nutrientes e desencadeia estresse oxidativo, com sérias consequências para culturas de base alimentar como o milho (*Zea mays* L.). O primeiro capítulo, uma revisão integrativa, discute os avanços no entendimento da tolerância ao Al em cereais, com foco em milho, arroz, trigo, cevada, sorgo e, especialmente, no milho-pipoca. A revisão detalha os dois principais mecanismos de tolerância: (i) exclusão via exsudação de ácidos orgânicos e fenóis, e (ii) tolerância interna baseada em sequestro vacuolar, modificação da parede celular e ativação do sistema antioxidante. A análise aborda os papéis de genes como MATE, ALMT, STOP1, WRKY e transportadores ABC, assim como a contribuição das abordagens ômicas, como a genômica, transcriptômica, proteômica, metabolômica e epigenômica, para a identificação de vias regulatórias, alvos funcionais e marcadores moleculares. O estudo ressalta que a integração multi-ômica tem revelado respostas coordenadas entre tecidos em níveis moleculares, tornando-se base para a aplicação de ferramentas modernas como seleção assistida por marcadores, predição genômica e edição gênica. O segundo capítulo, de natureza experimental, descreve uma abordagem integrada de proteômica e metabolômica em uma linhagem de milho-pipoca previamente selecionada por sua tolerância ao Al. Plântulas foram submetidas por 72 horas a soluções nutritivas com e sem Al, e tecidos radiculares e foliares foram analisados por LC-MS/MS e GC×GC/TOF-MS. Foram identificadas 423 proteínas diferencialmente acumuladas e 68 metabólitos diferencialmente acumulados, com a maioria das alterações ocorrendo nas raízes. A resposta à tolerância incluiu a indução de enzimas do ciclo do ácido cítrico (TCA), citrato sintase, vias de biossíntese de compostos fenólicos, malato e glutationa, além da ativação de sistemas antioxidantes. Nas folhas, observaram-se ajustes no metabolismo energético, rotas do selênio e sinalização redox. A análise integrada revelou convergência funcional entre proteínas e metabólitos, destacando estratégias-chave como exsudação de ácidos orgânicos,

detoxificação de espécies reativas de oxigênio e reforço estrutural. Conjuntamente, esses estudos ampliam significativamente a compreensão dos mecanismos moleculares de tolerância ao Al em milho-pipoca, fornecendo biomarcadores promissores para programas de melhoramento genético. Eles demonstram o poder da biologia integrativa para transformar dados moleculares em aplicações práticas, impulsionando o desenvolvimento de cultivares mais resilientes e adaptadas a ambientes com alta acidez do solo.

Palavras-chave: proteômica; metabolômica; mecanismos de tolerância ao Al; metabolismo antioxidante; ciclo do TCA

SUMMARY

General Introduction

Soil acidity is one of the major constraints to agricultural productivity in tropical and subtropical regions, directly affecting nutrient availability, soil structure, and plant health. In soils with pH below 5.5, aluminum (Al), which is naturally present as inert silicates and oxides, becomes soluble and highly phytotoxic in its trivalent form (Al^{3+}). This ion interacts with the cell walls of roots, inhibits cell elongation, impairs ion transport, and activates oxidative stress pathways, leading to physiological dysfunction and yield loss (Kochian et al., 2015; Ofoe et al., 2023; Silva, 2012). It is estimated that more than 40% of arable land in the Americas shows some degree of acidity, making Al stress one of the most important abiotic limitations to global food security (Tandzi et al., 2018; Tyagi et al., 2020).

Exposure to Al^{3+} triggers a cascade of physiological, biochemical, and molecular responses that vary according to genotype, tissue, and stress intensity (Chakraborty et al., 2024). In strategic crops like maize (Zea mays L.), sensitivity to Al compromises root system development, reduces water and nutrient uptake, impairs photosynthesis, and causes significant metabolic imbalances. These effects are particularly critical in special maize types such as popcorn (Zea mays var. everta), a high-value commercial crop whose expansion in acidic soils is limited by its elevated sensitivity to Al toxicity (Mutimaamba et al., 2020; Yoshida et al., 2023). Brazil is currently the second-largest producer of popcorn worldwide, with increasing production aimed at both domestic consumption and export (Kist, 2019; USDA, 2025), reinforcing the urgency of developing genotypes better adapted to the country's edaphoclimatic conditions.

Despite its economic relevance, most of the knowledge in the context of functional genomics, with limited availability of reference genome assemblies, stress-responsive gene annotations and publicly available omics data, is extrapolated from maize, which may overlook specific genotypic responses and regulatory mechanisms unique to popcorn. This highlights the

importance of dedicated multi-omics studies on popcorn to reveal its distinct physiological and molecular characteristics under abiotic stress conditions, such as Al toxicity.

Al tolerance in plants involves two main strategies: exclusion, which prevents Al^{3+} entry into root cells via exudation of chelating compounds such as citrate, malate, and phenolics; and internal tolerance, which relies on intracellular sequestration, vacuolar compartmentalization, cell wall remodeling, and antioxidant system activation (Chakraborty et al., 2024; Kochian et al., 2015; Ofoe et al., 2023). These mechanisms are regulated by complex gene networks involving transporters such as ALMT, MATE, and ABC, transcription factors (STOP1, WRKY, NAC, MYB), antioxidant enzymes, cell wall modifiers, and hormonal signaling components (Guan et al., 2023; Liu et al., 2023; Piñeros et al., 2008).

Historically, breeding programs relied on phenotypic selection to improve Al tolerance, based on traits such as root elongation and biomass under stress. However, these traits are highly polygenic and strongly influenced by environmental interactions, making them difficult to select efficiently. The advent of systems biology and omics technologies has made it possible to unravel the molecular basis of stress adaptation. In this scenario, proteomics and metabolomics offer a high-resolution functional perspective, directly accessing the outputs of active metabolic pathways under Al stress (Rahman et al., 2022; Roychowdhury et al., 2023; Varadharajan et al., 2025).

Proteomics reveals differentially abundant proteins, including key enzymes, membrane transporters, chaperones, and regulatory proteins that play critical roles in Al tolerance. Metabolomics, in turn, detects changes in metabolites associated with detoxification, redox balance, cell wall reinforcement, stress signaling, and energy metabolism. Integrating these data layers enables the identification of coordinated pathways, central regulatory hubs, and biochemical tolerance signatures (Araujo et al., 2023; Pinto et al., 2023; Yoshida et al., 2023). Moreover, omics studies have shown that Al stress responses are highly tissue-specific: roots

prioritize chelation, structural reinforcement, and energy reprogramming, while leaves maintain photosynthesis, manage oxidative damage, and support systemic adaptation (Pinto et al., 2021b; Wang et al., 2023).

In popcorn, recent studies applied to a previously selected Al-tolerant inbred line (Rahim et al., 2019) have revealed induction of pathways such as the tricarboxylic acid (TCA) cycle, cysteine and methionine metabolism, phenylpropanoid biosynthesis, and glutathione metabolism in roots. The presence of enzymes like citrate synthase and the accumulation of malate, combined with the repression of malate dehydrogenase, reflect a tightly regulated control of organic acid exudation as a key tolerance mechanism (Pinto et al., 2021a, 2021b, 2023; Yoshida et al., 2023).

In this study, it was conducted an integrated proteomic and metabolomic analysis of roots and leaves from an Al-tolerant popcorn inbred line under Al stress conditions. The results revealed a coordinated reprogramming of primary and secondary metabolism, highlighting adaptive functional networks with potential applications in plant breeding.

In this context, plant breeding programs gain a solid molecular foundation to accelerate the identification and introgression of alleles associated with Al tolerance. Omics-derived data provide candidate genes, regulatory proteins, and key metabolites with validated roles in stress adaptation. These findings can be directly applied to marker-assisted selection (MAS), genomic prediction, and precision gene editing approaches, significantly enhancing the efficiency, predictability, and speed of breeding cycles (Azeem et al., 2025; Derbyshire; Batley; Edwards, 2022; Roychowdhury et al., 2023). Additionally, the construction of gene–protein–metabolite networks enables modeling of phenotype–genotype associations. Recent advances in artificial intelligence and machine learning offer powerful tools to integrate and analyze multi-omics datasets, increasing the resolution of stress-response prediction and accelerating decision-making in cultivar development (Varadharajan et al., 2025).

Moreover, the development of Al-tolerant genotypes reduces the need for intensive liming, thereby minimizing production costs and the environmental impacts associated with excessive chemical inputs. This aligns with sustainability goals and promotes low-input agriculture in resource-constrained regions.

By integrating omics technologies with breeding tools, this research not only advances our understanding of Al stress adaptation in popcorn but also elevates this underutilized crop to a new frontier in plant biotechnology and sustainable food production.

References

ARAUJO, Alda Neis Miranda *et al.* Integration of proteomic and metabolomic analyses: New insights for mapping informal workers exposed to potentially toxic elements. **Frontiers in Public Health**, v. 10, 25 jan. 2023.

AZEEM, Asad *et al.* Integration of Multi-Omics Approaches and Speed Breeding for Plant Stress Tolerance. *In*: CHEN, Jen-Tsung (Org.). **Plant Stress Tolerance: Molecular Mechanisms and Breeding Strategies**. 1st. ed. Boca Raton: CRC Press, 2025. v. 2 p. 17.

CHAKRABORTY, Nilakshi *et al.* **Exploring Aluminum Tolerance Mechanisms in Plants with Reference to Rice and Arabidopsis: A Comprehensive Review of Genetic, Metabolic, and Physiological Adaptations in Acidic Soils**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jul. 2024.

DERBYSHIRE, Mark C.; BATLEY, Jacqueline; EDWARDS, David. **Use of multiple 'omics techniques to accelerate the breeding of abiotic stress tolerant crops**. **Current Plant Biology**Elsevier B.V., , 1 dez. 2022.

GUAN, Kexing *et al.* Two Sweet Sorghum (Sorghum bicolor L.) WRKY Transcription Factors Promote Aluminum Tolerance via the Reduction in Callose Deposition. **International Journal of Molecular Sciences**, v. 24, n. 12, p. 10288, 17 jun. 2023.

KIST, Benno Bernardo. **Anuário brasileiro do milho 2019**. Santa Cruz do Sul: Gazeta Santa Cruz, 2019.

KOCHIAN, Leon V. *et al.* Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. **Annual Review of Plant Biology**, v. 66, n. 1, p. 571–598, 29 abr. 2015.

LIU, Chan *et al.* Overexpression of ZmSTOP1-A Enhances Aluminum Tolerance in Arabidopsis by Stimulating Organic Acid Secretion and Reactive Oxygen Species Scavenging. **International Journal of Molecular Sciences**, v. 24, n. 21, p. 15669, 27 out. 2023.

MUTIMAAMBA, Charles *et al.* Line × tester analysis of maize grain yield under acid and non-acid soil conditions. **Crop Science**, v. 60, n. 2, p. 991–1003, 25 mar. 2020.

OFOE, Raphael *et al.* Aluminum in plant: Benefits, toxicity and tolerance mechanisms. **Frontiers in Plant Science**, v. 13, 13 jan. 2023.

PIÑEROS, Miguel A. *et al.* Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of ZmALMT1 – an anion-selective transporter. **The Plant Journal**, v. 53, n. 2, p. 352–367, 17 jan. 2008.

PINTO, Vitor Batista *et al.* Uncovering the transcriptional response of popcorn (Zea mays L. var. everta) under long-term aluminum toxicity. **Scientific Reports**, v. 11, n. 1, p. 19644, 4 out. 2021a.

PINTO, Vitor Batista *et al.* Deciphering the major metabolic pathways associated with aluminum tolerance in popcorn roots using label-free quantitative proteomics. **Planta**, v. 254, n. 6, p. 132, 25 dez. 2021b.

PINTO, Vitor Batista *et al.* Transcriptome-based strategies for identifying aluminum tolerance genes in popcorn (Zea mays L. var. everta). **Scientific Reports**, v. 13, n. 1, p. 19400, 8 nov. 2023.

RAHIM, Faisal *et al.* Identification of contrasting tropical popcorn inbreds for studying aluminum toxicity tolerance inheritance. **Euphytica**, v. 215, n. 3, 1 mar. 2019.

RAHMAN, Shafeeq Ur *et al.* **State-of-the-art OMICS strategies against toxic effects of heavy metals in plants: A review**. **Ecotoxicology and Environmental Safety**Academic Press, , 1 set. 2022.

ROYCHOWDHURY, Rajib *et al.* **Multi-Omics Pipeline and Omics-Integration Approach to Decipher Plant's Abiotic Stress Tolerance Responses**. **Genes**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jun. 2023.

SILVA, Sónia. Aluminium Toxicity Targets in Plants. **Journal of Botany**, v. 2012, p. 1–8, 2 jul. 2012.

TANDZI, Liliane Ngoune *et al.* Breeding Maize for Tolerance to Acidic Soils: A Review. **Agronomy**, v. 8, n. 6, p. 84, 29 maio 2018.

TYAGI, Wricha *et al.* Root transcriptome reveals efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype. **Scientific Reports**, v. 10, n. 1, p. 4580, 12 mar. 2020.

USDA. **United States Department of Agriculture. Foreign Agricultural Service. Grain: world markets and trade**. Disponível em: <https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads>. Acesso em: 18 jun. 2024.

VARADHARAJAN, Venkatramanan *et al.* **Multi-Omics Approaches Against Abiotic and Biotic Stress—A Review**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 mar. 2025.

WANG, Jingbo *et al.* Transcriptomics and metabolomics reveal tolerance new mechanism of rice roots to Al stress. **Frontiers in Genetics**, v. 13, 10 jan. 2023.

YOSHIDA, Camila Hatsu Pereira *et al.* Tolerance mechanisms to aluminum in popcorn inbred lines involving aluminum compartmentalization and ascorbate–glutathione redox pathway. **Planta**, v. 257, n. 2, p. 28, 2 fev. 2023.

CHAPTER I - ADVANCES IN UNDERSTANDING AND MITIGATING ALUMINUM TOXICITY IN GRAIN CROPS: OMICS APPROACHES AND GENETIC IMPROVEMENT STRATEGIES

Advances in understanding and mitigating aluminum toxicity in grain crops: omics approaches and genetic improvement strategies

Matheus Pereira Ribeiro[1*], José Marcelo Soriano Viana[1], Cleberson Ribeiro[1], Vitor Batista Pinto[2]

[1] Department of General Biology, Federal University of Viçosa, 36570-900, Viçosa, MG, Brazil

[2] State University of North Fluminense, Biosciences and Biotechnology Center, 28013-602, Campos dos Goytacazes, RJ, Brazil

[*]Corresponding author: matheus.p.ribeiro@ufv.br. ORCID 0000-0002-0486-0505

Abstract

Aluminum (Al) toxicity in acidic soils is a major abiotic constraint to agricultural productivity, particularly in tropical and subtropical regions. In its trivalent form (Al^{3+}), Al becomes soluble and phytotoxic, impairing root growth, nutrient uptake, and overall plant development. This review provides a comprehensive synthesis of the physiological, biochemical, and molecular mechanisms underlying Al tolerance in grain crops, with emphasis on maize and popcorn. Two main tolerance strategies are described: exclusion, based on the exudation of organic acids and phenolics; and internal tolerance, involving vacuolar sequestration, antioxidant defense, and cell wall modifications. Recent advances in genomics, transcriptomics, proteomics, metabolomics, and epigenomics have enabled the identification of regulatory networks, key transporters (MATE, ALMT, ABC), and transcription factors (STOP1, WRKY, NAC, MYB) involved in Al stress responses. Multi-omics integration has revealed tissue-specific and genotype-dependent responses, highlighting the role of convergent metabolic and signaling pathways. Studies in maize and rice demonstrated functional convergence across omics layers, reinforcing the importance of citrate and malate exudation, lignin biosynthesis, and redox homeostasis. Furthermore, omics-based insights are now being translated into marker-assisted selection, genomic prediction, and gene editing tools for breeding Al-tolerant cultivars. We also highlight current gaps, including the need for functional validation, exploration of epigenetic memory, and incorporation of underrepresented crops like popcorn. Altogether, this review underscores the transformative potential of omics technologies for improving crop resilience to Al stress and guides future efforts in sustainable agriculture on acid soils.

Key-words: Multi-omics integration; Popcorn; Plant Breeding; Al tolerance.

Introduction

Aluminum (Al) is the third most abundant element in the Earth's crust after oxygen and silicon. In most soils, Al exists as non-toxic Al silicates and oxides to which plant roots are exposed and have no deleterious effects (Ofoe et al., 2023). However, in soils with a pH below 5.5, Al becomes soluble and bioavailable as Al^{3+} causing phytotoxicity. These ions react with water (Al^{3+} hydrolysis) in a process that releases H^{+} ions, further increasing soil acidity. Soil acidity is one of the main barriers to global agricultural production. It is estimated that more than 40% of potentially arable soils on the American continent have some degree of acidity (Tyagi et al., 2020).

The Al^{3+} compromises root growth, interferes with cell metabolism and severely impairs the physiological performance of plants (Banet et al., 2020; Shetty et al., 2021). This ion interacts rapidly with the cell wall and plasma membrane of root cells, promoting structural changes, blocking ion channels and inducing oxidative stress (Kocjan; Kwasniewska; Szurman-Zubrzycka, 2024; Ofoe et al., 2023; Yoshida et al., 2023). As a consequence, there is inhibition of cell elongation, loss of membrane integrity, damage to the absorption of essential nutrients and reduced photosynthetic efficiency, impacting directly agricultural productivity (Chakraborty et al., 2024; Cheng et al., 2020; Hajiboland et al., 2023) . In crops of great socioeconomic relevance such as grain crops, these effects are particularly serious (Ofoe et al., 2023).

Sets of genotypes of various crops have shown varying degrees of tolerance to Al, which has prompted research aimed at identifying the physiological, molecular and biochemical mechanisms involved in this adaptation. Al tolerance mechanisms are generally classified into two groups: (i) exclusion: acts externally to the plant, preventing Al from entering the root cells by exuding chelating compounds such as malic, citric and oxalic acids, and (ii) tolerance: acts internally to the plant and is based on vacuolar sequestration of Al, intracellular complexation with organic ligands and modulation of the cell wall and antioxidant network (Ahammed et al.,

2024; Chakraborty et al., 2024; Peng et al., 2023). These mechanisms are controlled by complex regulatory networks involving transporters such as MATE (multidrug and toxic compound extrusion), ALMT (Aluminum-Activated Malate Transporter) and ABC (ATP binding cassette), transcription factors such as STOP1 (sensitive to protein rhizotoxicity 1), WRKY, NAC and MYB, as well as antioxidant enzymes, cell wall modifiers and hormonal regulators (Chakraborty et al., 2024; Guan et al., 2023; Liu et al., 2023; Maron et al., 2010; Ofoe et al., 2023; Piñeros et al., 2008). In addition, recent studies suggest that epigenomic modulation, including RNA modifications such as m6A, playing an essential role in the dynamic regulation of gene expression during Al-stress (Wu et al., 2024), enabling rapid and coordinated responses to environmental changes.

The advancement of integrated multi-omics approaches, encompassing transcriptomics, proteomics, metabolomics, epigenomics and high-resolution phenotypic analyses, has enabled a much more comprehensive understanding of the mechanisms involved in Al tolerance. These technologies reveal not only the individual molecular components of the response, but also their interactions in functional networks, offering a systemic view of adaptations to stress (Anani et al., 2022; Derbyshire; Batley; Edwards, 2022; Rahman et al., 2022; Varadharajan et al., 2025; Wu et al., 2024) . Models based on gene co-expression network analysis Weighted Gene Co-expression Network Analysis (WGCNA), GO/KEGG functional analysis, integration of bioinformatics pipelines and machine learning have been applied to identify core gene modules, priority targets for manipulation and robust molecular markers (Derbyshire; Batley; Edwards, 2022; Roychowdhury et al., 2023) . Such tools accelerate the translation of basic knowledge into practical applications in plant breeding and biotechnology, including the use of precision gene editing by CRISPR/Cas9 to modulate tolerance genes (Han et al., 2022; Liao et al., 2019; Santosh Kumar et al., 2020; Shi et al., 2017).

Cereals such as wheat, rice, maize, popcorn and sorghum form the basis of global food security, accounting for a large part of the caloric and nutritional intake of the world's population (Albahri et al., 2023). Particularly, in maize, wheat and rice are among the most widely produced and consumed crops, playing a central role in reducing hunger and malnutrition, especially in regions of high socioeconomic vulnerability (USDA, 2025). However, the development and productivity of these cereals are often limited by adverse soil factors, such as soil acidity and Al toxicity, a condition prevalent in tropical and subtropical areas (Carvalho et al., 2016; Jaiswal et al., 2024; Kochian et al., 2015; Rahim et al., 2019; Tandzi et al., 2018). This represents a significant threat to the stability of agricultural production and the maintenance of sustainable food systems (Kochian et al., 2015; Tandzi et al., 2018). In this context, the study of Al tolerance is strategically important, as it enables the identification of physiological, biochemical and genetic mechanisms that confer resistance to this stressor (Chakraborty et al., 2024; Kocjan; Kwasniewska; Szurman-Zubrzycka, 2024). Advances in this field are essential not only for preserving the productive potential of these crops in acidic environments, but also for guaranteeing the stability of agricultural systems, promoting adaptation to climate change and sustaining food supplies in vulnerable regions (Kocjan; Kwasniewska; Szurman-Zubrzycka, 2024).

In view of these advances, it is necessary to organize and synthesize the state of the art on the mechanisms of Al toxicity and tolerance in plants, with a main focus on maize, highlighting the role of omics tools and their practical implications for the development of more resilient cultivars. Multi-omics integration has revealed that Al tolerance is not the result of isolated pathways, but of a coordinated defense system activated in a manner dependent on genotype, tissue and stress intensity. This systemic vision is essential for guiding breeding and genetic engineering strategies, the subject of the next section. This review integrates the most relevant evidence for grain crops, discussing the main components of molecular regulation of

Al tolerance in an integrative biology perspective, as well as the opportunities offered by biotechnology and genomics applied to sustainable agriculture in acid soils.

Omics approaches applied to the study of Al tolerance

Elucidating the mechanisms of Al tolerance in plants requires a comprehensive and integrative approach capable of capturing the complexity of biological responses to stress. In this context, omics sciences, such as genomics, transcriptomics, proteomics, metabolomics and epigenomics, have established themselves as central tools for identifying genes, proteins, metabolites and regulatory changes involved in the response to Al (Anani et al., 2022; Rahman et al., 2022; Roychowdhury et al., 2023) . In grain crops, these approaches have been applied with increasing sophistication, allowing the construction of a robust functional landscape that combines basic discovery with practical application in breeding programs.

Genomics

Genomic analysis has become an essential tool for elucidating the molecular mechanisms underlying Al tolerance in agricultural crops. This approach enables the identification of candidate genes, regulatory elements, and genomic regions involved in stress perception, signaling, and adaptation (Raza et al., 2022). High-density genotyping has allowed the detection of quantitative trait loci (QTLs) associated with Al tolerance, providing a solid foundation for genetic improvement in cereals under acidic soil conditions (Guimaraes et al., 2014).

In maize, mapping studies have identified five major QTLs located on chromosomes 2, 6, and 8, which together account for approximately 63% of the phenotypic variance in root growth under Al stress. The most relevant QTL, *qALT6*, located on chromosome 6, co-localizes with the *ZmMATE1* gene, which encodes an Al-activated citrate transporter (Maron et al., 2010). A tandem duplication of *ZmMATE1* leads to its overexpression, promoting increased citrate exudation and enhanced Al tolerance (Maron et al., 2013; Matonyei et al., 2020). The *TaMATE1* gene, discovered by Garcia-Oliveira et al., (2014)., is positioned on the long arm of

the group 4 homologous chromosomes (*TaMATE1-4A*, *TaMATE1-4B*, *TaMATE1-4D*). Other important genes include *ZmNrat1*, which is Al-inducible and preferentially expressed in root tips, as well as transcription factors such as ZmART1 and ZmAT6, which coordinate defense responses and activate transporter expression (Du et al., 2020; Yamaji et al., 2009). Other authors have also described Al tolerance in maize by ROS elimination (*ZmALDH1* and *ZmAT6*) (Du et al., 2020, 2022), Al fixation in the cell wall (*ZmXTH1*) (Du et al., 2021b) and auxin transport (*ZmPGP1*) (Zhang et al., 2018).

In other crops, Al tolerance is primarily mediated by membrane transporters that enable organic-acid exudation or vacuolar sequestration, complemented by transcriptional regulators and QTL evidence. In wheat, TaALMT1 on chromosome 4D is a major contributor to Al exclusion via malate exudation (Farokhzadeh et al., 2019; Zeng et al., 2023a). In sorghum, the AltSB locus on chromosome 3, which includes SbMATE, plays a comparable role by promoting citrate exudation. In barley, HvAACT1 facilitates citrate secretion to detoxify Al, and HvALMT1 on chromosome 2H has also been reported (Huang; Sato; Ma, 2024). In rice, OsFRDL4 participates in citrate transport, while OsALS1, a half-size ABC transporter of the TAP subfamily, is rapidly and specifically up-accumulated by Al in roots and is expressed across root cell types (Huang et al., 2012). Transcriptional regulation further shapes these responses: in rice, ART1 is a central regulator of Al-tolerance genes; in sorghum, additional QTLs on chromosome 9 were refined to a locus harboring the transcription factors SbZNF and SbWRKY, initially identified through QTL and association mapping, supporting parallel and complementary regulatory pathways (Melo et al., 2019). Consistent with these gene-level findings, QTLs for Al tolerance in rice on chromosomes 1, 2, and 6 explain approximately 27% of the phenotypic variance (Jaiswal et al., 2024; Ma et al., 2002).

More recently, genome-wide association studies (GWAS) have revealed functional polymorphisms in transporter and regulatory genes (Famoso et al., 2011; Tao et al., 2018; Zeng

et al., 2023b; Zhang et al., 2019), significantly expanding the repository of molecular markers available for marker-assisted selection (MAS). Genomic analysis has revolutionized our understanding of Al tolerance in plants, not only by enabling the identification of specific genes and pathways but also by providing actionable targets for the development of stress-resilient cultivars. Tools like CRISPR/Cas9 and genomic prediction have made it possible to implement precision breeding strategies. While CRISPR/Cas9 allows targeted genome editing, creating specific mutations or inserting genes of interest, for example, modifying transcription factors like ART1 to increase Al tolerance in rice (Barrero et al., 2022). Genomic prediction relies on statistical models that use genomic marker data to predict breeding values, as successfully applied in rice breeding programs (Bartholomé et al., 2024). The fundamental difference between the two approaches is that CRISPR/Cas9 directly alters the DNA sequence at selected loci, while genomic prediction accelerates selection decisions without altering the genome itself. Furthermore, the integration of genomics with other omics approaches, such as transcriptomics, proteomics, and metabolomics, increases predictive power and supports the advancement of sustainable agriculture in acidic tropical soils.

Transcriptomics

Transcriptomics has been one of the most effective approaches for unraveling the molecular mechanisms underlying Al tolerance in plants, allowing for large-scale identification of differentially expressed genes under toxicity conditions. Through technologies such as RNA-Seq, it is possible to map regulatory networks, detect gene isoforms, non-coding RNAs, and signaling pathways associated with stress perception, response, and adaptation to Al (Pinto et al., 2023; Xu et al., 2018). Microarray and RNA-Seq technologies were developed in different periods, each representing a milestone in transcriptomic analysis. Microarrays provided the first large-scale insights into Al stress responses by enabling the simultaneous monitoring of thousands of genes, which helped identify candidate genes and pathways associated with

organic acid exudation and oxidative stress. RNA-Seq, as a subsequent technological evolution, expanded this capacity by allowing the discovery of novel transcripts, low-abundance genes, and alternative splicing events, offering a more comprehensive picture of transcriptional reprogramming under Al stress (Kloet et al., 2020; Zhao et al., 2014). Thus, rather than competing methods, both have contributed significantly to elucidating the molecular basis of aluminum tolerance in plants. According to Varadharajan et al., (2025), transcriptomics represents the first step toward building a functional architecture of the stress response, enabling integration with other omics and bioinformatics approaches for gene-to-phenotype network modeling. The use of functional enrichment analyses (GO/KEGG), co-expression networks (such as WGCNA), and multivariate analyses, like Principal Component Analysis (PCA) and clustering, has been crucial for prioritizing candidate genes for Al tolerance and adaptation to acidic soils.

In popcorn, study conducted by Pinto et al., (2021a) is among the most comprehensive to date, in which the Al-resistant line exhibited up-accumulated transporters, detoxification-related genes, and transcription factors, together with alterations in lipid and organic acid metabolism. Using RNA-Seq on roots of contrasting genotypes under long term Al exposure, the authors identified hundreds of differentially expressed genes related to crucial pathways such as organic acid exudation (via *ZmMATE*, *ZmALMT*), vacuolar transport (ABC transporters), cell wall remodeling, lignin biosynthesis, antioxidant responses, in addition to new players never been described to Al-tolerance, such as SWEET transporters. SWEETs are sugar transporters essential for development and stress response. In the Pinto et al., (2021a) study, SWEET genes were more active in the Al-resistant line, suggesting they help maintain energy balance during toxic stress. They may facilitate sugar storage in the vacuole and provide energy for detoxification mechanisms. This finding highlights their role in adaptation to acidic

soils, making them valuable targets for the development of more tolerant crops. In short, SWEETs are key regulators of sugar metabolism under adverse conditions.

Analysis of gene co-expression networks suggested that Al tolerance involves modules enriched in transcription factor families such as WRKY, NAC, MYB, and bZIP, and highlighted the central role of master regulators like STOP1 and STOP2. However, it is important to note that such network analyses provide insights into potential regulatory relationships, rather than direct validation of candidate genes. These factors control the expression of genes involved in cell wall modification, Al exclusion, and detoxification, playing a critical role in plant adaptation to acidic soils (Agrahari et al., 2021; Kobayashi et al., 2014; Li et al., 2020; Park et al., 2010; Tao et al., 2022). Notable examples include WRKY47, which regulates genes such as *ELP* and *XTH17* (Li et al., 2020); NAC factors like regulating *ANAC017*, which modulate cell wall components (Tao et al., 2022); MYBs regulating downstream genes such as *PGIP1* (Agrahari et al., 2021), of the cell wall defense pathway, where MYBs activate genes such as PGIP1 to protect the plant from damage; and master regulators STOP1/STOP2, which activate key genes like *ALS3*, *AtMATE*, and *PGIP1* (Agrahari et al., 2021; Kobayashi et al., 2014). Additionally, signaling molecules like nitric oxide further enhance the activity of these transcription factors (Agrahari et al., 2021). Altogether, co-expression modules represent a complex and coordinated regulatory network that underpins the Al-stress response.

In rice, genes involved in flavonoid metabolism, cell wall lignification, and hormonal signaling are differentially expressed under Al-stress (Wang et al., 2023), many of them are conserved orthologs in maize. *OsSTAR1* and *OsSTAR2* encode the NBD (nucleotide-binding domain) and TMD (transmembrane domain), respectively. When co-expressed, these genes form a protein complex localized in rice roots, which functions as a UDP-glucose transporter, as demonstrated in oocyte experiments. UDP-glucose-containing vesicles fuse with the plasma

membrane, releasing this metabolite into the root cell wall (Huang et al., 2009), a mechanism that helps reduce Al accumulation and toxicity (Kochian et al., 2015). Another key regulator of Al resistance in rice is *OsART1*, a transcription factor expressed in roots. Yamaji et al., (2009) identified 31 genes up-accumulated by *OsART1* in response to Al, including *OsSTAR1* and *OsSTAR2*. Subsequent studies leveraged this transcriptional profile to uncover novel resistance genes, such as *OsNrat1* (Al transporter), *OsMGT1* (Mg^{2+} transporter), *OsCDT3* (a metal-binding protein), and *OsFRDL4* (citrate exporter) (Kochian et al., 2015; Xia et al., 2010; Xia; Yamaji; Ma, 2013). Yokosho et al., (2016) demonstrated that, although *OsFRDL2* is involved in Al-induced citrate secretion, its overall contribution to high Al tolerance in rice is relatively limited. The *OsEXPA10* gene, associated with Al-induced cell elongation, was characterized by Che et al., (2016). Its expression, restricted to root tips, is regulated by the transcription factor ART1.

Transcriptomics also allows investigation of spatial and temporal responses. Genes expressed specifically in the elongation zone of the root, as demonstrated by Kocjan; Kwasniewska; Szurman-Zubrzycka, (2024) and Rahman et al., (2022), are directly linked to initial Al perception and activation of exclusion and structural reinforcement mechanisms, highlighting the importance of tissue-specific sampling for more precise analyses. In Al-sensitive rice, Al reduced root length without completely inhibiting elongation (Wang et al., 2023), while in maize, Al had a similar non-lethal effect (Kong et al., 2014). In contrast, barley is highly sensitive, with even micromolar Al concentrations causing strong root growth inhibition (Szurman-Zubrzycka et al., 2021). These contrasting responses highlight the importance of understanding Al toxicity in cereals.

Despite advances enabled by transcriptomics in identifying genes associated with Al tolerance, the field still faces gaps that cannot be addressed by any single technique. Outstanding challenges include the incomplete elucidation of regulatory mechanisms, limited

functional validation of candidate genes, and insufficient integration of expression profiles with genetic variation and phenotypic traits (Pinto et al., 2023) . Rather than a stand-alone pillar, transcriptomics should be viewed as one component within an integrated framework to dissect Al stress responses in crops (Pinto et al., 2023; Szurman-Zubrzycka et al., 2021). When combined with complementary layers, such as genomics, proteomics, metabolomics, epigenomics, and high-resolution phenotyping, it can support the discovery of candidate genes, enable marker development, and inform marker-assisted selection, transgenic approaches, and genome editing.

Proteomics

Proteomics is an indispensable tool in the post-genomic era, especially in the study of tolerance mechanisms to Al-stress. While transcriptomics provides information on the potential for gene expression, proteomics reveals the real functional activity of cellular systems by detecting expressed proteins, their relative amounts, post-translational modifications (PTMs) and functional interactions (Rahman et al., 2022) . The techniques used in proteomic studies range from traditional methods such as two-dimensional electrophoresis (2-DE) and Difference In-Gel Electrophoresis (DIGE) to new generation approaches such as LC-MS/MS (shotgun), iTRAQ, TMT and Orbitrap-based MS (Farooqi et al., 2022; Rahman et al., 2022; Roychowdhury et al., 2023; Varadharajan et al., 2025; Wu et al., 2024). These platforms allow the identification of low-abundance proteins, such as transcription factors, signaling proteins and vacuolar transporters, which are often crucial in the response to Al. Post-translational modifications such as phosphorylation, ubiquitination and acetylation also play central roles in the activation and regulation of proteins under stress. The phosphoproteomics approach, for example, has been instrumental in mapping signaling networks activated by ionic and oxidative stresses (Roychowdhury et al., 2023; Varadharajan et al., 2025).

The use of proteomic approaches has revealed central mechanisms in the response of cereal crops to Al-stress, pointing to the coordinated action of metabolic pathways, organic acid transporters, antioxidant systems and post-stress recovery processes (Dai et al., 2013; Han et al., 2019; Pinto et al., 2021b; Wang et al., 2014). One of the most recurrent discoveries concerns the reprogramming of energy metabolism, which is fundamental for sustaining root growth and Al detoxification. Enzymes associated with glycolysis, gluconeogenesis and the citric acid cycle appear to be up- accumulated under stress conditions, indicating a greater mobilization of energy for defense processes and cell maintenance (Pinto et al., 2021b; Wang et al., 2014). This strategy has been observed, for example, in tolerant popcorn genotypes, in which the high expression of sucrose synthase and TCA cycle enzymes favors the synthesis and secretion of organic acids, such as citrate, which are essential for Al complexation in the apoplast (Pinto et al., 2021b).

Another important axis of the protein response involves the activation of antioxidant systems. The presence of Al in the root environment promotes the accumulation of reactive oxygen species (ROS), requiring a rapid response to prevent oxidative damage to cell membranes and DNA. Proteins such as peroxidases, superoxide dismutase and other defense enzymes have been shown to be significantly more abundant in tolerant genotypes, as seen in barley (Han et al., 2019). In addition, some studies on Tibetan wild barley indicate that certain genotypes express specific proteins that are not present in sensitive inbred lines, which reinforces the importance of unique genetic variants adapted to naturally acidic environments (Niedziela et al., 2022).

The exclusion of Al from the root growth zone has also been associated with the expression of membrane transporters, especially members of the ABC and MATE families. These transporters facilitate the secretion of malate and citrate into the extracellular medium, a mechanism widely described in rice and barley. The functional conservation of these proteins

among different grain species highlights their central role in Al tolerance and points to promising molecular targets in genetic engineering and marker-assisted breeding programs (Dai et al., 2013; Pinto et al., 2021b).

In addition to the direct response to stress, the ability to recover after Al removal has emerged as an important differentiator between tolerant and sensitive genotypes. Studies on triticale reveal that tolerant cultivars show proteomic plasticity, quickly reacquiring their basal protein profile and resuming root growth. Sensitive genotypes, on the other hand, maintain persistent alterations, which compromises recovery and indicates a physiological limitation in reversing the damage (Han et al., 2019).

Despite these advances, there are still significant gaps in the understanding of protein regulation against Al, especially in cereals. One example of this is the scarcity of studies on post-translational modifications, such as phosphorylation, which are fundamental for the activation and regulation of protein function. Until now, analyses of this type have only been reported in soybeans under Al-stress, where the abundance of proteins involved in redox and hormonal signaling pathways was decisive for adaptation to stress (Han et al., 2020; He et al., 2024). The lack of similar research in species such as wheat, maize and rice represent an important gap in the proteomic characterization of Al tolerance.

This evidence reinforces the idea that Al tolerance is a multigenic trait, regulated by complex interaction networks that integrate primary metabolism, antioxidant defense, ion transport and post-translational regulation. The growing integration of proteomics with transcriptomic and genomic data has made it possible to map these networks with greater resolution, enabling the identification of more precise targets for biotechnological interventions (Derbyshire; Batley; Edwards, 2022; Roychowdhury et al., 2023; Varadharajan et al., 2025; Wu et al., 2024). Although challenges remain, especially with regard to functional validation in field conditions, the advances provided by proteomic analysis offer promising avenues for

the development of grain cultivars that are more resilient and productive in acidic soils. Critically, these protein-level insights gain full explanatory power when combined with metabolomic profiles, as metabolites represent the functional endpoints of both gene expression and protein activity.

Metabolomics

Metabolomics, the science that studies the complete set of metabolites produced by a cell, tissue, or organism, has gained prominence in elucidating plant responses to Al-stress. While genomic and transcriptomic analyses reveal potential cellular responses, metabolomics provides a direct functional readout of physiological states by quantifying the dynamic end-products of biochemical activity. This approach captures real-time metabolic fluctuations, offering an unparalleled window into the organism's adaptive responses to environmental challenges (Rahman et al., 2022). In the context of Al toxicity, metabolomics enables the identification of active defense, adaptation, and biochemical compensation pathways.

According to Roychowdhury et al., (2023), metabolomics occupies a strategic position in the multi-omics pipeline, serving as a direct bridge between molecular signals and phenotypic responses. By identifying differentially accumulated metabolites (DAMs), it is possible to associate them with functions such as Al chelation, cell wall reinforcement, antioxidant defense, osmoregulation, and stress signaling. Core metabolic pathways such as the TCA cycle, amino acid biosynthesis, and phenolic compound metabolism have been shown to be essential in Al tolerance.

Metabolomic analyses carried out on grain crops such as rice and maize have revealed distinct metabolic profiles between Al-tolerant and sensitive genotypes, pointing to metabolic pathways and key compounds involved in the stress response. In maize, analysis of the metabolites showed Al-stress leads to significant changes in primary metabolism. Al-sensitive genotypes accumulate higher levels of sugars, amino acids, proteins, and organic acids in

leaves, which is associated with impaired growth and increased oxidative damage. In contrast, Al-tolerant genotypes maintain stable primary metabolism and show less physiological disruption under Al stress (Khan; McNeilly; Collins, 2000; Siqueira et al., 2020). With regard to secondary metabolites, computational studies suggest that secondary metabolites like ascorbate, glutathione, jasmonic acid, and salicylic acid may interact with key enzymes, like dehydroascorbate reductase, to mitigate Al-induced oxidative stress, supporting the antioxidant defense system in maize (Choudhury et al., 2022).

Al tolerance in grain crops involves metabolic reprogramming that strengthens cell wall integrity, increases antioxidant capacity and favors detoxification mechanisms. While tolerant genotypes tend to activate pathways related to the biosynthesis of phenolic compounds, lignin and energy metabolism, sensitive ones accumulate amino acids and intermediates typical of cellular stress conditions (Table 1). These contrasting metabolic profiles represent promising signatures for the selection of biomarkers and the genetic improvement of cultivars with greater resistance to Al (Table 2) (Awasthi et al., 2017; Choudhury et al., 2022; Xie et al., 2022).

In rice, tolerant varieties, was detected show an increase in the biosynthesis of phenolic acids and alkaloids, while certain classes of lipids were reduced under Al toxicity. These compounds may be associated with antioxidant defense and direct complexation of Al in the apoplastic environment (Xie et al., 2022). On the other hand, Al-sensitive varieties tend to accumulate amino acids such as L-homoserine, L-methionine, L-ornithine and L-proline, generally related to stress signaling pathways and cellular detoxification mechanisms.

Metabolic markers could serve as powerful tools for selecting cultivars with enhanced resilience to acid stress in Al-toxicity environments. Using an UPLC-ESI-MS/MS system, Xie et al., (2022) demonstrated that elevated putrescine levels in rice – polyamines crucial for cellular function under heavy metal stress – may mediate Al neutralization in sensitive

genotypes. In addition, a reduced cysteine and S-(methyl)glutathione levels correlated with diminished detoxification capacity.

In addition, the activation of central energy supply pathways, such as glycolysis and gluconeogenesis, has been observed in Al-tolerant rice roots, suggesting that the redirection of energy metabolism is an important strategy for sustaining adaptive processes under stress (Wang et al., 2014). A striking example of this metabolic adjustment is the greater accumulation of glutathione reductase (GR) in tolerant rice cultivars, which reinforces its role in antioxidant defense against Al-induced oxidative stress (Awasthi et al., 2017). These findings underscore how targeted metabolomic profiling can reveal both protective mechanisms and vulnerability markers, providing a dual-strategy approach for crop improvement.

Multi-omics integration and predictive applications

The integration of genomic, transcriptomic, proteomic, and metabolomic data, commonly referred to as a multi-omics approach, has become essential to understanding the complexity of plant responses to Al toxicity. This systemic perspective allows researchers to move beyond the limitations of single-layer analyses, offering a holistic view of gene-to-phenotype regulation under stress conditions (Rahman et al., 2022; Roychowdhury et al., 2023; Varadharajan et al., 2025). By combining multiple data layers, multi-omics strategies enable the identification of regulatory hubs, convergence points of molecular pathways, and coordinated stress-response modules (Liu et al., 2021; Wang et al., 2023; Wu et al., 2024). This is particularly relevant in Al tolerance, where gene expression, protein abundance, and metabolite accumulation often do not correlate directly due to post-transcriptional regulation and differential tissue responses.

The application of bioinformatic tools such as WGCNA, PCA and GO/KEGG enrichment analyses has proven critical for interpreting complex datasets and identifying multi-omics markers of tolerance (Derbyshire; Batley; Edwards, 2022; Varadharajan et al., 2025). These tools have revealed that tolerant genotypes exhibit coordinated activation of organic acid

exudation pathways, like *ZmMATE*, *ZmALMT*, ROS detoxification systems, and lignin biosynthesis, all supported by transcriptomic, proteomic, and metabolomic evidence (Azeem et al., 2025; Liu et al., 2021; Roychowdhury et al., 2023; Varadharajan et al., 2025; Wang et al., 2023; Wu et al., 2024). Araujo et al., (2023) further demonstrated how omics integration can clarify the systemic nature of stress responses by identifying the overlap between differentially expressed transcripts, proteins, and metabolites. These shared components reflect central metabolic routes and structural defense mechanisms that are robustly expressed in tolerant genotypes and nearly absent in sensitive ones.

In recent years, the integration of multi-omics technologies, such as transcriptomics, proteomics and metabolomics, has significantly advanced our understanding of plant responses to Al stress. Studies in rice revealed that phenylpropanoid biosynthesis plays a central role in oxidative stress response, with a strong correlation between differentially expressed genes and metabolites (Wang et al., 2023). In popcorn, integrative analyses of transcriptome and proteome data from the Al-tolerant inbred line showed that although transcriptional regulation predominates, post-transcriptional adjustments, particularly in bioenergetic pathways, also contribute to the Al tolerance mechanism (Pinto et al., 2021b). Similar complexity was observed in barley, where m6A methylation profiling coupled with metabolomics highlighted that auxin and jasmonic acid suppress cell elongation while enhancing pectin demethylation, aiding Al detoxification through mechanisms such as Al exclusion, chelation, and ROS scavenging (Wu et al., 2024). Together, these findings underscore the intricate, multi-layered regulatory networks plants employ to mitigate Al toxicity, and demonstrate the value of integrated omics approaches in uncovering novel mechanisms of stress tolerance.

Integration of this kind not only validates individual findings but also enhances the predictive power of stress biomarkers. According to Varadharajan et al., (2025), multi-omics approaches represent a paradigm shift in plant biotechnology, especially when combined with

machine learning algorithms and artificial intelligence for large-scale pattern recognition. Such frameworks enable the prioritization of gene-metabolite-protein networks for functional validation, accelerating the development of stress-resilient cultivars. In summary, multi-omics integration is indispensable for unraveling the complex regulatory networks involved in Al tolerance, providing a strategic foundation for next-generation breeding programs targeting Al tolerance in acidic soils adaptation.

Future perspectives

The impacts of Al on plant morphology, biochemistry, and physiology, as well as the adaptive responses, highlight the complexity of this stress (Chakraborty et al., 2024; Tandzi et al., 2018). Over the past decades, advances and integration among omics have transformed the traditional research approach, enabling not only the identification of molecular components of tolerance but also the understanding of their interactions within interconnected regulatory networks. This has uncovered novel functional targets, coordinated defense pathways, and additional regulatory layers (Roychowdhury et al., 2023; Wu et al., 2024).

Despite these advances, important knowledge gaps remain. Most studies focus on early developmental stages or root tissues, neglecting the effects on aerial organs and during the reproductive phase, both essential to final yield. Furthermore, the intergenerational impact of Al exposure, potentially mediated by epigenetic mechanisms, remains largely unexplored in grain crops (Kocjan et al., 2024; Liu et al., 2021). The combination of multi-omics data with emerging technologies such as CRISPR/Cas9, epigenetic editing, high-resolution phenotyping, and machine learning points to a new frontier in the development of resilient cultivars. Predictive models based on gene networks and metabolic signatures will not only allow for the selection of superior genotypes but also enable simulations of their performance in varying edaphic environments, anticipating Al-stress responses even before field validation (Derbyshire et al., 2022; Varadharajan et al., 2025). In addition, innovative perspectives include: (i) Exploration of uncharacterized genetic diversity in traditional varieties and populations; (ii)

Adoption of participatory breeding strategies involving farmers in regions affected by acidic soils; (iii) Integration with agroecological practices, such as the use of growth-promoting microorganisms and regenerative soil management; (iv) Implementation of translational bioinformatics, bridging molecular data with digital phenotyping and marker-assisted selection.

References

AGRAHARI, Raj Kishan *et al.* Expression GWAS of PGIP1 Identifies STOP1-Dependent and STOP1-Independent Regulation of PGIP1 in Aluminum Stress Signaling in Arabidopsis. **Frontiers in Plant Science**, v. 12, 17 dez. 2021.

AHAMMED, Golam Jalal *et al.* Reactive oxygen species signaling in melatonin-mediated plant stress response. **Plant Physiology and Biochemistry**, v. 207, p. 108398, fev. 2024.

ALBAHRI, Ghosoon *et al.* Enhancing Essential Grains Yield for Sustainable Food Security and Bio-Safe Agriculture through Latest Innovative Approaches. **Agronomy**, v. 13, n. 7, p. 1709, 26 jun. 2023.

ANANI, Osikemekha Anthony *et al.* Insights to proteomics and metabolomics metal chelation in food crops. **Journal of Proteins and Proteomics**, v. 13, n. 3, p. 159–173, 20 jun. 2022.

ARAUJO, Alda Neis Miranda *et al.* Integration of proteomic and metabolomic analyses: New insights for mapping informal workers exposed to potentially toxic elements. **Frontiers in Public Health**, v. 10, 25 jan. 2023.

ARENHART, Rafael Augusto *et al.* Rice ASR1 and ASR5 are complementary transcription factors regulating aluminium responsive genes. **Plant, Cell & Environment**, v. 39, n. 3, p. 645–651, 14 mar. 2016.

AWASTHI, Jay Prakash *et al.* Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. **PLOS ONE**, v. 12, n. 4, p. e0176357, 27 abr. 2017.

AZEEM, Asad *et al.* Integration of Multi-Omics Approaches and Speed Breeding for Plant Stress Tolerance. *In*: CHEN, Jen-Tsung (Org.). **Plant Stress Tolerance: Molecular Mechanisms and Breeding Strategies**. 1st. ed. Boca Raton: CRC Press, 2025. v. 2 p. 17.

BANET, Travis *et al.* Assessing modified aluminum-based water treatment residuals as a plant-available phosphorus source. **Chemosphere**, v. 247, p. 125949, maio 2020.

BARRERO, Luz S. *et al.* Identifying genes associated with abiotic stress tolerance suitable for CRISPR/Cas9 editing in upland rice cultivars adapted to acid soils. **Plant Direct**, v. 6, n. 12, 10 dez. 2022.

BARTHOLOMÉ, Jérôme *et al.* Genomic selection for tolerance to aluminum toxicity in a synthetic population of upland rice. **PLOS ONE**, v. 19, n. 8, p. e0307009, 22 ago. 2024.

CARTES, P. *et al.* Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. **Annals of Applied Biology**, v. 156, n. 2, p. 297–307, 11 mar. 2010.

CARVALHO, Geraldo *et al.* Back to Acid Soil Fields: The Citrate Transporter SbMATE Is a Major Asset for Sustainable Grain Yield for Sorghum Cultivated on Acid Soils. **G3 Genes|Genomes|Genetics**, v. 6, n. 2, p. 475–484, 1 fev. 2016.

CHAKRABORTY, Nilakshi *et al.* **Exploring Aluminum Tolerance Mechanisms in Plants with Reference to Rice and Arabidopsis: A Comprehensive Review of Genetic, Metabolic, and Physiological Adaptations in Acidic Soils**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jul. 2024.

CHE, Jing *et al.* An Al-inducible expansin gene, OsEXPA10 is involved in root cell elongation of rice. **The Plant Journal**, v. 88, n. 1, p. 132–142, 22 out. 2016.

CHE, Jing *et al.* Functional characterization of an aluminum (Al)-inducible transcription factor, ART2, revealed a different pathway for Al tolerance in rice. **New Phytologist**, v. 220, n. 1, p. 209–218, 11 out. 2018.

CHENG, Xiaoqing *et al.* Protective roles of salicylic acid in maintaining integrity and functions of photosynthetic photosystems for alfalfa (Medicago sativa L.) tolerance to aluminum toxicity. **Plant Physiology and Biochemistry**, v. 155, p. 570–578, out. 2020.

CHOUDHURY, Shuvasish *et al.* A Computational Study of the Role of Secondary Metabolites for Mitigation of Acid Soil Stress in Cereals Using Dehydroascorbate and Mono-Dehydroascorbate Reductases. **Antioxidants**, v. 11, n. 3, p. 458, 25 fev. 2022.

CUADROS-INOSTROZA, Álvaro *et al.* TargetSearch - a Bioconductor package for the efficient preprocessing of GC-MS metabolite profiling data. **BMC Bioinformatics**, v. 10, 16 dez. 2009.

DAI, Huaxin *et al.* Comparative Proteomic Analysis of Aluminum Tolerance in Tibetan Wild and Cultivated Barleys. **PLoS ONE**, v. 8, n. 5, p. e63428, 14 maio 2013.

DAMERVAL, Catherine *et al.* Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. **ELECTROPHORESIS**, v. 7, n. 1, p. 52–54, 13 jan. 1986.

DAS, Abir *et al.* Mitigation of aluminum toxicity in rice seedlings using biofabricated selenium nanoparticles and nitric oxide: Synergistic effects on oxidative stress tolerance and sulfur metabolism. **Chemosphere**, v. 370, p. 143940, fev. 2025.

DE SOUSA, Alexandra *et al.* Subcellular compartmentalization of aluminum reduced its hazardous impact on rye photosynthesis. **Environmental Pollution**, v. 315, p. 120313, dez. 2022.

DERBYSHIRE, Mark C.; BATLEY, Jacqueline; EDWARDS, David. **Use of multiple 'omics techniques to accelerate the breeding of abiotic stress tolerant crops**. **Current Plant Biology**Elsevier B.V., , 1 dez. 2022.

DISTLER, Ute *et al.* Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. **Nature Methods**, v. 11, n. 2, p. 167–170, 15 fev. 2014.

DONNELLY, Chase P. *et al.* Malate production, sugar metabolism, and redox homeostasis in the leaf growth zone of Rye (Secale cereale) increase stress tolerance to aluminum stress: A biochemical and genome-wide transcriptional study. **Journal of Hazardous Materials**, v. 464, p. 132956, fev. 2024.

DU, Hanmei *et al.* A Maize ZmAT6 Gene Confers Aluminum Tolerance via Reactive Oxygen Species Scavenging. **Frontiers in Plant Science**, v. 11, 9 jul. 2020.

DU, Hanmei *et al.* ZmMATE6 from maize encodes a citrate transporter that enhances aluminum tolerance in transgenic Arabidopsis thaliana. **Plant Science**, v. 311, p. 111016, out. 2021a.

DU, Hanmei *et al.* ZmXTH, a xyloglucan endotransglucosylase/hydrolase gene of maize, conferred aluminum tolerance in Arabidopsis. **Journal of Plant Physiology**, v. 266, p. 153520, nov. 2021b.

DU, Han-Mei *et al.* Overexpression of the Aldehyde Dehydrogenase Gene ZmALDH Confers Aluminum Tolerance in Arabidopsis thaliana. **International Journal of Molecular Sciences**, v. 23, n. 1, p. 477, 1 jan. 2022.

FAMOSO, Adam N. *et al.* Development of a Novel Aluminum Tolerance Phenotyping Platform Used for Comparisons of Cereal Aluminum Tolerance and Investigations into Rice Aluminum Tolerance Mechanisms. **Plant Physiology**, v. 153, n. 4, p. 1678–1691, 3 ago. 2010.

FAMOSO, Adam N. *et al.* Genetic Architecture of Aluminum Tolerance in Rice (Oryza sativa) Determined through Genome-Wide Association Analysis and QTL Mapping. **PLoS Genetics**, v. 7, n. 8, p. e1002221, 4 ago. 2011.

FANG, Qiu *et al.* Degradation of STOP1 mediated by the F-box proteins RAH1 and RAE1 balances aluminum resistance and plant growth in Arabidopsis thaliana. **The Plant Journal**, v. 106, n. 2, p. 493–506, 10 abr. 2021.

FAROKHZADEH, Sara *et al.* Mapping QTLs of flag leaf morphological and physiological traits related to aluminum tolerance in wheat (Triticum aestivum L.). **Physiology and Molecular Biology of Plants**, v. 25, n. 4, p. 975–990, 6 jul. 2019.

FAROOQI, Muhammad Qudrat Ullah *et al.* **Recent developments in multi-omics and breeding strategies for abiotic stress tolerance in maize (Zea mays L.)**. **Frontiers in Plant Science**Frontiers Media S.A., , 23 set. 2022.

FOYER, Christine H.; NOCTOR, Graham. Ascorbate and Glutathione: The Heart of the Redox Hub. **Plant Physiology**, v. 155, n. 1, p. 2–18, jan. 2011.

GAO, Jie *et al.* Identification of a bacterial-type ATP-binding cassette transporter implicated in aluminum tolerance in sweet sorghum (Sorghum bicolor L.). **Plant Signaling & Behavior**, v. 16, n. 7, p. 1916211, 3 jul. 2021.

GAO, Li Jun *et al.* ART1 and putrescine contribute to rice aluminum resistance via OsMYB30 in cell wall modification. **Journal of Integrative Plant Biology**, v. 65, n. 4, p. 934–949, 18 abr. 2023.

GARCIA-OLIVEIRA, A. L. *et al.* Molecular cloning of TaMATE2 homoeologues potentially related to aluminium tolerance in bread wheat (Triticum aestivum L.). **Plant Biology**, v. 20, n. 5, p. 817–824, 17 set. 2018.

GARCIA-OLIVEIRA, Ana *et al.* Molecular characterization of TaSTOP1 homoeologues and their response to aluminium and proton (H+) toxicity in bread wheat (Triticum aestivum L.). **BMC Plant Biology**, v. 13, n. 1, p. 134, 2013.

GARCIA-OLIVEIRA, Ana Luísa *et al.* Molecular characterization of the citrate transporter gene TaMATE1 and expression analysis of upstream genes involved in organic acid transport under Al stress in bread wheat (Triticum aestivum). **Physiologia Plantarum**, v. 152, n. 3, p. 441–452, 2 nov. 2014.

GE, Steven Xijin; JUNG, Dongmin; YAO, Runan. ShinyGO: a graphical gene-set enrichment tool for animals and plants. **Bioinformatics**, v. 36, n. 8, p. 2628–2629, 15 abr. 2020.

GRUBER, Benjamin D. *et al.* HvALMT1 from barley is involved in the transport of organic anions. **Journal of Experimental Botany**, v. 61, n. 5, p. 1455–1467, mar. 2010.

GUAN, Kexing *et al.* Two Sweet Sorghum (Sorghum bicolor L.) WRKY Transcription Factors Promote Aluminum Tolerance via the Reduction in Callose Deposition. **International Journal of Molecular Sciences**, v. 24, n. 12, p. 10288, 17 jun. 2023.

GULLBERG, Jonas *et al.* Design of experiments: an efficient strategy to identify factors influencing extraction and derivatization of Arabidopsis thaliana samples in metabolomic studies with gas chromatography/mass spectrometry. **Analytical Biochemistry**, v. 331, n. 2, p. 283–295, ago. 2004.

GUPTA, Aditi; RICO-MEDINA, Andrés; CAÑO-DELGADO, Ana I. The physiology of plant responses to drought. **Science**, v. 368, n. 6488, p. 266–269, 17 abr. 2020.

HAJIBOLAND, Roghieh *et al.* Aluminum Toxicity in Plants: Present and Future. **Journal of Plant Growth Regulation**, v. 42, n. 7, p. 3967–3999, 28 jul. 2023.

HAN, Lei *et al.* Proteomic profiling sheds light on alkali tolerance of common wheat (Triticum aestivum L.). **Plant Physiology and Biochemistry**, v. 138, p. 58–64, maio 2019.

HAN, Rongrong *et al.* Quantitative phosphoproteomic analysis provides insights into the aluminum-responsiveness of Tamba black soybean. **PLOS ONE**, v. 15, n. 8, p. e0237845, 19 ago. 2020.

HAN, Yingying *et al.* One Novel Mitochondrial Citrate Synthase from Oryza sativa L. can Enhance Aluminum Tolerance in Transgenic Tobacco. **Molecular Biotechnology**, v. 42, n. 3, p. 299–305, 27 jul. 2009.

HE, Ying *et al.* Comparative quantitative phosphoproteomic and parallel reaction monitoring analysis of soybean roots under aluminum stress identify candidate phosphoproteins involved in aluminum resistance capacity. **Journal of Hazardous Materials**, v. 479, p. 135485, nov. 2024.

HOFFMAN, Daniel E. *et al.* Changes in diurnal patterns within the Populus transcriptome and metabolome in response to photoperiod variation. **Plant, Cell & Environment**, v. 33, n. 8, p. 1298–1313, 5 ago. 2010.

HORST, Walter J.; WANG, Yunxia; ETICHA, Dejene. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. **Annals of Botany**, v. 106, n. 1, p. 185–197, jul. 2010.

HUANG, Chao Feng *et al.* A Bacterial-Type ABC Transporter Is Involved in Aluminum Tolerance in Rice. **The Plant Cell**, v. 21, n. 2, p. 655–667, 30 mar. 2009.

HUANG, Chao-Feng *et al.* A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. **The Plant Journal**, v. 69, n. 5, p. 857–867, 29 mar. 2012.

HUANG, Sheng *et al.* Identification of STOP1-Like Proteins Associated With Aluminum Tolerance in Sweet Sorghum (Sorghum bicolor L.). **Frontiers in Plant Science**, v. 9, 28 fev. 2018.

HUANG, Sheng; SATO, Kazuhiro; MA, Jian Feng. Breeding for an elite malting barley cultivar with acid soil tolerance. **Communications Biology**, v. 7, n. 1, p. 1203, 28 set. 2024.

JAISWAL, Sandeep *et al.* Deciphering genetic mechanisms of Al toxicity tolerance through meta-QTL analysis in rice (Oryza sativa L.). **Environmental and Experimental Botany**, v. 228, p. 106030, dez. 2024.

JEZ, Joseph M. Structural biology of plant sulfur metabolism: from sulfate to glutathione. **Journal of Experimental Botany**, v. 70, n. 16, p. 4089–4103, 19 ago. 2019.

KHAN, A. A.; MCNEILLY, T.; COLLINS, J. C. Accumulation of amino acids, proline, and carbohydrates in response to aluminum and manganese stress in maize. **Journal of Plant Nutrition**, v. 23, n. 9, p. 1303–1314, set. 2000.

KIST, Benno Bernardo. **Anuário brasileiro do milho 2019**. Santa Cruz do Sul: Gazeta Santa Cruz, 2019.

KLOET, Frans M. van der *et al.* Increased comparability between RNA-Seq and microarray data by utilization of gene sets. **PLOS Computational Biology**, v. 16, n. 9, p. e1008295, 30 set. 2020.

KOBAYASHI, Yuriko *et al.* STOP2 Activates Transcription of Several Genes for Al- and Low pH-Tolerance that Are Regulated by STOP1 in Arabidopsis. **Molecular Plant**, v. 7, n. 2, p. 311–322, fev. 2014.

KOCHIAN, Leon V. *et al.* Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. **Annual Review of Plant Biology**, v. 66, n. 1, p. 571–598, 29 abr. 2015.

KOCJAN, Anna; KWASNIEWSKA, Jolanta; SZURMAN-ZUBRZYCKA, Miriam. **Understanding plant tolerance to aluminum: exploring mechanisms and perspectives**. **Plant and Soil**Springer Science and Business Media Deutschland GmbH, , 1 fev. 2024.

KOHORN, Bruce D.; KOHORN, Susan L. The cell wall-associated kinases, WAKs, as pectin receptors. **Frontiers in Plant Science**, v. 3, 2012.

KONG, Xiangpei *et al.* System analysis of microRNAs in the development and aluminium stress responses of the maize root system. **Plant Biotechnology Journal**, v. 12, n. 8, p. 1108–1121, out. 2014.

KOPKA, Joachim *et al.* GMD@CSB.DB: The Golm metabolome database. **Bioinformatics**, v. 21, n. 8, p. 1635–1638, 15 abr. 2005.

KOVÁČIK, Jozef; KLEJDUS, Bořivoj. Dynamics of phenolic acids and lignin accumulation in metal-treated Matricaria chamomilla roots. **Plant Cell Reports**, v. 27, n. 3, p. 605–615, 8 mar. 2008.

LASKY, Jesse R. *et al.* Natural Variation in Abiotic Stress Responsive Gene Expression and Local Adaptation to Climate in Arabidopsis thaliana. **Molecular Biology and Evolution**, v. 31, n. 9, p. 2283–2296, set. 2014.

LENG, Ya-Jun *et al.* Arabidopsis ERdj3B coordinates with ERECTA-family receptor kinases to regulate ovule development and the heat stress response. **The Plant Cell**, v. 34, n. 10, p. 3665–3684, 27 set. 2022.

LI, Chun Xiao *et al.* A WRKY transcription factor confers aluminum tolerance via regulation of cell wall modifying genes. **Journal of Integrative Plant Biology**, v. 62, n. 8, p. 1176–1192, 13 ago. 2020.

LI, Ge Zi *et al.* Transcription factor WRKY22 promotes aluminum tolerance via activation of OsFRDL4 expression and enhancement of citrate secretion in rice (Oryza sativa). **New Phytologist**, v. 219, n. 1, p. 149–162, 15 jul. 2018.

LI, Hongjie *et al.* ZmNRAMP4 Enhances the Tolerance to Aluminum Stress in Arabidopsis thaliana. **International Journal of Molecular Sciences**, v. 23, n. 15, p. 8162, 25 jul. 2022.

LI, Xiao Feng; MA, Jian Feng; MATSUMOTO, Hideaki. Pattern of Aluminum-Induced Secretion of Organic Acids Differs between Rye and Wheat. **Plant Physiology**, v. 123, n. 4, p. 1537–1544, 1 ago. 2000.

LIU, Chan *et al.* Overexpression of ZmSTOP1-A Enhances Aluminum Tolerance in Arabidopsis by Stimulating Organic Acid Secretion and Reactive Oxygen Species Scavenging. **International Journal of Molecular Sciences**, v. 24, n. 21, p. 15669, 27 out. 2023a.

LIU, Jie *et al.* Altered Expression of the Malate-Permeable Anion Channel OsALMT4 Reduces the Growth of Rice Under Low Radiance. **Frontiers in Plant Science**, v. 9, 3 maio 2018.

LIU, Siyan *et al.* MsDjB4, a HSP40 Chaperone in Alfalfa (Medicago sativa L.), Improves Alfalfa Hairy Root Tolerance to Aluminum Stress. **Plants**, v. 12, n. 15, p. 2808, 28 jul. 2023b.

LIU, Weixiao *et al.* Integrated proteomics and metabolomics analysis of transgenic and gene-stacked maize line seeds. **GM Crops and Food**, v. 12, n. 1, p. 361–375, 2021.

LIU, Xiang P. *et al.* A novel kinase subverts aluminium resistance by boosting ornithine decarboxylase-dependent putrescine biosynthesis. **Plant, Cell & Environment**, v. 45, n. 8, p. 2520–2532, 20 ago. 2022.

LIU, Yinghui *et al.* Characterization of a Stress-induced NADP-isocitrate Dehydrogenase Gene in Maize Confers Salt Tolerance in Arabidopsis. **Journal of Plant Biology**, v. 53, n. 2, p. 107–112, 12 abr. 2010.

LOU, He Qiang *et al.* A NAC-type transcription factor confers aluminium resistance by regulating cell wall-associated receptor kinase 1 and cell wall pectin. **Plant, Cell & Environment**, v. 43, n. 2, p. 463–478, 2 fev. 2020.

LU, Muxue *et al.* Functional characterization of the SbNrat1 gene in sorghum. **Plant Science**, v. 262, p. 18–23, set. 2017.

MA, Jian Feng *et al.* Response of Rice to Al Stress and Identification of Quantitative Trait Loci for Al Tolerance. **Plant and Cell Physiology**, v. 43, n. 6, p. 652–659, 15 jun. 2002.

MAGALHAES, Jurandir V *et al.* A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. **Nature Genetics**, v. 39, n. 9, p. 1156–1161, 26 set. 2007.

MAGNAVACA, R.; GARDNER, C. O.; CLARK, R. B. Inheritance of aluminum tolerance in maize. *In*: **Genetic Aspects of Plant Mineral Nutrition**. Dordrecht: Springer Netherlands, 1987. p. 201–212.

MARON, Lyza G. *et al.* Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. **The Plant Journal**, v. 61, n. 5, p. 728–740, mar. 2010.

MARON, Lyza G. *et al.* Aluminum tolerance in maize is associated with higher MATE1 gene copy number. **Proceedings of the National Academy of Sciences**, v. 110, n. 13, p. 5241–5246, 26 mar. 2013.

MATONYEI, Thomas K. *et al.* Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1. **Scientific Reports**, v. 10, n. 1, p. 7320, 30 abr. 2020.

MATTIELLO, Lucia *et al.* Transcriptional profile of maize roots under acid soil growth. **BMC Plant Biology**, v. 10, n. 1, p. 196, 9 dez. 2010.

MELO, Janaina O. *et al.* Repeat variants for the SbMATE transporter protect sorghum roots from aluminum toxicity by transcriptional interplay in cis and trans. **Proceedings of the National Academy of Sciences**, v. 116, n. 1, p. 313–318, 2 jan. 2019.

MIFTAHUDIN, Miftahudin *et al.* OsGERLP: A novel aluminum tolerance rice gene isolated from a local cultivar in Indonesia. **Plant Physiology and Biochemistry**, v. 162, p. 86–99, maio 2021.

MUTIMAAMBA, Charles *et al.* Line × tester analysis of maize grain yield under acid and non-acid soil conditions. **Crop Science**, v. 60, n. 2, p. 991–1003, 25 mar. 2020.

NANJO, Yohei *et al.* Mass Spectrometry-Based Analysis of Proteomic Changes in the Root Tips of Flooded Soybean Seedlings. **Journal of Proteome Research**, v. 11, n. 1, p. 372–385, 1 jan. 2012.

NIEDZIELA, Agnieszka *et al.* Aluminum Stress Induces Irreversible Proteomic Changes in the Roots of the Sensitive but Not the Tolerant Genotype of Triticale Seedlings. **Plants**, v. 11, n. 2, p. 165, 8 jan. 2022.

NINAMANGO-CÁRDENAS, Fernando Enrique *et al.* Mapping QTLs for aluminum tolerance in maize. **Euphytica**, v. 130, n. 2, p. 223–232, 2003.

NIU, Hong *et al.* Toxicity of soil labile aluminum fractions and aluminum species in soil water extracts on the rhizosphere bacterial community of tall fescue. **Ecotoxicology and Environmental Safety**, v. 187, p. 109828, jan. 2020.

NUNES-NESI, Adriano *et al.* The complex role of mitochondrial metabolism in plant aluminum resistance. **Trends in Plant Science**, v. 19, n. 6, p. 399–407, jun. 2014.

OFOE, Raphael *et al.* Aluminum in plant: Benefits, toxicity and tolerance mechanisms. **Frontiers in Plant Science**, v. 13, 13 jan. 2023.

PANG, Zhiqiang *et al.* MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. **Nucleic Acids Research**, v. 52, n. W1, p. W398–W406, 5 jul. 2024.

PARK, Myoung-Ryoul *et al.* Supra-optimal expression of the cold-regulated OsMyb4 transcription factor in transgenic rice changes the complexity of transcriptional network with major effects on

stress tolerance and panicle development. **Plant, Cell & Environment**, v. 33, n. 12, p. 2209–2230, 28 dez. 2010.

PENG, Xiaohua *et al.* Reactive oxygen species signaling is involved in melatonin-induced reduction of chlorothalonil residue in tomato leaves. **Journal of Hazardous Materials**, v. 443, p. 130212, fev. 2023.

PIÑEROS, Miguel A. *et al.* Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of ZmALMT1 – an anion-selective transporter. **The Plant Journal**, v. 53, n. 2, p. 352–367, 17 jan. 2008.

PINTO, Vitor Batista *et al.* Uncovering the transcriptional response of popcorn (Zea mays L. var. everta) under long-term aluminum toxicity. **Scientific Reports**, v. 11, n. 1, p. 19644, 4 out. 2021a.

PINTO, Vitor Batista *et al.* Deciphering the major metabolic pathways associated with aluminum tolerance in popcorn roots using label-free quantitative proteomics. **Planta**, v. 254, n. 6, p. 132, 25 dez. 2021b.

PINTO, Vitor Batista *et al.* Transcriptome-based strategies for identifying aluminum tolerance genes in popcorn (Zea mays L. var. everta). **Scientific Reports**, v. 13, n. 1, p. 19400, 8 nov. 2023.

QIN, Yu *et al.* A phenylalanine ammonia lyase from Fritillaria unibracteata promotes drought tolerance by regulating lignin biosynthesis and SA signaling pathway. **International Journal of Biological Macromolecules**, v. 213, p. 574–588, jul. 2022.

R CORE TEAM. **A Language and Environment for Statistical Computing.** R Foundation for Statistical Computing. Viena, Austria, , 2025. Disponível em: <https://www.R-project.org/>. Acesso em: 12 abr. 2025

RAHIM, Faisal *et al.* Identification of contrasting tropical popcorn inbreds for studying aluminum toxicity tolerance inheritance. **Euphytica**, v. 215, n. 3, 1 mar. 2019.

RAHMAN, Shafeeq Ur *et al.* **State-of-the-art OMICS strategies against toxic effects of heavy metals in plants: A review**. **Ecotoxicology and Environmental Safety**Academic Press, , 1 set. 2022.

RANJAN, Alok *et al.* Alleviating aluminum toxicity in plants: Implications of reactive oxygen species signaling and crosstalk with other signaling pathways. **Physiologia Plantarum**, v. 173, n. 4, p. 1765–1784, 17 dez. 2021.

RAZA, Ali *et al.* **Advances in "Omics" Approaches for Improving Toxic Metals/Metalloids Tolerance in Plants**. **Frontiers in Plant Science**Frontiers Media S.A., , 4 jan. 2022.

ROSA, Sílvia B. *et al.* Cytosolic APx knockdown indicates an ambiguous redox responses in rice. **Phytochemistry**, v. 71, n. 5–6, p. 548–558, abr. 2010.

ROYCHOWDHURY, Rajib *et al.* **Multi-Omics Pipeline and Omics-Integration Approach to Decipher Plant's Abiotic Stress Tolerance Responses**. **Genes**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jun. 2023.

SCHAUER, Nicolas *et al.* GC-MS libraries for the rapid identification of metabolites in complex biological samples. **FEBS Letters**, v. 579, n. 6, p. 1332–1337, 28 fev. 2005.

SHANNON, Paul *et al.* Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. **Genome Research**, v. 13, n. 11, p. 2498–2504, nov. 2003.

SHETTY, Rajpal *et al.* Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. **Science of The Total Environment**, v. 765, p. 142744, abr. 2021.

SILVA, Sónia. Aluminium Toxicity Targets in Plants. **Journal of Botany**, v. 2012, p. 1–8, 2 jul. 2012.

SIQUEIRA, João A. *et al.* 1Metabolic and physiological adjustments of maize leaves in response to aluminum stress. **Theoretical and Experimental Plant Physiology**, v. 32, n. 2, p. 133–145, 6 jun. 2020a.

SIQUEIRA, João A. *et al.* Metabolic and physiological adjustments of maize leaves in response to aluminum stress. **Theoretical and Experimental Plant Physiology**, v. 32, n. 2, p. 133–145, 6 jun. 2020b.

SIVAGURU, Mayandi *et al.* Aluminum-Induced Gene Expression and Protein Localization of a Cell Wall-Associated Receptor Kinase in Arabidopsis. **Plant Physiology**, v. 132, n. 4, p. 2256–2266, 1 ago. 2003.

SONG, Jianling *et al.* Differential expressions and enzymatic properties of malate dehydrogenases in response to nutrient and metal stresses in Stylosanthes guianensis. **Plant Physiology and Biochemistry**, v. 170, p. 325–337, jan. 2022.

ŠUKALOVIĆ, Vesna Hadži-Tašković *et al.* Cell wall-associated malate dehydrogenase activity from maize roots. **Plant Science**, v. 181, n. 4, p. 465–470, out. 2011.

SUN, Chengliang *et al.* Aluminum Induces Distinct Changes in the Metabolism of Reactive Oxygen and Nitrogen Species in the Roots of Two Wheat Genotypes with Different Aluminum Resistance. **Journal of Agricultural and Food Chemistry**, v. 65, n. 43, p. 9419–9427, 1 nov. 2017.

SZKLARCZYK, Damian *et al.* STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. **Nucleic Acids Research**, v. 47, n. D1, p. D607–D613, 8 jan. 2019.

SZURMAN-ZUBRZYCKA, Miriam *et al.* Aluminum or Low pH – Which Is the Bigger Enemy of Barley? Transcriptome Analysis of Barley Root Meristem Under Al and Low pH Stress. **Frontiers in Genetics**, v. 12, 19 maio 2021.

TANDZI, Liliane Ngoune *et al.* Breeding Maize for Tolerance to Acidic Soils: A Review. **Agronomy**, v. 8, n. 6, p. 84, 29 maio 2018.

TAO, Ye *et al.* The NAC transcription factor ANAC017 regulates aluminum tolerance by regulating the cell wall-modifying genes. **Plant Physiology**, v. 189, n. 4, p. 2517–2534, 1 ago. 2022.

TAO, Yonghong *et al.* Genome-wide association mapping of aluminum toxicity tolerance and fine mapping of a candidate gene for Nrat1 in rice. **PLOS ONE**, v. 13, n. 6, p. e0198589, 12 jun. 2018.

TESFAYE, M. *et al.* Overexpression of Malate Dehydrogenase in Transgenic Alfalfa Enhances Organic Acid Synthesis and Confers Tolerance to Aluminum. **Plant Physiology**, v. 127, n. 4, p. 1836–1844, 1 dez. 2001.

TYAGI, Wricha *et al.* Root transcriptome reveals efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype. **Scientific Reports**, v. 10, n. 1, p. 4580, 12 mar. 2020a.

TYAGI, Wricha *et al.* 1Root transcriptome reveals efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype. **Scientific Reports**, v. 10, n. 1, p. 4580, 12 mar. 2020b.

USDA. **United States Department of Agriculture. Foreign Agricultural Service. Grain: world markets and trade**. Disponível em: <https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads>. Acesso em: 18 jun. 2024.

VARADHARAJAN, Venkatramanan *et al.* **Multi-Omics Approaches Against Abiotic and Biotic Stress—A Review**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 mar. 2025.

WANG, Jingbo *et al.* Transcriptomics and metabolomics reveal tolerance new mechanism of rice roots to Al stress. **Frontiers in Genetics**, v. 13, 10 jan. 2023.

WANG, Mei *et al.* The auxin influx carrier, OsAUX3, regulates rice root development and responses to aluminium stress. **Plant, Cell & Environment**, v. 42, n. 4, p. 1125–1138, 29 abr. 2019.

WANG, Yuqi *et al.* An exclusion mechanism is epistatic to an internal detoxification mechanism in aluminum resistance in Arabidopsis. **BMC Plant Biology**, v. 20, n. 1, p. 122, 18 dez. 2020.

WANG, Yuqi *et al.* The plasma membrane-localized OsNIP1;2 mediates internal aluminum detoxification in rice. **Frontiers in Plant Science**, v. 13, 12 set. 2022.

WANG, Zhan Qi *et al.* Root proteome of rice studied by iTRAQ provides integrated insight into aluminum stress tolerance mechanisms in plants. **Journal of Proteomics**, v. 98, p. 189–205, fev. 2014.

WU, Liyuan *et al.* Multi-omics analysis unveils early molecular responses to aluminum toxicity in barley root tip. **Plant Physiology and Biochemistry**, v. 217, p. 109209, dez. 2024.

XIA, Jixing *et al.* Plasma membrane-localized transporter for aluminum in rice. **Proceedings of the National Academy of Sciences**, v. 107, n. 43, p. 18381–18385, 26 out. 2010.

XIA, Jixing; YAMAJI, Naoki; MA, Jian Feng. A plasma membrane-localized small peptide is involved in rice aluminum tolerance. **The Plant Journal**, v. 76, n. 2, p. 345–355, 6 out. 2013.

XIE, Lihua *et al.* Metabolome Analysis under Aluminum Toxicity between Aluminum-Tolerant and -Sensitive Rice (Oryza sativa L.). **Plants**, v. 11, n. 13, p. 1717, 28 jun. 2022.

XIE, Wenxiang *et al.* PP2C.D phosphatase SAL1 positively regulates aluminum resistance via restriction of aluminum uptake in rice. **Plant Physiology**, v. 192, n. 2, p. 1498–1516, 31 maio 2023.

YAMAJI, Naoki *et al.* A Zinc Finger Transcription Factor ART1 Regulates Multiple Genes Implicated in Aluminum Tolerance in Rice. **The Plant Cell**, v. 21, n. 10, p. 3339–3349, 1 dez. 2009.

YOKOSHO, Kengo *et al.* Functional Analysis of a MATE Gene OsFRDL2 Revealed its Involvement in Al-Induced Secretion of Citrate, but a Lower Contribution to Al Tolerance in Rice. **Plant and Cell Physiology**, v. 57, n. 5, p. 976–985, maio 2016.

YOSHIDA, Camila Hatsu Pereira *et al.* Tolerance mechanisms to aluminum in popcorn inbred lines involving aluminum compartmentalization and ascorbate–glutathione redox pathway. **Planta**, v. 257, n. 2, p. 28, 2 fev. 2023.

YOU, Jun; CHAN, Zhulong. ROS Regulation During Abiotic Stress Responses in Crop Plants. **Frontiers in Plant Science**, v. 6, 8 dez. 2015.

ZENG, Meng *et al.* Genome-wide association studies and transcriptome sequencing analysis reveal novel genes associated with Al tolerance in wheat. **Chemosphere**, v. 317, p. 137885, mar. 2023a.

ZENG, Meng *et al.* Genome-wide association studies and transcriptome sequencing analysis reveal novel genes associated with Al tolerance in wheat. **Chemosphere**, v. 317, p. 137885, mar. 2023b.

ZHAN, Meiqi *et al.* The transcription factor SbHY5 mediates light to promote aluminum tolerance by activating SbMATE and SbSTOP1s expression. **Plant Physiology and Biochemistry**, v. 205, p. 108197, dez. 2023.

ZHANG, Maolin *et al.* Auxin Efflux Carrier ZmPGP1 Mediates Root Growth Inhibition under Aluminum Stress. **Plant Physiology**, v. 177, n. 2, p. 819–832, 13 jun. 2018.

ZHANG, Peng *et al.* Mining candidate gene for rice aluminum tolerance through genome wide association study and transcriptomic analysis. **BMC Plant Biology**, v. 19, n. 1, p. 490, 12 dez. 2019.

ZHANG, Yanhong *et al.* Genome-Wide Identification of MDH Family Genes and Their Association with Salt Tolerance in Rice. **Plants**, v. 11, n. 11, p. 1498, 2 jun. 2022.

ZHAO, Daqiu *et al.* A Paeonia ostii caffeoyl-CoA O-methyltransferase confers drought stress tolerance by promoting lignin synthesis and ROS scavenging. **Plant Science**, v. 303, p. 110765, fev. 2021.

ZHAO, Shanrong *et al.* Comparison of RNA-Seq and Microarray in Transcriptome Profiling of Activated T Cells. **PLoS ONE**, v. 9, n. 1, p. e78644, 16 jan. 2014.

ZHENG, Lu *et al.* Proteomics of aluminum tolerance in plants. **PROTEOMICS**, v. 14, n. 4–5, p. 566–578, 4 mar. 2014.

Table 1. Main metabolic pathways associated with Al stress tolerance in plants

Species	Metabolic Pathway	Key Proteins / Metabolites	Function in Al Tolerance	References
Hordeum vulgare, Oryza sativa, Sorghum bicolor, Triticum aestivum, Zea mays	Al Exclusion via Organic Acids	Malate, Citrate, HvAACT1, ALMT1, Citrate synthase	Root exudation of organic acids that chelate Al^{3+} in the apoplast, preventing its uptake	Maron et al., (2013); Zeng et al., (2023a); Han et al., (2009)
Oryza sativa, Zea mays, Zea mays var. everta	Carbon and Sugar Metabolism	Fructose-6P, Glucose, ATP, Malate, Citrate, MDH, Sucrose synthase	Provides energy and metabolic intermediates; supports organic acid synthesis and defense compounds	Xie et al., (2023); Šukalović et al., (2011); Zhang et al., (2022); Pinto et al., (2021b)
Zea mays, Zea mays var. everta	Antioxidant Response	SOD, CAT, APX, POD, Ascorbic acid, Glutathione, GSH/GSSG	Detoxifies ROS generated under Al^{3+} stress, preventing oxidative damage to membranes and organelles	Yoshida et al., (2023); Peng et al., (2023); Du et al., (2022)
Oryza sativa, Sorghum bicolor, Triticum aestivum, Zea mays	Transcriptional Regulation	ART1, ASR5, WRKYs, STOP1	Controls expression of genes involved in Al exclusion, antioxidant defense, and cellular maintenance	Garcia-Oliveira et al., (2013); Li et al., (2020); Arenhart et al., (2016); Yamaji et al., (2009); Guan et al., (2023)
Medicago sativa, Zea mays var. everta	Amino Acid and Phenylpropanoid Metabolism	Phenylalanine, Proline, GABA, Ferulic acid, Lignin	Strengthens cell wall, contributes to signaling, antioxidant defense, and Al sequestration	Tesfaye et al., (2001); Pinto et al., (2021b); Yoshida et al., (2023)

Table 2. Key genes involved in Al tolerance in grain crops.

Species	Genes	Proteins/Metabolites	Functions	Regulations	References
Hordeum vulgare	*HvAACT1*	Citrate transporter (MATE)	Citrate exudation	Up	Huang et al. (2024)
	HvALMT1	Malate transporter (ALMT)	Malate exudation	Up	Gruber et al., (2010)
Oryza sativa	*OsA7*	H^+-ATPase	Interacts with *OsA7* to negatively regulate the PM H+-ATPase	Up	Xie et al., (2023)
	OsApx1 *OsApx1*	Ascorbate peroxidases	Cell defense	Up	Rosa et al., (2010)
	OsALMT4	Malate transporter (ALMT)	Malate exudation	Up	Liu et al., (2018)
	OsALS1	ABC transporter (ATPase)	Sequesters Al in vacuoles	Up	Huang et al. (2012)
	OsArPK	Al-related protein kinase		Up	Liu et al., (2022)
	OsART1	Transcription factor	Homolog of AtSTOP1; regulates STAR1, Nrat1	Up	Yamaji et al., (2009)
	OsART2	Transcription factor		Up	Che et al., (2018)
	OsASR1	Transcription factor	Complements *OsASR5* to regulate gene expression	Up	Arenhart et al., (2016)

	OsASR5	Transcription factor	Regulate genes related to photosynthesis	Up	Arenhart et al., (2016)
	OsAUX3	Auxin transporter	Involved in Al-induced inhibition of root growth	Up	Wang et al., (2019)
	OsCDT3	Cysteine-rich peptide	Binds Al in the cell wall, reducing cytosolic entry	Up	Xia et al., (2010; Xia; Yamaji; Ma, 2013)
	OsCS1	Citrate synthase		Up	Han et al., (2009)
	OsEXPA10	Al-inducible expansin gene	Cell elongation	Up	Che et al., (2016)
	OsFRDL2	Citrate transporter (FRDL)	Citrate efflux into the xylem	Up	Yokosho et al., (2016)
	OsFRDL4	Citrate transporter (FRDL)	Citrate efflux into the xylem	Up	Xia et al., (2010; Xia; Yamaji; Ma, 2013)
	OsGERLP	Ribossonal L32-like protein		Up	Miftahudin et al., (2021)
	OsMGT1	Mg transporter	Maintains Mg homeostasis	Up	Xia et al., (2010; Xia; Yamaji; Ma, 2013)
	OsMYB30	MYB transcription factor	Regulates modification of cell wall proteins	Up	Gao et al., (2023)
	OsNrat1	Al transporter (Nramp)	Al uptake and vacuolar storage	Up	Xia et al., (2010; Xia; Yamaji; Ma, 2013)
	OsNIP1;2	Aquaporins	Sequesters Al in vacuoles	Up	Wang et al., (2022)
	OsSAL1	PP2C.D phosphatase	Increased PM H+-ATPase activity and Al uptake	Up	Xie et al., (2023)
	OsSTAR1 *OsSTAR2*	ABC transporter complex	Cell wall repair via UDP-glucose transport	Up	Huang et al., (2009)
	OsWRKY22	WRKY transcription factor	Promote Al-induced increases in OsFRDL4 expression	Up	Li et al., (2018)
Sorghum bicolor	*SbGLU1*	β-1,3-glucanase enzyme	Regulate callose deposition	Up	Guan et al., (2023)
	SbHY5	MYB transcription factor	Active *SbMATE* and *SbSTOP1* expression	Up	Zhan et al., (2023)
	SbMATE	Citrate transporter (MATE)	Citrate exudation	Up	Magalhaes et al., (2007)
	SbNrat1	Al transporter (Nramp)	Selectively transports Al^{3+}	Up	Lu et al., (2017)
	SbSTAR1	ABC transporter	Regulating hemicellulose content in the root cell wall.	Up	Guan et al., (2023); Gao et al., (2021)
	SbSTOP1	Transcription factor	Complex regulatory mechanisms of STOP1-like proteins in response to Al toxicity	Up	Huang et al., (2018)

	SbZNF1	Transcription factor	Activation of SbMATE	Up	Melo et al., (2019)
	SbWRKY1	WRKY transcription factor	Activation of SbMATE	Up	Melo et al., (2019)
	SbWRKY22 *SbWRKY65*	WRKY transcription factor	Regulate callose deposition in roots	Up	Guan et al., (2023)
Triticum aestivum	*TaALMT1*	Malate transporter (ALMT)	Primary malate exudation mechanism in wheat	Up	Zeng et al., (2023a); Farokhzadeh et al., (2019)
	TaMATE1	Citrate transporter (MATE)	Complements *TaALMT1* in tolerant varieties	Up	Garcia-Oliveira et al., (2014)
	TaMATE2	Citrate transporter (MATE)	Citrate exudation	Up	Garcia-Oliveira et al., (2018)
	TaSTOP1	Transcription factor		Up	Garcia-Oliveira et al., (2013)
	TaWRKY47	Transcription factor	Activates detoxification genes (ALMT1, MATE)	Up	Li et al., (2020)
Zea mays	*ZmALDH*	Aldehyde dehydrogenase	Eliminates ROS under stress	Up	Du et al. (2022)
	ZmART1	Transcription factor	Associated with Al tolerance QTLs	Up	Matonyei et al., (2020)
	ZmAT6	Transcription factor	Associated with Al tolerance QTLs	Up	Du et al. (2020)
	ZmMATE1	Citrate transporter (MATE)	Citrate exudation	Up	Maron et al., (2010)
	ZmMATE6	Citrate transporter (MATE)	Citrate exudation	Up	Du et al., (2021a)
	ZmNRAMP4	Al transporter (Nramp)	Sequesters Al in vacuoles	Up	Li et al., (2022)
	ZmPGP1	ABC transporter	Associated with reduced auxin accumulation in root tips	Up	Zhang et al. (2018)
	ZmXTH	Xyloglucan endotransglucosylase/hydrolase	Reduces Al accumulation in roots and cell wall	Up	Du et al., (2021b)

CHAPTER II - PROTEOMIC AND METABOLOMIC SIGNATURES UNDERLYING ALUMINUM TOLERANCE IN POPCORN (*Zea mays* var. *everta*)

Proteomic and metabolomic signatures underlying aluminum tolerance in popcorn (*Zea mays var. everta*)

Matheus Pereira Ribeiro[1*], José Marcelo Soriano Viana[1], Lucas do Espírito Santo Gomes[2], Thais Regiani Cataldi[3], Allan de Marcos Lapaz[1], Cleberson Ribeiro[1], Vitor Batista Pinto[2]

[1] Department of General Biology, Federal University of Viçosa, 36570-900, Viçosa, MG, Brazil

[2] State University of North Fluminense, Biosciences and Biotechnology Center, 28013-602, Campos dos Goytacazes, RJ, Brazil

[3] Max Feffer Plant Genetics Laboratory, Department of Genetics, University of São Paulo - ESALQ, 13418-900, Piracicaba, SP, Brazil

[*]Corresponding author: matheus.p.ribeiro@ufv.br. ORCID 0000-0002-0486-0505

Abstract

Aluminum (Al) toxicity in acidic soils limits the productivity of many crops, including popcorn, a specialty maize with increasing economic relevance. In this study, an integrative proteomic and metabolomic analysis was conducted to uncover the molecular mechanisms underlying Al tolerance in a previously selected Al-tolerant popcorn inbred line. Seedlings were exposed for 72 hours to nutrient solution under -Al and +Al conditions in a controlled growth chamber. Root and leaf tissues were analyzed using LC-MS/MS and GC×GC/TOF-MS platforms following Al stress exposure. Across both tissues, a total of 423 differentially accumulated proteins (DAPs) and 68 differentially accumulated metabolites (DAMs) were detected, revealing tissue-specific responses. The highest number of DAPs (308) and DAMs (52) was observed in roots, where Al tolerance was associated with the upregulation of citrate synthase, TCA cycle enzymes, antioxidant proteins, and enhanced biosynthesis of phenylpropanoids, malate, and glutathione. In contrast, leaves exhibited metabolic adjustments involving energy metabolism, selenium pathways, and redox signaling aimed at preserving photosynthetic function. Integrated pathway analysis revealed a functional convergence between proteomic and metabolomic data, highlighting organic acid activity, ROS detoxification, and cell wall reinforcement as key tolerance strategies. These findings provide robust biochemical evidence of Al stress mitigation in popcorn and identify molecular signatures with potential applications in marker-assisted selection and precision breeding. This work demonstrates the value of multi-omics approaches in underutilized crops and contributes to the development of Al-tolerant cultivars adapted to acidic soils.

Key-words: Multi-omics integration; Al Tolerance mechanisms; antioxidant metabolism; TCA cycle.

Introduction

Maize (*Zea mays* L.) is the world's most produced cereal, with a global output of 1,158 million tons per season, considering all kinds of maize, like common maize, popcorn and others. Nearby half of this production comes from Americas, where the United States (31%), and Brazil (10%) rank as the first and third largest producers, respectively. It's playing a crucial role in the global supply of this crop, with a market worth billions of dollars (USDA, 2025). Thinking only of popcorn, a special type of corn, Brazil ranks second among the largest producers. Brazil's Midwest region is responsible for 80% of national production, with almost 300,000 tons produced in the 2019/20 season, with demand and productions increase year by year (Kist, 2019).

To address the dual challenges of rising global demand of food and climate change, understanding crop adaptability to suboptimal conditions, including stressed environments and low-fertility soils, becomes crucial. It is estimated that 40% of acid soils of the world (pH < 5.5), are distributed throughout the American continent (Tandzi et al., 2018). Modern agricultural practices, while essential for sustaining global food demand, often involve the intensive use of agrochemicals and pesticides, which contribute to the accumulation of heavy metals in soils and wastewater (Tandzi et al., 2018). Among these elements, aluminum (Al) stands out as one of the most abundant metals in the Earth's crust, and its concentration in soils is exacerbated by anthropogenic activities such as acidifying fertilization and land management strategies (Niu et al., 2020). In acidic soils, typically defined by a pH below 5.5, Al becomes solubilized into its phytotoxic ionic form (Al^{3+}), severely impairing root development and plant productivity (Silva, 2012).

Different environmental conditions influence gene expression in plants (Lasky et al., 2014). Genes activated under stress are responsible for triggering adaptive responses that will induce morphological, physiological and biochemical changes in different metabolic pathways (Gupta; Rico-Medina; Caño-Delgado, 2020). Plants exposed to Al stress use several survival

strategies, divided into two mechanisms: (i) resistance to Al, by reducing or preventing the binding of Al^{3+} to the root cell wall by the accumulation and/or exudation of organic ligands with a high affinity for Al^{3+}, such as organic acids (citrate, malate and others) and phenolic compounds (polypeptides and catechol); or (ii) tolerance to Al, by sequestering Al in the cell, forming a stable complex and stored in cell organelles, such as the vacuole, without any damage to the plant (Kochian et al., 2015; Nunes-Nesi et al., 2014). Due the natural Al sensibility in all kinds of maize, Al stress can cause yield losses of more than 30 % (Mutimaamba et al., 2020).

This ion binds to negatively charged groups in the cell wall and reduces mitotic activity in root apices, causing inhibition of cell elongation (Horst; Wang; Eticha, 2010). This inhibition results in damage to root morphology and architecture, with negative consequences for the ability to uptake water and nutrients (Silva, 2012). In maize, especially popcorn (*Zea mays* var. *everta*), Al^{3+} stress induces morphological changes such as cortical thickening, coif deformation, and altered lateral root patterning (Rahim et al., 2019; Yoshida et al., 2023). On a biochemical level, Al^{3+} triggers excessive reactive oxygen species (ROS) generation, leading to oxidative stress and cellular damage (Ranjan et al., 2021; You; Chan, 2015). Tolerant genotypes counteract this through antioxidant enzymes and compounds such as SOD, CAT, POD, ascorbate, and flavonoids (Tyagi et al., 2020a; Yoshida et al., 2023; You & Chan, 2015). Biochemical studies in popcorn under Al^{3+} stress have shown increased levels of amino acids (proline, arginine), phenolics, and sugars that support osmoprotection and redox balance (Pinto et al., 2021b; Yoshida et al., 2023). Therefore, the effects of Al stress result in a loss of plant productivity.

The process of refining knowledge about the genetic control of tolerance/resistance to stresses is crucial for the development of Al-tolerant genotypes. For many years, breeders have focused their efforts on obtaining tolerant plants through phenotypic changes. However, over

the years omics tools have emerged that are capable of assessing the genetic control of adaptive responses to stress-tolerance (Tandzi et al., 2018).

The development of popcorn adapted to Al toxicity has been the subject of several studies. Rahim et al., (2019) identified popcorn inbred lines contrasting for Al tolerance based on root damage and plant growth. Following, Yoshida et al., (2023) studied the compartmentalization of Al and the antioxidant defense system in these same popcorn inbred lines. High throughput transcriptomic and proteomic analyses were performed to evaluate the stress response mechanisms associated with Al-toxicity aiming to identify candidate players and pathways that confer Al-tolerance to advance in popcorn breeding (Pinto et al., 2021a, 2021b, 2023).

Modern plant breeding has integrated different high-throughput omics technologies to identify key genes that help crops resist environmental stresses. By combining insights from genomics, transcriptomics, proteomics, and metabolomics, researchers can now more accurately identify and select the genetic traits that make plants resilient to challenging growing conditions (Chen et al., 2022; Pinto et al., 2021b, 2023; Wang et al., 2023; Xie et al., 2022). The integration of multi-omics data has provided a comprehensive understanding of Al toxicity responses in crops, particularly in popcorn. These approaches have revealed that Al tolerance is governed by coordinated and genotype-specific molecular networks rather than isolated pathways, involving key mechanisms such as MATE and ALMT transporter activation, antioxidant responses, cell wall remodeling, and chelator accumulation (Pinto et al., 2021b, 2023; Yoshida et al., 2023). Multi-omics integration enables the identification of regulatory hubs and converging defense modules, highlighting the complexity of stress responses, which often include post-transcriptional modulation and tissue-specific dynamics (Rahman et al., 2022; Roychowdhury et al., 2023; Varadharajan et al., 2025).

Recently, several studies using an Al-tolerant popcorn inbred line have been conducted to understand the effects of Al stress on root systems, particularly at the metabolic and

biochemical levels. However, the adverse effects of Al on shoot development, energy synthesis processes, and other indirect impacts have not yet been fully elucidated from a proteomic and metabolic perspective (Pinto et al., 2021a, 2021b, 2023; Yoshida et al., 2023). In this study, proteomic and metabolomic analyses were carried out to investigate the response to Al stress in an Al-tolerant popcorn line, aiming to identify key molecular processes and potential candidate genes associated with Al tolerance. The results provide new insights into the molecular mechanisms underlying Al tolerance in popcorn, contributing to a deeper understanding of Al stress regulation and offering valuable support for sustainable agricultural development and the rational use of natural resources.

Material and Methods

Plant material and growth conditions

Seeds from Al-tolerant inbred line (11-133) after ten generations, selected previously by Rahim et al., (2019) developed by Popcorn Breeding Program at Federal University of Viçosa (Viçosa, Brazil) were used for this work. Seeds were treated with the fungicide Maxim® (Syngenta, Pratteln, Switzerland) and germinated on Germitest paper rolls in a growth chamber at 25 °C ± 1 °C at 16:8 h light:dark photoperiod for seven days. Then, seedlings with uniform growth were picked randomly, transferred to a ½ strength nutritive solution (pH 4.5) described by Magnavaca; Gardner; Clark, (1987) and modified by Famoso et al., (2010) with constant aeration for 24 h.

Posteriorly, the nutrient solution was changed to total strength and the seedlings were divided into two groups: control (-Al) and presence of Al (+Al). The nutrient solution composition consisted of: 1 mM KCl, 1.5 mM NH_4NO_3, 1 mM $CaCl_2$, 0.045 mM KH_2PO_4, 200 µM $MgSO_4$, 500 µM $Mg(NO_3)_2$, 155 µM $MgCl_2$, 11.8 µM $MnCl_2.4H_2O$, 33 µM H_3BO_3, 3.06 µM $ZnSO_4.7H_2O$, 0.8 µM $CuSO_4.5H_2O$, 1.07 µM $Na_2MoO_4.H_2O$, and 77 µM Fe-EDTA. In both, conditions the pH was adjusted to 4.5. For +Al treatment group, the nutritive solution was supplied to 540 µM of $AlCl_3$ (160 µM Al^{3+}). The seedlings were maintained in a growth

chamber under constant aeration, at 25 ± 1 °C, 200 μmol photons m^{-2} s^{-1} light intensity, 80% relative humidity, and photoperiod of 16:8 h (light:dark). After 72 h, the shoots and roots were collected and macerated and homogenized in liquid nitrogen were kept in an ultra-freezer (-80 °C). The experiment was performed in a completely randomized design with one seedling per pot.

Proteomic analysis

Protein extraction and tryptic digestion

For protein extraction, three biological replicates were established by pooling tissues from three individual plants per treatment. Shots and roots were separately collected for each condition (-Al and +Al), immediately frozen in liquid nitrogen, and individually macerated. Proteins were extracted from 300 mg (leaves) or 450 mg (roots) of powered fresh material (FM) via trichloroacetic acid (TCA)/acetone precipitation method (Damerval et al., 1986), with modifications. The samples were resuspended in 1 mL of cold extraction buffer containing 10% (w/v) of TCA (Sigma Aldrich, St. Louis, MO, USA) in acetone with 20 mM of dithiothreitol (DTT) (GE Healthcare, Piscataway, NJ, USA) and agitated for 5 minutes at 8 ºC. The mixture was kept at -20º C for 1 hour and subsequently centrifuged at 16.000 g for 30 minutes at 4 ºC. The resulting pellets were washed three times with cold acetone, with the addition of 20 mM of DTT, and centrifuged for 5 minutes per wash. The pellets were air-dried and resuspended in a buffer containing 7 M urea, 2 M of thiourea, 2 % of Triton X-100, 1 % of DTT, and 1 mM of fenilmetilsulfonil fluorete (PMSF) (Sigma-Aldrich). Samples were vortexed for 30 min at 4 °C in a refrigerator, followed by centrifugation at 16,000 × g for 20 min at 4 °C. The supernatants containing total proteins were collected, and the protein concentration was measured using Bradford reagent (Life Technologies, Carlsbad, CA, USA).

The proteins samples were precipitated using a methanol/chloroform extraction method to remove any interfering compounds from the samples (Nanjo et al., 2012). The samples were then resuspended in a solution consisting of 7 M urea and 2 M thiourea after which tryptic

protein digestion (1:100 enzyme:protein, V5111, Promega, Madison, WI, USA) was performed using Microcon-30 kDa filter units (Merck Millipore, Burlington, MA, USA) with the filter-aided sample preparation (FASP) methodology (Pinto et al., 2021b). The resulting peptides were quantified with the A205 nm protein and peptide method using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Mass spectrometry analysis and proteins identification by HPLC

The samples were injected into a nanoAcquity ultra-pure liquid chromatography (UPLC) mass spectrometer connected to a Q-TOF SYNAPT G2-Si instrument (Waters, Manchester, UK). The runs consisted of three biological replicates of 2 µg of peptide samples. The spectra processing and database search were performed using the ProteinLynx Global SERVER (PLGS) software v.3.02 (Waters) and the *Zea mays* protein databank (ID: UP000007305) available on UniProtKB (www.uniprot.org). The label-free quantification analyses were performed using ISOQuant software v.1.7 (Distler et al., 2014). To ensure the quality of the results after data processing, only proteins present in the three runs were accepted for differential abundance analysis. Proteins were deemed up-accumulated if the log2 value of the fold change (FC) was > 0.585 and down-accumulated if the log2 value of the FC was < -0.585, according to Student's t test (two-tailed, $P < 0.05$) and after P-value correction with the Benjamini–Hochberg test.

Functional analysis of proteomic data

Differentially accumulated proteins (DAPs) identified in root and leaf tissues underwent comprehensive functional annotation and enrichment analysis using ShinyGO (v0.82) (Ge; Jung; Yao, 2020). For both Gene Ontology (GO) and KEGG pathway analyses, was applied a p-value < 0.01 (Fisher's exact test with FDR correction) to identify significantly enriched terms. From the resulting datasets, were selected the top 15 most significantly enriched terms for Biological Process (GO-BP) and KEGG pathways based on their -log10 (p-value) scores. Plots

were constructed on R environment. The plots were generated with the RStudio (R Core Team, 2025) software.

For protein-protein interaction (PPI) network analysis, DAPs from roots and leaf were further examined using the *Zea mays* proteome reference in the STRING-DB plugin on Cytoscape software (Shannon et al., 2003) with a confidence score cutoff > 0.7 (high confidence), and selected KEGG pathways enriched with biological relevance for Al-tolerance. Cluster analysis was applied in the entire network with the ClusterMaker plugin with the MCODE algorithm to identify the modules highly connected to each other. Finally, topology analysis was performed in both PPI networks to identify the degree of each node using the NetworkAnalyzer plugin.

Metabolomic analysis

Metabolites extraction and derivatization

The extraction of metabolites, seven biological replicates were established by pooling tissues from three individual plants per treatment following Hoffman et al., (2010). In microtubes with macerated samples of roots (200 mg) and shoots (100 mg) was added three 3 mm tungsten beads and used a vibration mill (MM 301 Retch GmbH & Co, Haan, Germany) with a frequency of 30 Hz s^{-1} for 60 s. The metabolites were extracted by adding 500 µL of 6:2:2 methanol:chloroform:water ice-cold extraction solution was added. It was added 50 µL (1 mg ml^{-1} each of myristic acid (13C3), palmitic acid (13C4) and succinic acid (2H4)) stable isotope reference compounds in each sample which were used as external standards and used as external standard for quality control. These microtubes were vortexed for 30 s to shoots and 90 s to roots, and placed in an ultrasonic low-temperature bath at 30 Hz s^{-1} for 15 min. The samples were then centrifuged at 4°C for 10 min at 14000 x *g* (Eppendorf Centrifuge 5415R). Then, the supernatant was filtered (Millipore filter PVDF 0.22 µm) and 100 µL of the filtered was transferred to a chromatographic vial where the extracts were lyophilized until completely dry.

Finally, samples lyophilized were derivatized according to Gullberg et al., (2004) with 30 µL of methoxyamine hydrochloride (15 mg ml^{-1} in pyridine) for 16 h at room temperature in dark. The samples were trimethylsilylated by adding 30 µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS), the resulting mixture stand at room temperature for 1 h. Then, 30 µl of heptane with 15 ng $µl^{-1}$ de methyl-esterate was added. Blank control samples and a series of n-alkanes (C12–C40), which allowed retention indices to be calculated (Schauer et al., 2005) were also used.

Analysis and metabolites identification by GC-MS

One microliter of each derivatized sample was injected in splitless mode into a 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA), equipped with a Comb-xt Autosampler (Leap Technologies, Carrboro, USA). For compound separation, a DB-5 GC capillary column (20 m × 0.18 mm i.d. × 0.18 µm film thickness; Agilent Technologies) was employed as the primary column, while the secondary column consisted of an Rxi-17 capillary (0.69 m × 0.1 mm i.d. × 0.1 µm film thickness; Restek, Bellefonte, USA). The injector temperature was set to 280 °C, with a septum purge flow of 20 mL min^{-1}, activated 60 s after injection. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The column oven was programmed as follows: initial temperature of 80 °C for 2 min, ramped at 15 °C min^{-1} up to 305 °C, and held at this temperature for 10 min. The effluent was directed to the ion source of a GC×GC/TOF-MS system (Pegasus 4D, Leco Corp., St. Joseph, USA). The transfer line and ion source were maintained at 280 °C and 250 °C, respectively. Ionization was achieved using a 70-eV electron beam at an ionization current of 2.0 mA. Mass spectra were acquired at a rate of 10 spectra s^{-1}, covering a mass range of *m/z* 45–800.

Baseline correction and export of all mass spectrometry files in NetCDF format were performed using ChromaTOF software v. 4.51 (Leco Corp., St. Joseph, USA). Subsequent peak detection, retention time alignment, and compound identification were carried out using the TargetSearch package (Cuadros-Inostroza et al., 2009). Metabolites were annotated by

comparing retention indices (± 2 s) and spectral similarity (similarity score >600) against entries in the Golm Metabolome Database (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html) (Kopka et al., 2005). Metabolite intensities were normalized to both sample dry weight and the total ion chromatogram (TIC).

Metabolomic data analysis

The data analysis and graphics generations were done in MetaboAnalyst 6.0 (Pang et al., 2024). To reduce systematic variance and to improve the performance for downstream statistical analysis data were log-transformed and Pareto scaled prior to data analysis. Metabolites up- or down-accumulation was determined using the same FC analysis methodology used in the proteomic data analysis.

The metabolic pathways integrating proteomic and metabolomic data were constructed using the KEGG database (https://www.genome.jp/kegg/) by mapping the identified protein and metabolite IDs.

Results

Proteomic profile

A total of 308 differentially accumulated proteins (DAPs) were identified in the roots (Suppl. Table 1) and 115 in the leaves (Suppl. Table 2) of the Al-tolerant inbred line when comparing conditions with and without Al stress. In roots, were detected 68 proteins up-accumulated and 174 proteins down-accumulated, in addition to 36 proteins accumulated only in the -Al condition (down-related proteins), and 30 proteins accumulated only in +Al toxicity treatment (up-related proteins) (Suppl. Table 1). In leaves, were detected 14 and 69 DAPs up- and down-accumulated, respectively, in addition to 17 proteins accumulated only in -Al condition (down-related proteins), and 15 in the +Al toxicity treatment (up-related proteins) (Suppl. Table 2). Furthermore, 18 proteins were differentially accumulated under Al stress in both roots and leaves (Figure 1).

The GO analysis revealed distinct functional enrichment patterns between tissues, with leaf proteins predominantly associated with energy metabolic processes (e.g., glycogen metabolic process; UDP-glucose metabolic process; energy reserve metabolic process), while root proteins showed significant enrichment in detoxification pathways (including cellular detoxification; ROS metabolic process, and organic acid metabolic process) (Figure 2). The analysis of cellular component identified that most of the proteins are localized in photosystem and ribosomal components in leaves, and in cell wall in roots. For molecular function, in leaves were predominant proteins with transferase activity, while in roots were those with oxidoreductase and antioxidant activity (Suppl. Figure 1).

For KEGG enrichment analysis, leaf proteins exhibited high fold enrichment in pathways involved in the maintenance of energy and protein production, such as photosynthesis and antenna proteins, ribosome, selenocompound metabolism, and carbon metabolism (Figure 3). In roots, a greater number of proteins were associated with the pathways involved in energy generation, detoxification, and production of defense compounds, such as the tricarboxylic acid cycle (TCA) cycle, cysteine and methionine metabolism, phenylpropanoid biosynthesis, and glutathione metabolism (Figure 3).

In the PPI network from leaf proteins retrieved 57 physical interactions (Figure 4a) and three clusters – translation, photosynthesis and quinone binding, and protein folding (Figure 4b). For roots proteins, the network retrieved 151 interactions (Figure 5a) and nine clusters: translation, TCA cycle, proteasome, glycolysis, and pentose phosphate pathway, oxidative phosphorylation, protein folding, cysteine and methionine metabolism, glycolysis, and phagosome (Figure 5b). In leaf proteins the maximum degree of connections was equal 20 with the participation of ribosomal proteins (Suppl. Figure 2). In roots, the network topology revealed central hubs with maximum degree to 40S ribosomal proteins S2-1 (A0A804M605, and A0A804P6X5; degree = 19), and citrate synthase (B4FIC0; degree = 18) (Suppl. Figure 3).

Metabolite quantification

In the leaves of the Al-tolerant inbred line, 83 metabolites were identified, including 16 differentially accumulated metabolites (DAMs) (Suppl. Figure 4), with 8 up-accumulated and 8 down-accumulated (Figure 6) (Table 1). In the roots, a total of 65 metabolites were detected, among which 52 were DAMs (Suppl. Figure 5), including 40 up-accumulated and 12 down-accumulated (Figure 6) (Table 1). For KEGG enrichment analysis, Al-stress in the roots activated key metabolic pathways associated with detoxification and oxidative resistance, energy adjustments, cell protection, metabolic repair, and signaling under stress (Figure 7).

Integration of proteomic and metabolomic data

By integrating proteomic and metabolomic data, a metabolic regulation profile was observed with significant changes in metabolites and key enzymes in the roots. The main findings include: (i) the accumulation of nitrogenous intermediates (serine, glycine, alanine, aspartate, asparagine, threonine, tyrosine, tryptophan, valine, and leucine) and associated proteins (aspartate aminotransferase, serine hydroxymethyltransferase, glutamate synthetase, and glycine decarboxylase), identified in the amino acid biosynthesis pathway (Suppl. Figure 6); (ii) elevated levels of glucose-6-phosphate, phosphoenolpyruvate, pyruvate, phosphoserine, glycine, malate, oxaloacetate, and glyceraldehyde-3-phosphate in central carbon metabolism (Suppl. Figure 7); and (iii) the accumulation of malate with concomitant reduction in malate dehydrogenase activity and increase in citrate synthase, changes detected both in carbon metabolism (Suppl. Figure 7) and in the TCA cycle (Suppl. Figure 8).

Discussion

The integration of GO and KEGG functional enrichment analyses with PPI networks revealed a systemically coordinated and specialized response to Al stress, with roots and leaves playing complementary roles in plant survival and growth maintenance. While GO analysis classifies differentially expressed proteins according to their biological processes, cellular localization, and molecular functions, KEGG highlights the metabolic and signaling pathways

involved, revealing specific functional reprogramming. PPI analysis, in turn, reveals how these proteins interact functionally, forming clusters and identifying molecular hubs that are critical for cellular adaptation (Szklarczyk et al., 2019). When combined, these approaches contextualize each other's findings and also help uncover tissue-specific mechanisms, such as the prioritization of photosynthesis in leaves and detoxification in roots. The metabolic pathways integrate enabled a comprehensive visualization of the biochemical routes modulated under Al stress, highlighting the functional interactions between enzymes and their corresponding metabolic intermediates.

Photosynthetic Support and Metabolic Conservation

In leaves, the enrichment of the "photosynthesis" and "antenna protein" pathways indicate an attempt to preserve the functional integrity of the photosynthetic apparatus. At the same time, PPI analysis showed the repression of ribosomal proteins, reflecting a strategy to contain translation, possibly as an energy-saving mechanism in the face of adversity. The enrichment of the selenocompounds metabolism pathway, known for its association with the neutralization of ROS, highlights the role of selenium in antioxidant mechanisms (Cartes et al., 2010; Das et al., 2025).

The activation of the monobactam biosynthesis pathway, although with a low number of proteins but high enrichment value, suggests the recruitment of specific biosynthetic pathways. Intermediates of this pathway, such as serine and threonine, comprise the cytoplasmic kinase domain of WAK proteins (Kohorn; Kohorn, 2012), which are related to cell wall integrity. In this context, the strong up-accumulation of serine and the simultaneous down-accumulation of L-aspartate in metabolomic data reinforce the hypothesis of amino acid flux redirection toward WAK-related signaling. Additionally, the down-regulation of the enzyme sulfate adenylyltransferase (B6SRJ5), involved in sulfur metabolism (Jez, 2019), may impair sulfur-containing metabolite biosynthesis, indirectly affecting pectin structure and cell wall remodeling. This metabolic configuration can support a model where serine-driven WAK

activation contributes to maintaining cell wall integrity under Al stress. In fact, Sivaguru et al., (2003) demonstrated that overexpression of WAK1 increases Al resistance in *Arabidopsis*, while Lou et al., (2020) observed that this expression is associated with cell wall pectin content, contributing to tolerance in *Vigna umbellata*.

The predominance of repressed TCA cycle proteins in the leaves, with the exception of up-accumulated malate dehydrogenase (B4FVH1, MDH), whereas two other MDH isoforms were found down- accumulated, indicates a redirection of the energy pathway. This enzyme, involved in malate synthesis, may provide precursors for root exudation, as reported by Tesfaye et al., (2001) in alfalfa, and confirmed in corn (Šukalović et al., 2011) and *Stylosanthes* (Song et al., 2022). This pattern supports the hypothesis of intertissue metabolic collaboration, with leaves providing substrates for root defense.

Moreover, the metabolomic identification of up-accumulated coumaric acid in leaves suggests a potential aerial detoxification mechanism against Al. A possible mechanism by which phenolics contribute to ROS scavenging involves peroxidases. Specifically, phenolics can serve as electron donors for guaiacol-type peroxidase, facilitating the conversion of hydrogen peroxide into water (Kováčik; Klejdus, 2008). Its accumulation may thus represent a complementary strategy to root exudation, reinforcing the systemic nature of Al tolerance in maize.

Metabolic Reprogramming, Detoxification, and Defense

In contrast, roots exhibited significant enrichment in pathways related to metabolic reprogramming, antioxidant defense, and physical barrier formation. Integrated analysis revealed the accumulation of amino acids such as serine, glycine, aspartate, tyrosine, tryptophan, and phenylalanine, reflecting a strategic redistribution of carbon and nitrogen for the synthesis of defensive compounds. The induction of aspartate aminotransferase and glycine decarboxylase supports both amino acid production and the regeneration of reducing potential via folate and glutathione metabolism (Foyer; Noctor, 2011; Yoshida et al., 2023).

The positive modulation of cysteine and methionine pathways, glutathione biosynthesis, and phenylpropanoids highlights the coordinated mobilization of central metabolism to meet bioenergetic and antioxidant demands. The activation of citrate synthase (B4FIC0), highlighted as a central hub in the PPI network, due the large number of connections with other proteins (Suppl. Figure 3), reinforces its role as a key point in the TCA cycle and in the generation of citrate, an organic acid with Al^{3+} chelating action (Pinto et al., 2021b). Isocitrate dehydrogenase, also induced (B4FLJ3, A0A804QTB0), supports this function by contributing NADPH and specific intermediates (Li; Ma; Matsumoto, 2000; Liu et al., 2010). In parallel, MDH repression prevents the conversion of malate to oxaloacetate, suggesting its cytosolic accumulation and subsequent exudation (Zhang et al., 2022). Integration with the metabolomic profile confirmed the increase in malate in roots under stress, reinforcing its role as a chelating agent in popcorn under Al-exposure. Our results are consistent with those described by Pinto et al., (2021b), who demonstrated that Al toxicity triggers malate secretion in popcorn roots, while simultaneously inducing citrate synthase accumulation in the tolerant inbred line.

This classic tolerance mechanism is further complemented by glycolytic and anaplerotic pathways, with accumulation of pyruvate and phosphoenolpyruvate (Tesfaye et al., 2001), which provide precursors for the biosynthesis of organic acids and amino acids. The reduction of intermediates such as 3-phosphoglycerate and fructose-6-phosphate possibly reflects the diversion of carbon to more critical pathways.

Amino Acid and Phenolic Compound Metabolism

Amino acid biosynthesis pathways were found to be highly modulated, particularly the production of serine, glycine, aspartate, threonine, tryptophan, and phenylalanine. These compounds act as precursors to antioxidant molecules, such as glutathione, and phenolic compounds involved in strengthening the cell wall. The phenylpropanoid pathway, activated in response to stress, showed induction of phenylalanine ammonia-lyase (PAL) and caffeoyl-CoA O-methyltransferase 2 (CCoAOMT2), essential for the production of lignin and flavonoids (Qin

et al., 2022; Zhao et al., 2021). The increase in phenylalanine strengthens the functional convergence between the proteome and the metabolome.

These compounds not only act in antioxidant defense but also reinforce cell walls, limiting the entry of toxic ions. The GO annotation of proteins located in the cell wall, together with the presence of enzymes associated with callose deposition (Mattiello et al., 2010), corroborates this structural barrier as a defensive mechanism. Consistently, callose synthase (A0A1D6Q188) was found up- accumulated in the proteomic analysis, reinforcing the hypothesis of callose accumulation as a physical barrier against Al^{3+} intrusion. This enzyme plays a key role in synthesizing β-1,3-glucans, contributing to localized wall thickening and reduced cell wall porosity under stress conditions (Guan et al., 2023). The up-regulation of this isoform suggests an active reinforcement of cell walls in aerial tissues, complementing the metabolic production of lignin and phenolics.

PPI Networks and Functional Organization

The analysis of PPI networks reinforced the functional organization of the tissue. In the leaves, the formation of modules centered on translation, photosynthesis, and chaperones was observed, while in the roots, the topological complexity (with 151 interactions and nine clusters) reflected the diversity of activated responses, including proteasome, glycolysis, pentose phosphate pathway, and phagosome, related to damaged protein recycling, energy mobilization, and cell defense (De Sousa et al., 2022; Donnelly et al., 2024; Siqueira et al., 2020; Sun et al., 2017; Zheng et al., 2014).

Citrate synthase and ribosomal 40S S9-2 emerged as structuring hubs of responses in roots (Suppl. Figure 3) and leaves (Suppl. Figure 2), respectively, connecting energy metabolism, organic acid biosynthesis, and translation regulation. The high connectivity of oxidoreductase enzymes points to a global reorganization around redox stability, which is consistent with the enrichment of glutathione pathways and the accumulation of antioxidant amino acids (Foyer; Noctor, 2011).

In leaves, the enrichment of chaperone-related modules, especially involving heat shock proteins (HSPs), highlights the importance of protein folding and stabilization mechanisms under Al stress. Many HSPs identified were up- accumulated, suggesting their role in protecting the proteome from misfolding and aggregation caused by Al-induced oxidative damage (Liu et al., 2023). This protective function supports enhanced tolerance, as proper folding maintains cellular homeostasis and mitigates stress-induced proteotoxicity. The extensive interactions among HSPs also indicate a coordinated chaperone network actively responding to stress signals (Leng et al., 2022).

On the other hand, in roots, although a considerable number of proteins were down-accumulated, these nodes maintained interactions with classic al stress related proteins that were up- accumulated. This pattern suggests a complex regulatory balance, where some pathways are suppressed possibly to conserve energy (Tyagi et al., 2020), while key defense and detoxification (Ahammed et al., 2024; Das et al., 2025; You; Chan, 2015) proteins remain active to mitigate damage. The connectivity between down- and up-accumulated proteins emphasizes the fine-tuned coordination of stress responses in roots, integrating energy metabolism, protein turnover, and cellular defense mechanisms.

Functional Integration: A Multi-Strategic Defense System

The response to Al stress in maize is characterized by a functional division between tissues: leaves preserve photosynthetic capacity and contribute metabolic precursors (Siqueira et al., 2020), while roots coordinate the main tolerance mechanisms, exudation of organic acids, antioxidant defense, and structural barriers (Kochian et al., 2015). The integration of proteomic and metabolomic data revealed a coherent, efficient, and multidimensional response, operating from metabolic redirection to the reconfiguration of complex biosynthetic pathways.

This systemic reprogramming provides a solid foundation for the identification of key genes and target pathways for breeding and gene-editing strategies aimed at improving Al tolerance. Pathways associated with organic acid biosynthesis, ROS detoxification, cell wall

remodeling, and energy optimization are particularly promising, especially in maize and popcorn cultivars grown in acidic soils. Among the main candidate genes, those involved in citrate synthase activity and energy maintenance stand out. Additionally, genes related to cell wall modification, like those encoding pectin methylesterases and WAKs, play crucial roles in maintaining cell wall integrity under Al stress (Kohorn; Kohorn, 2012). Genes involved in antioxidant defense, including glutathione S-transferases (GSTs), superoxide dismutases (SODs), and heat shock proteins (HSPs), are also promising targets due to their role in mitigating oxidative damage caused by Al toxicity (Ahammed et al., 2024).

These genes serve as potential molecular markers for breeding programs and biotechnological applications aimed at improving Al tolerance. Integration of multi-omics data can facilitate the identification of novel candidate genes and regulatory networks, accelerating the development of tolerant maize and popcorn varieties.

Conclusion

These results show that Al-tolerant plants modulate their metabolism extensively, coordinating the synthesis of key compounds to optimize ionic detoxification, cellular maintenance, and redox balance. The strong convergence between proteome and metabolome, especially in roots, reveals an integrated defense involving organic acid exudation, antioxidant production, and structural reinforcement.

Notably, down-regulation of many proteins suggests energy conservation as a key tolerance strategy. This study is novel in profiling proteomic and metabolomic changes in both leaves and roots, providing a comprehensive view rarely addressed in the literature. Leaves exhibit a more conservative regulation with fewer differentially accumulated proteins and metabolites, while roots show more dynamic modulation, indicating ongoing acclimation after 72 hours of stress.

These findings highlight targets such as organic acid transport and redox control for genetic improvement of popcorn adapted to acidic soils.

References

AGRAHARI, Raj Kishan *et al.* Expression GWAS of PGIP1 Identifies STOP1-Dependent and STOP1-Independent Regulation of PGIP1 in Aluminum Stress Signaling in Arabidopsis. **Frontiers in Plant Science**, v. 12, 17 dez. 2021.

AHAMMED, Golam Jalal *et al.* Reactive oxygen species signaling in melatonin-mediated plant stress response. **Plant Physiology and Biochemistry**, v. 207, p. 108398, fev. 2024.

ALBAHRI, Ghosoon *et al.* Enhancing Essential Grains Yield for Sustainable Food Security and Bio-Safe Agriculture through Latest Innovative Approaches. **Agronomy**, v. 13, n. 7, p. 1709, 26 jun. 2023.

ANANI, Osikemekha Anthony *et al.* Insights to proteomics and metabolomics metal chelation in food crops. **Journal of Proteins and Proteomics**, v. 13, n. 3, p. 159–173, 20 jun. 2022.

ARAUJO, Alda Neis Miranda *et al.* Integration of proteomic and metabolomic analyses: New insights for mapping informal workers exposed to potentially toxic elements. **Frontiers in Public Health**, v. 10, 25 jan. 2023.

ARENHART, Rafael Augusto *et al.* Rice ASR1 and ASR5 are complementary transcription factors regulating aluminium responsive genes. **Plant, Cell & Environment**, v. 39, n. 3, p. 645–651, 14 mar. 2016.

AWASTHI, Jay Prakash *et al.* Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. **PLOS ONE**, v. 12, n. 4, p. e0176357, 27 abr. 2017.

AZEEM, Asad *et al.* Integration of Multi-Omics Approaches and Speed Breeding for Plant Stress Tolerance. *In*: CHEN, Jen-Tsung (Org.). **Plant Stress Tolerance: Molecular Mechanisms and Breeding Strategies**. 1st. ed. Boca Raton: CRC Press, 2025. v. 2 p. 17.

BANET, Travis *et al.* Assessing modified aluminum-based water treatment residuals as a plant-available phosphorus source. **Chemosphere**, v. 247, p. 125949, maio 2020.

BARRERO, Luz S. *et al.* Identifying genes associated with abiotic stress tolerance suitable for CRISPR/Cas9 editing in upland rice cultivars adapted to acid soils. **Plant Direct**, v. 6, n. 12, 10 dez. 2022.

BARTHOLOMÉ, Jérôme *et al.* Genomic selection for tolerance to aluminum toxicity in a synthetic population of upland rice. **PLOS ONE**, v. 19, n. 8, p. e0307009, 22 ago. 2024.

CARTES, P. *et al.* Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. **Annals of Applied Biology**, v. 156, n. 2, p. 297–307, 11 mar. 2010.

CARVALHO, Geraldo *et al.* Back to Acid Soil Fields: The Citrate Transporter SbMATE Is a Major Asset for Sustainable Grain Yield for Sorghum Cultivated on Acid Soils. **G3 Genes|Genomes|Genetics**, v. 6, n. 2, p. 475–484, 1 fev. 2016.

CHAKRABORTY, Nilakshi *et al.* **Exploring Aluminum Tolerance Mechanisms in Plants with Reference to Rice and Arabidopsis: A Comprehensive Review of Genetic, Metabolic, and Physiological Adaptations in Acidic Soils**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jul. 2024.

CHE, Jing *et al.* An Al-inducible expansin gene, OsEXPA10 is involved in root cell elongation of rice. **The Plant Journal**, v. 88, n. 1, p. 132–142, 22 out. 2016.

CHE, Jing *et al.* Functional characterization of an aluminum (Al)-inducible transcription factor, ART2, revealed a different pathway for Al tolerance in rice. **New Phytologist**, v. 220, n. 1, p. 209–218, 11 out. 2018.

CHENG, Xiaoqing *et al.* Protective roles of salicylic acid in maintaining integrity and functions of photosynthetic photosystems for alfalfa (Medicago sativa L.) tolerance to aluminum toxicity. **Plant Physiology and Biochemistry**, v. 155, p. 570–578, out. 2020.

CHOUDHURY, Shuvasish *et al.* A Computational Study of the Role of Secondary Metabolites for Mitigation of Acid Soil Stress in Cereals Using Dehydroascorbate and Mono-Dehydroascorbate Reductases. **Antioxidants**, v. 11, n. 3, p. 458, 25 fev. 2022.

CUADROS-INOSTROZA, Álvaro *et al.* TargetSearch - a Bioconductor package for the efficient preprocessing of GC-MS metabolite profiling data. **BMC Bioinformatics**, v. 10, 16 dez. 2009.

DAI, Huaxin *et al.* Comparative Proteomic Analysis of Aluminum Tolerance in Tibetan Wild and Cultivated Barleys. **PLoS ONE**, v. 8, n. 5, p. e63428, 14 maio 2013.

DAMERVAL, Catherine *et al.* Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. **ELECTROPHORESIS**, v. 7, n. 1, p. 52–54, 13 jan. 1986.

DAS, Abir *et al.* Mitigation of aluminum toxicity in rice seedlings using biofabricated selenium nanoparticles and nitric oxide: Synergistic effects on oxidative stress tolerance and sulfur metabolism. **Chemosphere**, v. 370, p. 143940, fev. 2025.

DE SOUSA, Alexandra *et al.* Subcellular compartmentalization of aluminum reduced its hazardous impact on rye photosynthesis. **Environmental Pollution**, v. 315, p. 120313, dez. 2022.

DERBYSHIRE, Mark C.; BATLEY, Jacqueline; EDWARDS, David. **Use of multiple 'omics techniques to accelerate the breeding of abiotic stress tolerant crops**. **Current Plant Biology**Elsevier B.V., , 1 dez. 2022.

DISTLER, Ute *et al.* Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. **Nature Methods**, v. 11, n. 2, p. 167–170, 15 fev. 2014.

DONNELLY, Chase P. *et al.* Malate production, sugar metabolism, and redox homeostasis in the leaf growth zone of Rye (Secale cereale) increase stress tolerance to aluminum stress: A biochemical and genome-wide transcriptional study. **Journal of Hazardous Materials**, v. 464, p. 132956, fev. 2024.

DU, Hanmei *et al.* A Maize ZmAT6 Gene Confers Aluminum Tolerance via Reactive Oxygen Species Scavenging. **Frontiers in Plant Science**, v. 11, 9 jul. 2020.

DU, Hanmei *et al.* ZmMATE6 from maize encodes a citrate transporter that enhances aluminum tolerance in transgenic Arabidopsis thaliana. **Plant Science**, v. 311, p. 111016, out. 2021a.

DU, Hanmei *et al.* ZmXTH, a xyloglucan endotransglucosylase/hydrolase gene of maize, conferred aluminum tolerance in Arabidopsis. **Journal of Plant Physiology**, v. 266, p. 153520, nov. 2021b.

DU, Han-Mei *et al.* Overexpression of the Aldehyde Dehydrogenase Gene ZmALDH Confers Aluminum Tolerance in Arabidopsis thaliana. **International Journal of Molecular Sciences**, v. 23, n. 1, p. 477, 1 jan. 2022.

FAMOSO, Adam N. *et al.* Development of a Novel Aluminum Tolerance Phenotyping Platform Used for Comparisons of Cereal Aluminum Tolerance and Investigations into Rice Aluminum Tolerance Mechanisms. **Plant Physiology**, v. 153, n. 4, p. 1678–1691, 3 ago. 2010.

FAMOSO, Adam N. *et al.* Genetic Architecture of Aluminum Tolerance in Rice (Oryza sativa) Determined through Genome-Wide Association Analysis and QTL Mapping. **PLoS Genetics**, v. 7, n. 8, p. e1002221, 4 ago. 2011.

FANG, Qiu *et al.* Degradation of STOP1 mediated by the F-box proteins RAH1 and RAE1 balances aluminum resistance and plant growth in Arabidopsis thaliana. **The Plant Journal**, v. 106, n. 2, p. 493–506, 10 abr. 2021.

FAROKHZADEH, Sara *et al.* Mapping QTLs of flag leaf morphological and physiological traits related to aluminum tolerance in wheat (Triticum aestivum L.). **Physiology and Molecular Biology of Plants**, v. 25, n. 4, p. 975–990, 6 jul. 2019.

FAROOQI, Muhammad Qudrat Ullah *et al.* **Recent developments in multi-omics and breeding strategies for abiotic stress tolerance in maize (Zea mays L.)**. **Frontiers in Plant Science**Frontiers Media S.A., , 23 set. 2022.

FOYER, Christine H.; NOCTOR, Graham. Ascorbate and Glutathione: The Heart of the Redox Hub. **Plant Physiology**, v. 155, n. 1, p. 2–18, jan. 2011.

GAO, Jie *et al.* Identification of a bacterial-type ATP-binding cassette transporter implicated in aluminum tolerance in sweet sorghum (Sorghum bicolor L.). **Plant Signaling & Behavior**, v. 16, n. 7, p. 1916211, 3 jul. 2021.

GAO, Li Jun *et al.* ART1 and putrescine contribute to rice aluminum resistance via OsMYB30 in cell wall modification. **Journal of Integrative Plant Biology**, v. 65, n. 4, p. 934–949, 18 abr. 2023.

GARCIA-OLIVEIRA, A. L. *et al.* Molecular cloning of TaMATE2 homoeologues potentially related to aluminium tolerance in bread wheat (Triticum aestivum L.). **Plant Biology**, v. 20, n. 5, p. 817–824, 17 set. 2018.

GARCIA-OLIVEIRA, Ana *et al.* Molecular characterization of TaSTOP1 homoeologues and their response to aluminium and proton (H+) toxicity in bread wheat (Triticum aestivum L.). **BMC Plant Biology**, v. 13, n. 1, p. 134, 2013.

GARCIA-OLIVEIRA, Ana Luísa *et al.* Molecular characterization of the citrate transporter gene TaMATE1 and expression analysis of upstream genes involved in organic acid transport under Al stress in bread wheat (Triticum aestivum). **Physiologia Plantarum**, v. 152, n. 3, p. 441–452, 2 nov. 2014.

GE, Steven Xijin; JUNG, Dongmin; YAO, Runan. ShinyGO: a graphical gene-set enrichment tool for animals and plants. **Bioinformatics**, v. 36, n. 8, p. 2628–2629, 15 abr. 2020.

GRUBER, Benjamin D. *et al.* HvALMT1 from barley is involved in the transport of organic anions. **Journal of Experimental Botany**, v. 61, n. 5, p. 1455–1467, mar. 2010.

GUAN, Kexing *et al.* Two Sweet Sorghum (Sorghum bicolor L.) WRKY Transcription Factors Promote Aluminum Tolerance via the Reduction in Callose Deposition. **International Journal of Molecular Sciences**, v. 24, n. 12, p. 10288, 17 jun. 2023.

GULLBERG, Jonas *et al.* Design of experiments: an efficient strategy to identify factors influencing extraction and derivatization of Arabidopsis thaliana samples in metabolomic studies with gas chromatography/mass spectrometry. **Analytical Biochemistry**, v. 331, n. 2, p. 283–295, ago. 2004.

GUPTA, Aditi; RICO-MEDINA, Andrés; CAÑO-DELGADO, Ana I. The physiology of plant responses to drought. **Science**, v. 368, n. 6488, p. 266–269, 17 abr. 2020.

HAJIBOLAND, Roghieh *et al.* Aluminum Toxicity in Plants: Present and Future. **Journal of Plant Growth Regulation**, v. 42, n. 7, p. 3967–3999, 28 jul. 2023.

HAN, Lei *et al.* Proteomic profiling sheds light on alkali tolerance of common wheat (Triticum aestivum L.). **Plant Physiology and Biochemistry**, v. 138, p. 58–64, maio 2019.

HAN, Rongrong *et al.* Quantitative phosphoproteomic analysis provides insights into the aluminum-responsiveness of Tamba black soybean. **PLOS ONE**, v. 15, n. 8, p. e0237845, 19 ago. 2020.

HAN, Yingying *et al.* One Novel Mitochondrial Citrate Synthase from Oryza sativa L. can Enhance Aluminum Tolerance in Transgenic Tobacco. **Molecular Biotechnology**, v. 42, n. 3, p. 299–305, 27 jul. 2009.

HE, Ying *et al.* Comparative quantitative phosphoproteomic and parallel reaction monitoring analysis of soybean roots under aluminum stress identify candidate phosphoproteins involved in aluminum resistance capacity. **Journal of Hazardous Materials**, v. 479, p. 135485, nov. 2024.

HOFFMAN, Daniel E. *et al.* Changes in diurnal patterns within the Populus transcriptome and metabolome in response to photoperiod variation. **Plant, Cell & Environment**, v. 33, n. 8, p. 1298–1313, 5 ago. 2010.

HORST, Walter J.; WANG, Yunxia; ETICHA, Dejene. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. **Annals of Botany**, v. 106, n. 1, p. 185–197, jul. 2010.

HUANG, Chao Feng *et al.* A Bacterial-Type ABC Transporter Is Involved in Aluminum Tolerance in Rice. **The Plant Cell**, v. 21, n. 2, p. 655–667, 30 mar. 2009.

HUANG, Chao-Feng *et al.* A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. **The Plant Journal**, v. 69, n. 5, p. 857–867, 29 mar. 2012.

HUANG, Sheng *et al.* Identification of STOP1-Like Proteins Associated With Aluminum Tolerance in Sweet Sorghum (Sorghum bicolor L.). **Frontiers in Plant Science**, v. 9, 28 fev. 2018.

HUANG, Sheng; SATO, Kazuhiro; MA, Jian Feng. Breeding for an elite malting barley cultivar with acid soil tolerance. **Communications Biology**, v. 7, n. 1, p. 1203, 28 set. 2024.

JAISWAL, Sandeep *et al.* Deciphering genetic mechanisms of Al toxicity tolerance through meta-QTL analysis in rice (Oryza sativa L.). **Environmental and Experimental Botany**, v. 228, p. 106030, dez. 2024.

JEZ, Joseph M. Structural biology of plant sulfur metabolism: from sulfate to glutathione. **Journal of Experimental Botany**, v. 70, n. 16, p. 4089–4103, 19 ago. 2019.

KHAN, A. A.; MCNEILLY, T.; COLLINS, J. C. Accumulation of amino acids, proline, and carbohydrates in response to aluminum and manganese stress in maize. **Journal of Plant Nutrition**, v. 23, n. 9, p. 1303–1314, set. 2000.

KIST, Benno Bernardo. **Anuário brasileiro do milho 2019**. Santa Cruz do Sul: Gazeta Santa Cruz, 2019.

KLOET, Frans M. van der *et al.* Increased comparability between RNA-Seq and microarray data by utilization of gene sets. **PLOS Computational Biology**, v. 16, n. 9, p. e1008295, 30 set. 2020.

KOBAYASHI, Yuriko *et al.* STOP2 Activates Transcription of Several Genes for Al- and Low pH-Tolerance that Are Regulated by STOP1 in Arabidopsis. **Molecular Plant**, v. 7, n. 2, p. 311–322, fev. 2014.

KOCHIAN, Leon V. *et al.* Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. **Annual Review of Plant Biology**, v. 66, n. 1, p. 571–598, 29 abr. 2015.

KOCJAN, Anna; KWASNIEWSKA, Jolanta; SZURMAN-ZUBRZYCKA, Miriam. **Understanding plant tolerance to aluminum: exploring mechanisms and perspectives**. **Plant and Soil**Springer Science and Business Media Deutschland GmbH, , 1 fev. 2024.

KOHORN, Bruce D.; KOHORN, Susan L. The cell wall-associated kinases, WAKs, as pectin receptors. **Frontiers in Plant Science**, v. 3, 2012.

KONG, Xiangpei *et al.* System analysis of microRNAs in the development and aluminium stress responses of the maize root system. **Plant Biotechnology Journal**, v. 12, n. 8, p. 1108–1121, out. 2014.

KOPKA, Joachim *et al.* GMD@CSB.DB: The Golm metabolome database. **Bioinformatics**, v. 21, n. 8, p. 1635–1638, 15 abr. 2005.

KOVÁČIK, Jozef; KLEJDUS, Bořivoj. Dynamics of phenolic acids and lignin accumulation in metal-treated Matricaria chamomilla roots. **Plant Cell Reports**, v. 27, n. 3, p. 605–615, 8 mar. 2008.

LASKY, Jesse R. *et al.* Natural Variation in Abiotic Stress Responsive Gene Expression and Local Adaptation to Climate in Arabidopsis thaliana. **Molecular Biology and Evolution**, v. 31, n. 9, p. 2283–2296, set. 2014.

LENG, Ya-Jun *et al.* Arabidopsis ERdj3B coordinates with ERECTA-family receptor kinases to regulate ovule development and the heat stress response. **The Plant Cell**, v. 34, n. 10, p. 3665–3684, 27 set. 2022.

LI, Chun Xiao *et al.* A WRKY transcription factor confers aluminum tolerance via regulation of cell wall modifying genes. **Journal of Integrative Plant Biology**, v. 62, n. 8, p. 1176–1192, 13 ago. 2020.

LI, Ge Zi *et al.* Transcription factor WRKY22 promotes aluminum tolerance via activation of OsFRDL4 expression and enhancement of citrate secretion in rice (Oryza sativa). **New Phytologist**, v. 219, n. 1, p. 149–162, 15 jul. 2018.

LI, Hongjie *et al.* ZmNRAMP4 Enhances the Tolerance to Aluminum Stress in Arabidopsis thaliana. **International Journal of Molecular Sciences**, v. 23, n. 15, p. 8162, 25 jul. 2022.

LI, Xiao Feng; MA, Jian Feng; MATSUMOTO, Hideaki. Pattern of Aluminum-Induced Secretion of Organic Acids Differs between Rye and Wheat. **Plant Physiology**, v. 123, n. 4, p. 1537–1544, 1 ago. 2000.

LIU, Chan *et al.* Overexpression of ZmSTOP1-A Enhances Aluminum Tolerance in Arabidopsis by Stimulating Organic Acid Secretion and Reactive Oxygen Species Scavenging. **International Journal of Molecular Sciences**, v. 24, n. 21, p. 15669, 27 out. 2023a.

LIU, Jie *et al.* Altered Expression of the Malate-Permeable Anion Channel OsALMT4 Reduces the Growth of Rice Under Low Radiance. **Frontiers in Plant Science**, v. 9, 3 maio 2018.

LIU, Siyan *et al.* MsDjB4, a HSP40 Chaperone in Alfalfa (Medicago sativa L.), Improves Alfalfa Hairy Root Tolerance to Aluminum Stress. **Plants**, v. 12, n. 15, p. 2808, 28 jul. 2023b.

LIU, Weixiao *et al.* Integrated proteomics and metabolomics analysis of transgenic and gene-stacked maize line seeds. **GM Crops and Food**, v. 12, n. 1, p. 361–375, 2021.

LIU, Xiang P. *et al.* A novel kinase subverts aluminium resistance by boosting ornithine decarboxylase-dependent putrescine biosynthesis. **Plant, Cell & Environment**, v. 45, n. 8, p. 2520–2532, 20 ago. 2022.

LIU, Yinghui *et al.* Characterization of a Stress-induced NADP-isocitrate Dehydrogenase Gene in Maize Confers Salt Tolerance in Arabidopsis. **Journal of Plant Biology**, v. 53, n. 2, p. 107–112, 12 abr. 2010.

LOU, He Qiang *et al.* A NAC-type transcription factor confers aluminium resistance by regulating cell wall-associated receptor kinase 1 and cell wall pectin. **Plant, Cell & Environment**, v. 43, n. 2, p. 463–478, 2 fev. 2020.

LU, Muxue *et al.* Functional characterization of the SbNrat1 gene in sorghum. **Plant Science**, v. 262, p. 18–23, set. 2017.

MA, Jian Feng *et al.* Response of Rice to Al Stress and Identification of Quantitative Trait Loci for Al Tolerance. **Plant and Cell Physiology**, v. 43, n. 6, p. 652–659, 15 jun. 2002.

MAGALHAES, Jurandir V *et al.* A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. **Nature Genetics**, v. 39, n. 9, p. 1156–1161, 26 set. 2007.

MAGNAVACA, R.; GARDNER, C. O.; CLARK, R. B. Inheritance of aluminum tolerance in maize. *In*: **Genetic Aspects of Plant Mineral Nutrition**. Dordrecht: Springer Netherlands, 1987. p. 201–212.

MARON, Lyza G. *et al.* Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. **The Plant Journal**, v. 61, n. 5, p. 728–740, mar. 2010.

MARON, Lyza G. *et al.* Aluminum tolerance in maize is associated with higher MATE1 gene copy number. **Proceedings of the National Academy of Sciences**, v. 110, n. 13, p. 5241–5246, 26 mar. 2013.

MATONYEI, Thomas K. *et al.* Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1. **Scientific Reports**, v. 10, n. 1, p. 7320, 30 abr. 2020.

MATTIELLO, Lucia *et al.* Transcriptional profile of maize roots under acid soil growth. **BMC Plant Biology**, v. 10, n. 1, p. 196, 9 dez. 2010.

MELO, Janaina O. *et al.* Repeat variants for the SbMATE transporter protect sorghum roots from aluminum toxicity by transcriptional interplay in cis and trans. **Proceedings of the National Academy of Sciences**, v. 116, n. 1, p. 313–318, 2 jan. 2019.

MIFTAHUDIN, Miftahudin *et al.* OsGERLP: A novel aluminum tolerance rice gene isolated from a local cultivar in Indonesia. **Plant Physiology and Biochemistry**, v. 162, p. 86–99, maio 2021.

MUTIMAAMBA, Charles *et al.* Line × tester analysis of maize grain yield under acid and non-acid soil conditions. **Crop Science**, v. 60, n. 2, p. 991–1003, 25 mar. 2020.

NANJO, Yohei *et al.* Mass Spectrometry-Based Analysis of Proteomic Changes in the Root Tips of Flooded Soybean Seedlings. **Journal of Proteome Research**, v. 11, n. 1, p. 372–385, 1 jan. 2012.

NIEDZIELA, Agnieszka *et al.* Aluminum Stress Induces Irreversible Proteomic Changes in the Roots of the Sensitive but Not the Tolerant Genotype of Triticale Seedlings. **Plants**, v. 11, n. 2, p. 165, 8 jan. 2022.

NINAMANGO-CÁRDENAS, Fernando Enrique *et al.* Mapping QTLs for aluminum tolerance in maize. **Euphytica**, v. 130, n. 2, p. 223–232, 2003.

NIU, Hong *et al.* Toxicity of soil labile aluminum fractions and aluminum species in soil water extracts on the rhizosphere bacterial community of tall fescue. **Ecotoxicology and Environmental Safety**, v. 187, p. 109828, jan. 2020.

NUNES-NESI, Adriano *et al.* The complex role of mitochondrial metabolism in plant aluminum resistance. **Trends in Plant Science**, v. 19, n. 6, p. 399–407, jun. 2014.

OFOE, Raphael *et al.* Aluminum in plant: Benefits, toxicity and tolerance mechanisms. **Frontiers in Plant Science**, v. 13, 13 jan. 2023.

PANG, Zhiqiang *et al.* MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. **Nucleic Acids Research**, v. 52, n. W1, p. W398–W406, 5 jul. 2024.

PARK, Myoung-Ryoul *et al.* Supra-optimal expression of the cold-regulated OsMyb4 transcription factor in transgenic rice changes the complexity of transcriptional network with major effects on stress tolerance and panicle development. **Plant, Cell & Environment**, v. 33, n. 12, p. 2209–2230, 28 dez. 2010.

PENG, Xiaohua *et al.* Reactive oxygen species signaling is involved in melatonin-induced reduction of chlorothalonil residue in tomato leaves. **Journal of Hazardous Materials**, v. 443, p. 130212, fev. 2023.

PIÑEROS, Miguel A. *et al.* Not all ALMT1-type transporters mediate aluminum-activated organic acid responses: the case of ZmALMT1 – an anion-selective transporter. **The Plant Journal**, v. 53, n. 2, p. 352–367, 17 jan. 2008.

PINTO, Vitor Batista *et al.* Uncovering the transcriptional response of popcorn (Zea mays L. var. everta) under long-term aluminum toxicity. **Scientific Reports**, v. 11, n. 1, p. 19644, 4 out. 2021a.

PINTO, Vitor Batista *et al.* Deciphering the major metabolic pathways associated with aluminum tolerance in popcorn roots using label-free quantitative proteomics. **Planta**, v. 254, n. 6, p. 132, 25 dez. 2021b.

PINTO, Vitor Batista *et al.* Transcriptome-based strategies for identifying aluminum tolerance genes in popcorn (Zea mays L. var. everta). **Scientific Reports**, v. 13, n. 1, p. 19400, 8 nov. 2023.

QIN, Yu *et al.* A phenylalanine ammonia lyase from Fritillaria unibracteata promotes drought tolerance by regulating lignin biosynthesis and SA signaling pathway. **International Journal of Biological Macromolecules**, v. 213, p. 574–588, jul. 2022.

R CORE TEAM. **A Language and Environment for Statistical Computing.** R Foundation for Statistical Computing. Viena, Austria, , 2025. Disponível em: <https://www.R-project.org/>. Acesso em: 12 abr. 2025

RAHIM, Faisal *et al.* Identification of contrasting tropical popcorn inbreds for studying aluminum toxicity tolerance inheritance. **Euphytica**, v. 215, n. 3, 1 mar. 2019.

RAHMAN, Shafeeq Ur *et al.* **State-of-the-art OMICS strategies against toxic effects of heavy metals in plants: A review**. **Ecotoxicology and Environmental Safety**Academic Press, , 1 set. 2022.

RANJAN, Alok *et al.* Alleviating aluminum toxicity in plants: Implications of reactive oxygen species signaling and crosstalk with other signaling pathways. **Physiologia Plantarum**, v. 173, n. 4, p. 1765–1784, 17 dez. 2021.

RAZA, Ali *et al.* **Advances in "Omics" Approaches for Improving Toxic Metals/Metalloids Tolerance in Plants**. **Frontiers in Plant Science**Frontiers Media S.A., , 4 jan. 2022.

ROSA, Sílvia B. *et al.* Cytosolic APx knockdown indicates an ambiguous redox responses in rice. **Phytochemistry**, v. 71, n. 5–6, p. 548–558, abr. 2010.

ROYCHOWDHURY, Rajib *et al.* **Multi-Omics Pipeline and Omics-Integration Approach to Decipher Plant's Abiotic Stress Tolerance Responses**. **Genes**Multidisciplinary Digital Publishing Institute (MDPI), , 1 jun. 2023.

SCHAUER, Nicolas *et al.* GC-MS libraries for the rapid identification of metabolites in complex biological samples. **FEBS Letters**, v. 579, n. 6, p. 1332–1337, 28 fev. 2005.

SHANNON, Paul *et al.* Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. **Genome Research**, v. 13, n. 11, p. 2498–2504, nov. 2003.

SHETTY, Rajpal *et al.* Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. **Science of The Total Environment**, v. 765, p. 142744, abr. 2021.

SILVA, Sónia. Aluminium Toxicity Targets in Plants. **Journal of Botany**, v. 2012, p. 1–8, 2 jul. 2012.

SIQUEIRA, João A. *et al.* 1Metabolic and physiological adjustments of maize leaves in response to aluminum stress. **Theoretical and Experimental Plant Physiology**, v. 32, n. 2, p. 133–145, 6 jun. 2020a.

SIQUEIRA, João A. *et al.* Metabolic and physiological adjustments of maize leaves in response to aluminum stress. **Theoretical and Experimental Plant Physiology**, v. 32, n. 2, p. 133–145, 6 jun. 2020b.

SIVAGURU, Mayandi *et al.* Aluminum-Induced Gene Expression and Protein Localization of a Cell Wall-Associated Receptor Kinase in Arabidopsis. **Plant Physiology**, v. 132, n. 4, p. 2256–2266, 1 ago. 2003.

SONG, Jianling *et al.* Differential expressions and enzymatic properties of malate dehydrogenases in response to nutrient and metal stresses in Stylosanthes guianensis. **Plant Physiology and Biochemistry**, v. 170, p. 325–337, jan. 2022.

ŠUKALOVIĆ, Vesna Hadži-Tašković *et al.* Cell wall-associated malate dehydrogenase activity from maize roots. **Plant Science**, v. 181, n. 4, p. 465–470, out. 2011.

SUN, Chengliang *et al.* Aluminum Induces Distinct Changes in the Metabolism of Reactive Oxygen and Nitrogen Species in the Roots of Two Wheat Genotypes with Different Aluminum Resistance. **Journal of Agricultural and Food Chemistry**, v. 65, n. 43, p. 9419–9427, 1 nov. 2017.

SZKLARCZYK, Damian *et al.* STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. **Nucleic Acids Research**, v. 47, n. D1, p. D607–D613, 8 jan. 2019.

SZURMAN-ZUBRZYCKA, Miriam *et al.* Aluminum or Low pH – Which Is the Bigger Enemy of Barley? Transcriptome Analysis of Barley Root Meristem Under Al and Low pH Stress. **Frontiers in Genetics**, v. 12, 19 maio 2021.

TANDZI, Liliane Ngoune *et al.* Breeding Maize for Tolerance to Acidic Soils: A Review. **Agronomy**, v. 8, n. 6, p. 84, 29 maio 2018.

TAO, Ye *et al.* The NAC transcription factor ANAC017 regulates aluminum tolerance by regulating the cell wall-modifying genes. **Plant Physiology**, v. 189, n. 4, p. 2517–2534, 1 ago. 2022.

TAO, Yonghong *et al.* Genome-wide association mapping of aluminum toxicity tolerance and fine mapping of a candidate gene for Nrat1 in rice. **PLOS ONE**, v. 13, n. 6, p. e0198589, 12 jun. 2018.

TESFAYE, M. *et al.* Overexpression of Malate Dehydrogenase in Transgenic Alfalfa Enhances Organic Acid Synthesis and Confers Tolerance to Aluminum. **Plant Physiology**, v. 127, n. 4, p. 1836–1844, 1 dez. 2001.

TYAGI, Wricha *et al.* Root transcriptome reveals efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype. **Scientific Reports**, v. 10, n. 1, p. 4580, 12 mar. 2020a.

TYAGI, Wricha *et al.* 1Root transcriptome reveals efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype. **Scientific Reports**, v. 10, n. 1, p. 4580, 12 mar. 2020b.

USDA. **United States Department of Agriculture. Foreign Agricultural Service. Grain: world markets and trade**. Disponível em: <https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads>. Acesso em: 18 jun. 2024.

VARADHARAJAN, Venkatramanan *et al.* **Multi-Omics Approaches Against Abiotic and Biotic Stress—A Review**. **Plants**Multidisciplinary Digital Publishing Institute (MDPI), , 1 mar. 2025.

WANG, Jingbo *et al.* Transcriptomics and metabolomics reveal tolerance new mechanism of rice roots to Al stress. **Frontiers in Genetics**, v. 13, 10 jan. 2023.

WANG, Mei *et al.* The auxin influx carrier, OsAUX3, regulates rice root development and responses to aluminium stress. **Plant, Cell & Environment**, v. 42, n. 4, p. 1125–1138, 29 abr. 2019.

WANG, Yuqi *et al.* An exclusion mechanism is epistatic to an internal detoxification mechanism in aluminum resistance in Arabidopsis. **BMC Plant Biology**, v. 20, n. 1, p. 122, 18 dez. 2020.

WANG, Yuqi *et al.* The plasma membrane-localized OsNIP1;2 mediates internal aluminum detoxification in rice. **Frontiers in Plant Science**, v. 13, 12 set. 2022.

WANG, Zhan Qi *et al.* Root proteome of rice studied by iTRAQ provides integrated insight into aluminum stress tolerance mechanisms in plants. **Journal of Proteomics**, v. 98, p. 189–205, fev. 2014.

WU, Liyuan *et al.* Multi-omics analysis unveils early molecular responses to aluminum toxicity in barley root tip. **Plant Physiology and Biochemistry**, v. 217, p. 109209, dez. 2024.

XIA, Jixing *et al.* Plasma membrane-localized transporter for aluminum in rice. **Proceedings of the National Academy of Sciences**, v. 107, n. 43, p. 18381–18385, 26 out. 2010.

XIA, Jixing; YAMAJI, Naoki; MA, Jian Feng. A plasma membrane-localized small peptide is involved in rice aluminum tolerance. **The Plant Journal**, v. 76, n. 2, p. 345–355, 6 out. 2013.

XIE, Lihua *et al.* Metabolome Analysis under Aluminum Toxicity between Aluminum-Tolerant and -Sensitive Rice (Oryza sativa L.). **Plants**, v. 11, n. 13, p. 1717, 28 jun. 2022.

XIE, Wenxiang *et al.* PP2C.D phosphatase SAL1 positively regulates aluminum resistance via restriction of aluminum uptake in rice. **Plant Physiology**, v. 192, n. 2, p. 1498–1516, 31 maio 2023.

YAMAJI, Naoki *et al.* A Zinc Finger Transcription Factor ART1 Regulates Multiple Genes Implicated in Aluminum Tolerance in Rice. **The Plant Cell**, v. 21, n. 10, p. 3339–3349, 1 dez. 2009.

YOKOSHO, Kengo *et al.* Functional Analysis of a MATE Gene OsFRDL2 Revealed its Involvement in Al-Induced Secretion of Citrate, but a Lower Contribution to Al Tolerance in Rice. **Plant and Cell Physiology**, v. 57, n. 5, p. 976–985, maio 2016.

YOSHIDA, Camila Hatsu Pereira *et al.* Tolerance mechanisms to aluminum in popcorn inbred lines involving aluminum compartmentalization and ascorbate–glutathione redox pathway. **Planta**, v. 257, n. 2, p. 28, 2 fev. 2023.

YOU, Jun; CHAN, Zhulong. ROS Regulation During Abiotic Stress Responses in Crop Plants. **Frontiers in Plant Science**, v. 6, 8 dez. 2015.

ZENG, Meng *et al.* Genome-wide association studies and transcriptome sequencing analysis reveal novel genes associated with Al tolerance in wheat. **Chemosphere**, v. 317, p. 137885, mar. 2023a.

ZENG, Meng *et al.* Genome-wide association studies and transcriptome sequencing analysis reveal novel genes associated with Al tolerance in wheat. **Chemosphere**, v. 317, p. 137885, mar. 2023b.

ZHAN, Meiqi *et al.* The transcription factor SbHY5 mediates light to promote aluminum tolerance by activating SbMATE and SbSTOP1s expression. **Plant Physiology and Biochemistry**, v. 205, p. 108197, dez. 2023.

ZHANG, Maolin *et al.* Auxin Efflux Carrier ZmPGP1 Mediates Root Growth Inhibition under Aluminum Stress. **Plant Physiology**, v. 177, n. 2, p. 819–832, 13 jun. 2018.

ZHANG, Peng *et al.* Mining candidate gene for rice aluminum tolerance through genome wide association study and transcriptomic analysis. **BMC Plant Biology**, v. 19, n. 1, p. 490, 12 dez. 2019.

ZHANG, Yanhong *et al.* Genome-Wide Identification of MDH Family Genes and Their Association with Salt Tolerance in Rice. **Plants**, v. 11, n. 11, p. 1498, 2 jun. 2022.

ZHAO, Daqiu *et al.* A Paeonia ostii caffeoyl-CoA O-methyltransferase confers drought stress tolerance by promoting lignin synthesis and ROS scavenging. **Plant Science**, v. 303, p. 110765, fev. 2021.

ZHAO, Shanrong *et al.* Comparison of RNA-Seq and Microarray in Transcriptome Profiling of Activated T Cells. **PLoS ONE**, v. 9, n. 1, p. e78644, 16 jan. 2014.

ZHENG, Lu *et al.* Proteomics of aluminum tolerance in plants. **PROTEOMICS**, v. 14, n. 4–5, p. 566–578, 4 mar. 2014.

Table 1. Differentially Accumulated Metabolites (DAMs) identified in roots and leaves. DAMs with log2(FC) value > 0.585 was up-accumulated and down-accumulated if the log2(FC) < -0.585.

Tissue	Metabolite	log2(FC)	p.ajusted
Roots	1,2,3-Trihydroxybenzene	-5.3998	0.00102
	Methyl mannofuranoside	-4.776	9.97E-06
	Rhamnose	-3.825	0.01819
	Fructopyranose/Sorbopyranose/Fructopyranose/Psicopyranose/Tagatopyranose/Sorbose pyranose	-3.7776	0.0069
	2-Allyl-1,4-dimethoxybenzene	-3.5109	7.24E-08
	Erythronic acid lactone	-3.4802	0.00616
	Arabinonic acid,1,4-lactone	-3.3791	6.29E-08
	2',5'-Dimethoxypropiophenone	-3.2432	0.00542
	Threono-1,4-lactone/Erythronic acid,1,4-lactone	-2.906	5.13E-08
	3,4-Dihydroxydihydro-2(3H)-furanone	-2.4119	0.00331
	Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	-1.3748	0.03128
	Mannopyranose/Galactopyranose/Allopyranose/Talopyranose/Glucopyranose	-0.9860	0.04809
	Lactic acid	2.0294	0.00332
	Glycolic acid	2.0894	0.00034
	Xylopyranose/Lyxopyranose/Ribopyranose/Arabinopyranose/Lyxosepyranose	2.3151	0.03601
	Asparagine	2.7126	1.86E-09
	3-Hydroxybutyric acid	2.7502	0.00879
	Aspartic acid	2.7546	1.38E-09
	Tyrosine	2.7633	0.00049
	Serine	2.7668	1.38E-09
	Gluconic acid	2.7956	1.30E-08
	Threonine	2.8775	1.38E-09
	Malic acid	2.8924	3.44E-09
	Glycine	2.9296	0.00034
	Uracil	2.9461	1.38E-09
	Itaconic acid	2.9461	1.38E-09
	Phosphoric acid, monomethyl ester	3.0186	1.13E-05
	Aminobutyric acid	3.0537	3.20E-07
	Uridine	3.2121	0.01414
	Pyroglutamic acid	3.267	8.18E-08
	Erythritol	3.5224	0.00044
	Galactaric acid/Allaric acid/Idaric acid/Mannaric acid/Mucic acid/Saccharic acid	3.5562	1.43E-08
	Methyl galactoside	3.6488	0.0049
	Trypophan	3.6552	0.00039
	Galacturonic acid/Glucuronic acid	3.7234	7.48E-08
	Mannopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate	3.779	0.033308
	2-Keto-gluconic acid	3.8535	7.38E-08
	Pyrrolidinone	3.9503	8.43E-07
	Glyceric acid	4.0564	1.26E-06
	Methylmaleic acid	4.0749	0.002422
	Aucubin	4.1107	0.00375
	Leucine	4.5295	0.00063
	Aconitic acid	4.6317	0.00223
	Valine	4.7044	1.25E-06

	Putrescine	5.6106	2.96E-06
	Phenylalanine	5.9263	0.00156
	Thymine	6.1627	4.55E-05
	Xylose	6.6234	0.00406
	Lyxose	6.8419	0.01067
	Mannose/Galactose/Glucose/Talose/Allose	7.3991	1.09E-05
	Benzoic acid	7.4798	0.04354
	Urea	9.1263	0.00313
	Serine	1.0651	0.00731
Leaves	Myoinositol	0.71304	0.00731
	1-Glucopyranosylpiperidine	4.4934	0.00957
	N-(Trifluoroacetyl)-norepinephrine	2.2291	0.02105
	Erythrofuranose/Erythrofuranose/Threose/Erythrose	3.1079	0.02693
	Octadecanoic acid	1.9961	0.02934
	Coumaric acid	0.8293	0.02941
	2,5-Dimethoxymandelic acid	1.5054	0.02999
	Thymine	-5.1104	0.00731
	Deoxyadenosine	-3.9745	0.0074
	Aspartic acid	-0.7131	0.0139
	Xylopyranose/Lyxopyranose/Ribopyranose/Arabinopyranose/Lyxose, pyranose/Xylose	-3.9841	0.02092
	2-Methyl-1,2,3-propanetriol	-7.0726	0.02105
	3-Carbomethoxycyclopentanone	-4.4611	0.02934
	2-(Acetylamino)-2-deoxyhexopyranose	-3.9117	0.03237
	Mannopyranose/Galactopyranose/Allopyranose/Talopyranose/Glucopyranose	-0.8972	0.04652

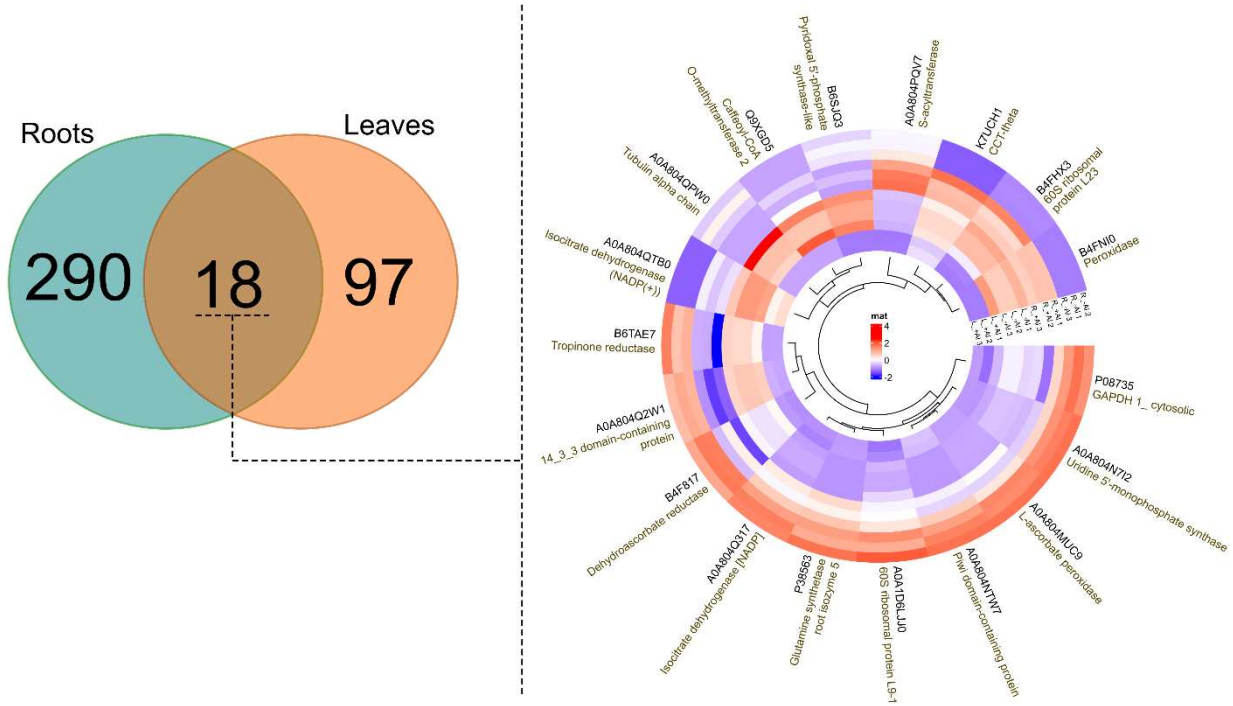


Figure 1. Co-DAPs in roots and leaves.

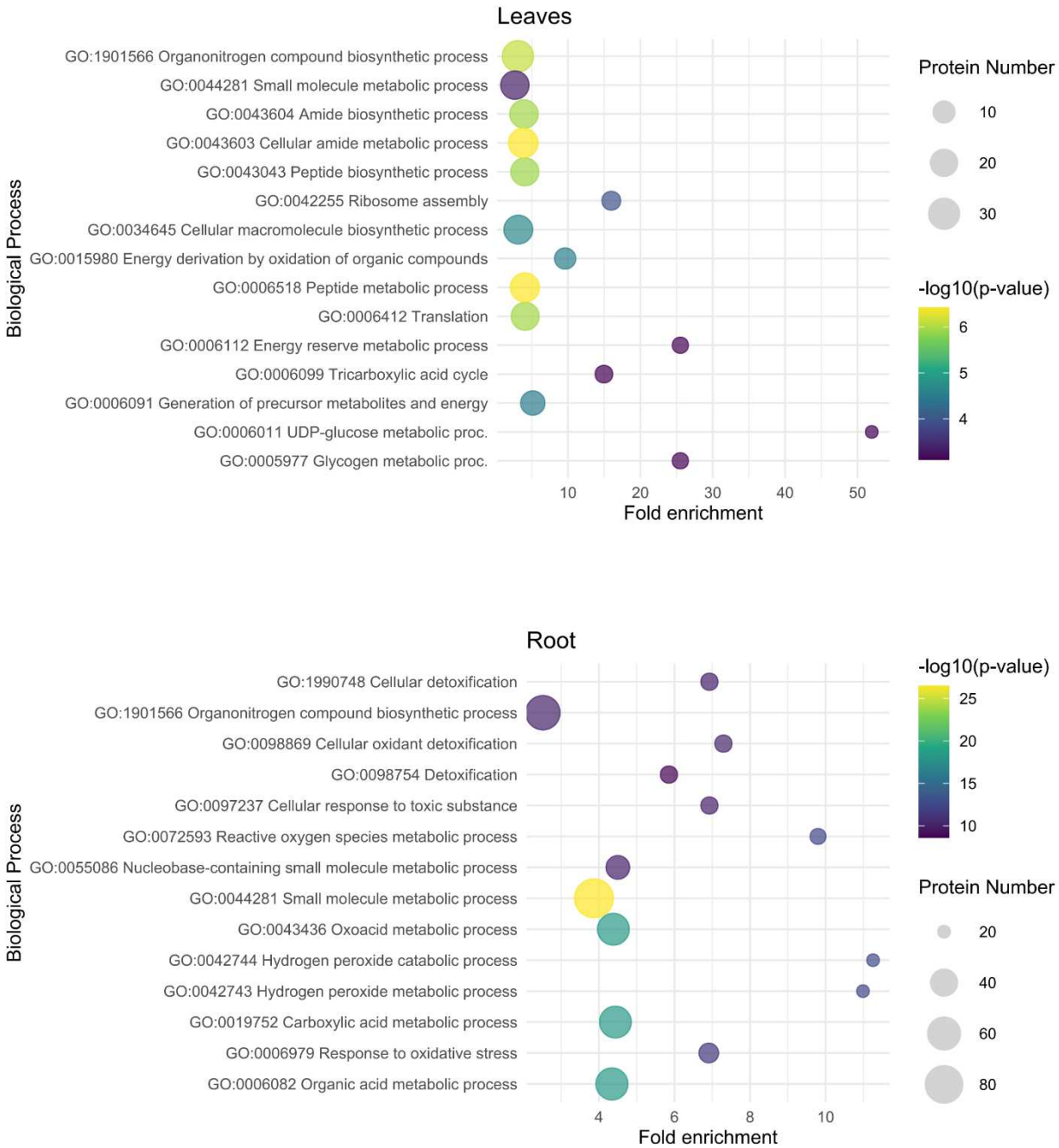


Figure 2. Gene Ontology enrichment analysis identified functional terms related to DAPs in leaves and roots, based on the *Zea mays* reference genome.

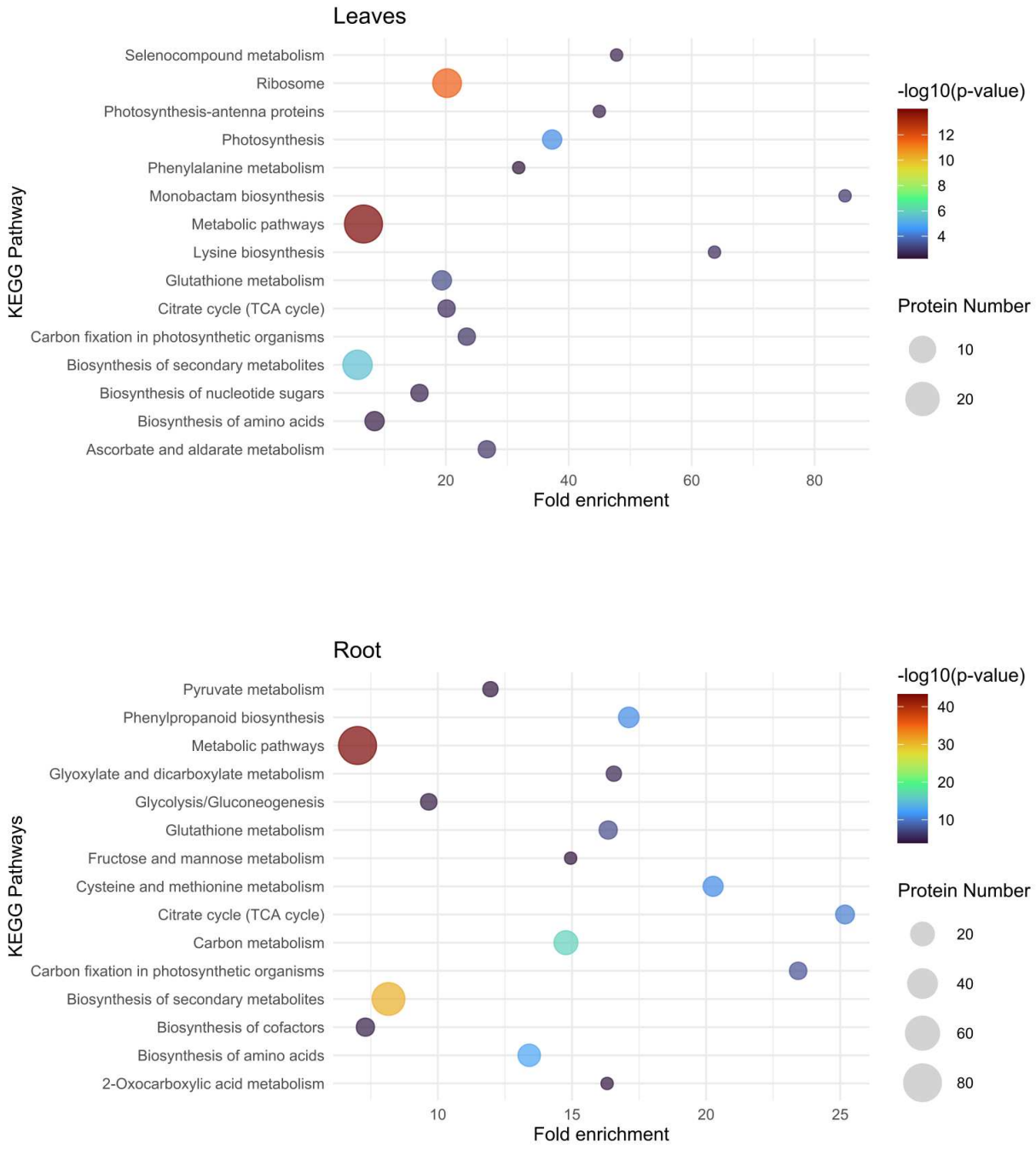


Figure 3. KEGG pathway analysis of DAPs in leaves and roots.

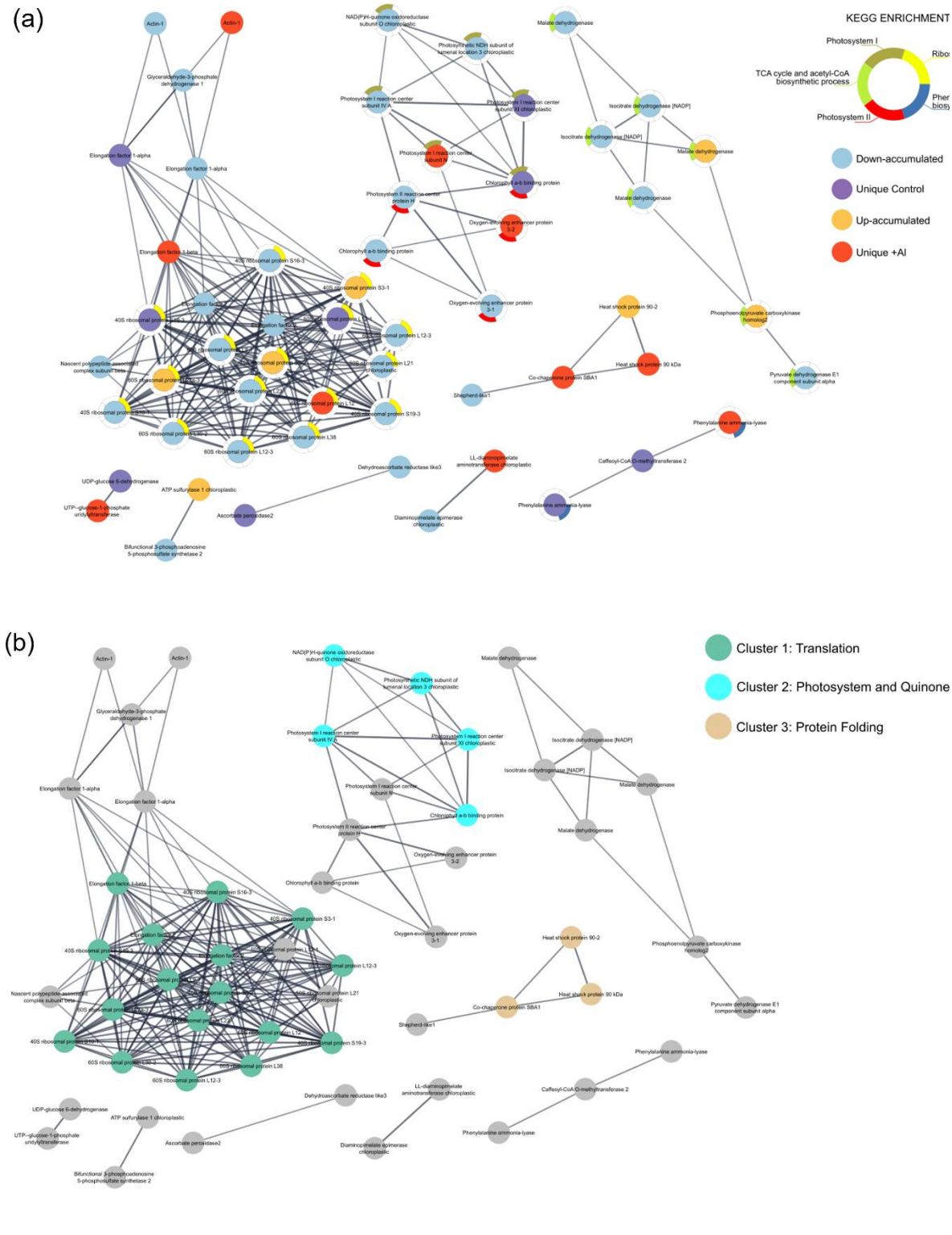


Figure 4. PPI enriched network **(a)** and clusters **(b)** of DAPs in leaves.

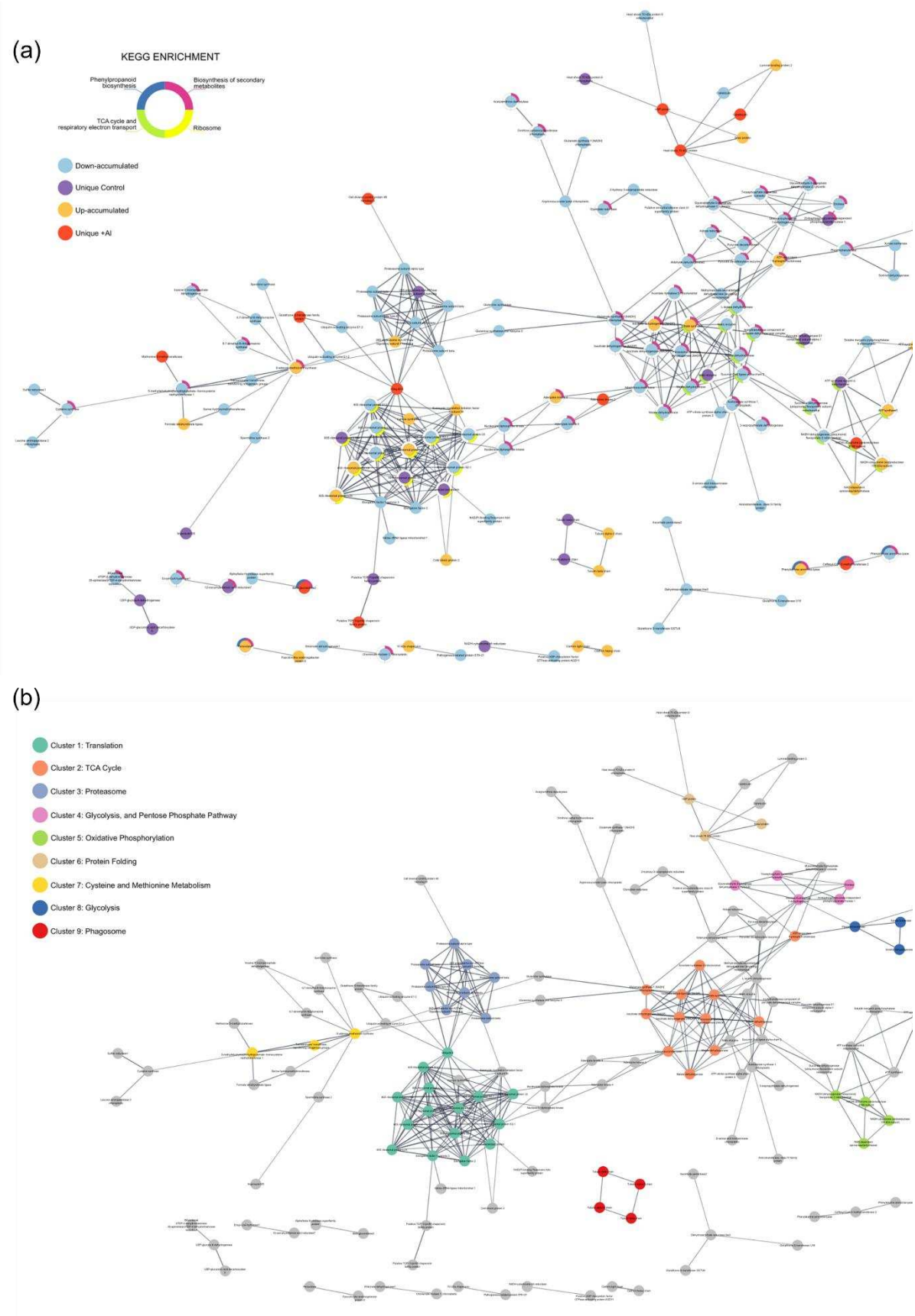


Figure 5. PPI enriched network (a) and clusters (b) of DAPs in roots

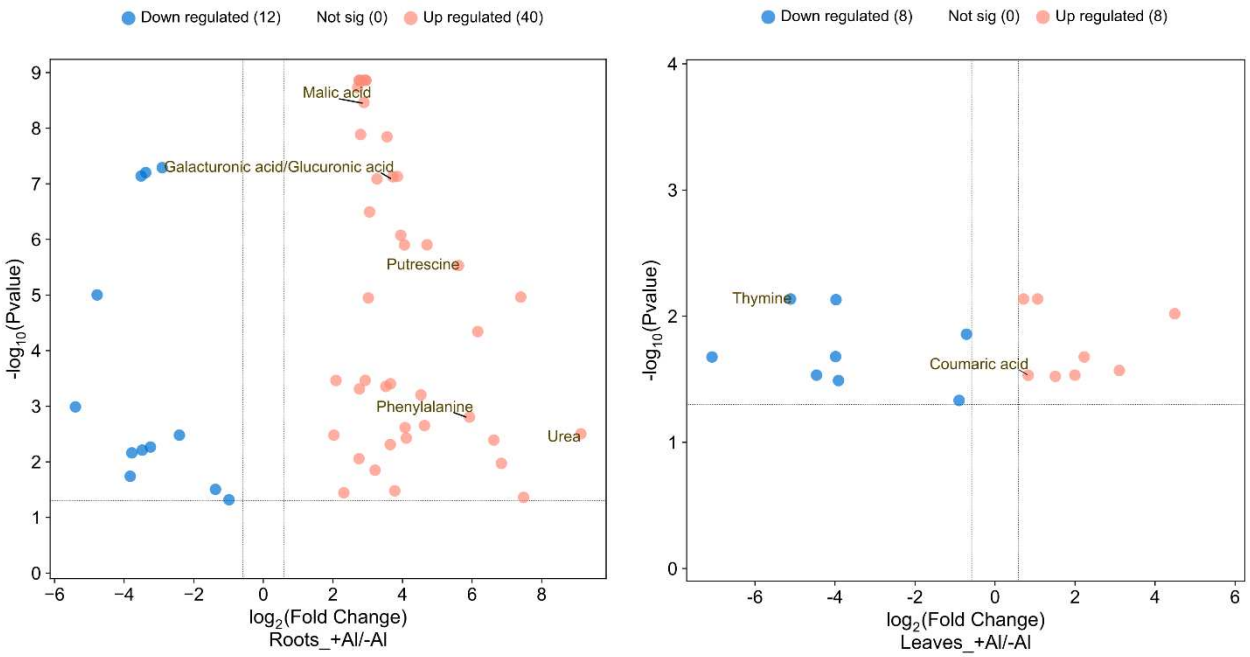


Figure 6. Volcano plot of DAMs up- (orange) and down-accumulated (blue) quantified at the roots (left) and leaves (right) in an Al-tolerant inbred line.

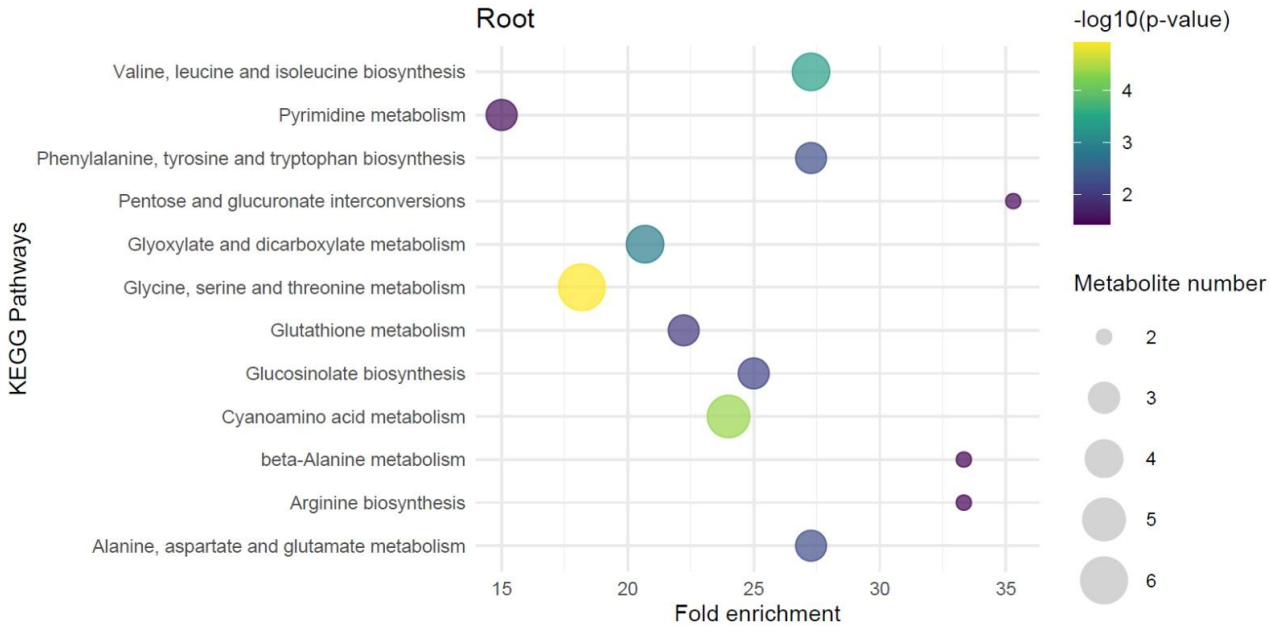


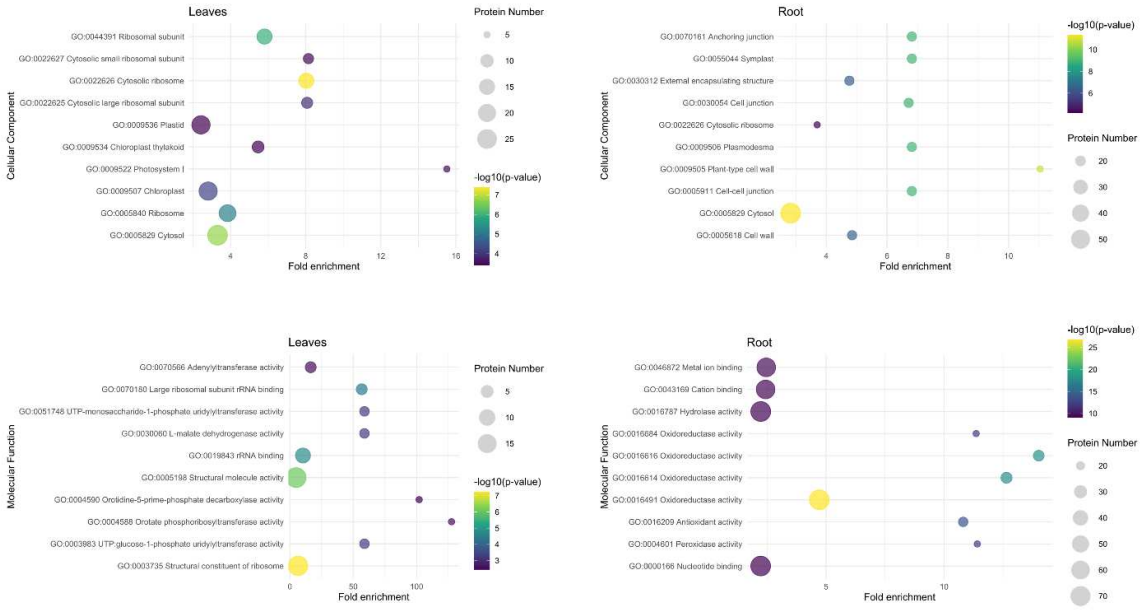
Figure 7. KEGG pathway analysis of DAMs in roots.

Supplementary materials

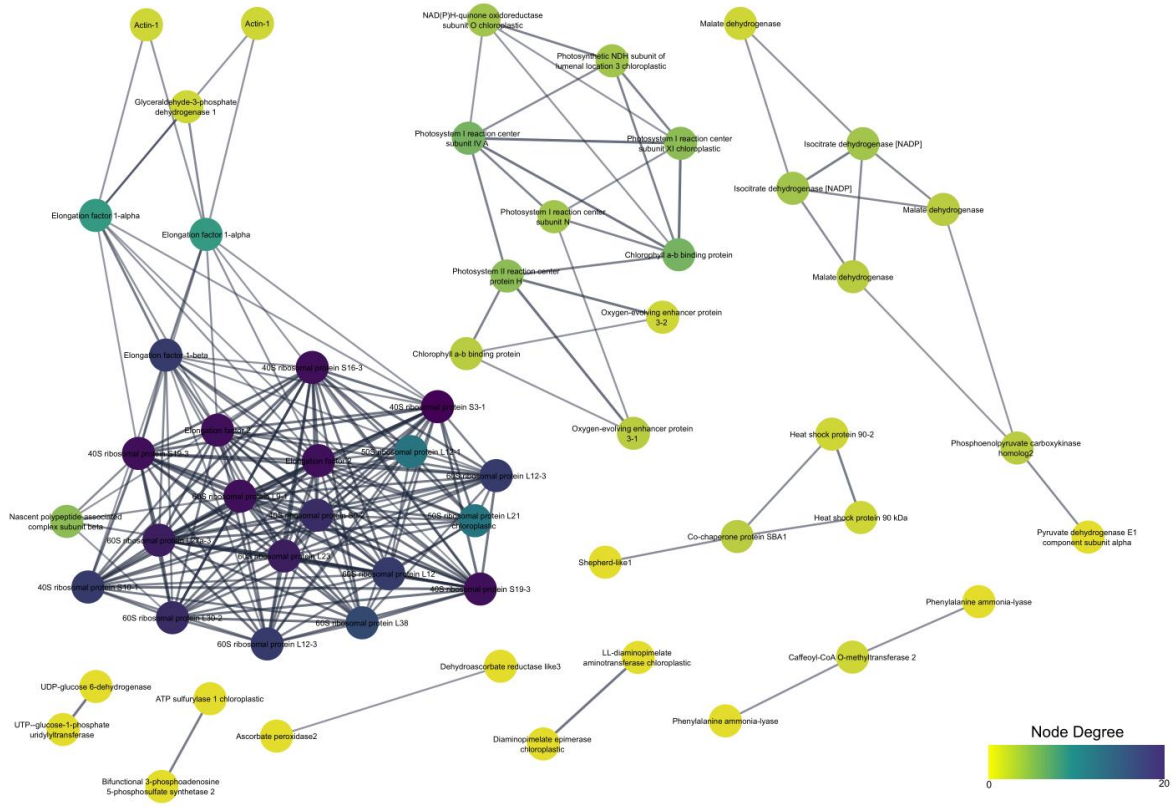
Suppl. Table 1. https://docs.google.com/spreadsheets/d/1dyS9ZPW634-mEg-dy0A0YC0yyu1pE2f8/edit?usp=drive_link&ouid=112605248685174005647&rtpof=true&sd=true

Suppl. Table 2.

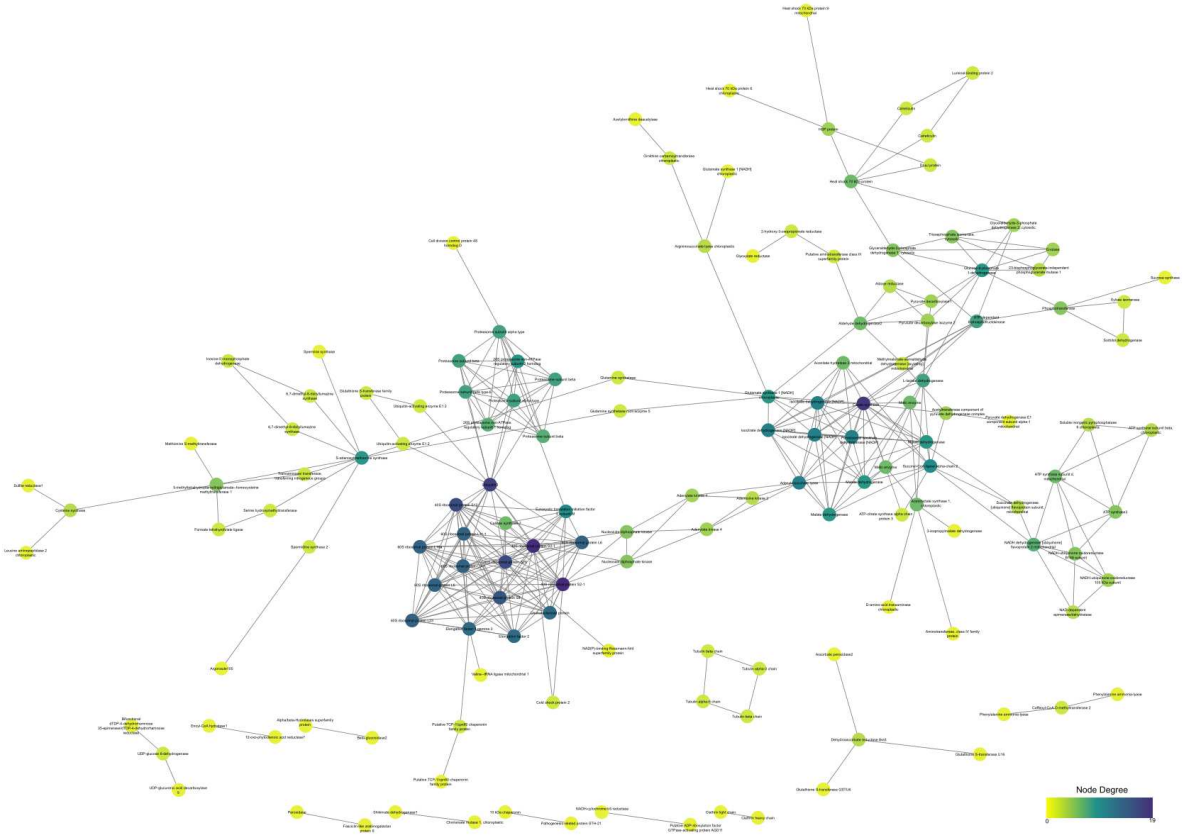
https://docs.google.com/spreadsheets/d/1O50_yZvuOMweNuhX4owTx45VEI1ZENyr/edit?usp=drive_link&ouid=112605248685174005647&rtpof=true&sd=true



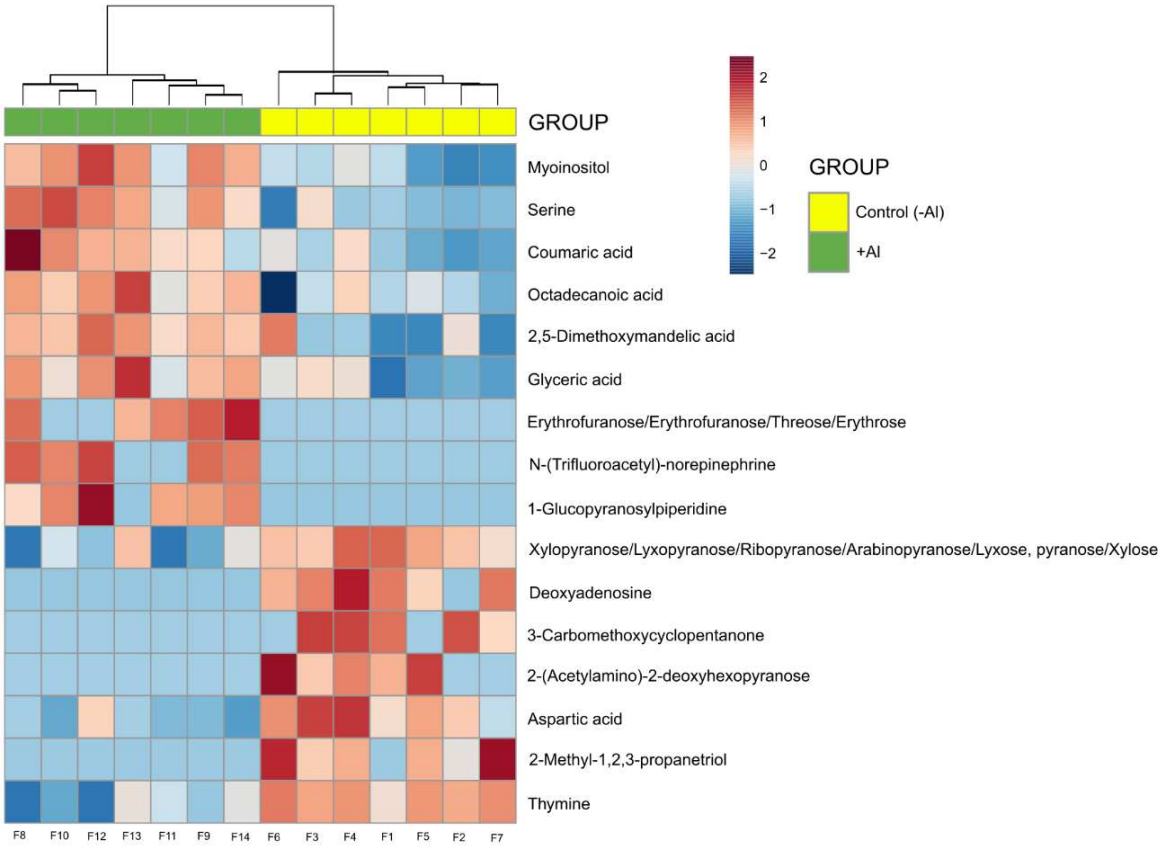
Suppl. Figure 1. Gene Ontology enrichment analysis identified functional terms related to proteins in leaves and roots, based on the *Zea mays* reference genome.



Suppl. Figure 2. PPI enriched network degrees of DAPs in leaves.

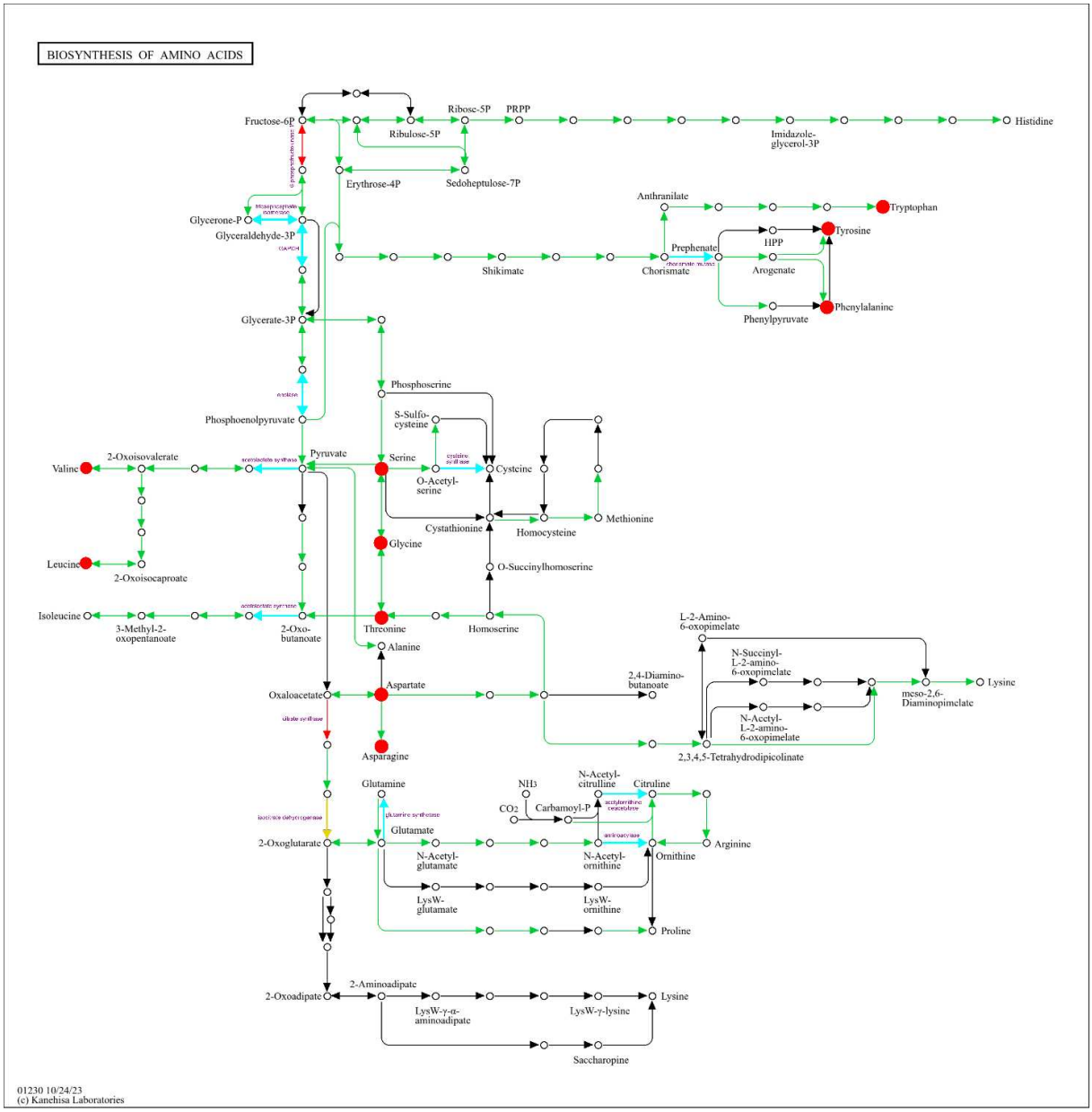


Suppl. Figure 3. PPI enriched network degrees of DAPs in roots.

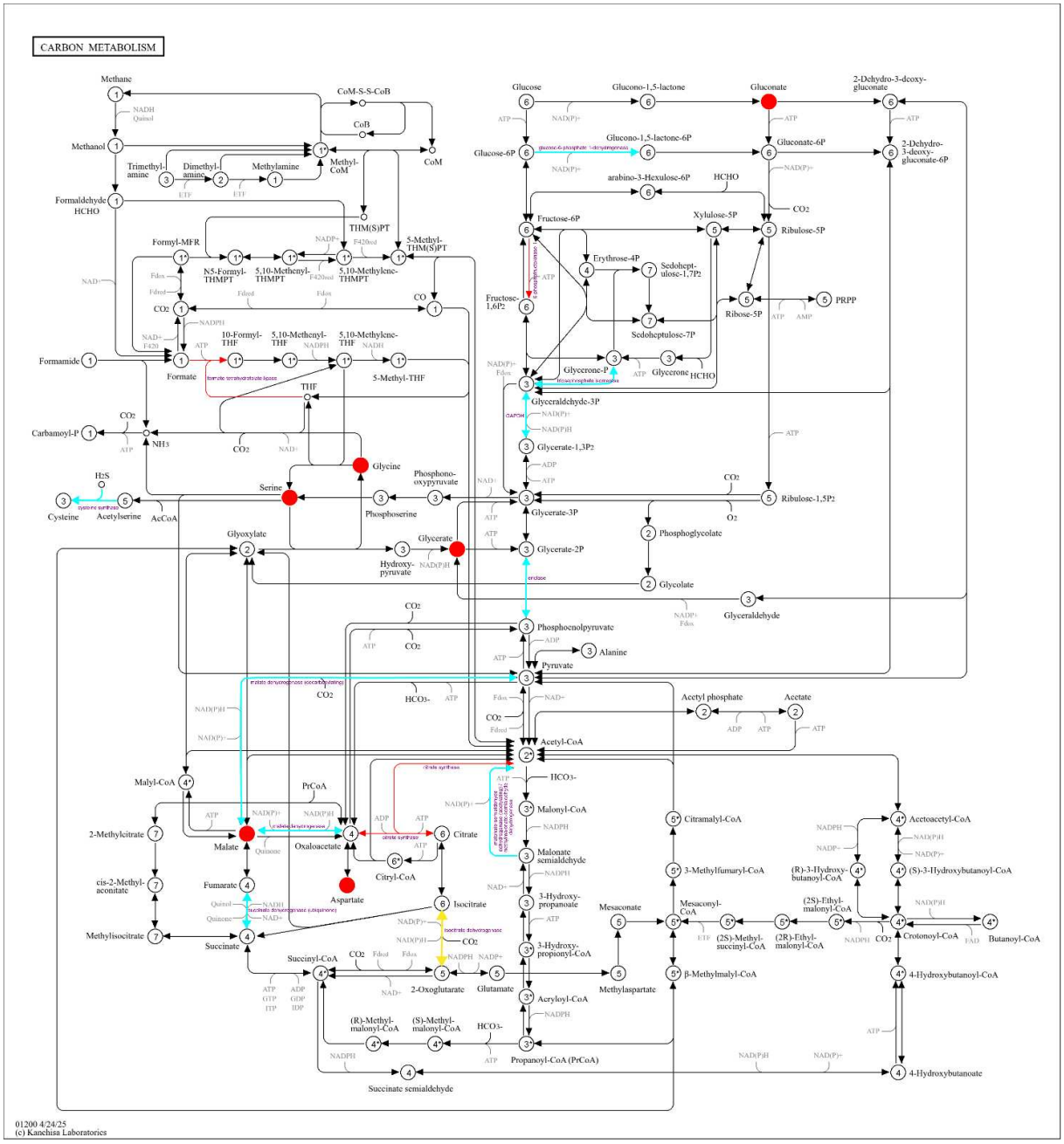


Suppl. Figure 4. Heatmap of the 16 DAMs identified in leaves. The color gradient from blue (less) to red (more) indicates the metabolite concentration.

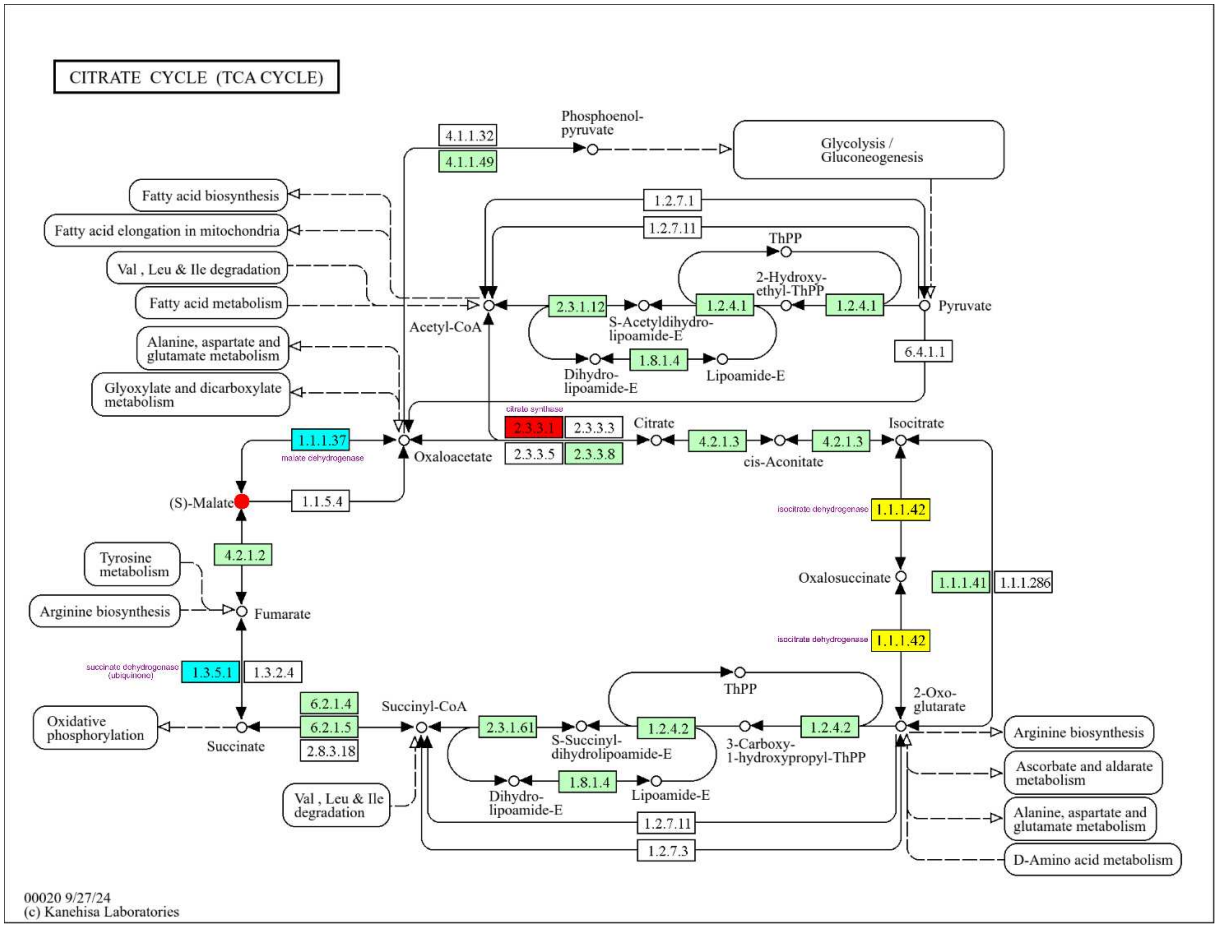
Suppl. Figure 5. Heatmap of the 52 DAMs identified in roots. The color gradient from blue (less) to red (more) indicates the metabolite concentration.



Suppl. Figure 6. Integration of proteomic and metabolomic data in the amino acid biosynthesis pathway of popcorn roots under +Al. Arrows represent proteins; circles represent metabolites. Red indicates up-accumulated elements, blue indicates down-accumulated elements, and yellow denotes elements showing both up- and down-regulation.



Suppl. Figure 7. Integration of proteomic and metabolomic data in the central carbon metabolism of popcorn roots under +Al. Arrows represent enzymes; circles represent metabolites. Red indicates up-accumulated elements, blue indicates down-accumulated elements, and yellow denotes elements showing both up- and down-regulation.



Suppl. Figure 8. Integration of proteomic and metabolomic data in the TCA cycle of popcorn roots under +Al. Squares represent proteins; circles represent metabolites. Red indicates up-accumulated elements, blue indicates down-accumulated elements, and yellow denotes elements with both up- and down- accumulated entries.