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Jover da Silva Alves

Alumínio alivia a clorose causada pela deficiência de ferro interferindo na homeostase de fósforo em gramíneas

Tese de Doutorado

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Jover da Silva Alves

Tese submetida ao Programa de Pós-Graduação em Biologia Celular e Molecular do Centro de Biotecnologia da UFRGS como requisito parcial para a obtenção do título de Doutor em Ciências

Orientador: Felipe Klein Ricachenevsky

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"The important thing is not to stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery every day."

Albert Einstein

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Ao primeiro amante das
plantas que conheci, ao meu avô
Antônio Solon Serpa da Silva.

(*in memorian*)

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LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

ABC	*ATP-Binding Cassette*
Al	Alumínio
ALMT	*Al-Activated Malate Transporter*
ART1	*Al resistance transcription factor 1*
ASR	*ABA-Stress and Ripening*
ASR1	*ABA-Stress and Ripening 1*
ASR5	*ABA-Stress and Ripening 5*
AtAHA2	*Arabidopsis H+-pump ATPase*
AtbHLH100	*basic Helix-Loop-Helix* 100 de *Arabidopsis thaliana*
AtbHLH1001	*basic Helix-Loop-Helix* 101 de *Arabidopsis thaliana*
AtbHLH38	*basic Helix-Loop-Helix* 38 de *Arabidopsis thaliana*
AtbHLH39	*basic Helix-Loop-Helix* 39 de *Arabidopsis thaliana*
AtFRO2	*Ferric Reductase Oxidase* de *Arabidopsis thaliana*
AtIRT1	*iron-regulated transporter* de *Arabidopsis thaliana*
AtMATE1	*Multidrug and Toxic Compound Extrusion 1* de Arabidopsis thaliana
AtNIP1;2	*NOD26-like intrinsic protein 1-2* de *Arabidopsis thaliana*
ATP	Adenosina trifosfato
AtSTOP1	*SENSITIVE TO PROTON RHIZOTOXICITY 1* de *Arabidopsis thaliana*
AtWRKY46	*WRKY transcription factor 46* de *Arabidopsis thaliana*
bHLH	*basic Helix-Loop-Helix*
bZIP58	*Basic leucine-zipper 5*
C	Carbono
Ca	Cálcio
CAT	Catalase
COPT2	*Copper Transporter* 2
Cu	Cobre
DMA	*Deoxymugineic acid*
DMAS	*Deoxymugineic acid synthase*
EROs	espécies reativas de oxigênio
FDR3	*Ferric Reductase-Deficient 3*

Fe	Ferro
Fe(III)-PS	Complexo FeIII quelado à fitossideróforos
FIT	*FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR*
FRO2	*Ferric Reduction Oxidase 2*
GOE	Grande Evento de Oxigenação
HmPALT1	*Plasma membrane aluminum transporter 1* de *Hydrangea macrophylla*
HmVALT	*Vacuolar aluminum transporter* de *Hydrangea macrophylla*
HvYS1	*Yellow Stripe 1* de *Hordeum vulgare*
IAA	ácido indol-3-acético
K	Potássio
MATE	*Multidrug and Toxic Compound Extrusion*
Mb	Pares de base
Mg	Magnésio
Mn	Manganês
N	Nitrogênio
NA	Nicotianamina
NAAT	*Nicotianamine aminotransferase*
NADPH	Fosfato de dinucleótido de nicotinamida e adenina
NAS	*Nicotianamine synthase*
NAS4	*Nicotianamine Synthase 4*
NRAMP1	*Natural Resistance-Associated Macrophage Protein 1*
NRAMP1	*Natural Resistance-Associated Macrophage Protein 1*
ODS2	Objetivo de Desenvolvimento Sustentável 2
ONU	Organização das Nações Unidas
OsALMT4	*Al-Activated Malate Transporter 4* de *Oryza sativa*
OsALS1	*Aluminum sensitive 1* de *Oryza sativa*
OsbHLH156	*basic Helix-Loop-Helix* 156 de *Oryza sativa*
OsCDT3	*Cadmium Tolerance 3* de *Oryza sativa*
OsDMAS1	*Deoxymugineic acid synthase 1* de *Oryza sativa*
OsEXPA10	Expansina A de *Oryza sativa*
OsFRDL4	*Ferric reductase defective-like 4* de *Oryza sativa*
OsIRO2	*IRON-RELATED bHLH TRANSCRIPTION FACTOR 2*
OsIRT1	*Iron-Regulated Transporter1* de *Oryza sativa*
OsIRT2	*Iron-Regulated Transporter 2* de *Oryza sativa*

OsMGT1	*Magnesium Transporter 1* de *Oryza sativa*
OsNAAT1	*Nicotianamine aminotransferase 1* de *Oryza sativa*
OsNAS1	*Nicotianamine synthase 1* de *Oryza sativa*
OsNAS2	*Nicotianamine synthase 2* de *Oryza sativa*
OsNRAMP1	*Natural Resistance-Associated Macrophage Protein 1*de *Oryza sativa*
OsNRAMP5	*Natural Resistance-Associated Macrophage Protein 5* de *Oryza sativa*
OsNrat	*Nramp aluminum transporter 1* de *Oryza sativa*
OsSTAR1	*Sensitive to Al Rhizotoxicity 1* de *Oryza sativa*
OsSTAR2	*Sensitive to Al Rhizotoxicity 2* de *Oryza sativa*
OsTOM1	*DMA efflux transporter1* de *Oryza sativa*
OsWRKY22	*WRKY transcription factor 22* de *Oryza sativa*
OsYSL15	*Yellow Stripe like 15* de *Oryza sativa*
OsZIFL4	*ZINC-INDUCED FACILITATOR-LIKE 4* de *Oryza sativa*
P	Fósforo
PHT4;4	*Phosphate Transporter 4;4*
POD	Peroxidases
PS	*Phytosiderophores*
S	Enxofre
SAMS	*S-adenosyl-methionine synthetase*
Si	Silício
SOD	Superóxido dismutase
TaALMT1	*Al-Activated Malate Transporter 1* de *Triticum aestivum*
TaMATE1	*Multidrug and Toxic Compound Extrusion 1* de *Triticum aestivum*
VTC4	*Vitamin C 4*
VuMATE1	*Multidrug and Toxic Compound Extrusion*
YS	*Yellow Stripe*
YS1	*Yellow Stripe 1*
ZmALMT1	*Al-Activated Malate Transporter 1* de *Zea mays*
ZmALMT2	*Al-Activated Malate Transporter 2* de *Zea mays*
ZmMATE1	*Multidrug and Toxic Compound Extrusion 1* de *Zea mays*
ZmYS1	*Yellow Stripe 1* de *Zea mays*
Zn	Zinco

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RESUMO

O alumínio (Al), amplamente reconhecido por seus efeitos tóxicos em plantas, especialmente em solos ácidos, tem sido associado a benefícios pontuais em determinadas condições. Este trabalho estuda como o Al pode influenciar a deficiência de ferro (Fe) e sua interação com o fósforo (P), explorando seus efeitos no crescimento e na fisiologia de culturas agrícolas importantes, como o arroz (*Oryza sativa* L.) e o milho (*Zea mays* L.). Os achados mostram que o Al alivia a clorose causada por deficiência de Fe, promovendo a recuperação da fotossíntese e mantendo o teor de clorofila em ambas as espécies. No arroz, verificou-se que o Al reduz a expressão de genes marcadores de deficiência de Fe, mas esse efeito positivo é indiretamente mediado pela indução de deficiência de P nas folhas. Quando o P foi fornecido em excesso, os benefícios do Al foram revertidos, destacando a interação dinâmica entre esses nutrientes. Nossos resultados sugerem que o papel do Al na mitigação da clorose por deficiência de Fe é conservado entre espécies da família Poaceae, ocorrendo por meio de mecanismos que envolvem a interação com P e outros nutrientes. Apesar do efeito positivo na clorose, o Al apresentou toxicidade, como redução do crescimento radicular. Este estudo contribui para uma compreensão mais ampla das interações entre elementos do ionoma.

ABSTRACT

Aluminum (Al), widely recognized for its toxic effects on plants in acidic soils, has been associated with specific beneficial roles under certain conditions. This study investigates how Al influences iron (Fe) deficiency and its interaction with phosphorus (P), focusing on their effects on growth and physiology in key agricultural crops, rice (*Oryza sativa L.*) and maize (*Zea mays L.*). The findings reveal that Al alleviates chlorosis caused by Fe deficiency, improving photosynthesis and maintaining chlorophyll content in both species. In rice, Al reduced the expression of Fe deficiency marker genes, an effect indirectly mediated by P deficiency induced in leaves. Excess P supplementation reversed Al's benefits, highlighting the dynamic nutrient interactions. These results suggest that Al mitigates Fe deficiency-induced chlorosis through mechanisms conserved within the Poaceae family, involving nutrient interplay. However, Al toxicity was evident, causing reduced root growth. This study enhances our understanding of ionomic interactions and nutrient dynamics in plants.

INTRODUÇÃO

1. A cultura do arroz

O arroz *(Oryza sativa* L.) é uma das principais culturas globais, e o terceiro cereal mais cultivado e consumido no mundo, superado apenas pelo milho (*Zea mays*) e pelo trigo (*Triticum aestivum*) (Elert 2014). Na safra 2023/2024, a produção mundial de arroz foi de aproximadamente 521,52 milhões de toneladas ("Rice | USDA Foreign Agricultural Service"), enquanto que para a safra de 2024/2025, o Departamento de Agricultura dos Estados Unidos (USDA) projeta o recorde de 530 milhões de toneladas em uma área de cultivada de 168 milhões de hectares[1]. O arroz é considerado alimento base para mais da metade da população mundial, uma vez que o grão fornece cerca de 19 à 21% das calorias consumidas diariamente pelos humanos (Elert 2014; Fukagawa & Ziska 2019).

Com origem na Ásia (Callaway 2014), o arroz foi difundido globalmente, adaptando-se a diferentes climas e sistemas de cultivo, incluindo regiões tropicais, subtropicais e temperadas. Fora do continente asiático, o Brasil é o maior produtor e consumidor de arroz (Wander & Ferreira 2020). Na safra 2023/24, o Brasil alcançou uma produção de 10.585,5 mil toneladas, cultivados em uma área de 1.607,8 mil hectares. Para a safra de 2024/25, a produção está estimada em 12.046,07 mil toneladas em 1.767,5 hectares, representando um aumento na produção de 13,8% em relação à safra anterior[2]. Na última safra, o estado do Rio Grande do Sul produziu 7.159,8 mil toneladas, o que representa aproximadamente 68% da produção nacional[3]. Esses números reforçam a importância do arroz para a economia e a segurança alimentar do

[1] USDA Foreign Agricultural Service (FAS). Production, Commodity, 0422110. Disponível em: https://fas.usda.gov/data/production/commodity/0422110. Acessado em 23/10/2024.

[2] CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. Acompanhamento da Safra Brasileira de Grãos, Brasília, DF, v. 12, safra 2024/25, n. 1 primeiro levantamento, outubro 2024.

[3] CONAB- COMPANHIA NACIONAL DE ABASTECIMENTO. Previsão de safra por produto – Grãos – Safra 2024/2025- 1º Levantamento, outubro 2024.

Brasil, evidenciando seu papel estratégico na produção global e no combate aos desafios nutricionais.

Estima-se que a desnutrição humana aumentou de 7,5% para 9,1% entre 2019 e 2023 (FAO *et al.* 2024). Diante desse cenário, a Organização das Nações Unidas (ONU) implementou o Objetivo de Desenvolvimento Sustentável (ODS) 2, que visa erradicar a fome até 2030 (FAO *et al.* 2021). A subnutrição afeta principalmente a população de países em desenvolvimento, onde o arroz está associado à segurança alimentar. Ao comparar os mapas de desnutrição global (Figura 1A) com o de produção de arroz (Figura 1B), observa-se uma considerável sobreposição entre as áreas de alta desnutrição e aquelas onde a produção de arroz é predominante. Portanto, o arroz é considerado uma das *commodities* estratégicas para o mundo, servindo como uma das mais importantes táticas para segurança alimentar e nutrição, além de fomentar o crescimento econômico, emprego e estabilidade social (Bin Rahman & Zhang 2023).

Para além da importância socioeconômica, o arroz também possui grande relevância científica como planta-modelo para estudos em monocotiledôneas. O genoma do arroz é um dos menores entre as espécies cultivadas, com cerca de 389 - 430 milhões de pares de bases, contrastando com o genoma de outros cereais, como o do trigo, que pode chegar a 16.000 milhões de pares de bases (Eckardt 2000b; Burr 2002; Chen *et al.* 2002; Jadhav & Ingale 2023; Sukegawa *et al.* 2023). Seu genoma compacto contribuiu para o arroz tornar-se a primeira planta cultivada a ter seu genoma completamente sequenciado (Eckardt 2000a), um avanço fundamental que forneceu uma referência importante para estudos genéticos em cereais e auxiliou em melhoramentos agrícolas subsequentes (Li *et al.* 2014; Gu *et al.* 2022). Além disso, as variedades de arroz *indica* e *japonica* têm seus genomas completamente sequenciados (Yu *et al.* 2002; Kikuchi *et al.* 2003; Sasaki 2005). A comunidade científica também conta com bancos de mutantes, ferramentas para análise de expressão gênica, proteômica e interatômica, além de

protocolos para edição de genes por CRISPR-Cas9 (Stein *et al.* 2018; Wang *et al.* 2018; Lian *et al.* 2024).

A)

B)

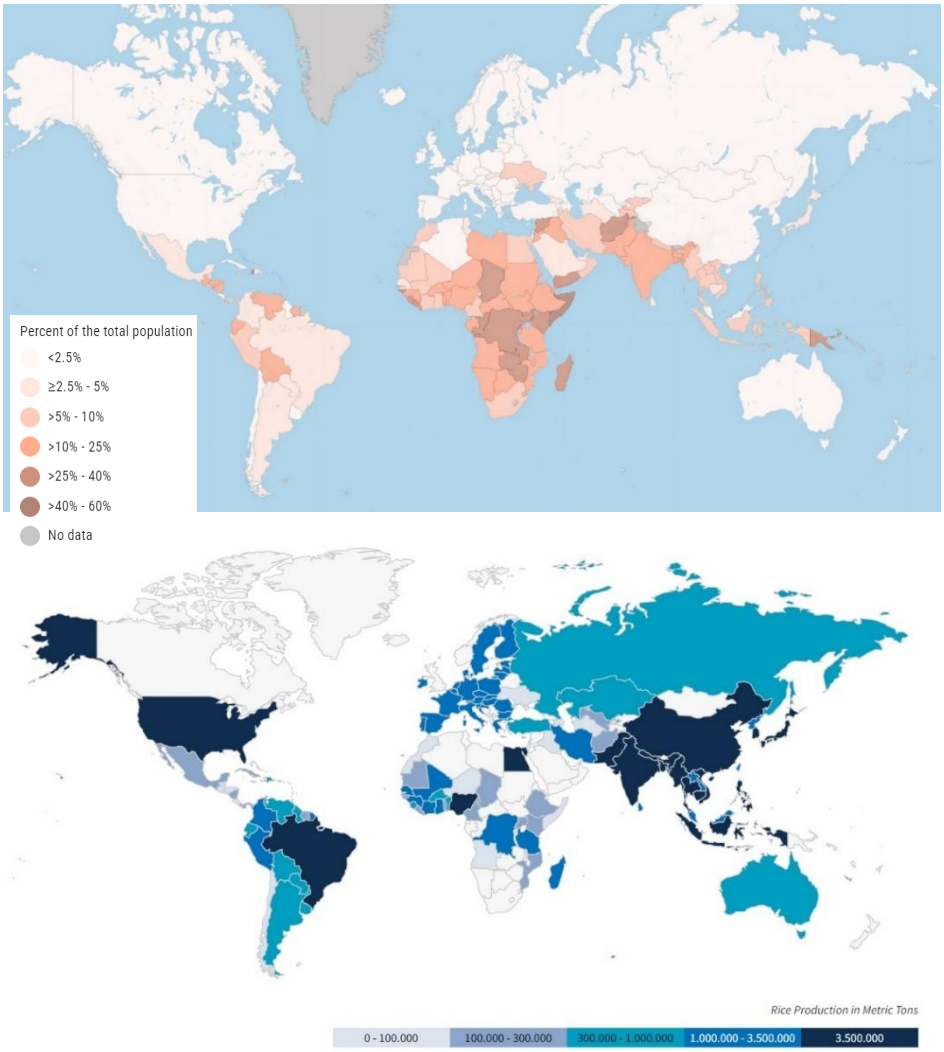


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2. Ferro: características gerais e funções

O ferro (Fe) é o quarto elemento e o segundo metal mais abundante na crosta terrestre (Frey & Reed 2012; Chen *et al.* 2016; Wang *et al.* 2020). É amplamente encontrado em aerossóis atmosféricos, corpos d'água, organismos vivos e no solo (Al-Abadleh 2015; A. Al-Abadleh 2021). A relevância desse metal no meio ambiente e em processos biológicos é notoriamente conhecida devido à sua capacidade de alterar reversivelmente seu estado de valência. Essa característica permite que o Fe forme complexos octaédricos com diversos ligantes, contribuindo para uma ampla gama de reações químicas (Römheld & Nikolic 2006). A capacidade do Fe de variar o potencial redox em resposta a condições ambientais o torna um elemento geologicamente ativo, importante para a ciclagem de outros elementos, como carbono (C), nitrogênio (N) e fósforo (P) (Yan *et al.* 2022). Além disso, sua habilidade de formar complexos impacta significativamente processos metabólicos em organismos vivos (Guerinot & Yi 1994a; Römheld & Nikolic 2006). Sendo assim, o Fe é essencial para o crescimento e desenvolvimento de quase todos os seres vivos.

Apesar de sua abundância geológica, o Fe frequentemente é um fator limitante para o crescimento de organismos, uma vez que se encontra em formas poucos solúveis, dificultando sua absorção (Crichton & Pierre 2001; Abbaspour *et al.* 2014). Essa limitação é crítica, pois para a maioria dos organismos, desequilíbrios na homeostase de Fe podem resultar em danos celulares (Koleini *et al.* 2021). No corpo humano o metal desempenha um papel importante na formação da hemoglobina, a proteína responsável pelo transporte de oxigênio nos tecidos do corpo (Beard 2001; Pires *et al.* 2023). Além disso, o ferro é fundamental em várias reações enzimáticas que sustentam o metabolismo energético e a síntese de DNA (Puig *et al.* 2017; Boinska *et al.* 2024).

A deficiência de Fe na nutrição humana ocorre quando o Fe disponível não atende às necessidades metabólicas do organismo, com ou sem anemia (Cappellini *et al.* 2017) . Estima-

se que a deficiência de Fe afete 30% da população mundial, mais de 2 bilhões de pessoas, especialmente mulheres em idade reprodutiva, crianças e indivíduos com doenças inflamatórias crônicas, como insuficiência cardíaca e doenças renais (Baker *et al.* 2010; Rizzo *et al.* 2021; Tawfik *et al.* 2024). Cerca de 50% dos casos de anemia no mundo são causados por deficiência de ferro, reforçando sua relevância como problema de saúde pública em diferentes contextos econômico (Camaschella 2015). A subnutrição de Fe, junto com a de zinco (Zn) e vitamina A, caracterizam o fenômeno conhecido como "fome oculta", que não apresenta sintomas claros inicialmente, mas afeta o sistema imunológico, o crescimento físico e mental, podendo até levar à morte (Lowe 2021; Weffort & Lamounier 2024). Dentro desse contexto, a biofortificação tem como objetivo aumentar a concentração de Fe em cultivos como arroz e trigo, usando modificações genéticas, manejo agronômico ou adição industrial (Majumder *et al.* 2019; Teklu *et al.* 2023; Zulfiqar *et al.* 2024). Essa estratégia pode melhorar o valor nutricional de alimentos e ajudar na saúde pública em áreas com alta desnutrição.

Em plantas, o Fe desempenha funções vitais em processos como a fotossíntese e a respiração celular, onde atua como um cofator em diversas enzimas que facilitam reações bioquímicas fundamentais (Chen *et al.* 2018a; Shi *et al.* 2018a; Yoneyama 2021). Na fotossíntese, o Fe participa ativamente na cadeia de transferência de elétrons, onde atua como um centro redox, alternando entre os estados de oxidação Fe^{2+} e Fe^{3+} (Römheld & Nikolic 2006). Essa capacidade é fundamental para a transferência eficiente de elétrons, permitindo a síntese de ATP e NADPH, compostos essenciais que armazenam energia e são cruciais para a fase bioquímica da fotossíntese, onde ocorre a fixação de carbono (Noctor & Foyer 1998; Vanlerberghe *et al.* 2020; Smith, Strand, *et al.* 2024). Além disso, mesmo não sendo um componente da molécula de clorofila, o Fe é essencial para a sua biossíntese e estabilidade, contribuindo para a eficiência fotossintética das plantas (Römheld & Nikolic 2006). Sendo assim, a deficiência de ferro pode comprometer esses processos, resultando em clorose e

diminuição da produtividade agrícola. Além de suas funções no metabolismo, o Fe influencia a homeostase de outros nutrientes, influenciando a absorção e o metabolismo de elementos como manganês (Mn), cobre (Cu) e Zn (Jeong & Guerinot 2009; Connorton *et al.* 2017; Rai *et al.* 2021). Do mesmo modo, a homeostase de Fe é afetada por outros elementos, como o enxofre (S) (Astolfi *et al.* 2021a), N (Song *et al.* 2023a), magnésio (Mg) (Rajonandraina *et al.* 2023a) e P (Therby-Vale *et al.* 2022a).

3. Ferro no complexo solo-planta

Embora o Fe seja um dos elementos mais abundantes da crosta terrestre, a disponibilidade desse nutriente para as plantas é muitas vezes limitada, principalmente em solos com pH neutro a alcalino e em condições aeróbicas. Nessas condições, o Fe predomina na forma férrica (Fe^{3+}), que é caracterizada por baixa solubilidade e tende a formar óxidos-hidróxidos insolúveis, como o oxihidróxidos de ferro (FeOOH) (Schwertmann 1988). Essas condições levam a uma drástica redução na disponibilidade do Fe na solução do solo, onde a concentração do elemento pode atingir níveis tão baixos quanto 10^{-17} M, um valor insuficiente para atender às necessidades de crescimento das plantas, que geralmente requerem concentrações entre 10^{-9} e 10^{-4} M (Guerinot & Yi 1994b).

Em função da baixa disponibilidade de Fe para absorção, as plantas desenvolveram diferentes estratégias para absorver Fe na rizosfera. Espécies dicotiledôneas e monocotiledôneas não gramíneas adotam a estratégia I, caracterizada pela redução de Fe^{3+} em Fe^{2+}, uma forma solúvel e mais facilmente absorvida pelas raízes. Já as gramíneas utilizam a estratégia II, baseada na quelação, que envolve a liberação de fitossideróforos para quelar o Fe^{3+} no solo, possibilitando sua absorção (Römheld & Marschner 1986a; Marschner *et al.* 1986). Essas estratégias evoluíram como respostas adaptativas fundamentais para garantir o suprimento de Fe.

3.1. Estratégia I

Dicotiledôneas e monocotiledôneas não-gramíneas empregam a estratégia I (Figura 2A), que é baseada na redução de Fe. Esse processo inicia com a acidificação da rizosfera, mediada pela extrusão de prótons por H^+-ATPases presentes na membrana plasmática das raízes, como a AtAHA2 (do inglês *Arabidopsis H+-pump ATPase*) em Arabidopsis (*Arabidopsis thaliana*). Essa acidificação diminui o pH do solo ao redor das raízes, aumentando a solubilidade de Fe^{3+} e facilitando a disponibilidade do nutriente para a planta (Santi & Schmidt 2009).

A etapa subsequente envolve a redução do Fe^{3+} a Fe^{2+}, realizada por redutases férricas, como a AtFRO2 (do inglês *Ferric Reductase Oxidase*), que estão ligadas à membrana plasmática (Robinson *et al.* 1999). Esse processo é fundamental, pois o Fe^{2+} é uma forma mais solúvel e biodisponível do elemento. Essa redução é favorecida pelo ambiente ácido criado na rizosfera, que, além de aumentar a solubilidade do Fe, otimiza a atividade das redutases, facilitando ainda mais a absorção do Fe pela planta.

Por fim, o Fe^{2+} é absorvido pelas células epidérmicas das raízes através de transportadores específicos, como o AtIRT1 (do inglês *iron-regulated transporter*), que pertence à família de transportadores ZIP de alta afinidade para a absorção de Fe^{2+} em condições de baixa disponibilidade. A expressão dos genes que codificam esses transportadores é induzida pela deficiência de ferro, tornando o AtIRT1 um dos principais mecanismos de resposta adaptativa para captação de ferro em plantas (Eide *et al.* 1996; Vert *et al.* 2002). Além do IRT1, o NRAMP1 (do inglês *Natural Resistance-Associated Macrophage Protein 1*) também participa da absorção de Fe como um transportador de Fe de baixa afinidade (Castaings *et al.* 2016; Tergemina *et al.* 2022).

3.2. Estratégia II

A estratégia II, utilizada pelas gramíneas, é baseada na quelação de Fe^{3+} (Figura 2B). Essa estratégia envolve a exsudação de fitossideróforos (PS, do inglês *phytosiderophores*) na rizosfera, compostos quelantes de Fe que formam complexos Fe(III)-PS, os quais são posteriormente absorvidos pelas células epidérmicas através de transportadores específicos da família YS (do inglês *Yellow Stripe*) (Curie *et al.* 2001b; Murata *et al.* 2006; Inoue *et al.* 2009; Yamagata *et al.* 2022). A eficiência dessa estratégia permite que as gramíneas estejam melhores adaptadas a solos com baixos níveis de Fe, como solos alcalinos (Li *et al.* 2023).

A biossíntese e secreção dos PS em gramíneas é regulada geneticamente e ocorre através de um conjunto de enzimas específicas. Inicialmente, a rota de síntese dos PS foi elucidada em cevada e envolve a enzima S-adenosil metionina sintase (SAMS, do inglês *S-adenosyl-methionine synthetase*), que catalisa a adenosilação da metionina (Takizawa *et al.* 1996). A nicotianamina sintase (NAS, do inglês *nicotianamine synthase*) converte a adenosil metionina em nicotianamina (NA) (Higuchi *et al.* 1999), que é posteriormente transformada pelo complexo enzimático nicotianamina aminotransferase (NAAT, do inglês *nicotianamine aminotransferase*) (Takahashi *et al.* 1999) e ácido desoximugineico sintase (DMAS, do inglês *deoxymugineic acid synthase*) (Bashir *et al.* 2006) em ácido desoximugineico (DMA, do inglês *deoxymugineic acid*), um dos principais PS nas gramíneas (Römheld & Marschner 1990). Nas raízes, a secreção de DMA para o solo ocorre pelo transportador OsTOM1/OsZIFL4, responsável por promover o efluxo de PS em resposta à deficiência de Fe (Ricachenevsky *et al.* 2011; Nozoye *et al.* 2011).

Ao alcançar a rizosfera, os PS se ligam ao Fe^{3+}, formando complexos hexadentados altamente estáveis que são transportados para as células radiculares por proteínas transportadoras da família YS, como o gene ZmYS1 em milho, responsável pela absorção eficiente do complexo Fe(III)-PS (Curie *et al.* 2001b). Estudos com mutantes do gene YS1 indicam que plantas que perdem a funcionalidade desse gene apresentam sintomas de clorose

internerval, sintoma típico de deficiência de Fe. Em arroz e cevada, genes ortólogos como *OsYSL15* e *HvYS1* participam desse processo de absorção primária do Fe na rizosfera, demonstrando a conservação funcional desse mecanismo entre espécies de gramíneas (Murata *et al.* 2006; Inoue *et al.* 2009; Lee *et al.* 2009).

3.3. Estratégia combinada

O arroz, apesar de pertencer a família das gramíneas (*Poaceae*), possui um aparato de absorção de Fe que combina características da estratégia I e da estratégia II (Figura 2C). Além de absorver Fe através da quelação através do complexo Fe (III)-PS, típico de gramíneas, o arroz também absorve Fe^{2+}, similar ao que ocorre na estratégia I, utilizando o transportador OsIRT1, porém sem atividade de redutase (Wairich et al 2019). O genoma do arroz codifica os transportadores OsIRT1, OsIRT2, OsNRAMP1 e OsNRAMP5, os quais desempenham papéis específicos na aquisição de Fe em condições variadas de disponibilidade e forma do metal, permitindo que o arroz se adapte a solos inundados, onde a forma predominante de ferro é o Fe^{2+} devido à condição anaeróbica (Takahashi *et al.* 2011; Kobayashi *et al.* 2014).

Em resposta à deficiência de Fe, as raízes do arroz aumentam a expressão dos genes que codificam os transportadores OsIRT1 e OsIRT2, o que indica que esses genes têm um papel crucial na captação de Fe^{2+} sob baixa disponibilidade do nutriente (Ishimaru *et al.* 2006b; Walker & Connolly 2008). O gene *OsNRAMP5*, por outro lado, é expresso de forma constitutiva, sem depender da deficiência de ferro para ser ativado, o que sugere que ele garante um suprimento basal de ferro independente das condições de disponibilidade externa do metal (Ishimaru *et al.* 2012).

O primeiro estudo demonstrando que o arroz domesticado é capaz de, além transportar Fe^{3+}-PS, também transporta Fe^{2+} foi publicado em 2006 (Ishimaru *et al.* 2006a). A partir desse

trabalho, se estabeleceu que o arroz utiliza a estratégia combinada. Esse modelo propõe que o mecanismo combinado foi uma evolução como adaptação à condição de alagamento dos campos de arroz, onde o Fe está predominante na forma de Fe^{2+} (Ricachenevsky & Sperotto 2014). No entanto, um estudo mais recente (Wairich *et al.* 2019) mostrou que outras espécies gramíneas, como o milho, também podem utilizar IRT1 para absorver Fe, sugerindo que a estratégia combinada tenha uma origem mais antiga e comum dentro da família *Poaceae*.

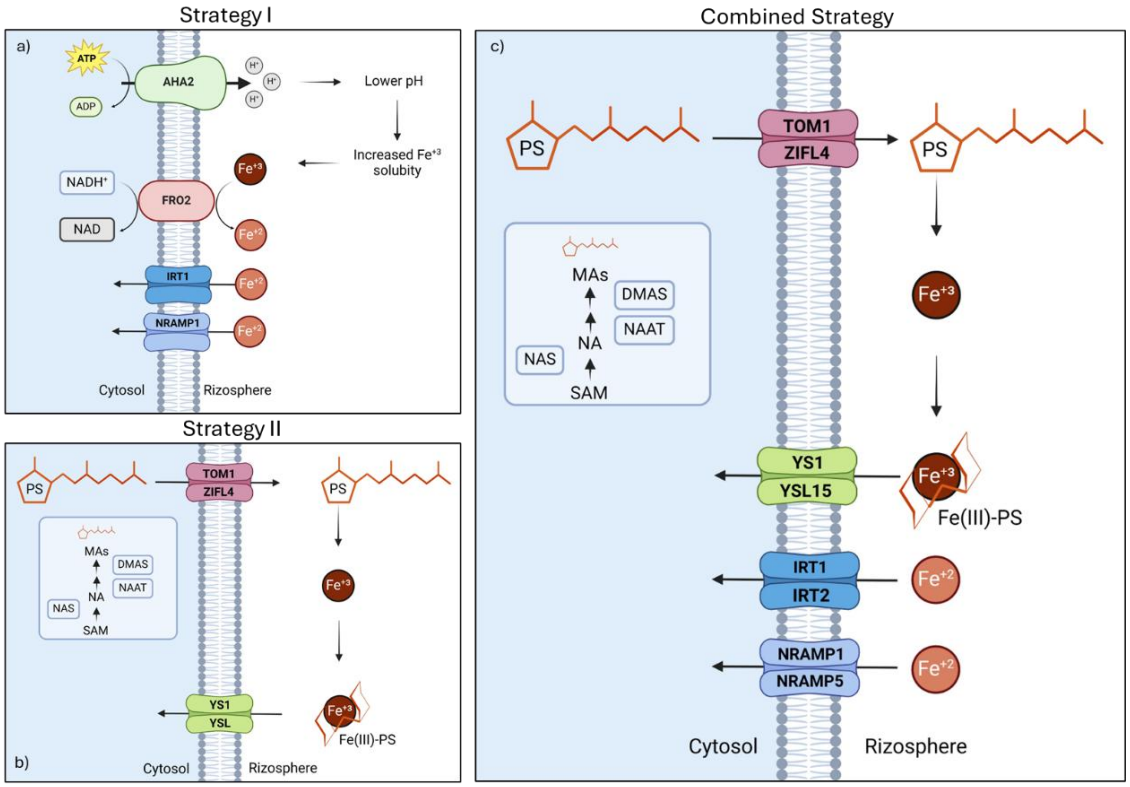


Figura 2. Estratégias para absorção de Fe a partir do solo. (a) Estratégia I – estratégia baseada na redução do Fe^{3+}a Fe^{2+}. *Proton-ATPases* (AHA2) realizam a extrusão de prótons e acidificam a rizosfera, aumentando a solubilidade do Fe^{3+}. A forma oxidada do Fe solubilizado é reduzida por redutases férricas ligadas à membrana plasmática (FRO2), e o Fe reduzido é absorvido por transportadores de metal transmembrana (IRT1); (b) Estratégia II – estratégia baseada na quelação do Fe^{3+}. Após a síntese e secreção de fitossideróforos na rizosfera por TOM1/OsZIFL4, o Fe^{3+} da rizosfera é

quelado pelos PSs, e o complexo Fe(III)-PSs é absorvido para o interior das células das raízes por transportadores da família *Yellow Stripe*; (c) A estratégia combinada, empregada por plantas de arroz, permite que essa gramínea absorva não apenas Fe^{3+} quelado a PSs, como classicamente proposto, mas também absorva Fe^{2+} pela atividade de transportadores de metal transmembrana IRT1/2, NRAMP1/5. Figura criada: https://BioRender.com

3.4. Deficiência de ferro

No início da vida na Terra, a atmosfera apresentava um caráter redutor, o que favorecia a alta disponibilidade de Fe para os organismos. Entretanto, há cerca de 2,4 bilhões de anos, com o surgimento da fotossíntese, ocorreu o primeiro Grande Evento de Oxigenação (GOE) (Figura 3). Esse evento promoveu a rápida liberação de oxigênio molecular (O_2), alterando drasticamente as condições atmosféricas e oceânicas no planeta. Após a evolução da fotossíntese, os níveis de O_2 aumentaram de 0,001% para 21% (Lyons *et al.* 2014). Esse acúmulo de O_2 levou à oxidação do Fe solúvel (Fe^{2+}), transformando-o em Fe insolúvel (Fe^{3+}), resultando em uma diminuição drástica na biodisponibilidade de Fe na terra (Watanabe & Tajika 2021; Wade *et al.* 2021). Atualmente, aproximadamente 30% dos solos cultiváveis no mundo são calcários e suscetíveis a apresentarem deficiência de Fe para o cultivo (Wallace & Lunt 1960; Römheld & Marschner 1986a).

Plantas precisam adquirir Fe em quantidades suficientes para atender as suas necessidades metabólicas, mas sem ultrapassar níveis que posam causar toxicidade. Para isso, elas regulam cuidadosamente os processos de absorção, utilização e armazenamento de Fe, ajustando-os às condições ambientais. Nos últimos anos, a homeostase de Fe em plantas tem sido explorada com maior profundidade devido à combinação dos avanços em biologia molecular e bioinformática com os campos tradicionais da fisiologia vegetal, como a bioquímica vegetal, nutrição mineral e ciência do solo (Kobayashi & Nishizawa 2012). Pesquisas nessa área

utilizam *A. thaliana* como modelo de plantas não gramíneas e arroz como planta modelo para as gramíneas.

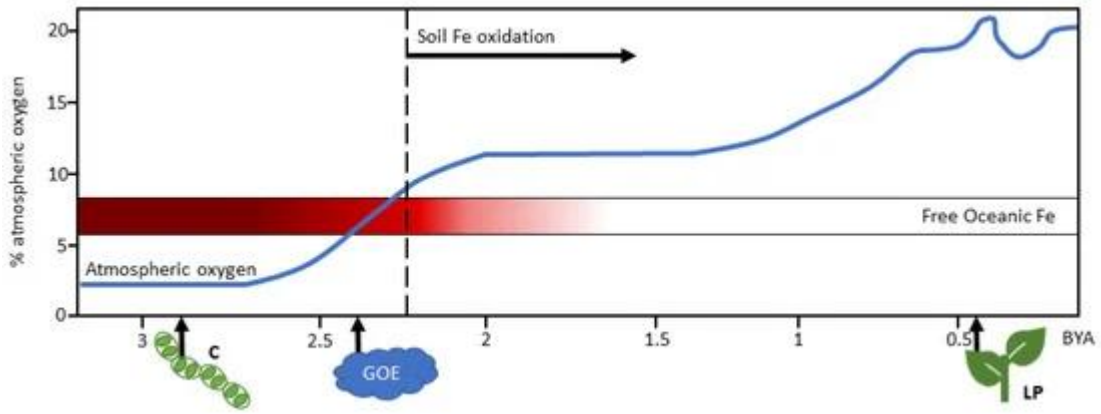


Figura 3- Modelo da relação entre o oxigênio atmosférico e a disponibilidade de Fe ao longo da história da Terra. A porcentagem de oxigênio atmosférico é apresentada no eixo Y, enquanto o tempo é mostrado no eixo X. A atmosfera primitiva da Terra continha cerca de 0,001% de oxigênio até o início da fotossíntese em cianobactérias, aproximadamente 2,7 bilhões de anos atrás (BYA). A linha azul representa a tendência geral de aumento dos níveis de oxigênio até os valores atuais. O gradiente vermelho reflete a quantidade estimada de Fe^{2+} livre com base na frequência dos padrões de formações ferríferas bandadas nas camadas, sendo que áreas mais escuras indicam maior quantidade de Fe^{2+} livre. A linha pontilhada marca o início da oxidação do Fe no solo no registro geológico. O primeiro surgimento de cianobactérias (C), o Grande Evento de Oxigenação (GOE) e as plantas terrestres (LP) estão destacados no eixo X. Figura disponível em (Kroh & Pilon 2020).

Quando em deficiência de Fe, ocorrem diversas alterações fisiológicas e morfológicas nas plantas, as quais alteram seu desenvolvimento. Haja vista que, fisiologicamente, o Fe é essencial para síntese de clorofila e processos fotossintéticos, o sintoma típico da deficiência de Fe é a ocorrência de clorose internerval nas folhas jovens, caracterizada pelo amarelecimento dessas folhas (Brown & Holmes 1955; Römheld & Nikolic 2006) – folhas jovens com cor amarela enquanto as nervuras permanecem verdes (Curie & Briat 2003; Mahender *et al.* 2019a).

O Fe é um elemento com baixa mobilidade dentro das plantas, o que dificulta sua translocação para as folhas mais jovens. Como resultado, os órgãos mais jovens e em desenvolvimento são os mais afetados pelo estresse (Römheld & Nikolic 2006). Diversas mudanças morfológicas ocorrem nas folhas em deficiência de Fe, especialmente no que diz respeito ao crescimento e à estrutura celular. Um dos principais efeitos é a redução na espessura das folhas, que resulta em menor área foliar. Esse fenômeno é uma adaptação que a planta faz para minimizar os danos causados pela redução da fotossíntese, que ocorre devido à baixa disponibilidade de Fe (Akmakjian *et al.* 2021).

Nas raízes, ocorrem alterações morfológicas e arquitetônicas com o objetivo de aumentar a superfície de absorção e facilitar uma maior captação de Fe no solo. Na deficiência de Fe, há um aumento na densidade de pelos radiculares e da espessura do córtex (Briat 2006; Li, Kronzucker, *et al.* 2016; Li & Lan 2017; Fu *et al.* 2017). Esse estresse estimula a formação de pelos radiculares em locais normalmente ocupados por células não pilosas, além de desencadear pelos radiculares bifurcados (Schmidt *et al.* 2000). Plantas de *A. thaliana* sob deficiência moderada de Fe (5 e 10 μM de Fe) aumentam o comprimento da raiz primária e o número de raízes laterais; no entanto, sob deficiência severa (sem adição de Fe no meio de cultivo) essas estruturas radiculares são drasticamente reduzidas (Gruber *et al.* 2013). A deficiência de Fe modifica a arquitetura do sistema radicular ao regular os níveis de auxina nas pontas das raízes para promover o alongamento de raízes laterais em busca de zonas que contenham Fe (Giehl *et al.* 2012, 2012).

A deficiência de Fe ativa a expressão de genes envolvidos na absorção de Fe. Vários fatores de transcrição, em particular os da família bHLH (do inglês *basic Helix-Loop-Helix*), uma das maiores famílias de fatores de transcrição em plantas, desempenham papéis centrais na resposta à deficiência de Fe (Li, Chen, *et al.* 2020; Gao & Dubos 2021). Em Arabidopsis, o fator de transcrição FIT (do inglês *FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION*

FACTOR), membro da família bHLH, forma heterodímeros com os fatores de transcrição AtbHLH38, AtbHLH39, AtbHLH100 e AtbHLH101 e regulam a expressão de genes como o FRO2 e IRT1 (Yuan *et al.* 2008a; Wang, Cui, *et al.* 2013a). Em arroz, OsbHLH156, um órtológo do FIT de Arabidopsis, interage fisicamente com OsIRO2 (do inglês *IRON-RELATED bHLH TRANSCRIPTION FACTOR 2*). OsIRO2 regula vários genes envolvidos na estratégia II, incluindo genes relacionados à síntese de fitossideróforos, como OsNAS1, OsNAS2, OsNAAT1 e OsDMAS1, do transportador de DMA OsTOM1/OsZIFL4 e do transportador de Fe(III)-PSs OsYSL15 (Ogo *et al.* 2006, 2007, 2011). Embora gramíneas e não-gramíneas utilizem estratégias diferentes para absorção de Fe, as cascatas de sinalizações dos fatores de transcrição ativados em resposta à deficiência de Fe compartilham semelhanças em seus mecanismos, uma vez que, tanto OsbHLH126 em arroz, quanto FIT em Arabidopsis, regulam as respostas à deficiência de Fe através da formação de heterodímeros com membros da família bHLH (Riaz & Guerinot 2021).

4. Características gerais do alumínio

O alumínio (Al) é classificado como um metal leve, amplamente utilizado na indústria e no nosso cotidiano. Compõe cerca de 7,7 a 9% da crosta terrestre, sendo o metal mais abundante e o terceiro elemento químico mais presente, atrás apenas do oxigênio e do silício (Si) (Sigel & Sigel 1988). Apesar de sua abundância, o Al é encontrado sempre combinado com outros elementos, principalmente na forma de minerais aluminosilicatos como os feldspatos em rochas metamórficas e ígneas, e em minerais argilosos em solos altamente intemperizados (Sigel & Sigel 1988; Frank *et al.* 2009).

Sua solubilidade é baixa em pH neutro (6,0 a 8,0), mas aumenta em condições ácidas (pH < 6,0) (Dong *et al.* 1999). Embora o alumínio metálico seja atóxico, suas formas iônicas, como Al^{3+} e $Al(OH)_4^-$, podem ser tóxicas para organismos vivos (Ma 2007). Em humanos, sua

toxidez está associada a potenciais efeitos neurotóxicos, com estudos sugerindo uma ligação entre o acúmulo de Al no cérebro e doenças neurodegenerativas, como o Alzheimer (Crapper *et al.* 1973; Dey & Singh 2022). A principal exposição humana ao Al ocorre principalmente pelo consumo de alimentos não processados e água potável que naturalmente contem Al, pela migração de materiais de contato à base de alumínio, assim como por aditivos alimentares aprovados (Exley 2013). No entanto, sua presença em alimentos cultivados em solos ácidos e sua relação com a toxicidade iônica continuam sendo temas de preocupação ambiental e científica.

Para as plantas, o Al é um dos maiores problemas da agricultura mundial (von Uexküll & Mutert 1995a). Em solos ácidos o Al se encontra em sua forma iônica solúvel (Al^{3+}) (Ginocchio *et al.* 2009). Nessas condições, o alumínio interfere diretamente no desenvolvimento das raízes, inibindo sua elongação e comprometendo a absorção de água e nutrientes essenciais (Ma *et al.* 2005; Yamamoto 2019). Estratégias para mitigar a toxicidade do Al incluem o uso de cultivares resistentes, a aplicação de calcário, para neutralizar a acidez do solo e a adoção de práticas de manejo que promovam a qualidade do solo (Rahman *et al.* 2018a; Rahman & Upadhyaya 2021). Assim, a toxidez é reconhecida como um obstáculo crítico na agricultura.

5. Solos ácidos e a toxidez por alumínio em plantas

Os solos com pH inferior a 5,5 são classificados como ácidos e representam aproximadamente 50% das terras aráveis do mundo (von Uexküll & Mutert 1995b; Kochian *et al.* 2015a) (Figura 4). Aproximadamente 60% desses solos ácidos encontram-se em regiões subtropicais e tropicais, como a América Latina, Caribe, África subsaariana, sudeste e leste Asiático e algumas partes da América do Norte (Wood *et al.* 2000). Nas Américas, um total de 1,616 bilhões de hectares de terra são ácidos, principalmente na América do Sul (von Uexküll & Mutert 1995b). Essa condição é amplamente influenciada por fatores naturais e

antropogênicos, incluindo lixiviação de cátions básicos devido a altas taxas de precipitação, práticas agrícolas intensivas, uso excessivo de fertilizantes nitrogenados e a deposição de poluentes atmosféricos, como N e S (Kennedy 1987; Ma & Ryan 2010a; Sade *et al.* 2016; Slessarev *et al.* 2016a; Smith, Poch, *et al.* 2024). A acidificação também é agravada por mudanças climáticas, que afetam o balanço hídrico dos solos, promovendo a transição de pH alcalino para ácido em regiões onde a precipitação anual excede a evapotranspiração potencial anual (Slessarev *et al.* 2016b). A ocorrência de solos ácidos e do processo de acidificação, especialmente em países em desenvolvimento, destaca a necessidade de ações coordenadas que promovam práticas agrícolas sustentáveis, garantindo a produtividade sem comprometer a qualidade do solo e reforçando a segurança alimentar em regiões vulneráveis.

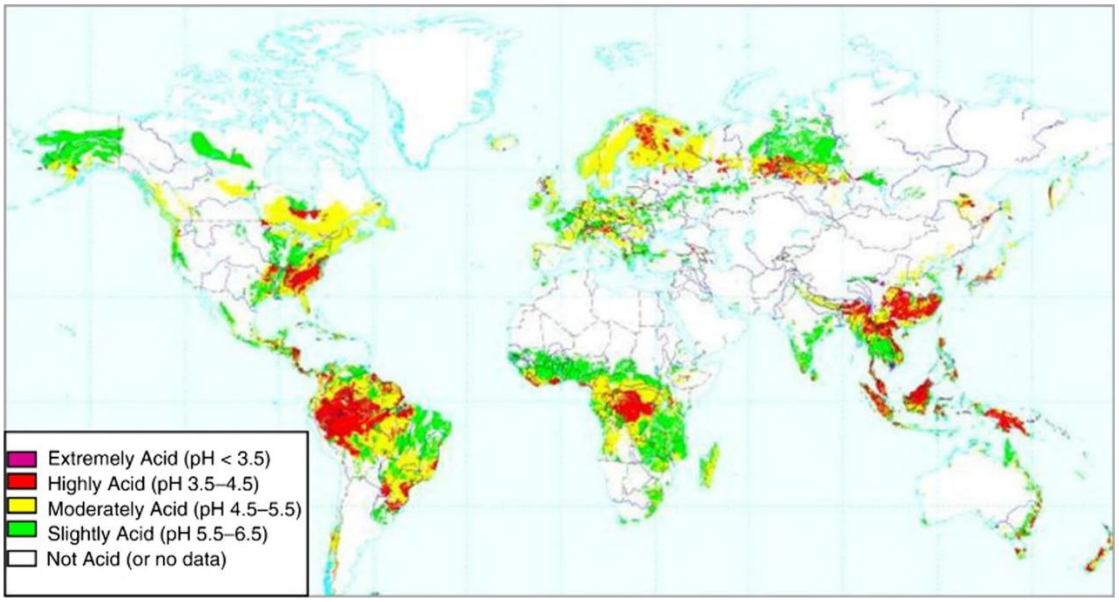


Figura 4 – Distribuição mundial dos solos ácidos. (USDA, NRCS, world soil resources, Washington, D.C.).

À medida que o pH do solo diminui, a disponibilidade de nutrientes é afetada negativamente, devido a altos níveis de íons H^+ (Wang *et al.* 2023). Em condições ácidas, os

íons de Al e os constituintes do solo, como óxidos de Fe, ligam-se fortemente ao P, reduzindo sua disponibilidade para as plantas (Kochian *et al.* 2005; Sade *et al.* 2016), podendo levar à deficiência desse mineral (Quartin *et al.* 2001; Jemo *et al.* 2007; Zheng 2010; Chen *et al.* 2016, 2022). A deficiência de P, combinada com a menor disponibilidade de outros nutrientes como cálcio (Ca) (Adams & Hathcock 1984; Ryan & Kochian 1993; Jones *et al.* 1998; Silva *et al.* 2005), Mg (Silva *et al.* 2010; Cunha Neto *et al.* 2020) e potássio (K) (Liu & Luan 2001; Silva *et al.* 2010; Goulding 2016), exacerbadas pela presença de Al, prejudicam o desenvolvimento saudável das plantas, além de causar problemas estruturais que dificultam o crescimento das raízes e a absorção de água e nutrientes (Kochian *et al.* 2005; Ma & Ryan 2010b). Portanto, a maior problemática associada aos solos ácidos é a toxidez por Al, que se torna solúvel em condições de baixa acidez, atingindo níveis tóxicos para as plantas.

O efeito mais característico da toxicidade do Al é a interrupção do alongamento radicular, que pode ocorrer minutos após a exposição à concentrações baixas do metal (Poschenrieder *et al.* 1995; Ma *et al.* 2004; Ma 2007). O Al^{3+} acumula-se primariamente no apoplasto radicular, onde se liga a pectinas através de grupos carboxílicos negativamente carregados. Este processo afeta a estrutura da parede celular, aumentando sua rigidez e reduzindo a extensibilidade necessária para o alongamento celular (Doncheva *et al.* 2005; Eticha, Thé, *et al.* 2005; Delhaize *et al.* 2007a). Além disso, o Al interfere na divisão celular nas pontas das raízes e nas raízes laterais e diminui a deposição de polissacarídeos na parede celular (Sade *et al.* 2016). A rigidez excessiva da parede celular, promovida pela ligação do Al às pectinas e hemiceluloses, também está associada à formação de calose no apoplasto. A calose, composta por β-1,3-glucanos, bloqueia o transporte apoplástico e simplástico de solutos, como o ácido indol-3-acético (IAA), essencial para o crescimento radicular (Sivaguru *et al.* 2000). Na membrana plasmática, o Al desloca cátions de Ca, essenciais para a integridade da membrana, alterando sua estrutura (Piñeros & Kochian 2001). Outro efeito deletério é a

peroxidação lipídica, tornando a membrana plasmática menos fluída e comprometendo o transporte de íons, como Ca, Mg e K (Keltjens & Tan 1993; Ahn *et al.* 2002).

O Al causa disfunções no citoesqueleto, essencial para a divisão celular e a expansão das células radiculares (Schwarzerová *et al.* 2002; Singh *et al.* 2017; Kocjan *et al.* 2024). Ele desorganiza microtúbulos e filamentos de actina, impedindo a polimerização adequada desses elementos e alterando a dinâmica celular (Matsumoto *et al.* 1976; Sade *et al.* 2016). A exposição ao Al leva à desintegração dos microtúbulos corticais e ao bloqueio da divisão celular na metáfase, devido à formação de complexos estáveis de Al^{3+}–ATP e Al^{3+}–ADP, que substituem o Mg^{2+} em funções enzimáticas críticas (Rengel & Zhang 2003; Silva *et al.* 2012). Essas alterações estruturais resultam em raízes curtas, espessas e com desenvolvimentos anormais. Somado a isso, o Al interage com o núcleo celular, prejudicando a composição do DNA e a estrutura da cromatina (Chen *et al.* 2019). Além disso, o Al aumenta a rigidez da dupla hélice, o que reduz a replicação de DNA (Pan *et al.* 2017).

O excesso de Al provoca um aumento na produção de espécies reativas de oxigênio (EROs), como superóxido, peróxido de hidrogênio e radicais hidroxila (Yamamoto *et al.* 2002; Du *et al.* 2020; Ranjan *et al.* 2021; Huang *et al.* 2024). Essas moléculas são geradas em organelas como mitocôndrias, cloroplastos e peroxissomos, e embora as EROs desempenhem papéis sinalizadores, o excesso dessas moléculas leva à morte celular programada e a danos aos lipídeos de membrana, proteínas e ao DNA (Wang, Loake, *et al.* 2013; Petrov *et al.* 2015; Ye *et al.* 2021). O estresse oxidativo resultante da exposição ao Al, pode ser mitigado pela ativação de mecanismos antioxidantes, como a produção de enzimas tais como a Superóxido dismutase (SOD), catalase (CAT) e peroxidases (POD) (Zhao *et al.* 2017; Ranjan *et al.* 2021; Silva *et al.* 2023). Essas defesas neutralizam os radicais livres, prevenindo danos à membrana celular e organelas. Em arroz e trigo, genótipos com maior produção de enzimas antioxidantes demonstram maior tolerância à toxidez por Al (Bhoomika *et al.* 2013; Yu *et al.* 2019).

5.1. Estratégias de resistência e tolerância ao alumínio

Devido à sua natureza séssil, as plantas estão constantemente expostas a diversos estresses ambientais, incluindo a toxidez por Al em solos ácidos. Para mitigar esse estresse, as plantas desenvolveram estratégias altamente especializadas, que podem ser classificadas em dois principais mecanismos: (1) mecanismo de exclusão de Al, que impede a entrada do Al na planta, principalmente na região apical da raiz (Figura 5A), e (2) mecanismo interno de tolerância ao Al (Figura 5B), que permite a entrada do Al nos tecidos das plantas, mas o desintoxica e o sequestra de forma eficiente (Ma 2007; Kochian *et al.* 2015a; Chauhan *et al.* 2021).

A exclusão de Al é amplamente mediada pela exsudação de compostos orgânicos, como ácidos orgânicos (citrato, malato e oxalato) e fenóis, que quelam o Al no apoplasto, convertendo-o em formas não tóxicas. Essa estratégia é regulada por proteínas transportadoras localizadas na membrana plasmática, destacando-se os membros das famílias ALMT (do inglês *Al-Activated Malate Transporter*) (Sasaki *et al.* 2004) e MATE (do *inglês Multidrug and Toxic Compound Extrusion*) (Furukawa *et al.* 2007; Magalhaes *et al.* 2007). Na presença do Al, esses transportadores promovem a liberação de ácidos orgânicos pelas células radiculares para a rizosfera, onde formam complexos estáveis e não tóxicos, como o Al-citrato, Al-malato e Al-oxalato, reduzindo a absorção do Al pelas raízes (Ma 2007; Kochian *et al.* 2015a; Chauhan *et al.* 2021). Além disso, esses compostos contribuem para a manutenção da homeostase redox, o equilíbrio dos gradientes iônicos e a proteção contra danos oxidativos nas células. Embora menos eficazes na quelação de Al em comparação aos ácidos orgânicos, compostos fenólicos (Kochian *et al.* 2015a), como catecol, catequina e quercetina, também desempenham um papel importante na exclusão de Al em algumas espécies, como milho (Kidd *et al.* 2001). Esses compostos não apenas quelam o Al, mas também participam de processos de proteção estrutural,

como o acúmulo de flavonoides na camada externa das raízes, que pode se desprender para minimizar a exposição ao Al (Osawa *et al.* 2011).

Quando o Al é absorvido pelas raízes e entra nos tecidos internos, as plantas ativam os mecanismos de tolerância para minimizar seus efeitos tóxicos (Ma 2007; Kochian *et al.* 2015a; Chauhan *et al.* 2021). Essa estratégia envolve modificação da parede celular, o transporte intracelular e o sequestro no vacúolo (Huang *et al.* 2009, 2012; Xia *et al.* 2010). A parede celular é a primeira estrutura interna a interagir com o Al, pois os íons Al^{3+} possuem alta afinidade pelos grupos carboxílicos negativos da pectina. Plantas tolerantes costumam apresentar um maior grau de metilação da pectina, o que reduz a carga negativa da parede celular e, consequentemente, a ligação de Al (Gall *et al.* 2015). Outra estratégia adotada pelas plantas tolerantes é o transporte do Al do apoplasto para o citoplasma, seguido pelo sequestro no vacúolo (Kochian *et al.* 2015a). Transportadores específicos, como os membros das famílias ABC (do inglês *ATP-Binding Cassette*) e NRAMP, desempenham papéis cruciais nesse processo, contribuindo para a detoxificação eficiente do Al (Xia *et al.* 2010).

A resistência e a tolerância ao Al não são mutuamente exclusivas, muitas plantas combinam esses mecanismos para uma maior adaptação ao estresse (Hajiboland *et al.* 2023). Por exemplo, o arroz, apesar de utilizar exsudação de citrato e malato, depende fortemente da modificação da parede celular e do sequestro vacuolar para remediar a toxidez por Al (Huang *et al.* 2009; Xia *et al.* 2010; Huang *et al.* 2012; Delhaize *et al.* 2012a). Avanços em estudos moleculares e genéticos continuam a identificar novos genes e transportadores associados à resistência ao Al. Compreender esses processos é fundamental para o desenvolvimento de cultivares adaptadas a solos ácidos, contribuindo para uma agricultura sustentável em regiões suscetíveis à toxidez por Al.

5.2. Mecanismos moleculares em resposta à toxidez de alumínio

Os mecanismos de resistência ao Al que utilizam de ácidos orgânicos para quelar os íons de Al, utilizam transportadores de membrana, como os da família ALMT, uma família de canais aniônicos específicos de planta. Estes transportadores desempenham um papel crucial na resistência ao Al ao mediar a exsudação de malato (Delhaize *et al.* 2007b). O primeiro gene caracterizado dessa família foi o *TaALMT1*, em trigo (Sasaki *et al.* 2004; Piñeros, Cançado, & Kochian 2008; Zhang *et al.* 2008). Ortólogos desse gene foram identificados em várias espécies, incluindo arroz (Liu *et al.* 2017), milho (Piñeros, Cançado, Maron, *et al.* 2008; Ligaba *et al.* 2012) e *A. thaliana* (Hoekenga *et al.* 2006; Kobayashi *et al.* 2007a). No arroz, a superexpressão de *OsALMT4* demonstrou aumentar o efluxo de malato nas raízes, contribuindo para maior resistência ao Al (Liu *et al.* 2017). No milho, os transportadores ZmALMT1 e ZmALMT2 possuem funções complementares: enquanto ZmALMT1 atua no transporte de ânions inorgânicos, como cloreto (Cl^-) e nitrato (NO_3^-), auxiliando na homeostase iônica, ZmALMT2 é permeável tanto ao malato quanto ao citrato, além de outros ânions orgânicos, participando da regulação do equilíbrio osmótico e na nutrição mineral (Piñeros, Cançado, Maron, *et al.* 2008; Ligaba *et al.* 2012). Já em *A. thaliana*, o ortólogo *AtALMT1* apresenta uma função similar ao *TaALMT1* do trigo (Hoekenga *et al.* 2006; Kobayashi *et al.* 2007a).

O citrato, no entanto, é exsudado para a rizosfera, majoritariamente, através dos transportadores da família MATE (Kochian *et al.* 2015a). Os primeiros genes MATE associado à resistência ao Al foram identificados em sorgo (*SbMATE*) (Magalhaes *et al.* 2007) e na cevada (*VuMATE1*) (Furukawa *et al.* 2007). Desde então, genes ortólogos têm sido encontrados em várias espécies, como Arabidopsis (*AtMATE1*) (Liu *et al.* 2009), milho (*ZmMATE1*) (Maron *et al.* 2013), arroz (*OsFRDL4*) (Yokosho *et al.* 2011) feijão-de-arroz (*VuMATE1*) (Yang *et al.* 2011) e no trigo (*TaMATE1*) (Ryan *et al.* 2009). Cultivares de arroz da subespécie *japonica*

apresentam uma inserção de transposon no promotor de *OsFRDL4*, resultando em maior expressão e, consequentemente, maior tolerância ao Al (Yokosho *et al.* 2016).

Enquanto isso, os mecanismos de tolerância interna ao Al envolvem transportadores que atuam na absorção, sequestro e translocação de íons Al^{3+}, sendo especialmente relevantes em culturas como arroz (Chauhan *et al.* 2021). Nessa gramínea, a tolerância está associada a expressão dos genes *OsNrat1* (do inglês *Nramp aluminum transporter 1*) (Xia *et al.* 2010) e *OsALS1* (do inglês *Aluminum sensitive 1*) (Huang *et al.* 2012). O OsNrat1 foi o primeiro transportador de Al caracterizado que está relacionado com mecanismos de resistência ao Al, localizado na membrana plasmática das células radiculares, atua transportando o metal da parede celular para o vacúolo, onde é neutralizado com auxílio do OsALS1 (Xia *et al.* 2010; Huang *et al.* 2012). A função de OsNrat1 é altamente seletiva, transportando exclusivamente íons Al^{3+}, sem afinidade por metais divalentes como Mn^{2+}, Fe^{2+} e Cd^{2+} (Xia *et al.* 2010). Estudos genômicos identificaram haplótipos de Nrat1 na subpopulação de arroz *aus*, responsáveis por até 40% da variação fenotípica na tolerância ao Al (Huang & Han 2014). Os transportadores ABC, amplamente distribuídos em diversos organismos, também têm papel fundamental na tolerância ao Al. Em arroz, além do gene *OsALS1*, os genes *OsSTAR1* e *OsSTAR2* codificam um complexo que transporta UDP-glicose para a parede celular, modificando sua estrutura e reduzindo a acumulação de Al (Huang *et al.* 2009).

As aquaporinas também estão envolvidas no transporte e armazenamento de Al em plantas tolerantes (Kochian *et al.* 2015a), como hortênsia (*Hydrangea macrophylla*) (Ma *et al.* 1997) e trigo-sarraceno (*Fagopyrum esculentum*) (Ma & Hiradate 2000), que acumulam Al nas partes aéreas sem apresentar toxicidade. Em *H. macrophylla*, os transportadores HmPALT1 e HmVALT facilitam a entrada e o sequestro de Al no vacúolo (Negishi *et al.* 2012). Em *A. thaliana*, o transportador AtNIP1;2 transporta complexos Al-malato, ajudando a remover Al da parede celular e promovendo sua translocação para as partes aéreas (Wang *et al.* 2017).

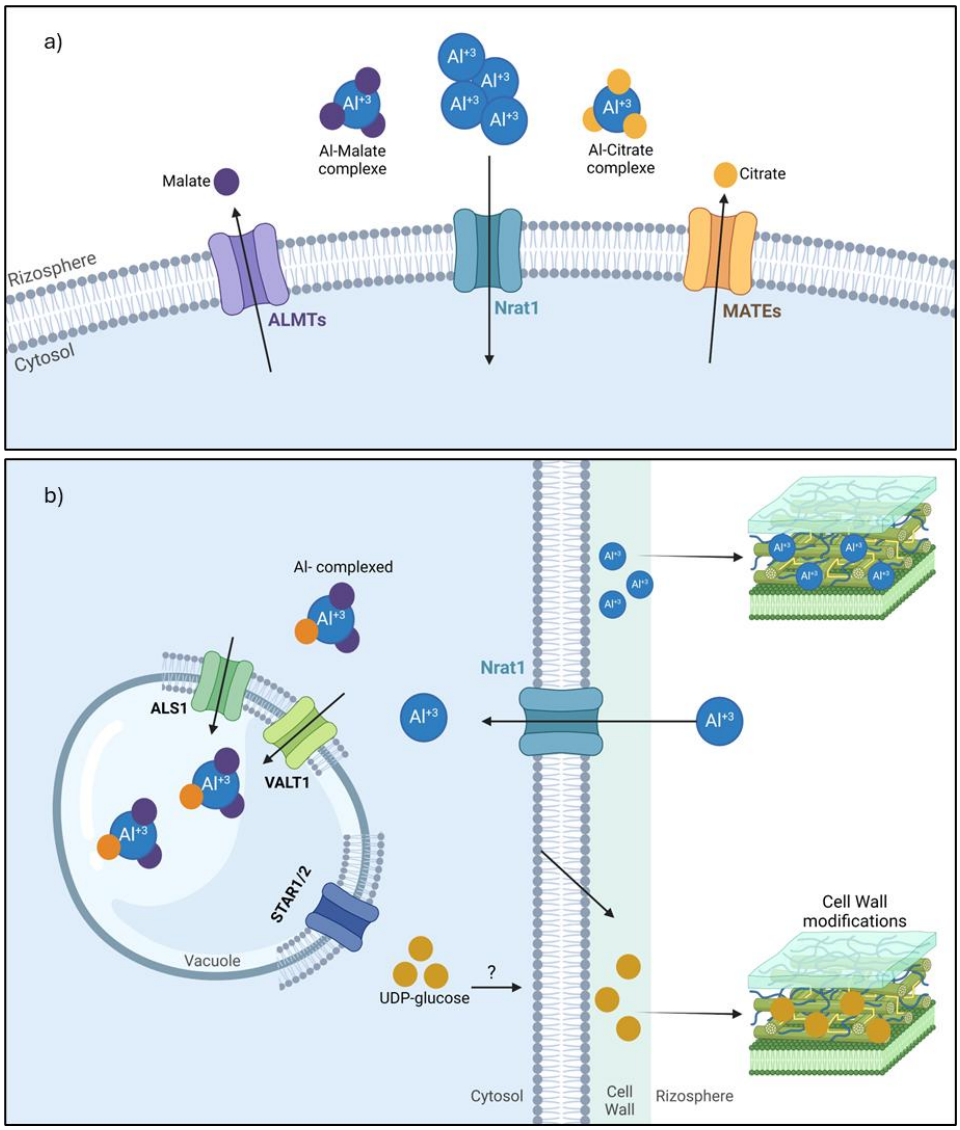


Figura 5 - Estratégias de resistência e tolerância ao Al. (a) Mecanismo de exclusão de Al envolvem a exsudação de ácidos orgânicos mediado pelos transportadores ALMTs e MATEs. (b) Mecanismo interno de tolerância ao Al envolve a absorção do Al pelo transportador Nrat, no citosol o Al é quelado com ácidos orgânicos e transportado para dentro do vacúolo pelos transportadores ALS1 e VALT1. STAR1 e STAR2 parecem mediar o efluxo de UDP-glicose na parede celular, alterando sua composição e limitando o acúmulo de Al na parede celular. Figura criada em: https://BioRender.com

A regulação da expressão gênica em resposta à toxicidade por Al é controlada por fatores de transcrição que desempenham papéis cruciais na ativação de genes relacionados à resistência e tolerância ao Al. Essas proteínas regulam a expressão de genes que codificam esses transportadores ao se ligarem diretamente na região promotora desses genes. Sua função principal é controlar quando, onde e por quanto tempo o gene será transcrito em RNA mensageiro. Em *A. thaliana*, o fator de transcrição *AtSTOP1* desempenha um papel crucial na indução de genes resistentes ao Al, como *AtALMT1*, *AtMATE1* e *AtALS3.* (Iuchi *et al.* 2007; Liu *et al.* 2009; Sawaki *et al.* 2009). O *AtSTOP1* é constitutivamente expresso, mas sua ativação depende de mecanismos pós-traducionais, como fosforilação, que são induzidos pela presença de Al (Kobayashi *et al.* 2007b). Além disso, o fator de transcrição AtWRKY46 atua como regulador negativo do *AtALMT1*, controlando a liberação de malato para evitar perdas excessivas de carbono (Ding *et al.* 2013).

No arroz, o primeiro fator de transcrição identificado como essencial para a tolerância ao Al foi o ART1 (do inglês *Al resistance transcription factor 1*) (Yamaji *et al.* 2009; Gao *et al.* 2023), ortólogo do STOP1 em Arabidopsis (Yamaji et al., 2009; Tsutsui et al., 2011). ART1 regula pelo menos 31 genes relacionados à tolerância ao Al, como *STAR1, STAR2, OsFRDL4, OsCDT3, OsMGT1, Nrat1, OsALS1* e *OsEXPA10* (Tsutsui et al., 2011). Esses genes desempenham funções na detoxificação do Al tanto interna quanto externamente. A família de fatores de transcrição WRKY também desempenha papéis significativos na resposta ao Al. Em arroz, o *OsWRKY22* regula a expressão de *OsFRDL4*, promovendo a secreção de citrato e aumentando a resistência ao Al. Este fator de transcrição atua em conjunto com o *ART1*, formando uma rede regulatória que otimiza a resposta ao estresse (Li et al., 2018).

Os genes *ASR* (do inglês *ABA-Stress and Ripening*) codificam fatores de transcrição que desempenham um papel central na regulação da resposta à toxidez por Al em arroz (Arenhart *et*

al. 2013). Entre os membros dessa família, o ASR5 é o mais estudado, sendo responsável por regular uma série de genes envolvidos na detoxificação do Al. O ASR5 se liga ao promotor e induz a expressão de *STAR1* e *STAR2*, genes que codificam subunidades de um transportador tipo ABC, responsável pelo transporte de UDP-glucose para modificação da parede celular. O gene *ASR5* também regula o gene *Nrat1*, um transportador que sequestra Al^{3+} nos vacúolos, evitando sua toxicidade intracelular. Além disso, o ASR5 controla *OsFRDL4*, um gene que codifica um transportador de citrato que exsuda citrato para a rizosfera, onde o citrato quela o Al e impede sua entrada nas células (Arenhart *et al.* 2014). O fator de transcrição ASR1, embora compartilhe funções semelhantes ao *ASR5* e regule genes como *STAR1* e *OsFRDL4*, exerce um papel complementar, podendo substituir o *ASR5* em na regulação gênica em resposta à toxidez por Al (Arenhart *et al.* 2016).

6. Efeitos benéficos do alumínio em plantas

O Al é amplamente relatado como um elemento tóxico para plantas, com a inibição do crescimento radicular sendo seu efeito mais evidente, principalmente em solos ácidos, onde sua solubilidade aumenta (Kochian *et al.* 2015a; Kopittke *et al.* 2016). No entanto, estudos indicam que, em baixas concentrações, o Al pode apresentar efeitos benéficos, promovendo o crescimento radicular e o desenvolvimento em algumas espécies vegetais (Arasimowicz-Jelonek *et al.* 2014a; Liu *et al.* 2016; Muhammad *et al.* 2019). Esses efeitos variam conforme as condições de crescimento, concentração do metal, tempo de exposição, espécie, genótipo e idade fisiológica (Bojórquez-Quintal *et al.* 2017; Aguilera *et al.* 2019; Ofoe *et al.* 2022). Por exemplo, concentrações entre 0,25 e 0,5 mM de Al não afetam o crescimento radicular de espécies de trevo (*Trifolium vesiculosum* e *Trifolium repen*) e tomate (*Solanum lycopersicum*). No entanto, 1,25 mM restrigem significativamente o crescimento das raízes (Bortolin *et al.* 2020; Ofoe *et al.* 2022). Em arroz, a concentração de 200 μM $AlCl_3$ pode promover o

crescimento radicular (Moreno-Alvarado *et al.* 2017). Já em *A. thaliana*, 50 μM $AlCl_3$ são suficientes para reduzir o crescimento radicular em mais de 30% (Li, Ma, *et al.* 2020). Esses dados sugerem que diferentes espécies toleram diferentes concentrações de Al.

Diversas espécies arbóreas, como *Camellia sinensis*, *Camellia japonica, Coffea arabica*, *Symplocos paniculata*, *Quercus serrata*, *Melastoma malabathricum* e *Vochysia tucanorum*, são conhecidas por serem hiper-acumuladoras de Al, mostrando maior tolerância e até crescimento estimulado em ambientes ácidos (Hajiboland, Barceló, *et al.* 2013a; de A. Bojórquez-Quintal *et al.* 2014a; Schmitt, Watanabe, *et al.* 2016; Sun *et al.* 2020; Liu *et al.* 2020; Bressan *et al.* 2021). Em *Camellia japonica*, concentrações entre 0,5-1mM de Al aumentaram a biomassa radicular, o conteúdo de clorofila e a capacidade fotossintética, além de melhorar a acumulação de nutrientes como N e P nas raízes (Liu *et al.* 2020). Outro estudo com essa mesma espécie mostrou que o Al não apenas é essencial para o estímulo do crescimento e desenvolvimento radicular, promovendo a formação de novas raízes, como também mantém a integridade do DNA em células meristemáticas (Sun *et al.* 2020).

Efeitos benéficos no crescimento vegetal também foram documentados em espécies de interesse econômico. Em arroz, variedades tolerantes apresentaram um aumento no crescimento das raízes quando expostas à 160 μM (Famoso *et al.* 2010a) e 200 μM de Al, além de aumentar o conteúdo de clorofila, carotenoides, altura da pare áerea e biomassa fresca e seca de raízes (Moreno-Alvarado *et al.* 2017). Em cultivares de soja tolerantes ao Al, 25 μM de Al por 24, 36 e 48 horas induziram de forma significativa a produção de calos e crescimento radicular (Du *et al.* 2010). Em tomate, a concentração de 500 μM de Al estimulou crescimento radicular e o peso fresco total de plântulas (Ofoe *et al.* 2022). No milho, concentrações baixas de Al (48 μM) inibiram o crescimento radicular, porém promoveram o crescimento foliar, dependendo do tempo de exposição (Wang, Fan, *et al.* 2015a).

A literatura sugere dois mecanismos de estímulo ao crescimento induzido pelo Al em plantas: (1) a longo prazo, com crescimento duradouro e observado em espécies hipertolerantes e acumuladoras de Al; (2) a curto prazo, com crescimento momentâneo, identificado na maioria dos estudos realizados em laboratório (Poschenrieder *et al.* 2015). Estudos realizados com hiper-acumuladores de Al sugerem que tais estímulos estão relacionados a diversos processos fisiológicos e bioquímicos, como o aumento na absorção de nutrientes (Watanabe *et al.* 2005a; Liu *et al.* 2020), ativação de enzimas antioxidantes (Hajiboland, Barceló, *et al.* 2013b; Xu *et al.* 2016), manutenção e integridade do DNA (Sun *et al.* 2020) e aumento no acúmulo de carboidratos e compostos fenólicos (Moriyama *et al.* 2016; Xu *et al.* 2016; Maejima *et al.* 2017). Embora os efeitos do Al no estímulo ao crescimento vegetal sejam amplamente documentados, os mecanismos exatos responsáveis por esse evento ainda não foram completamente compreendidos (Ofoe *et al.* 2023).

A estimulação do crescimento induzida pelo Al pode ser associada à promoção da absorção de nutrientes. Em plantas hiper-acumuladoras, o Al pode estimular ou não ter efeitos na absorção de nutrientes (Malta *et al.* 2016). Em *Camellia sinensis,* o crescimento induzido pelo Al foi atribuído ao aumento na absorção de macronutrientes (Fung *et al.* 2008). Além disso, em espécies como *Melastoma malabathricum* e *Quercus serrata* verificou-se um aumento na absorção e acúmulo de P nas raízes e folhas na presença de Al, provavelmente devido à precipitação do complexo Al-P na superfície das raízes ou no apoplasto, permitindo o uso desse P pelas plantas (Konishi 1992; Osaki *et al.* 1997; Tomioka & Takenaka 2007). Em *Quercus serrata*, o crescimento radicular foi associado à ativação da redutase de nitrato e ao aumento na absorção de NO_{3-} (Tomioka *et al.* 2007, 2012), enquanto em espécies do gênero *Symplocos*, a absorção de cálcio (Ca) foi positivamente correlacionada com os níveis de Al (Schmitt, Boras, *et al.* 2016). Em raízes de *Coffea arabica*, o Al aumentou os teores de K e Ca (de A. Bojórquez-Quintal *et al.* 2014b). Em *A. thaliana*, o Al induz a despolarização da membrana, o influxo de

K e H^+ e reduz o efluxo de K^+. (Bose *et al.* 2010a, 2010b). Em um mutante de *A. thaliana* (*alr-140*), observou-se um aumento no influxo e no conteúdo intracelular de Mg induzido pelo Al, sugerindo que o metal pode ativar canais e transportadores de Mg em plantas resistentes ao Al (Bose *et al.* 2013).

O Al também apresenta efeitos benéficos com relação à resistência das plantas, atuando em mecanismos associados à estresses bióticos e abióticos. Além de melhorar absorção de nutrientes, o Al pode mitigar toxidez causadas pelo excesso de outros elementos, como Mn e Fe (Watanabe *et al.* 2005b; Hajiboland, Barceló, *et al.* 2013a; Wang, Fan, *et al.* 2015a). Em plantas de arroz tratadas com excesso de Mn, o Al reduziu a toxidez por Mn ao limitar a absorção radicular e a translocação para os caules, modificando o potencial da membrana e a ligação às paredes celulares (Wang, Zhao, *et al.* 2015). Da mesma forma, o Al aliviou a toxidez por Fe em *Camellia sinensis* e *Melastoma malabathricum*, prevenindo a formação de EROS e o bronzeamento foliar (Watanabe *et al.* 2005b; Hajiboland, Bahrami Rad, *et al.* 2013). Estresses bióticos como resistências a patógenos e herbívoros também podem ser mitigados pelo Al. Em batata (*Solanum tuberosum*), o Al aumentou a resistência a patógenos como *Phytophthora infestans* e *Thielaviopsis basicola*, inibindo a germinação de esporos e promovendo respostas de defesa mediadas por óxido nítrico e ácido salicílico (Fichtner *et al.* 2006; Arasimowicz-Jelonek *et al.* 2014b). Em gramíneas como *Festuca arundinacea*, a aplicação de Al reduziu a biomassa de herbívoros e a oviposição de insetos, formando barreiras sensoriais que limitam a infestação (Potter *et al.* 1996)

Além disso, o Al influencia positivamente o metabolismo vegetal ao regular a expressão de genes que codificam enzimas relacionadas à tolerância ao estresse oxidativo, à deficiência de P e à secreção de ácidos orgânicos (Muhammad *et al.* 2019). Em espécies como o milho e a laranjeira (*Citrus sinensis*), o Al induz a expressão de genes relacionados à atividade antioxidante, como SOD e ascobarto peroxidase, que reduzem os danos causados por ROS

(Maron *et al.* 2008; Li, Yang, *et al.* 2016). Somado a isso, o Al estimula a absorção de P ao aumentar a expressão gênica de transportadores e enzimas como fosfatases ácidas e ribonucleases, que são cruciais para a adaptação a solos pobres em P (Li, Yang, *et al.* 2016).

7. Interação entre a deficiência de ferro e outros estresses nutricionais

Plantas terrestres por serem sésseis estão constantemente expostas às mais diferentes combinações de estresses nutricionais, os quais ocorrem naturalmente no solo. A deficiência ou o excesso de elementos minerais, mesmo os nutrientes, pode desencadear distúrbios fisiológicos nas plantas, comprometendo seu desenvolvimento (White & Brown 2010). A disponibilidade de nutrientes é amplamente limitada pelas propriedades físico-químicas do solo e dos próprios elementos, e até mesmo pela concentração e interação entre eles (Kim *et al.* 2016; Fan *et al.* 2021). Em resposta a essas variações, as plantas desenvolveram mecanismos adaptativos altamente especializados, permitindo que as plantas co-regulem estresses nutricionais, garantindo a manutenção da homeostase (Saenchai *et al.* 2016a; Bouain *et al.* 2018; Xie *et al.* 2019).

A homeostase de Fe é afetada pela concentração de outros elementos, como P (Dong *et al.* 2017; Therby-Vale *et al.* 2022b), N (Song *et al.* 2023b), S (Astolfi *et al.* 2021b), Mn (Rajonandraina *et al.* 2023b), Cu (Garcia-Molina *et al.* 2020; Gong *et al.* 2023), Zn (Zargar *et al.* 2015) e outros metais pesados (Lešková *et al.* 2017). Condições balanceadas e adequadas do suprimento de N e Fe aumenta o rendimento de grãos, perfilhamento e eficiência do uso de N em arroz e trigo (Song *et al.* 2023b). Há uma drástica redução no rendimento de grãos na deficiência de Fe, mesmo havendo N suficiente. A expressão de genes envolvidos na absorção e sinalização de N é inibida em condição de deficiência de Fe mesmo em suficiência de N. Já em condições de deficiência de N a expressão de genes relacionados à homeostase de Fe é

ligeiramente maior que em condições suficientes de N, independente da concentração de Fe (Song *et al.* 2023b).

A disponibilidade de S no solo ou no meio de cultivo influencia a absorção e o acúmulo de Fe. Vários estudos mostram que plantas em deficiência de S acumulam menos Fe em seus tecidos. No milho, plantas expostas à deficiência de S apresentam menor teor de Fe na parte área em comparação às plantas cultivadas na presença de S (Astolfi *et al.* 2004). Em cevada, o transportador de Fe HvYSL1 tem sua expressão gênica modulada por S, indicando que o S afeta principalmente a parte de aquisição de Fe (Astolfi *et al.* 2010). No arroz, a redução do teor de Fe na parte aérea induzida por deficiência de S foi associada a uma diminuição do nível de nicotianamina, sugerindo que S não é apenas crucial para a aquisição de Fe, mas também para a sua translocação para a parte aérea (Wu *et al.* 2020). Curiosamente, a deficiência simultânea de Fe e S pode mitigar sintomas típicos de clorose induzida por falta de Fe (Robe *et al.* 2020), a deficiência de S reprime a expressão de genes responsivos à deficiência de Fe, como *IRT1, FRO2, FDR3, NRAMP1* e *NAS4* (Robe *et al.* 2020; Astolfi *et al.* 2021b). Apesar dos avanços, ainda há lacunas na compreensão de como as plantas percebem e respondem a variações ambientais de Fe e S, integrando sinais para otimizar a assimilação e o metabolismo.

A homeostase de Fe, Cu, Mn e Zn são cruciais para o desenvolvimento vegetal, pois regulam a concentração desses micronutrientes para evitar lesões oxidativas causadas pelo acúmulo excessivo, bem como a deficiência dos mesmos, os quais são nutrientes essenciais (Rai *et al.* 2016). Esses micronutrientes frequentemente competem por transportadores de membrana, por proteínas de ligação com especificidades sobrepostas para cofatores metálicos e por sistemas compartilhados de transporte e armazenamento intracelular. Por exemplo, embora IRT1 seja o principal transportador de Fe em Arabidopsis, ele também transporta Zn e Mn (Grotz & Guerinot 2006). Apesar dos avanços com abordagens integradas que combinam dados de

transcriptoma, metaboloma e atividade enzimática, os mecanismos dessa complexa regulação precisam ser mais bem elucidados.

Os elementos Fe e Cu apresentam características semelhantes, especialmente em relação às propriedades redox, desempenhando papéis essenciais em vários processos fisiológicos vitais, como fotossíntese e respiração (Puig *et al.* 2007). Tanto o excesso de Cu quanto a deficiência de Fe resultam em clorose (Puig *et al.* 2007; Marschner 2011). Em arroz, excesso de Cu regula positivamente genes relacionados com a resposta à deficiência de Fe, no entanto, genes relacionados à resposta ao excesso e à desintoxicação de Cu não são induzidos pela deficiência de Fe (Gong *et al.* 2023). Ou seja, o excesso de Cu pode causar deficiência de Fe. Já a interação entre a deficiência de Fe e Cu compartilha a regulação de alguns genes, além de apresentar adaptação metabólica às limitações desses metais. Em Arabidopsis, o gene que codifica o transportador de Cu COPT2 (do inglês *Copper Transporter* 2) é induzido tanto em deficiência de Fe quanto em deficiência de Cu, de maneira dependente do fator de transcrição FIT1, o que sugere uma interação entre as vias de sinalização (Colangelo & Guerinot 2004). Em plantas sob limitação de Cu, ocorre substituição funcional de proteínas dependentes de Cu por equivalentes que utilizam Fe, como a troca de Cu/ZnSOD por FeSOD nos cloroplastos, economizando Cu para funções essenciais (Quinn & Merchant 1995; Merchant *et al.* 2006).

A atividade fotossintética requer o fornecimento constante de Fe e Mn. Nas últimas décadas, diversos estudos voltados à identificação de genes que codificam transportadores de Fe e Mn para dentro do cloroplasto têm ganhado atenção (Therby-Vale *et al.* 2022b). Relatos sobre a interação entre esses dois elementos apontam que plantas em deficiência de Fe hiperacumulam Mn (Robe *et al.* 2020). Esse fenótipo pode ser explicado pela ativação da resposta à deficiência de Fe, a qual regula positivamente a expressão de IRT1 (Alam *et al.* 2000; Führs *et al.* 2010; Eroglu *et al.* 2016). Devido à sua baixa especificidade, o transportador aumenta a absorção de Mn^{2+} a níveis potencialmente tóxicos para a planta (Korshunova *et al.*

1999; Fecht-Christoffers *et al.* 2003). Por sua vez, a toxidez por Mn afeta negativamente a fotossíntese, inibindo a atividade do PSI e reduzindo levemente a eficiência do PSII, além de desencadear respostas fisiológicas que simulam a deficiência de Fe, mesmo quando este está disponível (Kohno *et al.* 1984; Millaleo *et al.* 2013; Robe *et al.* 2020). Essa interação complexa evidencia a necessidade de um controle rigoroso das concentrações de Fe e Mn, uma vez que o desequilíbrio entre esses elementos pode comprometer a funcionalidade dos sistemas fotossintéticos e resultar em sintomas como clorose.

Os sintomas de deficiência de Fe em plantas frequentemente se assemelham aos causados pela toxidez por Zn, evidenciando a interação entre esses dois metais. Sob deficiência de Fe, ocorre a expressão gênica do transportador IRT1, que tem afinidade por outros metais, como o Zn, resultando em maior acúmulo de Zn nas plantas (Arrivault *et al.* 2006). Em condições de deficiência combinada de Fe e Zn, as plantas ativam o fator de transcrição FIT, que regula positivamente a expressão de *FRO2* e *IRT1/2*, para aumentar absorção e o transporte de desses metais (Yuan *et al.* 2008b; Wang, Cui, *et al.* 2013b). Por outro lado, o excesso de Zn pode induzir a deficiência de Fe, reduzindo seu acúmulo tanto nas folhas (Le¡ková *et al.* 2017) quanto nas raízes (Fukao *et al.* 2011). Além disso, plantas expostas ao excesso de Zn apresentam um padrão transcricional semelhante ao observado em condições de deficiência de Fe (Shanmugam *et al.* 2011; Lešková *et al.* 2017; Fan *et al.* 2021).

A interação entre P e Fe é uma das interações nutricionais mais estudadas nos últimos anos. P e Fe podem influenciar a disponibilidade do outro, tanto no solo quanto na regulação da homeostase de ambos os nutrientes nas plantas (Yang *et al.* 2024). Em casos de deficiência de Fe, a aquisição de P é estimulada em folhas e raízes, enquanto a deficiência de P aumenta significativamente a disponibilidade de Fe nas raízes (Zheng *et al.* 2009a; Briat, Dubos, *et al.* 2015; Briat, Rouached, *et al.* 2015). Na rizosfera, a formação do complexo Fe-P torna ambos os elementos indisponíveis para a absorção das plantas. Um dos mecanismos desenvolvidos pelas

plantas em resposta à deficiência de P é a exsudação de citrato e malato, a fim de liberar o P ligado aos complexos formados no solo de Al-P, Fe-P e Ca-P. Esses ácidos orgânicos possuem alta afinidade com o Al, Fe e Ca, podendo facilmente realocar o P dessas formas insolúveis e deixá-lo precipitado para a absorção pelas raízes (Delhaize *et al.* 2012b; de Bang *et al.* 2021). Em condições de deficiência de Fe e excesso de P, a absorção de Fe é ativada, enquanto que em condições contrárias (excesso de Fe e deficiência de P) há acumulo de Fe e Al (Ward *et al.* 2008a; Briat, Dubos, *et al.* 2015; Briat, Rouached, *et al.* 2015).

Interessantemente, em condições de baixo Fe, a deficiência de P promove o acúmulo de Fe nas raízes (Misson *et al.* 2005; Li & Lan 2015; Hoehenwarter *et al.* 2016). Em contrapartida, excesso de P causa deficiência de Fe. O aumento da disponibilidade de P no meio reduz a absorção e translocação de Fe, agravando os sintomas induzidos pela deficiência de Fe, como a clorose, por exemplo (Elliott & Läuchli 1985; Romera *et al.* 1992; Sánchez-Rodríguez *et al.* 2014; Zhang *et al.* 2020). No arroz, a deficiência de P reprime a expressão de genes associados à absorção de Fe (Zheng *et al.* 2009b), mas regula positivamente a expressão dos genes VITs, os quais codificam transportadores vacuolares de Fe, resultando no acúmulo de Fe nos vacúolos e na parede celular (Ding *et al.* 2018).

Fe e Pi (fósforo inorgânico) também podem formar complexos insolúveis no apoplasto das raízes, impedindo a translocação de Fe para as folhas e contribuindo para a ocorrência de clorose (Shi *et al.* 2018b). Nam et al. (Nam *et al.* 2021a) identificaram uma via de sinalização envolvendo a regulação da fotossíntese em condições combinadas de deficiência de Fe e P. Nesse estudo, foram identificados genes como o do transportador de Pi e ascorbato PHT4;4, e o fator de transcrição bZIP58 (do inglês *Basic leucine-zipper 58*), que regula a expressão de genes relacionados à fotossíntese, prevenindo a clorose induzida pela deficiência de Fe. O fator de transcrição bZIP58 regula a expressão do gene *VTC4* (do inglês *Vitamin C 4*), envolvido na biossíntese de ácido ascórbico, enquanto o transporte de ácido ascórbico para o cloroplasto é

mediado por PHT4;4. No cloroplasto, o transporte de ácido ascórbico é crucial para prevenir a clorose, pois a deficiência de Fe causa o acumulo de EROs nos cloroplastos, e o ácido ascórbico possui propriedades antioxidantes (Nam *et al.* 2021a). O fenótipo de plantas não cloróticas em deficiência combinada de Fe e P também foi observado em diversas espécies, como o arroz (Saenchai *et al.* 2016b; Guo *et al.* 2022a), *Lemna gibba* (Nam *et al.* 2021a), cevada, tomate (Tiziani *et al.* 2023a) e até mesmo em cianobactérias (Qiu *et al.* 2024). Esses resultados indicam que esse fenótipo é amplamente conservado entre diferentes espécies vegetais e organismos fotossintéticos.

Tanto o Al como o Fe regulam positivamente a abundância de STOP1 dentro do núcleo de células radiculares em *A. thaliana* (Godon *et al.* 2019). O fator de transcrição STOP1 é crucial na resposta à toxidez por Al, e regula a expressão de genes relacionados à resistência ao Al, como o do transportador de malato ALMT1 (Liu *et al.* 2009; Sawaki *et al.* 2009). Sob deficiência de P, o Fe inibe o crescimento radicular. Essa inibição é dependente do malato exsudado via ALMT1 (Svistoonoff *et al.* 2007; Ward *et al.* 2008b; Müller *et al.* 2015). Fe e Al ativam de maneira independente a expressão de *ALMT1*, mas a presença dos dois metais possui efeitos sinérgicos, com o Al intensificando a inibição do crescimento radicular mediada por Fe e aumentando a expressão de *ALMT1* no ápice radicular. Essas respostas parecem estar ligadas à acumulação de STOP1 no núcleo em condições de estresse combinado, enquanto Fe e Al isoladamente não induzem essa resposta em mutantes *stop1* (Le Poder *et al.* 2022). Plantas de *A. thaliana* expostas à 10μM de Fe e 10μM Al apresentam um fenômeno chamado parada de extensão da raiz, o mesmo fenótipo não é observado quando as plantas são expostas a esses tratamentos de forma isolada (Kaur *et al.* 2024). Nesse mesmo estudo, não houve redução no crescimento radicular em plantas cultivadas sob deficiência de Fe (sem adição de Fe) e excesso de Al (20 μM de Al).

OBJETIVOS

1. Objetivo Geral

Investigar o impacto do Al na resposta à deficiência de Fe em plantas de arroz (*Oryza sativa*).

2. Objetivos Específicos

- Investigar o papel do Al na mitigação dos efeitos da deficiência de Fe em plantas de arroz, como o alívio na clorose induzida pela deficiência de Fe, na redução de pigmentos fotossintéticos e na diminuição precoce da fotossíntese.
- Avaliar como o Al modula a expressão de genes marcadores de deficiência de Fe e P em plantas de arroz, em busca de elucidar os mecanismos envolvidos na interação entre esses nutrientes.
- Investigar como o excesso de P interfere na capacidade do Al e aliviar a clorose causada pela deficiência de Fe em plantas de arroz.
- Explorar os mecanismos pelos quais o Al e o P modulam a fotossíntese de arroz em condições de deficiência de Fe, avaliando os efeitos opostos dessas interações.
- Verificar se os mecanismos pelos quais o Al alivia a clorose causada pela deficiência de Fe em arroz são conservados em milho (*Zea mays*) validando a aplicabilidade dos resultados em outras gramíneas.

CAPÍTULO 1

ALUMINUM ALLEVIATES IRON DEFICIENCY CHLOROSIS BY INTERFERING WITH PHOSPHORUS HOMEOSTASIS IN RICE (*Oryza sativa* L.)

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Aluminum alleviates iron deficiency chlorosis by interfering with phosphorus homeostasis in rice (*Oryza sativa* L.)

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for more than half of the human population. Rice plants are cultivated in several different environments, and face various abiotic stresses, including nutritional imbalance in soils. The ionome, the inorganic composition of an organism, is known to be tightly regulated, as changes in concentration of one element affect concentrations of others. Iron (Fe) is an essential element that is involved in redox reactions, nitrogen metabolism and chlorophyll synthesis. The hallmark of Fe deficiency in plants is leaf chlorosis, a phenotype known to be alleviated by deficiencies of other elements, such as phosphorus (P). Aluminum (Al) is abundant in soils and limits plant growth in acidic soils. Despite its well-established detrimental effects, Al has been proposed to have a positive effect on growth for some species, but little is known about this phenomenon. Here we aim to understand whether Al affects Fe homeostasis in rice. We found that Al alleviated Fe deficiency-induced chlorosis. +Al–Fe treatment decreased expression of Fe deficiency marker genes and partially recovered photosynthesis. We also observed that Al induced expression of a P deficiency marker gene, and addition of excess P to nutrient solution reversed effects of Al on chlorosis. Our data show that Al alleviates Fe deficiency-induced chlorosis, and suggests that this occurs indirectly by inducing P deficiency in leaves.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most cultivated and consumed cereals in the world and is considered a staple food for more than half of the world's population, providing 21% of the calories consumed by humans (Elert, 2014). Rice is cultivated on all continents, with rice genotypes and cultivars adapted to different environmental, climatic, and agronomic conditions. Due to the large cultivation area, rice fields are often exposed to abiotic stresses, such as flooding, drought, salinity, heat, cold, deficiency of essential nutrients, and toxicity of hazardous elements, which can have detrimental impacts in yield and quality (Ganie et al., 2024; Ray et al., 2015). These stresses can occur simultaneously, forcing plants to respond to combinations of stresses (Priya et al., 2023). Diverse edaphic conditions also result in combined mineral element-related stresses (DeLoose et al., 2024), since changes in the concentration of one element can affect the homeostasis of others (Huang and Salt, 2016; Pita-Barbosa et al., 2019; Ricachenevsky et al., 2018). Therefore, it is crucial to understand how accumulation of one element might affect the ionome balance.

Aluminum (Al) is one of the most abundant elements in the Earth's crust and a major limiting factor for plant growth worldwide (Imadi et al., 2016; Ofoe et al., 2023). This is because Al^{3+} becomes highly soluble and causes phytotoxicity under acidic conditions, which are prevalent in approximately 50% of arable soils (Kochian et al., 2015). Most plants are sensitive to Al, which inhibits root growth even when present at micromolar concentrations, thereby decreasing water and

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nutrient uptake and impairing growth (Famoso et al., 2010; Yamamoto, 2019). Interestingly, rice is one of the most Al-tolerant crops, and a good model for understanding tolerance mechanisms (Arenhart et al., 2013; Kochian et al., 2015). Although Al has no known function in any organism, there is evidence that Al might be beneficial depending on the plant species, developmental stage, concentration, and exposure time (reviewed by (Ofoe et al., 2023). For example, the root growth of *Camellia* sp. is stimulated by Al, and this element has been proposed to be beneficial (Sun et al., 2020) or essential (Liu et al., 2020) for this species. For rice, evidence suggests that Al may induce root growth and increase uptake of macronutrients (Moreno-Alvarado et al., 2017). However, there is little information on how these potential beneficial effects might occur and whether they involve Al-induced changes in the homeostasis of essential elements.

Iron (Fe) is an essential nutrient for plants that plays crucial roles in electron transfer reactions in photosynthesis and respiration, nitrogen and sulfate metabolism and chlorophyll synthesis (Chen et al., 2018; Li et al., 2021; Shi et al., 2018). Plants employ two mechanisms for Fe uptake: one based on the reduction of ferric (Fe^{3+}) ion prior to uptake (Strategy I), found in all non-Poaceae species; and the other, common to Poaceae, relying on the import of intact Fe^{3+}-phytosiderophore chelates (Strategy II). Notably, rice employs a combined strategy, which has all the features of Strategy II and some of the features of Strategy I (Kobayashi and Nishizawa, 2012; Ricachenevsky and Sperotto, 2014; Wairich et al., 2019, 2022). Well-established molecular and physiological responses are induced in rice plants under Fe deficiency. At the molecular level, expression of several Fe deficiency-associated genes is markedly increased in roots, such as the bHLH transcription factor OsIRO2 (Ogo et al., 2011), which is a positive regulator of the Fe deficiency response; and membrane transporters such as OsYSL15 (Inoue et al., 2009; Lee et al., 2009), involved in uptake of Fe^{3+}-phytosiderophore chelates; and OsNRAMP1 (Chang et al., 2020), involved in manganese (Mn) and cadmium Cd transport. At the physiological level, leaf chlorosis is a hallmark of Fe deficiency, and is typically associated with decreased Fe concentration in leaves (Tran et al., 2023; Wairich et al., 2019). Interestingly, deficiencies of other elements, such as phosphorus (P) and sulphur (S), were shown to revert chlorosis and decrease the expression of Fe-deficiency markers in different plant species (Astolfi et al., 2021; Nam et al., 2021; Robe et al., 2020; Therby-Vale et al., 2022), suggesting that the Fe deficiency phenotype might be finely regulated by nutrient balances.

Fe homeostasis is affected by concentrations of other elements, such as S (Astolfi et al., 2021), nitrogen (Song et al., 2023), magnesium (Rajonandraina et al., 2023), and phosphorus (P) (Therby-Vale et al., 2022). Fe and P homeostasis are interconnected at different levels. In *Arabidopsis thaliana* roots, local P deficiency leads to shorter roots due to decreased cell elongation and arrested cell division at the tip (Sánchez-Calderón et al., 2005). The phenotype is dependent on the presence of Fe, which is remobilized from the soil under P deficiency to generate reactive oxygen species and induce cell wall stiffening, resulting in impaired cell elongation (Balzergue et al., 2017; Müller et al., 2015). Moreover, Fe deficiency-induced chlorosis is dependent on the presence of P, as leaves of *A. thaliana* plants exposed to combined Fe and P deficiency are less chlorotic compared to Fe-deficient plants (Nam et al., 2021). The same phenomenon has been observed in several plant species, including rice, *Lemna gibba*, barley, tomato and cyanobacteria (Guo et al., 2022; Qiu et al., 2024; Saenchai et al., 2016; Tiziani et al., 2023), suggesting conservation in the plant lineage. Although Al excess and P deficiency are known to have partially overlapping genetic factors (Magalhaes et al., 2018), whether Al interferes with the Fe-P interaction remains unknown.

Here we investigated the impact of Al stress on the response to Fe deficiency in rice. Since (1) Al excess and P deficiency share overlapping mechanisms; and (2) P deficiency is known to alleviate Fe deficiency chlorosis; we hypothesized that Al excess could induce secondary P deficiency and alleviate Fe deficiency-induced chlorosis. We found that shoot chlorosis derived from Fe deficiency is alleviated by Al, suggesting that Al may affect Fe utilization in rice plants and could account for a beneficial effect of Al in rice. Furthermore, our findings indicate that the Al effect on Fe deficiency chlorosis is reversed by P excess, indicating that Al alleviates Fe deficiency chlorosis by inducing P deficiency.

2. Results

2.1. Al alleviates Fe deficiency-induced chlorosis

To investigate the possible effects of Al in Fe deficiency response in rice, we conducted hydroponics experiments comparing plants under control (sufficient Fe and no Al), 300 μM Al (hereafter + Al), Fe deficiency (−Fe) and combined Fe deficiency and 300 μM Al (hereafter −Fe + Al). After 21 days of treatment, we observed that plants under + Al, −Fe, and −Fe + Al treatments had reduced root length compared to control plants (Fig. 1A and B). The treatments did not significantly affect root dry mass (Fig. 1C). The presence of Al in the Fe-sufficient nutrient solution did not induce visible symptoms on the shoots such as chlorosis or necrosis, while −Fe resulted in the expected chlorosis associated with Fe deficiency (Fig. 1D). Strikingly, plants grown under −Fe + Al treatment were not chlorotic, with shoots resembling those of control and +Al-treated plants (Fig. 1D). All three treatments resulted in decreased culm height compared to control plants (Fig. 1E), whereas the whole shoot dry mass was not affected (Fig. 1F). Taken together these data suggest that while + Al, −Fe, and −Fe + Al treatments affect plant growth, with decreases in root and culm length, Al alleviates Fe deficiency-associated chlorosis.

To gain insight into the timing of the observed effect of Al on Fe deficiency, we analyzed SPAD values of leaves from plants under control, +Al, −Fe, and −Fe + Al treatments in a time-course experiment (Fig. 2). While plants grown under control and +Al had similar SPAD values throughout the whole experiment, −Fe-treated plants exhibited drastically decreased SPAD values, starting at 7 days of treatment, remaining low at day 17 and further decreasing at 21 days (Fig. 2A). −Fe + Al plants, on the other hand, had a less pronounced reduction in SPAD values (Fig. 2A and B). This result matched the visual aspect of the leaves (Fig. 2C). Taken together, these data show that Al drastically alleviates Fe deficiency chlorosis, delaying the onset of the symptom in Fe-deficient plants.

2.2. Early decrease in photosynthesis caused by Fe deficiency is alleviated by Al

Since we observed Al alleviates and delays chlorosis in rice (Fig. 2), we investigated how photosynthesis was affected at the early stage of treatments (i.e., seven days). Compared to control, net photosynthesis was not affected by + Al alone but significantly decreased under −Fe (Fig. 3A). In plants grown under combined −Fe + Al treatment, net photosynthesis was also lower compared with control, but higher than in −Fe-treated plants, suggesting that −Fe effects on photosynthesis are alleviated by Al (Fig. 3A). Carboxylation efficiency was decreased by −Fe, and improved in −Fe + Al treated plants, similar to net photosynthesis (Fig. 3B). Water use efficiency was also decreased in −Fe plants compared to controls but was not significantly improved by the supply of Al (Fig. 3C). Taken together, these results indicate that Al can alleviate the deleterious effects of Fe deficiency on photosynthesis in rice leaves.

2.3. Al decreases the expression of Fe deficiency markers and increases the expression of P deficiency marker

To understand whether Al is affecting Fe sensing in rice, we analyzed the expression of genes encoding the transcription factor OsIRO2 and the transporters OsYSL15 and OsNRAMP1, since these genes are good markers of Fe deficiency in rice (De Oliveira et al., 2020; Wairich et al.,

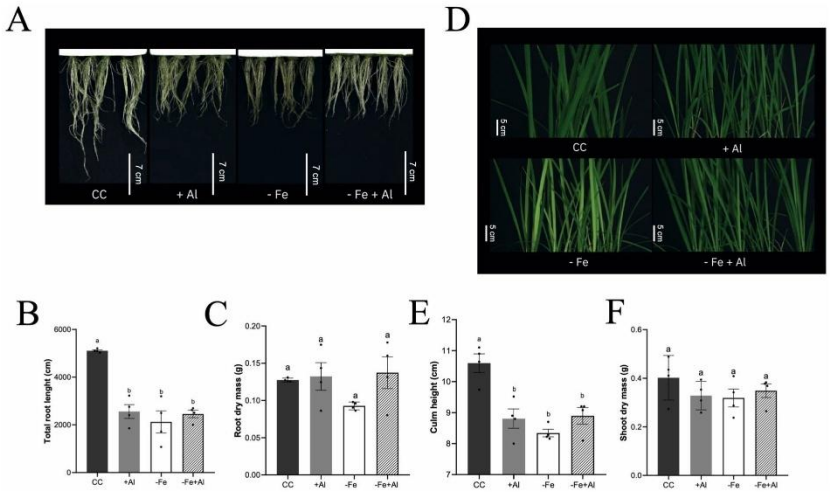


Fig. 1. Phenotype and growth of plants cultivated under control, +Al, −Fe and −Fe + Al treatments. (A) Root phenotypes. (B) Total root length. (C) Root dry mass. (D) Shoot phenotypes. (E) Culm height. (F) Shoot dry mass. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

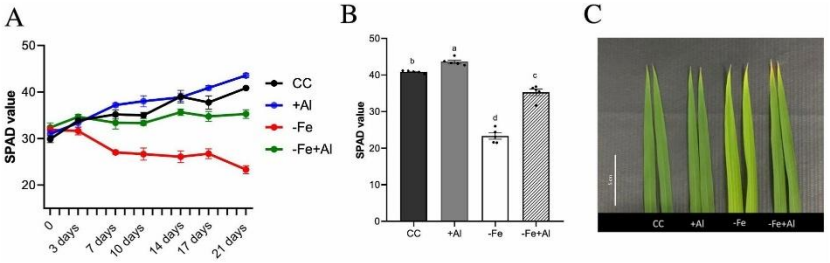


Fig. 2. Leaf chlorosis in leaves of plants cultivated under control, +Al, −Fe and −Fe + Al treatments. (A) SPAD measurements throughout the experiment. (B) SPAD values from leaves of plants at the end of the experiment. (C) Leaf phenotype. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

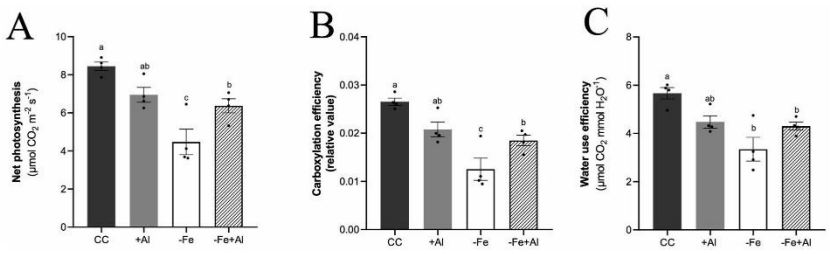


Fig. 3. Photosynthetic measurements from leaves of plants cultivated under control, +Al, −Fe and −Fe + Al treatments. (A) Net photosynthesis. (B) Carboxylation efficiency. (C) Water use efficiency. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

2019). All three genes exhibited were strongly up-regulated under −Fe compared to control condition (Figs. 4A–5C). Interestingly, in roots of plants exposed to the combined −Fe + Al treatment, the expression of the three genes was not up-regulated, and were similar to control.

The phenotypic effects of Al on Fe-deficient plants observed here are similar to that of P deficiency, which also leads to alleviation of Fe deficiency chlorosis (Saenchai et al., 2016; Zheng et al., 2009). To test whether Al-treated plants were mimicking P deficiency, we used *OsSPX1* as a P-deficiency marker (Gonçalves et al., 2020; Guo et al., 2022). The + Al treatment led to *OsSPX1* up-regulation in roots compared to plants grown under control conditions (Fig. 4D). The expression of *OsSPX1* was not significantly altered under −Fe compared to control. However, in roots of plants under −Fe + Al treatment, *OsSPX1* expression was higher than that of −Fe plants, while comparable to that of control plants (Fig. 4D). These results suggest that Al treatment decreases expression of Fe deficiency markers, and induces P deficiency markers in roots.

2.4. Alleviation of chlorosis under low Fe by Al is reversed by P excess

Given that P homeostasis is disturbed by Al (Fig. 4D) and that chlorosis under Fe-limiting conditions could be alleviated by Al similarly as by lowering the concentration of supplied P, as demonstrated previously (Guo et al., 2022; Saenchai et al., 2016), we reasoned that Al might be inducing P deficiency. To test that hypothesis, we grew rice plants under control conditions (CC), +Al, P excess (x4 the P concentration of that in the control condition; ++P), −Fe, and combinations of these treatments (+Al++P, −Fe++P, −Fe + Al and −Fe + Al++P). The presence of Al, the excess of P or the combination of both did not cause leaf chlorosis (Fig. 5A). Under −Fe, the excess of P did not alter the chlorosis of plants but, when supplied together with Al, prevented the Al-dependent chlorosis alleviation. Compared to control conditions, shoot dry mass was significantly decreased when plants were cultivated under + Al, −Fe, −Fe++P, −Fe + Al, and −Fe + Al++P, with Al or excess P not decreasing further the dry mass of plants grown under −Fe (Fig. 5B).

Roots of rice plants under all treatments were visually similar (Fig. 5C), while root dry mass was reduced in plants cultivated under −Fe, −Fe++P, −Fe + Al, and −Fe + Al++P (Fig. 5D), suggesting that Fe deficiency is the major factor decreasing biomass accumulation.

To quantitatively analyze the differences in chlorosis observed, we measured SPAD values in the leaves of plants treated as described above in a time-course experiment for 21 days. Plants from the control condition, +Al, ++P, and +Al++P had comparable SPAD values and similar symptoms of chlorosis and SPAD values at harvest (Fig. 6A–C). Again, the supply of Al to the −Fe nutrient solution maintained higher SPAD values compared to −Fe or supplied with excess P throughout the experiment, with chlorosis symptoms almost absent at 21 days. −Fe + Al++P treatment showed chlorosis comparable to Fe-deficient plants (Fig. 6A–C), again suggesting that the Al-dependent effect on chlorosis is likely to involve secondary P deficiency. We also quantified chlorophyll *a*, chlorophyll *b*, and total chlorophyll, and found that plants under −Fe, −Fe++P and −Fe + Al++P treatments had decreased chlorophyll concentrations (Fig. 7D–F), in agreement with SPAD measurements. Taken together, these results reinforced that Al alleviates Fe deficiency chlorosis, an effect that was reversed by an excess of P.

2.5. Al alleviation effect is dependent on phosphorus but not on Fe concentration

To understand how the ionome is affected by treatments, we measured elemental composition in roots and leaves of rice plants cultivated in all eight nutritional conditions described above. We sampled the last fully expanded leaf, which showed clear phenotypes (Fig. 6A) after 21 days of treatment. We found that leaves from plants under + Al and +Al++P treatments had similar Fe concentrations compared to the control, whereas ++P led to a significant increase in Fe (Fig. 7A). As expected, leaves from plants grown in −Fe had lower Fe concentration compared to the control. However, all combined treatments with no added Fe, including −Fe + Al, had similar Fe concentration to those found in plants under −Fe condition (Fig. 7A), suggesting that chlorosis was not entirely dependent on Fe concentration of the whole leaf tissue. In roots, Fe concentration had less pronounced changes compared to leaves (Fig. 7B). Excess P increased Fe concentration in roots, whereas only −Fe++P and −Fe + Al decreased (Fig. 7B). Interestingly, Al concentration in leaves barely changed, with only + Al++P treatment showing increased concentration compared to the control (Fig. 7C). Al concentrations, as expected, were higher in treatments containing Al, with −Fe + Al and −Fe + Al++P showing the highest values (Fig. 7D).

Given that our data and others (Nam et al., 2021) suggested that the Al effect might involve P homeostasis, we then compared P concentration in leaves from plants under the same treatments. Clearly, Al treatment reduced P accumulation in leaves, as both + Al and −Fe + Al treatments had the lowest P concentration (Fig. 7E). As expected, extra P in the growth media increased P concentration in leaves; however, the highest concentration was found in −Fe + Al++P, which was = significantly higher than + Al++P (Fig. 7E). Leaves from plants under −Fe also accumulated higher P concentration, suggesting that Fe deficiency facilitates P accumulation. P concentration in roots was increased in all treatments with extra P, as well as in −Fe treatments, with the three combined treatments showing the highest concentration (Fig. 7F). Taken together, these results support the hypothesis that Al is causing P deficiency in shoots and resulting in chlorosis reversion in Fe-deficient plants.

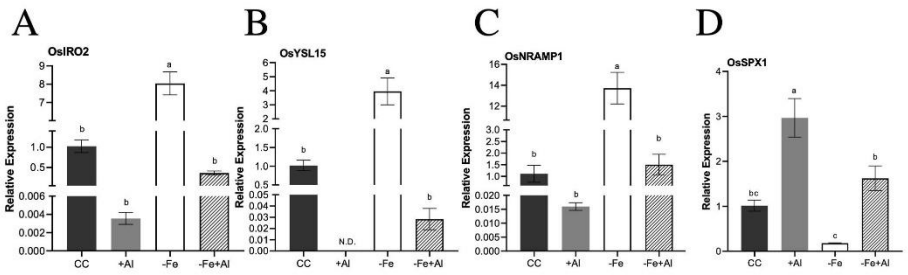


Fig. 4. Gene expression of Fe deficiency and P deficiency markers in roots of plants cultivated under control, +Al, −Fe and −Fe + Al treatments. (A) Gene expression of Fe deficiency marker OsIRO2. (B) Gene expression of Fe deficiency marker OsYSL15. (C) Gene expression of Fe deficiency marker OsNRAMP1. (D) Gene expression of P deficiency marker OsSPX1. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

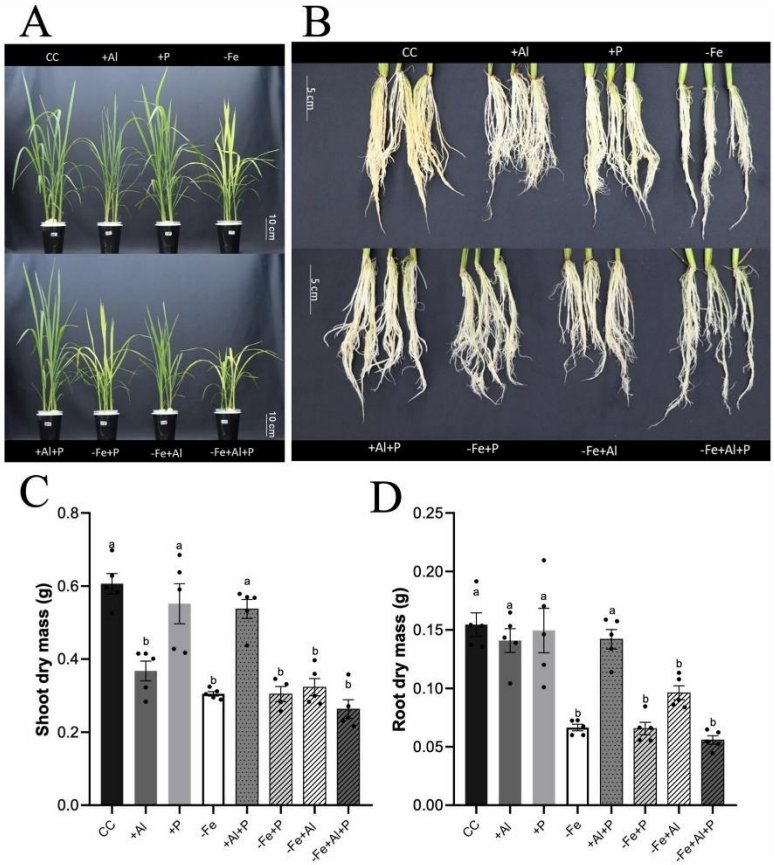


Fig. 5. Phenotype and growth of plants cultivated under control, +Al, ++P, −Fe, +Al++P, −Fe++P, −Fe + Al and −Fe + Al++P treatments. (A) Shoot phenotype. (B) Shoot dry mass. (C) Root phenotype. (D) Root dry mass. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

2.6. Photosynthesis impact by Fe deficiency is partially recovered by Al excess and impaired by P excess

Given the observation that P excess reverses the Al effect on chlorosis, we analyzed how photosynthesis was affected by these treatments after 21 days of treatment (Fig. 8). We conducted experiments as above, with all eight treatments, and measured photosynthetic parameters in shoots of plants at the end of the experiment. Net photosynthesis was not affected by + P and +Al + P, since plants under those treatments had values comparable to plants under control conditions (Fig. 8A). Both + Al and −Fe led to decreased net photosynthesis, although −Fe values were even lower than those of +Al plants. Interestingly, −Fe + Al treated plants showed values similar to those of +Al alone, and higher than −Fe, again showing the alleviation effect. Strikingly, −Fe++P and −Fe + Al++P showed negative net photosynthesis (Fig. 8A). The same pattern was observed for carboxylation efficiency (Fig. 8B) and water use efficiency (Fig. 8C). We also found that Fv/Fm values, which are a good proxy for stress in plants, were reduced to the same extent in all three treatments that induced chlorosis, −Fe, −Fe++P, and −Fe + Al++P (Fig. 8D). Taken together, these data show that Al can alleviate −Fe effects on photosynthesis, but those are reversed with P excess.

3. Discussion

Al is a well-known toxic element that reduces plant growth (Kochian et al., 2015). Paradoxically, beneficial effects of Al in plants were reported in some species, including crops, at specific concentrations (Moreno-Alvarado et al., 2017; Ofoe et al., 2023; Sun et al., 2020). However, there is no clear understanding of how Al might mechanistically improve plant growth and/or other traits. Since Al has no described

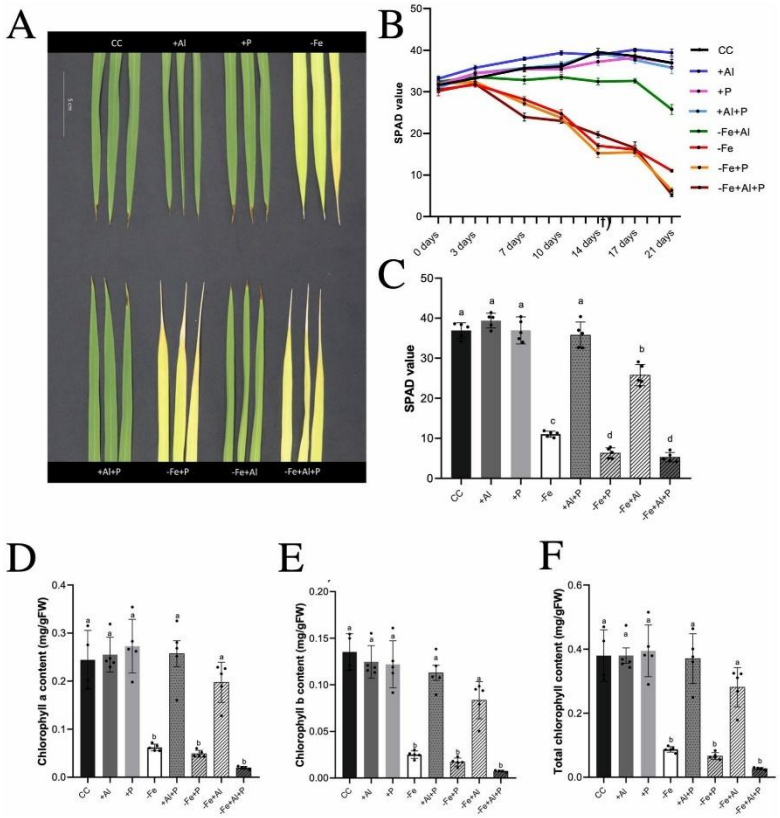


Fig. 6. Leaf chlorosis and photosynthetic pigments concentration in leaves of plants cultivated under control, +Al, ++P, −Fe, +Al++P, −Fe++P, −Fe + Al and −Fe + Al++P treatments. (A) Leaf phenotype. (B) SPAD measurements throughout the experiment. (C) SPAD values from leaves of plants at the end of the experiment. (D) Chlorophyll *a* concentration. (E) Chlorophyll *b* concentration. (F) Total chlorophyll concentration. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

function in plant physiology, it is possible that positive effects of Al are caused indirectly by interfering with other processes. Here, we demonstrate that Al alleviates Fe deficiency chlorosis in rice, and raise evidence that this Al effect might be caused by indirectly inducing P deficiency.

Fe deficiency-induced chlorosis is classically linked to low Fe concentration in leaves. Fe participates in chlorophyll synthesis and is a cofactor of electron transport chain components in chloroplasts, which presumably explains leaf yellowing when Fe concentration is low (Therby-Vale et al., 2022; Wairich et al., 2019). Experimental evidence has shown that low Fe-induced chlorosis is alleviated in distinct species by combined treatments, such as the addition of silicon (Si) (Pavlovic et al., 2013) or ammonium (NH_4^+) to rice (Zhang et al., 2019), and deficiencies of sulphur (S) (Robe et al., 2020) or P (Nam et al., 2021) in more than one species. From these, the interaction of Fe and P is probably the most well-studied. Transcriptome analyses of roots showed an extensive reversion of Fe deficiency-induced changes when plants were exposed to combined Fe and P deficiencies (Zheng et al., 2009). However, leaf Fe concentrations were similar in rice plants grown under −Fe−P and −Fe (Guo et al., 2022; Saenchai et al., 2016; Zheng et al., 2009), a result also observed in *A. thaliana* when Fe and P deficiency are combined (Nam et al., 2021), suggesting that chlorosis is not entirely dependent on bulk Fe leaf concentration. In our study, Fe concentrations were similar in leaves of −Fe + Al and −Fe plants (Fig. 7), and the upregulation of Fe-deficiency markers was reversed by the supply of Al to −Fe (Fig. 4), indicating that Al alleviates chlorosis without significantly changing bulk Fe concentrations in the leaf tissue. However, we cannot rule out that changes in tissue and cellular-level distribution are involved, such as Fe precipitation in the apoplast or alteration in subcellular Fe localization.

Our results raised evidence that Al is inducing P deficiency. First, the addition of extra P in the nutrient solution reversed the Al-mediated alleviation of Fe deficiency symptoms (Figs. 5 and 6). Second, Al treatment alone resulted in decreased P concentration in leaves compared to the control condition, irrespective of the supplied Fe concentration and

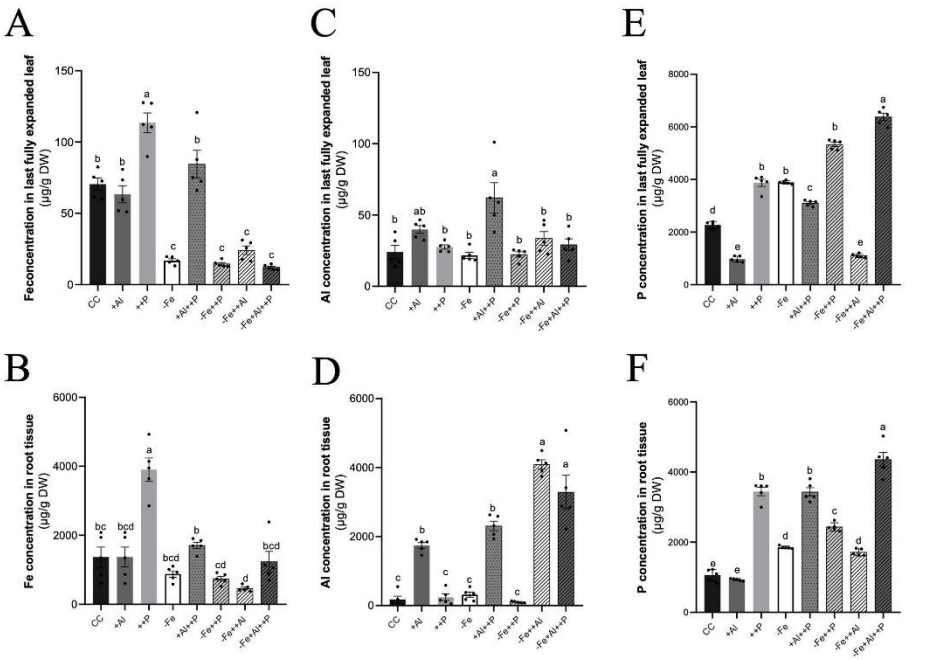


Fig. 7. Concentrations of Fe, P and Al in the last fully expanded leaf and roots of plants cultivated under control, +Al, ++P, −Fe, +Al++P, −Fe++P, −Fe + Al and −Fe + Al++P treatments. (A) Fe concentration in last fully expanded leaf. (B) Fe concentration in roots. (C) Al concentration in last fully expanded leaf. (D) Al concentration in roots. (E) P concentration in last fully expanded leaf. (F) P concentration in roots. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

even when excess P was supplied to the nutrient solution (Fig. 7E). Importantly, this effect was not observed in roots (Fig. 7F), suggesting that Al, despite being accumulated in roots (Fig. 7F), affects leaf P concentration. Third, *OsSPX1*, a P deficiency-responsive gene (Guo et al., 2022), was induced by Al (Fig. 4D). OsSPX1 is a negative regulator of OsPHR2, a transcription factor that controls P deficiency systemic responses, and is induced in response to low P as part of a negative feedback control mechanism (Gonçalves et al., 2020; Liu et al., 2010; Wang et al., 2014). Fe deficiency repressed *OsSPX1* expression whereas −Fe + Al treatment reestablished expression to the control level (Fig. 4D), again suggesting that Al induces P deficiency. Moreover, Fe deficiency markers were downregulated by Al, recapitulating what is observed under low P (Zheng et al., 2009). Fe and P are known to be antagonistic, with several players being oppositely regulated (Gonçalves et al., 2020). Taken together, our data indicate that we recapitulated −Fe−P phenotype by adding Al to the nutrient solution.

Distinct mechanisms could underlie the effects of Al/P deficiency on Fe deficiency-induced chlorosis. Pleiotropy between Al resistance and P efficiency is well-known (Magalhaes et al., 2018), with mechanisms of Al resistance clearly being involved in local P sensing and P deficiency response in roots, mainly through changes in root system architecture (Gutiérrez-Alanís et al., 2018). In *A. thaliana*, Al induces shortening of the primary root and increased lateral root density, and mutants for genes involved in P deficiency-associated changes in root system architecture are less sensitive to Al effects, suggesting common mechanisms are used for both responses (Ruíz-Herrera and López-Bucio, 2013). Both Al excess and P deficiency activate AtSTOP1 pathway to induce AtALMT1, which secretes malate to the rhizosphere (Balzergue et al., 2017; Tokizawa et al., 2015). Malate can bind to Al^{3+}, avoiding its detrimental effects, but also bind to Fe^{3+}, which might be abundant in acidic soils that are low in P, therefore increasing P availability (Abel, 2017). Interestingly, mobilization of Fe^{3+} is key for ROS generation and callose deposition, which in turn lead to cell wall stiffening and inhibited elongation that is typical of P deficiency in *A. thaliana* (Balzergue et al., 2017; Müller et al., 2015).

One plausible hypothesis to explain our observations is that Al directly and/or indirectly (i.e, by causing P deficiency) could stimulate organic acid secretion, increasing the mobilization of cell wall-bound Fe and, hence, decreasing chlorosis. Indeed, combined −Fe−P was shown to decrease Fe bound to root hemicellulose in *A. thaliana*, increasing Fe transfer to shoots (Li et al., 2023). However, in our experiments, Al did not significantly increase Fe concentration in leaves (Fig. 8A) that would be expected if Fe was being mobilized from roots and translocated to shoots. Similar results were observed for rice (Saenchai et al., 2016) and *A. thaliana* (Nam et al., 2021). Therefore, Al-mediated remobilization of Fe from root cell wall and transport to shoots may not be causing the alleviation of chlorosis.

Other mechanisms linking Fe and P homeostasis might be involved in the observed phenotype. In rice, it was shown that OsPHR2, a transcription factor that is a central regulator of systemic P sensing, works in

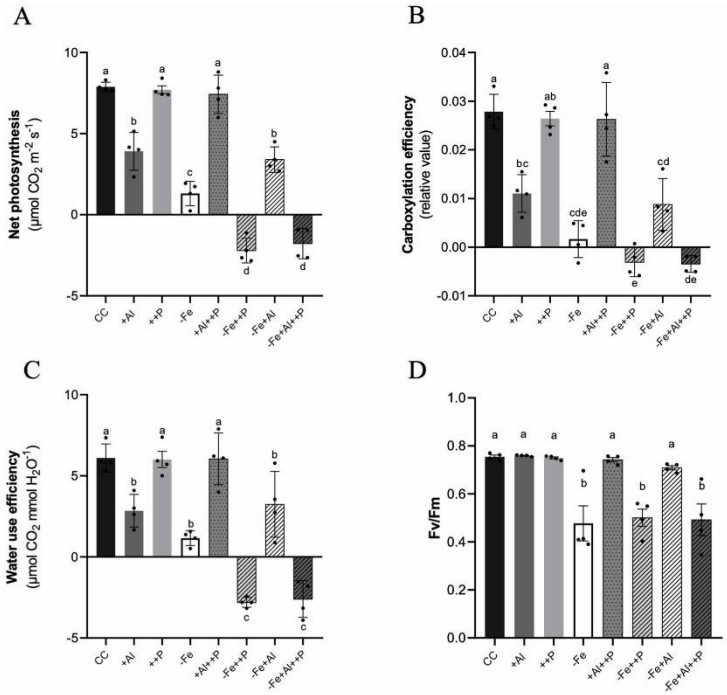


Fig. 8. Photosynthetic measurements from leaves of plants cultivated under control, +Al, ++P, −Fe, +Al++P, −Fe++P, −Fe + Al and −Fe + Al++P treatments. (A) Net photosynthesis. (B) Carboxylation efficiency. (C) Water use efficiency. (D) Fv/Fm. Different letters denote statistically significant values ($p \leq 0.05$) using ANOVA following by Tukey HSD.

a reciprocal inhibitory module with OsHRZ2, an E3 Ubiquitin Ligase that is an Fe sensor (Guo et al., 2022). OsPHR2 is stable at the protein level, but is ubiquitinated by OsHRZ2, which is up-regulated under Fe deficiency, partially explaining why P concentration decreases under low Fe. Interestingly, OsHRZ2 also ubiquitinates key components of the Fe uptake pathway as part of a negative feedback loop. OsPHR2 repressed *OsHRZ2* under P deficiency, increasing Fe uptake. Authors showed that in combined Fe and P deficiency OsPHR2 can transcriptionally down-regulate *OsHRZ2*, therefore allowing more efficient Fe acquisition, reducing chlorosis (Guo et al., 2022). If Al induces P deficiency through the same pathway, it is possible that the OsPHR2-OsHRZ2 module could be involved.

In *A. thaliana*, Fe deficiency induces reactive oxygen species formation in the chloroplasts, which act a retrograde signal to the nucleus for down-regulation of bZIP58, a transcription factor that positively regulates the expression of photosynthesis-related genes (Nam et al., 2021). P deficiency alleviates chlorosis by increasing ascorbate transport into chloroplasts, which scavenge Fe deficiency-induced reactive oxygen species, reestablishing bZIP58 expression and its downstream photosynthetic genes. Similar to our data, authors also observed no difference in Fe concentration in leaves comparing −Fe and −Fe−P (Nam et al., 2021). Although the existence of a homologous mechanism in rice is yet unknown, it will be interesting to test whether Al has the same effect in *A. thaliana* and whether the same pathway is involved. Moreover, it will also be important to test the possible role of the OsPHR2-OsHRZ2 module in the Al effect on Fe deficiency in the future.

Finally, our data also indicates that alleviation of chlorosis by Al occurs alongside toxic effects. Al-treated rice plants showed decreased root growth (Figs. 1 and 5) and decreased net photosynthesis (Fig. 8), which were not different from plants exposed to combined Al excess and Fe deficiency. The toxic effects of Al depend on concentration, pH and exposure time. In our work, we used 300 μM Al, which is lower than other works focusing on Al tolerance (450 μM) (Arenhart et al., 2013, 2014), but higher than the concentration in which only positive effects were observed (200 μM) (Moreno-Alvarado et al., 2017). Interestingly, Fv/Fm values were not altered by our 300 μM treatment alone, were clearly decreased by −Fe, and were similar to control in +Al−Fe plants (Fig. 8). It is possible to speculate that the Al effect on chlorosis might be independent of toxicity, and that subtoxic concentration could decrease chlorosis without major effects on plant growth, at least in a relatively tolerant crop such as rice. Moreover, it will be interesting to test whether Al-positive effects such as those observed for rice (Moreno-Alvarado et al., 2017) and other crops (Magalhaes et al., 2018; Ofoe et al., 2023) are due to mild P deficiency that could be priming plants to be more P efficient.

4. Conclusion

We described the Al alleviation effect on Fe deficiency chlorosis and showed evidence that Al is inducing P deficiency, which is known to cause chlorosis alleviation in several plants. This effect of Al suggests a new interaction between the three elements in rice, and opens the possibility to explore how Al might have beneficial effects in low concentration in rice and other plants by inducing low-grade P deficiency.

5. Materials and methods

5.1. Plant material and growth

Seeds of *Oryza sativa* L., cultivar IRGA 429, were imbibed in distilled water, sodium hypochlorite and liquid detergent to minimize contamination, and germinated at 25 °C in the dark for 24 h. Seeds were maintained for six days in Petri dishes with filter paper soaked in distilled water at 25 °C. After germination, plants were transferred to vermiculite for 10 days and irrigated with nutrient solution, as described (Ricachenevsky et al., 2011), until the third fully expanded leaf stage. Briefly, the solution consisted of 700 μM K_2SO_4, 100 μM KCl, 100 μM KH_2PO_4, 2 mM $Ca(NO_3)_2$, 500 μM $MgSO_4$, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 100 μM Fe^{3+}-EDTA, with a pH adjusted to 5.4. Then, plants were transferred to hydroponics 0.4 L plastic cups containing nutritive solution.

After eleven days of acclimation, plants were submitted to treatments: control, aluminum (Al) stress (300 μM $AlCl_3$ added to nutrient solution), Fe deficiency (no Fe added) and combined −Fe + Al. The nutrient solutions were changed every three days. Experiments with excess P corresponded to a concentration of 400 μM P in the solution (the control concentration was 100 μM). Experiments were conducted for 21 days.

5.2. Growth measurements

After 21 days of treatment root length and culm height were measured using a millimeter ruler. Shoots and roots were collected and dried in the oven at 65 °C until they reached constant mass. Dry matter was measured using a precision balance. Roots samples were suspended in distilled water thin layer of 0.5 cm, and scanned with an EPSON 11000 scanner at 600 dpi resolution. WinRHIZO© Pro 2007 software was used to measure total root system length.

5.3. Chlorophyll measurements

Leaf samples of plants exposed to treatments for 21 days were collected (n = 3) and chlorophyll *a* and chlorophyll *b* concentrations were analyzed as described (Schlindwein et al., 2006), with minor modifications. Rice leaves (50 mg) were pulverized in liquid nitrogen and homogenized with 1 mL of acetone/0.1 M tris buffer (80/20, v/v) (pH 7.8). Extracts were ultrasonicated in ice water for 15 min, placed in the dark at 4 °C for 24 h, and ultrasonicated for another 15 min. The extracts were centrifuged for 5 min at 10,000×*g*, and the supernatant was recovered and diluted with 1 mL of acetone/0.1 M tris buffer (1/3 ratio). Absorbances were measured using a SpectraMax M2 UV–Vis spectrophotometer at 470, 537, 647 and 663 nm. Total chlorophyll concentration, chlorophyll *a* and chlorophyll *b* ratio were estimated according to the equations proposed by (Sims and Gamon, 2002). Time-course measurements of relative leaf chlorophyll levels were performed with Soil Plant Analysis Development (SPAD-502, Minolta, Japan) every three or four days during our experiments to monitor Fe deficiency symptoms. In each experiment, n = 5 plants were used.

5.4. Determination of gas exchange and chlorophyll a fluorescence

We performed experiments using plants treated for seven and 21 days, as mentioned in the Results section. The CO_2 assimilation rate (A) and intercellular CO_2 partial pressure (C_i) were measured with a portable infrared gas analyzer (IRGA) system equipped with a red-blue LED source and a leaf chamber (LCpro-SD, ADC BioScientific, Hoddesdon, UK). Each sample comprised of the younger fully expanded leave from two different plants and each measurement was performed for 8 min per sample (n = 4). The internal parameters in the IRGA chamber during gas exchange measurements were 1000 μmol m^{-2} s^{-1} PPFD, 420 ppm CO_2, at 28 °C, and ambient relative humidity (≈75%). The water use efficiency (WUE) was calculated as A/transpiration and the carboxylation efficiency as A/C_i.

In vivo chlorophyll *a* fluorescence was measured using a chlorophyll fluorometer (OS-30p, Opti-Sciences, Hudson, NH, USA) following the saturation pulse method (Schreiber et al., 1995). The maximum quantum yield of photosystem II (Fv/Fm = (Fm-Fo)/Fm) was measured in the younger fully expanded leaves of 30-min-dark-adapted plants (n = 4). The Fm and Fo parameters correspond to the maximum and minimum fluorescence of dark-adapted leaves, respectively. The intensity and duration of the saturation light pulse were 3000 μmol m^{-2} s^{-1} and 0.7 s, respectively.

5.5. Gene expression analyses

For RT-qPCR analyses, plants were treated for seven days, and root samples (n = 3) were collected. Gene expression analyses were performed as described (Ricachenevsky et al., 2011). Samples were pulverized in liquid nitrogen and total RNA was extracted using Trizol Plant RNA Reagent (Invitrogen®), following the manufacturer's instructions. Quantification was performed Nanodrop®. cDNA synthesis was performed with Reverse Transcriptase MMLV (Invitrogen®), following the manufacturer's instructions, after treatment with DNAse I (Invitrogen®). Relative gene expression analyses were conducted in StepOne RealTimes PCR Systems (Applied Biosystems®, Foster City, CA, USA). Data was analyzed following the method by (Livak and Schmittgen, 2001). The OsUBQ5 gene (Jain et al., 2006) was used as a normalizer. Primers for all genes analyzed are listed in Supplementary Table 1.

5.6. Mineral element concentration

For mineral element analysis with ICP-MS, whole root or shoot samples were dried at 65 °C and weighted into polytetrafluoroethylene tubes. Samples were then digested with concentrated HNO_3 (67–69%; Bernd Kraft) and pressurized in a high-performance microwave reactor (UltraCLAVE IV, MLS GmbH). Digested samples were diluted with deionized water (Milli-Q Reference A+, Merck Millipore). Element analysis was carried out by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) (ELEMENT 2, Thermo Fisher Scientific, Germany). We analyzed the elements Fe, P, Al, zinc (Zn), manganese (Mn), copper (Cu), calcium (Ca), magnesium (Mg), sulphur (S) and potassium (K) (Supplementary Fig. 1).

5.7. Statistical analyses

The data were analyzed with analysis of variance (ANOVA), and the means were compared by Tukey HSD using SISVAR software (Ferreira, 2011).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.109427.

Data availability

Data will be made available on request.

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CAPÍTULO 2

ALUMINUM-INDUCED ALLEVIATION OF IRON DEFICIENCY IN MAIZE SUPPORTS MECHANISM CONSERVATION IN GRASSES

Aluminum-Induced Alleviation of Iron Deficiency in Maize Supports Mechanism Conservation in Grasses

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ABSTRACT

This study investigates the role of aluminum (Al) in alleviating iron (Fe) deficiency-induced chlorosis in maize (*Zea mays* L.), a phenomenon previously observed in rice (*Oryza sativa* L.). Using hydroponic experiments, we found that Al mitigates Fe deficiency-induced chlorosis in maize by maintaining chlorophyll content and photosynthetic efficiency, similar to its effects in rice. Under Fe-deficient conditions, Al treatment restored net photosynthesis, stomatal conductance, and carboxylation efficiency to levels comparable to control plants. Additionally, Al reduced root elongation independently of Fe deficiency, although root biomass remained unaffected. Our findings suggest that the mechanism through which Al alleviates Fe deficiency symptoms is conserved in *Poaceae* species, providing insights into potential strategies for improving nutrient efficiency in staple crops. This study also highlights the complex interactions between Al, Fe, and other nutrients, underlining the importance of understanding ionome balance in plant stress responses. Future research will focus on the molecular mechanisms underlying this process, including the expression of key Fe and phosphorus (P) homeostasis genes in maize.

Keywords: aluminum, iron, iron deficiency, maize, chlorosis, grasses.

Introduction

Maize (*Zea mays* L.) is a globally significant cereal crop, providing over 20% of the total caloric intake in numerous countries where it serves as a dietary staple (Ekpa *et al.* 2018; Santpoort 2020). With an annual cultivation area of approximately 197 million hectares worldwide, maize ranks as the second most widely grown crop globally, after wheat (Erenstein

et al. 2022). Its role extends beyond human consumption, contributing to livestock production cycles and serving as a raw material in various industries, such as biofuel and ethanol production, highlighting its versatility and essential role in ensuring global food security (Oikeh *et al.* 2003; Agarwal *et al.* 2019; Prasanna *et al.* 2020). Due to this extensive and heterogeneous cultivation range, maize is grown in a wide variety of soil environments. Such variability in soil types often results in complex stress related to mineral disorders as variations in the concentration of one element can impact the balance of others (Huang & Salt 2016; Ricachenevsky *et al.* 2018; Pita-Barbosa *et al.* 2019; DeLoose *et al.* 2024).

Approximately 40–50% of the world's potentially arable land is acidic, with aluminum (Al) toxicity being a major factor limiting crop productivity in these soils (von Uexküll & Mutert 1995; Kochian *et al.* 2015). In acidic conditions, Al exists in its soluble ionic form (Al^{+3}), which inhibits root elongation and reduces the absorption of water and essential nutrients, leading to stunted plant growth (Ma 2005; Ginocchio *et al.* 2009; Yamamoto 2019). Sensitive crops, such as maize, can experience growth impairment and decreased photosynthesis even at low Al concentrations (Peixoto *et al.* 2002; Wang *et al.* 2015). Al toxicity also triggers reduced leaf expansion, stomatal closure, and diminished photosynthetic efficiency, further impairing water transport and stomatal conductance (Yang *et al.* 2013; Ahmed *et al.* 2016; Silva *et al.* 2020). Strategies to mitigate these effects include breeding Al-tolerant cultivars, applying lime to neutralize soil acidity, and adopting sustainable soil management practices to improve soil quality (Rahman *et al.* 2018; Rahman & Upadhyaya 2021). As such, Al toxicity remains a critical barrier to achieving sustainable agriculture.

On the other hand, despite iron (Fe) being the fourth most abundant element, it is estimated that one-third of Earth's soils are deficient in this mineral (Mahender *et al.* 2019). The availability and solubility of Fe in soil are influenced by various factors, including soil pH, redox potential, microbial activity, organic matter content, and soil aeration (Becker & Asch 2005).

Fe is a key nutrient for plants, being involved in crucial functions such as electron transfer during photosynthesis and respiration, as well as in the metabolism of nitrogen and sulfur and the synthesis pathway of chlorophyll (Chen *et al.* 2018; Shi *et al.* 2018; Li *et al.* 2021). Due to its importance, plants have evolved specific mechanisms for Fe uptake, which are named Strategy I, or reduction strategy, common to all non-*Poaceae* species; and Strategy II, or chelation strategy, common to *Poaceae* (Römheld & Marschner 1986; Marschner & Römheld 1994). In the chelation strategy, when Fe levels are low, grasses produce and release Fe(III)-chelating molecules called phytosiderophores (PS), also referred to as mugineic acids. These PS in the rhizosphere chelate insoluble Fe, forming highly stable hexadentate Fe(III)-PS complexes that are taken up by root cells via Yellow Stripe transporters located on the plasma membrane (Römheld & Marschner 1986; von Wiren *et al.* 1994; Curie *et al.* 2001; Schaaf *et al.* 2004; Murata *et al.* 2006). In maize, the *ZmYS1* gene plays a crucial role in the efficient uptake of Fe(III)-PS complexes. Studies on *zmys1* mutants have shown that plants lacking functional ZmYS1 transporters exhibit interveinal chlorosis, a typical symptom of Fe deficiency, highlighting the importance of this mechanism in Fe acquisition (Curie *et al.* 2001). Among the main symptoms occurring due to the Fe deficiency, the interveinal chlorosis in young leaves is considered one of the major symptoms, as Fe plays a vital role in photosynthesis. Affected leaves turn yellow, while the veins remain green, highlighting the impact of the Fe deficiency. (Chen *et al.* 2018; Shi *et al.* 2018; Mahender *et al.* 2019).

The uptake and homoeostasis of Fe can be negatively impacted by other nutrients (Fageria *et al.* 2008). While plants under Fe deficiency alone typically develop chlorosis, plants subjected to combined Fe and phosphorus (P) deficiencies remain green and do not exhibit chlorosis (Nam *et al.* 2021). This same response has been observed in species like rice, barley, tomato, *Lemma gibba* and cyanobacteria (Saenchai *et al.* 2016; Guo *et al.* 2022; Tiziani *et al.* 2023; Qiu *et al.* 2024). Understanding how the concentration of one element influences the

overall ionome balance is therefore crucial. For example, Al resistance may have pleiotropic effects on P acquisition through modifications in root system architecture, mediated by both organic acid (OA) transporters and uptake transporters. These changes in root system architecture involve an Fe-dependent interplay between Al resistance and enhanced P uptake (Magalhaes *et al.* 2018).

Our previous studies demonstrated that Al alleviates Fe deficiency-induced chlorosis in rice (*Oryza sativa* L.), reducing the expression of Fe deficiency marker genes, partially restores photosynthesis, and indirectly induces P deficiency, which reverses the effects of Al when excess P is supplied (Alves *et al.* 2024). Here, we show that a similar phenomenon occurs in maize, another *Poaceae* species. Fe deficiency in maize typically leads to interveinal chlorosis in young leaves, and the addition of Al mitigates these symptoms, suggesting that the mechanisms through which Al alleviates Fe deficiency in rice may be conserved among grasses. This aligns with our goal of understanding whether these mechanisms operate similarly in other important cereal crops.

Results

Al alleviates Fe deficiency-induced chlorosis in maize leaves

To explore the potential effects of Al excess on the response to Fe deficiency in maize, we performed hydroponic experiments comparing plants under control (100 µM Fe), +Al (300 µM Al), -Fe (no Fe added) and combined treatment (300 µM Al + no Fe added – hereafter – Fe+Al). After 9 days of treatment, aluminum alone (+Al) did not induced any visible symptoms in the shoots. In contrast, plants subjected to -Fe treatment displayed characteristic chlorosis associated with Fe deficiency (Fig. 1A). Interestingly, plants in -Fe+Al group did not exhibit

chlorosis, resembling control and +Al treatments. This observation indicates a potential role of Al in mitigating chlorosis under Fe-deficient conditions (Fig. 1A).

To further investigate this hypothesis, we analyzed SPAD values, an indirect indicator of chlorophyll content, in leaves of plants under control, +Al, -Fe and -Fe+Al treatments (Fig 1B). Plants grown under control, +Al, and -Fe+Al conditions had similar SPAD values, reflecting higher chlorophyll content and absence of chlorosis. In contrast, -Fe plants had significantly lower SPAD values, confirming the chlorotic phenotype observed (Fig 1A and 1B). Together, these findings demonstrate that Al alleviates chlorosis induced by Fe deficiency.

Maize plants under +Al treatment showed a significant reduction in both the number of leaves and shoot dry mass compared to control conditions, demonstrating clear sensitivity to Al. However, plants under -Fe and -Fe+Al treatments exhibited values for both parameters that were different to the control (Fig. 1C and 1D). Taken together, these results suggest that Al reduces shoot dry biomass and number of leaves in maize but mitigates Fe deficiency-induced chlorosis.

Al reduces maize root length independently of Fe deficiency without affecting root biomass

Root length was significantly affected by Al excess. Both +Al and -Fe+Al treatments resulted in a reduction in root length compared to the control. Notably, plants under -Fe conditions alone showed no statistical difference from the control, maintaining root length similar to non-stressed plants (Figure 2A and 2B). These findings suggest that Al excess reduces root elongation independently of Fe deficiency, as the effect is observed in the absence or in presence of -Fe conditions. Despite the evident phenotype and the clear reduction in root length observed under Al treatments, no statistical differences were observed in root dry mass among the treatments (Fig. 2A and 2C). This result indicates that the overall biomass allocation to roots was maintained, even when Al-induced root shortening occurred.

All mitigates Fe Deficiency-Induced reduction in Photosynthesis in Maize.

Considering that we observed Al alleviates chlorosis in maize (Fig 1A and 1B), we investigate how photosynthesis was affected. After 6 days of treatment, we measured net photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and carboxylation efficiency. Plants in +Al reduced net photosynthesis compared to control conditions. However, -Fe resulted in greater reduction. Interestingly, the combined -Fe+Al treatment did not statistically differ from the control, indicating that Al alleviated the negative effects of Fe deficiency on net photosynthesis (Fig. 3A). The stomatal conductance was affected by +Al and -Fe stress separately, showing a reduction compared to the control. However, when tested under combined -Fe+Al conditions, the observed stomatal conductance values were stastistically similar to control (Fig 3B), closely mirroring the pattern observed for net photosynthesis (Fig. 3A). This observation suggests that the presence of Al under Fe-deficient conditions mitigates the impact of Fe deficiency on stomatal function, paralleling its effect on net photosynthesis. Carboxylation efficiency further supported these findings, with -Fe treatment being the only group to differ significantly from the control (Fig. 3C). This reduction indicates decreased photosynthesis due to reduced carbon fixation under Fe-deficient conditions. Importantly, +Al and -Fe+Al treatments showed no significant differences from the control, implying that Al, even under Fe deficiency, helped sustain carboxylation efficiency at levels comparable to control plants. Analysis of internal CO_2 concentration reinforced these interpretations (Fig 3D). Only the -Fe treatment displayed significantly higher internal CO_2 concentration values compared to the control, reflecting an accumulation of CO_2 due to impaired carbon fixation. In contrast, the +Al and -Fe+Al treatments exhibited values similar to the control, indicating that the presence of Al under Fe-deficient conditions restored the plant's capacity for effective carbon assimilation. Together, these findings demonstrate that Al plays a

crucial role in mitigating the negative effects of Fe deficiency on photosynthesis. While -Fe conditions severely impacted net photosynthesis, stomatal conductance, carboxylation efficiency, and CO_2 assimilation, the addition of Al (-Fe+Al) effectively alleviated these disruptions, maintaining photosynthetic performance comparable to the one observed in control conditions.

Discussion

The findings presented here build upon our previous study with rice (Alves *et al.* 2024) by demonstrating that Al can also alleviate Fe deficiency-induced chlorosis in maize, another important crop from *Poaceae* species. This evidence strengthens the hypothesis of a conserved mechanism among grasses where Al mitigates the phonotype and functional symptoms of Fe deficiency (Fig. 1, 2 and 3). In maize, as observed in rice (Alves *et al.* 2024), the addition of Al under Fe-deficient conditions reversed the characteristic chlorosis and restored photosynthetic efficiency. The alleviation effect was evident both in maintenance of chlorophyll content (Fig. 1) and in photosynthetic parameters (Fig. 3), as addition of Al under Fe-deficient conditions restored net photosynthesis rates, stomatal conductance, and carboxylation efficiency to levels comparable to Fe-sufficient controls, suggesting that similar regulatory networks underlie the interplay between Fe and Al across *Poaceae* species.

The mechanisms underlying Al role in alleviating Fe deficiency symptoms remain an open question. In rice, Alves *et al.* (2024) suggested that Al alleviates Fe deficiency-induced chlorosis indirectly, potentially driven by its ability to induce P deficiency in leaves, which mitigates the symptoms of Fe deficiency. Al could be indirectly alleviating Fe deficiency symptoms by modulating P homeostasis, a well-documented interaction in plants, several studies have documented the phenotype of non-chlorotic plants under combined Fe and P

deficiencies (Saenchai *et al.* 2016; Nam *et al.* 2021; Guo *et al.* 2022; Tiziani *et al.* 2023; Qiu *et al.* 2024).

To further contextualize these findings, it is noteworthy that rice exhibits significantly higher Al tolerance compared to maize and other cereals, with levels 2- to 6-fold greater across varying Al concentration (Famoso *et al.* 2010). Our results demonstrate that 300 mM Al significantly reduced root length in both maize (Fig. 3) and rice (Alves *et al.* 2024). This aligns with findings by (Famoso *et al.* 2010), who reported that Al-induced reductions in maize occurred at much lower concentrations. However, their study also showed that rice growth was unaffected at concentrations up to 160 mM Al. The use of 300 mM Al in our study provides a robust framework to assess comparative tolerance, as this concentration clearly exceeds thresholds previously established for maize while effectively challenging rice's known tolerance to Al stress.

In terms of root development, Al reduced root length in both +Al and -Fe+Al treatments, indicating that this effect occurs independently of Fe status. Despite this reduction, root dry mass was unaffected, suggesting that biomass allocation to roots remained stable under Al stress (Fig. 2). Studies indicate that Al disrupts root elongation by binding to negatively charged carboxyl groups in pectins, leading to increased rigidity of the cell wall and reduced extensibility, as observed in maize (Eticha *et al.* 2005; Doncheva *et al.* 2005; Delhaize *et al.* 2007). Furthermore, Al interferes with cellular processes by accumulating in the root apoplast, affecting the division and structure of root cells, and inducing callose deposition, which blocks essential solute transport, such as indole-3-acetic acid (IAA), required for root growth (Sivaguru *et al.* 2000). Studies on maize have demonstrated that low exposure, such 48 mM Al, inhibited root growth (Wang *et al.* 2015). Here we used 300 mM Al, which collaborates and justifies the reduction we found in plants treated with Al.

Al is widely recognized as a toxic element that limits plant growth, particularly in acid soils where its bioavailability increases (Kochian *et al.* 2015). However, our findings highlight a dual role for Al in maize, demonstrating that it can alleviate Fe deficiency-induced chlorosis while also exerting inhibitory effects on root elongation and shoot biomass. Despite its well-documented toxicity, mainly in the root system (Kochian *et al.* 2015; Chauhan *et al.* 2021; Hajiboland *et al.* 2023), beneficial effects of Al have been observed in certain plants under specifics conditions (Moreno-Alvarado *et al.* 2017; Bojórquez-Quintal *et al.* 2017; Muhammad *et al.* 2019; Sun *et al.* 2020; Ofoe *et al.* 2023). Therefore, these findings emphasize the complex ionomic interactions in plants, where the availability of one nutrient can significantly influence the physiological roles of others (Ricachenevsky *et al.* 2018).

To further investigate the underlying mechanisms, future studies will focus on the expression of key Fe and P homeostasis genes, such as *ZmYS1*, alongside the maize homologous genes *OsIRO2*, *OsYSL15*, and OsNRAMP1, previously analyzed in rice. Comparisons with the molecular dynamics observed in rice (Alves *et al.* 2024) could provide insights into the conservation and divergence of these mechanisms within grasses, offering novel strategies for enhancing nutrient efficiency in staple crops.

Conclusion

This study demonstrates that Al alleviates Fe deficiency-induced chlorosis in maize, a phenomenon previously observed in rice, thus supporting the hypothesis of conserved mechanisms among grasses.

Materials and Methods

Plant Material and Growth

Biomatrix hybrid maize seeds – VTPRO3 were sterilized with distilled water, sodium hypochlorite and liquid detergent to minimize contamination, and germinated at 25ºC in the dark for 24 hours. Seeds were germinated for five days in gerbox with filter paper soaked in distilled water at 25 °C. After germination, plants were transferred to vermiculite and cultivated in nutrient solution, as described (Ricachenevsky *et al.* 2011; Wairich *et al.* 2019) until the third leaf expands. Then, plants were transferred to 2L plant pots containing nutritive solution and aerated by air pumps. Each experimental unit contained 15 plants.

After three days of acclimatation, plants, were submitted to the following treatments: control, Al toxicity (+Al; 300 µM $AlCl_3$ added to nutrient solution), Fe deficiency (-Fe; no Fe added) and combined Fe deficiency + Al toxicity (-Fe+Al). Plants were grown in a growth room at 26 °C ± 1 °C under photoperiod of 16 h/8 h light/dark (150 µmol.m^{-2}.s^{-1}). The pH was set to 4.5, and solutions were change every three days.

Growth measurements

After nine days of treatment root length was measured using a millimeter ruler (n=4). Shoots and roots were collected and dried in the oven at 65ºC until they reached constant mass. Dry matter was measured using a precision balance (n=7). For number of leaves was used n =10.

Chlorophyll measurements

After nine days of treatment, measurements of relative leaf chlorophyll level were performed with Soil Plant Analysis Development (SPAD-502, Minolta, Japan; n=4).

Determination of gas exchange parameters

The CO_2 assimilation rate (A), stomatal conductance (g_s) and intercellular CO_2 partial pressure (C_i) were measured with a portable infrared gas analyzer (IRGA) system equipped with a red-blue LED source and a leaf chamber (LCpro-SD, ADC BioScientific, Hoddesdon, UK). Each sample comprised the younger fully expanded leave and each measurement was performed for 15 min per sample (n = 4). The internal parameters in the IRGA chamber during gas exchange measurements were 1,000 μmol m^{-2} s^{-1} PPFD (Photosynthetic Photon Flux Density), 420 ppm CO_2, at 28 °C, and ambient relative humidity (≈ 75%). The carboxylation efficiency was calculated as A/C_i.

Statistical analyses

Mean values were compared by the Student's *t* test ($p \leq 0.05$) using the GraphPad Prism 7 (GraphPad Software) for Windows.

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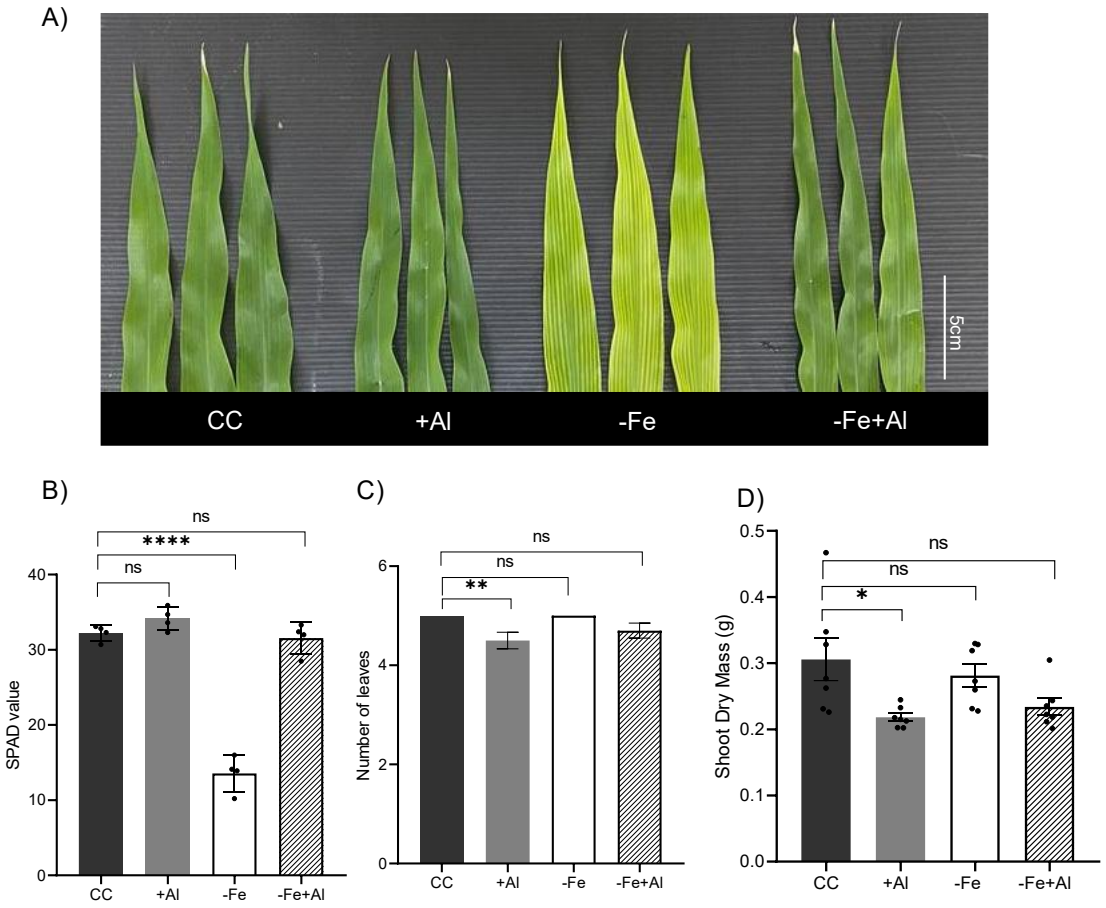


Figure 1 – Leaf phenotype and growth parameters of maize plants cultivated under control, +Al, -Fe and -Fe+Al treatments. (A) Leaf phenotype. (B) SPAD values from leaves (n=4). (C) Number of leaves (n=10). (D) Shoot dry mass (n=7). Asterisks indicate statistical difference between plants grown under CC and different treatments (Student *t*-test, *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001).

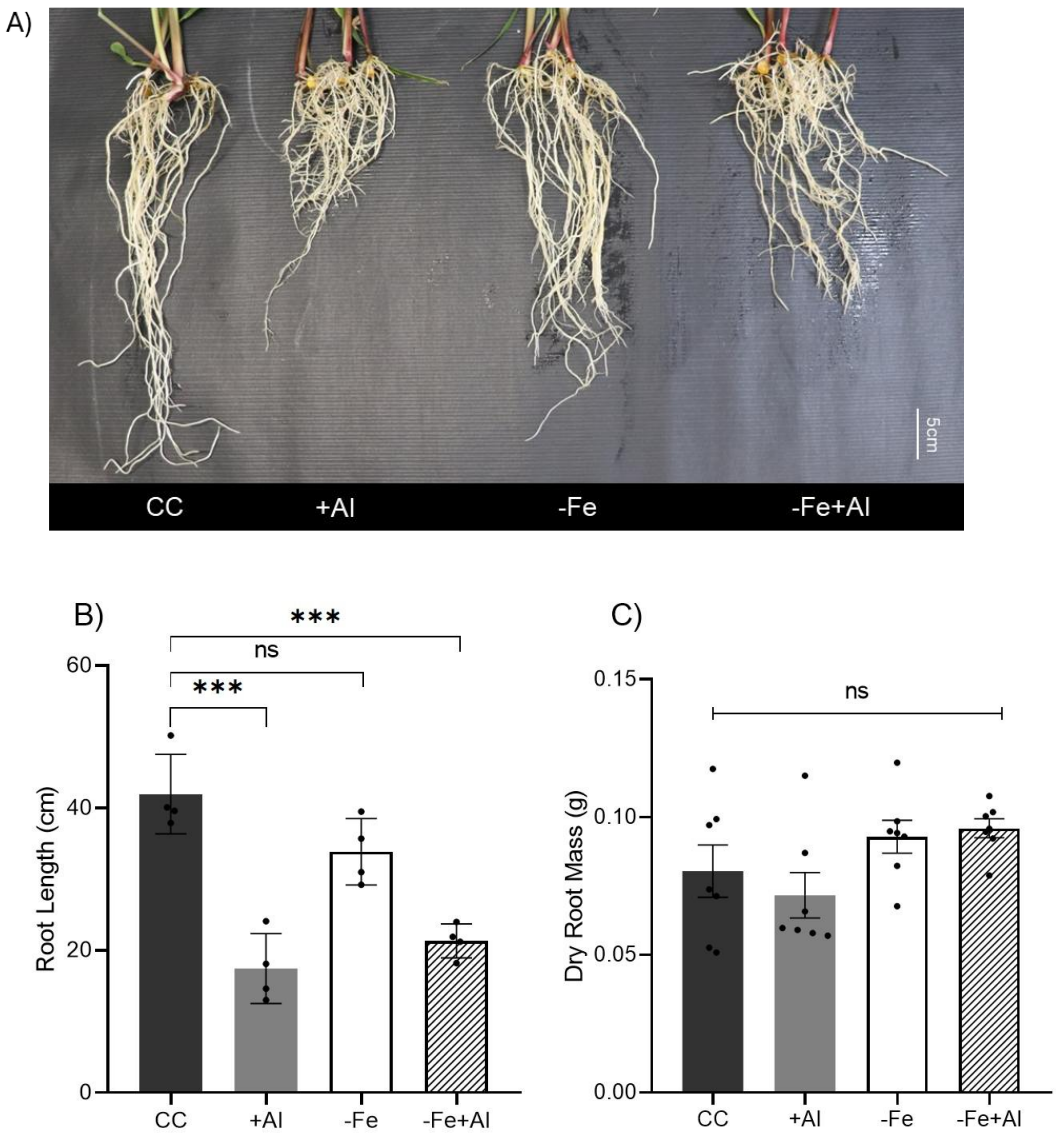


Figure 2 – Root phenotype and growth of maize plants cultivated under control, +Al, -Fe and -Fe+Al treatments. (A) Root phenotypes. (B) Root length (n=4). (C) Root dry mass (n=7). Asterisks indicate statistical difference between plants grown under CC and different treatments (Student *t*-test, *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001).

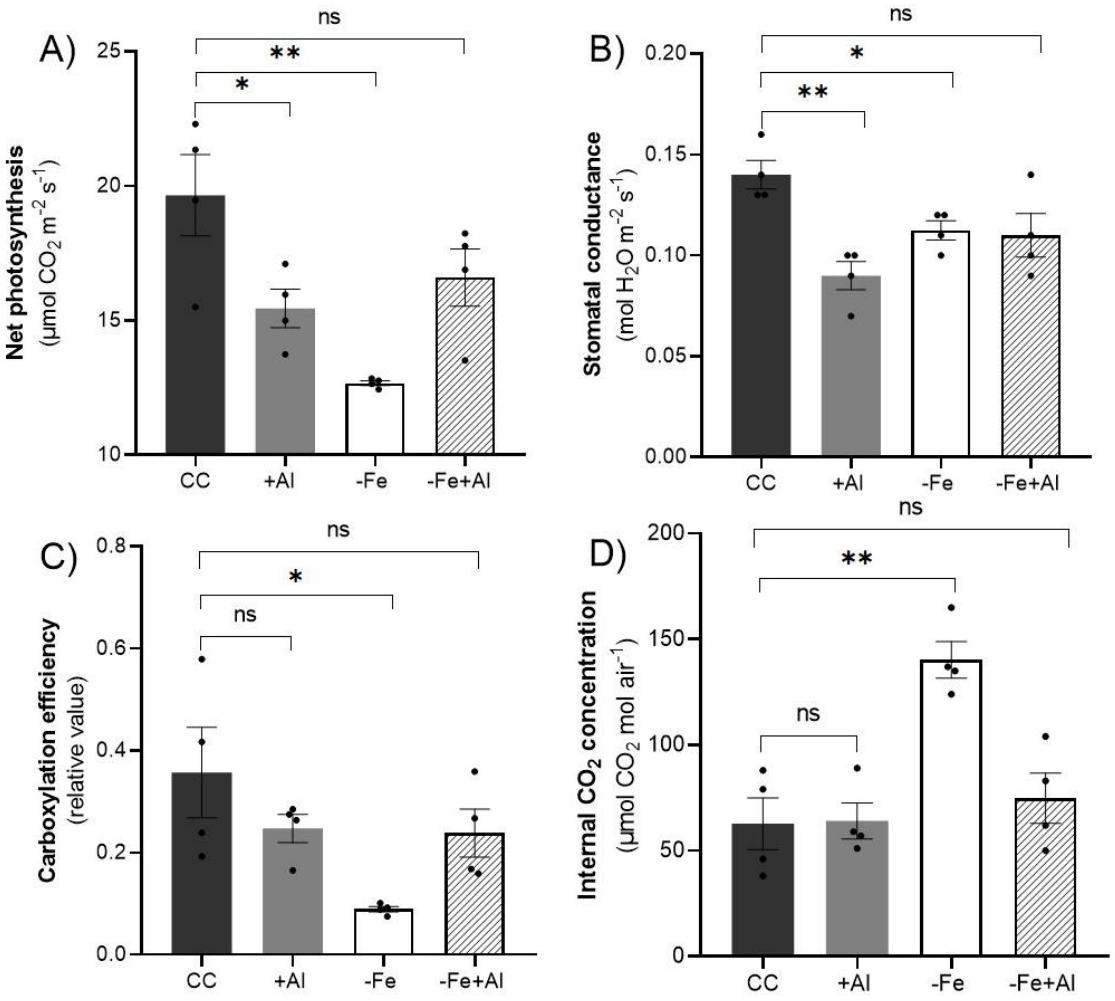


Figure 3 - Photosynthetic measurements from leaves of plants cultivated under control, +Al, −Fe and −Fe+Al treatments. (A) Net Photosynthesis. (B) Stomatal conductance. (C) Internal CO_2 concentration in leaf Cells. (D) Carboxylation efficiency (n=4). Asterisks indicate statistical difference between plants grown under CC and different treatments (Student *t*-test, *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001, ****P-value < 0.0001).

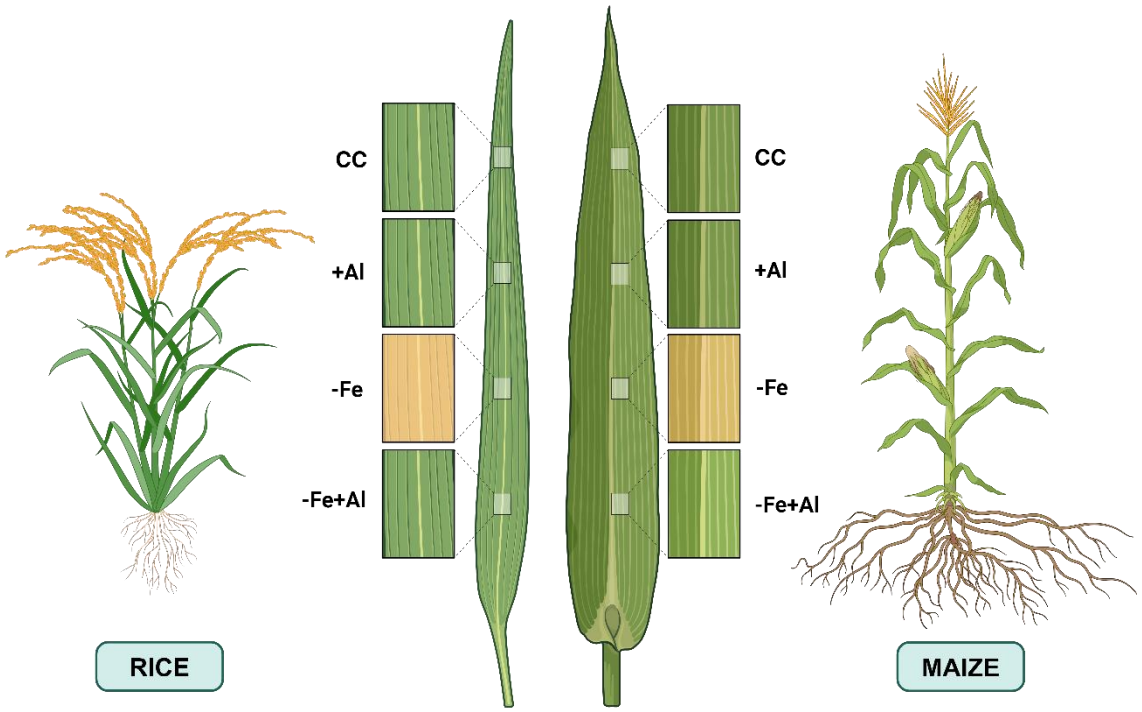


Figure 4 - Aluminum-induced alleviation of iron deficiency-induced chlorosis in rice and maize provides support for conserved mechanisms in grasses. Created using: https://BioRender.com

DISCUSSÃO GERAL

Este estudo expande as evidências sobre os efeitos do Al em plantas, demonstrando que ele pode aliviar a clorose causada por deficiência de Fe não apenas em arroz (Alves *et al.* 2024). mas também em milho, uma cultura essencial da família Poaceae. A adição de Al sob condições de deficiência de Fe aliviou a clorose, restaurando os níveis de clorofila e parâmetros fotossintéticos, como taxas de fotossíntese e eficiência de carboxilação, para valores próximos aos de plantas com suprimento adequado de Fe. Esses resultados sugerem que mecanismos conservados podem modular a interação entre Fe e Al em gramíneas

Embora o Al seja amplamente reconhecido como tóxico em solos ácidos (Kochian *et al.* 2015b), ele também apresenta efeitos benéficos sob condições específicas (Moreno-Alvarado *et al.* 2017; Ofoe *et al.* 2023). Nos capítulos dessa tese, mostramos que o Al alivia os sintomas de deficiência de Fe sem alterar significativamente as concentrações de Fe nas folhas, o que pode ser um mecanismo indireto relacionado à homeostase de P. Em arroz, foi proposto que o Al induz deficiência de P, o que alivia os sintomas de deficiência de Fe (Alves *et al.* 2024). Essa interação entre Fe e P é bem documentada em plantas, com fenótipo de alívio da clorose similares observados sob deficiências combinadas desses nutrientes (Nam *et al.* 2021b; Guo *et al.* 2022b; Tiziani *et al.* 2023b; Qiu *et al.* 2024).

No entanto, o Al também apresentou efeitos adversos, como redução do crescimento radicular em ambas as espécies estudadas. Em milho, a concentração de 300 µM de Al reduziu significativamente o comprimento das raízes, corroborando estudos que atribuem essa redução à interação do Al com componentes da parede celular, como pectinas, aumentando a rigidez e dificultando o alongamento (Eticha, Stass, *et al.* 2005; Delhaize *et al.* 2007b). Ademais, o Al pode interferir no transporte de solutos essenciais, como o ácido indol-3-acético (IAA), necessário para o crescimento radicular (Sivaguru *et al.* 2000).

Nossos achados destacam o papel dual do Al, que pode tanto limitar o crescimento radicular quanto aliviar sintomas de deficiência de Fe. Efeitos benéficos foram relatados em outras espécies sob condições específicas (Sun *et al.* 2020; Ofoe *et al.* 2023), evidenciando as complexas interações iônicas entre nutrientes e suas funções fisiológicas (Ricachenevsky *et al.* 2018). Estudos futuros devem focar na expressão de genes relacionados à homeostase de Fe e P no milho, como ZmYS1, e em genes homólogos ao OsIRO2, OsYSL15 e OsNRAMP1 em arroz. Além disso, pretendemos verificar se tal fenômeno é compartilhado por espécies de plantas não-gramíneas.

ANEXOS

ARTIGO CIENTÍFICO SUBMETIDO AO PERIÓDICO PLANT PHYSIOLOGY AND BIOCHEMISTRY (2025)

Plant Physiology and Biochemistry

Abscisic Acid/Stress/Ripening Proteins Play a Role in Iron (Fe) Homeostasis in Rice (Oryza sativa L.)

--Manuscript Draft--

Manuscript Number:	PLAPHY-D-25-00309
Article Type:	VSI:Plant stress responses: Research Paper (for only Brazil authors)
Section/Category:	heavy metal stress; ion transport
Keywords:	iron; abscisic acid/stress/ripening; chlorosis; rice; ionome

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Selenium does not ease growth inhibition caused by aluminum in *Schinus terebinthifolius* seedlings

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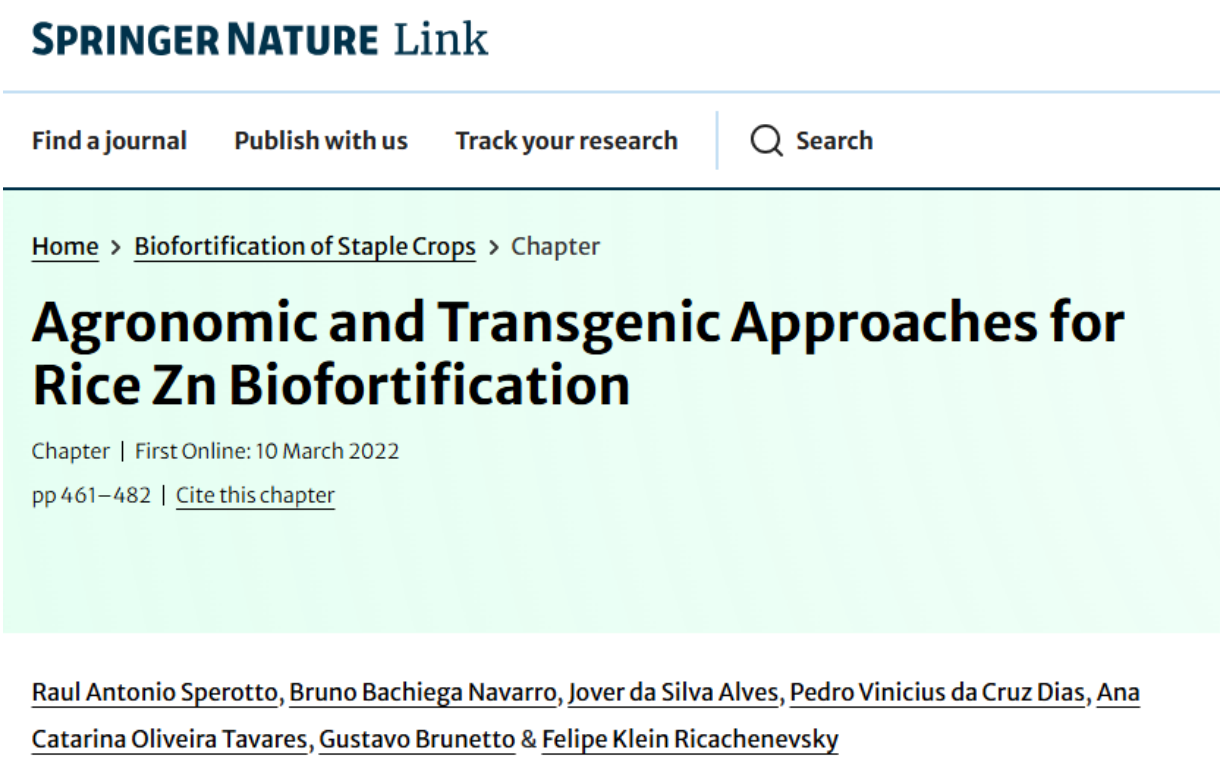
ABSTRACT

Aluminum (Al) toxicity is observed in several crops in the world and affects the yield of many cultures. Using beneficial elements such as selenium (Se) has been the object of studies focused on finding an alternative to mitigate the toxic effects of metals on crops. Thus, the aims of the current study were to assess the tolerance/sensitivity to Al, and Se mitigating effects on morphological, photosynthetic and biochemical variables recorded for *Schinus terebinthifolius* seedlings exposed to Al. *Schinus terebinthifolius* seedlings in hydroponics were subjected to the following Al and Se combinations added to the nutrient solution in mM: 0 Al + 0 Se; 0 Al + 0.0025 Se; 1.85 Al + 0 Se; 1.85 Al + 0.0025 Se; 3.71 Al + 0 Se and 3.71 Al + 2.5 Se. The study followed the completely randomized experimental design, with three repetitions. Al reduced the leaf area, shoot and root dry mass, root increment, as well as root length, surface area, volume and diameter. Seedlings' photosynthetic activity decreased possibly due to the presence of Al in them; and even when Se was present, it was not able to mitigate the effects of Al. The enzymatic and non-enzymatic antioxidant system was not able to avoid the oxidative stress caused by the presence of Al, as well as the Se was not able to minimize the effects from different Al concentrations on *S. terebinthifolius* seedlings. Therefore, species *S. terebinthifolius* is sensitive to Al in hydroponic systems and Se addition, in general, did not mitigate the toxic effects from different Al concentrations.

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KEYWORDS
Antioxidant system; metal toxicity; morphophysiological variables; oxidative stress

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Programa de Pós-Graduação em Biologia Celular e Molecular

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Curso: BIOLOGIA CELULAR E MOLECULAR - Doutorado
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Situação: ATIVO
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Período	Código	Atividade de Ensino/Professor	Créditos	Carga Horária	Conceito	Frequência (%)	Situação	Instituição
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2024/02	BCM10301	Seminário do Centro de Biotecnologia - 60 horas	4	60	-	-	Matriculado	UFRGS
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2021/01	BCM13045	Biologia Molecular e Funcional de Plantas Professor: Professor: ARTHUR GERMANO FETT NETO Titulação: Doutorado Professor: Professor: FELIPE KLEIN RICACHENEVSKY Titulação: Doutorado Professor: Professor: JANETTE PALMA FETT Titulação: Doutorado	4	60	B	100.00	Aprovado	UFRGS
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Universidade Federal do Rio Grande do Sul
Programa de Pós-Graduação em Biologia Celular e Molecular

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