MOISÉS ALVES DE SOUZA

MECANISMOS FOTOSSINTÉTICOS EM CULTIVARES DE ARROZ SOB EXCESSO DE FERRO E ONDAS DE CALOR

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Agronomia/Fisiologia Vegetal, área de concentração em Fisiologia Vegetal, para obtenção de título de Doutor.

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PHOTOSYNTHETIC MECHANISMS IN RICE CULTIVARS UNDER IRON EXCESS AND HEATWAVES

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APROVADA em 16 de março de 2023.
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Agradece a Deus pela vida, saúde, proteção. Dedico a minha filha Maysa, aos meus pais Maria Genilda e José João pelo apoio em todos as etapas.

AGRADECIMENTOS

À Deus, que é Poderoso para fazer infinitamente mais do que pedi ou pensei.

Minha princesinha, minha vida, meu pacotinho de felicidade, minha filha, Maysa, amor maior que inspira cada passo meu.

Aos meus pais, José João e Maria Genilda, pela segurança de seus passos sempre guiando os meus, pela crença nos meus sonhos e pelo amor e educação que me deram e que fez com que esse momento fosse possível.

A minha irmã Marília, e minha sobrinha Milena por me proporcionarem sempre os melhores momentos e serem luz na minha vida.

A meu orientador, professor Eduardo Gusmão, por ter me acolhido no momento que mais precisei, pela amizade e principalmente por ter feito todo o possível para que eu sempre trabalhasse com entusiasmo. Seu amor à ciência e à docência que me inspira todos os dias a ser uma profissional melhor. Obrigado pelos ensinamentos que me fizeram amadurecer, pelo carinho e pela paciência. Serei sempre grata por tudo e vou levar os seus ensinamentos para vida.

Aos meus amigos Marina, Talita, Karina, Luziene e Paulo, minha família em Florestal, aqueles que me abrigaram quando cheguei, que me apoiaram nos momentos mais difíceis dessa jornada e que ficam felizes com a minha felicidade. Obrigado pela amizade, cumplicidade e a capacidade de transformar lágrimas em sorrisos. Obrigado por tudo!

Aos meus amigos de laboratório Isadora, Leticia e Iuly, pela grande ajuda durante a execução do experimento.

Aos meus amigos Marcos Jose e Lissa Izabel, pela grande ajuda durante a execução do experimento.

A Rosy e Nalva, por terem sido as grandes incentivadoras dessa caminhada, pela amizade e por tudo que têm feito por mim.

Ao Laboratório de Fisiologia do Estresse Abiótico pela realização dos experimentos e contribuição para o meu amadurecimento profissional

Universidade Federal de Lavras – UFLA e Universidade Federal de Viçosa – UFV Campus-Florestal, pela oportunidade de realização deste trabalho, e aos seus professores pela contribuição à minha formação profissional.

À Pós-Graduação em Agronomia/Fisiologia Vegetal, por toda contribuição na minha formação.

A Prof. Dra. Elisa Bicalho pelos bons conselhos e por ter permitido que eu realizasse parte deste trabalho no Laboratório Crescimento e Desenvolvimento de Plantas

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa.

A todos que direta ou indiretamente contribuíram para conclusão desta etapa.

Muito obrigado!

RESUMO

As cultivares de arroz (*Oriza sativa* L.) diferem quanto a sua tolerância ao excesso de ferro (Fe). Nas cultivares sensíveis, a toxidez por excesso de Fe pode causar aumento da produção de espécies reativas de oxigênio, redução na taxa de assimilação de carbono, redução na eficiência quântica do fotossistema II e concomitantemente a incapacidade de suprimir o excesso de energia por dissipação não fotoquímica. A eficiência hídrica, a utilização de energia luminosa e os mecanismos de fotoproteção nas cultivares de arroz, podem variar de acordo com as concentrações de Fe, ou entre a interação do excesso de Fe com aumento de temperatura. Desta forma, o objetivo do trabalho é compreender como as cultivares de arroz contrastantes na tolerância ao excesso de Fe são afetadas pelas limitações fotoquímicas, bioquímicas ou estomáticas, e a eficiência fotossintética em temperaturas mais elevadas. Nas cultivares de arroz contrastante, as concentrações de Fe e o tempo de exposição ao Fe, foram essenciais para determinar a sensibilidade e tolerância. A cultivar tolerante IRGA 424 apresentou maior eficiência fotossintética com maior tempo de exposição ao Fe e não alterou seus parâmetros fotossintéticos, além de apresentar mecanismos fotoprotetores como extinção não fotoquímica e fotorrespiração. A cultivar sensível IRGA 417, apresentou maior dano oxidativo, redução nos rendimentos quânticos e eficiências do PSII, e nos fluxos específicos de energia do PSII, no qual aumentou os processos fotoinibitórios. As concentrações de Fe reduziram o fluxo de seiva do xilema e o potencial hídrico foliar. A exposição das cultivares ao aumento de temperatura, resultou em maior concentração de Fe na parte aérea da cultivar tolerante e menor taxa de carboxilação da rubisco em ambas as cultivares. A limitação estomática ocorreu apenas como resposta tardia, reduzindo o acúmulo de Fe com o aumento da temperatura, na cultivar sensível IRGA 417. A ativação da fotorrespiração como dreno de elétrons sob excesso de Fe, também foi eficiente com o aumento da temperatura durante as ondas de calor, e impediu maiores danos à membrana. A sensibilidade à toxicidade do ferro e ao estresse térmico está associada à dissipação não fotoquímica ineficiente. Assim, a compressão dos eventos envolvidos no papel do Fe e no aumento da temperatura irá facilitar o desenvolvimento de novas cultivares tolerantes às alterações climáticas.

Palavras-chave: *Oryza sativa.* Toxidez de ferro. Stress térmico. Fotoinibição

ABSTRACT

Rice cultivars differ in their tolerance to excess iron (Fe). In sensitive cultivars, Fe excess toxicity can cause increased production of reactive oxygen species, reduced carbon assimilation rate, reduced quantum efficiency of photosystem II, and concomitant inability to suppress excess energy by non-photochemical dissipation. The water and light energy use efficiency, and photoprotection mechanisms in rice cultivars may vary according to Fe concentrations, or between the interaction of excess Fe with increasing temperature. Thus, the objective of the work is to understand how rice cultivars contrasting in tolerance to Fe excess are affected by photochemical, biochemical, or stomatal limitations, and photosynthetic efficiency at higher temperatures. In the contrasting rice cultivars, Fe concentrations and Fe exposure time were essential to determine sensitivity and tolerance. The tolerant cultivar IRGA 424 showed higher photosynthetic efficiency with longer exposure time to Fe and did not alter its photosynthetic parameters, and showed photoprotective mechanisms such as non-photochemical extinction and photorespiration. The sensitive cultivar IRGA 417 showed greater oxidative damage, reduced quantum yields and efficiencies, and specific energy fluxes of PSII, in which increased photoinhibitory processes. Fe concentrations reduced xylem sap flow and leaf water potential. Exposure of the cultivars to increased temperature, resulted in higher Fe concentration in the aerial part of the tolerant cultivar and lower carboxylation rate of rubisco in both cultivars. Stomatal limitation occurred only as a late response, reducing Fe accumulation with increasing temperature in the sensitive cultivar IRGA 417. Activation of photorespiration as an electron drain under excess Fe was also efficient with increasing temperature during heat waves and prevented further membrane damage. Sensitivity to iron toxicity and heat stress is associated with inefficient non-photochemical dissipation. Thus, compression of the events involved in the role of Fe and temperature increase will facilitate the development of new cultivars tolerant to climate change.

Keywords: *Oryza sativa*. Iron toxicity. Heat stress. Photoinhibition

SUMÁRIO

PRIMEIRA PARTE

1- INTRODUÇÃO GERAL

O arroz (*Oryza sativa* L.) é um dos cereais mais importantes do mundo devido à sua relevância como um dos alimentos básicos, sendo fonte significativa de ingestão calórica diária para mais da metade da população mundial. As cultivares de arroz também se destacam pela grande variabilidade genética que diferem amplamente em relação à capacidade de tolerar o excesso de ferro (Fe) (MAHENDER et al., 2019). A toxidez por excesso de Fe é o estresse nutricional mais difundido que afeta a produção de arroz em solos alagados. Ocorre como consequência das altas quantidades de Fe redutível, baixo pH e baixa capacidade de troca catiônica (DOBERMANN & FAIRHURST, 2000; BECKER & ASCH, 2005).

A toxidez por excesso de Fe pode afetar diferentes aspectos fisiológicos da planta. Nos tecidos vegetais, pode levar ao aumento do estresse oxidativo, uma vez que aumenta a produção de espécies reativas de oxigênio (EROs). Dentre as EROs, pode ocorrer a formação do radical hidroxila ($HO^{\bullet}$), por meio da reação do Fe^{2+} com o peróxido de hidrogênio (H_2O_2) através da reação de Fenton (FANG et al., 2001; MAJERUS et al. 2007; KOBAYASHI & NISHIZAWA, 2012). O Fe^{2+} pode ainda reagir com o oxigênio e formar radical superóxido ($O_2^{\bullet -}$), e juntamente com outras EROs levar a danos na estrutura das membranas celulares, modificando suas atividades enzimáticas e resultando na perda da permeabilidade seletiva, com consequente extravasamento de eletrólitos. Isso afeta a maioria dos processos metabólicos, incluindo a diminuição do transporte de elétrons na etapa fotoquímica, a redução na taxa de assimilação fotossintética de carbono, dentre outros (NENOVA, 2009; PEREIRA et al., 2013).

O cultivo de arroz em condições de excesso de Fe merece uma atenção especial principalmente em regiões tropicais e subtropicais, que estão propensas ao aumento de temperaturas provocando ondas de calor. Com o aquecimento global as ondas de calor serão mais frequentes e duradouras (XU et al., 2018). As ondas de calor aumentam a expressão de genes para proteases intracloroplásticas que perturbam a estrutura e função do cloroplasto por meio da degradação de proteínas (SINVANY-VILLALOBO et al., 2004). O estresse térmico também é prejudicial ao complexo de desenvolvimento de oxigênio do fotossistema II (PSII) (MURATA et al., 2007). O mecanismo de reparo do PSII é inibido pela produção de EROs com as ondas de calor, levando ao aumento da fotoinibição (ALLAKHVERDIEV et al., 2008). No entanto, cultivares de arroz, com tolerância contrastante ao Fe, pode apresentar diferentes mecanismos fotoprotetores que previnam ou reparem os efeitos deletérios da fotoinibição.

Os efeitos do aumento de temperatura em combinação como excesso de Fe no aparato fotossintético de cultivares de arroz contrastantes quanto à sensibilidade ao metal, ainda não são bem compreendidos. Entretanto, sabe-se que o comprometimento da fotossíntese em cultivares sensíveis ao excesso de Fe está relacionada a limitações não estomáticas, desencadeadas por danos oxidativos e incapacidade de suprir o excesso de energia por dissipação não fotoquímica (PINTO et al., 2016). Nas limitações estomáticas, os mecanismos que explicam como o Fe induzem o fechamento estomático, permanecem desconhecidos. Entretanto, a redução do teor de água, em condições excessivas de Fe, pode estar relacionada à redução da absorção de água na raiz, devido aos danos oxidativos. Consequentemente, o potencial hídrico e os danos oxidativos provocam o fechamento dos estômatos, causando alterações na limitação difusiva.

Mais informações sobre como as cultivares de arroz contrastantes ao excesso de Fe são afetadas pelas limitações fotoquímicas, bioquímicas ou estomáticas, e a modulação da fotossíntese em decorrência das ondas de calor são fundamentais para novas estratégias de manipulação genética visando o aumento de resistência a fatores abióticos. Desta forma, o objetivo geral do trabalho é elucidar quais as causas das limitações fotossintéticas (bioquímica, fotoquímica e difusiva) que são determinantes nas cultivares de arroz contrastantes, quanto à sensibilidade ao excesso de ferro, e como ocorre a modulação fotossintética em decorrência da fotoinibição e ondas de calor nestas cultivares de arroz.

2- REFERENCIAL TEÓRICO

2.1- Mecanismos de absorção e tolerância ao excesso de ferro em arroz

O ferro (Fe) é um nutriente essencial em diversos processos fisiológicos nas plantas, devido à sua capacidade de mudança no estado de oxidação-redução. É requerido em diversos processos enzimáticos durante o desenvolvimento das plantas, como na biossíntese da clorofila, nos processos fotossintéticos, na respiração e na fixação do N_2 atmosférico (JEONG & CONNOLLY, 2009; KOBAYASHI & NISHIZAWA, 2012). No entanto, a toxidez por excesso de Fe em plantas pode ser observada naturalmente em solos ácidos constantemente alagados. A toxidez por ferro é o principal fator abiótico que limita a produtividade de arroz em cultivo alagado (DOBERMANN & FAIRHURST, 2000).

O arroz apresenta duas estratégias para absorção de Fe (ISHIMARU et al., 2006) (Figura 1). A primeira estratégia, comum entre eudicotiledôneas e monocotiledôneas não-gramíneas, envolve a acidificação do solo pela extrusão de prótons, aumentando a solubilidade do Fe^{3+} e subsequente a sua redução à Fe^{2+} pela redutase férrica da membrana

plasmática e absorção para o citosol radicular, via proteína da família de transportadores de metais ZIP (SANTI et al., 2005; SANTI & SCHMIDT, 2009).

A segunda estratégia, utilizada pelas gramíneas em geral, consiste na produção e liberação de fitossideróforos (FS) que quelam o Fe^{3+} na rizosfera, sendo o complexo Fe^{3+}-fitossideróforo absorvido pelo transportador específico nas raízes (HELL & STEPHAN, 2003). Os FS são compostos de baixo peso molecular, com alta afinidade para o Fe e para outros metais. Os FS pertencem à família do ácido muginéico (MA) e são sintetizados através da via S-adenosil-L-metionina. Esta via inclui três reações enzimáticas sequenciais mediadas pela nicotianamina sintase (NAS), nicotianamina aminotransferase (NAAT) e ácido desoximugineico sintase (DMAS), gerando ácido 2'-desoximugineico (DMA), o precursor de todos os outros MAs.

Especialmente sob condições de estresse, estabelecendo ligações com o Fe oxidado, forma que prevalece em condições de aerobiose existente nos solos aráveis. No arroz, a libertação dos FS para a rizosfera é mediada por vários transportadores de ácido muginéico, cuja ação é regulada pela disponibilidade de Fe (NOZOYE et al., 2015). A formação destes complexos Fe^{3+}- FS aumenta o movimento de Fe na solução do solo (UENO et al., 2007).

Figura 1 - Estratégias de absorção de ferro pelas raízes de plantas de arroz. Ácido muginéico (MA); NA, nicotianamina; SAM, S -adenosil-l-metionina; fitosideróforos (PS).
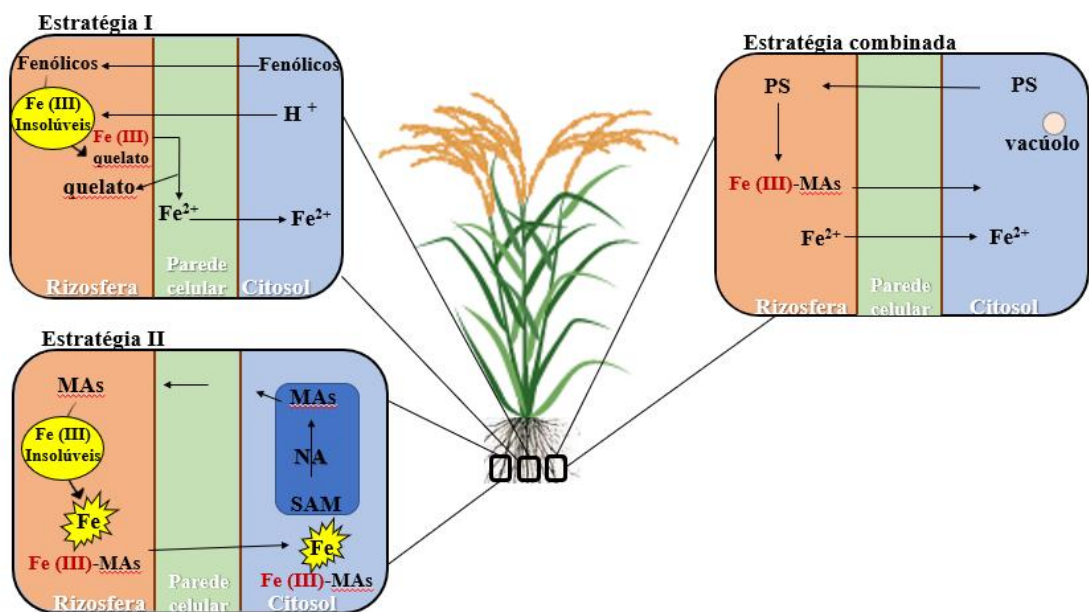
Fonte: adaptado de SANTOS et al., 2017

Uma vez absorvido pela raiz, a translocação de Fe nas plantas envolve várias etapas, incluindo o transporte radial através dos tecidos da raiz, que compreende também o transporte simplástico para passar pelas estrias de Caspary; carregamento na seiva do xilema na forma de complexos com ácidos orgânicos, especialmente citrato; movimento simplástico em direção ao local da demanda; e/ou retranslocação a partir de origem ou tecido senescente (KIM & GUERINOT, 2007). Uma vez no mesofilo, via fluxo transpiratório (CURIE & BRIAT, 2003), o Fe pode ser armazenado nos vacúolos ou imobilizado pela proteína ferritina, a qual ocorre principalmente nos plastídios (ZANCANI et al., 2004).

Genótipos de arroz variam muito em seu mecanismo de resistência à toxidez de Fe, e o uso de cultivares tolerantes é uma das estratégias eficazes para a prevenção da perda do rendimento. As cultivares de arroz podem apresentar diferentes estratégias de resistência ao excesso de Fe: (I) evitando a absorção do Fe pelo sistema radicular; (II) aumentando a tolerância, com absorção de Fe e posterior compartimentalização em proteínas de armazenamento de Fe, como ferritinas, ou em vacúolos; ou associada a mecanismos de reparo aos danos atribuídos às EROs, e indução dos sistemas antioxidantes (MAJERUS et al., 2007; WU et al., 2014; SIQUEIRA-SILVA et al., 2019).

Em relação à evitação radicular, nas raízes das plantas de arroz geralmente ocorre a formação de uma placa de ferro, servindo como mecanismo de exclusão pois pode limitar a absorção do excesso de Fe (SNOWDEN & WHEELER, 1995; DENG et al., 2009; PEREIRA et al., 2014).

Os sistemas antioxidantes incluem componentes enzimáticos e não enzimáticos. Os antioxidantes enzimáticos incluem superóxido dismutase (SOD), a catalase (CAT) e a peroxidase do ascorbato (APX) (MAJERUS et al., 2007; SAIKIA & BARUAH, 2012). Mecanismos não enzimáticos podem neutralizar espécies reativas de oxigênio através de compostos orgânicos, como glutationa reduzida, α-tocoferol, ácido ascórbico e carotenoides (SMIRNOFF, 2005). Dentre os componentes não enzimáticos, o ascorbato e a glutationa são os antioxidantes hidrossolúveis mais abundantes, além de atuarem no ciclo ascorbato-glutationa. O ácido ascórbico é responsável pela eliminação de H_2O_2, $^1O_2$ e $O_2^{\bullet-}$, ainda inibe reações desencadeadas por EROs, protegendo os lipídios de membrana (NOCTOR & FOYER, 1998).

As EROs contribuem para a produção OH• através da chamada reação de Fenton com o envolvimento do catalisador Fe^{2+}, que ocorre excessivamente sob condições tóxicas de Fe

(BECANA et al., 1998). As EROs, especialmente OH•, pode oxidar lipídios, proteínas e DNA, e assim, causar necrose. No arroz, a toxicidade de Fe leva aos sintomas visíveis como bronzeamento foliar, que ocorrem junto com reduções de crescimento de brotações e raízes (PONNAMPERUMA et al., 1955; WU et al., 2014).

A compartimentalização em organelas ou proteínas específicas é um passo importante no metabolismo de minerais. O armazenamento de ferro em plantas de arroz pode ocorrer nos apoplastos, vacúolos e plastídeos e dentro da cavidade central da proteína especializada conhecida como ferritina (MAJERUS et al., 2009; SILVEIRA et al., 2009; STEIN et al., 2009; BRIAT et al., 2010).

As EROs mais importante são os ânions superóxido ($O_2^{\bullet-}$), os radicais perhidroxila ($HO_2^{\bullet}$), peróxido de hidrogênio (H_2O_2), radical hidroxila ($HO^{\bullet}$) e o oxigênio singleto ($^1O_2$). O $O_2^{\bullet-}$, H_2O_2 e $HO^{\bullet}$ são produzidos como resultado do transporte de elétrons, enquanto o $^1O_2$ é produzido como resultado da transferência de energia de excitação (ASADA, 1999). O $^1O_2$ é produzido pela clorofila triplet na transferência de energia para o oxigênio molecular no complexo de antenas PSII ou pela recombinação do par radical separado de carga no centro de reação PSII (POSPÍŠIL, 2016). Em nível de PSII pode ocorrer a formação do $O_2^{\bullet-}$ e do $OH^{\bullet}$ (POSPÍŠIL & PRASAD, 2014) (Figura 2).

Em casos graves de toxidez por Fe, plantas de arroz apresentaram redução do teor relativo de água, do índice de clorofila, aumento da resistência estomática, e da concentração de Fe nas raízes e na parte aérea (DUFEY et al., 2009; PEREIRA et al., 2013). O fechamento estomático, em reposta ao excesso de Fe, pode variar de acordo com o genótipo, sendo relatado como um mecanismo tardio de resposta, após uma alta concentração de ferro ter sido atingida no tecido foliar, uma vez que as plantas mais sensíveis apresentaram maior resistência estomática e alta concentração de Fe (DUFEYET et al., 2009; PINTO et a., 2016).

2.2- Aspectos limitantes do metabolismo fotossintético sob excesso de ferro e fotoinibição

Sabe-se que a fotossíntese do arroz é afetada pelo excesso de ferro através de um aumento inicial na resistência estomática, diminuindo a concentração de CO_2 no mesofilo e através da limitação não estomática, devido ao comprometimento bioquímico e fotoquímico após estresse severo (PEREIRA et al., 2013). Estudos com diferentes cultivares de arroz relatam que a toxicidade por Fe diminui a eficiência quântica máxima do fotossistema II (PSII) (DUFEY et al., 2009; PEREIRA et al. 2013; PINTO et al., 2016).

Como os processos fotossintéticos estão inteiramente interligados, de acordo com o modelo fotossintético de Farquhar et al. (1980), a redução da capacidade de transporte de elétrons, devido à limitação no PSII, pode levar a uma diminuição na capacidade de regeneração de ribulose-1,5-bifosfato (RuBP). A limitação na condutância mesofílica (g_m), também é um dos fatores responsáveis pela redução da fotossíntese. A g_m regula o fluxo de CO_2 a partir dos espaços intercelulares (C_i) para o sítio de carboxilação da Rubisco no estroma dos cloroplastos (C_c) (GRASSI et al., 2005). A taxa de transporte de elétrons ($J_{máx}$) apresenta relação com as respostas da condutância estomática (g_s), em função de condições de luz ou CO_2 (MARTINS et al., 2014). A inibição do ciclo de Calvin-Benson e a redução na taxa de transporte de elétrons cloroplastídica, pela imposição do excesso de Fe, nas plantas de arroz, pode estar associada à diminuição no transporte de elétrons atribuídos às reações da carboxilase (J_c) da Rubisco.

Plantas de arroz cultivadas sob excesso de Fe apresentam incremento no transporte de elétrons atribuído às reações da oxigenase (J_o) da Rubisco, o que demonstra que a fotorrespiração pode ser o principal dreno de elétrons no processo fotossintético em resposta à toxidez por excesso de Fe (PEREIRA et al., 2013). A fotorrespiração é resultado da atividade oxigenativa da Rubisco, observada principalmente em plantas C3, que, em excesso, acarreta em perdas significativas de CO_2 dependente de luz (OGREN, 1984). Dentre outros fatores, as taxas fotorrespiratórias aumentam quando a relação CO_2/O_2 diminui (MURAOKA et al., 2000).

Os efeitos no aparato fotossintético do arroz sob excesso de Fe, podem resultar em danos fotoxidativos e causar a fotoinibição (PINTO et al., 2016). A fotoinibição do fotossistema II (PSII) ocorre quando a taxa de inativação induzida pela luz (fotodano) excede a taxa de reparo (MURATA et al., 2012). A inibição do reparo está associada à superprodução de EROs, uma vez que o centro de reação do PSII, o fotossistema I (PSI) e o complexo coletor de luz (LHCII) do PSII, são principais locais na geração de espécies reativas de oxigênios (PINNOLA & BASSI, 2018).

Estressores abióticos, como o excesso de Fe, podem resultar em estresse luminoso (GURURANI et al., 2015). Sob excesso de luz, a produção de EROs no PSII está associada à transferência de energia e o transporte de elétrons. A limitação na transferência de energia ocorre quando o excesso de energia absorvido pela clorofila no complexo de antenas PSII não é totalmente utilizado no centro de reação do PSII. Isso pode resultar no dano à proteína D1, de ligação ao centro de reação do PSII. A proteína D1 danificada é removida por proteases específicas e substituída por uma nova cópia produzida através de uma nova síntese.

Entretanto, quando a intensidade da luz se torna excessiva, ou em função da combinação com outros estressores, como altas temperaturas, ocorre agregação irreversível de D1 e o seu reparo é impedido. Uma vez que os produtos agregados se acumulam nos complexos do PSII, é difícil removê-los por proteases e ocorre uma fotoinibição irreversível.

Figura 2 - Esquema de produção de EROs e fotoinibição nos PSII e PSI. Setas vermelhas representam as vias ativadas quando o fluxo de fótons excede a capacidade de transporte de elétrons e assimilação de CO$_2$.
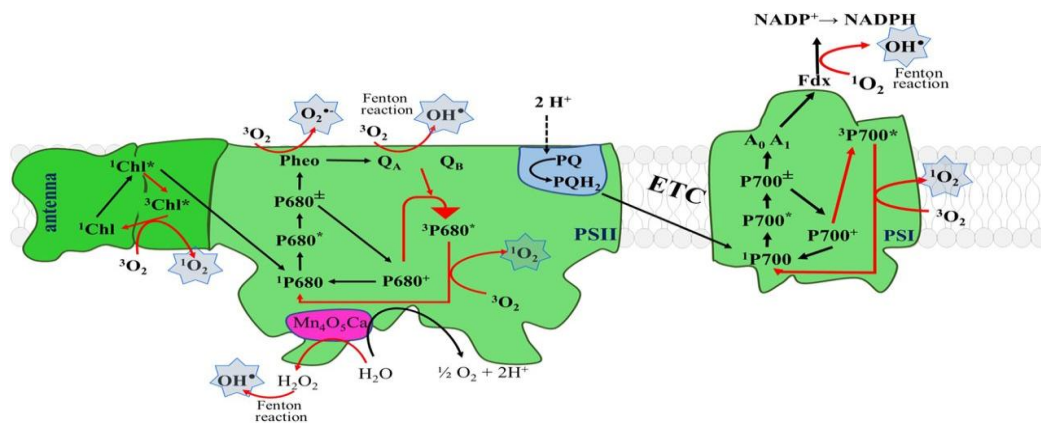
Fonte: PINNOLA & BASSI (2018)

As plantas apresentam diferentes mecanismos fotoprotetores que permite a redução na produção de EROs e, assim, evitar a fotoinibição (PINNOLA & BASSI, 2018). A dissipação do excesso de energia luminosa inclui várias alternativas como o movimento foliar, redução do tamanho dos complexos antena, ativação do estado de transição e ciclo das xantofilas (JALEEL et al., 2009).

Dentre os mecanismos fotoprotetores localizados na membrana do tilacoide, a dissipação do excesso de energia de excitação como calor é conhecida como quenching não fotoquímico (NPQ) (ROCHAIX, 2011; CAZZANIGA et al., 2013; SPETEA et al., 2014). O NPQ é um mecanismo importante em plantas que protege os centros de reação do PSII de danos por meio da dissipação do excesso de energia luminosa na forma de calor. O principal componente do NPQ, o quenching dependente de energia (q_E), é desencadeado por uma diminuição do pH dentro do lúmen do tilacoide, induzindo a interconversão de pigmentos xantofilas específicas que estão principalmente ligados às proteínas do LHC (MÜLLER et al., 2001).

O NPQ é comprometido pelo aumento das EROs, produzidas por altas concentrações de Fe em cultivares de arroz. O comprometimento dos pigmentos da antena, indicado pelo aumento da fluorescência mínima, em um estado aclimatado à luz (F_0) e menor conteúdo de clorofila, também pode ter influência no comprometimento do NPQ, uma vez que um sistema de antena PSII perfeitamente organizado e funcional é necessário para o acúmulo máximo de NPQ (LOKSTEIN et al., 2002; PEREIRA et al., 2014)

2.3- Impactos fisiológicos do estresse térmico causado pelo aumento de temperatura

A temperatura elevada compromete a forma e a função da membrana plasmática, altera a proporção de ácidos graxos saturados para insaturados e causa desnaturação de proteínas, resultando em maior fluidez e permeabilidade, afeta integridade da membrana e maior vazamento de íons orgânicos e inorgânicos das células (NIU et al., 2017; ZHU et al., 2017; HIGASHI et al., 2019). Portanto, o valor de vazamento de eletrólito serve como um indicador de danos à membrana e reflete mudanças induzidas por estresse e tem sido usado para avaliar a termoestabilidade da membrana sob condições de estresse de alta temperatura (LIU & HUANG, 2000). A temperatura mais alta reduz a taxa fotossintética, diminuindo os teores de clorofila e nitrogênio nas folhas.

A fotossíntese é muito sensível ao aumento de temperatura, sendo o componente mais sensível o fotossistema II (WANG et al., 2017). A alta temperatura resulta na destruição da permeabilidade da membrana do tilacoide, ou mesmo na desintegração do grana do tilacoide e desencadeia uma diminuição no teor de clorofila, levando a alterações das reações fotoquímicas com redução na proporção de fluorescência variável para fluorescência máxima (F_v/F_m). A inativação pelo aumento de temperatura do PSII é principalmente devido à dissociação do complexo de evolução do oxigênio (CEO) no PSII, resultando na inibição do transporte de elétrons do CEO para o lado aceptor do PSII. (SAILAJA et al., 2015, WANG et al., 2018).

Temperaturas acima do ideal para a funcionalidade típica da planta interrompem a fixação fotossintética do CO_2. A atividade da ribulose-1,5-bifosfato carboxilase/oxigenase (Rubisco) é inibida (PERDOMO et al., 2017). Essa interrupção da fotossíntese comumente ocorre com o início precoce e acelerada senescência foliar, que resulta da degradação da clorofila causada por EROs (KHANNA-CHOPRA, 2012; JAJIC et al., 2015), A redução na fixação de CO_2, sob estresse térmico, deve-se à baixa produção de ATP e NADPH a partir da fotoquímica, bem como à diminuição da concentração intercelular de CO_2 (WANG et al., 2017). As atividades das principais enzimas fotossintéticas, como anidrase carbônica, Rubisco, Rubisco Ativase (RCA) e fosforibuloquinase são afetadas pelo estresse térmico.

Além da ativação de Rubisco, a atividade de transporte de elétrons, causa redução na regeneração da Rubisco, com o aumento de temperatura (SCHRADER et al., 2004, YAMORI et al., 2008, CARMO-SILVA et al., 2011).

O campo mais importante para estudos futuros é o desenvolvimento de cultivares tolerantes ao aumento de temperaturas. A identificação das características fisiológicas e bioquímicas das cultivares de arroz contrastante à toxidez por excesso de Fe, quando submetidas ao aumento de temperaturas, serão etapas essenciais para o melhoramento da termotolerância. O desenvolvimento de novas variedades é demorado e caro; portanto, a compreensão dos mecanismos de tolerância ao calor facilitaria o desenvolvimento de estratégias para triagem de germoplasma das cultivares de arroz quanto a características relacionadas à tolerância ao calor.

Sob excesso de Fe e altas temperaturas o metabolismo fotossintético é limitado pela difusão do CO_2 até a carboxilação, e pelo transporte de elétrons na fotoquímica para a regeneração da Rubisco. Nessas condições ambientais, cultivares contrastante de arroz podem apresentar diferentes mecanismos de fotoproteção como: dissipação do excesso de energia (NPQ), fotorrespiração (R_l), restrição na condutância estomática (g_s) e respiração (R_d). Esses mecanismos serão essenciais para manter a eficiência fotossintética.

Para desvendar esses mecanismos em plantas de arroz, foram realizados diversos ensaios que são descritos em dois manuscritos. No manuscrito 1, foram avaliadas a tolerância e sensibilidade das cultivares quanto ao tempo de exposição e às concentrações de Fe, e como as diferentes concentrações de Fe afetam as limitações fotoquímicas, bioquímicas e estomáticas. No manuscrito 2, foram avaliadas a eficiência fotossintética das duas cultivares na interação entre o excesso de Fe e o aumento de temperaturas, e a eficiências dos mecanismos de fotoproteção na interação dos fatores.

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SEGUNDA PARTE – MANUSCRITOS

ARTIGO 1: Contrasting diffusive and photochemical limitations in rice cultivars with different levels of sensitivity to excess iron.

Manuscrito nas normas do periódico Theoretical and Experimental Plant Physiology (Fator de impacto: 2.656)

ARTIGO 2: Photoprotective mechanisms and higher photorespiration are key points for iron stress tolerance under heatwaves in rice plants.

Manuscrito nas normas do periódico Plant Cell and Environment.
(Fator de impacto: 7.947)

Contrasting diffusive and photochemical limitations in rice cultivars with different levels of sensitivity to excess iron

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ABSTRACT

Toxicity due to excess iron can result in oxidative stress, impacting photosynthetic processes, especially those related to the electron transport chain and CO_2 assimilation. However, the limitations of photosynthesis in rice cultivars may vary according to the concentration and time of exposure to iron and the tolerance level of the cultivars. The present study investigated the relationship between oxidative damage caused by excess iron and the photosynthetic efficiency of two contrasting rice cultivars regarding iron sensitivity. Two rice cultivars, IRGA 424 (tolerant to excess iron) and IRGA 417 (sensitive to excess iron), reached the V6 growth stage when they were grown in four concentrations of Fe^{2+} (0.019 control, 2, 4, and 7 mM) in nutrient solution for eight days. The concentrations and time of exposure to Fe were essential to determining the sensitivity and tolerance of both cultivars. Fe concentrations reduced Ψ_{leaf} and xylem sap flow in both cultivars. The tolerant cultivar IRGA 424 showed increased photosynthetic efficiency with a longer exposure time and did not change its photosynthetic parameters up to 2 mM of Fe. While the sensitive cultivar IRGA 417 experienced greater oxidative damage, which reduced the quantum yields and specific efficiencies and fluxes of PSII, increasing photoinhibitory processes. Photoprotective mechanisms such as non-photochemical quenching, photorespiration and dark respiration were more efficient in the tolerant cultivar IRGA 424 than in the sensitive cultivar with increased Fe concentrations. These mechanisms will improve the development of tolerant cultivars and increase the productivity of rice in soils with low pH values and excess Fe.

Keywords: *Oryza sativa*, iron toxicity, photosynthetic limitation, chlorophyll *a* fluorescence.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population (Yang et al. 2008). Improving rice cultivars is crucial for ensuring global food security, and there is an urgent need for high-yield phenotypes and improvements to the mechanistic understanding of plant responses to environmental limitations (Pleban et al. 2020). Iron toxicity is one of the main limitations affecting floodplain rice production (Audebert and Fofana 2009). Excess Fe in cells impairs biological processes and leads to leaf bronzing symptoms as a result of cell death. Leaf bronzing induced by Fe toxicity is closely associated with loss of yield (Wu et al. 2014).

In flooded soils, Fe^{2+} is rapidly absorbed by plant roots and can lead to Fe cellular overload, inducing numerous metabolic disorders and causing a reduction in the main photosynthetic processes (Briat and Lobréaux 1997). Although Fe is an essential micronutrient involved in various aspects of plant metabolism (photosynthesis, respiration, and chlorophyll synthesis) (Kar and Panda 2018), excess Fe can result in the potentiation of oxidative stress, with increased production of reactive oxygen species (ROS) by the Fenton reaction (Kobayashi and Nishizawa 2012).

At the same time, through genetic improvement, rice cultivars have different levels of tolerance and sensitivity to Fe concentrations (Fageria and Rabelo 1987; Sahrawat et al. 1996). This tolerance to excess Fe in rice cultivars is a complex mechanism that is related to the control of oxidative damage (Pinto et al. 2016). Excess Fe toxicity in sensitive rice cultivars leads to impaired photosynthesis by nonstomatal limitation, involving the inability to dissipate excess energy by photoprotective mechanisms (Pereira et al. 2013; Pinto et al. 2016). One of the plant's responses to excess Fe is related to stomatal closure, and in comparison, to those sensitive cultivars to excess Fe, tolerant ones have shown greater stomatal resistance that occurred late due to toxicity (Dufey et al. 2009; Pereira et al. 2013). However, the physiological response to which stomatal limitation is attributed has not yet been elucidated.

Reduced water uptake in the roots may contribute to the late response of stomatal limitation under excessive Fe, as observed for other plant species under metal stress (Rucińska-Sobkowiak 2016; Gavassi et al. 2020; Chen et al. 2022). The reduction in xylem sap flow rate in the roots may occur due to oxidative damage to the root with exposure to high Fe concentrations. The loss of root conductivity associated with stomatal closure would limit the Fe uptake in the roots and its translocation to the shoot. Stomatal closure in rice under

excess Fe has been considered as an avoidance mechanism (Becker and Asch 2005; Dufey et al. 2009), affecting diffusive limitations.

In addition to stomatal conductance (g_s), diffusive limitations of the photosynthetic capacity is also influenced by mesophyll conductance (g_m), which is related to the diffusion of CO_2 from the intercellular spaces to the carboxylation sites in the chloroplast stroma (Flexas et al. 2012). Consequently, despite the photobiochemical limitations with a reduction in CO_2 assimilation in the Calvin-Benson cycle (Pereira et al. 2013), contrasting rice cultivars regarding the tolerance to Fe excess, would be affected differently in hydraulic and diffusive traits, in the mechanisms of dissipating excess energy and photoprotection.

Improving the development of resistant varieties will require knowledge about their photosynthetic responses under different Fe concentrations and exposure times to excess Fe. Photosynthetic variables are useful for selecting genotypes and varieties of rice with higher productivity in environments where biomass production is the main limiting factor of yield (Hubbart et al. 2007). Although some characteristics related to stomatal and nonstomatal limitations in rice cultivars under excess Fe conditions are known, the objective of this present study is to elucidate if the oxidative damage in the roots, and the diffusive limitation linked to stomatal and mesophyll conductances (g_s and g_m) affects the extent tolerance and sensitivity to excess Fe in rice cultivars.

MATERIAL AND METHODS

Cultivars and cultivation conditions

Two rice (*Oryza sativa L.*) cultivars (IRGA 424 and IRGA 417) were used. IRGA 417 was previously characterized as sensitive to excess Fe, and IRGA 424 was characterized as tolerant to excess Fe (SOSBAI 2007; 2012). IRGA 417 was the first cultivar of the modern agronomic type derived from a cross between parents of the Indica and Japonica subspecies. It stands out for its precocity, high yield, excellent grain quality, high initial seedling vigor, and good adaptability to all rice-producing regions. It presents a susceptibility reaction to Fe toxicity and panicle blast (EMBRAPA 2014). IRGA 424 CL stands out for its high productive potential and good industrial and grain cooking quality, except for the white center index, which is considered intermediate. It has a medium cycle and hairy leaves, and it is short. It is tolerant to Fe toxicity from excess Fe levels and is resistant to blast (EMBRAPA 2014).

The seeds were sterilized with 10% sodium hypochlorite for 10 minutes and then washed in deionized water. Then, they were transferred to germination paper and placed in a germination chamber for 72 h under a photoperiod of 12 h (200-250 µmol m^{-2} s^{-1}) at a temperature of 28 °C and relative humidity of 100%. After germination, the seedlings were transferred to hydroponic cultivation in a nonaerated Hoagland nutrient solution at half strength (Hoagland and Arnon 1938). The nutrient solution was adjusted to a pH of 5.0 ± 0.1 daily. After the seedlings reached the V6 growth stage (Counce et al. 2000), four concentrations of Fe^{2+} (0.019 (control) 2, 4, and 7 mM) were applied and were supplied as $FeSO_4$-EDTA.

Measurements of chlorophyll *a* fluorescence

Chlorophyll fluorescence was evaluated by using a portable fluorometer (FluorPen FP 100, Photon Systems Instruments, Brno, Czech Republic). Measurements were performed at 0, 2, 4, 6, and 8 days after the application of Fe, and the variables described in Table 1 were calculated.

Table 1. Equations and definitions of the variables OJIP obtained from chlorophyll *a* fluorescence emission analyses (OJIP) according to Strasser et al. (2000).

At eight days after application of the treatments, the light response curves were evaluated to determine the instantaneous capacity of the photosynthetic response to radiation,

Variables	Name and Description
Quantum yields and efficiencies	Derivatives of rapid fluorescence kinetics
$F_v/F_m = \Phi P_o = TR_o/ABS = [1 - (F_o/F_m)]$	Maximum quantum yield for PSII primary photochemistry
$\Psi_o = ET_o/TR_o = (1 - V_J)$	Probability of electron transport after reduction of Q_A^- (at t = 0)
$\Phi E_o = ET_o/ABS = [1 - (F_o/F_m)] * \psi_o = \Phi P_o * \Psi_o$	Quantum efficiency for the transfer of electrons from Q_A^- to the electron transport chain (at t = 0)
$\Phi D_o = 1 - \phi P_o = (F_o/F_m)$	Quantum efficiency for energy dissipation (at t = 0)
Specific energy flows	By active reaction centers (RCs) of PSII
ABS/RC	Energy flow absorbed per active RC unit
TR_o/RC	Excitation energy flow captured (reduced Q_A^-) per active RC unit at the beginning of the illumination of a dark-adapted sample (at t = 0)
ET_o/RC	Flow of electrons transferred per unit of active RC (at t = 0)
DI_o/RC	Total energy dissipated as heat, fluorescence and energy transfer to the PSI (in t = 0), per unit of active RC

using a Mini-PAM modulated pulse fluorometer (Heinz Walz, Efeltrich, Germany). The leaves were acclimated in the dark for 30 minutes to obtain the minimum (F_0) and maximum (F_m) fluorescence values. Then, after acclimation to ambient light, the leaves were exposed to increasing intensities of actinic light (0-2000 µmol m^{-2} s^{-1}) for nine intervals of 40 s to measure Fs and F_m'. From these parameters were calculated: the effective quantum yield of PSII in the illuminated plant tissue ($\phi_{PSII} = (Fm'- F_s)/F_m'$), the nonphotochemical quenching (NPQ = Fm - Fm'/Fm'), and the apparent rate of electron transport (ETR $=\phi_{PSII} \times$ PPFD $\times$ l$_A \times$ 0.5; where PPFD = photosynthetic photon flux density, l$_A$ = leaf absorbance coefficient will be assumed as 0.85 (LI et al. 2009), and 0.5 = partition of the quanta absorbed between the two photosystems (Bilger and Björkman 1990). From data on the light curves we calculated the maximum values for ETR$_{max}$ and NPQ$_{max}$.

Measurements of gas exchange

Gas exchange measurements were performed between 08:00 am and 12:00 pm using an infrared (IR) gas analyzer (model LI-6400XT, Li-Cor Inc., Lincoln, NE, USA), at 0, 2, 4, 6, and 8 days after treatment application of Fe. The following were determined: net assimilation of CO_2 (A_n, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), and a ratio between intracellular/extracellular CO_2 concentrations (C_i/C_a). The measurements were obtained using an irradiance source of 1500 µmol photon m^{-2} s^{-1}, a temperature of 25 °C, and an atmospheric CO_2 concentration of 400 µmol mol^{-1}. The dark respiration measurements (R_d, µmol m^{-2} s^{-1}) were estimated before dawn, at 8 days after application of the treatments, at a temperature of 25 °C and an atmospheric CO_2 concentration of 400 µmol mol^{-1}.

At eight days after the application of the treatments, the A_n/C_i curves were determined by the responses of the assimilation of CO_2 (A_n) as a function of the variation in the intercellular concentration of CO_2 (C_i) of the measuring chamber (400, 300, 200, 150, 50, 400, 400, 500, 600, 700, 1000, 1100, and 1200 µmol mol^{-1}) (Long and Bernacchi 2003). From the A_n/Ci curves, the maximum carboxylation rate of Rubisco (Vc$_{max}$, µmol m^{-2} s^{-1}), the electron transport rate for carboxylation (J$_{max}$, µmol m^{-2} s^{-1}), and the triose phosphate utilization rate (TPU) were determined (Sharkey 2007).

Based on the measurements of gas exchange and chlorophyll a fluorescence, the mesophyll conductance (g$_m$, mol CO_2 m^{-2} s^{-1}) and chloroplast CO_2 concentration (C$_c$, µmol CO_2 mol^{-1}) were estimated according to the method described by Harley et al. (1992):

$$g_m = \frac{A_n}{C_i - \dfrac{\Gamma^*[\,ETR + 8\,(A_n + R_d)\,]}{ETR - 4\,(A_n + R_d)}}$$

$$C_C = C_i \frac{A_n}{g_m}$$

where Rd, C_i, and A_n were determined from measurements of gas exchange and the A_n/C_i curves. The value of the CO_2 compensation point related to C_i (Γ^* µmol mol^{-1}) for rice was obtained from Li et al. (2009).

From these data, it was possible to calculate the photorespiration rate (P_r), which was obtained using the following equation (Valentini et al. 1995):

$$P_r = \frac{1}{12}[ETR - 4\,(A + R_d)]$$

Relative water content, leaf water potential (Ψ_{leaf}) and xylem sap flow rate

The leaf water potential and relative water content of the leaves were evaluated eight days after the application of the treatments. In the morning and at noon, the measurements were obtained for the same leaves as those used for the gas exchange analyses, using a pressure chamber (Model 1000, PMS Instruments Company, Corvallis, OR, USA). Xylem root sap flow rate (J_v) was evaluated at 8 days after the application of the treatments using the sap flow method induced by root pressure. The plants were placed in the same pressure chamber described previously, with their roots submerged in a nutrient solution, the shoots were removed, and a pressure (0.1 MPa) was applied so that the sap flow rate was determined. The collection of sap on the cutting surface was performed every 120s with the aid of absorbent paper, whose mass was previously measured. After collecting the sap, the wet absorbent paper mass was immediately measured on an analytical scale.

Lipid peroxidation in leaves and roots of rice cultivars

At eight days after the application of the treatments, lipid peroxidation in the leaves and roots was estimated by the concentration of thiobarbituric acid reactive substance (TBARS) according to Hodges et al. (1999). For this, 0.1 g of leaf and root tissue was weighed and then macerated with liquid nitrogen. After maceration, 1 mL of 80% ethanol was

added, and the samples were placed in a refrigerated ultrasonic bath (Elmasonic S30 H) for 10 minutes. Subsequently, the samples were centrifuged for 10 minutes at 10,000 °C at 4 °C (Megafuge 16R, Thermo Fisher Scientific, Waltham, USA), the supernatant was collected, and the same extraction process was repeated two more times. One milliliter of the extract was added to 1 mL of TBA+ solution (20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid), and another 1 mL of the sample was added to 1 mL of TBA solution (trichloroacetic acid 20%, butylated hydroxytoluene 0.01%). Then, the samples were placed in a water bath (Model 521-3D, Ethik Technology, Brazil) at 95 °C for 25 minutes, and the reaction was stopped in an ice bath for 10 minutes. Subsequently, the samples were centrifuged for 10 minutes at 25 °C and 10,000 g. After centrifugation, readings were performed in a spectrophotometer (UV-1800 Shimadzu, Japan) at three wavelengths: 440 nm, 532 nm, and 600 nm. The calculations were performed using the equation $\{[(A523 - A600) - (A440 - A600) (MA \text{ sucrose at } 532/MA \text{ sucrose at } 440)]/157000\} \, 10^6$, where A = absorbance, MA = molar absorbance, and 157000 = molar extinction coefficient.

Iron concentration and morphological variables

The concentrations of Fe in the shoots and roots were evaluated eight days after the application of the treatments. The roots were washed with a dithionite-citrate-bicarbonate (DCB) solution to eliminate the iron adhered to the root surface (Pereira et al. 2014). The concentrations of Fe were evaluated according to the methodology of Tedesco et al. (1997) by atomic absorption spectrophotometry (AA-7000, Shimadzu, Japan) after digestion with nitric-perchloric acid solution (3:1) at 200 °C. At 8 days after application of the treatments, the following morphological variables were analyzed: total leaf area and the dry weight of leaves and roots.

Statistical analyses and experimental design

A randomized block experimental design was used in a factorial scheme (2 x 4), with two cultivars and four Fe concentrations, with four replicates. For the variables that involved sampling time (0, 2, 4, 6, and 8 days after Fe application), the data were grouped into subdivided plots. Analyses of variance were performed and, when significant, Tukey's test ($P < 0.05$) was performed. The analyses were conducted using the statistical software R version R i386 3.1.2.

RESULTS

Temporal dynamics in gas exchange measurements reflect the susceptibility of rice cultivars to Fe toxicity

Evaluating gas exchange (A_n, g_s, and E) in plants up to eight days after application of Fe concentrations (Fig. 1), the sensitive cultivar (IRGA 417) showed a reduction in gas exchange two days after application, at the highest concentration of Fe (7 mM). In general, throughout the evaluation period, the tolerant cultivar (IRGA 424) showed no difference between the Fe concentrations of 0.019 and 2 mM for gas exchange variables. The most substantial reductions were observed only after four days with the application of the highest Fe concentration.

IRGA 424   IRGA 417
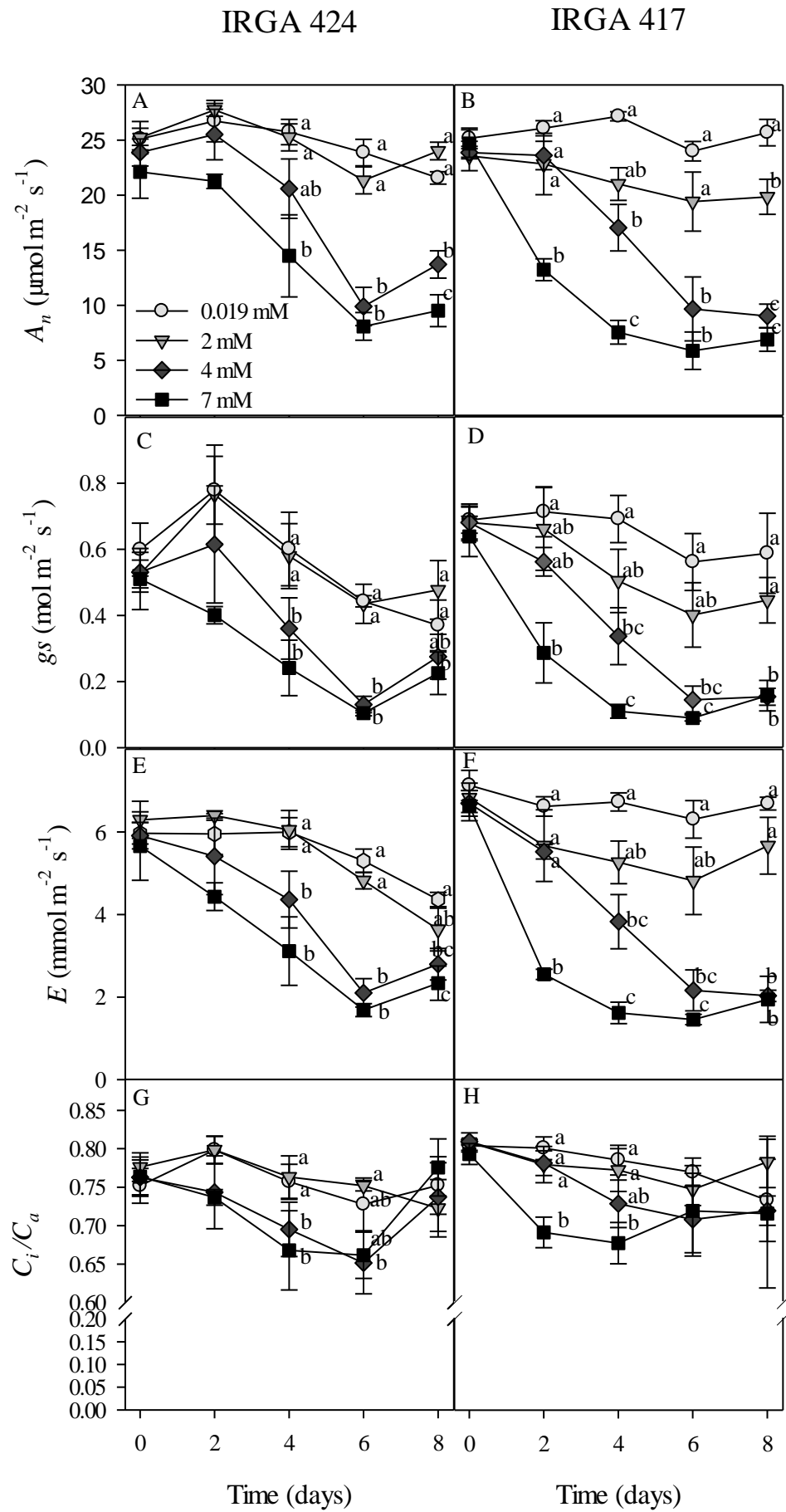
Figure 1 Net photosynthetic rate (A_n) (A, B), stomatal conductance (g_s) (C, D), transpiration (E) (E, F) and the ratio between internal and external CO_2 concentration (Ci/Ca) (G, H) in two contrasting rice cultivars under four concentrations of Fe^{2+}. Different letters indicate significant differences (p <0.05) between treatments with $FeSO_4$-EDTA within days after application. The data are presented as the means ± standard errors of four replicates.

Temporal dynamics in specific PSII energy fluxes and quantum efficiency for energy dissipation reflect the susceptibility of rice cultivar to Fe toxicity

The tolerant rice cultivar showed no effect of Fe concentrations on the PSII quantum efficiencies (Fig. 2) or on the specific PSII energy fluxes (Fig. 3) during the eight days after Fe excess application. The sensitive cultivar after six days of the Fe application showed a F_v/F_m reduction at a concentration of 7 mM (Fig. 2B). However, from the days following the application of Fe concentrations, the sensitive cultivar at the 7 mM Fe concentration already showed a decrease in the probability of electron transport after the reduction in Q_{A-} (at t = 0) (Ψ_o) and in quantum efficiency for the transfer of electrons from Q_{A-} to the electron transport chain (at t = 0) (ΦE_o) (Fig. 2D & F). At the same time, this cultivar increased the quantum efficiency for energy dissipation (ΦD_o) within six days of application of the highest Fe concentration (Fig. 2H).

IRGA 424 IRGA 417
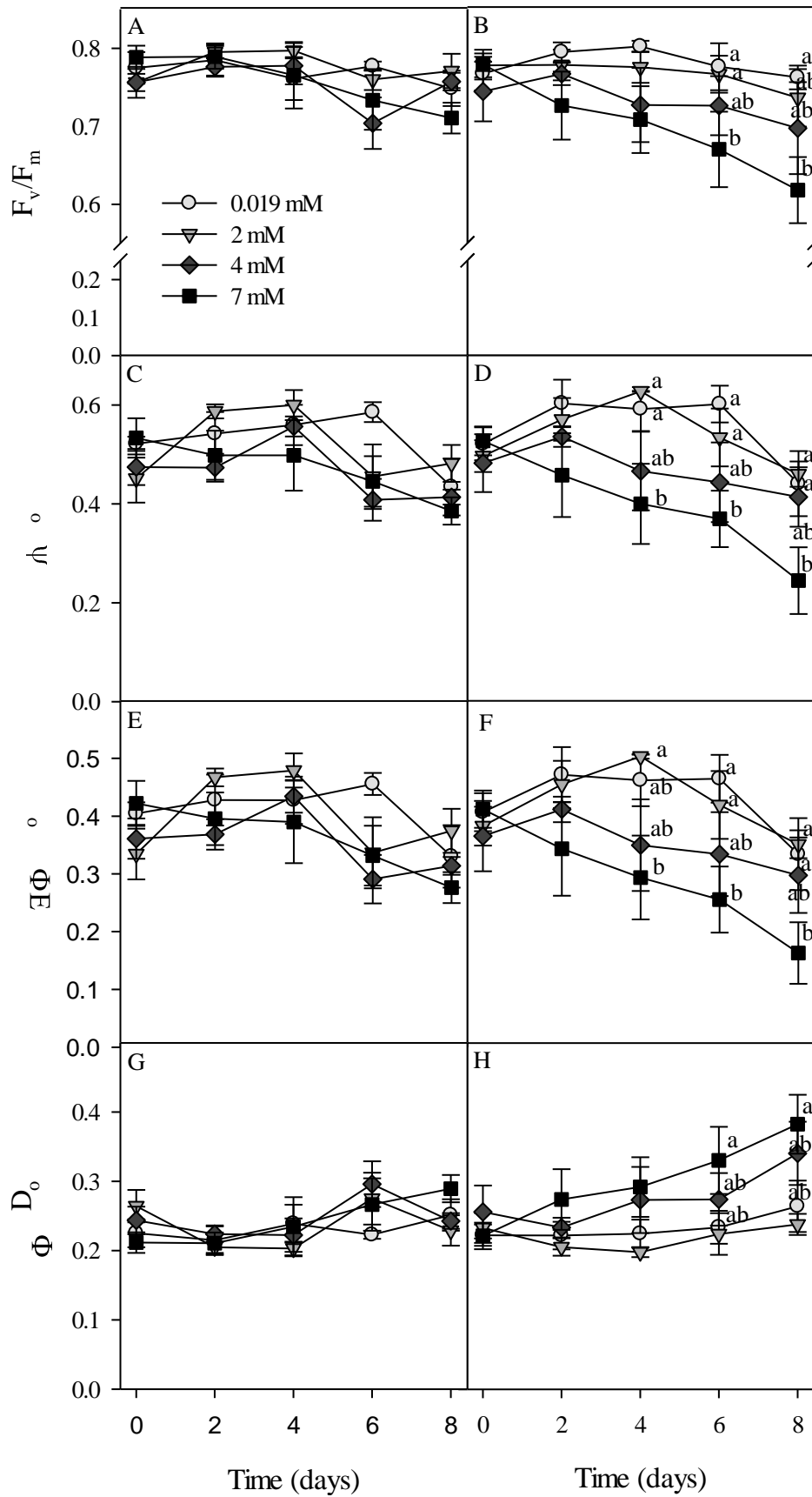
Figure 2 Maximum quantum yield for PSII primary photochemistry (F_v/F_m), probability of electron transport after reduction of QA^- (at t = 0) (Ψ_o), quantum efficiency for the transfer of electrons from QA^- to the electron transport chain (at t = 0) (ΦEo), quantum efficiency for energy dissipation (at t = 0) (ΦD_o) in two contrasting rice cultivars under four concentrations of Fe^{2+}. Different lowercase letters indicate significant differences (p <0.05) between treatments with $FeSO_4$-EDTA within days after application. The data are presented as the means ± standard errors of four replicates.

The sensitive cultivar showed a higher energy flow per unit of active RC (light energy absorption (ABS/RC) and excitation energy trapping (TRo/RC) with 7 mM of Fe after two days of application (Fig. 3B & D). A greater difference was observed between the Fe concentrations of 0.019 and 2 mM when compared to 4 and 7 mM in the ABS/RC and TRo/RC for the sensitive cultivar. Six days after Fe application the sensitive cultivar showed a difference between the Fe concentrations, with lower values for the flow of electrons transferred per active RC unit (ETo/RC) and higher values for the total energy dissipated as heat, fluorescence and energy transfer to the PSI per active RC unit (DIo/RC) in the concentration of 7 mM of Fe (Fig. 3F & H).

IRGA 424   IRGA 417
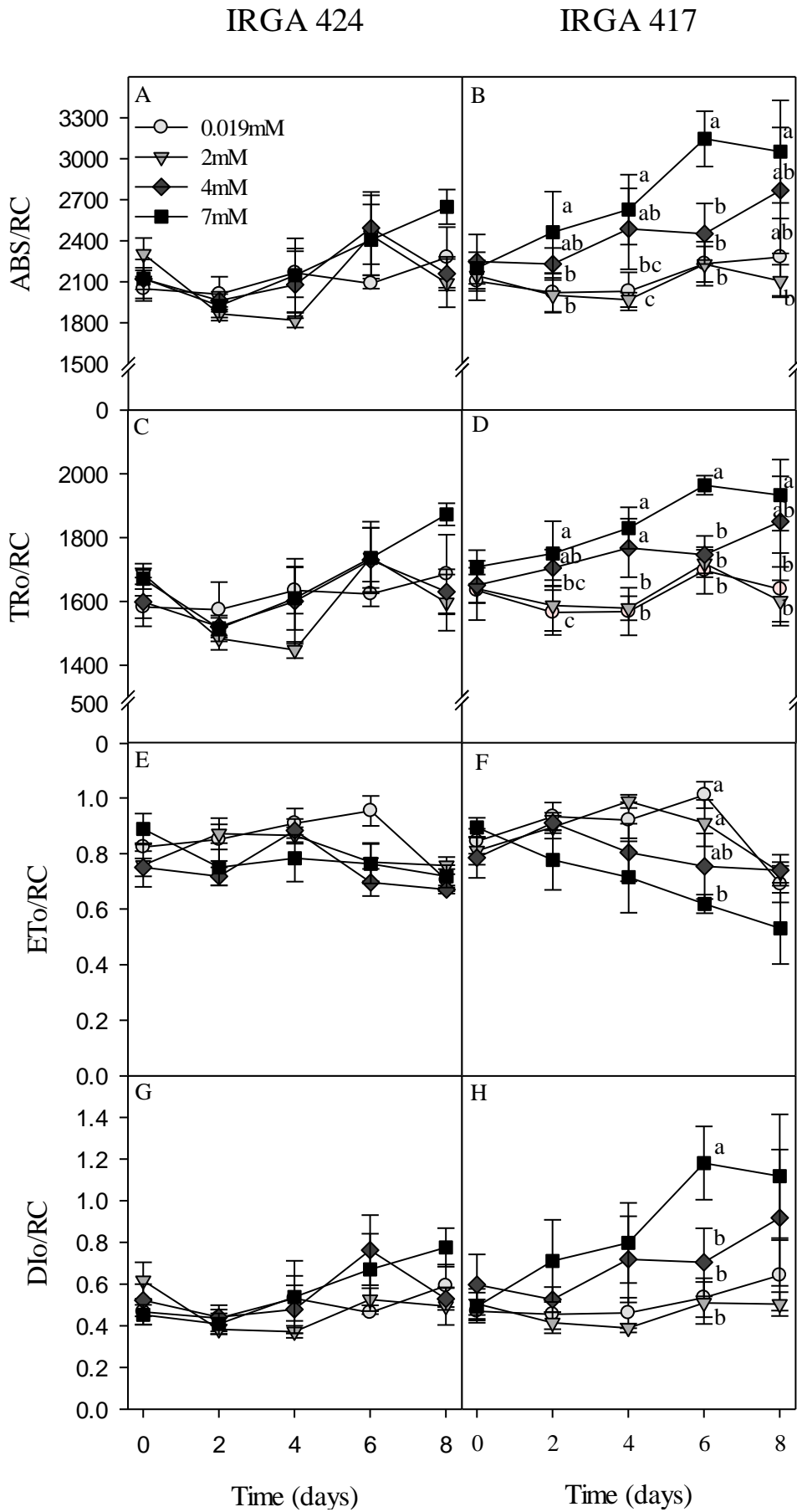
Figure 3 Energy flow absorbed per active RC unit (ABS/RC), excitation energy flow captured (reduced QA) per active RC unit at the beginning of the illumination of a dark-adapted sample (at t = 0) (TRo/RC), flow of electrons transferred per unit of active RC (at t = 0) (ETo/RC), total energy dissipated as heat, fluorescence and energy transfer to the PSI (in t = 0), per unit of active RC (DIo/RC) in two rice cultivars under four concentrations of Fe^{2+}. Different lower case letters indicate signi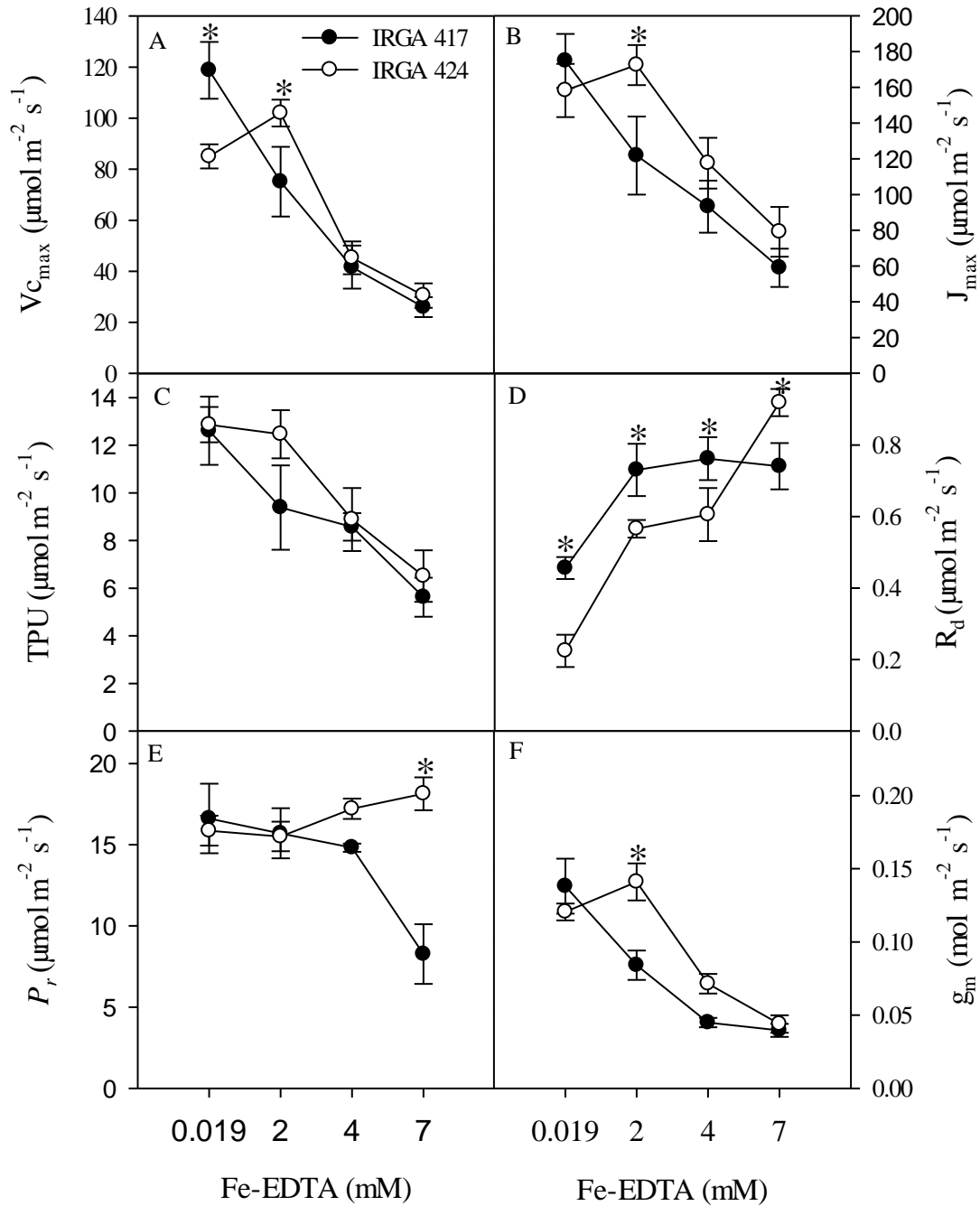ficant differences (p <0.05) between treatments with FeSO4-EDTA within days after application. The data are presented as the means ± standard errors of four replicates.

The contrasting rice cultivars under four concentrations of Fe^{2+} showed great differences in carbon metabolism and their ability to cope with excess light energy

After eight days of Fe excess application, the A_n–ci response curve was evaluated. In this period, the cultivars already showed a significant reduction in gas exchange (Figure 5). Evaluating the genotypic differences between the cultivars with the Fe concentration, the tolerant cultivar showed no difference in V_{cmax}, J_{max}, TPU and g_m between the control and the 2 mM Fe concentration (Fig. 4). At a concentration of 2 mM, V_{cmax}, *Jmax,* and g_m in the sensitive cultivar were significantly lower than those in the tolerant cultivar. The increase in the Fe concentrations in the nutrient solution reduced the TPU in both cultivars, with no interaction regarding the genotypic differences (Fig. 4C).

R_d was higher in the sensitive cultivar than in the tolerant cultivar until the Fe concentration of 4 mM, and after this concentration was applied, the tolerant cultivar showed a higher Rd (Fig. 4D). The Rd in the tolerant cultivar showed an increase proportional to the concentrations of Fe. This response was not observed in the sensitive cultivar, in which there was no difference observed with the application of Fe concentrations (2, 4, and 7 mM). At the same time, the P_r of the sensitive cultivar decreased with increasing Fe concentrations (Fig. 4E). However, the tolerant cultivar had an increase in P_r at the highest concentration of Fe.

Figure 4 The maximum carboxylation rate of Rubisco (Vc_{max}) (A), electron transport rate for carboxylation (J_{max}) (B), and utilization of trioses phosphate (TPU) (C), dark respiration (Rd) (D), photorespiration (P_r) (E) and mesophyll conductance (g_m) (F), in two contrasting rice cultivars under four concentrations of Fe^{2+}. Asterisks indicate significant differences ($p < 0.05$) between treatments with $FeSO_4$-EDTA. The data are presented as the means ± standard errors of four replicates.

Eight days after the Fe application, the light-response curve of fluorescence variables was also evaluated (Table 2). There were no interactions among the cultivars and Fe doses for the variables ϕPSII and ETRmax. Fe excess at 7mM reduced the ϕPSII and ETR_{max} values in both cultivars when compared to the control treatment (0.019 mM). The NPQ_{max} did not differ between iron concentrations in the sensitive cultivar IRGA 417. However, NPQ_{max} increased in the tolerant cultivar when exposed to excess Fe concentrations (Table 2).

Oxidative damage at excessive Fe doses is associated with differences in root xylem sap flow and water status in susceptible and tolerant cultivars

The MDA concentration in roots and leaves showed higher values at Fe concentrations of 2, 4, and 7 mM than at the control concentration in both cultivars (Table 2). Evaluating the separate effect, the sensitive cultivar showed a higher MDA concentration in its roots and leaves than the tolerant cultivar in the Fe concentrations of 2 and 4 mM.

Table 2 The quantum yield of photochemical energy conversion in PSII (ϕPSII), the maximum non-photochemical quenching (NPQ_{max}), maximum apparent electron transport rate (ETR_{max}), malondialdehyde (MDA) concentration in leaves, and root in two rice cultivars

Cultivar	Fe-EDTA	ϕPSII	NPQ_{max}	ETR_{max}	MDA_{Root} $(nmol.g^{-1}MF)$	MDA_{Leave} $(nmol.g^{-1}MF)$
IRGA 417	0.019mM	0.272 ± 0.01^{Ba}	1.89 ± 0.4^{Aa}	275.13 ± 15.00^{Ba}	10.10 ± 1.49^{Ab}	39.78 ± 8.28^{Ab}
	2mM	0.215 ± 0.02^{Ba}	2.45 ± 0.21^{Aa}	217.41 ± 24.93^{Ba}	27.37 ± 4.19^{Aa}	57.67 ± 2.69^{Aa}
	4mM	0.188 ± 0.02^{Bab}	2.27 ± 0.27^{Aa}	188.12 ± 23.34^{Bab}	45.72 ± 4.69^{Aa}	60.91 ± 4.21^{Aa}
	7mM	0.110 ± 0.00^{Bb}	2.17 ± 0.13^{Ba}	110.03 ± 09.72^{Bb}	39.60 ± 1.69^{Aa}	56.81 ± 7.00^{Aa}
IRGA 424	0.019mM	0.293 ± 0.01^{Aa}	1.61 ± 0.08^{Ac}	296.63 ± 17.42^{Aa}	9.21 ± 0.47^{Ab}	38.27 ± 7.17^{Ab}
	2mM	0.275 ± 0.01^{Aa}	1.78 ± 0.18^{Abc}	280.20 ± 9.24^{Aa}	17.28 ± 0.46^{Ba}	48.64 ± 1.17^{Ba}
	4mM	0.242 ± 0.01^{Aab}	3.51 ± 0.45^{Aab}	246.19 ± 11.54^{Aab}	28.11 ± 2.70^{Ba}	46.66 ± 4.21^{Ba}
	7mM	0.198 ± 0.02^{Ab}	3.99 ± 1.03^{Aa}	199.53 ± 28.28^{Ab}	40.68 ± 7.36^{Aa}	55.55 ± 2.82^{Aa}

under four concentrations of Fe^{2+}.

Capital letters compare cultivars, and small letters compare Fe–EDTA treatments. The data are presented as the means $\pm$ SE, n = 4.

Iron excess reduced sap flow rate and leaf water potential in both cultivars (Table 2) after exposition to Fe concentration of 4 mM, with the lowest values were observed at concentration of 7 mM. The two cultivars showed no significant differences between them in

the distinct treatments with Fe. Leaf relative water content showed no significant difference, irrespective of Fe concentration or cultivar.

Table 3 Xylem sap flow rate (J_v), leaf water potential (Ψ_{leaf}), relative water content (RWC) and dry weights of the root (RDW) and shoot (SDW) and leaf area (LA) of two rice cultivars under four concentrations of Fe^{2+}.

Cultivar	Fe-EDTA	J_v (mg s^{-1})	Ψ_{leaf} (MPa)	RWC (%)	RDW (g)	SDW (g)	LA (cm^2)
IRGA 417	0.019mM	0.074±0.02[Aa]	0.57±0.07[Ab]	58.25±4.14Aa	0.32±0.06[Aa]	0.71±0.09[Aa]	122.92±29.09[Aa]
	2mM	0.048±0.00[Aab]	0.41±0.05[Ab]	60.27±8.92Aa	0.19±0.01[Aab]	0.52±0.06A[ab]	77.92±3.64A[ab]
	4mM	0.040±0.00[Aab]	0.50±0.07[Ab]	59.59±5.14Aa	0.23±0.01[Aab]	0.58±0.02[Aab]	75.41±6.05[Ab]
	7mM	0.032±0.00[Ab]	0.66±0.02[Aa]	54.01±3.82Aa	0.15±0.02[Ab]	0.35±0.02[Ab]	56.20±7.42[Ab]
IRGA 424	0.019mM	0.078±0.03[Aa]	0.40±0.05[Ab]	68.15±4.86Aa	0.36±0.03[Aa]	0.72±0.06[Aa]	103.34±4.10[Aa]
	2mM	0.046±0.00[Aab]	0.39±0.06[Ab]	58.97±6.12Aa	0.30±0.01[Aa]	0.61±0.05[Aa]	90.99±4.62[Aab]
	4mM	0.039±0.00[Aab]	0.58±0.06[Ab]	57.54±8.69Aa	0.19±0.02[Aa]	0.40±0.05[Ab]	68.60±8.36[Ab]
	7mM	0.022±0.00[Ab]	0.95±0.17[Aa]	55.19±3.58Aa	0.12±0.00[Ab]	0.32±0.05[Ab]	51.23±8.98[Ab]

Capital letters compare cultivars, and small letters compare Fe–EDTA treatments. The data are presented as the means ± SE, n = 4.

Susceptible and tolerant cultivars presented distinct iron accumulation capacities at excessive Fe doses but the same morphological responses

The two cultivars experienced a reduction in biomass of roots and shoots and leaf area with increasing Fe concentration in the nutrient solution, and the lowest values were observed at concentrations of 4 and 7 mM. However, there was no significant differences between cultivars in these variables within different Fe concentrations (Table 2).

Eight days after Fe application, the cultivars developed typical symptoms of Fe toxicity, with the appearance of tanned and necrotic lesions on the leaves especially in the sensitive cultivar, while the roots became brown/orange (Fig. 5). The increase in Fe concentration in the nutrient solution resulted in the accumulation of the metal in the shoots and roots. In comparison to the tolerant cultivar, the sensitive cultivar IRGA 417 showed a higher concentration of Fe in the roots and leaves only with the application of 7 mM (Fig. 5).
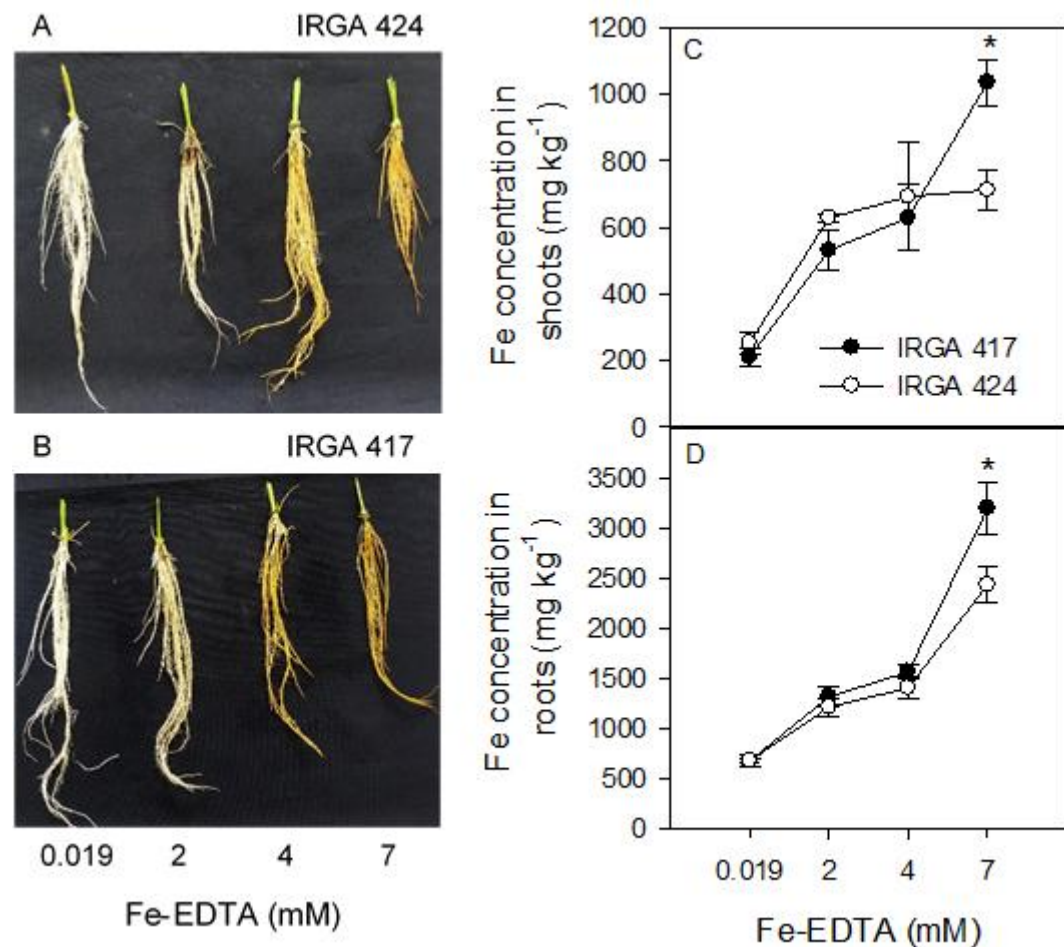
Figure 5 Effects of excess iron in roots from the rice cultivars IRGA 424 and IRGA 417 (A, B) exposed for eight days to iron treatments. Fe concentration in shoots (C), Fe concentration in roots (D) in two contrasting rice cultivars under four concentrations of Fe^{2+}. Asterisks indicate significant differences ($p < 0.05$) between treatments with $FeSO_4$-EDTA. The data are presented as the means ± standard errors of four replicates.

DISCUSSION

Knowledge of the contrasting photosynthetic mechanisms of rice cultivars will be of great importance for genetically improving new cultivars under excess Fe. These two cultivars showed different mechanics concerning the increase in Fe in the nutrient solution. Cultivars were previously selected as tolerant (IRGA 424) and sensitive (IRGA 417) to excess Fe (SOSBAI 2007; 2012). In comparison to the sensitive cultivar, in addition to presenting lower iron content in the root and leaf at the highest Fe concentration, the tolerant cultivar was more efficient in gas exchange and photoprotection mechanisms. These mechanisms, which

includes also the control of oxidative damage, were efficient in ensuring greater photosynthetic performance in PSII and preventing photoinhibitory processes.

Mechanisms of Fe tolerance in rice cultivars involve the prevention of oxidative damage and adjustments in hydraulic traits

The two cultivars showed levels of Fe in the leaves and roots above those considered critical for Fe toxicity ($\geq$300–500 mg kg^{-1} dry weight) (Dobermann and Fairhurst 2000). Rice cultivars under conditions of excess Fe employ different defense mechanisms, and these mechanisms will vary according to the concentration of Fe and the tolerance level of the cultivars. Plants can resist Fe toxicity through mechanisms such as avoidance and tolerance (Zhang et al. 2016); these mechanisms are based on absorption restriction, formation of iron plaque on the root surface, cell compartmentalization, and antioxidant defense system (Deng et al. 2010; Li et al. 2017; Müller et al. 2015). Glutathione reductase (GR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and ascorbate (AsA), superoxide dismutase (SOD) may act against ROS and eliminate the tissue damage caused by Fe toxicity (Rios et al., 2021; Ahammed et al., 2020; Verma & Pandey, 2019), and also able to prevent biological damage caused by ROS (Müller et al., 2015; Stein et al., 2014). The antioxidant defense system is activated under Fe toxicity to maintain Fe homeostasis at cellular level (Pinto et al., 2016).

The translocation of Fe in the xylem follows the transpiration flow (Becker and Asch 2005). The variation in tolerance to iron toxicity among rice cultivars may be related to the composition of the root cell wall. Increased lignification in the outer layers of the cortex and in the vascular bundle (Pereira et al., 2014) may alter Fe permeability, radial diffusion, and translocation of the Fe from the roots to the shoots, leading to a greater tolerance to high external iron levels (Siqueira-Silva et al., 2019; Stein et al. 2019). In addition, the closure of the stomata can also be seen as a preventive mechanism to limit the uptake and translocation of iron in the shoots (Dufey et al. 2009; Pereira et al. 2013), but since it occurs as a late response after Fe toxicity (Dufey et al. 2009; Pereira et al. 2013) it was expected to be a secondary response triggered by oxidative damage and loss of connectivity with the root tissues as observed in our study. A significant correction between oxidative damage in the root and J_v was seen in both cultivars with increasing Fe concentrations (R^2 values of 0.92 and 0.84 for cultivar IRGA 417 and IRGA 424, respectively).

Reduced xylem sap flow rate and leaf water potential under excess iron conditions, may be related to reduced water uptake at the root level caused by oxidative stress dysfunction resulting from Fe^{2+} excess. Elevated Fe^{2+} concentration in the substrate strongly decreases radicle development (Rodrigues Filho et al., 2020). Despite, the oxidative damage in the root vascular cylinder and the increased stomatal resistance, as found for both cultivars in our study, the decrease in water transport from the root to shoot at higher concentrations of Fe may be also related to several factors, as found by other authors, such as less developed and smaller protoxylem vessels or even root structure, and increased thickness of iron plaque (Hose et al. 2001; Dufey et al. 2009; Pereira et al. 2014; Gavassi et al. 2020).

The suberization and lignification of root exodermis represent an adaptation that effectively limits the oxygen diffusion out of the aerenchyma in lowland cultivars, leading to a low capacity of rice to take up water (Colmer et al. 1998; Armstrong et al. 2000; Hose et al. 2001). Increased cell wall lignification in the root cortex layers (endodermis and exodermis), as well as in the central cylinder (primary xylem and pith parenchyma cell), which would be a new mechanism for rice tolerance to high Fe concentrations (Stein et al 2019). At the same time, low root surface area and biomass with increasing Fe in the nutrient solution, may limit water and nutrient uptake. This decrease in root growth has a severe effect on all processes related to development, including loss of root hairs and reduction in root volume, which will drastically reduce the plant's total water content (De Dorlodot et al. 2005; Dufey et al. 2009).

Diffusive limitations linked to coupled reductions in gs and gm impair the photosynthetic efficiency of rice cultivars under excess Fe

The stomatal pore and the path through the cell wall mesophyll to Rubisco are two primary resistance forces to CO_2 diffusion. The mesophyll pathway serves a series of physical barriers to the diffusion of CO_2, including cell walls, lipid membranes, liquid cytoplasm, and stroma (Flexas et al. 2012; Terashima et al. 2011). The decreased net CO_2 assimilation rate (A_n) under Fe toxicity has been attributed to impairment in photochemical reactions, as well as to stomatal and nonstomatal limitations of photosynthesis (Pereira et al. 2013; Pinto et al. 2016). However, our study demonstrates novel information that not only g_s but also g_m limits CO_2 flux towards the chloroplast stroma in response to Fe excess. Furthermore, in the sensitive cultivar, the reduction in g_m occurs before the reduction in g_s (Fig. 1). This may be associated with oxidative damage to the roots and loss of flow capacity in the root xylem (Table 2).

The biochemical limitations of photosynthesis are related to impairments in the carboxylation rate by Rubisco (V_{cmax}), the regeneration rate of RuBP (J_{max}), and the use of triose-phosphate (TPU) (Lawlor and Tezara 2009). Under conditions of excess Fe, the maintenance of leaf water potential and RWC achieved by cultivars due to diffusive restriction (g_s and g_m), also inhibited the carboxylation of RuBP (V_{cmax}) (Fig. 4). Furthermore, the photochemical limitations (Fig. 2 and 3, Table 2) proved to be more responsive for the sensitive cultivar. The higher sensitivity in the IRGA 417 cultivar in carboxylation processes when exposed to excess Fe, may have been related to oxidative damage in this cultivar. Photochemical limitations of photosynthesis can increase oxidative damage in sensitive cultivars, leading to impairments in biochemical processes.

The photoprotective mechanisms and photorespiration were essential for tolerance of the IRGA 424 cultivar under excess Fe

The decline in carbon metabolism is mainly due to feedback inhibition by ATP and NADPH, which are not adequately utilized in the Calvin-Benson cycle under excess Fe (Siedlecka et al. 1997), the production of ROS increases, which blocks the repair of photodamaged PSII (Takahashi and Murata 2005). If the recycling of glycolate-2-phosphate into glycerate-3-phosphate through photorespiration (P_r) is not increased, then the regeneration of RuBP is restricted, and the operation of the Calvin-Benson cycle is even more inhibited. The photorespiratory pathway can aid in avoiding inhibition of the repair of photodamaged PSII by maintaining the energy utilization in the Calvin-Benson cycle, which is important for reducing the generation of ROS, under conditions where the supply of CO_2 is limited (Takahashi and Badger 2011) as occurs under Fe toxicity with stomata closure. Pr was the main alternative sink for electrons in the tolerant rice cultivar IRGA 424 under iron excess (Fig. 4). Oxidative damage and the decrease in ϕPSII and ETRmax may have caused the inefficiency of Pr in the sensitive cultivar.

The increase in ΦDo in the last days after Fe application observed only for the sensitive cultivar IRGA 417 (Figure 2) is related to the loss of photosynthetic efficiency. The variables F_v/F_m and ΦD_o can be considered inverse to each other, and all energy that is not transferred results in the photochemical step being lost through energy dissipation in a regulated way, in the form of heat, or even in an unregulated way or fluorescence because they involve concurrent processes. The former dissipative process occurred only in the tolerant rice cultivar which invested in photoprotective mechanisms. The reduction of Ψ_o and

ΦE_o in the sensitive cultivar IRGA 417 was observed before the F_v/F_m reduction. The lower the excitation energy needed to reduce Q_{A-}, the lower the probability that the electron can be transported beyond Q_{A-} to reduce the intersystem acceptors and, consequently, the lower the quantum yield for the transport of electrons through the transport chain (Fig. 2).

The initial stage of the photosynthetic activity of an RC is regulated by three functional steps: light energy absorption (ABS), excitation energy trapping (TR_o), and excitation energy conversion into electron transport (ET_o) (Thach et al. 2007). Changes in specific PSII energy fluxes for the sensitive cultivar IRGA 417, such as the increase in ABS/CR, mean that a fraction of the RC is inactivated (non-QA reducing centers). These results were corroborated by TR_o/RC, suggesting that Fe concentrations in the sensitive cultivar caused changes both in the fraction of RCs transformed into nonreducing centers of QA and in the functional size of the antennae complex (Fig. 3) (Yusuf et al. 2010).

NPQ is a mechanism related to nonphotochemical energy dissipation in the form of heat and is mainly controlled by changes in the xanthophyll cycle. The higher sensitivity to excess Fe in some rice cultivars, including the sensitive cultivar IRGA 417, is related to the inability of the plant to dissipate excess energy through photochemistry or photoprotection mechanisms (Pinto et al. 2016). The potentially oxidative stress suggests that the sensitive cultivar was not able to completely dissipate the excess light energy absorbed in LHCII through NPQ, increasing the photooxidative damage in the thylakoid membrane. Greater oxidative damage in the thylakoid membrane may hinder the formation of a transmembrane electrochemical potential gradient in chloroplasts through lipid peroxidation, preventing NPQ build up. Other energy dissipation mechanisms, such as the water-water cycle (Asada, 2000) or cyclic flow of electrons within PSII (Heber et al., 2001; Pereira et al. 2013), could also have played a role in dissipating the excess reducing power under Fe stress in the tolerant cultivar IRGA 424.

CONCLUSION

Oxidative damage in the roots with Fe application, led to reduced water transport from root to leaf, especially in the sensitive cultivar, as evidenced by low values of Ψ_{leaf} and xylem sap flow rate. The oxidative damage to the roots can lead to the closure of stomata, causing diffusive limitations which initiate with g_m constraints. Cultivars respond initially through diffusive limitation, and later through photochemical restriction, with more severity in the

sensitive cultivar. The results showed that the tolerance and sensitivity of the two rice cultivars are directly linked to Fe concentrations and exposure time to Fe. The tolerant cultivar IRGA 424 was more photosynthetically efficient with longer exposure time to excess Fe and did not change its photosynthetic parameters between the initial Fe concentrations (0.019 and 2 mM Fe). The sensitive cultivar IRGA 417, reduced the quantum efficiency for the transfer of electrons from Q_{A^-} to the electron transport chain, resulting in photoinhibitory processes. The IRGA 424 cultivar showed photoprotective mechanisms, such as increased NPQ, P_r, and greater R_d, that contribute to the tolerance after exposure to the Fe concentrations.

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Photoprotective mechanisms and higher photorespiration are key points for iron stress tolerance under heatwaves in rice plants

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ABSTRACT

Considering the current climate change scenario, the development of heat-tolerant rice cultivars (*Oryza sativa* L.) is paramount for cultivation in waterlogged systems affected by iron (Fe) excess. The objective of this work is to investigate the physiological basis of tolerance to excess Fe in rice cultivars that would maintain photosynthetic efficiency at higher temperatures. In an experimental approach, two rice cultivars (IRGA424 - tolerant and IRGA417- susceptible to Fe toxicity) were exposed to two concentrations of $FeSO_4$-EDTA, control (0.019 mM) and excess Fe (7 mM) and subsequent exposition to heatwaves at different temperatures (25 °C – control, 35, 40, 45, 50, and 55 °C). In addition, we compiled a data set of photosynthetic responses to excess Fe or heat stress in 23 rice cultivars. The increase in temperatures resulted in a higher Fe concentration in shoots in the tolerant cultivar and a lower Rubisco carboxylation rate in both cultivars. Stomatal limitation only occurred as a late response, reducing Fe accumulation with increasing temperature in the sensitive cultivar. The Fe tolerant cultivars presented efficient photoprotective mechanisms. The activation of photorespiration as electron sink under Fe excess with increasing temperature during heatwaves, prevented higher membrane damage. The sensitivity to iron toxicity and heat stress is associated with inefficient non-photochemical dissipation.

Keywords: *Oryza sativa*, iron toxicity, heat tolerance, photosynthesis, photoprotection.

1- INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crop, being used as a staple food for more than 50% of the world population (Xu et al., 2021). It is cultivated mainly in summer, in tropical and subtropical areas, however, one of the major problems for its cultivation in waterlogged systems is the excess of iron (Fe), which is transported through the transpiration stream and can reach toxic levels in rice leaves promoting oxidative stress. Fe excess toxicity causes the overproduction of reactive oxygen species (ROS), especially the hydroxyl radical ($*OH$), via the Fenton reaction, which irreversibly impairs the cellular structure and damages membranes and proteins (Kobayashi & Nishizawa, 2012). At the same time, the global warming scenario may intensify the toxicity caused by excess Fe in waterlogged cropping systems with a reduction of photosynthesis, and consequently the productivity of rice cultivars.

With global warming, heatwaves will be more frequent and with higher intensity (Xu et al. 2018). The frequent exposure of rice plants to multiple stress factors influences photosynthesis, the damage caused by Fe toxicity can be potentiated when added to other stressful factors such as heatwaves. The occurrence of heatwaves can impair the structure and function of plasma membranes, altering the proportion of saturated and unsaturated fatty acids and triggering protein denaturation. This can impair general membrane structure and functionality and lead to increased leakage of organic and inorganic cell ions (Sita et al., 2017).

It is well-known that the higher photosynthetic impairment of rice cultivars sensitive to Fe excess, when compared with tolerant ones, is caused mainly by the non-stomatic limitation, triggered by oxidative damage and consequent inability to dissipate the excess energy by regulated processes (Pereira et al., 2013; Pinto et al., 2016). The tolerance mechanisms of rice cultivars under Fe toxicity protect photosystem II (PSII) reaction centers against oxidative damage (Pereira et al., 2013; Pinto et al., 2016). Some tolerant rice cultivars are capable of preventing, or limiting, such damage by safe thermal dissipation of light energy (non-photochemical quenching, NPQ) or energy consumption of photochemistry in the form of ATP, through alternative sinks for electrons, such as the photorespiratory pathway (Derks et al., 2015; Pereira et al., 2013; Wingler et al., 2000).

The tolerance mechanisms of Fe toxicity would also be important to prevent photoinhibitory processes and maintain photosynthetic efficiency and grain yield in conditions of higher temperatures. Moreover, the regulation of the respiration process is considered a mechanism of tolerance to temperature increases. However, changes in respiration rate are more sensitive to thermal stress than changes in photosynthesis, and heat-

tolerant rice cultivars generally present lower increments in respiration rate after exposure to temperature (Ferguson et al., 2020). Despite the effects on respiration under heatwaves, the photosynthetic aspects can be decisive for rice tolerance mechanisms under the combination of stresses, especially the toxicity of Fe.

Increasing global population and climate change uncertainties have compelled increased photosynthetic efficiency and yields to ensure food security over the coming decades (Hussain et al., 2021). The breeding of adaptive traits is required for increasing the resilience of crops to current climate change conditions to help sustain productivity (Kumari et al., 2021). Due to the complexity of assessing heat tolerance both in controlled environments (Wang et al., 2012) and in field conditions (Thomey et al., 2019), there are no data on the physiological mechanisms related to the interaction of excess Fe and thermal stress in rice cultivars. In this context, this study aimed to investigate whether the mechanisms of tolerance to excess Fe in rice cultivars can maintain photosynthetic efficiency at higher temperatures.

2- MATERIAL AND METHODS

2.1 - Plant material and experimental conditions

Seeds of two rice cultivars were used in the experimental approach, IRGA 424 (tolerant to excess iron), and IRGA 417 (sensitive to excess iron). Both cultivars were developed by the Breeding Group of the Rio Grandense Rice Institute (IRGA) in Brazil and were previously characterized as susceptible and tolerant to Fe toxicity (SOSBAI, 2018) following the methodology proposed by Bacha & Ishiy (1986). The IRGA 417 was the first rice cultivar of the modern agronomic type derived from a cross between parents of the indica and japonica subspecies. It stands out for its precocity, high productivity, excellent grain quality and high initial seedling vigor (SOSBAI, 2018). The cultivar IRGA 424 stands out for its high yield potential and good industrial and cooking quality of the grains. It has a medium cycle, low stature and hairy leaves. It is also known that this cultivar shows acclimative responses when grown in regions with low average temperature conditions during the seedling stage (SOSBAI, 2018).

The seeds of both cultivars were disinfected with 10% sodium hypochlorite for 10 minutes and then washed in deionized water and transferred to germination paper and placed in a germination chamber, in a photoperiod of 12 h (40 μmol m^{-2} s^{-1}), at a temperature of 25ºC and relative humidity of 100%. After germination, the seedlings were transferred to 500

mL pots with a 50% Hoagland nutrient solution (Hoagland & Arnon, 1938) without aeration at half strength, in a greenhouse. The pH of the nutrient solution was adjusted daily to 5.0 and the solution was renewed every five days. During the experiment, the average temperature in the greenhouse was 19.7 °C (minimum of 7.4 °C and maximum of 38 °C), relative humidity minimum of 20% and maximum of 90% and maximum day-accumulated solar radiation incident of 57071.88 W m^{-2}.

When the seedlings reached the growth stage V6 (Counce et al., 2000) the solution was adjusted for full strength, and two Fe^{2+} concentrations provided by $FeSO_4$-EDTA (0.019; control and 7 mM; excess Fe) were applied (Pinto et al., 2016). Five days after Fe excess application, between 8:00 and 12:00 h, the plants were transferred to a temperature chamber (Fig. S1) where heatwaves were applied at different temperatures (35, 40, 45, 50, and 55 °C) for 30 minutes, in addition to the control (25 °C), the application was in the order of increasing temperature. Immediately after heatwave application the plants were removed from the chamber and evaluated for gas exchange and chlorophyll a fluorescence.

2.2 - Gas exchange measurements

Gas exchange measurements were performed with an infrared gas analyzer (LI-6400; Li-Cor Inc., Lincoln, NE, USA), shortly after each application of temperatures between 8:00 and 12:00 h, in fully expanded leaves. We measured the net photosynthetic rate (A_n, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and the ratio between the intern and extern CO_2 concentration (C_i/C_a). Measurements were performed using irradiance of 1500 μmol m^{-2} s^{-1}, flow rate of 500 μmol s^{-1} and atmospheric CO_2 concentration of 400 μmol mol^{-1}. The temperature in the IRGA chamber was adjusted before measurements according to the applied temperature (except for the temperature of 55 °C, in which the IRGA chamber was adjusted to 50°C). The means of vapor pressure deficit and leaf temperature during gas exchange measurements are presented in Fig. S2.

Before and 24 h after the application of the heatwave at the temperature of 45°C, A_n-C_i curves (Long & Bernacchi, 2003), and respiration measurements in the dark (R_d, μmol m^{-2}s^{-1}) were performed. The A_n-C_i curves were performed by changing the reference CO_2 concentration in 12 steps (400, 300, 200, 100, 50, 400, 600, 700, 800, 1000, 1200, and 1300 μmol CO_2 mol^{-1}) and using an irradiance of 1500 μmol photon m^{-2} s^{-1}, flow rate of 500 μmol s^{-1}, and a temperature of 25 ºC for all treatments. From the A_n-C_i curves, the maximum carboxylation rate of Rubisco (V_{cmax}, μmol m^{-2} s^{-1}), and the electron transport rate for carboxylation (J_{max}, μmol m^{-2} s^{-1}), the triose phosphate utilization (TPU) (Sharkey,

2007) were determined. R_d measurements were performed using a nocturnal temperature of 25 ºC and an atmospheric CO_2 concentration of 400 μmol mol^{-1}. In addition to control (25 ºC) and 45 ºC, the *Rd* measurements were also performed in plants exposed to a temperature of 55°C.

2.3 - Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed immediately after heatwave application, using the Mini-PAM modulated pulse fluorometer (Heinz Walz, Efeltrich, Germany). The rice leaf was exposed to a light source (1500 μmol m^{-2} s^{-1}) for 30 seconds to achieve fluorescence in steady-state (F_s), and a saturation pulse was applied for 0.8 seconds to obtain the maximum fluorescence of light-acclimatized leaf tissue ($F_{m'}$) (Genty et al., 1989). Then, the leaves were acclimated in the dark for at least 30 minutes to obtain the minimum (F_0) and maximum (F_m) fluorescence. The maximum quantum yield of photosystem II (PSII) was calculated by the following equation: $F_v/F_m = (F_m - F_0)/F_m$ (Genty et al., 1989). From these parameters, it was possible to calculate: the effective quantum yield of PSII in illuminated plant tissue ($\phi_{PSII} = (F_{m'} - F_s)/F_{m'}$); non-photochemical quenching (NPQ = $F_m - F_{m'}/F_{m'}$), and the apparent rate of electron transport (ETR = $\phi_{PSII} \times$ PPFD $\times lA \times 0.5$). Where, PPFD = photosynthetic photon flow density, lA = leaf absorbance coefficient assumed to be 0.85 for rice and 0.5 = the partition of quanta absorbed between the two photosystems (Melis et al., 1987; Bilger & Björkman, 1990; Genty et al., 1996; Li et al., 2009).

2.4 - Assessing photorespiration in vivo

The photorespiration rate (P_r) was estimated before and after the application of heatwaves at the temperature of 45°C by using the following formula (Valentini et al., 1995):

$$P_r = \frac{1}{12}[ETR - 4(A + R_d)]$$

2.5 - Leaf relative water content (RWC)

The RWC was performed according to the methodology described by Weatherley (1950). Two leaf segments of approximately 2 cm each were collected from expanded leaves and immediately weighed to obtain the fresh mass (FM). Leaf segments were placed in falcon tubes containing deionized water and kept in refrigeration for 24 hours. After this period, the segments were placed on paper towels for the removal of excess water and weighed to

determine the turgid mass (TM). For the dry mass (DM) the segments were dried at 65°C to constant weight. The equation for calculating RWC was:

$$RWC\ (\%) = \frac{(FM - DM)}{(TM - DM)}\ x\ 100$$

2.6 - Membrane permeability

After the application of thermal treatments, membrane permeability was evaluated by electrolyte leakage. In fully expanded leaves, two leaf segments of approximately 2 cm each were collected in 6 mL of deionized water. Samples were infiltrated under vacuum for 5 min. Tubes were shaken and the conductivity of the water in each tube was immediately measured with a conductivity meter (Quimis, Q405M). The tubes were then heated for 20 min in a water bath at 100 °C and after cooling the final conductivity of the solution was measured. Electrolyte leakage or percent damage was calculated as:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Conductivity after exposure to temperature (°C)}}{\text{Conductivity after exposure to 100 °C}}\ x100$$

2.7 - Morphological variables and iron concentration

The evaluation of the dry weights of the root and shoots was performed 24 h after the application of the heatwaves. The plants were dried at 75°C until reaching constant weight. The Fe concentrations were determined in the shoots of the two cultivars according to the methodology of Tedesco et al. (1997), through atomic absorption spectrophotometry after digestion with a nitric-perchloric acid solution (3:1) at 200 °C.

2.8 - Statistical analyses

The experimental design consisted of randomized blocks with four replicates. Data processing and analysis were performed within the R software environment (R Core Team, 2014). The effect of temperature and Fe concentrations, and their interaction, on the cultivars, was determined using an analysis of variance (ANOVA). The 'anova_test()' function from the rstatix package was used. Subsequently, Tukey tests were performed for multiple comparations between Fe concentrations within temperatures, performed using the 'tukey_hsd() function'.

2.8. Data set compilation

Research papers were searched from the Wiley Online Library, ScienceDirect, SpringerLink, and Frontiers using four search terms: 'Iron toxicity +photorespiration + rice', 'Iron toxicity + photosynthesis + rice', 'Heat stress + photosynthesis + rice', and 'Heat stress + photorespiration + rice' in November 2022. An initial search resulted in 32.947 articles (total results from the four databases), which were reduced to 3.044 by limiting research to agriculture, environmental science, physiology, and biochemistry. Then, 14 published articles describing the results of 23 different rice cultivars on V_{cmax}, J_{max}, NPQ, F_v/F_m, P_r, and ETR, were compiled and data were extracted from tables or the text, if possible, otherwise digitized from figures using WebPlotDigitizer v.4.6 (https://automeris.io/WebPlotDigitizer). The employed data are detailed in Supplemental Data Set S1.

The different rice cultivars were classified according to their susceptibility (tolerant, sensitive, not identified) to excess Fe or heat stress. The results of the meta-analysis of cultivars regarding their sensitivity and treatments for iron toxicity or heat stress were presented on the heat map. Data were presented as means of replicates.
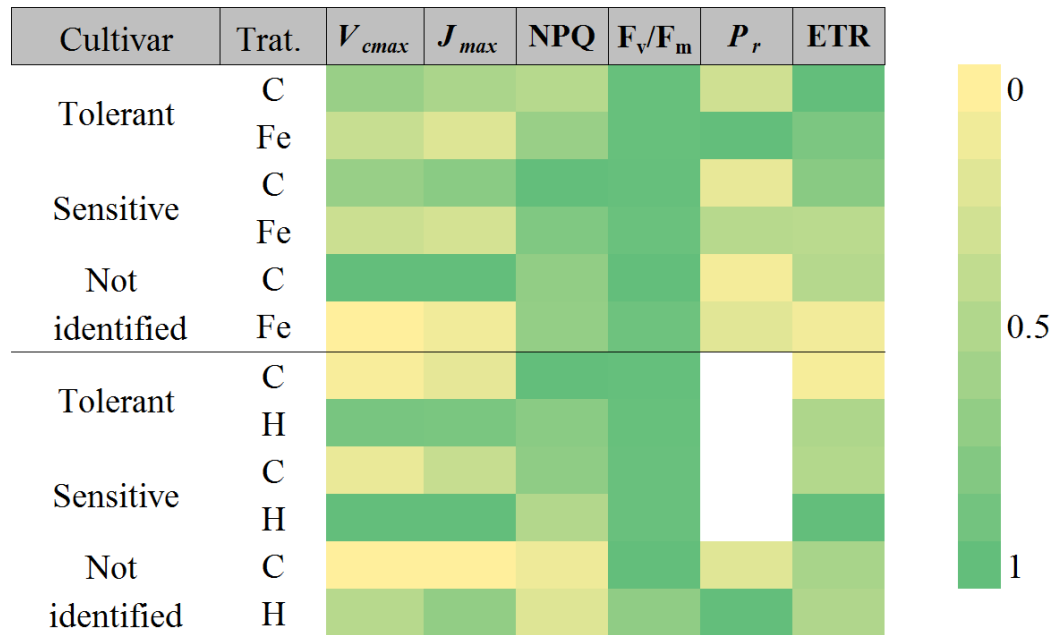
3- RESULTS

3.1 – Cultivars sensitive to iron toxicity or heat stress are inefficient in absorbing and dissipating excess light energy.

The sensitivity of the twenty-five cultivars, including the two used in the experimental approach, was distributed as follows: iron toxicity (four tolerant, three sensitive, and seven not identified) heat stress (two tolerant, two sensitive, and nine not identified). Fig. 1, illustrates a heatmap of the summarized results of the cultivars when submitted to treatments with Fe toxicity or heat stress. In general, cultivars sensitive and non-identified when exposed to iron toxicity showed reductions in the variables related to photosynthetic efficiency. Reductions in F_v/F_m and ETR in the sensitive cultivar were linked to a small increase in Pr and low efficiency in the NPQ mechanisms.

In general, heat stress increases V_{cmax}, and J_{max} and decreases NPQ in rice cultivars. However, reduced NPQ values were observed in the tolerant cultivars under heat stress.

The amount of data available for modeling the relationship between photoprotective mechanisms and photosynthetic efficiency is limited. Few papers have presented a response to Pr, mainly considering heat stress. However, it was observed that increased Pr occurs in not-identified cultivars regarding heat stress, and in Fe-tolerant cultivars under iron excess (Fig.

1). In addition to the present study, no articles were found for cultivars tolerant to both factors, excess iron and heat stress.

Cultivar	Trat.	V_{cmax}	J_{max}	NPQ	F_v/F_m	P_r	ETR
Tolerant	C						
	Fe						
Sensitive	C						
	Fe						
Not identified	C						
	Fe						
Tolerant	C						
	H						
Sensitive	C						
	H						
Not identified	C						
	H						

Fig. 1 - Heatmap of the maximum carboxylation rate of Rubisco (V_{cmax}), electron transport rate for carboxylation (J_{max}), *non-photochemical quenching* (NPQ), the maximum quantum efficiency of photosystem II (F_v/F_m), photorespiration (P_r) and the apparent rate of electron transport (ETR) of twenty-five cultivars under iron toxicity (Fe) or heat stress (H). Data were presented as means of replicates found in the studies, according to Sup. Data Set S1. The data in publications were transformed according to the false color code scale, ranging from 0 to 1 in response to the treatments with iron excess (Fe), heat (H) or control conditions (C).

3.2 - The increase in heatwaves temperature provided a higher concentration of Fe in the shoot of the tolerant cultivar.

The excess of Fe in the nutrient solution promoted a greater accumulation of Fe in the shoot of the two rice cultivars, regardless of heatwaves treatments (Fig. 2). However, the two cultivars presented different responses to the accumulation of Fe in the shoots. Only the cultivar tolerant to excess Fe (IRGA 424) proportionally increased the concentration of Fe with the application of heatwaves meanwhile the sensitive cultivar remains stable (IRGA 417) (Fig. 2A and 2B). No significant effects of the treatments were observed on the shoot and root biomass (Fig. S3) in both cultivars.
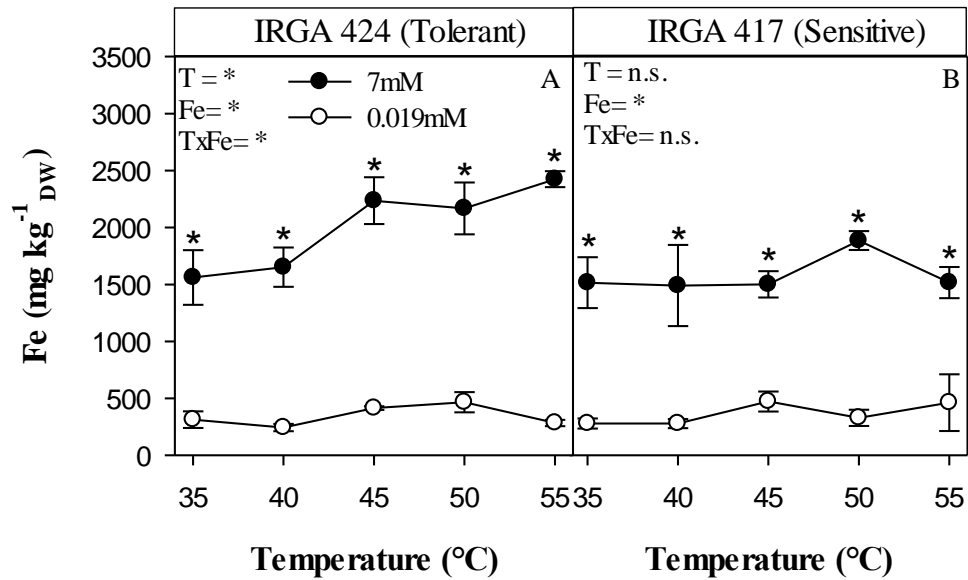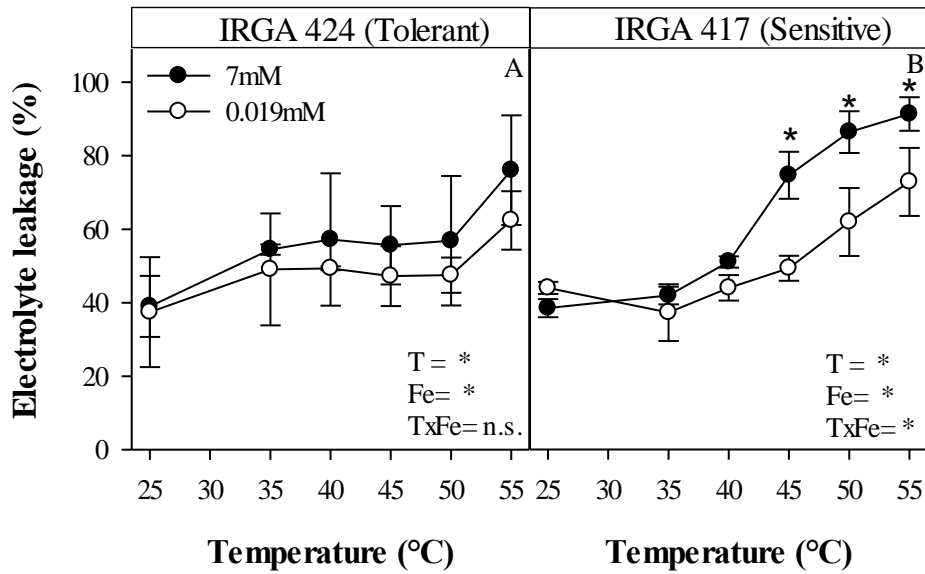
Figure 2 – Iron (Fe) concentration in the shoot of two rice cultivars under control conditions (0.019 mM; open symbols) and 7 mM (closed symbols) at different temperatures. Asterisks indicate significant differences (p <0.05) between treatments with $FeSO_4$-EDTA within temperatures.

3.3 - High temperatures during heatwaves promoted greater damage to the membrane in the sensitive cultivar under excess of Fe

The excess of Fe in the nutrient solution promoted greater extravasation of electrolytes in the two rice cultivars (Fig. 3). However, the tolerant cultivar IRGA 424 did not present a significant effect on the interaction of temperatures and Fe concentration, being the highest percentage of electrolyte leakage observed at 55°C at both treatments (Fig. 3A). Meanwhile, the sensitive cultivar (IRGA 417), presented significant higher electrolyte leakage from the application of the temperature of 45°C under Fe excess, with significantly higher values than the control treatment, and also including a significant interaction between the heatwave and temperature that was not observed in the tolerant cultivar (Fig. 3B).

Figure 3 - Electrolyte leakage in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures. Asterisks indicate significant differences (p <0.05) between treatments with $FeSO_4$-EDTA within each temperature.

3.4 – Fe excess and increased heatwaves temperatures provokes A_n reductions associated with stomatal closure

Under conditions of excess Fe, the two cultivars presented a lower net photosynthetic rate (A_n). With the increase in heatwave temperature associated with higher vapor pressure deficit (VPD) (Fig. S2), the A_n showed a decrease for both cultivars (Fig. 4), with a significant reduction, from the application of temperatures of 45°C and 40°C for cultivars IRGA 424 and IRGA 417, respectively.

The significant reduction in g_s for both cultivars under increased heatwave temperature (Fig. 4) was not caused by changes in the relative water content, since no significant effects of the treatments were observed (Fig. S4), The reduction of A_n with a lower temperature in the cultivar IRGA 417, is related to the lower g_s after the application of the heatwaves. Although the two cultivars reduced the g_s in the first heatwave temperature (35°C), only the sensitive cultivar IRGA 417 showed also a significant reduction of 72% due to the interactive effects with the excess Fe.

The transpiration rate (E) in both rice cultivars was lower with exposure to excess Fe, but there was no isolated effect of temperature. The exposure of cultivars at

temperatures 35, 40, and 45°C decreased the C_i/C_a ratio. However, as well as at ambient temperature of 25°C, there were increases in this ratio at the highest temperatures of 50 and 55°C.
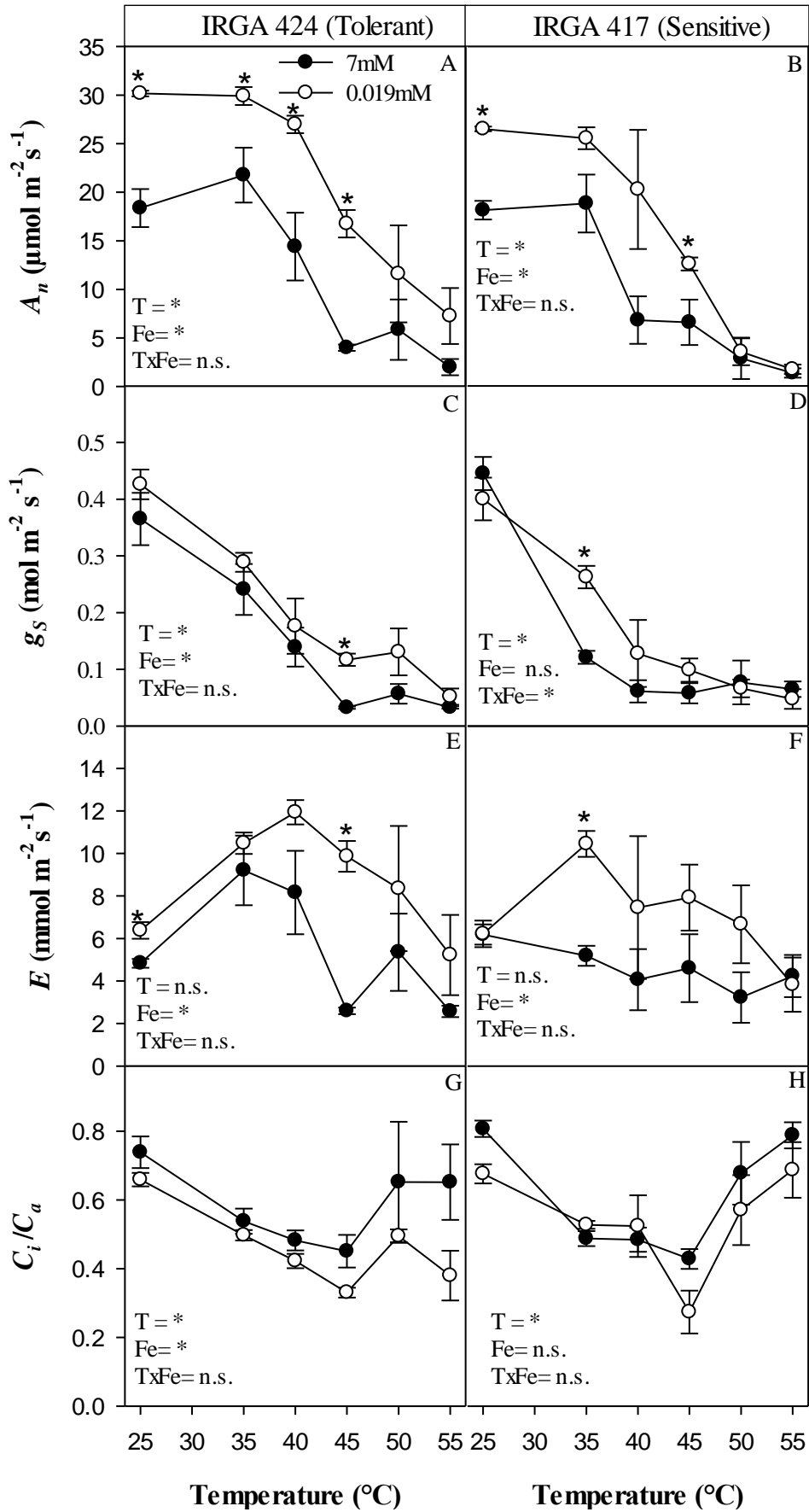
Figure 4 - Net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration (E) and the relationship between internal and external CO_2 concentration (C_i/C_a) in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures. Asterisks indicate significant differences ($p < 0.05$) between treatments with $FeSO_4$-EDTA within temperatures.

3.5 - The cultivar tolerant to Fe excess was more efficient in mechanisms such as NPQ and P_r for the protection of photosystems with the increase in heatwave temperature, even with reductions in V_{cmax}

The maximum carboxylation rate of Rubisco (V_{cmax}), the electron transport rate for carboxylation (J_{max}), and the utilization of trioses phosphate (TPU) showed no interactive effect of temperature and Fe concentrations (Fig. 5). However, there was an isolated effect of excess Fe in these variables for both cultivars. The two cultivars reduced the V_{cmax} after the application of temperature and also in a condition of excess Fe.

The J_{max} and the TPU did not change after the application of the temperature, but were reduced in plants exposed to the condition of excess Fe. The J_{max} in the Fe-sensitive cultivar before the application of the temperature showed no difference between the concentrations of Fe, only after temperature application.
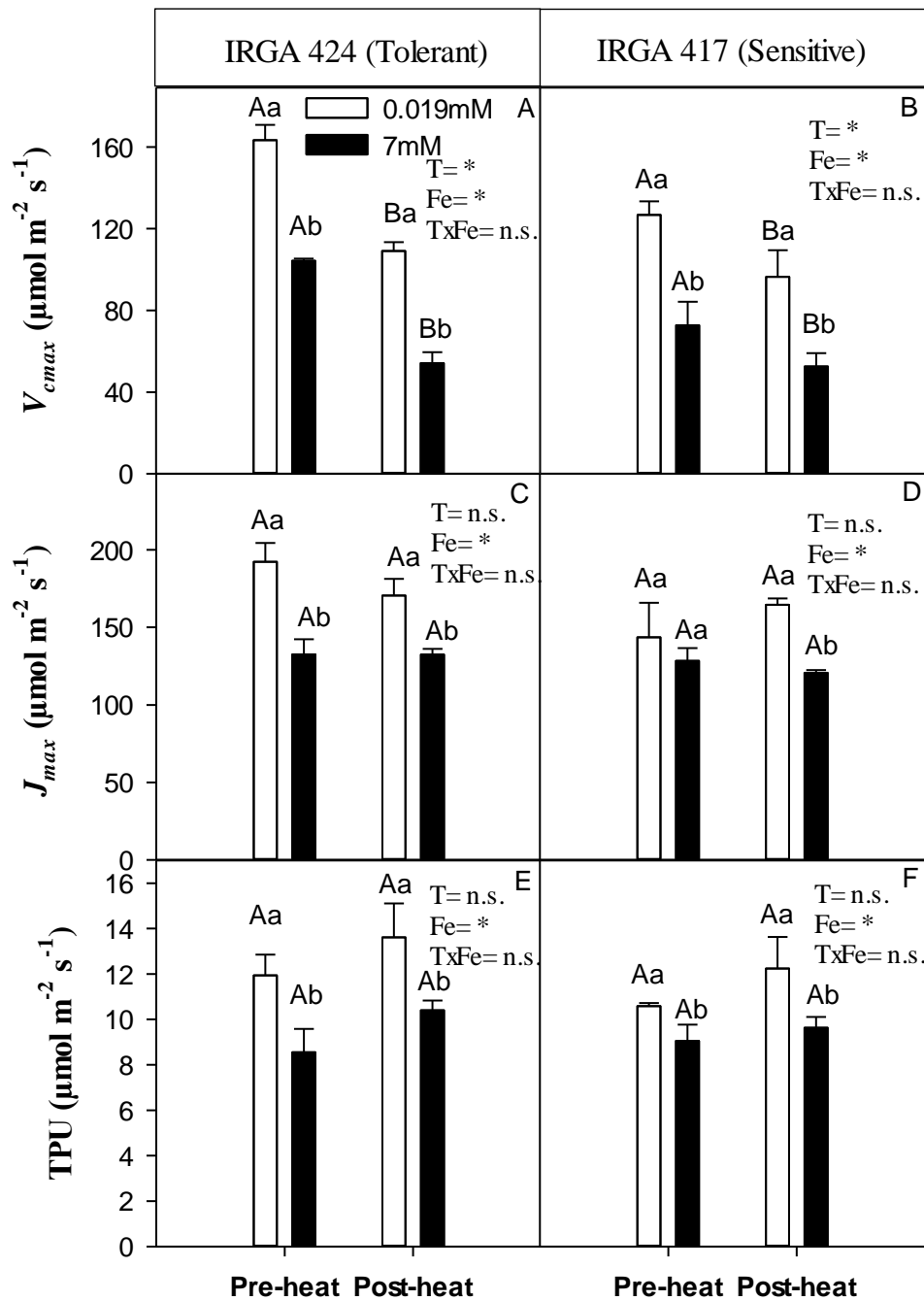
Figure 5 - The maximum carboxylation rate of Rubisco (V_{cmax}), electron transport rate for carboxylation (J_{max}), and utilization of trioses phosphate (TPU) in two rice cultivars under two Fe conditions (0.019 mM and 7 mM) before and after temperature application (45°C). Upper case letters compare pre-heat and post-heat, and lower case letters compare the iron treatments (p <0.05).

The increase in Fe concentrations in the nutrient solution did not present a significant effect on the maximum quantum efficiency of photosystem II (F_v/F_m) in both cultivars (Fig.

6). However, temperature application showed a similar response in F_v/F_m in both cultivars, in which a reduction was observed from the temperature of 45°C. The reduction of F_v/F_m in the highest temperature of heatwave (55°C) compared to control temperature (25°C) was more pronounced in the sensitive cultivar, being 55.4% and 34.9% for cultivar IRGA 417 and IRGA 424, respectively.

Under excess Fe, the effective quantum yield of PSII (ϕ_{PSII}) and the apparent rate of electron transport (ETR) decreased in both cultivars. Regarding temperature, cultivars IRGA 424 and IRGA 417 maintained ϕ_{PSII} and ETR up to 45 and 35°C, respectively (Fig. 6). The non-photochemical quenching (NPQ) increased in cultivar IRGA 424 (Fig. 6) under the condition of excess Fe, and exposure to high temperatures. On the contrary, the cultivar IRGA 417 did not present a significant difference in the mechanism of energy dissipation in a condition of excess Fe. However, this cultivar under control treatment (0.019 mM Fe) at 55 ºC presented a higher NPQ (Fig. 6). Despite no interaction between excess Fe and increasing temperature, the sensitive cultivar showed more accentuated reductions in ϕ_{PSII} and ETR under excess Fe at each applied temperature.
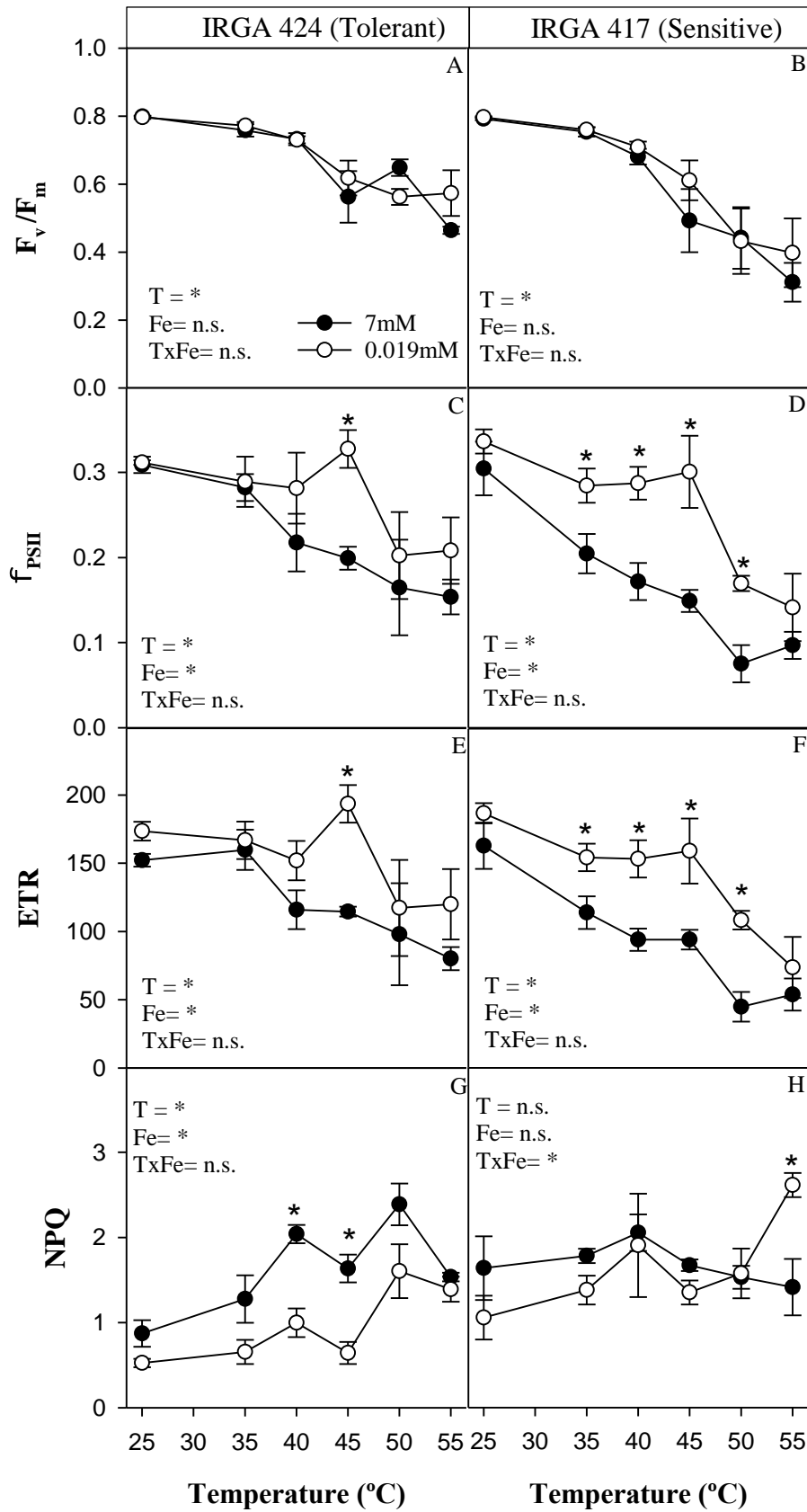
Figure 6 - The maximum quantum efficiency of photosystem II (F_v/F_m), the effective quantum yield of PSII in illuminated plant tissue (ϕ_{PSII}), the apparent rate of electron transport

(ETR), and *non-photochemical quenching* (NPQ) in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures. Asterisks indicate significant differences (p <0.05) between Treatments with FeSO$_4$-EDTA with each temperature.

Dark respiration (R_d) did not present a significative effect before and after the application of the heatwave at a temperature of 45°C, and regarding conditions of Fe in both cultivars. However, when the cultivars were submitted to a temperature of 55°C, the highest R_d was verified in cultivar IRGA 417 in the condition of excess Fe (Fig. S5). Even before the exposition to the heatwave, the tolerant cultivar under excess of Fe presented higher photorespiration (P_r) than the control plants. With temperature application, the sensitive cultivar in excess Fe condition reduced P_r. However, the tolerant cultivar showed no difference from the control treatment. Under adequate conditions of Fe, both cultivars increased the P_r after the application of the temperature (Fig. 7).
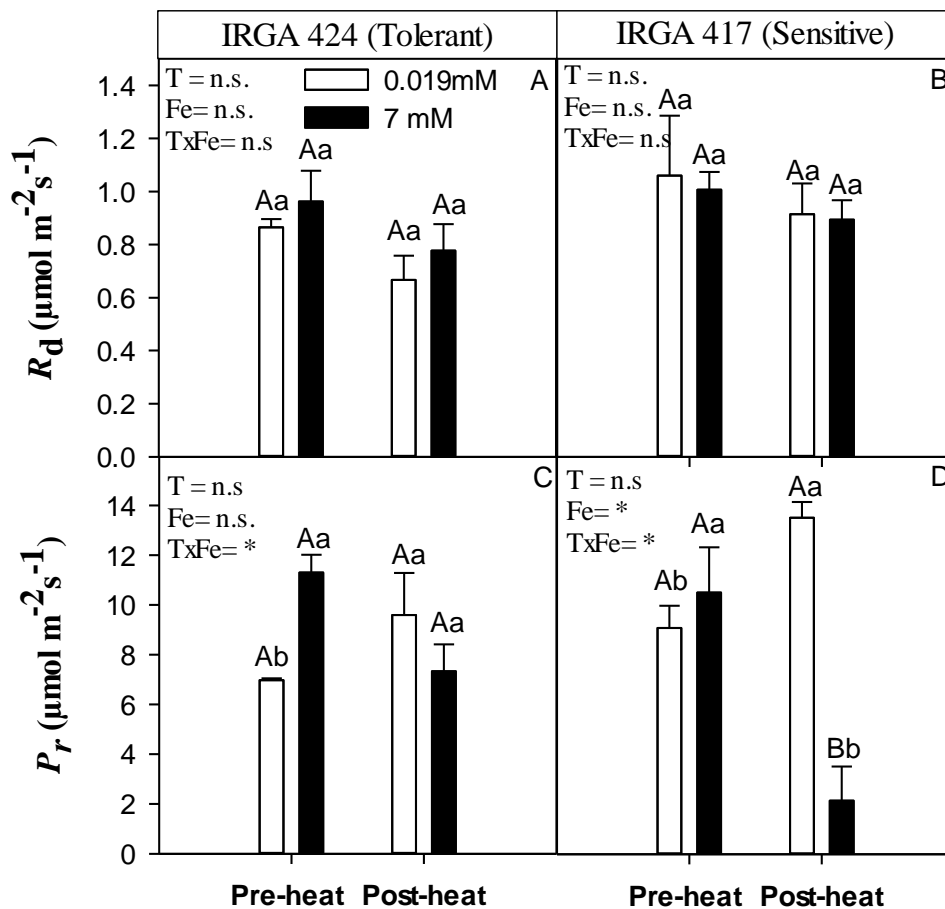
Figure 7 - Dark respiration (R_d), and photorespiration (P_r) in two rice cultivars under two conditions Fe (0.019 mM and 7 mM) before and after application of temperature (45°C).

Upper case letters compare pre-heat and post-heat, and lower case letters compare the iron treatments (p <0.05).

4- DISCUSSION

The Fe toxicity enhances with increased temperature during heatwaves in rice cultivars, which showed different thermotolerance mechanisms concerning the degree of tolerance to Fe excess. The tolerant cultivars to excess Fe presented strategies that provided efficient adjustments at higher temperatures. Photoprotective mechanisms and photorespiration are key points for iron stress tolerance in rice and heatwaves. These results would contribute to the selection of new cultivars, or even indicate a tolerant cultivar in places where excess Fe and temperatures are harmful factors for photosynthetic efficiency and yield.

The two cultivars used in the experimental approach, IRGA 417 and IRGA 424, were previously characterized as sensitive and tolerant to Fe excess, respectively (SOSBAI, 2018). Under excess of Fe, the two cultivars accumulated the metal above the critical levels for toxicity (>300 - 500 mg kg^{-1} DW) (Dobermann & Fairhurst, 2000). One of the proposed mechanisms of tolerance to Fe excess in rice plants consists of excluding Fe from the roots and using oxygen derived from aerenchyma or enzymatic activity to oxidize Fe^{2+} in Fe^{3+}, which precipitates as a plate of Fe on the root surface (Wu et al., 2014). However, the increase in temperature promoted Fe accumulation in the shoot of the tolerant cultivar (Fig. 2), which, even if the Fe plaque would be considered as mechanism of resistance (Pereira et al., 2014), it did not prevent Fe absorption during heatwaves.

This accumulation of Fe in the tolerant cultivar does not necessarily indicate the state of physiological resistance. The tolerant cultivar could show other resistance strategies. Fe can be absorbed and later compartmentalized in proteins such as ferritins or stored on the vacuoles, or repair mechanisms to damage attributed to EROs and induction of antioxidant systems (Majerus et al., 2007; Stein et al., 2014; Wu et al., 2014; Siqueira-Silva et al., 2019). Low molecular mass antioxidants (ascorbic acid, glutathione) and ROS-interacting enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX) dehydroascorbate reductase (DHAR) may act as tissue tolerance mechanisms to eliminate or reduce the damaging effects caused by Fe overload (Stein et al., 2014; Wu et al., 1998).

The higher accumulation of Fe in tolerant cultivar due to higher temperatures did not result in higher membrane damage (Fig. 3). It indicates that together with the photoprotective

mechanisms and P_r maintenance, the antioxidant system are sufficient to prevent damage mediated by ROS, to keep the deleterious effects to a minimum (Müller et al., 2015; Stein et al., 2014). At the same time, the damage to the membrane of the sensitive cultivar with exposition at heatwaves above 45°C may have occurred due to the direct effects of high temperature, photooxidation of chlorophyll pigments (Fig. 6B), impaired electron flow (Fig. 6D) and inhibition of carbon fixation (Fig. 4B).

Reduction in A_n may occur by decreasing the intercellular concentration of CO_2, as well as the low production of ATP and NADPH from photochemical reactions (Wang et al., 2017). Thermal stress reduces A_n when g_s decreases with increasing VPD (Berry & Björkman, 1980.) At the same time, under excessive Fe the rice plants close their stomata and consequently limit the absorption of Fe through transpiration flow, which can affect the exchange rate of CO_2 and the related photosynthetic functions, causing the growth of the plant to be reduced (Goicoechea et al., 2001). However, until 45°C, g_s relates tightly with A_n, but after that temperature, other factors are the limiting ones, such as the photobiochemistry (Fig. 4).

Under excess Fe and increasing temperature during heatwaves, A_n and g_s were reduced in both cultivars. However, the maintenance of higher g_s in tolerant cultivar under excess Fe and, consequently, the higher photosynthetic rates at temperatures between 35-40°C compared to the sensitive cultivar (Fig. 4), indicates a difference between cultivars for the speed of response in stomatal closure. This delayed resistance response concerning stomatal closure observed for tolerant cultivars under Fe excess conditions (Dufey et al., 2009; Pereira et al., 2013) was also influenced by temperature.

The closure of stomata in the sensitive cultivar under excess Fe, from the first applied temperature (35°C) may have contributed indirectly to oxidative damage together with reduced ETR and ϕ_{PSII} (Fig. 6). Oxidative stress induced by high temperatures causes the dissociation of the oxygen evolution complex (OEC) in the PSII. This results in the inhibition of electron transport of the OEC towards the accepting side of the PSII and subsequently photoinhibitory processes, contributing to the non-stomatic effects in photosynthesis (Essemine et al., 2017), which were observed at temperatures above 40°C. Although many studies report that cultivars more sensitive to Fe excess have a higher susceptibility to photoinhibition (Müller et al., 2015, Pinto et al., 2016; Pereira et al., 2013), the changes in F_v/F_m were not significant for cultivars concerning Fe applications (Fig. 1 and 6). This response may be related to the period of 5 days of exposure to excess Fe since some studies present a difference in F_v/F_m occurring from 7 days after application, as observed in the

results from Sup. Data Set S1. This indicates the robustness of F_v/F_m as a physiological indicator of severe stress conditions, in which its impairment only occurred after significant changes in electrolyte leakage due to heatwaves, mainly in the sensitive cultivar.

Under thermal stress and Fe toxicity plants are potentially exposed to excess excitation energy, which will inevitably result in damage to the photosynthetic apparatus if this excess energy cannot be safely dissipated (Yin et al., 2010: Pereira et al., 2013; Pinto et al., 2016). The ability to overcome the effects of reduced photosynthetic capacity by dissipating the excess energy through non-photochemical quenching (NPQ) (Rochaix, 2014), was evident in tolerant cultivars (Fig. 1 and 6). The earlier activation of NPQ in the tolerant cultivar IRGA 424, when exposed to heatwaves and Fe excess, was an important mechanism that protected PSII reaction centers from damage by dissipating excess light energy in the form of heat (Fig. 6G). However, the inefficiency of excess energy dissipation in sensitive cultivars at higher temperatures may be related to a higher percentage of electrolyte extravasation (Fig. 3B), which caused damage mainly to the thylakoid membrane and may prevent the formation of a gradient of transmembrane electrochemical potential in chloroplasts, avoiding dissipation of excess energy in the form of NPQ.

The two cultivars showed a negative regulation in V_{cmax} for both Fe excess and heatwave exposure at 45ºC. However, the reduction due to the temperature rise is probably linked to the thermo-sensitivity of *Rubisco Activase* (Feller et al., 1998; Makino and Sage, 2007) and general metabolic reprogramming in chloroplasts (Wang et al., 2018). The reduction of V_{cmax} and the electron transport rate for carboxylation (J_{max}) (Fig. 1, 5) proved to be more responsive to Fe toxicity than to heatwaves. Iron excess inside the plant can lead to the enhancement of oxidative stress, resulting from the overproduction of reactive oxygen species, compromising most metabolic processes, including photochemical impairment of photosynthesis, reduction in the ETR and J_{max}, with a consequent reduction in the A_n and V_{cmax} as observed also for other authors in the Sup. Data Set S1 (Fang et al., 2001; Majerus et al., 2009; Nenova 2009; Pereira et al., 2013).

Under ideal conditions, the vast majority of energy is provided for photosynthetic carbon reduction (Dani et al., 2014). However, considering that the internal CO_2 levels were restricted by stomatic closure until the temperature of 45ºC during heatwaves (Fig. 3), the demand for the carbon reduction cycle decreases and with this, the fraction of energy used in A_n and also affected the V_{cmax}. At higher heatwave temperatures the damage to enzymatic (including Rubisco) and other cell components highlighted by the higher electrolyte leakage, mainly in sensitive cultivar, surpassed the diffusive and photobiochemistry limitations of

photosynthesis. P_r has been indicated as an important mechanism to relieve photodamage under conditions of high light and low CO_2 (Dani et al., 2014), as well as under excess of Fe (Pereira et al., 2013), as confirmed by the present study, mainly for tolerant rice cultivars exposed to the isolated effects of Fe excess and heatwaves. The impairment of the photorespiratory pathway, as observed in plants under excess Fe at high temperatures, would lead to the suppression of the repair of the photodamaged PSII by inhibiting the synthesis of protein D1 in the translation stage (Takahashi et al., 2007), being much more expressive in sensitive rice cultivars. The P_r proved to be an efficient alternative electron sink (Pereira et al., 2013) for the tolerant cultivar under excess of Fe. After heatwaves in both cultivars under Fe excess, the electron draining capacity in the P_r was affected, and the greater reduction in the sensitive cultivar may be related to greater electrolyte leakage and lower ETR.

The maximum temperature values in the experimental period may have resulted in R_d acclimatization in both cultivars before and after the application of temperature (45ºC) (Fig. 6). However, the acclimatization process in the sensitive cultivar did not lead to the balance between gain (A_n) and carbon release (R_d) upon 45ºC temperature application (Fig. 4B, 7D). Fe excess showed to be a more responsive factor than temperature up to 45°C for both rice cultivars (Fig. S5). R_d can be divided into growth respiration which is determined by the amount and chemical composition of the new biomass generated, and by maintenance respiration which covers the costs of keeping existing tissues functional (McCree, 1970). Thus, taking into account that there was no change in dry matter in the roots and leaf (Fig. S4), the increase of Rd in cultivars above Fe is at 55ºC in the sensitive cultivar under excess of Fe, which was intended for cell maintenance and repair after stress. The increase in respiration at the leaf and whole plant level was attributed to substantial losses of yield in rice (Mohammed and Tarpley, 2009).

5- CONCLUSIONS

The tolerance mechanisms of rice cultivars to Fe excess contributed to better responses during heatwaves without compromising their photosynthetic efficiency. Mechanisms such as non-photochemical quenching (NPQ) and photorespiration (P_r) were of great importance for the protection of photosystems under Fe toxicity and heatwaves, but can be impaired by increasing temperature mainly in sensitive rice cultivars. Such evidence indicates that the Fe-tolerant cultivar IRGA 424 presented better physiological dynamics under excess of Fe and did not compromise A_n at a temperature of 40 °C.

At the same time, a great challenge for genetic improvement of rice cultivars under excess of Fe. Thus, the compression of the events involved in the role of Fe in the response to temperature increase will facilitate the development of new cultivars tolerant to climate change.

Acknowledgments

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SUPPORTING INFORMATION

Photoprotective mechanisms and higher photorespiration are key points for iron stress tolerance under heatwaves in rice plants

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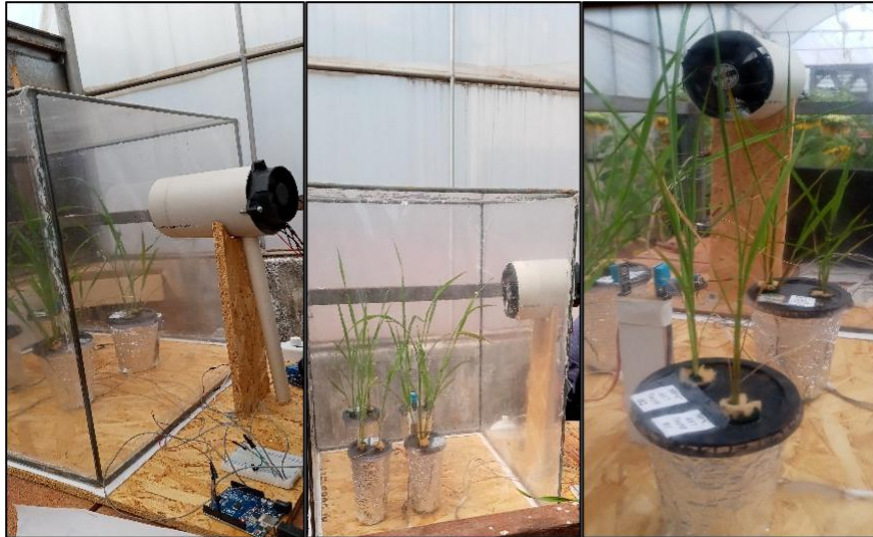
Figure S1. Temperature chamber (50x50cm), made of steel frame, covered with plastic film. The temperature was controlled by an arduino board and a resistance. The programming on the arduido board setted the desired chamber temperature and the resistance was turned on or off, when it deviated by 1° C from the programmed temperature.
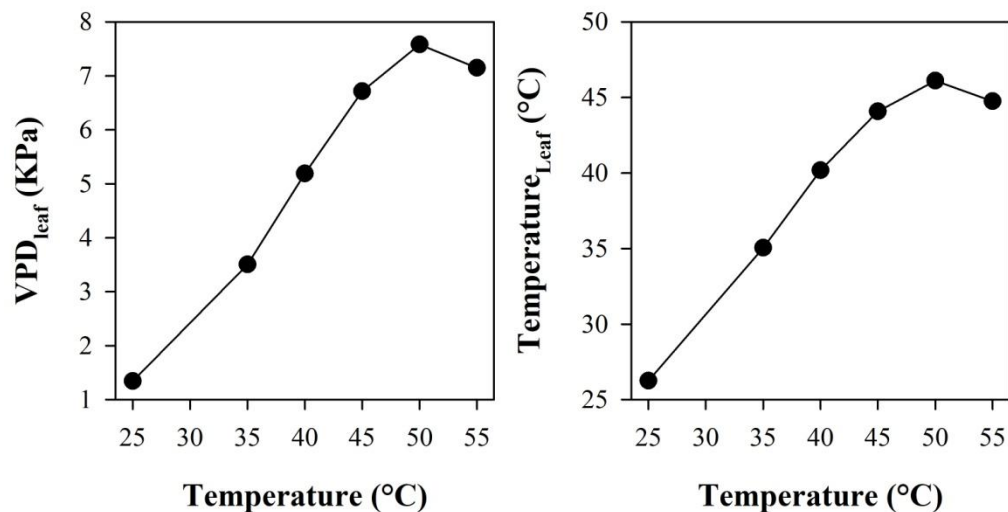

Figure S2 – Estimated vapor pressure deficit and temperature of leaves under heatwaves during gas exchange evaluations.
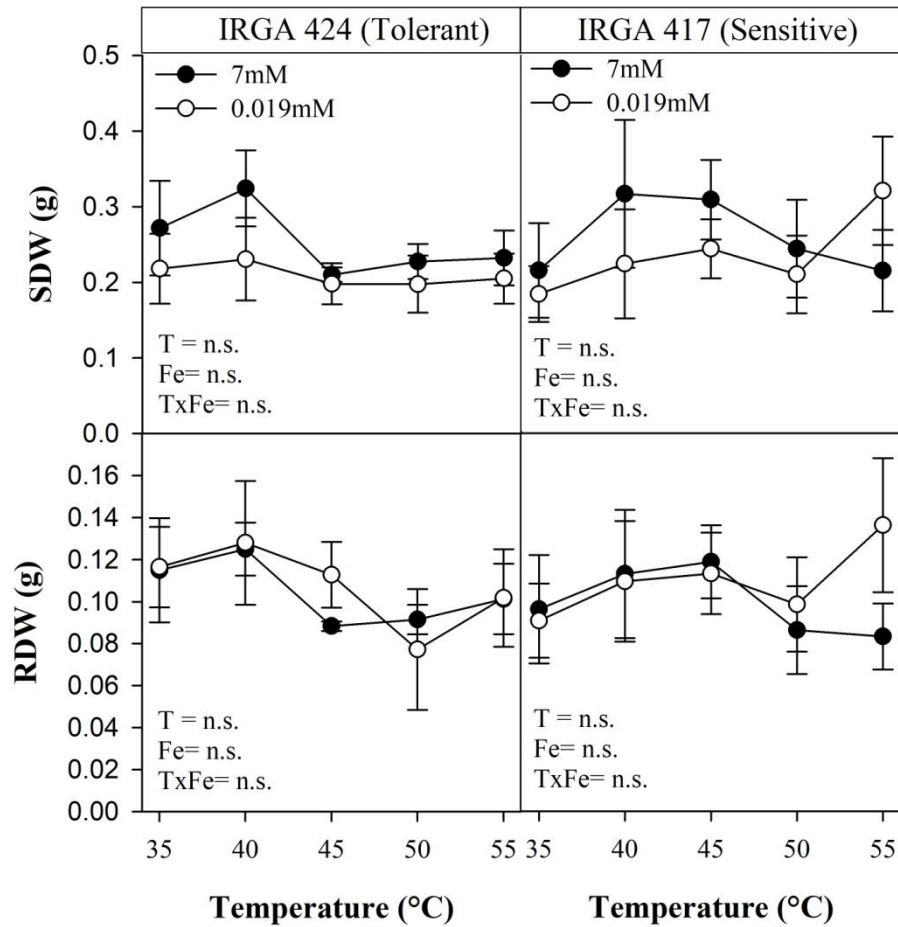
Figure S3 - Dry shoot (SDW) and root (RDW) in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures.
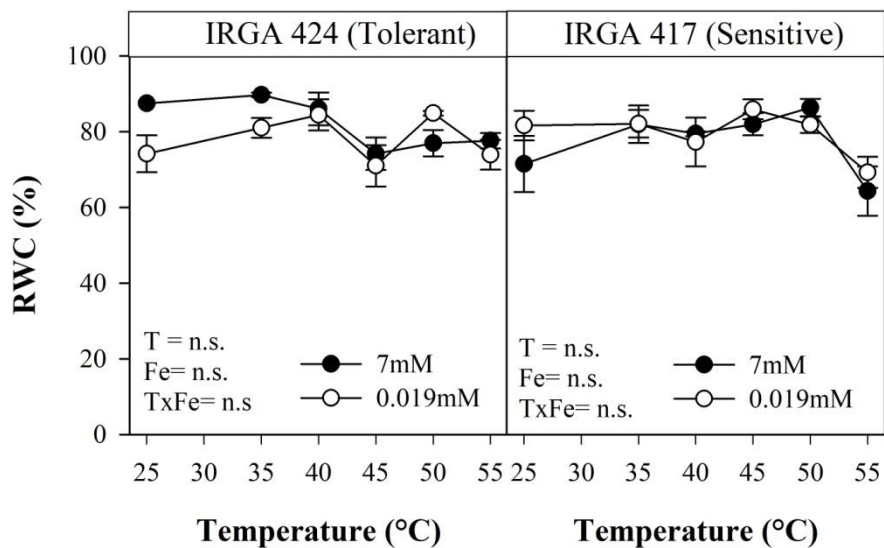
Figure S4- Relative water content (TRA) in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures.
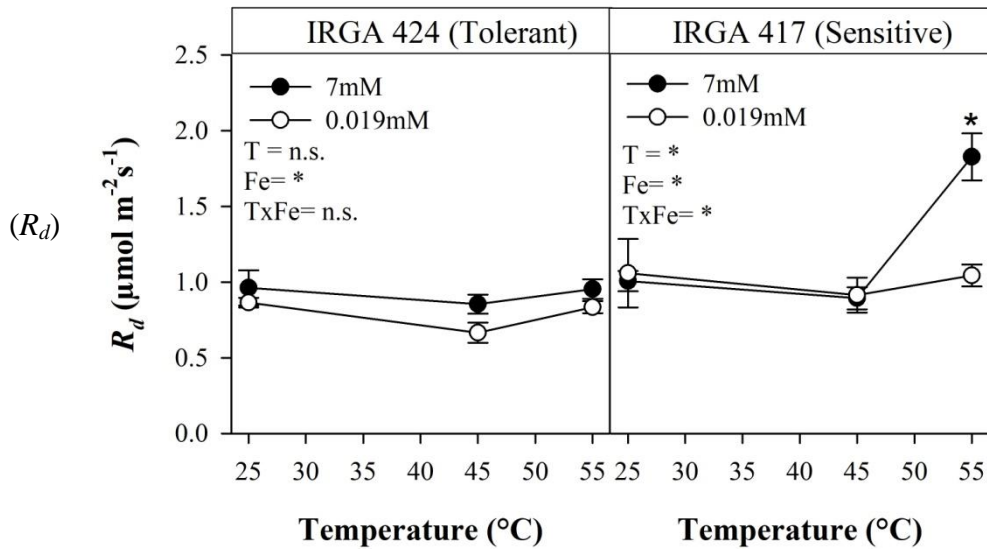
Figure S5 - Dark respiration in two rice cultivars under control conditions (0.019 mM; open symbols) and Fe-excess (7 mM; closed symbols) at different temperatures. Asterisks indicate significant differences ($p < 0.05$) between FeSO4-EDTA treatments within temperatures.

CONSIDERAÇÕES FINAIS

Os resultados mostraram que a tolerância e a sensibilidade das duas cultivares de arroz estão diretamente ligadas às concentrações de Fe e ao tempo de exposição ao Fe. E que os danos oxidativos nas raízes com aumento das concentrações de Fe, levaram a uma redução do transporte de água da raiz para a folha, especialmente na cultivar sensível. E provavelmente, os danos oxidativos nas raízes levou ao fechamento dos estomas, causando limitações difusivas que se iniciam com restrições de gm. As cultivares respondem inicialmente através de limitação difusiva, e mais tarde através de restrição fotoquímica, com maior severidade na cultivar sensível. Os processos fotoinibitórios foram mais severos na cultivar sensível IRGA 417. A cultivar tolerante IRGA 424 mostrou mecanismos fotoprotectores, tais como aumento NPQ, Pr e Rd, que contribuem para a tolerância após a exposição às concentrações de Fe.

Esses mecanismos de tolerância da cultivares de arroz cultivar IRGA 424 sob ao Fe, excesso contribuíram para melhores respostas durante as ondas de calor sem comprometer a sua eficiência fotossintética. Tais evidências indicam que a cultivar IRGA 424 apresentou uma melhor dinâmica fisiológica sob excesso de Fe e não comprometeu A_n a uma temperatura de 40 °C. Assim, a compressão dos eventos envolvidos no papel do Fe na resposta ao aumento da temperatura irá facilitar o desenvolvimento de novas cultivares tolerantes às alterações climáticas. O grande desafio para o melhoramento genético das cultivares de arroz sob excesso de Fe e ondas de calor.