

Transcriptome-derived networks reconstruct distinct immune strategies to counteract fungal infection under different iron availability between *Arabidopsis* and rice[☆]

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ABSTRACT

Fe, an essential element for plants to sustain central biological functions, recently emerged as a modulator of immunity. However, our understanding of the crosstalk between Fe and defence is limited. Here, we report antagonistic immune phenotypes in *Arabidopsis thaliana* and *Oryza sativa* infected by *Colletotrichum higginsianum* and *Magnaporthe oryzae* under different Fe nutritional status. Functional and topological analysis of transcriptional networks unveiled differences in complexity and hierarchy for similar biological functions, as well as in Fe usage and distribution, between the two pathosystems, with a focus on the plant side. Mechanistically, our results are consistent with a regulatory role of Fe in the biosynthesis of the defensive metabolites glucosinolates in *A. thaliana* (joint effect hypothesis), whereas *O. sativa* uses Fe to trigger oxidative stress at infection sites (metal defence). Nevertheless, common regulatory features might differently coordinate the transcriptome responses of both species. An evolutionary interpretation of divergences in the interplay between Fe and plant immunity is discussed.

1. Introduction

Iron (Fe) is a fundamental nutrient for plants since oxidoreductions between ferrous (Fe^{+3}) and ferric (Fe^{+2}) forms under physiological conditions sustain central reactions in photosynthesis, respiration, and alleviation of oxidative burst (Connorton et al., 2017). Despite being highly abundant on Earth's crust, Fe accessibility is immensely restricted as it is predominantly present in the insoluble form Fe^{+3} due to the oxygenic conditions of the atmosphere, and many biological processes depend on Fe^{+2} . Fe bioavailability is further impeded in calcareous and alkaline soils, by the presence of chelating agents such as organic matter, or continuous leaching (Guerinot and Yi, 1994). Consequently, Fe deficiency represents a major agricultural constraint

worldwide for plant distribution and optimal yield. Under this adverse scenario, plants evolved two main mechanisms to acquire Fe from the soil. On the one hand, dicots and non-gramineous monocots induce soil acidification along with Fe^{+3} to Fe^{+2} reductive steps prior to Fe uptake by high-affinity transporters (strategy I). In *Arabidopsis thaliana* (hereafter *Arabidopsis*), the master regulator FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT), in concert with other basic-helix-loop-helix (bHLH) transcription factors, orchestrates the induction of H^{+} -ATPase 1 (AHA1), FERRIC REDUCTION OXIDASE 2 (FRO2) and IRON-REGULATED TRANSPORTER 1 (IRT1) in roots under Fe deficiency (Liang, 2022; Briat et al., 2007; Morrissey and Guerinot, 2009). On the other hand, gramineous monocots secrete phytosiderophores to the soil, i.e., organic compounds capable of chelating Fe as

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nicotianaminic and mucigenic acids, and the resulting complexes are internalised via YELLOW STRIPE1 (YS1) and YS1-LIKE (YSL) transporters (strategy II) (Liang, 2022; Briat et al., 2007; Morrissey and Gueriot, 2009). Despite this generalisation, certain species present singularities in Fe uptake mechanisms indicating that strategies I and II are not fully exclusive. Arabidopsis, representative of strategy I, was recently reported to trigger strategy II-related mechanisms under alkalinity (Li et al., 2023; Robe et al., 2021), whereas the monocot *Oryza sativa* (hereafter rice) uses both strategies simultaneously (Kobayashi et al., 2014). Importantly, Fe can cause cytotoxicity if accumulated in excess since its redox properties lead to the generation of reactive oxygen species (ROS) via Fenton reactions. Therefore, the regulation of plant homeostatic networks is finely-tuned to maintain Fe within proper physiological ranges and, therefore, Fe is rapidly complexed by chelators and specialised proteins like FERRITINS (FERS), delivered to final acceptors, or stored in organelles (Briat et al., 2007; Morrissey and Gueriot, 2009, 2010).

Besides Fe imbalances, plants in nature are simultaneously threatened by numerous unfavourable conditions, e.g., other nutritional limitations, fluctuations in temperature and light intensity, water shortage, or pathogen infection. From a systemic perspective, plants articulate counteracting responses to adverse environmental cues by transient reconfigurations in homeostatic networks, which sophisticatedly readjust cross-talks among biological functions. The components mediating acclimation to stressors have been mainly dissected in isolation, however, homeostatic responses can be largely tailored by the Fe nutritional status. Indeed, Fe-responsive features occupy prevalent positions in plant signalling networks at the interplay of relevant processes like copper (Cu) and phosphate (Pi) homeostasis, hormone signalling, or pathogen defence (Chutia et al., 2019; Nam et al., 2021; Perea-García et al., 2013; Riaz and Gueriot, 2021; Verbon et al., 2017; Herlihy et al., 2020). The role of Fe as a key player to sustain plant immunity attracted researchers' interest during the last decade (Herlihy et al., 2020; Liu et al., 2021; Verbon et al., 2017; Trapet et al., 2021). Fe interferes with the outcome of plant-pathogen interactions depending on the Fe usage by the plant and the capacity of both players to compete for the metal. Arabidopsis tends to promote Fe withholding to defend against a wide range of pathogens with different lifestyles such as the bacteria *Dickeya dadantii*, *Pseudomonas syringae*, and *Pectobacterium carotovorum*, the biotrophic fungi *Hyaloperonospora arabidopsidis*, or the necrotrophic *Botrytis cinerea* (Hsiao et al., 2017; Trapet et al., 2021; Lu and Liang, 2023; Aznar et al., 2015; Kieu et al., 2012). On the other side, pathogens hijack hosts' Fe uptake and distribution mechanisms and secrete siderophores to feed from plant Fe sources and thus sustain virulence (Greenshields et al., 2007; Rai et al., 2015; Oide et al., 2006; L., L. Liu et al., 2019; Lu and Liang, 2023). Unlike Arabidopsis, other plant species deviate elevated amounts of Fe at foci of infection to locally trigger ROS-derived cytotoxicity and, ultimately, a hypersensitive response that restricts pathogen progression, a process termed ferroptosis (Distéfano et al., 2021). Ferroptosis has been reported in rice, *Zea mays* (maize), and *Nicotiana benthamiana* upon infection by the respective pathogens *Magnaporthe oryzae*, *Blumeria graminis*, and *Tobacco mosaic virus* (Liu et al., 2007; Dangol et al., 2019; Macharia et al., 2020; Sánchez-Sanuy et al., 2022).

Arabidopsis and rice are model plants for different Fe uptake strategies that apparently adopted opposite Fe-depending nutritional immunity. However, the molecular basis underlying the interaction between Fe homeostasis and defence in both species remains elusive. Agronomically, the characterisation of divergent nutritional immunity strategies is necessary since fertilisation of arable lands might compromise defence depending on the species, as reported for Pi and nitrogen (N) when exceed certain levels (Val-Torregrosa et al., 2022; Campos-Soriano et al., 2020; Veresoglou et al., 2013; Ballini et al., 2013). To shed some light on this issue, we conducted a systemic-wise comparison between Arabidopsis and rice transcriptional responses during infection by hemibiotrophic fungi, namely *Colletotrichum higginsianum* and

M. oryzae, under different Fe statuses. The results presented herein corroborated the antagonistic effect of Fe in immunity between Arabidopsis and rice and recapitulated divergences in the reconfiguration and prioritisation of biological functions mediating defence, although the regulatory elements are preserved. Collectively, this study illustrates differences in Fe usage and host transcriptional responses in the two pathosystems that should be considered to avoid undesired payoffs when it comes to designing biotechnological strategies.

2. Material and methods

2.1. Plant cultivation

Arabidopsis thaliana ecotype Col-0 and *Oryza sativa* subsp. *japonica* cv. *Nipponbare* were used as wild types. Arabidopsis seeds and rice grains were stratified at 4 °C for two days or germinated at 28 °C for two days, respectively, and cultivated on semi-hydroponic substrate consisting in a 1:1 (w/w) mixture of inert substrate (vermiculite 33 % and 66 % peat supplemented with 1 g/L CaCO₃) and drained dorsolite (0.6 a 1.2 mm grain diameter). Plants were irrigated with 1/10 Hoagland solutions (Hermans and Verbruggen, 2005) supplemented with 1 µM CuSO₄ and 2, 20, or 200 µM Fe-EDTA and grown under a 12 h light (22 °C and 120 µmol m⁻² s⁻¹) / 12 h dark (18 °C) cycle with a relative humidity of 65 % in FitoClima 1200 PLH LED cabinets (Aralab, Rio de Mouro, Portugal). Experiments were conducted in five independent replicates including non-less than ten plants per Fe condition. Samples from shoots and roots were harvested 6 h after the onset of light and flash-frozen in liquid nitrogen.

2.2. Fungal infection assays

C. higginsianum (O'Connell et al., 2012) was cultivated on oatmeal plates at room temperature in the dark for two weeks and mycelia were prepared in water at 10⁶ spores mL⁻¹. *M. oryzae* strain Guy-11 was grown on Complete Media Agar at 25 °C under illumination for two weeks and mycelia were resuspended in water at 10⁵ spores mL⁻¹ and Tween 0.025 %. Eighteen-day-old Arabidopsis and rice plants were mock-treated or infected by surface spraying and kept under high humidity. Fungal biomass was quantified from entire shoots seven dpi by quantitative real-time PCR (qPCR) on approx. 10 ng gDNA using specific fungus primers and the Arabidopsis *ACTIN2* (*ACT2*) or rice *UBQ10* genes as references (Supplementary Data 1).

2.3. Transcriptome profiling and analysis

Transcriptome analyses were conducted two dpi to address responses related to defence while avoiding interferences by necrosed tissues. Total RNA from shoots of mock-treated or infected Arabidopsis and rice plants (*n* = 3) were prepared from approx. 50 mg of plant material with the Maxwell® RSC Plant RNA kit (Promega, Wisconsin, USA) according to the manufacturer's indications. RNA-Seq libraries were prepared, and pair-end sequenced (2 × 150 bp) at Sistemas Genómicos (València, Spain) with standard Illumina protocols. RNA-Seq datasets were analysed on the Galaxy platform (Afgan et al., 2022). Reads were cleaned from adapters using *Trimomatic* (Bolger et al., 2014) and mapped to the Arabidopsis or reference genomes TAIR10 or IRGSP-1.0 with STAR (Dobin et al., 2013). Read counts were computed with *featureCounts* (Liao et al., 2014) and to *DESeq2* (Love et al., 2014) was employed to filter changing transcripts (adjusted *P* ≤ 0.05, Likelihood ratio test) and DEGs between infections and mock treatments under each Fe condition (absolute log₂ fold-change ≥ 1, adjusted *P* ≤ 0.05, Wald test). Functional annotation of transcripts was conducted based on GO terms enrichment with a fold enrichment ≥ 2 containing ≤ 1000 genes (adjusted *P* ≤ 0.05, Fisher's Exact Test) in Panther (<http://www.pantherdb.org/>). GSEA was carried out on GO terms ranked based on enrichment scores (adjusted *P* ≤ 0.05, Fisher's Exact Test) to the total

number of genes in the reference genomes TAIR10 or IRGSP-1.0 with the *clusterprofiler* package in RStudio. Transcriptome datasets are deposited at the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE231365.

2.4. Gene expression analysis

Total RNA was retrotranscribed with the *NZY M-MuLV Reverse Transcriptase* kit (NZYTech, Lisbon, Portugal) according to the manufacturer's instructions. Transcript expression levels were quantified by qPCR with SYBR Green I dye using specific primers (Supplementary Data 1) in a Roche Light Cycler 480 (Roche, Basel, Switzerland) according to the $2^{-(Ct \text{ gene of interest} - Ct \text{ reference gene})}$ formula.

2.5. Element analysis

Roots and shoots were gently rinsed with milliQ water and lyophilised. Samples were pre-digested in 65 % (v/v) HNO_3 and H_2O_2 and digested at 120 °C for 2 h and diluted in milliQ. Element concentration was determined by ICP-OES and ICP-MS at Serveis Tècnics d'Investigació (Universitat d'Alacant, Spain).

2.6. Bioinformatic and statistical analysis

Networks were elaborated for changing transcripts in response to Fe abundance, fungal infection, or both ($P \leq 0.05$, Likelihood ratio test). For correlation networks, pair-wised Pearson correlation matrices were computed, and Random Matrix Theory was applied to determine a cut-off that avoids network saturation with a resolution of 0.01 using the *RMThreshold* package in RStudio. For GRNs, transcription factor-target associations were inferred with the *GENIE3* package in RStudio using the DEGs under pathogen infection for each Fe condition and selecting the lists of Arabidopsis and rice transcription factors as regulators. GRNs were filtered to retain for each gene the 15% most relevant transcription factors and thus avoid spurious connections adapted as from previously described (Cuesta-Astroz et al., 2021). The *Igraph* package in RStudio was used to assess topological information of networks and community composition according to the *FastGreedy* algorithm. Networks were visualised in Cytoscape version 3.9.1 (Shannon et al., 2003). Ortholog pairs for Arabidopsis and rice genes were explored in the PLAZA interface (Van Bel et al., 2022) using the integrative method and default parameter based on at least one criterion of evidence. Transcription factor enrichments on selected transcripts for overrepresentation of families were manually calculated and filtered according to adjusted $P \leq 0.05$ (Fisher's exact test). The *networkD3* package in RStudio was used to elaborate flow networks that depict transcription factor-target transcripts relationships in GRNs. Unsupervised gene clustering was conducted according to the *k*-means algorithm with the *kmeans* package in RStudio. Statistical analysis of data, and elaboration of plots were carried out in RStudio using the base interface and *pheatmap* and *ggpubr* packages. The Bonferroni-Hochberg method was used to correct *p*-values for multiple testing.

3. Results

3.1. Fe availability exerts an antagonistic effect on Arabidopsis and rice immune responses against infection by hemibiotrophic fungi

To investigate the interplay between Fe and defence, *Arabidopsis thaliana* and *Oryza sativa* subsp. *japonica* (hereafter Arabidopsis and rice) were selected as evolutionary distant species that employ distinct Fe uptake strategies. Arabidopsis and rice plants were semi-hydroponically cultivated on 1/10 Hoagland supplemented with 2 μM , 20 μM , or 200 μM Fe-EDTA to achieve low Fe, sufficient Fe, or high Fe conditions (hereafter -Fe, suf and +Fe). After 18 days, Fe concentration in shoots tended to increase from 130 $\mu\text{g/g}$ DW under -Fe to 200 $\mu\text{g/g}$

DW under +Fe in Arabidopsis and from 80 to 250 $\mu\text{g/g}$ DW in rice, whereas Zn and Mn – two Fe competitors – displayed opposite trends in both species (Supplementary Figures 1a, b). Despite the increase in Fe concentration in Arabidopsis not being as pronounced as in rice, the levels of the Fe-storage marker *FERRITIN1* (*FER1*) significantly decreased 0.6-fold under -Fe and rose 2.7-fold under +Fe compared with suf (Supplementary Figure 1c). To note, the growth conditions did not lead to severe stress as neither root and shoot biomass nor photosynthesis performance were importantly impaired (Supplementary Figure 2). Thus, our setup is valid to simulate different Fe nutritional statuses with similar intensity in both plants.

Infection assays were conducted with the hemibiotrophic fungi *Colletotrichum higginsianum* and *Magnaporthe oryzae* as Arabidopsis- and rice-compatible pathogens with comparable lifestyles. To avoid age-dependent defence mechanisms, infections were carried out on 18-day-old plants and fungal progression monitored after seven days. Interestingly, Arabidopsis plants exhibited remarkable symptoms of leaf lesions under -Fe and +Fe compared with suf, whereas the same treatments conferred a protective effect in rice (Figs. 1a-b). Quantitatively, infected Arabidopsis leaves accumulated approx. 2.5-fold more *C. higginsianum* biomass under -Fe and +Fe than plants under suf (Fig. 1c). Conversely, -Fe and +Fe treatments led to approx. three-fold less *M. oryzae* biomass in rice compared with standard conditions (Fig. 1d). Hence, our results demonstrate an opposite effect of Fe in Arabidopsis and rice under fungal infection. These observations further support that, in addition to being an essential nutrient for plant growth, Fe can differentially interfere with the plant immune phenotype during pathogen infection, a response that is dependent on the pathosystem.

3.2. Arabidopsis and rice transcriptome networks recapitulate different organisations of biological functions in the crosstalk between Fe and immunity

The antagonistic impact of Fe on Arabidopsis and rice disease resistance motivated us to monitor systemic responses to fungal infection depending on Fe availability on the plant side. The biotrophic phase of both *C. higginsianum* and *M. oryzae* occurs within the first 48 h post-infection (Jones et al., 2021; Jayawardena, 2021). Consistently, levels of the infection markers Arabidopsis *PATHOGENESIS RELATED1* (*PR1*) and rice *PR10a/PROBENAZOLE-INDUCIBLE PROTEIN* (*PBZ1*) increased three to five-fold compared with mocks at this time-point (Supplementary Figure 1d), supporting that infection was perceived by plants. Therefore, RNA-Seq profiling was conducted on shoots from 48 h mock and pathogen-inoculated plants to consider transcriptional changes related to defence reactions while avoiding interferences by necrosed tissues. The likelihood ratio test (adjusted $P \leq 0.05$) was employed to identify transcripts responsive to either Fe availability or fungal infection across samples. Arabidopsis and rice displayed approx. 4700 altered transcripts (19 % and 15 % of the total covered genes), having Fe availability imposed less transcriptional changes than fungal infection (approx. 1000 vs. 4500 transcripts) (Supplementary Figure 3a; Supplementary Data 2 and 3). Then, this study revealed global transcriptomic changes of the same intensity in the response of Arabidopsis and rice plants to pathogen infection.

To obtain a holistic view of the interplays among biological events during defence under different Fe nutritional statuses, transcriptome networks for Arabidopsis and rice (hereafter *AthNet* and *OsNet*) were constructed based on pair-wise correlations between levels of changing transcripts across all samples (see Methods) (Fig. 2). Networks were first investigated for community composition and functionally annotated based on significantly overrepresented gene ontology (GO) terms (adjusted $P \leq 0.05$, Fisher's exact test) (Supplementary Data 4). *AthNet* communities recapitulated a wide range of responses to abiotic (heat, cold, hypoxia, salt, reactive oxygen species) and biotic stressors (defence, cell wall modification, glucosinolates), secondary metabolism (phenylpropanoids and anthocyanins), hormone biosynthesis

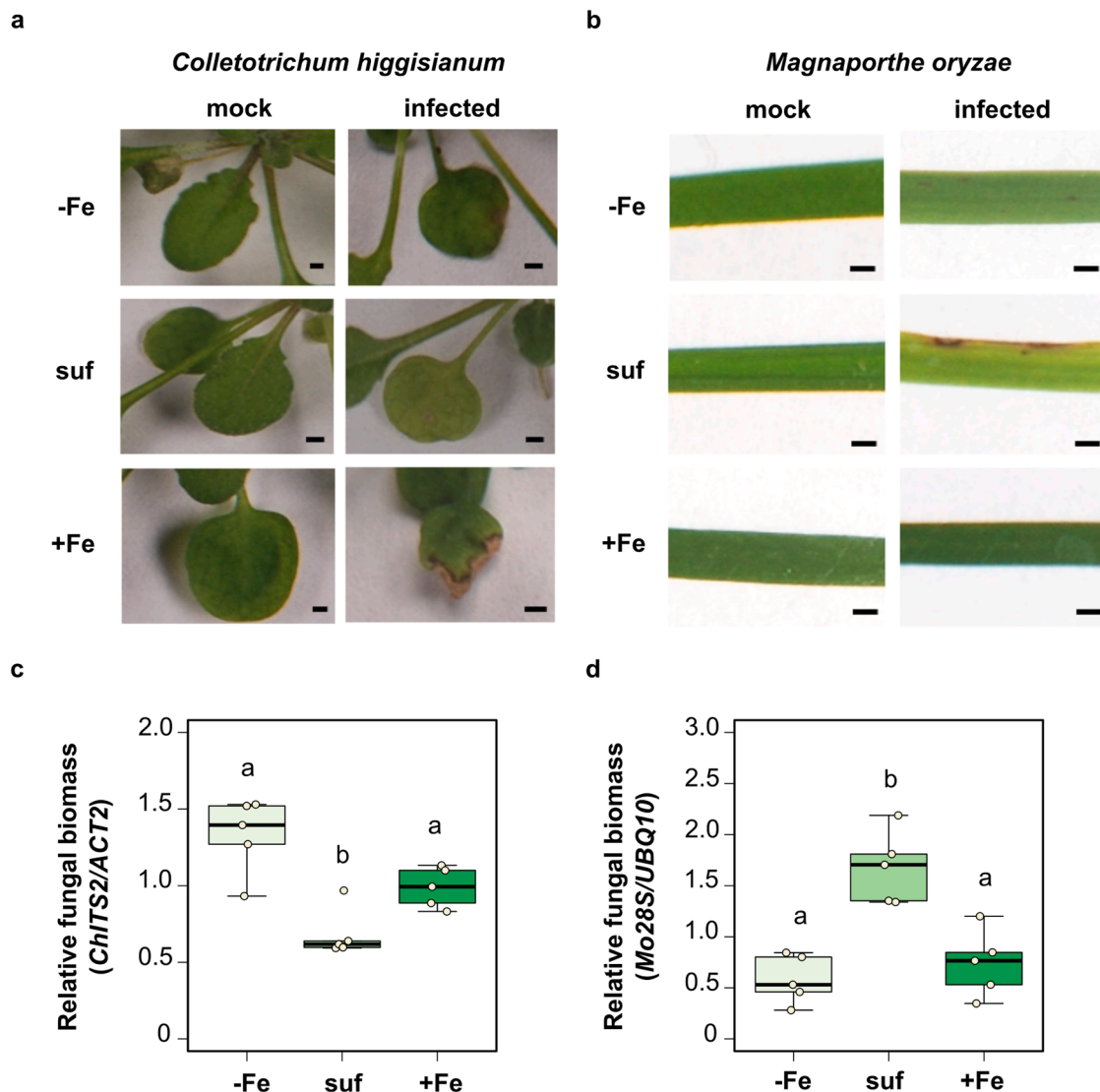


Fig. 1. Immune phenotypes of Arabidopsis and rice under different Fe availability conditions. Representative pictures of 18-day-old Arabidopsis (a) and rice (b) plants cultivated in semi-hydroponic conditions for low (2 μ M Fe-EDTA: -Fe), sufficient (20 μ M Fe-EDTA: suf), and high (200 μ M Fe-EDTA: +Fe) iron availability and either mock-treated or infected with *Colletotrichum higginsianum* (Arabidopsis) or *Magnaporthe oryzae* (rice) suspensions at 10^5 spores/mL. Scale bar, 1 mm. Relative fungal biomass under sufficient conditions in infected plants was determined by qPCR on total aerial tissues with specific fungal primers and normalised to Arabidopsis ACT2 (c) or rice UBQ10 (d) house-keeping genes. Statistically significant groups according to one-way ANOVA (adjusted $P \leq 0.05$; $n = 5$ independent experiments) are indicated with letters.

[cytokinin, jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene] and central processes (chloroplast biology, translation, protein folding) (Fig. 2a). Similarly, OsNet communities were related to central functions, namely photosynthesis and translation, as well as response to oxidative stress and hormones [JA, SA, and gibberellic acid (GA)], although a smaller number of terms was detected (Fig. 2b). As expected, both networks included communities representing Fe homeostasis and defence processes (Fig. 2). However, *AthNet* transcripts related to defence were mainly co-expressed with features responding to cold, SA, hypoxia, and chloroplast function but to SA, JA, and ROS in *OsNet* (Fig. 2). Hence, primary functional analysis of *AthNet* and *OsNet* anticipated that fungal infections under different Fe conditions rely on similar biological functions in both species, although the patterns of response in Arabidopsis plants infected by *C. higginsianum* - inferred by the heterogeneity and distribution of biological processes in communities - would be more diversified compared with rice under *M. oryzae* infection.

AthNet and *OsNet* were further contrasted for topological properties.

Both networks included a similar number of nodes (transcripts), however, *OsNet* encompassed almost two-fold more edges than *AthNet* and, consequently, the average connectivity, diameter, path length, density, and clustering coefficient was higher (Fig. 3a). As nodes in correlation networks are connected based on co-expression patterns, the high interconnectivity of transcripts in *OsNet* is consistent with the minor heterogeneity of the response in rice. Indeed, *AthNet* depicted more communities than *OsNet* (14 vs. 11) (Fig. 3a). Node connectivity was used to assess preservation in responses between species. Briefly, nodes of similar degrees in different networks might have conserved functions, whereas changes in connectivity could be attributed to functional rewiring. Remarkably, Arabidopsis and rice ortholog pairs showed an absolute lack of correlation in node degree (number of edges per node) (Fig. 3b), suggesting the different relevance of equivalent transcripts in the response in each pathosystem. Likewise, *AthNet* and *OsNet* local hubs - nodes with the top five highest degree per community - only overlapped for two orthologs (Fig. 3c and Supplementary Data 5). As a result, Arabidopsis and rice are expected to differently coordinate and

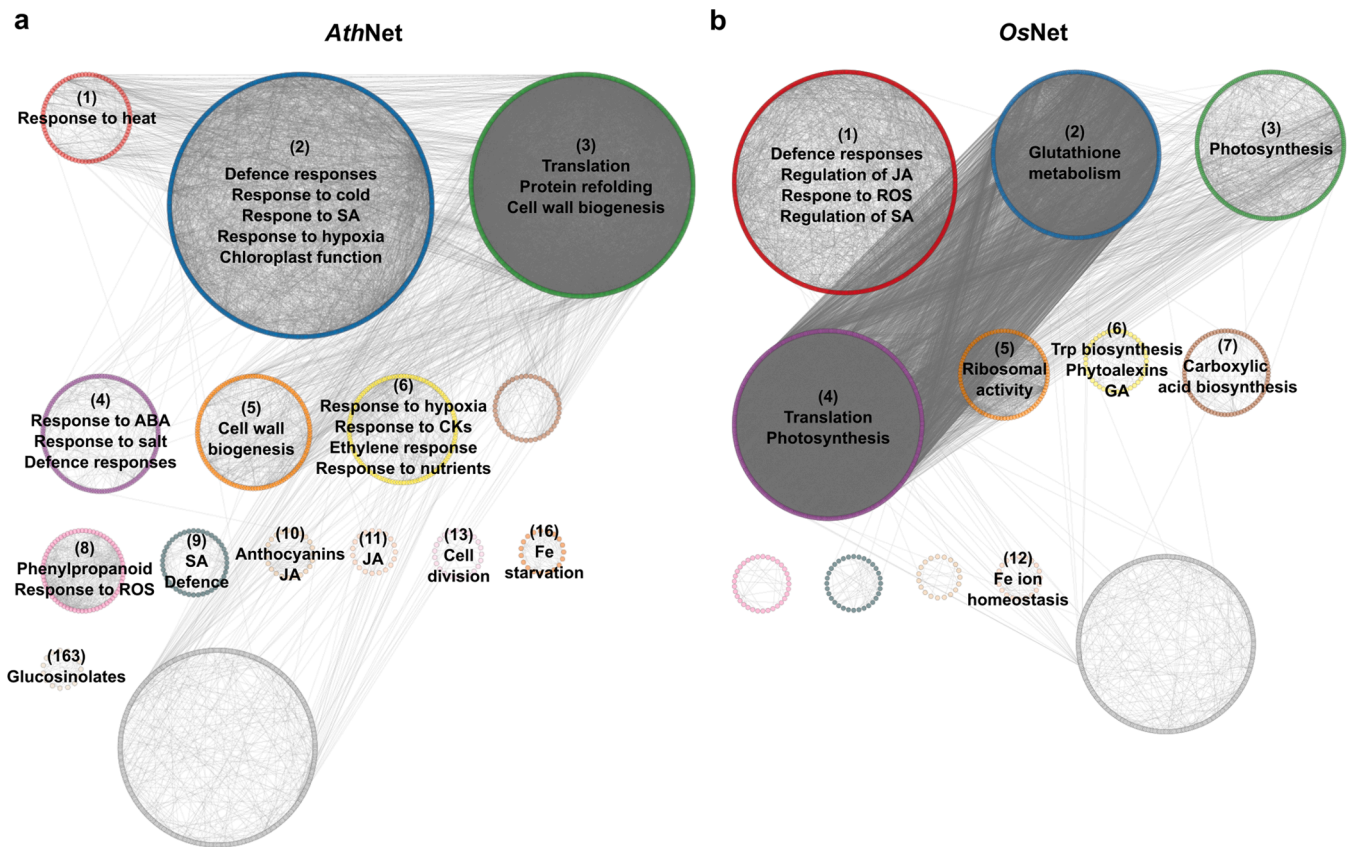


Fig. 2. Arabidopsis and rice transcriptome networks for response to fungal infection depending on Fe availability. Arabidopsis (AthNet, a) and rice (OsNet, b) networks were constructed using normalised counts from RNA-Seq in plants cultivated under different Fe conditions either mocked-treated or infected (2 dpi) based on pair-wise Pearson coefficients among transcripts (see Methods). Network communities were determined according to the *FastGreedy* method and functionally annotated for significant for GO terms related to biological processes (adjusted $P \leq 0.05$, Fisher's exact test). The identifiers of the communities are indicated in brackets.

prioritise the biological functions articulating the response against their respective fungal infection under different Fe availability.

Next, to dissect interspecific divergences in Fe homeostasis and defence processes, local hubs in the main AthNet and OsNet communities representative of these two functions were used to retrieve the corresponding subnetworks based on first neighbours (Fig. 3d). Both Fe homeostasis communities were governed by hubs operating as central regulators of Fe deficiency responses: the Arabidopsis Fe sensor *BRUTUS* (*BTS*) and the rice transcription factor *IRON-RELATED TRANSCRIPTION FACTOR 2* (*IRO2*) (Rodríguez-Celma et al., 2019). Moreover, Fe communities encompassed orthologs for Fe regulation and transport such as *POPEYE* (*PYE*), *BHLH100/1001*, *IRONMAN1/2/3* (*IMA1/2/3*), *OBP3-RESPONSIVE GENE1* (*ORG1*), *NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 4* (*NRAMP4*), *OLIGOPEPTIDE TRANSPORTER 3* (*OPT3*), and *ZINC INDUCED FACILITATOR 1* (*ZIF1*) in Arabidopsis and *HAEMERYTHRIN MOTIF-CONTAINING RING AND ZINC-FINGER PROTEIN 1/2* (*HRZ1/2*), *IMA1/2*, *FRO1*, and *NRAMP1* in rice (Fig. 3d). On the other hand, some Arabidopsis hubs for defence were the components of the glucosinolate biosynthetic pathway *MYB51*, *INDOLE GLUCOSINOLATE O-METHYLTRANSFERASE* (*IGMT1*), and *CYTOCHROME P450 FAMILY 81 SUBFAMILY F2* (*CYP81F2*) that moreover attracted a subcommunity of additional features in glucosinolate metabolism, defence to fungus, callose deposition, and response to ROS (Fig. 3e, left panel). In contrast, the general stress regulators *WRKY24*, *RECEPTOR-LIKE CYTOPLASMIC KINASE 63/238* (*RLCK63/238*) and *CALCIUM-DEPENDENT PROTEIN KINASE 4* (*CPK4*) (Wang et al., 2018; Bundo and Coca, 2016) were hubs for defence in rice that co-expressed with transcripts responsive to ROS and JA (Fig. 3e, right panel). Thus, both species recruit similar players to sustain Fe homeostasis but coordinate defence strategies differently via the preferential

use of glucosinolates in Arabidopsis, whereas rice would rather rely on general stress mechanisms mediated by JA and ROS.

3.3. Fungal infections impose distinct Fe responses in Arabidopsis and rice

After completing the network analysis, we wondered about the trajectories underlying the transcripts responsive to fungal infection under different Fe supply. Thus, unsupervised *k*-means clustering of normalised transcript levels was used to discriminate expression patterns (Figs. 4a, b and Supplementary Figure 3b, c). The major coreset of changing transcripts in Arabidopsis and rice exhibited responsiveness to fungal infection regardless of Fe availability, being approx. 35 % of all changing transcripts raised (clusters Ath1 and Os4) and approx. 30–40 % decreased (clusters Ath2 and Os1) (Figs. 4a–c). Functionally, pathogen infection mainly led to changes in general defence in Arabidopsis but to ethylene signalling and ROS response in rice (Fig. 4d and Supplementary Data 6 and 7). In Arabidopsis, 28 % of all transcripts reflected interactive effects between Fe status and infection since Fe-responsive transcripts under mock remained induced under infection (clusters Ath3) or conversely only responded to Fe under infection (Ath4 and Ath5) (Figs. 4a and c). These patterns were functionally related to additional immune features, Fe homeostasis and secondary metabolites with defensive roles (glucosinolates, xyloglucans, and anthocyanins) (Fig. 4d). On the contrary, Fe-responsive genes in rice (20 % of all transcripts) behaved similarly under mock and infection clusters (Os3 and Os5) and included terms for defence and responses to ROS (Figs. 4b–d). These results support the existence of common and predominant trends in transcriptome reconfigurations in both species aimed at preparing plants to counteract fungal progression independently of Fe

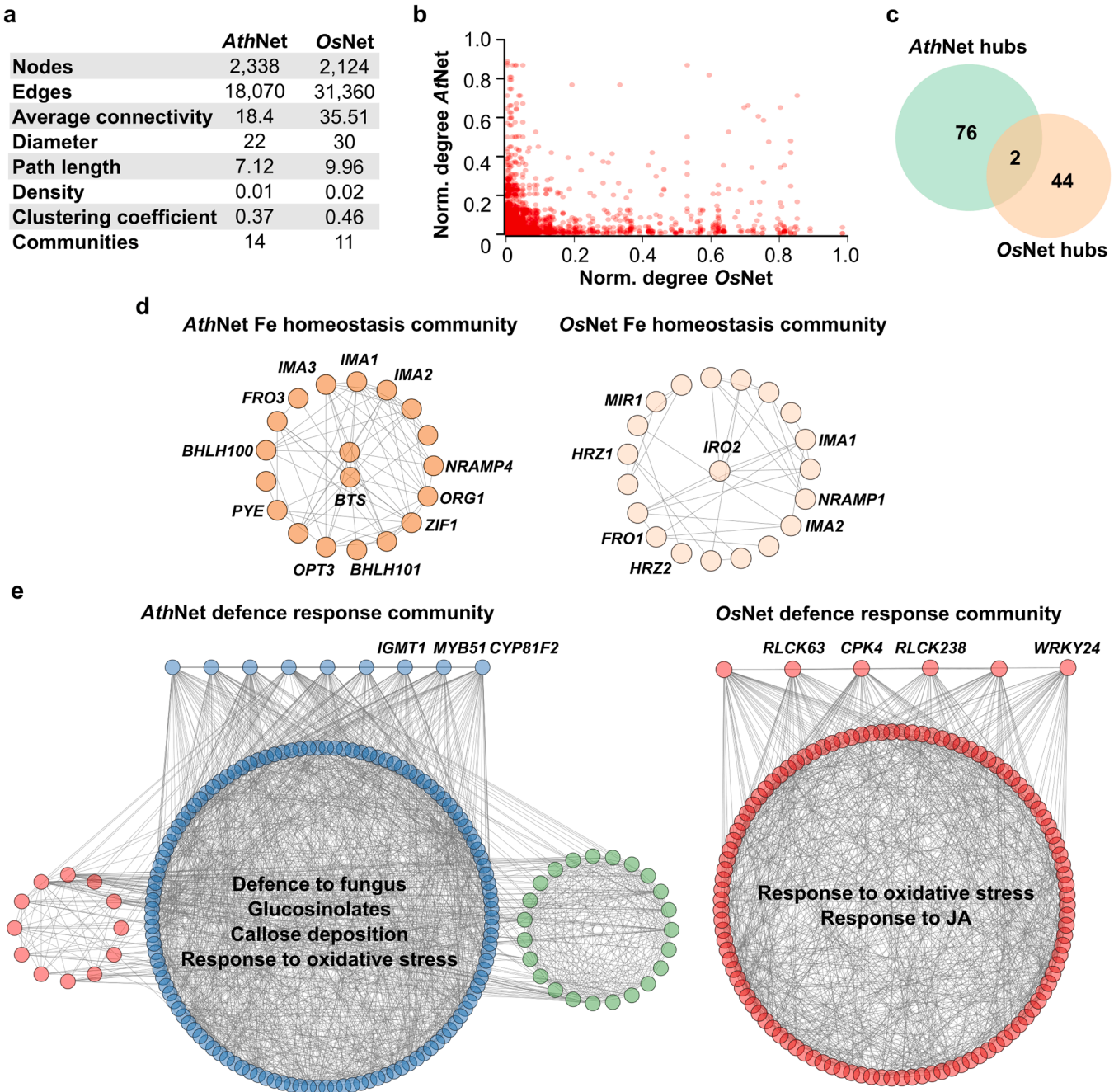


Fig. 3. Comparison of Arabidopsis and rice transcriptome networks. Central topological metrics for *AthNet* and *OsNet* (a). Comparison in node connectivity (degree) of potential pairs of transcript homologues in *AthNet* and *OsNet* (b). Different hub composition between *AthNet* and *OsNet* (c). The top five highly connected nodes in each community were declared local hubs and used for comparisons based on homology between Arabidopsis and rice. Local hubs in *AthNet* and *OsNet* communities related to Fe homeostasis (d) and defence (e) were used as seeds to extract the corresponding subnetworks based on first neighbours. The name of the most relevant hubs and the significantly enriched GO terms for biological processes (adjusted $P \leq 0.05$, Fisher's exact test) are indicated.

availability. In contrast, Fe responses were conditioned by the presence of fungi in Arabidopsis, whereas in rice they were mainly unrelated to fungal perception.

Since Fe homeostatic components appeared significantly enriched in two different expression patterns in Arabidopsis but only one in rice (clusters Ath6 and Ath3 vs. Os2) (Fig. 4d; Supplementary Data 6 and 7), we interrogated whether fungal infections could impose distinct Fe usage between species. Heatmaps for expression levels of representative features for Fe homeostasis depicted that the principal sensors and regulators behaved as expected in both mock and infected plants, i.e., the Arabidopsis *BTS*, *PYE*, and *bHLH100/1* and the rice *HRZ1/2*, *IRO2/*

3, and *IMA1/2* lowered as Fe availability increased, although a more pronounced repression was found in infected plants under +Fe (Figs. 5a, b). Similar trends were found in several Fe uptake and distribution components including the Arabidopsis *NRAMP4*, *FRO2*, *OPT3*, and *YSL2* and the rice *NRAMP1*, *FRO1*, *IRT2* and *NICOTIANAMINE SYNTHASE* (*NAS1/2*) (Figs. 5a, b). Conversely, markers of Fe sequestration and storage like the Arabidopsis *FER3/4* and *VACUOLAR TRANSPORTER LIKE 1/2* (*VTL1/2*) or the rice *FER* and *FER2*, the tonoplast Fe transporters *VACUOLAR MUGINEIC ACID TRANSPORTER* (*VMT*) and *VIT2*, as well as *YSL9*, increased from -Fe to +Fe in mock-treated plants (Figs. 5a, b). However, these markers in infected plants under +Fe were

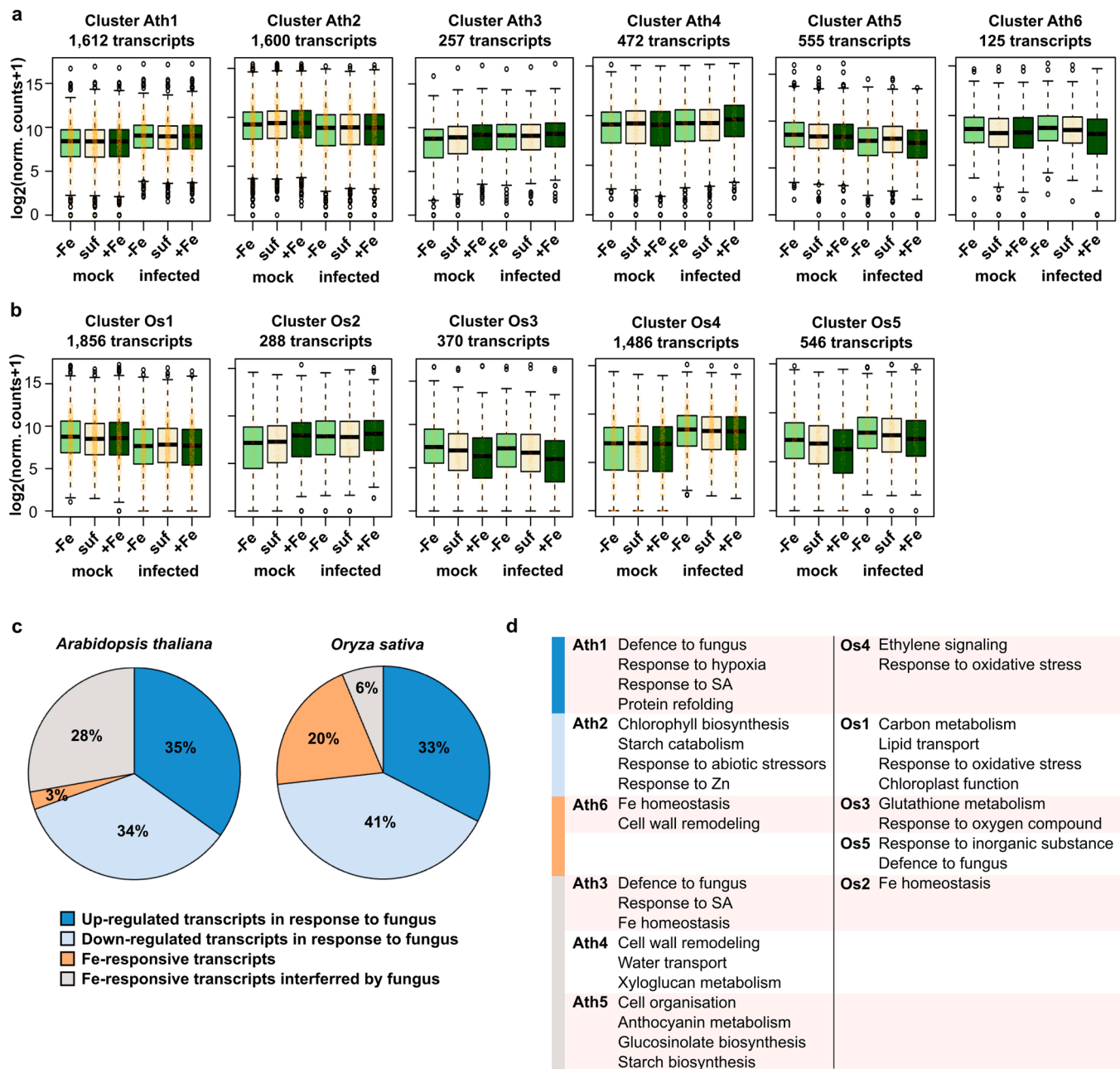


Fig. 4. Expression patterns of transcripts responsive to fungal infection in combination with different Fe supply. Boxplots summarising the trajectories of changing transcripts in Arabidopsis (a) and rice (b) (adjusted $P \leq 0.05$, Likelihood ratio test). Transcripts were clustered according to the WardD2 method on normalised counts from RNA-Seq data. The optimal number of expression patterns was determined according to the k -means method for the highest number of clusters (k) that rendered the minimal total within-cluster sum of square measure (Supplementary Figure 3c, d). Circular plots (c) describe the fraction of transcripts in Arabidopsis and rice describing the patterns identified in (a) and (b). Functional comparison of transcripts with similar expression patterns based on main enriched GO terms (Supplementary Data 6 and 7) (d).

remarkably enhanced in Arabidopsis, while repressed in rice (Figs. 5a, b). Further divergences were detected in features for Zn/Fe transport and distribution, since the Arabidopsis *IRT3* and several *ZIPs* failed to increase in infected plants under +Fe compared to mocks, whereas the rice *ZIP1/2/8*, *YSL12/16*, or *NRAMP2* exclusively accumulated upon infection regardless of Fe status (Figs. 5a, b). Then, infected plants could properly sense Fe availability but would employ distinct Fe usage strategies, whereby Arabidopsis prioritised Fe storage, while rice would rather mobilise it.

To corroborate our observations, the mineral composition of roots and shoots was monitored two and seven days post-infection (dpi). The Fe concentration in Arabidopsis and rice tended to raise over time in

both roots and shoots of mock plants as the Fe supply increased (Figs. 5c, d). However, Fe concentration remained steady in Arabidopsis upon fungal infection within ranges of mock plants under Fe sufficiency (approx. 1000 and 150 $\mu\text{g Fe/g DW}$ in roots and shoots, respectively), whereas it accumulated in both rice shoots and roots, especially two dpi (Figs. 5c, d). The concentration of other related metals, e.g., Cu, Zn, or Mn, displayed antagonistic Fe trends in both species, which were more evident in Arabidopsis, proving the competence of divalent ions for Fe uptake and distribution systems (Supplementary Figures 4–7). Collectively, our analysis supports opposite ways of handling Fe ions by the host plant during each of the fungal infections, consisting of restricting (Arabidopsis) or increasing (rice) Fe availability in shoots. Nonetheless,

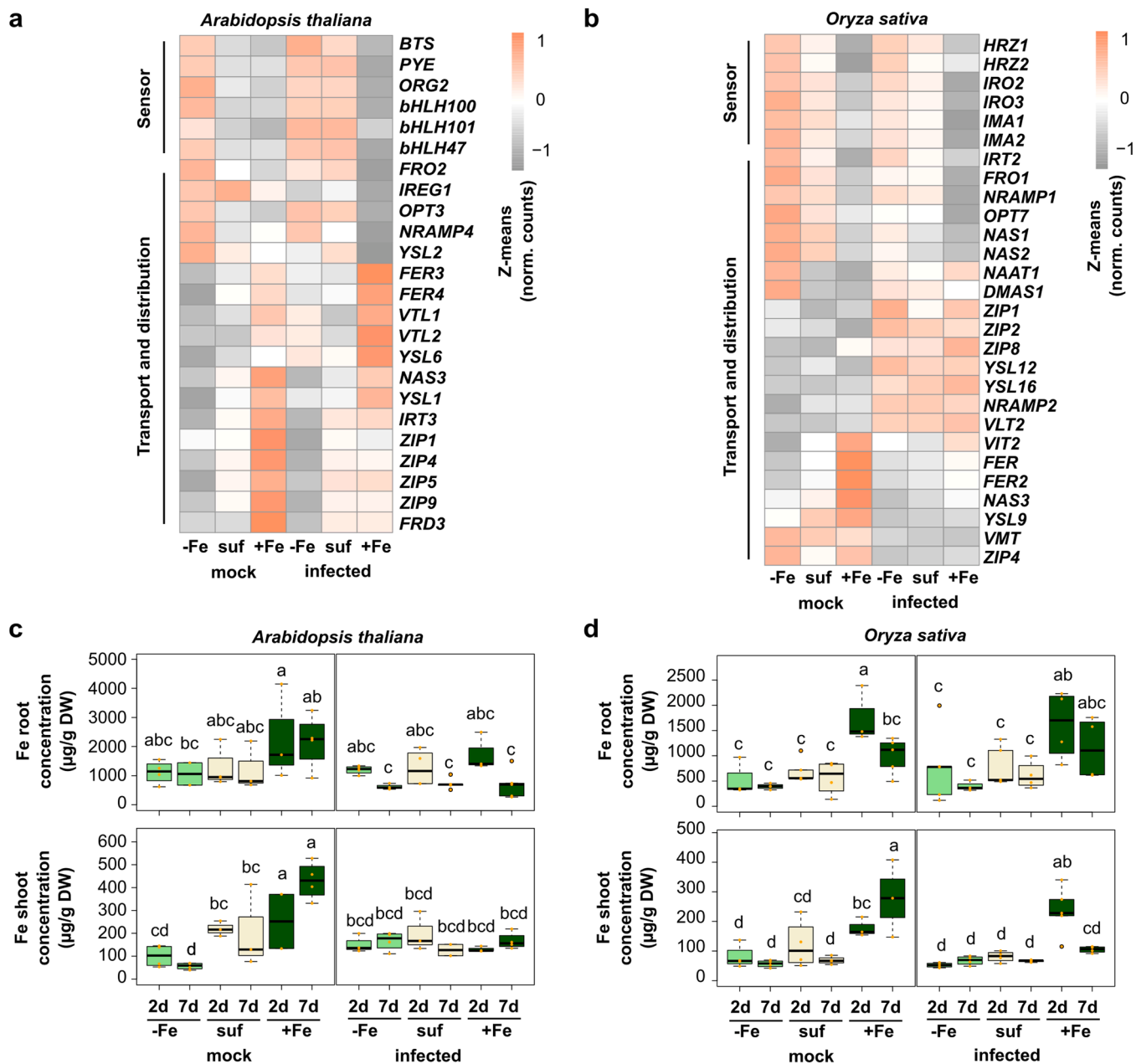


Fig. 5. Arabidopsis and rice display different Fe homeostatic strategies upon fungal infection. Heatmaps of Z-means of normalised counts from RNA-Seq experiments for representative Fe-responsive genes in Arabidopsis (a) and rice (b). Total Fe content in shoots and roots from Arabidopsis (c) and rice (d) cultivated under low, sufficiency and high Fe (-Fe, suf, +Fe) was determined 2 and 7 days after mock treatment or pathogen infection. Box plots represent data from $n = 5$ independent experiments and statistically significant groups according to Duncan's test (adjusted $P \leq 0.05$) are indicated with letters.

it is worth mentioning that whether those differences are motivated by the use of Fe of each pathogen deserves further investigation.

3.4. Defence responses to fungal infection under several fe conditions operate via different biological functions in Arabidopsis and rice

Since pathogen infection imposed the major fraction of Arabidopsis and rice transcriptomes changes but Fe usage differed between species (Figs. 4c, 5 and Supplementary Figure 3a), we aimed to dissect how the Fe status could refine the defence strategies. Thereto, transcripts displaying absolute $\log_2$ fold-change ≥ 1 in infected plants to respective mocks for each Fe nutritional status (adjusted $P \leq 0.05$, Wald's test) qualified as differentially expressed genes (DEGs). In total, 575, 474 and 681 Arabidopsis DEGs and 2237, 1015 and 1441 rice DEGs were detected under -Fe, suf, and +Fe, respectively (Supplementary Data 8

and 9). Multiple comparisons unveiled common coresets of DEGs (168 in Arabidopsis vs. 665 in rice), as well as transcripts at the intersection of two Fe treatments (50–100 DEGs in Arabidopsis vs. 70–350 DEGs in rice), which collectively surpassed the fraction of transcripts particularly altered under each Fe condition (Figs. 6a, b).

Functional annotation of Arabidopsis DEGs based on over-represented GO terms revealed a common program implementing systemic acquired responses, defence to fungus and related hormones (JA, SA, and ABA), although more evident under -Fe and suf (adjusted $P \leq 0.05$, Fisher's exact test) (Fig. 6b, blue terms, and Supplementary Data 10). Additional terms for defence to fungus, ethylene, and protein refolding on the one hand and to carbon metabolism on the other were only found under -Fe and +Fe, respectively (Fig. 6c). Interestingly, -Fe and +Fe - the treatments leading to susceptibility in Arabidopsis - coincidentally caused alterations in the metabolism of glucosinolates and

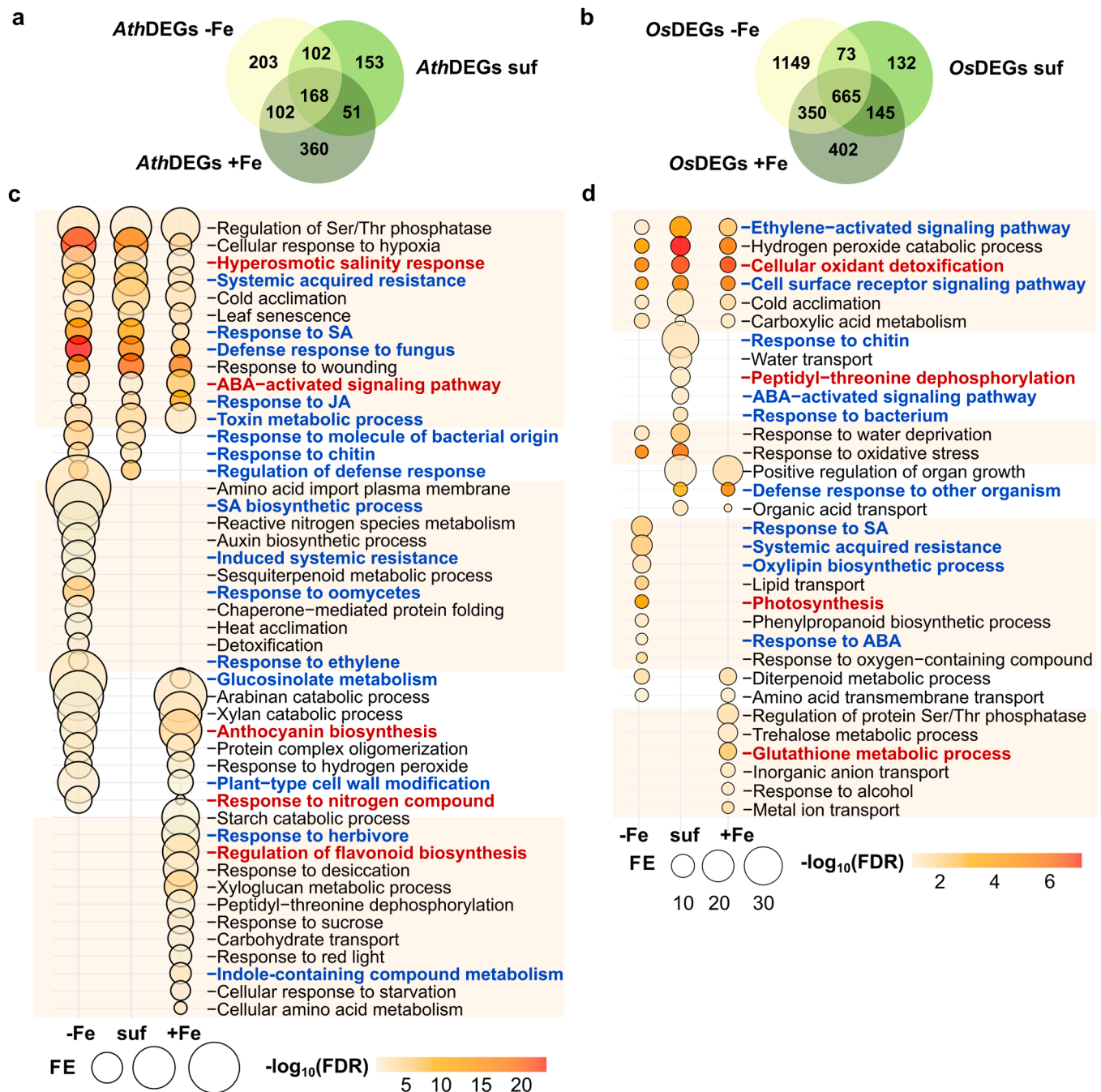


Fig. 6. Functional annotation of transcriptional responses to fungal infection depending on Fe availability. Venn diagrams for multiple comparisons among differentially expressed genes (DEGs, absolute $\log_2$ fold-change ≥ 1 for infections under Fe conditions to the respective mock, adjusted $P \leq 0.05$, Wald's test) in Arabidopsis (a) and rice (c). Bubble comparing functional categories for Arabidopsis (b) and rice (d) DEGs based on significant enrichment in GO terms (adjusted $P \leq 0.05$, Fisher's exact test). The size of the bubble is proportional to the fold-enrichment (FE) to random expectation and the intensity of the orange colour indicates the significance of the enrichment as the $-\log_{10}(\text{FDR})$.

anthocyanins and the cell wall (Fig. 6b). As this finding was consistent with the prevalent position of glucosinolate biosynthesis in the *Ath*Net defence community (Figs. 3e and 7a), transcript levels of representative components in these pathways were investigated in detail. Some intermediates in the synthesis of aliphatic and indolic glucosinolates, such as *FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE* (*FMO GS-OX8*), *ISOPROPYLMALATE ISOMERASE 2* (*IPMI2*), *CYTOCHROME P450 79F1* (*CYP79F1*), *EPITHIOSPECIFIER PROTEIN* (*ESP*) or *INDOLE GLUCOSINOLATE O-METHYLTRANSFERASE 5* (*IGMT5*) overall tended to lower under infection. However, the fold-change with mock conditions was only significantly enhanced under +Fe (adjusted $P \leq 0.05$,

Duncan's test) (Fig. 7a). Conversely, *CYP81F2*, *CYP71B15*, *MYB51*, and *IGMT1/2* commonly rose upon infection under -Fe and Fe sufficiency but displayed similar levels in mock and infected plants under +Fe (Fig. 7a). Anthocyanins are secondary metabolites contributing to dealing with a broad range of stressors (Garcia-Molina and Pastor, 2023), whose biosynthesis was also altered under -Fe and +Fe. Further Gene Set Enrichment Analysis (GSEA) on Arabidopsis DEGs corroborated the relevance of anthocyanin biosynthesis under -Fe and +Fe to respond to fungal infection (adjusted $P \leq 0.05$, Fisher's exact test) (Fig. 6b, red terms, and Supplementary Data 11). Indeed, -Fe and +Fe imposed significant decrease of markers for anthocyanin biosynthesis, namely

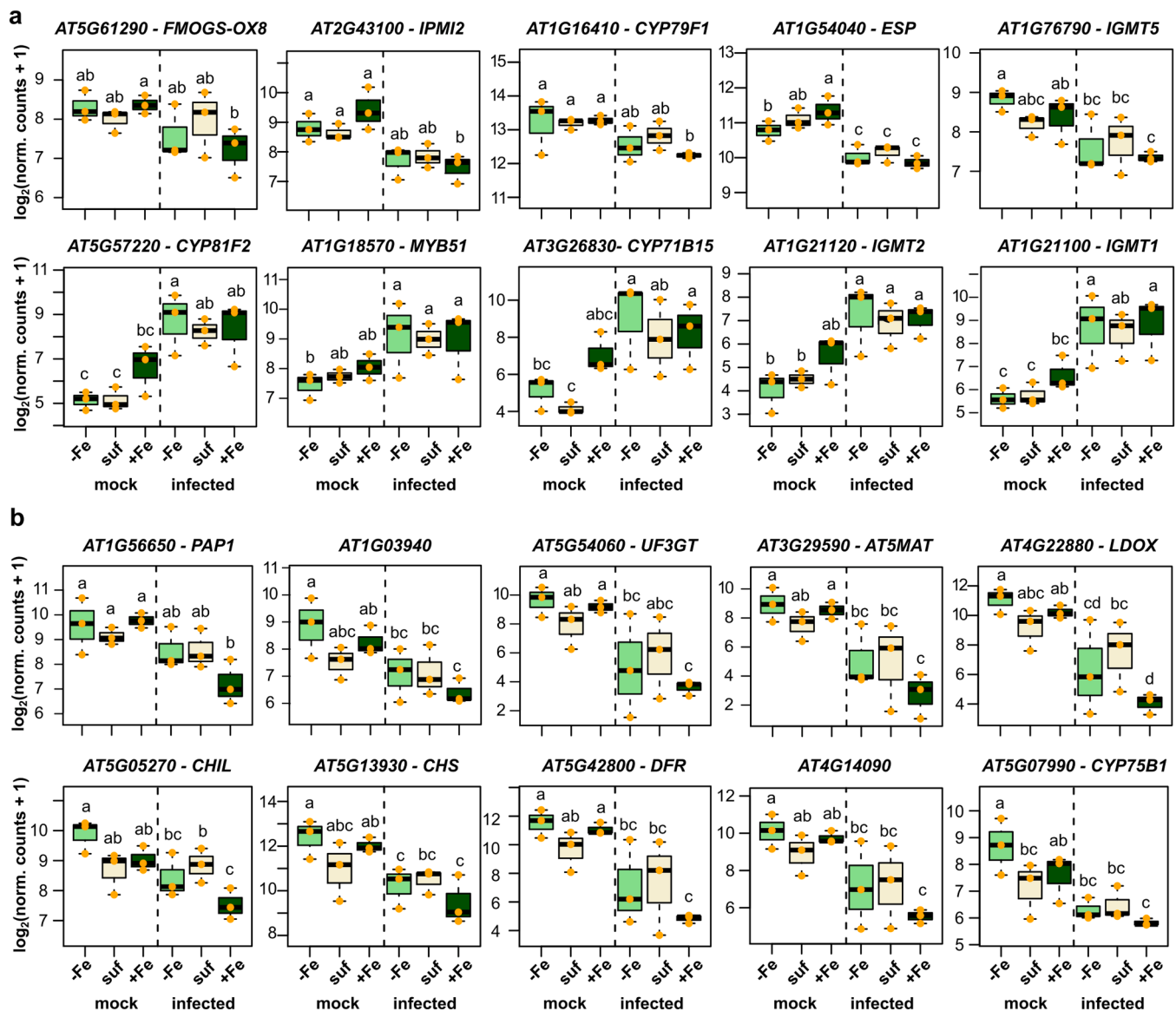


Fig. 7. Relative expression of markers of glucosinolate and anthocyanin biosynthesis upon fungal infection in *Arabidopsis* plants with different Fe availability. Normalised read counts from RNA-Seq experiments were used to elaborate boxplots for representative transcripts involved in glucosinolate (a) and anthocyanin (b) biosynthesis ($n = 3$ independent experiments). Statistically significant groups according to Duncan's test (adjusted $P \leq 0.05$) are indicated with letters.

CALCHONE SYNTHASE (CHS), *DIHYDROFLAVONOL 4-REDUCTASE (DFR)*, *LEUCOANTHOCYANIDIN DIOXYGENASE (LDOX)*, or *CHALCONE ISOMERASE LIKE (CHIL)* among others, under infection compared to mock (adjusted $P \leq 0.05$, Duncan's test), whereas under Fe sufficiency remained unaltered (Fig. 7b). Notably, mapping the specific *Arabidopsis* DEGs on the *AthNet* allowed to distinguish up-regulation of particular components in the main defence community (community 2) under -Fe and +Fe (Supplementary Figure 8a). Conversely, complementary defence communities related to phenylpropanoid metabolism and response to ROS (community 8) under -Fe, and anthocyanin and glucosinolate metabolism, as well as defence-related hormones (ABA, SA, JA) (communities 4, 9, 10, 11, 163) under +Fe included down-regulated transcripts (Supplementary Figure 8a). Consequently, the Fe status could modulate the composition of secondary metabolites with defence capacity in infected *Arabidopsis* plants by *C. higginsianum*, although at different level depending on the availability.

In rice, the core of biological processes responding to fungal infection included terms related to immune defence, also suggested by GSEA (raw

$P \leq 0.05$, Fisher's exact test), and ROS production (Fig. 6d, blue and red terms and Supplementary Data 10 and 11). Further defence functions against biotic stressors appeared singularly under suf or -Fe (Fig. 6d, blue terms), whereas metal transport categories related to Fe and Zn homeostasis were exclusively detected under +Fe (Fig. 6d). Remarkably, specific rice DEGs were mainly included in the defence *OsNet* community (community 1) as up-regulated, regardless of the Fe availability, whereas components of the glutathione metabolism community (community 2) were detected with the set of specific DEGs under -Fe and +Fe (Supplementary Figure 8b). Transcripts involved in counteracting ROS were monitored due to the centrality found in the *OsNet* defence community (Fig. 3d). We mainly found members of the *PEROXIDASES (PRXs)* family, whose expression increased upon infection and for some of them (*PRX29/37/88*), also under +Fe in mock (Fig. 8). These results support the uniform defence response in rice against *M. oryzae* anticipated by network analysis and provide a hint towards the potential induced resistance by +Fe. Together, immune responses in both species rely on central components distinctly implemented according to Fe

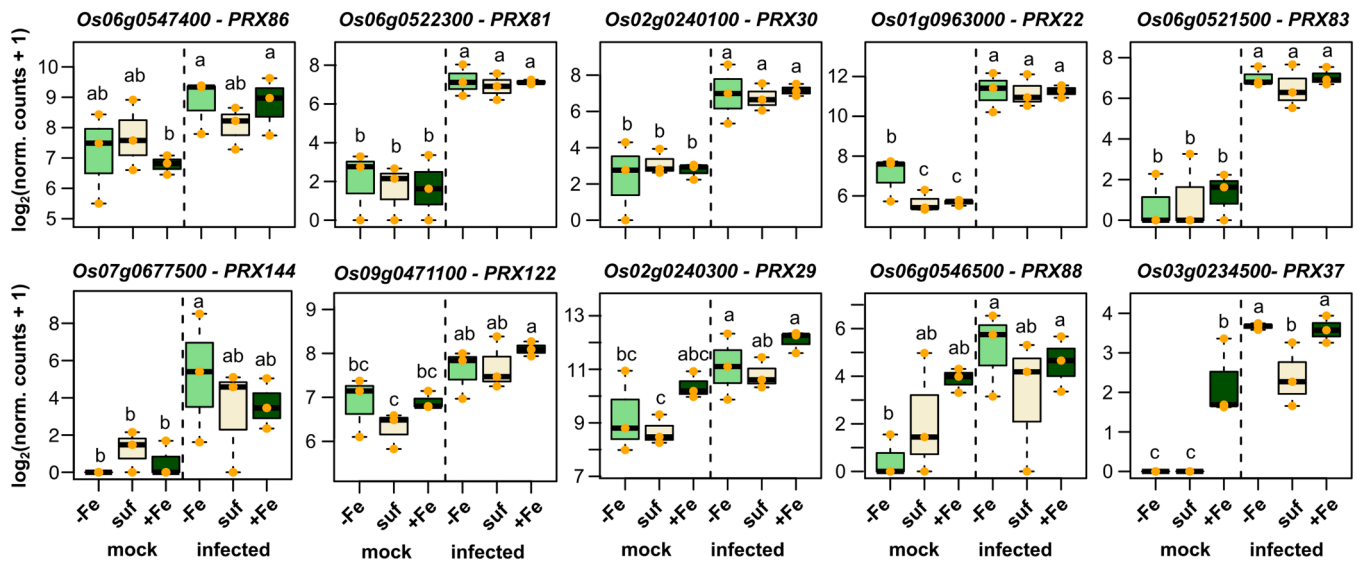


Fig. 8. Relative expression of markers of oxidative stress upon fungal infection in rice plants with different Fe availability. Normalised read counts from RNA-Seq experiments were used to elaborate boxplots for representative transcripts involved in response to oxidative stress ($n = 3$ independent experiments). Statistically significant groups according to Duncan's test (adjusted $P \leq 0.05$) are indicated with letters.

availability. Thus, *Arabidopsis* challenged by *C. higginsianum* would prioritise the proper accumulation of secondary metabolites with defensive properties, i.e., glucosinolates and anthocyanins, whereas rice infected by *M. oryzae* would rather trigger mechanisms involving oxidative stress production, consistent with ferroptosis (Dangol et al., 2019).

3.5. *Arabidopsis* and rice employ partially conserved regulatory circuits with different hierarchies to activate immune responses depending on Fe availability

Despite the divergences in Fe usage and defence strategies between pathosystems (Figs. 2 and 6), the coincidences in the principal trajectories of changing transcripts (Fig. 4) and fractions of DEGs (Supplementary Figure 3a) in both species suggested the potential preservation of regulatory mechanisms. Therefore, Gene Regulatory Networks (GRNs) for each Fe condition were inferred using the *Arabidopsis* and rice DEGs with the *GENIE3* method (Huynh-Thu et al., 2010) to retrieve transcription factors potentially governing transcriptome responses (*AthTFs* and *OsTFs*) (see Methods, Supplementary Figure 9 and Supplementary Data 12 and 13). *AthTFs* and *OsTFs* exhibited a positive correlation trend ($r > 0.5$) in degree and betweenness in the respective GRNs, and low correlations for closeness centrality (Fig. 9a). However, comparative analysis between *AthTFs* and *OsTFs* based on homology showed low and negative correlations for the three metrics (Fig. 9a, Supplementary Data 14). These results point to equal relevance of transcription factors in GRNs intraspecies, but to profound divergences between pathosystems. Nevertheless, approx. 60 % of *AthTFs* and 40 % of *OsTFs* ortholog pairs (82) commonly participated in the *Arabidopsis* and rice GRNs (Figs. 9b and Supplementary Data 14). Next, to ascertain the degree of specialisation of transcription factors, common *AthTFs* and *OsTFs* were used as seeds to capture first neighbours in the respective networks and compute the connectivity of targeted nodes (Fig. 9c). In both cases, *AthTFs* and *OsTFs* targets displayed approx. two-times lower average degree than expected in 1000 permutations using the same number of random nodes ($P \leq 0.01$; Kolmogorov-Smirnov test) (Fig. 9c). Hence, the restricted connectivity of common *AthTFs* and *OsTFs* targets could obey selective pressures and be indicative of functional specialisation. Finally, *AthTF* and *OsTF* categorisation indicated similar distribution of the largest number of represented transcription factors, being the common fraction of *AthTFs* and *OsTFs* significantly enriched in

members that belonged to the APETALA2 (AP2), homeobox (HOME), MYB, WRKY and ZINC FINGER TRANSCRIPTION FACTOR (ZTF) families in at least one of the species (adjusted $P \leq 0.05$, Fisher's exact test) (Fig. 9d). Only members of the ERF and HEAT SHOCK TRANSCRIPTION FACTOR (HSF) families were significantly enriched for specific *AthTFs* (Fig. 9d). Therefore, our analysis highlights equivalences in the transcription factors operating in the GRNs of both species, but important reorganisation in the hierarchy of regulatory events coordinating the responses to each fungal infection depending on Fe status in *Arabidopsis* and rice.

GRNs were further inspected to assess differential reprogramming of immune-related pathways liable to Fe availability. Thereto, transcripts involved in principal defence responses of each species, namely general defence (both in *Arabidopsis* and rice), secondary metabolism of anthocyanins, phenylpropanoids, glucosinolates (*Arabidopsis*), cell wall remodelling (*Arabidopsis*), oxidative stress (both), cellular detoxification, and glutathione (rice) were linked to the common and specific fractions of *AthTFs* and *OsTFs* among GRNs. *AthTFs* specifically detected under conditions that led to enhanced susceptibility against fungus (-Fe, +Fe and -Fe and +Fe, approx. 45 % of all *AthTFs*) targeted higher number of defence-related transcripts for all selected categories compared to those specific for suf (approx. 20 %) or detected in common (approx. 16 %) (Fig. 9e, left panel). Conversely, the specific *OsTFs* detected under conditions that led to enhanced tolerance (-Fe, +Fe and -Fe and +Fe, approx. 50 % of all *OsTFs*) attracted more defensive transcripts than those under suf (approx. 9 % of all *OsTFs*) and, in a certain measure, than those commonly found in the three GRNs (approx. 26 % of all *OsTFs*) (Fig. 9e, right panel). These results suggest the need of *Arabidopsis* to implement additional reconfiguration of defence features to counteract the susceptibility imposed by low and high Fe statuses, whereas in rice the same conditions trigger higher orchestration of oxidative stress responses to limit fungal progression.

4. Discussion

The Fe status of plants interferes with immunity and provokes opposite effects in *Arabidopsis* and rice. In our setup, -Fe and +Fe weakened the capacity of *Arabidopsis* to counteract *C. higginsianum* infection but reinforced rice immunity against *M. oryzae* (Fig. 1). Consistently, infected *Arabidopsis* plants limited Fe in shoots and stimulated components for Fe storage (*FERs*, *VTLs*), whereas rice promoted

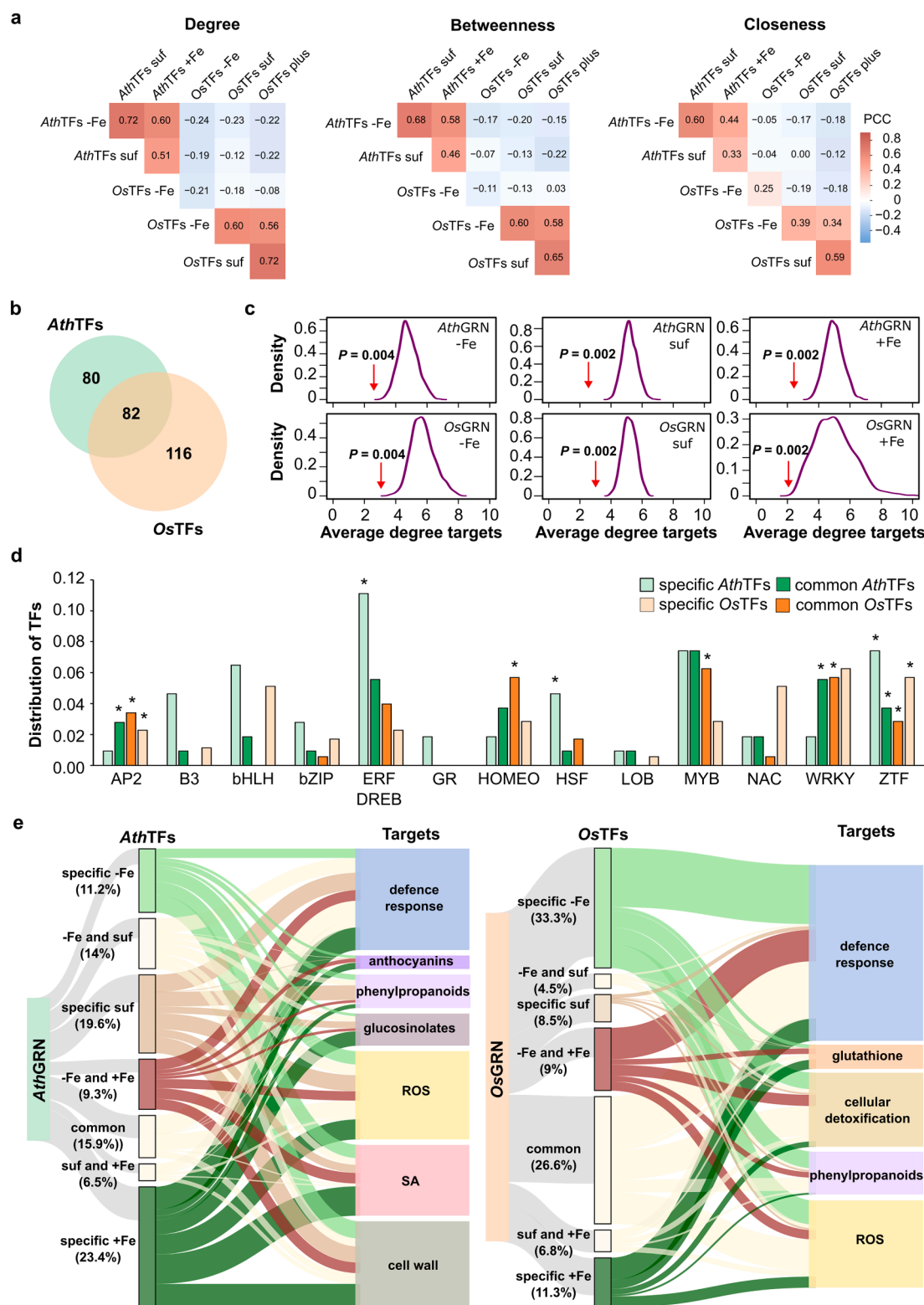


Fig. 9. Analysis of regulatory components of the transcriptome response to fungal infection under different Fe conditions. Transcription factors operating in Arabidopsis and rice (*AthTFs* and *OsTFs*) were retrieved from the Gene Regulatory Networks (GRNs) inferred for transcriptional changes under infection depending on Fe availability. Correlation between normalised number of targets (degree), betweenness and closeness centrality (closeness) between *AthTFs* and *OsTFs* in GRNs (a). Relevance of *AthTFs* and *OsTFs* targets. *AthTFs* and *OsTFs* were used as seeds in GRNs to identify first neighbours and calculate the average degree (red arrow) (b). Density plots depict the average connectivity of virtual targets in 1000 permutations using the same number of random nodes targeted by *AthTFs* and *OsTFs*. Statistical differences to randomised nodes are computed according to the Kolmogorov-Smirnov test. Identification of equivalent *AthTFs* and *OsTFs* based on comparisons of ortholog pairs (c). Categorisation of transcription factors (d). Bar plot depicts the fraction of specific and common *AthTFs* and *OsTFs* that belong to each family of transcription factors. Statistically significant enriched families are calculated according to the Fisher's exact test (adjusted $P \leq 0.05$). Common and specific *AthTFs* and *OsTFs* among the corresponding GRNs were connected to targeted transcripts involved in selected defence-related pathways (e). The width of the edges is proportional to the number of elements in each category (fraction of transcription factors or targeted transcripts).

Fe accumulation and mobilisation in leaves upon infection (Fig. 5). The antagonistic effect of +Fe - detrimental to Arabidopsis but beneficial to rice - agrees with previous studies reporting Fe withdrawal in infected Arabidopsis leaves to restrict Fe availability to pathogens, while rice uses Fe for ferroptosis (Hsiao et al., 2017; Trapet et al., 2021; Lu and Liang, 2023; Aznar et al., 2015; Kieu et al., 2012; Dangol et al., 2019; Sánchez-Sanuy et al., 2022). However, why -Fe reproduces the immune phenotypes observed under +Fe is more arguable. Evidence that -Fe strengthened Arabidopsis immunity is supported by studies where Fe deficiency was imposed before pathogen challenge and infections were more invasive (Trapet et al., 2021; Lu and Liang, 2023; Aznar et al., 2015; Kieu et al., 2012). Then, lowering pools of stored Fe at infected areas may be advantageous for Arabidopsis defence, however, sustained and general Fe starvation might rather constrain plant fitness and immunity, at least against fungi like *C. higginsianum*. In rice, -Fe could lead to generalised ROS production that activates signalling for defence, impedes Fe feeding by pathogen, and/or partially mimics ferroptosis. In this work, we focused on Fe-regulated host defence responses, however additional investigation would be necessary to disentangle how changes in fungal biomass could partially depend on the pathogen's capacity to compete for the host's Fe pools as a fundamental nutrient.

Systemically, divergences in immune responses might obey different rewiring in homeostatic networks at the levels of node and/or edge

composition and functionality of groups of nodes (Pham et al., 2016; Sun and Kim, 2011; Beleva Guthrie et al., 2018; Gu et al., 2019). Node expansion in AthNet or OsNet was ruled out as both networks encompassed similar fractions of transcripts that, moreover, were organised into communities related to central biological functions involved in defence (Figs. 2, 3a). Nevertheless, Arabidopsis and rice ortholog pairs displayed distinct connectivity and, therefore, revealed different hierarchies in the processes mediating immune responses. Indeed, Arabidopsis prioritised defence on anthocyanins and, especially, glucosinolates (Figs. 3e, 6b, and 7), in agreement with the fundamental role of these secondary metabolites in *Brassicaceae* to counteract biotic stressors (Ishida et al., 2014; Essoh et al., 2020; Seo and Kim, 2017; Abuyusuf et al., 2023). However, rice defence relied on general stress regulators and responses to JA and oxidative stress (Figs. 3 and 8), which is consistent with rice's dependence on JA to trigger ROS production to counteract *M. oryzae* progression (De Vleeschauwer et al., 2013; Trang Nguyen et al., 2019; Ma et al., 2022). Collectively, we propose that Arabidopsis reorganised the crosstalks for Fe-immunity to base defence on secondary metabolites and relegated the role of Fe to regulate their biosynthesis (joint effect hypothesis), whereas rice redirected central stress pathways and Fe usage to trigger cytotoxicity (metal defence hypothesis) (Fig. 10).

Regarding the selective pressures underpinning the different

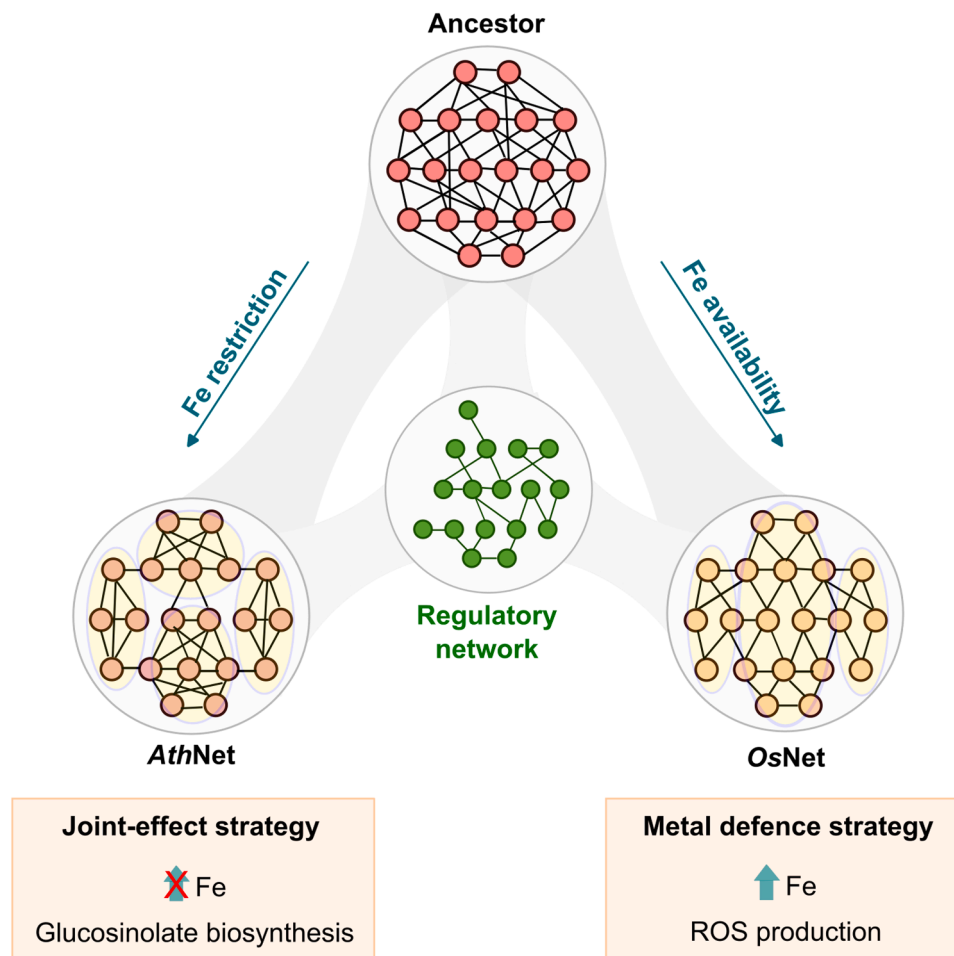


Fig. 10. Scheme summarising the divergence in systemic responses between Arabidopsis and rice. Arabidopsis and rice have evolved from a common ancestral species in habitats with different nutrient bioavailability, being Fe restricted for Arabidopsis in calcareous and alkaline soils but highly accessible to rice under flooding conditions. The homeostatic network of Arabidopsis has been reconfigured accordingly to increase the number of communities and intermodular connectivity to diversify the response. In rice, fewer communities, but higher overall connectivity occurs as the response is more uniform. Ultimately, Arabidopsis triggers a “joint effect” strategy where Fe accumulation in shoots upon fungal infection is restricted while the biosynthesis of glucosinolates is differentially regulated depending on the Fe status. Conversely, rice tends to mobilise Fe pools to activate a “metal defence” strategy consisting of ferroptosis. Despite these divergences, responses are similarly orchestrated by conserved regulatory mechanisms in both species that operate with different priority.

interplay between Fe and immunity, distinct nutrient availability in natural habitats could have set interspecific barriers conditioning Fe usage. Arabidopsis is widely distributed in calcareous and alkaline soils (Terés et al., 2019) and, therefore, must economise Fe for essential functions, whereas rice is generally cultivated in flooded and acidic conditions, where Fe is less limited (Quinet et al., 2012; Neue et al., 1998; Fageria et al., 2008). Nevertheless, ferroptosis occurs in Arabidopsis to promote controlled cell death during heat shock conditions (Conlon and Dixon, 2017; Distéfano et al., 2017) but not as a defence strategy. However, (Escudero et al., 2022) reported an equivalent mechanism based on Zn in Arabidopsis leaves infected by *Plectosphaerella cucumerina*. Then, it seems plausible that strategies for nutritional immunity would be evolutionarily conserved but adapted to mineral availability by metal co-option. In the same line, similarities in *AthTF* and *OsTF* composition and the specialisation of targeted genes pointed to the likely preservation of the regulatory features operating in GRNs between Arabidopsis and rice, although with different prioritisation (Fig. 9a-d). Remarkably, the principal equivalent *AthTFs* and *OsTFs* belonged to general stress-responsive families, namely WRKY, MYB, HSF and HOME0 (Wang et al., 2021; Jain et al., 2008; Jiang et al., 2017; Liu and Chang, 2013) (Fig. 9d). Conservation of GRNs governing abiotic stress responses occurred across evolution in the process of adaption to dry environments (Artur and Kajala, 2021) or between *Marchantia polymorpha* and Arabidopsis to acclimate to cold, heat, and salinity (Wu et al., 2021; Tan et al., 2023). Due to the complexity of interplays among biological processes in networks, substitutions and perturbations on regulatory nodes can lead to profound restructuration, endangering the entire system. Thus, adaptation to different conditions would be more efficient if GRNs are preserved and network rewiring occurs by changes in the hierarchy of transcription factors and/or exploring co-options at the level of regulated transcripts (Fig. 10). Further investigation is required to understand whether mechanisms similar to those described in this work also operate during interaction of rice and Arabidopsis plants with pathogens other than *M. oryzae* and *C. higginsianum*.

Preparing plants for variable and adverse environments is of utmost urgency in a context of climate change. However, increasing evidence warns us about the risk of compromising plant fitness when engineering individual biological pathways or translating advantageous strategies characterised in model plants to species of agronomical interest due to interspecific barriers (M. Liu et al., 2019; Ke et al., 2020; García-Molina and Leister, 2020). Our study uncovers trade-offs for nutritional immunity but, at the same time, may inspire biotechnological strategies to promote resilience to pathogens by manipulating Fe homeostasis.

Data availability

The transcriptome datasets generated for the current study are available in the Gene Expression Omnibus repository (GEO) (<http://www.ncbi.nlm.nih.gov/geo>) under accession number GSE231365.

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CRediT authorship contribution statement

Antoni García-Molina: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Héctor Martín-Cardoso:** Writing – review & editing, Methodology, Data curation.

María Ribaya: Writing – review & editing, Methodology. **Blanca San Segundo:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Silvia Busoms:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.stress.2025.100756](https://doi.org/10.1016/j.stress.2025.100756).

Data availability

Data will be made available on request.

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