Modulation of defence and iron homeostasis genes in rice roots by the diazotrophic endophyte Herbaspirillum seropedicae

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Rice is staple food of nearly half the world’s population. Rice yields must therefore increase to feed ever larger populations. By colonising rice and other plants, Herbaspirillum spp. stimulate plant growth and productivity. However the molecular factors involved are largely unknown. To further explore this interaction, the transcription profiles of Nipponbare rice roots inoculated with Herbaspirillum seropedicae were determined by RNA-seq. Mapping the 104 million reads against the Oryza sativa cv. Nipponbare genome produced 65 million unique mapped reads that represented 13,840 transcripts each with at least two-times coverage. About 7.4% (1,014) genes were differentially regulated and of these 255 changed expression levels more than two times. Several of the repressed genes encoded proteins related to plant defence (e.g. a putative probenazole inducible protein), plant disease resistance as well as enzymes involved in flavonoid and isoprenoid synthesis. Genes related to the synthesis and efflux of phytosiderophores (PS) and transport of PS-iron complexes were induced by the bacteria. These data suggest that the bacterium represses the rice defence system while concomitantly activating iron uptake. Transcripts of H. seropedicae were also detected amongst which transcripts of genes involved in nitrogen fixation, cell motility and cell wall synthesis were the most expressed.

To answer the ever increasing demand for cereals, genetic improvement of rice and the concomitant development of bio-fertilisers are promising, low environmental-impact solutions. After water, the most limiting nutrient in plant development is nitrogen and nitrogenous fertilisers have been heavily used in rice cultivation. Heavy use of nitrogen fertilisers causes environmental damage including contamination of ground-water and the release of nitrogen oxides.

An alternative to the use of nitrogenous fertilisers is to employ plant-associated micro-organisms that fix nitrogen. Herbaspirillum seropedicae is an endophytic diazotroph that can colonise many plants and improve their productivity (reviewed by Monteiro et al. and Chubatsu et al.). Inoculation of rice with H. seropedicae increased root and shoot biomass by 38 to 54% and 22 to 50% respectively, part of which was attributable to biological nitrogen fixation. Pankievicz et al. showed that the nitrogen fixed by H. seropedicae and Azospirillum brasilense was rapidly incorporated into Setaria viridis. Other factors, including production of phyto-hormones by...
the bacteria stimulate plant growth and several authors have observed that the increase in biomass of inoculated plants is dependent on the plant genotype49.

Transcriptome based studies are powerful tools to detect differentially expressed genes (DEG) and discover novel molecular processes10–16. Expression analyses (using EST sequencing and RT-qPCR) of rice roots inoculated with *H. seropedicae* suggested that genes related to auxin and ethylene syntheses as well as defence are modulated by the microorganism in a cultivar dependent manner17. Therefore, the purpose of the present study was to determine the effect of *H. seropedicae* on the gene expression of rice roots by RNA-seq transcriptome analyses. The data showed a genome wide repression of plant defence genes and activation of iron uptake systems, which seems an important feature for successful root colonization.

Results and Discussion

Transcriptional analyses. *H. seropedicae* enters rice roots via cracks at the points of lateral root emergence and later (three to 15 days) colonises the intercellular spaces, aerenchyma, cortical cells and vascular tissue4,6,7,18. 14 days after inoculation we observed an increase of weight of roots and leaf but this increase was not statistically significant. The number of endophytic *H. seropedicae* reached approximately $10^5$ to $10^6$ CFU per gram of fresh root weight one to two days after inoculation (DAI), with a peak at three DAI (Supplementary Fig. 1). For this reason roots were collected for RNA-seq analyses three DAI when the population of endophytic bacteria stabilised (1 of fresh roots and $4.4 \times 10^8$ epiphytic CFU.

Sixty-four percent of the reads (103,563,118) were finally used for mapping to the reference rice and *H. seropedicae* genomes. Illustration of RNA-seq analyses is shown in Supplementary Fig. 2 and the numbers of reads mapped to each reference genome are listed in Supplementary Table 1. Mapping on the rice genome database (http://rice.plantbiology.msu.edu/) produced 22 million unique mapped reads representing 13,837 expressed genes. 1TCA cycle/organic acids: 2oxidative pentose phosphate pathway; 3mitochondrial electron transport/ATP synthesis; 4gluconeogenesis/glyoxylate cycle; 5cofactor and vitamin synthesis.

Differentially expressed genes. Statistical analyses were performed using DESeq software19 and comparison of non-inoculated with inoculated samples revealed 1,014 differentially expressed transcripts (P < 0.05) (Supplementary Table 2), with 255 having fold-changes higher than two. A heat map constructed using the expression profiles of these transcripts showed the inoculated sample clustering clearly separated from the control samples (Supplementary Fig. 3). In addition, functional categorisation of these set of genes was performed using MapMan (http://mapman.gabipd.org/) (Fig. 1B) and Blast2GO20 followed by GO enrichment with Gene Set Enrichment Analysis (GSEA)21. In the Biological Process category the following GOs terms were enriched: homeostatic process, drug metabolic process, carboxylic acid metabolic process, transmembrane transport, ion transport, response to chemical, regulation of transcription, DNA-templated, oxidation-reduction process and small molecule biosynthetic process (Supplementary Table 3).

MapMan analyses also revealed several rice roots pathways regulated by *H. seropedicae* colonisation. Considering the number of regulated genes in relation to the number of expressed genes in each category, the main MapMan categories down-regulated by *H. seropedicae* were: metal handling (9.0%; 4/41), polyamine metabolism (9.1%; 1/11), secondary metabolism (9%; 15/167), hormone metabolism (4.7%; 11/233); stress (4.8%; 24/503) and nucleotide metabolism (4.9%; 6/123). Among genes up-regulated by bacteria were those involved in gluconeogenesis/glyoxylate cycles (28.6%; 02/07), polyamine metabolism (27.3%; 03/11), metal handling (24.4%; 10/41), fermentation (23.1%; 03/13) and nitrogen metabolism (18.8%; 03/16) (Fig. 1A). Since MapMan was
specifically developed to cover plant-specific pathways and is widely used by plant researchers, some of genes of these regulated categories are analysed in more detail below.

**Biotic and abiotic stresses.** Among the 255 differentially expressed genes (>2-fold), 59 were stress-related (30 repressed and 29 induced) in the following categories: secondary metabolites, hormone signalling, cell-wall, proteolysis, PR-proteins, signalling, transcription factors, redox-state, abiotic stress and peroxidases (Fig. 1B and Supplementary Table 4).

**Secondary metabolism.** Amongst the secondary metabolic pathways with higher numbers of regulated genes were those involved in phenylpropanoid and isoprenoid synthesis (Supplementary Table 4). Phenylpropanoids synthesised by deamination of L-phenylalanine are eventually converted to p-coumaric acid, a precursor of flavonoids and lignin. Additionally, *H. seropedicae* modulated expression of four genes involved in flavonoid synthesis. Amongst them, the gene encoding chalcone isomerase (CHI), which catalyses the synthesis of naringenin from tetrahydroxy-chalcone, was repressed 2.1-fold. Naringenin is a key intermediate in the synthesis of other compounds including flavonol and anthocyanins. In rice, *H. seropedicae* repressed genes of the MEP pathway (Supplementary Table 4 and Fig. 3). The most repressed transcript (5.9 times) encodes 1-deoxy-D-xylulose 5-phosphate synthase (DXS) the first enzyme of the pathway that synthesises 1-deoxy-D-xylulose 5-phosphate (DXP) from pyruvate and D-glyceraldehyde 3-phosphate. DXP is a precursor of antimicrobial compounds including phytoalexins.耐根菌诱导的MEP途径（MEP）（Fig. 3）和two-fold when infected with *Azospirillum* spp.25. It thus seems as if *H. seropedicae* represses production of defence-related isoprenoids in rice roots perhaps to allow the bacteria to enter and rapidly colonise the intercellular spaces and xylem.
Oxidative stress responses. In this study *H. seropedicae* modulated expression of five redox state genes including those for non-symbiotic hemoglobin 2 and peroxiredoxin. One peroxiredoxin transcript (LOC_Os07g44440.1) induced 3.7 and the other LOC_Os01g16152.1 was repressed 2.1 fold. Peroxiredoxins are a group of H$_2$O$_2$-decomposing antioxidant enzymes related to the redox state. In addition to the reduction of H$_2$O$_2$, peroxiredoxin proteins also detoxify alkyl hydroperoxides and peroxinitrite$^{30,31}$. Plants respond to attacks by pathogens with rapid increases in reactive oxygen species (ROS) such as superoxide and H$_2$O$_2$.$^{32}$ Peroxidases produce ROS that could cause oxidative damage to proteins, DNA, and lipids.

**Figure 3.** Isoprenoid synthesis genes down-regulated in rice roots colonised by *H. seropedicae*. The names of the genes differentially expressed are shown and the numbers in parentheses represent the fold change. The components of the MEP pathway leading to geranylgeranyl diphosphate synthesis and the diterpenoid-phytoalexin pathway are: G3P, glyceraldehyde-3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol; CDP-ME2P, 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBDP, 1-hydroxy-2-methyl-2-butenyl 4-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGDP, geranylgeranyl diphosphate; and CDP, copalyl diphosphate. Enzymes are indicated in rose coloured circles: DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, DXP reductoisomerase; CMS, CDP-ME synthase; CMK, CDP-ME kinase; MCS, MECDP synthase; HDS, HMBDP synthase; HDR, HMBDP reductase; IPI, IPP isomerase; GGPS, GGDP synthase; OsCyc1, syn-CDP synthase; OsCyc2, ent-CDP synthase; OsDTC2, stemar-13-ene synthase.
Many defects in the immune system of mature Arabidopsis thaliana plants with reduced expression of two key peroxidase genes, PRX33 or PRX34, were observed. Silencing the French-bean class III peroxidase (FBP1) in A. thaliana impaired the oxidative burst and rendered plants more susceptible to bacterial and fungal pathogens. Proteomic studies of rice roots seven days after inoculation with H. seropedicae showed induction of ascorbate peroxidases, though the genes encoding these enzymes were not affected in our RNA-seq analyses. Moreover, seven days after inoculation, ROS levels in Herbaspirillum rubrisubalbicans attached to rice roots had increased suggesting that the bacteria were subject to oxidative stresses. In this work, two peroxidase genes were induced by H. seropedicae in inoculated roots.

**Cell wall.** A variety of diazotrophic microorganisms such as H. seropedicae Z67, H. rubrisubalbicans and A. brasilense produce cell-wall degrading enzymes. Interestingly, the H. seropedicae SmR1 genome did not reveal genes coding for known cellulases, pectinases or any other cell-wall degrading enzymes. Nevertheless, rice roots inoculated with H. seropedicae induced a gene (2.0-fold) encoding a β-D-xylosidase and repressed 2.4-fold a gene coding for a polygalacturonase suggesting remodelling of plant cell wall. In A. thaliana, β-D-xylosidase has been shown to be involved in secondary cell-wall hemi-cellulose metabolism and plant development, but little is known about the function of this enzyme.

Other differentially expressed genes involved in cell-wall metabolism code for proteins similar to an expansin (induced 2.6-fold). Expansins have a loosening effect on plant cell-walls and function in cell enlargement as well as in diverse developmental processes in which cell-wall modification occurs including elongation. In addition, they promote elongation of root-hairs and root-hair initiation.

Expansins have been correlated with plant-bacteria interactions. In tobacco, Bacillus subtilis G1, a plant growth promoting bacterium, induced the expression of two expansins NTEXP2 and NTEXP6. Also, inoculation of Melilotus alba with Sinorhizobium meliloti lead to enhanced MaEXP1 mRNA levels in roots and nodules. Together, these results suggest that inoculation with H. seropedicae also led to modification of plant cell wall which may facilitate bacterial colonization of inner tissue by loosening cell wall.

**Plant immune responses.** The plant immune system can sense and respond to pathogen attacks in two manners: the first involves recognition of pathogen/microbe associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs) by surface pattern-recognition receptors (PRRs) resulting in pattern-triggered immunity (PTI). Second, resistance proteins (R) that recognize pathogen effectors are expressed leading to effector-triggered immunity (ETI). The receptors that perceive PAMPs or DAMPs have an ectodomain potentially involved in ligand binding, a single transmembrane domain and, most of times, an intracellular kinase domain. Amongst the genes with the highest expression differences seen in RNA-seq data was a wall-associated receptor kinase-like (WAK) 22 precursor (LOC_Os10g07556.1), repressed 93-fold (6.5-fold by RT-qPCR) in H. seropedicae inoculated roots. Cell wall-associated receptor kinases (WAKs) contain an extracellular domain composed of one or more epidermal growth factor (EGF) repeats. Animal proteins containing these repeats are known to bind small peptides. In A. thaliana, WAKs bind to cross-linked pectin cell wall, pathogen- or damage-induced pectin fragments and oligogalacturonides, thus regulating cell expansion or stress response depending on the state of the pectin.

Several studies have described the role of WAK genes in rice resistance to pathogens. Plant proteins domain architecture consist of cell-wall pectin binding extracellular region, the EGF-like domain, and a kinase domain. Analyses of rice loss-of-function mutants of WAK genes showed that individual genes are important for resistance against M. oryzae. OsWAK14, OsWAK91 and OsWAK92 positively regulate resistance while OsWAK112d is a negative regulator of blast resistance. Cayrol et al. (2016) demonstrated that OsWAK14, OsWAK91 and OsWAK92 can form homo- and hetero-complexes and hypothesized that the loss of function of any of these proteins may destabilize the complex and affect their functioning. In A. thaliana the WAK22 orthologous gene (AtWAK110) encodes a functional guanylyl cyclase which is co-expressed with pathogen defense related genes. Thus, the wall-associated receptor kinase-like 22 gene of rice could be a candidate for surface pattern-recognition receptor and its repression may allow H. seropedicae to evade activation of the plant-defense system.

A cysteine-rich receptor-like protein kinase (CRK) (LOC_Os04g56430.1) and a serine/threonine-protein kinase At1g18390 precursor (LOC_Os05g47770.1) were induced 3.2-fold and 2-fold, respectively, while a lectin-like receptor kinase 7 (LOC_Os07g03790.1) and SHR5-receptor-like kinase (LOC_Os08g10320.1) were repressed 2.7 and 2.5-fold respectively in the presence of H. seropedicae (Supplementary Table 4). The latter protein has 75% identity with sugarcane SHRs receptor kinase repressed by colonization with diazotrophic endophytes. The authors suggested that the expression levels of this gene were inversely related to the efficiency of beneficial plant-bacterial interactions. Besides in Arabidopsis cysteine rich receptor-like kinase 5 protein is involved in regulation of growth, development, and acclimatory responses.

Among the regulated genes that have functions involved in defence (in dark grey squares in Fig. 1B), as determined by MapMan, three genes encoding PR-proteins were repressed (LOC_Os10g25870.1, LOC_Os11g07680.1, LOC_Os02g38392.1, 2.5, 3.4 and 2.7-fold respectively) while one transcript that encoded a lipase called EDS1 (enhanced disease susceptibility 1) was induced 2-fold. The role of EDS1 in defence is well described in A. thaliana and is required for resistance conditioned by R genes which encode proteins that contain nucleotide-binding sites and leucine-rich repeats (NBS-LRR). Mutant eds1 seedlings exhibited enhanced susceptibility to the biotrophic oomycete Peronospora parasitica. Analysis of EDS1 and PR-gene expression showed induction of both genes after inoculation with Pseudomonas syringae or treatment with salicylic acid (SA). In addition, in the A. thaliana eds1 mutant, the expression of PR-proteins was undetectable. When the eds1 mutant was treated with SA however, expression of PR was detected. The authors suggest that EDS1 functions upstream of PRI1-mRNA accumulation. Among the 3 transcript for PR-proteins repressed, one (LOC_Os02g38392.1) is a NBS-LRR disease
resistance protein. Therefore, in *H. seropedicae*–rice interaction an inverse correlation between PR-protein and EDS1 expression was observed, perhaps suggesting a fine regulation of these defence systems by protein that occurs in a broad range of plant species. Thionins are known for their toxicity to plant pathogens, as seen with *H. seropedicae* in rice roots infected with *M. graminicola* et al.71 in rice roots infected with *Os07g24830.1* was induced 7.2-fold in the presence of *H. seropedicae*. Kawahara et al.65 using an RNA-seq approach to study the transcriptome of rice inoculated with the blast fungus *M. oryzae* observed that the same PBZ1 genes detected in our study were induced 273 and 233-fold upon inoculation with the pathogen. The induction of PBZ1 gene by pathogens has been considered as a molecular marker for rice defence response.

Another gene regulated by *H. seropedicae* that is related to defence codes for a thionin, a small cysteine-rich protein that occurs in a broad range of plant species. Thionins are known for their toxicity to plant pathogens and several studies showed that their over-expression is related to increased resistance to diseases. Brusamarello-Santos et al.17 showed 5-fold repression of thionin genes from chromosome 6 seven days after inoculation with *H. seropedicae* but these thionins from chromosome 6 were not regulated in roots three days after inoculation with *H. seropedicae*. Since there are 15 thionin genes (according to the Rice Genome Annotation Project RGA/27) sharing high identity in chromosome 6, the unmapped reads were mapped to the rice genome as well as to chromosome 6 separately. An average of 480 reads of control libraries and 136 of inoculated libraries mapped on thionin genes, a result that is in accordance with the repression pattern observed in rice roots seven days after inoculation with *H. seropedicae*. Interestingly, a thionin transcript from chromosome 7 (*LOC_Os07g24830.1*) was induced 7.2-fold in the presence of *H. seropedicae* three DAI. Time-dependent regulation of thionin was also observed by Li et al.71 in rice roots infected with *Meloidogyne graminicola*. Straub et al.72 observed only a few defence-related genes induced in the transcriptome of *Miscanthus sinensis* inoculated with *Herbaspirillum frisingense* helping to explain why this bacterium can effectively invade and colonise plants.

The repression of genes related to defence is necessary perhaps to allow the bacteria to enter and rapidly colonise the intercellular space and xylem. However, it seems as if a balance between induction and repression of defence-related genes allows *H. seropedicae* to survive inside rice tissues while concomitantly activating some defence responses to protect the plant from pathogens.

### Phytohormones

Auxins regulate diverse physiological processes such as vascular tissue differentiation, lateral root initiation and have also been linked to defence in plant-pathogen interactions. Auxin response elements (AuxREs), when bound to auxin response factors (ARFs), control auxin-dependent gene expression. The Aux/IAA protein family members that inhibit ARFs mediate this regulation.

Four differentially expressed transcript related to auxin signalling were identified in rice roots inoculated with *H. seropedicae*. The transcript *LOC_Os05g01570.1* encoding an auxin-responsive protein was repressed 2.1-fold (Table 1) whereas the transcript coding for auxin-induced proteins, *LOC_Os09g25770.1* and *LOC_Os05g01570.1*, were repressed 1.8 and 2.5-fold, respectively. Repression of genes related to auxin signalling was reported in rice roots inoculated with *H. seropedicae*. Brusamarello-Santos et al.17 found that ARF2-like, IAA 11 and IAA18 were repressed 1.4, 1.5 and 2.8-fold, respectively, in the presence of *H. seropedicae* 7 days after inoculation. These genes were not regulated in our data probably due to the time difference of the cDNA library construction and expression levels too low.

| Locus name          | Gene product/description* | Fold-change | Pvalue     |
|---------------------|---------------------------|-------------|------------|
| LOC_Os06g02220.1    | Auxin-induced protein 5NG4 | 2.6         | 1.83E-11   |
| LOC_Os04g57400.1    | Methylthioribose kinase   | 6.1         | 3.20E-18   |
| LOC_Os04g57410.1    | Methylthioribose kinase   | 10.2        | 1.16E-12   |
| LOC_Os06g04510.1    | Similar to enolase 1      | 1.9         | 0.03       |
| LOC_Os03g63900.1    | 1-aminoacyclopropane-1-carboxylate oxidase 2 (ACC oxidase) | 0.50 | 7.88E-04 |
| LOC_Os01g22010.3    | S-adenosylmethionine synthetase (SAM) | 1.9 | 1.72E-05 |
| LOC_Os03g06620.1    | 1,2-dihydroxy-3-keto-5-methylpentene dioxygenase | 7.5 | 5.94E-05 |
| LOC_Os10g28360.1    | 1,2-dihydroxy-3-keto-5-methylpentene dioxygenase | 0.47 | 6.14E-03 |

**Table 1.** Rice genes modulated by colonisation with *H. seropedicae* that are involved in phyto-hormone signalling and the MTA cycle. Gene product name/description of the both rice database were used: MSU (Rice Genome Annotation Project) and RAPDB (The Rice Annotation Project).
*H. seropedicae* can produce IAA in the presence of tryptophan, thus suggesting the plant growth promotion effect may be due to bacterial derived auxin\(^8\). In addition exogenous application of auxin lead to increase in lateral root numbers\(^2\). In the absence of clear regulation of auxin-dependent genes and phenotype, the results suggest that auxin does not play an important role in *Nipponbare* rice roots colonization by *H. seropedicae*. On the other hand the repression of auxin signalling has been correlated to defence responses. Auxin levels have been correlated with susceptibility to pathogens\(^78\) that are able to produce high levels of auxin\(^80\). It has also been shown that pathogen-associated molecular patterns (PAMPs) induce the expression of a miRNA that negatively regulates mRNAs for F-box auxin receptors leading to resistance to *P. syringae* in *Arabidopsis*\(^83\). The observed repression in several genes involved with defence in the rice- *seropedicae* interaction opens the question whether auxin could be important for bacteria survival inside the plant by down-regulating defence system. Further studies are needed to elucidate if and how auxin signalling participates in plant-bacterial interactions.

Ethylene is also involved in several biological processes that activate defence responses and adventitious root-growth in rice and other plants\(^82-84\). Ethylene can be synthesised by oxidation of 1-aminoacyclopropane-1-carboxylate (ACC) by ACC oxidase. ACC is synthesised from adenosylmethionine (AdoMet) by ACC synthase (ACS). ACS is up regulated 6-fold and ACC oxidase is repressed 2-fold by inoculation with *H. seropedicae*. An ethylene response factor (ERF) (LOC_Os01g04800.1) transcript was also induced 2.2-fold in inoculated roots. These data indicate that ethylene synthesis is attenuated in the presence of bacteria. Moreover Alberton et al.\(^26\) measured the level of ethylene in inoculated rice roots (seven days after inoculation) and found a decrease of ethylene levels. Furthermore, Valdameri et al.\(^35\) detected induction of ACC oxidase in rice plants inoculated with the pathogen *H. rubrisubalbicans*. These results suggest that the ethylene pathway is differentially modulated in the presence of pathogens and beneficial endophytic bacteria that promote plant growth.

Salicylic acid is derived from phenolic compounds and is involved in response to attack by pathogens\(^85,86\). In rice roots inoculated with *H. seropedicae*, a SA-dependent carboxyl methyltransferase family protein gene (LOC_Os11g15340.2) was repressed 40-fold. A member of this family is salicylic acid carboxyl methyltransferase (SAMT) that catalyses the formation of methyl salicylate (MeSA) from SA\(^87\). MeSA is an essential signal for systemic acquired resistance (SAR) in tobacco plants. In addition mutations in SAMT showed that this gene is required for SAR\(^88\).

SA signalling is differentially regulated by members of the WRKY transcription factor family\(^89\). In inoculated rice roots, two WRKY transcription factors were regulated, one repressed (2.3-fold) and one induced (2.0-fold). The induced gene encodes a WRKY51 similar to WRKY11 of *Arabidopsis*, whereas the repressed gene encodes WRKY46, which was shown to be induced by SA in *Arabidopsis*. WRKY11 is a negative regulator of resistance\(^90\) and *Arabidopsis* plants in which WRKY46 was over-expressed were more resistant to *P. syringae*\(^91\). These results are in agreement with the hypothesis that the SA signalling and defence system are attenuated in the presence of the *H. seropedicae*.

**Metal ion metabolism.** Several genes related to metal transport were differentially expressed, most of them up regulated in the presence of *H. seropedicae*. Amongst the 20 most highly regulated rice genes, eight were related to metal transport (Table 2 and 3). Phyto-siderophore synthesis starts with production of nicotianamine (NA) from S-adenosylmethionine, which in turn is derived from 5'-methylthioadenosine of the methionine salvage pathway (MTA cycle) (Fig. 4). Transcripts of enzymes of the MTA cycle encoding SAM, MTA nucleosidase, MTR kinase, E1 enolase/phosphatase and acireductone dioxygenase (ARD) were induced 1.9, 2.6, 6.0, 1.9 and 7.5-times, respectively, in roots colonised by *H. seropedicae* (Fig. 4). Using proteomic and RT-qPCR analyses, Alberton et al.\(^35\) observed similar expression patterns in rice roots inoculated with *H. seropedicae*.

The synthesis of NA and MAs involves a set of enzymes including S-adenosylmethionine synthase (SAM) that catalyses the adenylation of L-methionine to S-adenosylmethionine, nicotiamine synthase (NAS) that converts S-adenosylmethionine to nicotiamine, and nicotiamine aminotransferase (NAAT) that catalyses the amino transfer of NA to produce the 3'-keto intermediate that is reduced by deoxymugineic acid synthase (DMAS) to produce 2'-deoxymugineic acid (DMA)\(^92-95\).

Two nicotiamine synthase (NAS) genes of rice - LOC_Os03g19420.2 and LOC_Os03g19427.1 were induced (52- and 32-fold, respectively) in inoculated rice roots. LOC_Os03g19427.1 was also induced in rice roots inoculated with *Azospirillum*\(^29\). Increased levels of nicotiamine have been shown to increase Fe uptake in rice plants\(^89\). Furthermore, in *Lotus japonicus* inoculated with *Mesorhizobium loti*, nicotiamine synthase 2 was expressed only in nodules pointing to a role in symbiotic nitrogen fixation\(^96\). A tomato mutant defective in the expression of nicotiamine was affected in iron metabolism\(^88\). In addition, NAAT (LOC_Os02g20360.1) and DMAS (LOC_Os03g13390.2) were also induced 10-fold and 21-fold respectively in rice roots inoculated. Induction of *OsYSL15* was confirmed by RT-qPCR (Fig. 2).

Iron deficiency provoked induction of rice gene OsNAA1\(^98\).

Recently, members of a major facilitator super-family have been described as essential to the efflux of MA and NA in rice. TOM1 (transporter of mugineic acid 1) is involved in the efflux of MA, while ENA1 (efflux transporters of nicotiamine 1) and ENA2 in the efflux of NA\(^99\). TOM1 (LOC_Os11g04020.1) and ENA1 (LOC_Os11g05390.1) were induced 40 and 3-fold in colonised roots. Induction of TOM1 was confirmed by RT-qPCR (Fig. 2).

Fe\(^{3+}\) ions chelated by PS need to be transported inside the cell. In gramineous plants, two groups of Fe-MA transporters are present: ZmYSL1\(^100\) and the YSL (yellow strip-like) transporter family\(^101\). Inoue et al.\(^101\) analysed the expression of 18 YSL genes in rice and observed induction of *OsYSL15* and *OSYSL16* genes under iron-deficiency. Other studies have shown that OsYSL2 (LOC_Os02g43370) takes up Fe\(^{3+}\) -NA\(^98\) and OsYSL16 Fe\(^{3+}\) -DMA (LOC_Os04g45900.1)\(^103\). Here we showed induction (3.2-fold) of *OsYSL16* (LOC_Os04g45900.1) and 21-fold increase of transcripts encoding a gene similar to *OSYSL15* (LOC_Os02g43410.1). Induction of *OSYSL15* was confirmed by RT-qPCR (Fig. 2). Furthermore, OsIRT1 (iron-regulated transporter 1) and OsIRT2...
et al. non-symbiotic hemoglobin 2 could help buffer free oxygen and protect bacterial nitrogenase. Arredondo-Peter et al. in colonised roots codes for the non-symbiotic hemoglobin 2 (LOC_Os03g12510.1). High levels of colonised roots respond in such a manner as to accumulate Fe.

Table 2. Rice genes highly regulated (fold change > 10) by inoculation with *H. seropedicae*. Gene product name/description of the both rice database were used: MSU (Rice Genome Annotation Project) and RAPDB (The Rice Annotation Project).

| Locus name | Gene product/description* | Fold change | P-value |
|------------|---------------------------|-------------|---------|
| LOC_Os02g20360.1 | Tyrosine aminotransferase, putative, expressed, Similar to Nicotianamine aminotransferase A (RAPDB) | 10 | 5.24E-30 |
| LOC_Os04g57410.1 | Methylthioribose kinase, putative. Expressed | 10.2 | 1.16E-12 |
| LOC_Os03g06210.1 | Helix-loop-helix DNA-binding domain containing protein. Expressed | 12.6 | 4.10E-09 |
| LOC_Os01g23700.1 | Helix-loop-helix DNA-binding domain containing protein. Expressed | 16.4 | 3.36E-07 |
| LOC_Os11g15624.1 | Expressed protein | 17.7 | 1.22E-25 |
| LOC_Os03g13390.2 | Oxidoreductase. aldo/keto reductase family protein, putative. Expressed | 19.4 | 1.61E-10 |
| LOC_Os02g43410.1 | Transposon protein, putative. Unclassified. Expressed | 21 | 3.97E-01 |
| LOC_Os10g11889.2 | Expressed protein | 23 | 3.29E-113 |
| LOC_Os01g65110.1 | POT family protein. Expressed | 26 | 6.63E-14 |
| LOC_Os07g15640.1 | Metal transporter Nramp6, putative. Expressed | 27 | 3.56E-01 |
| LOC_Os03g03724.1 | Expressed protein | 28 | 0.02 |
| LOC_Os03g19427.1 | Nicotianamine synthase, putative. expressed | 32 | 1.02E-22 |
| LOC_Os03g04654.1 | Metal cation transporter, putative. expressed | 34 | 4.02E-08 |
| LOC_Os06g19095.1 | Expressed protein | 40 | 1.51E-14 |
| LOC_Os11g04020.1 | Major facilitator superfamily antiporter, putative. expressed | 40 | 8.84E-51 |
| LOC_Os03g19420.2 | Nicotianamine synthase, putative. expressed | 52 | 5.57E-80 |
| LOC_Os12g18410.1 | Expressed protein | 70 | 2.07E-05 |
| LOC_Os03g12510.1 | Non-symbiotic hemoglobin 2, putative. expressed | 104 | 5.58E-24 |
| LOC_Os04g51660.1 | Transferase family protein, putative. expressed | 0.08 | 2.79E-03 |
| LOC_Os04g59020.1 | Integral membrane protein, putative, expressed | 0.08 | 3.41E-03 |
| LOC_Os09g23300.1 | Integral membrane protein, putative, expressed | 0.06 | 3.63E-05 |
| LOC_Os11g15340.2 | SAM dependent carboxyl methyltransferase family protein, putative, expressed | 0.03 | 1.54E-03 |
| LOC_Os01g55690.1 | Glutelin, putative, expressed | 0.02 | 0.02 |
| LOC_Os12g38040.1 | Metallothionein family protein, expressed | 0.02 | 3.72E-22 |
| LOC_Os10g07556.1 | Wall-associated receptor kinase-like 22 precursor, putative, expressed | 0.01 | 5.22E-08 |

were also induced under low Fe conditions. We detected induction of OsIRT2 (LOC_Os03g46454.1) (34-fold in RNA-seq and 93-fold in RT-qPCR (Fig. 2)).

Other metal transporters such as Nramp6 (LOC_Os07g15460.1) were also up regulated (27-fold) in colonised rice roots, a result confirmed by RT-qPCR (Fig. 2). Members of the natural resistance-associated macrophage protein (NRAMP) family are transition metal cation/proton co-transporters or anti-porters of broad specificity. An NRAMP6 of *Arabidopsis* is up-regulated in response to iron deficiency and is involved with metal mobilization from vacuoles to cytosol. Induction of Nramp6 was observed in rice roots colonised by *H. seropedicae*. In a previous study in rice inoculated with *Azospirillum* spp the expression of this gene was also induced. In addition a gene for an integral membrane protein (LOC_Os09g23300.1) named OsVIT2 was 17.7-fold repressed by *H. seropedicae*. This gene is involved in transport of Fe/Zn into vacuoles and is up-regulated in rice roots with excess Fe. Knockout/ knockdown of this gene led to Fe accumulation in seeds. Together, these results suggest that colonised roots respond in such a manner as to accumulate Fe.

Interestingly the transcript with the highest fold change (104-times – a result confirmed using RT-qPCR (Fig. 2)) in colonised roots codes for the non-symbiotic hemoglobin 2 (LOC_Os03g12510.1). High levels of non-symbiotic hemoglobin 2 could help buffer free oxygen and protect bacterial nitrogenase. Arredondo-Peter et al. study the repression of two hemoglobins, Hb1 and Hb2, in rice. The Hb2 induced by *H. seropedicae* is very similar to the one described by Arredondo-Peter et al. (1997) (coverage 82% with an identity of 97%). In addition, Lira-Ruan, Sarath and Arredondo-Peter studied the synthesis of hemoglobins in rice under normal and stress conditions, coming to the conclusion that Hbs are not part of a generalised stress response. They demonstrated that Hb1 is expressed in different rice organs (root and leaves) during plant development. In etiolated rice plants under O₂ limiting conditions the Hb levels increase. This increase suggests that Hb expression maybe due to reduced O₂ levels in the presence of the bacteria which make the root environment microaerophilic. The higher requirement for Fe needed for incorporation into Hb may partially explain activation of siderophore synthesis and Fe accumulation. We also found a *H. seropedicae* bacterioferritin (Hsero_1195) gene induced (2.2-fold), but symptoms of Fe deficiency in colonised rice plants were not observed.

Iron homeostasis has been related to plant defence, ROS accumulation and immunity. Also, Fe deficiency triggers accumulation of antimicrobial phenolics compounds. It has been suggested recently that Fe sequestration by bacterial siderophore could be a signal for pathogen infection. However, bacterial genes involved in
siderophore biosynthesis were not observed among the *H. seropedicae* genes expressed in rice roots. Also, transcriptomic analysis of *H. seropedicae* attached to wheat and maize roots did not show iron metabolism genes up-regulated. These results suggest that the effect of bacteria on plant iron metabolism is more complex than those caused by iron sequestration.

Table 3. Differentially expressed genes in rice roots colonised by *H. seropedicae* involved in uptake and transport of metals. Gene product name/description of the both rice database were used: MSU (Rice Genome Annotation Project) and RAPDB (The Rice Annotation Project).

| Locus name     | Gene product/description*                               | Fold change | p-value  |
|----------------|--------------------------------------------------------|-------------|----------|
| LOC_Os01g22010.3 | S-adenosylmethionine synthetase, putative, expressed    | 1.9         | 1.72E-05 |
| LOC_Os03g19427.1 | Nicotianamine synthase, putative, expressed             | 32          | 1.02E-22 |
| LOC_Os03g19420.2 | Nicotianamine synthase, putative, expressed             | 52          | 5.57E-80 |
| LOC_Os02g20360.1 | Tyrosine aminotransferase, putative, expressed (MSU), Similar to Nicotianamine aminotransferase (RAPDB) | 10          | 5.24E-30 |
| LOC_Os03g13390.2 | Oxidoreductase, aldo/keto reductase family protein, putative, Expressed (MSU) Similar to NADPH-dependent codeinone reductase, gene name: deoxymugineic acid synthase1 (RAPDB) | 19.4        | 1.61E-10 |
| LOC_Os11g04020.1 | Major facilitator superfamily antipporter, putative, expressed (TOM1) | 40          | 8.84E-51 |
| LOC_Os11g05390.1 | Transporter, major facilitator family, putative, expressed (ENA1) | 3.1         | 5.80E-03 |
| LOC_Os03g46470.1 | Metal cation transporter, putative, expressed (OsIRT1) | 4.3         | 4.09E-14 |
| LOC_Os03g46454.1 | Metal cation transporter, putative, expressed (OsIRT2) | 34          | 4.02E-08 |
| LOC_Os04g45900.1 | Transposon protein, putative, unclassified, expressed (MSU) Similar to Metal-nicotianamine transporter YSL2, Gene symbol synonym: OsYSL16 (RAPDB) | 3.2         | 1.23E-07 |
| LOC_Os02g43410.1 | Transposon protein, putative. Unclassified, Expressed (MSU) Iron-phytosiderophore transporter; Iron homoeostasis (Os02t0650300-01); Similar to Iron-phytosiderophore transporter YSL15, (Os02t0650300-02) (RAP-DB) | 21          | 3.97E-81 |
| LOC_Os07g15460.1 | Metal transporter Nramp6, putative. Expressed (MSU)     | 27          | 3.56E-61 |

Figure 4. Differentially expressed genes in rice roots following colonisation by *H. seropedicae*. Genes involved in siderophore synthesis and transport, the methionine salvage pathway and ethylene synthesis are shown. Numbers in parentheses represent the fold change. *H. seropedicae* SmR1 induces methionine recycling and mugineic acid (MA) synthesis as well as the expression of transporters involved in iron metabolism. The expression of those genes marked with an asterisk was confirmed by RT-qPCR Abbreviations: AdoMet, S-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylate; ACS, 1-aminocyclopropane-1-carboxylate synthase; ACO, 1-aminocyclopropane-1-carboxylate oxidase; MTA, 5′-methylthioadenosine; MTR, 5′-methylthioribose; MTK, methylthioribose kinase; MTR-1-P, 5′-methylthioribose-1-phosphate; KMTB, 2-keto-4-methylthiobutyrate; ARD, acireductone dioxygenase; SAMS, S-adenosylmethionine synthetase; NAS, nicotianamine synthase; NAAT nicotianamine aminotransferase; DMAS, deoxymugineic acid synthase; Tom1, transporter of mugineic acid 1; ENA1 (efflux transporters of nicotianamine 1); Nramp6, Natural Resistance-Associated Macrophage Protein; IRT2(iron-regulated transporter 2); YSL16 (yellow strip-like gene 16); YSL15 (yellow strip-like gene 15).
with expression in culture. This gene was also found to be up-regulated in epiphytic flagellates.

A methyl-accepting chemotaxis trans-membrane protein (Hsero_2723) was induced 90-fold when compared to plants with bacterial genes expressed in culture revealed only 16 differences (p-value < 0.05 using the DESeq statistical package (Table 4)).

H. seropedicae transcripts detected in rice roots. The libraries from inoculated roots were mapped against the H. seropedicae genome (24,263 reads representing 0.06% of the total reads) (Supplementary Table 1). Amongst the 4,085 annotated genes of H. seropedicae, 287 were expressed in rice roots (at least one-fold coverage (this set of genes was called H. seropedicae expressed genes) (Supplementary Table 5)). After ribosomal genes, the most abundant functional classes found were unknown, energy production and conversion, amino acid transport and metabolism, cell motility and cell wall (Table 4). Comparison of expressed genes of H. seropedicae detected in plants with bacterial genes expressed in culture revealed only 16 differences [p-value < 0.05] as protein, turnover, chaperones.

| Locus_tag     | Fold Change | p-value | ID Feature      | Description                                           | COG                     |
|---------------|-------------|---------|-----------------|-------------------------------------------------------|-------------------------|
| HSERo_RS17470 | 0.4         | 7.0E-04 | qor             | qor NADPH:quinone oxidoreductase protein 4007887:4008915 forward | C-R Energy production and conversion;General function prediction only |
| HSERo_RS05670 | 30          | 6.20E-03| Hsero_1130      | Hsero_1130 ABC-type dipeptide transporter, periplasmic peptide-binding protein 1287409:1289031 forward | E - Amino acid transport and metabolism |
| HSERo_RS23580 | 27          | 7.70E-03| urtA            | UrtA ABC-type urea transport system, periplasmic component protein 5407999:5409252 forward | E - Amino acid transport and metabolism |
| HSERo_RS00420 | 43          | 0.02    | glnK            | GlnK nitrogen regulatory PII-like protein 99052:99090 forward | E - Amino acid transport and metabolism |
| HSERo_RS07390 | 0.2         | 2.0E-04 | trnC            | TrnC tRNA dehydrogenase protein 1690575:1691654 reverse | G - Carbohydrate transport and metabolism |
| HSERo_RS05635 | 84          | 0.02    | Hsero_1123      | Hsero_1123 family II amino transferase protein 1278563:1279939 reverse | H - Coenzyme transport and metabolism |
| HSERo_RS07590 | 0.1         | 2.0E-03 | rpsL            | RpsL SOS ribosomal subunit protein L19 5407999:1731915 forward | J - Translation, ribosomal structure and biogenesis |
| HSERo_RS03380 | 0.3         | 0.02    | ompW2           | OmpW2 outer membrane W protein 741960:741927 forward | M - Cell wall |
| HSERo_RS12905 | 0.4         | 5.0E-03 | lon             | Lon ATP-dependent protease LA protein 2940893:2943301 reverse | O - Posttranslational modification, protein turnover, chaperones |
| HSERo_RS00425 | 20          | 0.04    | amtB            | AmtB ammonium transporter transmembrane protein 99406:100938 forward | P - Inorganic ion transport and metabolism |
| HSERo_RS14575 | 0.1         | 1.10E-03| Hsero_2905      | Hsero_2905 conserved hypothetical protein 3301039:3301272 reverse | S - Function unknown |
| HSERo_RS00415 | 15          | 0.03    | Hsero_0083      | Hsero_0083 membrane protein 98251:99039 forward | S - Function unknown |
| HSERo_RS13665 | 90          | 1.90E-03| Hsero_2723      | Hsero_2723 methyl-accepting chemotaxis transmembrane protein 3101951:3103597 reverse | T-N Signal transduction mechanisms;Cell motility |
| HSERo_RS06095 | 0.3         | 0.04    | trnK            | TrnK tRNA-Lys 1374570:1374645 reverse | trnK |
| HSERo_RS07375 | 0.2         | 1.7E-03 | trnL            | TrnL tRNA-Leu 1689660:1689744 reverse | trnL |
| HSERo_RS07775 | 0.01        | 0.03    | trnS            | TrnS tRNA-Ser 1781783:1781873 forward | trnS |

Table 4. Genes of H. seropedicae regulated during interaction with rice roots.
Materials and Methods

Plant material and growth conditions. Testas were removed from seeds of rice (Oryza sativa ssp japonica cv. Nipponbare, kindly provided by the Instituto Riograndense do arroz, IRGA – Avenida Missões 342, Porto Alegre, RS, Brazil), then disinfected with 70% (v/v) ethanol for 5 min followed by 30 min soaking in 8% sodium hypochlorite (1 mL per seed) containing 0.1% (v/v) Triton-X100. After rinsing 20 times with sterile water, the seeds were treated with 0.025% (v/v) Vitavax-Thiram (Chentura, Avenida Nações Unidas 4777, Alto de Pinheiros, São Paulo, SP, Brazil) fungicide solution and sterilized (120 rpm) for 24 h in the dark at 30°C. The seeds were then transferred to 0.7% water-agar and left for two days to germinate after which the seedlings were inoculated with 1 mL of Herbaspirillum seropedicae strain SmR1 (10⁶ cells/seedling) for 30 minutes while control seedlings were treated with 1 mL of N-free NFbHP-malate medium (controls). Seedlings were washed with sterile water and transferred to glass tubes (25 cm long, 2.5 cm diameter) containing propylene beads and 25 mL of modified Hoagland's solution without nitrogen (1 mM KH₂PO₄, 1 mM KHPO₄, 2 mM MgSO₄·7H₂O, 2 mM CaCl₂·2H₂O, 1 mL/L micronutrient solution (H₃BO₃ 2.86 g/L⁻¹, MnCl₂·4H₂O 1.81 g/L⁻¹, ZnSO₄·7H₂O 0.22 g/L⁻¹, CuSO₄·5H₂O 0.08 g/L⁻¹, Na₂MoO₄·2H₂O 0.02 g/L⁻¹) and 1 mL/L Fe-EDTA solution (Na₂HEDTA·H₂O 13.4 g/L⁻¹ and FeCl₃·6H₂O 6 g/L⁻¹), pH 6.5–7.0. Plants were cultivated at 24°C under 14 h light and 10 h dark for 3 days. H. seropedicae was cultivated in NFbHP malate medium containing 5 mM glutamate as the nitrogen source. Cells were shaken (120 rpm) overnight at 30°C, then centrifuged, washed once with N-free NFbHP-malate and suspended in the same medium to OD₆₀₀ = 1 (corresponding to 10⁶ cells/mL⁻¹). Strain SmR1 is a spontaneous streptomycin resistant mutant of strain Z78. For colonisation assays roots from one plant were washed in 70% (v/v) ethanol for 1 min, 1% chloramidine T for 1 min followed by three washes with sterile water. At least 5 plants were used per data point. The roots were then crushed with a mortar and pestle in 1 mL of NFbHP-malate and serial dilutions (10⁻¹ to 10⁻⁶) were plated onto solid NFbHP-malate containing 20 mM NH₄Cl. Two days later the cells were counted to determine the colony forming units (CFU) per gram of fresh root.

RNA isolation and construction of libraries. Three days after inoculation with H. seropedicae (6 days after the disinfection procedure) the roots were separated from the aerial part and immediately stored in RNA later™ (Life Technologies, Foster City, CA, USA). Total RNA was extracted from roots of five rice plants for each biological replicates (using a RNAqueous kit (Ambion, Austin, TX, USA)). Contaminating genomic DNA was eliminated with RNase-free DNase I (Ambion) for 30 min at 37 °C. Total RNA (7 to 10 μg) was depleted of ribosomal RNA by treatment with a RiboMinus™ Plant Kit for RNA-Seq (Invitrogen, Carlsbad, CA, USA). The integrity and quality of the total RNA was checked spectrophotometrically and by agarose gel electrophoresis. Whole Transcriptome Analysis RNA Kits™ (Life Technologies) were used on 500 ng purified RNA to construct the libraries. The sequencing and data analysis were done in a SOLiD4 (Life Technologies) sequencer. Two independent libraries were constructed for each condition (control and inoculated). Three of these libraries (two from control sample and one from inoculated sample) were run twice to check for technical reproducibility.

Sequencing and data analysis. SOLiD sequencing produced 161 million 50 bp reads that were analysed by SAET software (Applied Biosystems – Foster City, CA, EUA) to improve base calling (command shown in Supplementary material 1), followed by quality trimming using the CLC Genomics Workbench (CLC bio, Qiagen, CA, USA) (quality scores higher than 0.05 and reads with less than 20 bp were discarded). Then the reads were mapped to the rice genome database from the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) using CLC Genomics Workbench and the following parameters: a minimum length fraction of 95%, minimum similarity of 90% and only one hit with less than 20 bp were discarded. Then the reads were mapped to the rice genome database from the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) using CLC Genomics Workbench and the following parameters: a minimum length fraction of 95%, minimum similarity of 90% and only one hit. Differential expression was analysed using DESeq. Genes covered at least twice were considered expressed and the following parameters: a minimum length fraction of 95%, minimum similarity of 90% and only one hit with less than 20 bp were discarded. Then the reads were mapped to the rice genome database from the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) using CLC Genomics Workbench and the following parameters: a minimum length fraction of 95%, minimum similarity of 90% and only one hit.

Quantification of mRNA levels using RT-qPCR. Reverse transcription quantitative PCR (RT-qPCR) analyses were used to evaluate gene expression under the conditions described above. Total RNA was isolated from rice roots using the TRI Reagent (Sigma, St. Louis, MO, USA) and contamination with genomic DNA was removed with DNAse I (Life Technologies). The integrity and quality of the total RNA was confirmed by spectrophotometric analyses and electrophoresis. cDNA was produced from 1 μg DNA-treated total RNA using high-capacity cDNA reverse transcription kits (Life Technologies). The cDNA reaction was diluted 60 times before quantitative PCR using Power SYBR-Green PCR Master Mix on a Step One Plus Real Time-PCR System (both from Life Technologies). Primer sequences are listed in Supplementary Table 6 and were designed with the Primer express 3.0 software (Applied Biosystems) and the NCBI primer designing tool using the genome sequence of O. sativa ssp. japonica cv. Nipponbare. Calibration curves for all primer sets were linear over four orders of magnitude (R² = 0.98 to 0.99) and efficiencies were 90% or higher. mRNA expression levels were normalised using the expression levels of actin 1, tubulin beta-2 chain (beta-2 tubulin) and a hypothetical protein (protein kinase) using geNorm 3.4 software. The relative expression level was calculated according to Pfaffl. Three independent samples were analysed for each condition and each sample was assayed in triplicate.
Data Availability

The data that support the findings of this study are openly available. RNA-Seq data from this study have been deposited at the NCBI under the BioProject accession No. PRJNA489273 and BioSample Nos. SAMN09942067, SAMN09942069, SAMN09942071 and SAMN09953899.

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Author Contributions

L.C.C.B.-S. did the plant growth experiments and RNA purification. L.C.C.B.-S., D.C.-N., M.Z.T.-S. and H.F. prepared the sequencing libraries and sequencing. L.C.C.B.-S., E.M.S., R.C., K.I. and A.B.-S. performed bioinformatics analyse. L.C.C.B.-S., D.A., R.A.M., W.J.B., E.O.P., R.W. and E.M.S. designed the project and wrote the main manuscript text. L.C.C.B.-S. and V.G. did the qRT-PCR experiments and analysis. All the authors reviewed the manuscript.
Additional Information

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