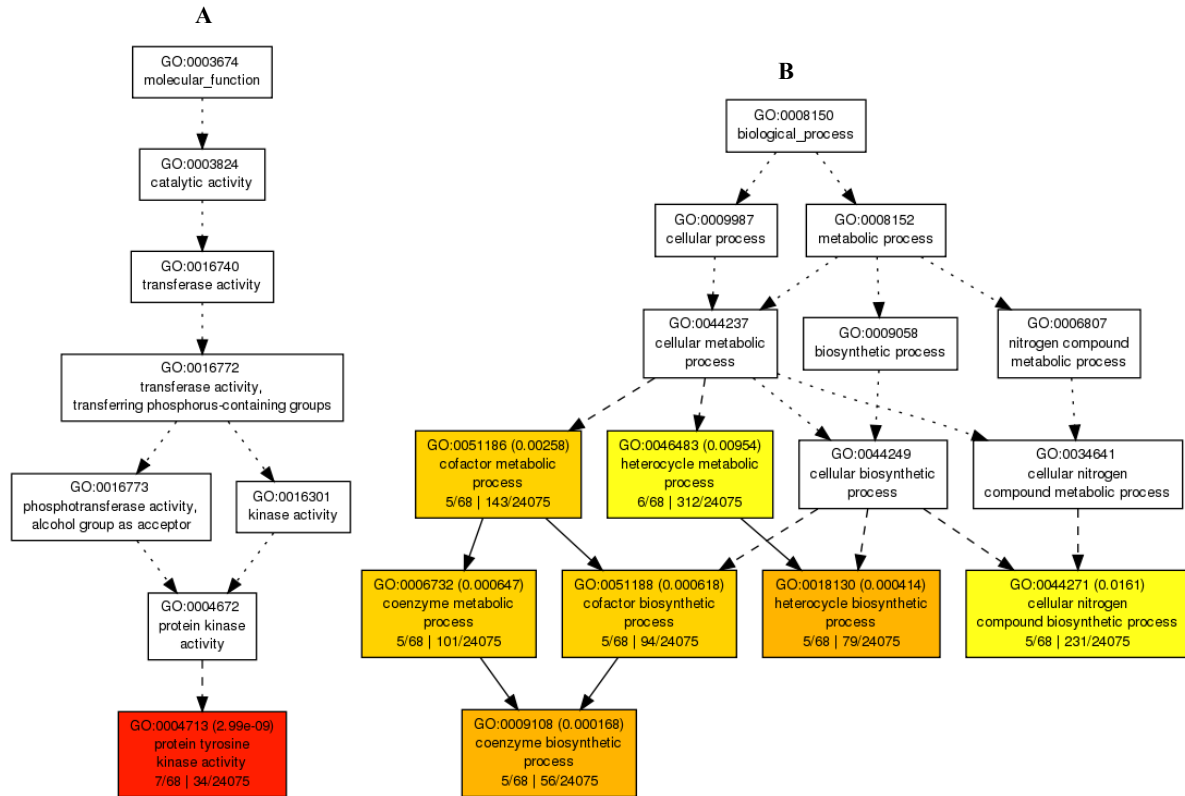


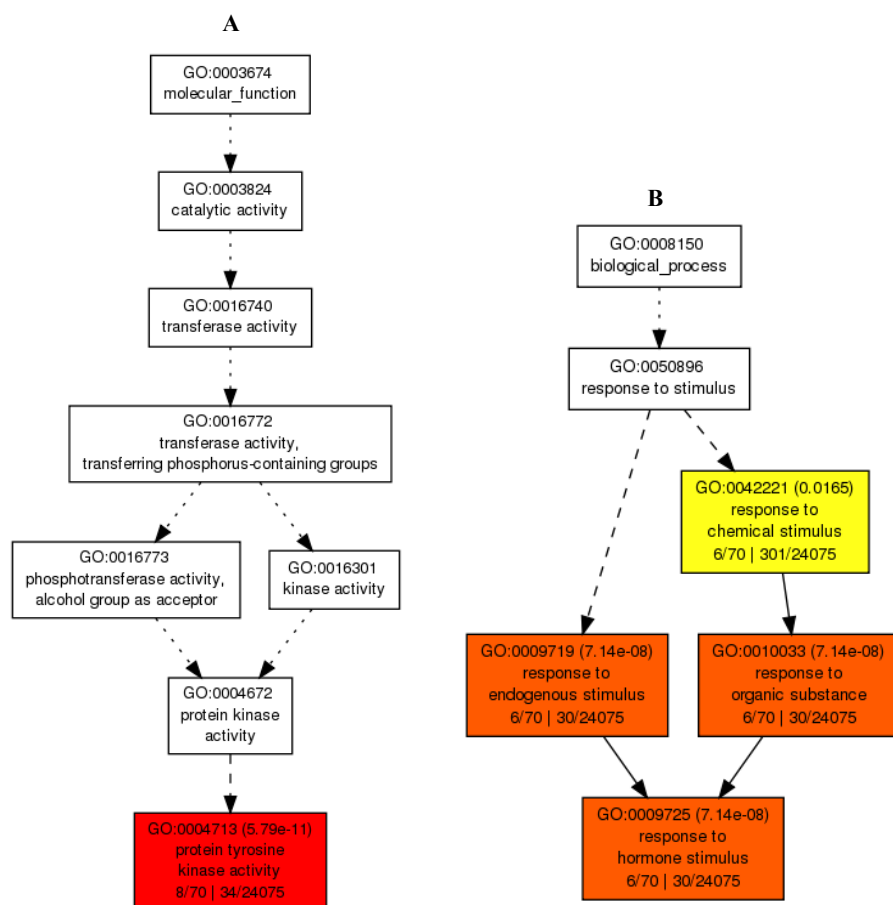
Rice miRNA-Mediated Trans-Kingdom Gene Regulation in Pathogenic and Symbiotic Fungal Interactions

Supplementary Information

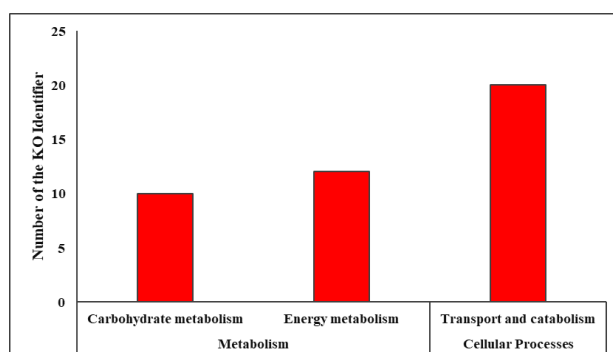
This PDF file includes: Supplementary figures S1-4, and tables S1– S4



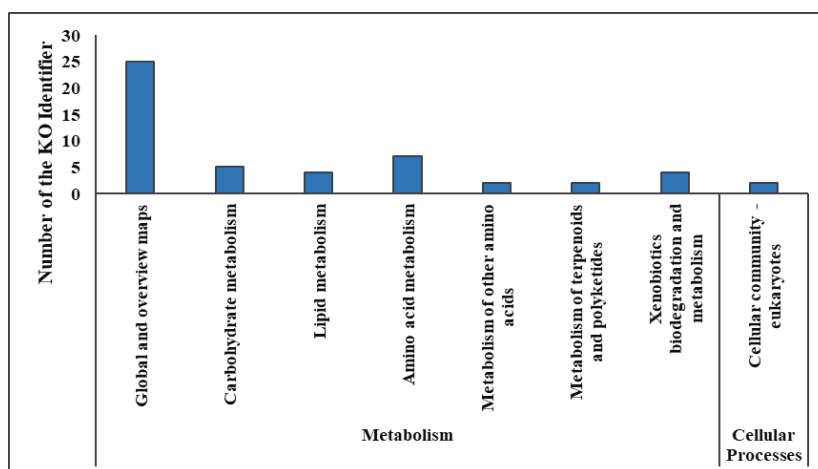
Supplementary figure S1. Potential functions of osa-miR171h coding *O. sativa* target genes. (A) Graphical results of the molecular function. (B) Graphical results of the biological process. The AgriGO generated graphical results.



Supplementary figure S2. Potential functions of osa-miR167d-5p coding *O. sativa* target genes. (A) Graphical results of the molecular function. (B) Graphical results of the biological process. The AgriGO generated graphical results.



Supplementary figure S3. KEGG annotation of the potential functions of osa-miR171h coding targets in the *R. irregularis* transcriptome.



Supplementary figure S4. KEGG annotation of the potential functions of osa-miR167d-5p coding targets in the *R. irregularis* transcriptome.

Supplementary table S1. Primers used for stem-loop RT-qPCR analysis of miRNAs.

miRNA	RT primer (5'-3')	(5'-3') Forward primer	(5'-3') Reverse primer
miRmiR167d-5p	CTCAACTGGTGTCTGGAGTCCGG-CAATTCAGTTGAGCAGATCAT	ACACTCCAGCTGGGTGAA-GCTGCC	AACTGGTGTCTGGAG
miR171h	CTCAACTGGTGTCTGGAGTCCGG-CAATTCAGTTGAGAGTGATAT	ACAC-TCCAGCTGGGGTGAGCCGAA	AACTGGTGTCTGGAG

Supplementary table S2. Target genes of the 26 and 21 osa-miRNAs against the *R. solani* and *M. oryzae* transcriptomes, respectively, that potentially play a role in fungal survival and pathogenicity.

Fungi Name	miRNA	Target gene	Potential Activity	Gene IDs	Maximum Expectation Score	Seed Mismatch
<i>R. solani</i>	osa-miR159c osa-miR167e-3p osa-miR167h-3p osa-miR169f.2 osa-miR319b osa-miR393b-3p osa-miR396d osa-miR535-3p osa-miR1423-3p osa-miR1429-3p osa-miR1850.3 osa-miR1857-5p osa-miR1861i osa-miR1863c osa-miR2863a osa-miR7693-5p osa-miR7693-5p	Cytochrome P450 family protein	Involved in secondary metabolism, production of fungal toxins, and counteracting plant defense mechanisms.	XM_043331959.1	3	0

	osa-miR159c osa-miR167d-5p osa-miR167e-3p osa-miR169f.2 osa-miR319b osa-miR393b-3p osa-miR396d osa-miR399j osa-miR535-3p osa-miR1423-3p osa-miR1429-3p osa-miR1850.3 osa-miR1863c osa-miR2863a osa-miR2873b osa-miR7693-5p	Glycoside hydrolase family protein 31 protein	Degradation of plant cell wall components	XM_043328893.1	3.5	2
	osa-miR1320-5p osa-miR1861i	Chitinase	Degradation of chitin in the fungal cell wall which can impact fungal growth, development, and its ability to interact with the host or evade plant defenses.	XM_043327070.1	5	2
	osa-miR167h-3p osa-miR393a osa-miR535-3p osa-miR1429-3p osa-miR1857-5p osa-miR2863a osa-miR2873b	Peptidase C14	Degradation of plant defense proteins or facilitation of fungal penetration into tissues.	XM_043325359.1	3.5	2
	osa-miR159c osa-miR167e-3p osa-miR319b osa-miR393a osa-miR393b-5p osa-miR396c-5p osa-miR396d osa-miR399j osa-miR535-3p osa-miR2873b	Glycosyltransferase family 1 protein	Involved in the synthesis of fungal cell wall carbohydrates or extracellular polysaccharides.	XM_043330528.1	4	0
	osa-miR167d-5p osa-miR167e-3p osa-miR393b-3p osa-miR396d osa-miR810b.2 osa-miR1429-3p osa-miR1850.3 osa-miR1861i osa-miR1863c	Fungal Zn(2)-Cys(6) binuclear cluster domain	Proteins with this domain are likely transcription factors that can regulate the expression of genes involved in pathogenicity.	XM_043320061.1	4.5	0
	osa-miR167h-3p osa-miR393b-3p osa-miR535-3p osa-miR1429-3p osa-miR7693-5p osa-miR7693-3p	Chitin synthase	Synthesis of chitin for fungal growth and development, and is potentially involved in interactions with the plant immune system.	XM_043324388.1	4	1
	osa-miR172a	ATP-dependent RNA helicase HAS1	Involved in RNA processing and may play a role in stress response and development necessary for infection.	XM_003716981.1	2	0
<i>M. oryzae</i>	osa-miR168a-5p	Branched-chain-amino-acid aminotransferase 1	Involved in amino acid metabolism, which is crucial for fungal growth and development during infection.	XM_003708849.1	2.5	0

osa-miR2118f	Sulfate permease 2	Sulfate uptake is essential for fungal growth and the synthesis of sulfur-containing compounds necessary for various metabolic processes, potentially including those involved in pathogenicity.	XM_003719656.1	2.5	1
osa-miR319b	6-phosphofructokinase	A key enzyme in glycolysis, providing energy for all cellular processes, including those required for infection and growth within the host.	XM_003721083.1	2.5	1
osa-miR160a-5p osa-miR160f-5p osa-miR167a-5p osa-miR398b	Gamma-glutamyl transferase	Involved in glutathione metabolism, which plays a role in detoxification and stress response, potentially important during plant-fungus interactions.	XM_003719949.1	3	1
osa-miR160a-5p osa-miR160f-5p	Nitrogen regulatory protein NUT1	Regulates the expression of genes involved in nitrogen assimilation, which is crucial for nutrient acquisition during infection.	XM_003720950.1	3	0
osa-miR162a osa-miR398b	Mating-type switching protein swi10	Involved in mating-type switching, which can contribute to genetic diversity and adaptation, potentially influencing virulence over time or in specific environments.	XM_003717902.1	3	0
osa-miR168a-5p	Adenylosuccinate lyase	Involved in purine biosynthesis, essential for nucleic acid production and overall growth during infection.	XM_003716196.1	3	
osa-miR169a osa-miR169h	Peptidase family M28 family protein	Peptidases can be involved in various processes during infection, such as nutrient acquisition, degradation of host defense proteins, or fungal development.	XM_003709418.1	3	1
osa-miR172a	Transcription elongation factor spt-4	Involved in regulating gene expression, which is crucial for the timely production of virulence factors during different stages of infection.	XM_003709635.1	3	1
osa-miR172a	Ornithine decarboxylase antizyme	Involved in polyamine metabolism, which has been implicated in various fungal processes, including development and stress response, potentially affecting pathogenicity.	XM_003718804.1	3	0
osa-miR394	Triacylglycerol lipase	Lipases can be involved in the breakdown of lipids for nutrient acquisition or signaling during infection.	XM_003718172.1	3	1
osa-miR396c-5p	Methionyl-tRNA synthetase	Essential for protein synthesis, which is fundamental for all cellular processes, including the production of virulence factors.	XM_003717848.1	3	2

osa-miR160a-5p osa-miR160f-5p	Cell morphogenesis protein PAG1	PAG1 is crucial for cell polarity and morphogenesis, particularly for the development of the appressorium, the specialized infection structure of <i>M. oryzae</i> .	XM_003715257.1	3.5	1
osa-miR166j-3p	Protein transporter SEC24	A component of the COPII complex involved in protein transport from the endoplasmic reticulum to the Golgi, crucial for the secretion of proteins, including potential virulence factors.	XM_003712129.1	3.5	0
osa-miR166k-3p osa-miR172a	Feruloyl esterase	Feruloyl esterases can degrade plant cell wall components, potentially aiding in penetration and nutrient acquisition.	XM_003717572.1	3.5	0
osa-miR167a-5p osa-miR167d-5p	Laccase-1	Laccases are involved in lignin degradation and melanin biosynthesis, which can contribute to appressorium function and protection against host defenses.	XM_016990464.1	3.5	2

Supplementary table S3. Target genes of the miR171 family against the *R. irregularis* transcriptome that potentially play a role in a symbiotic relationship.

miRNA	Target gene	Potential Activity	Gene IDs	Maximum Expectation Score	Seed Mismatch
osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p	Acyl-CoA dehydrogenase/oxidase	Involved in fatty acid metabolism, important for energy and signaling, potentially relevant during growth and interaction with plants.	XM_025323628.1	3	1
osa-miR171d-5p osa-miR171e-5p	Homocitrate synthase	Involved in homocitrate synthesis, a precursor of siderophores for iron uptake, which may be important in the soil environment and plant interaction.	XM_025316341.1	3.5	0
osa-miR171f-5p	Serine/threonine-protein kinase 6	Involved in signaling pathways regulating growth, development, and environmental responses, potentially including plant interaction.	XM_025314408.1	3.5	0
osa-miR171f-5p	Peptidase family C50-domain containing protein	Peptidases can be involved in protein turnover and nutrient acquisition, potentially relevant during plant interaction.	XM_025314128.1	3.5	2
osa-miR171a osa-miR171i-	Putative DEAD/DEAH box	Involved in RNA processing, crucial for gene expression	XM_025319471.1	3.5	1

3p	helicase	regulation in response to environmental changes, including interaction with plants.			
osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p	Glycosyltransferase family 20 protein	Involved in glycan synthesis, which can be important for cell wall structure and interaction with the environment, including the plant root surface.	XM_025316404.1	4	2
osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p	GPN-loop GTPase	Involved in cellular signaling, potentially regulating various processes including response to plant signals or environmental changes during symbiosis.	XM_025321960.1	4	1
osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p	Phosphatidylserine decarboxylase-related protein	Involved in phospholipid biosynthesis, crucial for cell membrane structure and function, potentially affecting membrane interactions with the plant.	XM_025326472.1	4	1
osa-miR171e-5p	DNA topoisomerase II	Involved in DNA topology changes, essential for DNA replication and gene transcription.	XM_025309003.1	3	1
osa-miR171b osa-miR171c-3p osa-miR171d-3p	Enoyl-CoA hydratase	Involved in fatty acid metabolism, important for energy and signaling, potentially relevant during growth and interaction with plants.	XM_025315536.1	4.5	1
osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p	Putative fatty acid desaturase	Involved in introducing double bonds into fatty acids, affecting membrane fluidity and function, potentially relevant during interaction with plants.	XM_025318861.1	4.5	2
osa-miR171h	OPT family small oligopeptide transporter	Involved in the uptake of small peptides, crucial for nutrient acquisition by the fungus from the host plant during symbiotic interaction	XM_025331042.1	4	2
osa-miR171i-3p	Disulfide isomerase PDI1 protein	Involved in the formation of disulfide bonds in proteins, crucial for the structure and function of secreted proteins, and potentially involved in plant interaction.	XM_025313386.1	4	0
osa-miR171i-3p	Protein transporter SEC13	A component of the SEC complex involved in protein transport from the ER, essential for secreting proteins that might be involved in plant interaction.	XM_025312875.1	4	1

osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p osa-miR171g osa-miR171h osa-miR171i-3p	Putative ubiquinol cytochrome-c reductase core protein 2	A component of the electron transport chain in mitochondria, involved in ATP production, is essential for all cellular processes, including symbiosis.	XM_025319964.1	4.5	2
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Supplementary table S4. Target genes of the miR171 family against the *O. sativa* transcriptome that potentially play a role in a symbiotic relationship.

miRNA	Target gene	Potential Activity	Gene IDs	Maximum Expectation Score	Seed Mismatch
osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p osa-miR171h osa-miR171i-3p	Nodulation signaling pathway 2 protein, putative, expressed [PFAM]	- Activator of the common symbiosis signaling pathway (CSSP) - Regulator of strigolactone biosynthesis	LOC_Os02g44370.1 12002.m09477 cDNA	0.5	0
osa-miR171b osa-miR171c-3p	Phosphate transporter 1, putative, expressed [PFAM]	- Mediates phosphate uptake from the mycorrhizal fungus to the plant roots.	LOC_Os02g56510.1 12002.m10672 cDNA	5	1
osa-miR171a osa-miR171b osa-miR171c3p osa-miR171d-5p osa-miR171d-3p osa-miR171e-3p osa-miR171f-5p osa-miR171i-3p	Sucrose-phosphate synthase 1, putative, expressed [PFAM]	- Regulation of carbon allocation to the symbiosis	LOC_Os08g20660.2 12008.m26651 cDNA	5	0
osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171e-3p osa-miR171f-3p osa-miR171g osa-miR171hosa-miR171i-3p	SCARECROW (SCR) gene regulator	- Influence via root architecture - Interaction with gibberellin signaling	LOC_Os02g44360.1 12002.m09476 cDNA	0.5	0
osa-miR171a osa-miR171b osa-miR171c-3p osa-miR171d-3p osa-miR171d-5p osa-miR171e-3p osa-miR171e-5p osa-miR171f-3p osa-miR171f-5p osa-miR171i-3p	Ankyrin repeat protein, chloroplast precursor	- Photosynthetic efficiency and carbon allocation	LOC_Os03g04300.1 12003.m05960 cDNA	0	0

osa-miR171c-5p	Pattern formation protein EMB30 (GNOM)	- Influence on root development and architecture - Potential involvement in membrane trafficking during arbuscule formation - Auxin signaling and mycorrhizal development - Potential for interaction with symbiotic signaling pathways	LOC_Os03g46330.1 12003.m09652 cDNA	3	1
osa-miR171d-5p osa-miR171e-5p	Serine/threonine protein kinase	- Phosphorylation events in the common symbiosis signaling pathway (CSSP) - Control of nutrient transporters	LOC_Os04g51950.1 12004.m10090 cDNA	3.5	0
osa-miR171i-5p	Receptor-like protein kinase precursor	- Perception of fungal signals - Activation of the Common Symbiosis Signaling Pathway (CSSP) - Regulation of fungal colonization within the root. - Control of nutrient exchange - Defense responses and symbiotic balance	LOC_Os11g46970.1 12011.m08477 cDNA	3	1
osa-miR171e-5p	Ethylene-responsive transcription factor 2 (ERF2)	- Regulation of ethylene signaling during mycorrhization - Modulation of root development relevant to mycorrhization - Interaction with other hormonal signaling pathways - Potential role in defense responses during colonization	LOC_Os04g46220.1 12004.m09573 cDNA	3	0
osa-miR171c-5p	Jasmonate O-methyltransferase (JOMT)	- Regulation of JA/MeJA balance during mycorrhizal establishment - Influence on root architecture and fungal colonization - Modulation of defense responses during established symbiosis - Potential role in signaling within the mycorrhizal interaction	LOC_Os06g13460.1 12006.m06061 cDNA	4	2