SUPPLEMENTS

Transcriptomic analysis of *sym28* and *sym29* supernodulating mutants of pea (*Pisum sativum* L.) under complex inoculation with beneficial microorganisms

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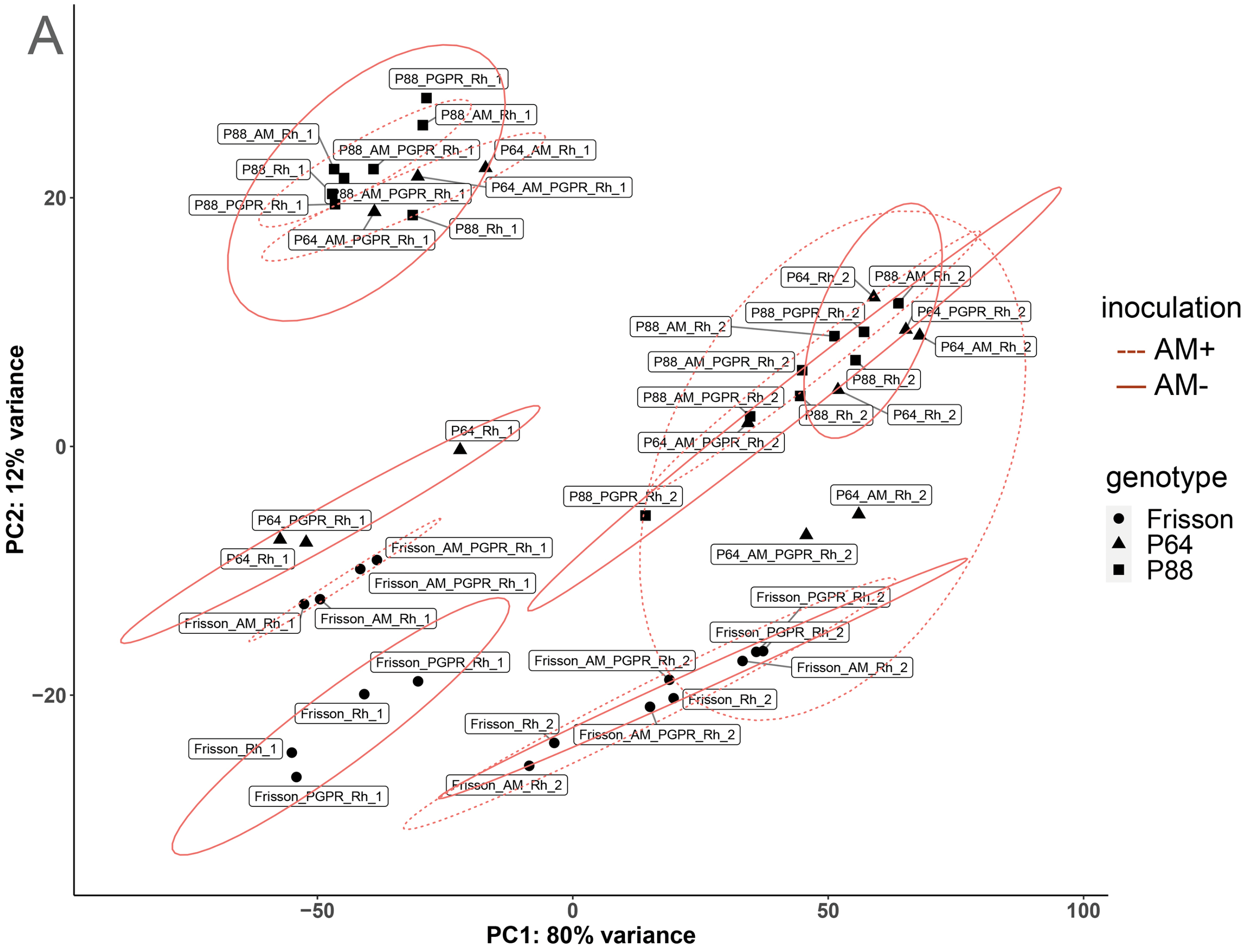
Table S1. Analyzed parameters and distribution families used for GLM fitting

Parameter	Units	Term	Type	Distribution family and link function
Shoot dry weigh	gram	2 wpi	continuous	Gamma, link=identity
		4 wpi		
Nodule number	-	2 wpi	discrete	quasipoisson link=log
Seed number	-	final	discrete	quasipoisson link=log
Seed weight per plant	gram	final	continuous	Gamma, link=identity

wpi — weeks after inoculation

Fig. S1. The PCA plots for root (A) and shoot (B) samples.



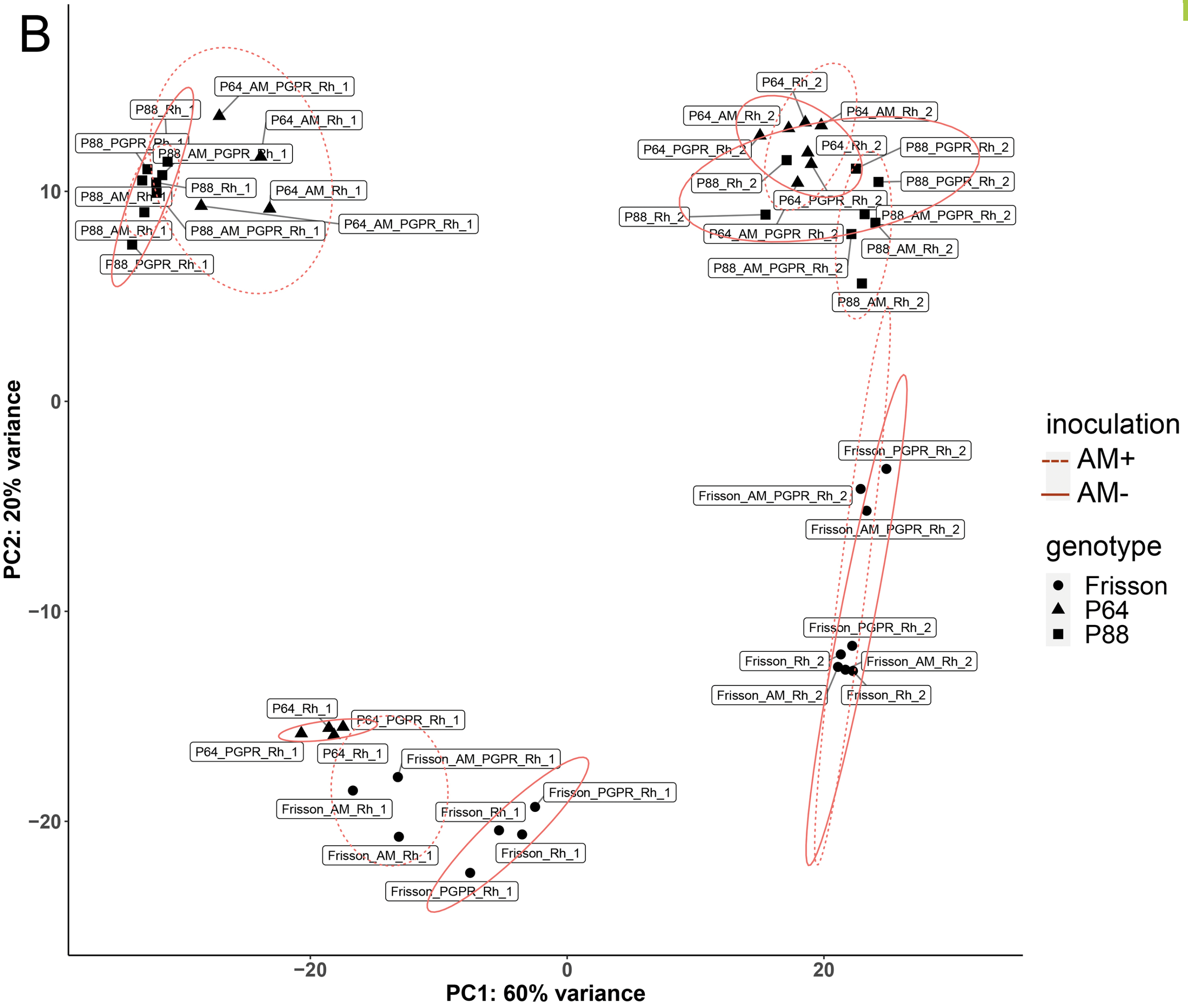
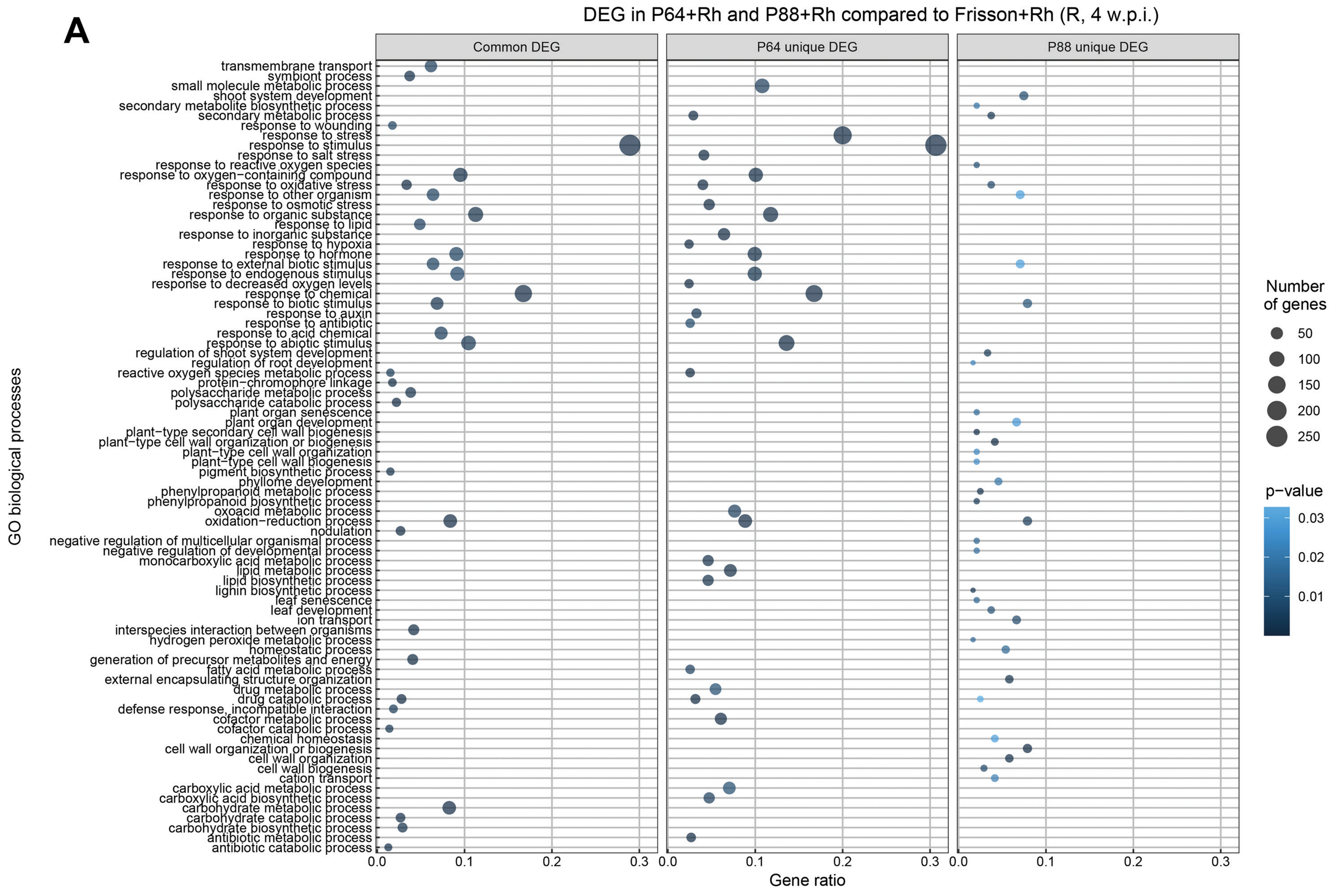
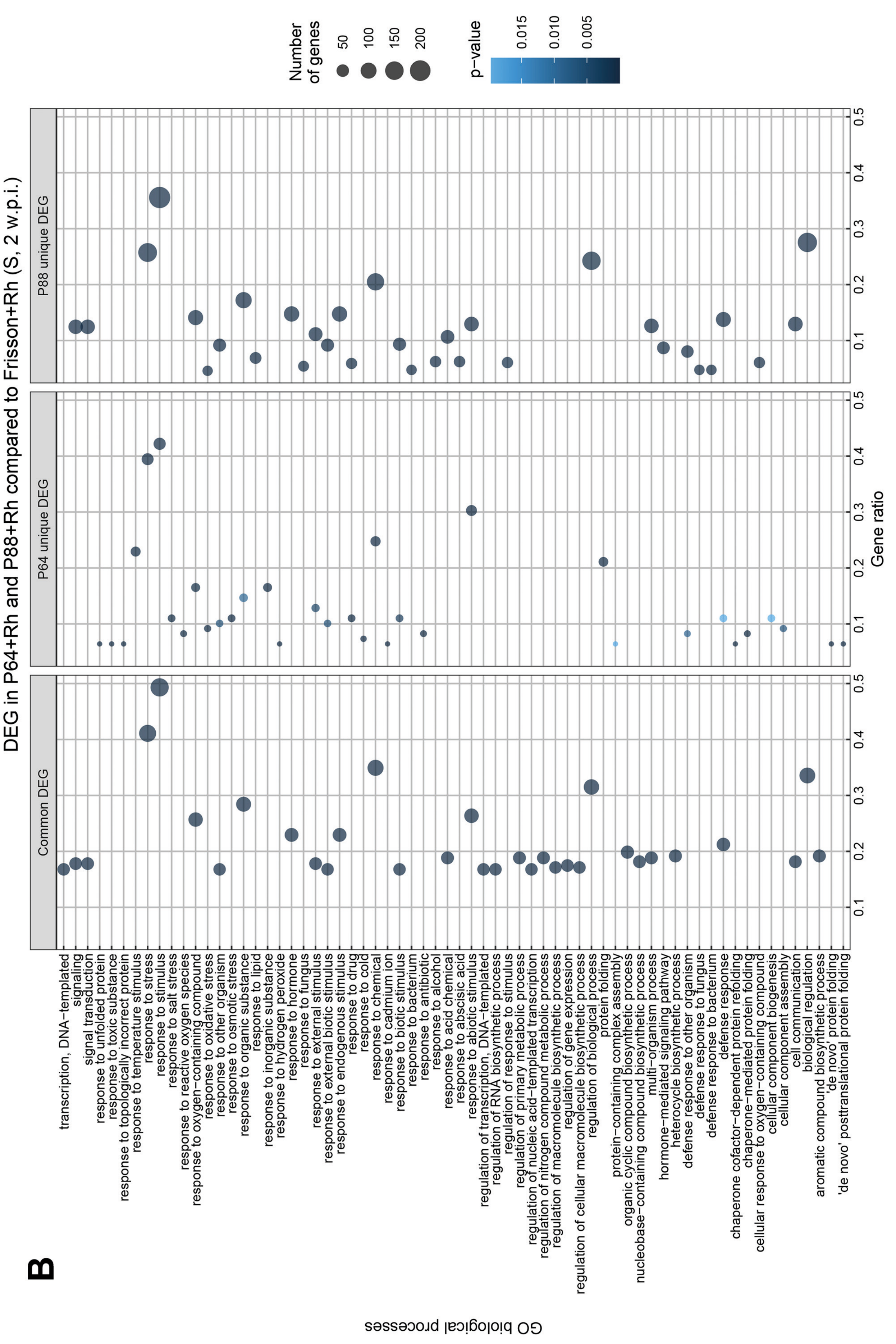

B
P64_AM_PGPR_Rh_1
P88_Rh_1
P64_AM_Rh_1
P88_PGPR_Rh_1
P88_AM_PGPR_Rh_1
P88_Rh_1
P64_AM_Rh_1
P88_AM_Rh_1
P64_AM_PGPR_Rh_1
P88_AM_Rh_1
P88_AM_PGPR_Rh_1
P88_PGPR_Rh_1
P64_Rh_2
P64_AM_Rh_2
P64_AM_Rh_2
P64_PGPR_Rh_2
P64_Rh_2
P88_PGPR_Rh_2
P88_Rh_2
P88_PGPR_Rh_2
P64_PGPR_Rh_2
P88_Rh_2
P88_AM_PGPR_Rh_2
P64_AM_PGPR_Rh_2
P88_AM_Rh_2
P88_AM_PGPR_Rh_2
P88_AM_Rh_2
Frisson_PGPR_Rh_2
Frisson_AM_PGPR_Rh_2
Frisson_AM_PGPR_Rh_2
Frisson_PGPR_Rh_2
Frisson_Rh_2
Frisson_AM_Rh_2
Frisson_AM_Rh_2
Frisson_Rh_2
P64_Rh_1
P64_PGPR_Rh_1
P64_PGPR_Rh_1
P64_Rh_1
Frisson_AM_PGPR_Rh_1
Frisson_AM_Rh_1
Frisson_Rh_1
Frisson_PGPR_Rh_1
Frisson_AM_Rh_1
Frisson_Rh_1
Frisson_PGPR_Rh_1
10
0
−10
−20
PC2: 20% variance
−20
0
20
PC1: 60% variance
inculation
AM+
AM-
genotype
● Frisson
▲ P64
■ P88

Fig. S2. Gene Ontology enrichment analysis of DEGs in roots (in 2 wpi with rhizobia) and shoots (in 2 and 4 wpi with rhizobia) of cv. 'Frisson', P64 (*sym28*) and P88 (*sym29*).




B
DEG in P64+Rh and P88+Rh compared to Frisson+Rh (S, 2 w.p.i.)
Common DEG
P64 unique DEG
P88 unique DEG
GO biological processes
Gene ratio
0.1
0.2
0.3
0.4
0.5
transcription, DNA−templated
signaling
signal transduction
response to unfolded protein
response to toxic substance
response to topologically incorrect protein
response to temperature stimulus
response to stress
response to stimulus
response to salt stress
response to reactive oxygen species
response to oxygen−containing compound
response to oxidative stress
response to other organism
response to osmotic stress
response to organic substance
response to lipid
response to inorganic substance
response to hydrogen peroxide
response to hormone
response to fungus
response to external stimulus
response to external biotic stimulus
response to endogenous stimulus
response to drug
response to cold
response to chemical
response to cadmium ion
response to biotic stimulus
response to bacterium
response to antibiotic
response to alcohol
response to acid chemical
response to abscisic acid
response to abiotic stimulus
regulation of transcription, DNA−templated
regulation of RNA biosynthetic process
regulation of response to stimulus
regulation of primary metabolic process
regulation of nucleic acid−templated transcription
regulation of nitrogen compound metabolic process
regulation of macromolecule biosynthetic process
regulation of gene expression
regulation of cellular macromolecule biosynthetic process
regulation of biological process
protein folding
protein−containing complex assembly
organic cyclic compound biosynthetic process
nucleobase−containing compound biosynthetic process
multi−organism process
hormone−mediated signaling pathway
heterocycle biosynthetic process
defense response to other organism
defense response to fungus
defense response to bacterium
defense response
chaperone cofactor−dependent protein refolding
chaperone−mediated protein folding
cellular response to oxygen−containing compound
cellular component biogenesis
cellular component assembly
cell communication
biological regulation
aromatic compound biosynthetic process
'de novo' protein folding
'de novo' posttranslational protein folding
Number of genes
50
100
150
200
p−value
0.015
0.010
0.005

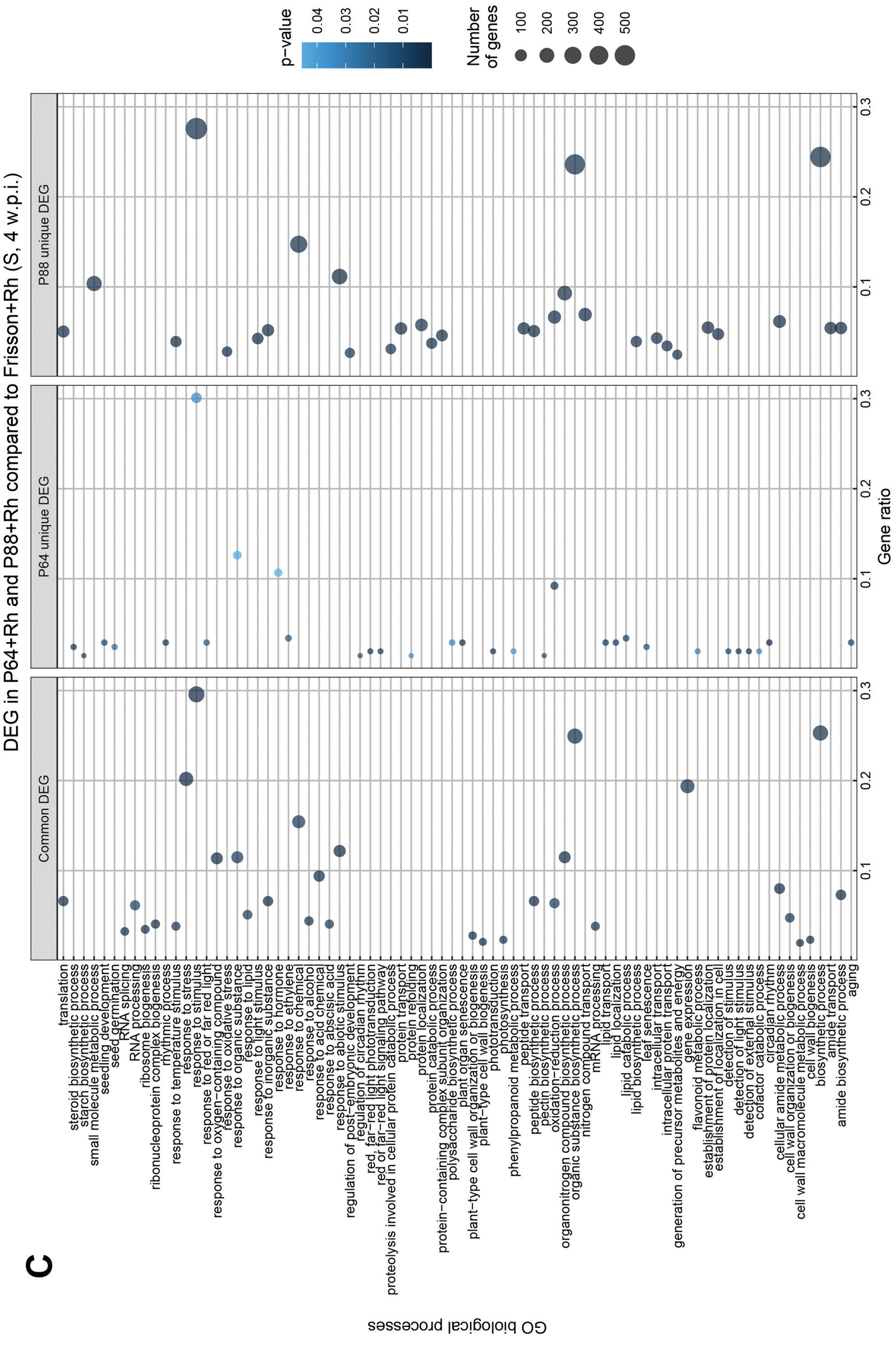

C
DEG in P64+Rh and P88+Rh compared to Frisson+Rh (S, 4 w.p.i.)
Common DEG
P64 unique DEG
P88 unique DEG
p−value
0.04
0.03
0.02
0.01
Number of genes
100
200
300
400
500
Gene ratio
0.1
0.2
0.3
GO biological processes
translation
steroid biosynthetic process
starch biosynthetic process
small molecule metabolic process
seedling development
seed germination
RNA splicing
RNA processing
ribosome biogenesis
ribonucleoprotein complex biogenesis
rhythmic process
response to temperature stimulus
response to stress
response to stimulus
response to red or far red light
response to oxygen−containing compound
response to oxidative stress
response to organic substance
response to lipid
response to light stimulus
response to inorganic substance
response to hormone
response to ethylene
response to chemical
response to alcohol
response to acid chemical
response to abscisic acid
response to abiotic stimulus
regulation of post−embryonic development
regulation of circadian rhythm
red, far−red light phototransduction
red or far−red light signaling pathway
proteolysis involved in cellular protein catabolic process
protein transport
protein refolding
protein localization
protein catabolic process
protein−containing complex subunit organization
polysaccharide biosynthetic process
plant organ senescence
plant−type cell wall organization or biogenesis
plant−type cell wall biogenesis
phototransduction
photosynthesis
phenylpropanoid metabolic process
peptide transport
peptide biosynthetic process
pectin biosynthetic process
oxidation−reduction process
organonitrogen compound biosynthetic process
organic substance biosynthetic process
nitrogen compound transport
mRNA processing
lipid transport
lipid localization
lipid catabolic process
lipid biosynthetic process
leaf senescence
intracellular transport
intracellular protein transport
generation of precursor metabolites and energy
gene expression
flavonoid metabolic process
establishment of protein localization
establishment of localization in cell
detection of stimulus
detection of light stimulus
detection of external stimulus
cofactor catabolic process
circadian rhythm
cellular amide metabolic process
cell wall organization or biogenesis
cell wall macromolecule metabolic process
cell wall biogenesis
biosynthetic process
amide transport
amide biosynthetic process
aging

Fig. S3. Gene Ontology enrichment analysis of DEGs in roots of cv. ‘Frisson’ and P88 (*sym28*) in 2 wpi with mycorrhizal fungus and rhizobia.

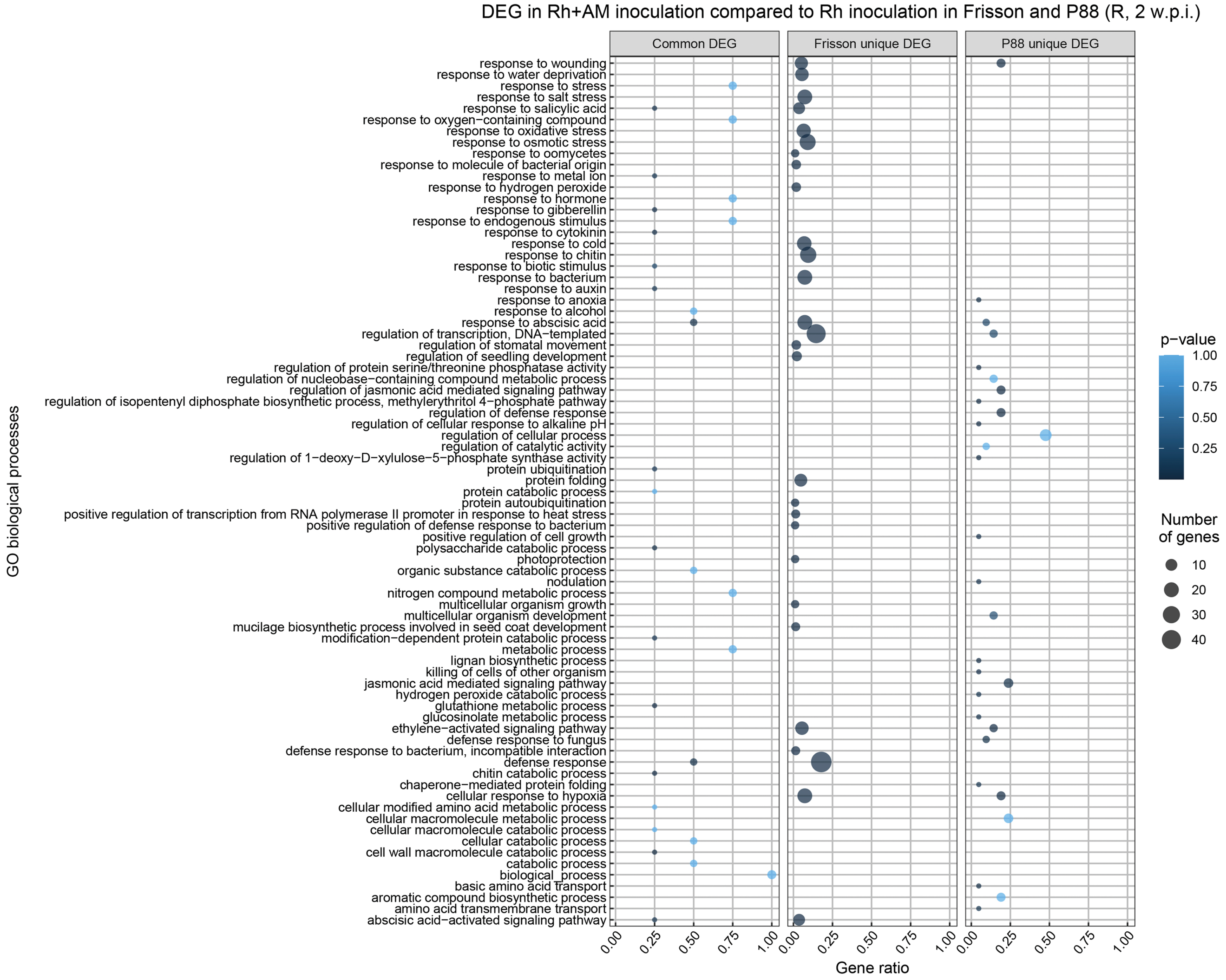
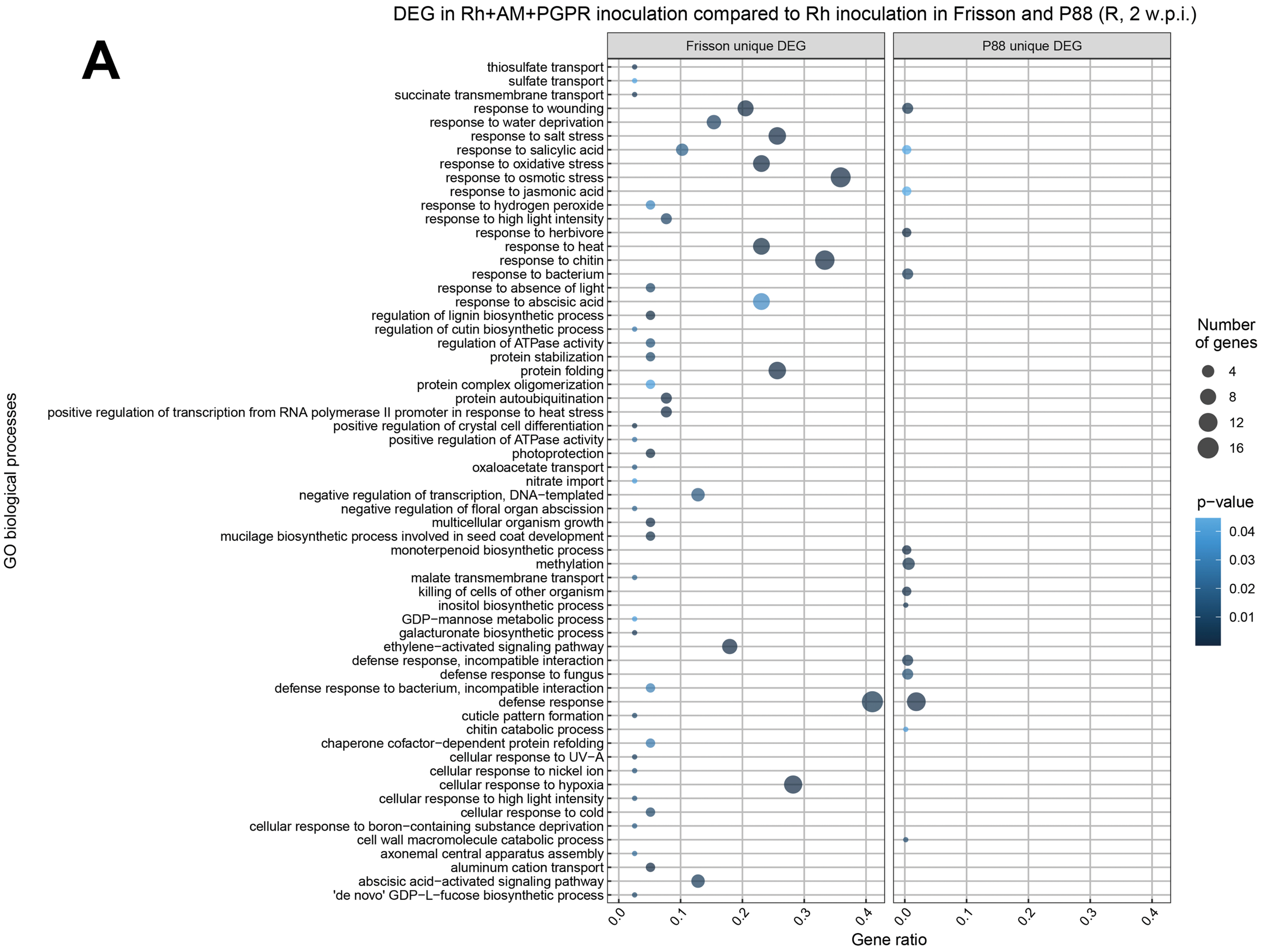
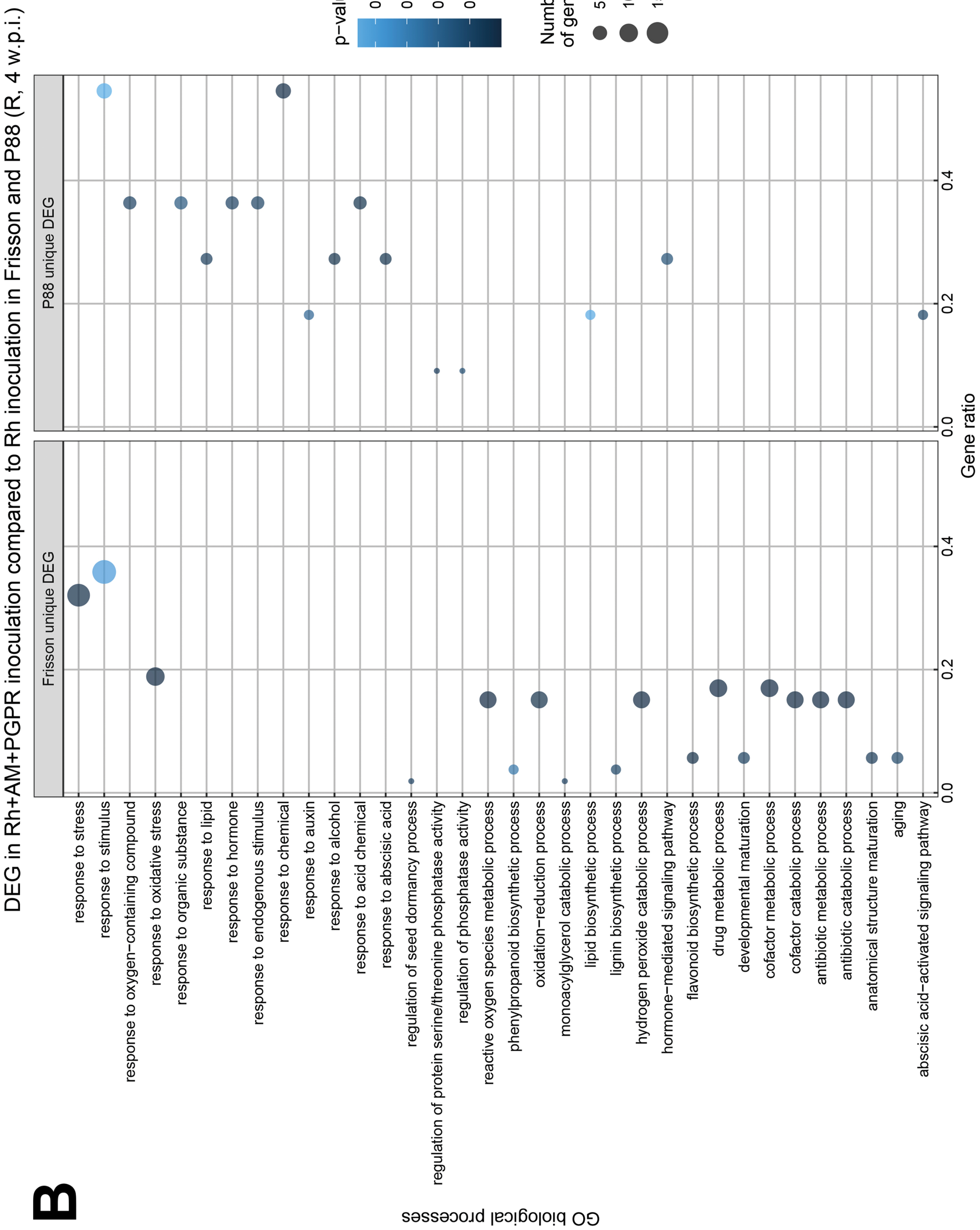
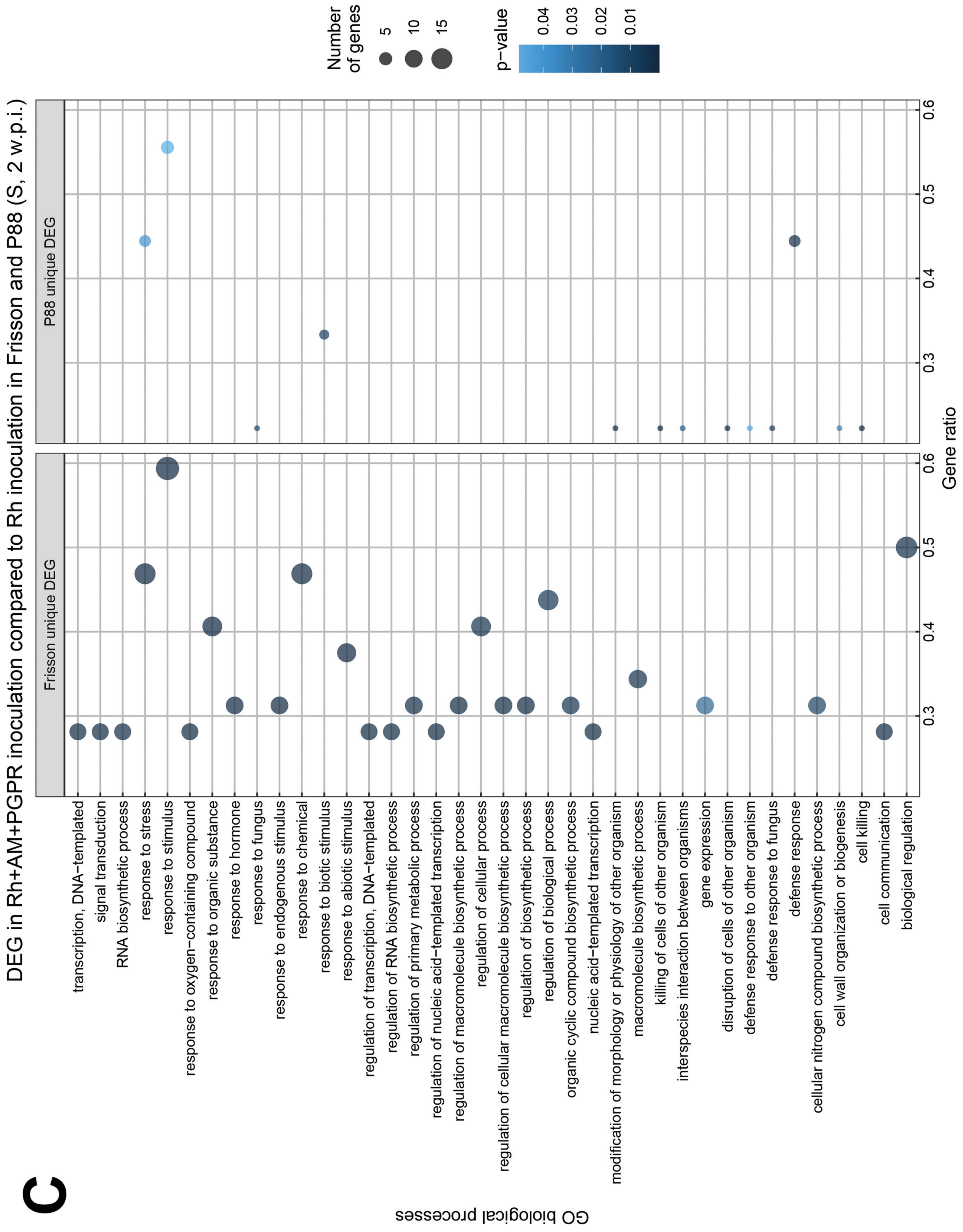


Fig. S4. Gene Ontology enrichment analysis of DEGs in roots (A, B) and shoots (C, D) of cv. ‘Frisson’ and P88 (*sym29*) in 2 and 4 wpi with triple inoculation (Rh + AM + PGPB).




B
DEG in Rh+AM+PGPR inoculation compared to Rh inoculation in Frisson and P88 (R, 4 w.p.i.)
Frisson unique DEG
P88 unique DEG
GO biological processes
response to stress
response to stimulus
response to oxygen−containing compound
response to oxidative stress
response to organic substance
response to lipid
response to hormone
response to endogenous stimulus
response to chemical
response to auxin
response to alcohol
response to acid chemical
response to abscisic acid
regulation of seed dormancy process
regulation of protein serine/threonine phosphatase activity
regulation of phosphatase activity
reactive oxygen species metabolic process
phenylpropanoid biosynthetic process
oxidation−reduction process
monoacylglycerol catabolic process
lipid biosynthetic process
lignin biosynthetic process
hydrogen peroxide catabolic process
hormone−mediated signaling pathway
flavonoid biosynthetic process
drug metabolic process
developmental maturation
cofactor metabolic process
cofactor catabolic process
antibiotic metabolic process
antibiotic catabolic process
anatomical structure maturation
aging
abscisic acid−activated signaling pathway
0.0
0.2
0.4
Gene ratio
p−value
0.04
0.03
0.02
0.01
Number of genes
5
10
15


C
DEG in Rh+AM+PGPR inoculation compared to Rh inoculation in Frisson and P88 (S, 2 w.p.i.)
Frisson unique DEG
P88 unique DEG
GO biological processes
transcription, DNA−templated
signal transduction
RNA biosynthetic process
response to stress
response to stimulus
response to oxygen−containing compound
response to organic substance
response to hormone
response to fungus
response to endogenous stimulus
response to chemical
response to biotic stimulus
response to abiotic stimulus
regulation of transcription, DNA−templated
regulation of RNA biosynthetic process
regulation of primary metabolic process
regulation of nucleic acid−templated transcription
regulation of macromolecule biosynthetic process
regulation of cellular process
regulation of cellular macromolecule biosynthetic process
regulation of biosynthetic process
regulation of biological process
organic cyclic compound biosynthetic process
nucleic acid−templated transcription
modification of morphology or physiology of other organism
macromolecule biosynthetic process
killing of cells of other organism
interspecies interaction between organisms
gene expression
disruption of cells of other organism
defense response to other organism
defense response to fungus
defense response
cellular nitrogen compound biosynthetic process
cell wall organization or biogenesis
cell killing
cell communication
biological regulation
0.3
0.4
0.5
0.6
0.3
0.4
0.5
0.6
Gene ratio
Number of genes
5
10
15
p−value
0.04
0.03
0.02
0.01

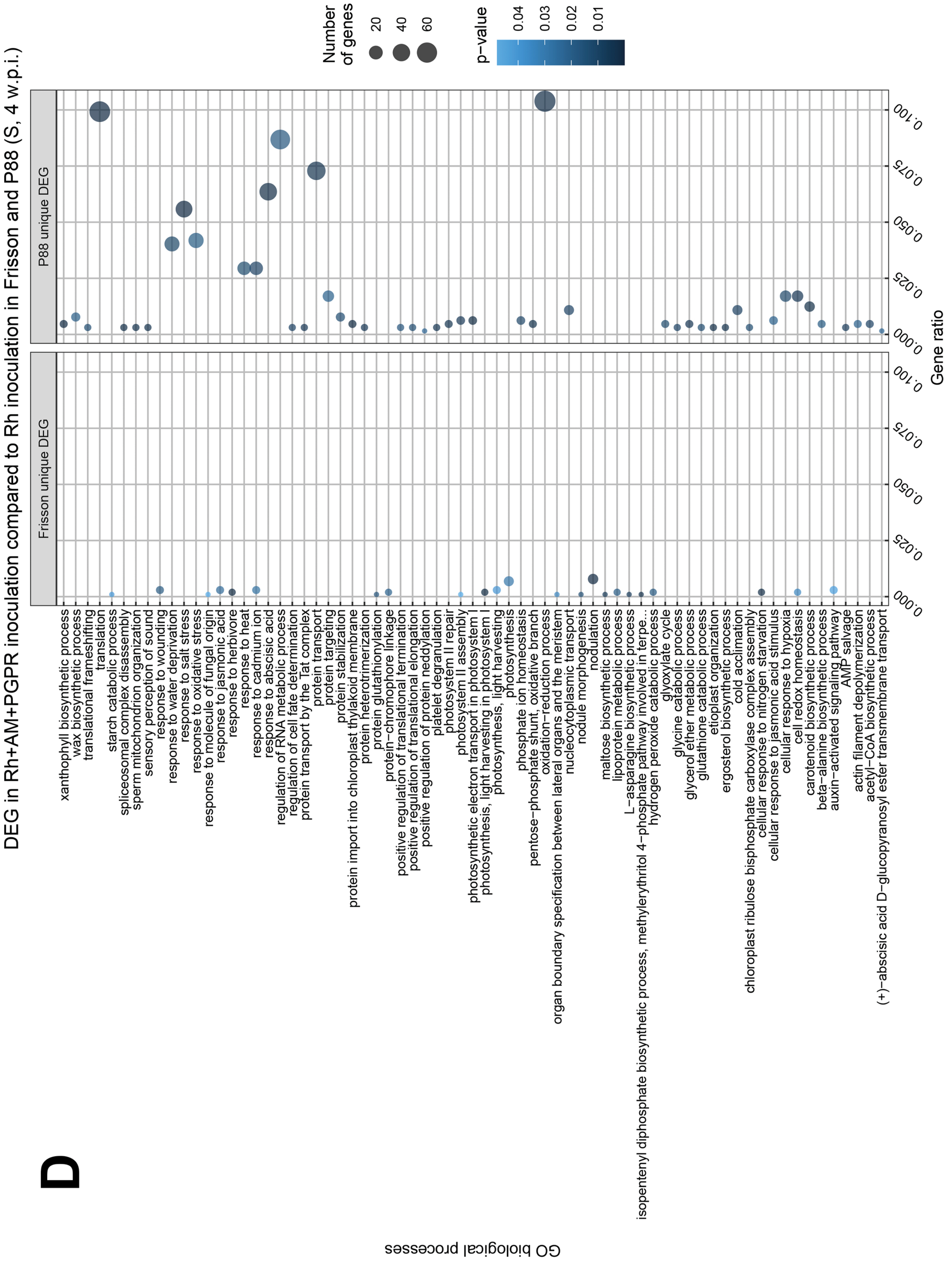
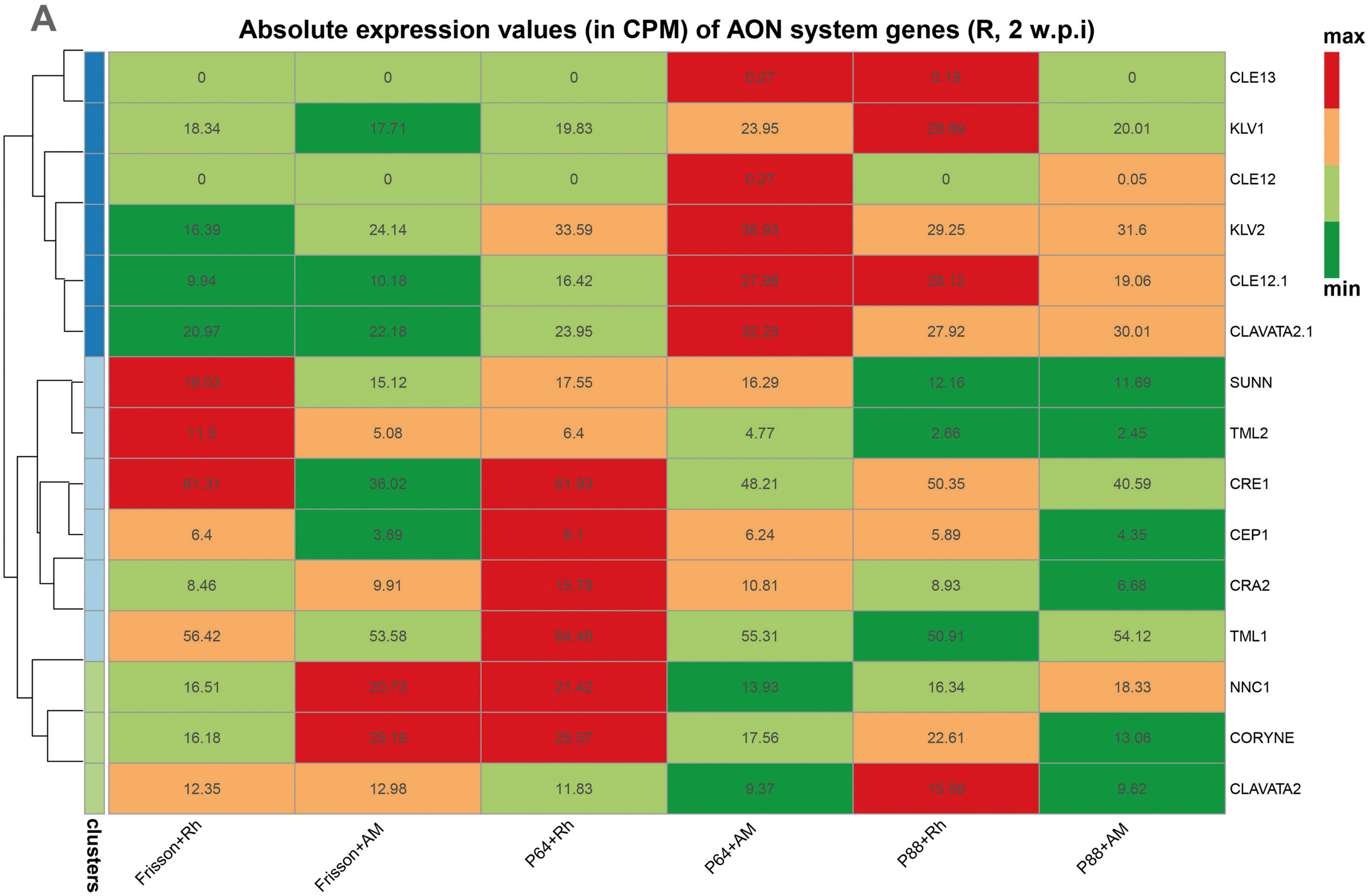

D
DEG in Rh+AM+PGPR inoculation compared to Rh inoculation in Frisson and P88 (S, 4 w.p.i.)
Frisson unique DEG
P88 unique DEG
GO biological processes
Gene ratio
0.000
0.025
0.050
0.075
0.100
Number of genes
20
40
60
p−value
0.04
0.03
0.02
0.01
xanthophyll biosynthetic process
wax biosynthetic process
translational frameshifting
translation
starch catabolic process
spliceosomal complex disassembly
sperm mitochondrion organization
sensory perception of sound
response to wounding
response to water deprivation
response to salt stress
response to oxidative stress
response to molecule of fungal origin
response to jasmonic acid
response to herbivore
response to heat
response to cadmium ion
response to abscisic acid
regulation of RNA metabolic process
regulation of cell fate determination
protein transport by the Tat complex
protein transport
protein targeting
protein stabilization
protein import into chloroplast thylakoid membrane
protein heterotrimerization
protein glutathionylation
protein−chromophore linkage
positive regulation of translational termination
positive regulation of translational elongation
positive regulation of protein neddylation
platelet degranulation
photosystem II repair
photosystem II assembly
photosynthetic electron transport in photosystem I
photosynthesis, light harvesting in photosystem I
photosynthesis, light harvesting
photosynthesis
phosphate ion homeostasis
pentose−phosphate shunt, oxidative branch
oxidation−reduction process
organ boundary specification between lateral organs and the meristem
nucleocytoplasmic transport
nodule morphogenesis
nodulation
maltose biosynthetic process
lipoprotein metabolic process
L−asparagine biosynthetic process
isopentenyl diphosphate biosynthetic process, methylerythritol 4−phosphate pathway involved in terpe...
hydrogen peroxide catabolic process
glyoxylate cycle
glycine catabolic process
glycerol ether metabolic process
glutathione catabolic process
etioplast organization
ergosterol biosynthetic process
cold acclimation
chloroplast ribulose bisphosphate carboxylase complex assembly
cellular response to nitrogen starvation
cellular response to jasmonic acid stimulus
cellular response to hypoxia
cell redox homeostasis
carotenoid biosynthetic process
beta−alanine biosynthetic process
auxin−activated signaling pathway
AMP salvage
actin filament depolymerization
acetyl−CoA biosynthetic process
(+)−abscisic acid D−glucopyranosyl ester transmembrane transport

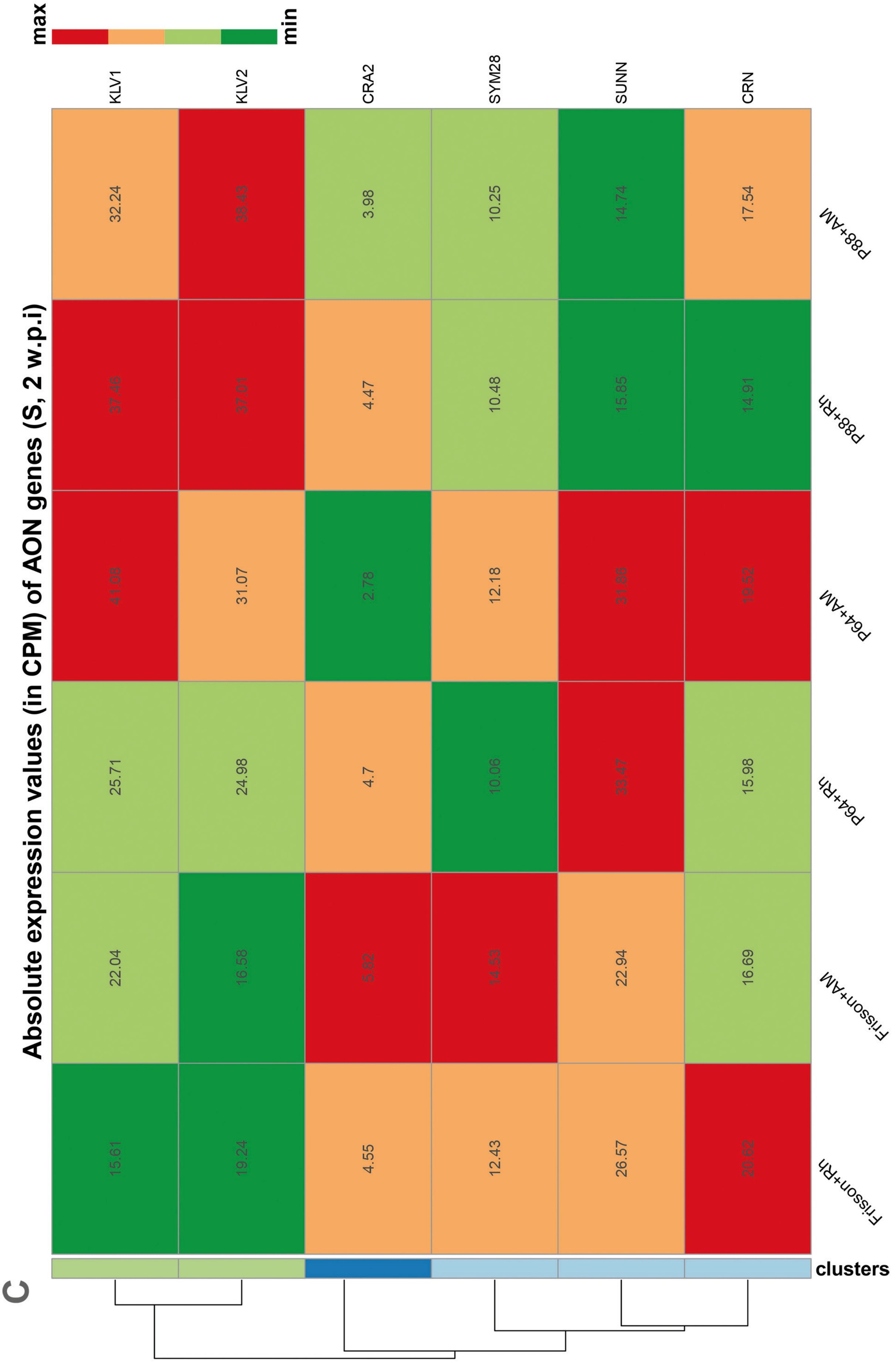
Fig. S5. Expression profiles of known AON-related genes in plant roots (A, B) and shoots (C, D) in Rh and Rh+AM variants.



	Frisson+Rh	Frisson+AM	P64+Rh	P64+AM	P88+Rh	P88+AM
CLE13	0	0	0	0.27	0.19	0
KLV1	18.34	17.71	19.83	23.95	28.69	20.01
CLE12	0	0	0	0.27	0	0.05
KLV2	16.39	24.14	33.59	36.93	29.25	31.6
CLE12.1	9.94	10.18	16.42	27.98	28.12	19.06
CLAVATA2.1	20.97	22.18	23.95	32.23	27.92	30.01
SUNN	19.03	15.12	17.55	16.29	12.16	11.69
TML2	11.5	5.08	6.4	4.77	2.66	2.45
CRE1	61.31	36.02	61.93	48.21	50.35	40.59
CEP1	6.4	3.89	8.1	6.24	5.89	4.35
CRA2	8.46	9.91	15.79	10.81	8.93	6.68
TML1	56.42	53.58	64.48	55.31	50.91	54.12
NNC1	16.51	20.73	21.42	13.93	16.34	18.33
CORYNE	16.18	25.19	25.57	17.56	22.61	13.06
CLAVATA2	12.35	12.98	11.83	9.37	15.58	9.62

Absolute expression values (in CPM) of AON genes (R, 4 w.p.i)

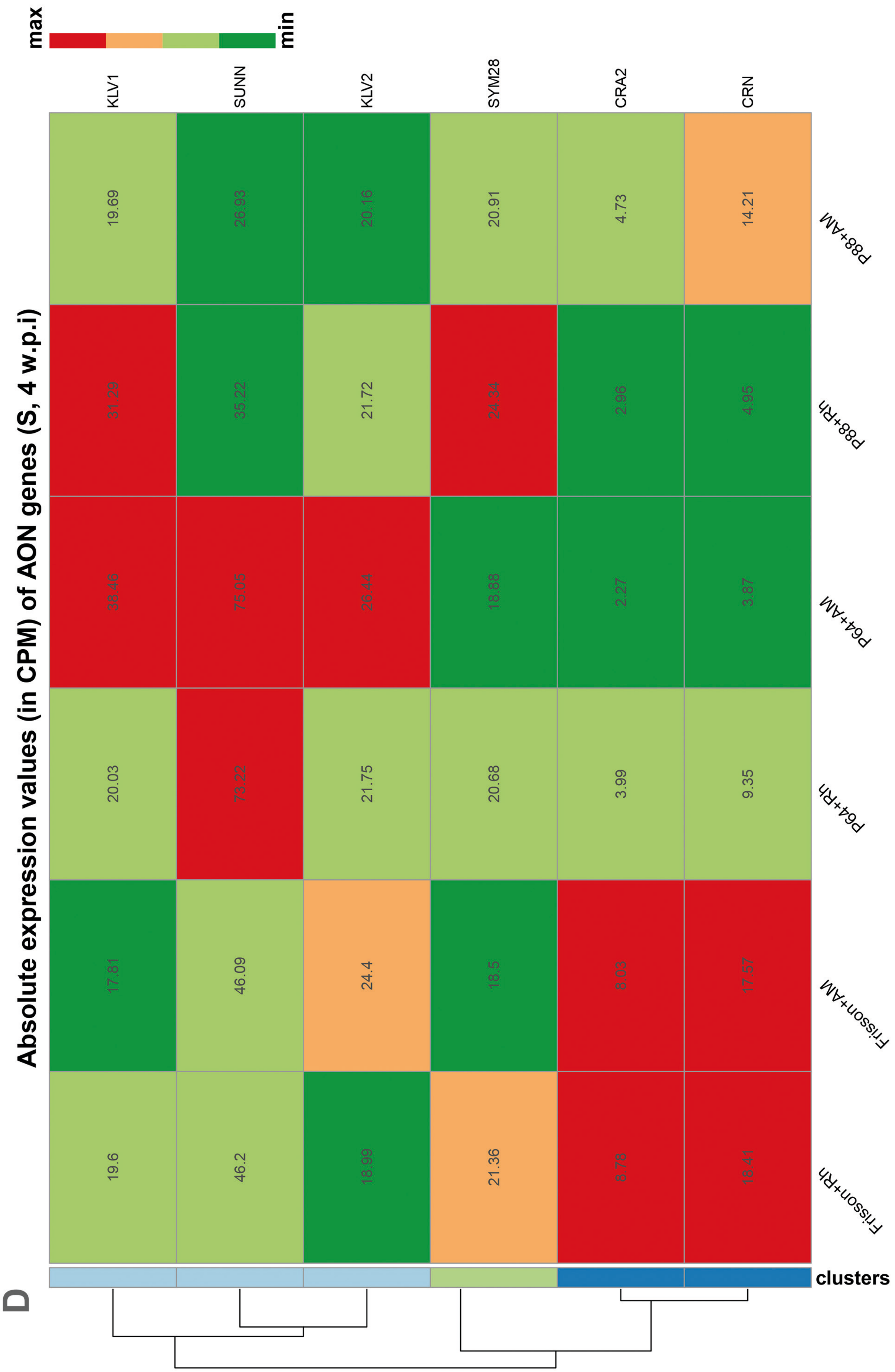
	Frisson+Rh	Frisson+AM	P64+Rh	P64+AM	P88+Rh	P88+AM
CRE1	61.69	55.75	65.6	67.99	93.23	66.62
CLE12	3.8	3.16	4.57	4.58	4.85	5.69
CLE13	0.24	0.12	2.58	2.24	1.21	1.62
CEP1	5.94	6.52	18.1	13.49	22.09	15.67
TML2	11.22	13.52	12.69	10.11	8.54	6.39
TML1	93.05	81.24	60.86	64.61	54.29	46.17
NNC	25.15	30.64	14.37	13.95	20.3	16.54
CORYNE	21.54	23.99	13.04	14.11	15.65	16.41
CLE12.1	12.09	16.37	12	11.86	12.23	13.15
CRA2	8.57	10.57	8.99	8.04	8.29	10.56



C

Absolute expression values (in CPM) of AON genes (S, 2 w.p.i)

	P88+AM	P88+Rh	P64+AM	P64+Rh	Frisson+AM	Frisson+Rh
KLV1	32.24	37.46	41.08	25.71	22.04	15.61
KLV2	38.43	37.01	31.07	24.98	16.58	19.24
CRA2	3.98	4.47	2.78	4.7	5.82	4.55
SYM28	10.25	10.48	12.18	10.06	14.53	12.43
SUNN	14.74	15.85	31.86	33.47	22.94	26.57
CRN	17.54	14.91	19.52	15.98	16.69	20.62


D
Absolute expression values (in CPM) of AON genes (S, 4 w.p.i)
max
min
KLV1
SUNN
KLV2
SYM28
CRA2
CRN
P88+AM
P88+Rh
P64+AM
P64+Rh
Frisson+AM
Frisson+Rh
19.69
26.93
20.16
20.91
4.73
14.21
31.29
35.22
21.72
24.34
2.96
4.95
38.46
75.05
26.44
18.88
2.27
3.87
20.03
73.22
21.75
20.68
3.99
9.35
17.81
46.09
24.4
18.5
8.03
17.57
19.6
46.2
18.99
21.36
8.78
18.41
clusters