The bZIP transcription factor BIP1 of the rice blast fungus is essential for infection and regulates a specific set of appressorium genes

Journées Jean Chevaugeon 15-19 janvier 2024

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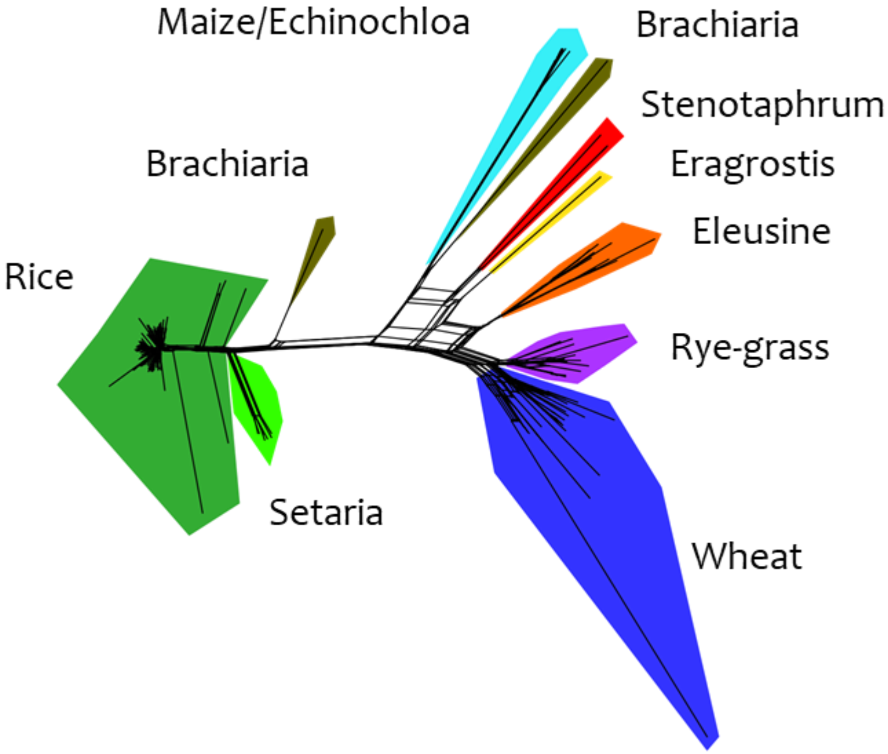
CIME - Thomas Kroj ‘s lab

The blast fungus *Magnaporthe oryzae* (*Pyricularia oryzae*)
a multihost and polyspecialist pathogen

More than 50 cereal and grass host plant species



Genetic differentation between specialized, host specific lineages

Frequent, ongoing host shifts

Rice blast is a major limitation for rice production

Rice (*Oryza sativa*)

- Most important crop for human nutrition
- Production has to double by 2050
- Model cereal species
- Rich genomic data and functional tools

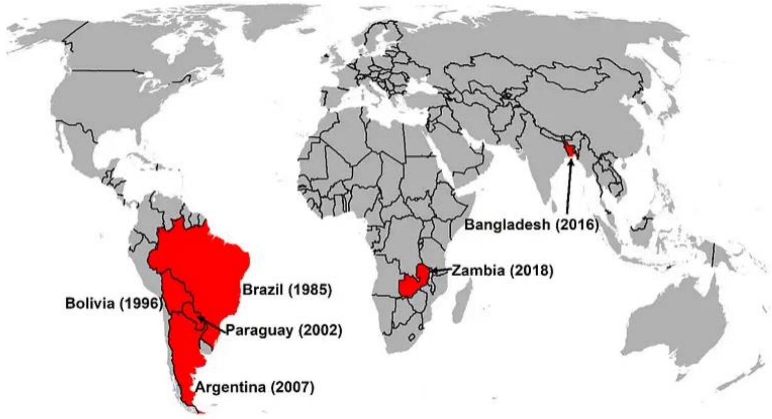
→ 440 Mb, ≈40000 genes, 12 chromosomes

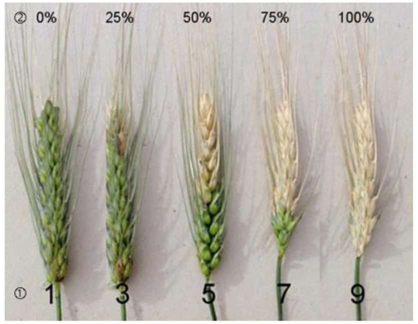
Rice Blast

- Present in all rice growing areas
- Frequent, strong and fast epidemics
- 5% global loss
- Model system for plant-fungus interactions

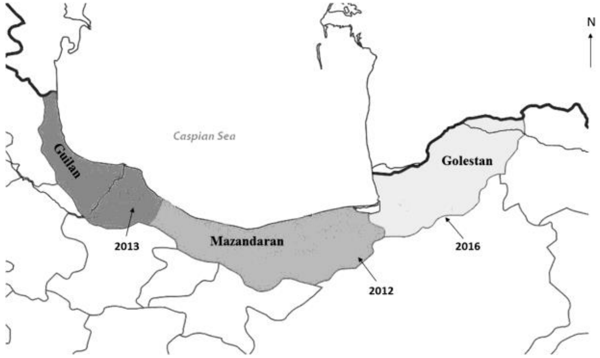
Magnaporthe oryzae a threat for global agriculture

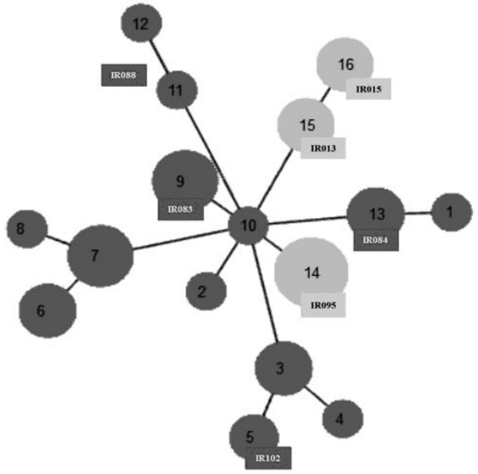
Wheat blast – a global problem

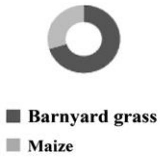




Blast epidemics on maize and barnayard grass (Eragrostis) in Northern Iran





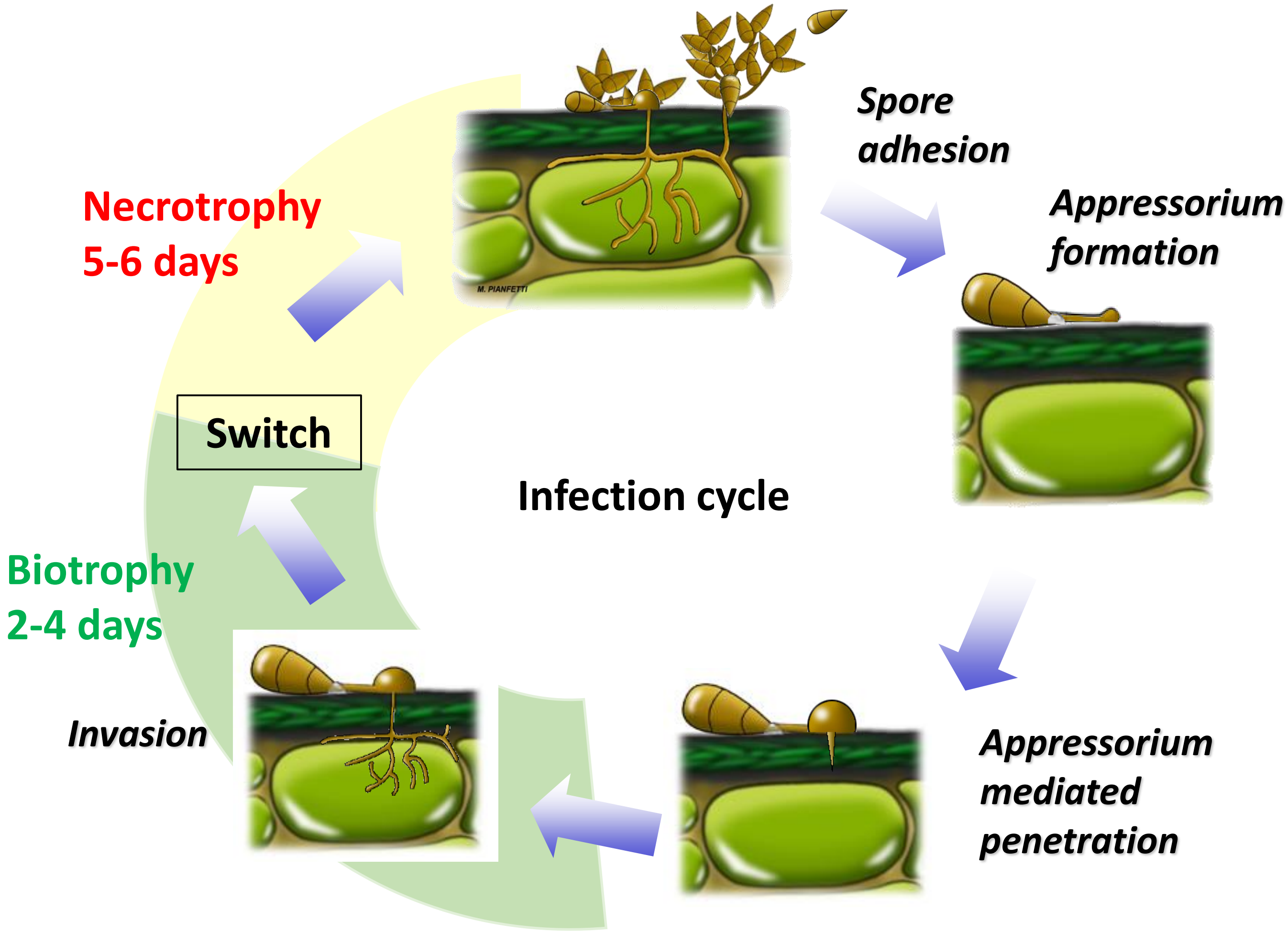


Didier Tharreau

M. oryzae host shifts and host range extensions are threat for major cereal crops

Pordel et al., Phytopathology, 2020

Magnaporthe oryzae (*Pyricularia oryzae*)
A fungal pathogen of rice hemi-biotroph



Infection cycle

Spore adhesion

Appressorium formation

Appressorium mediated penetration

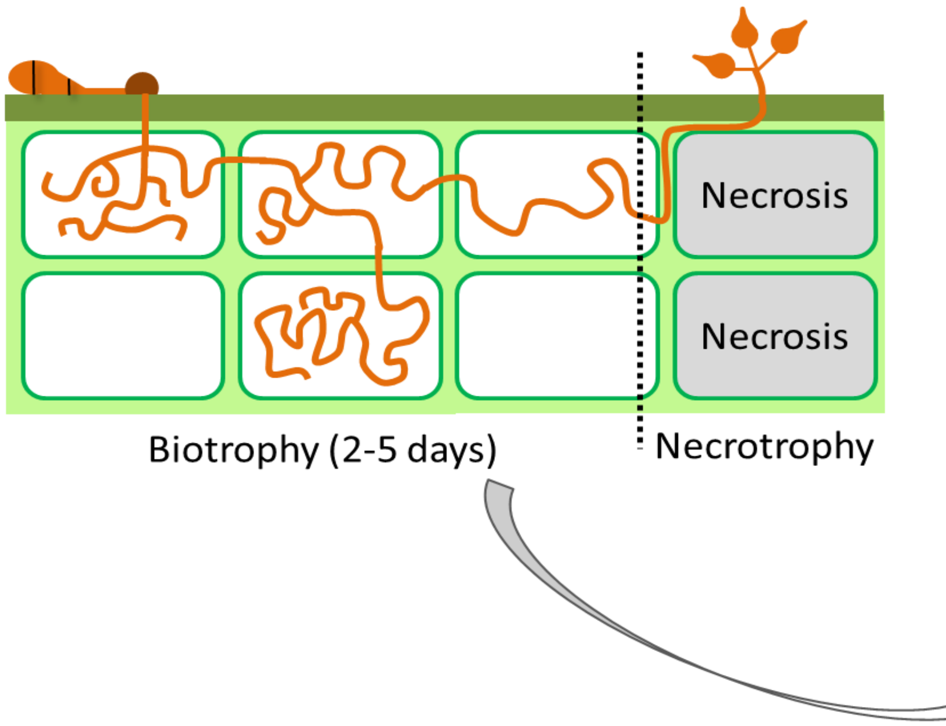
Invasion

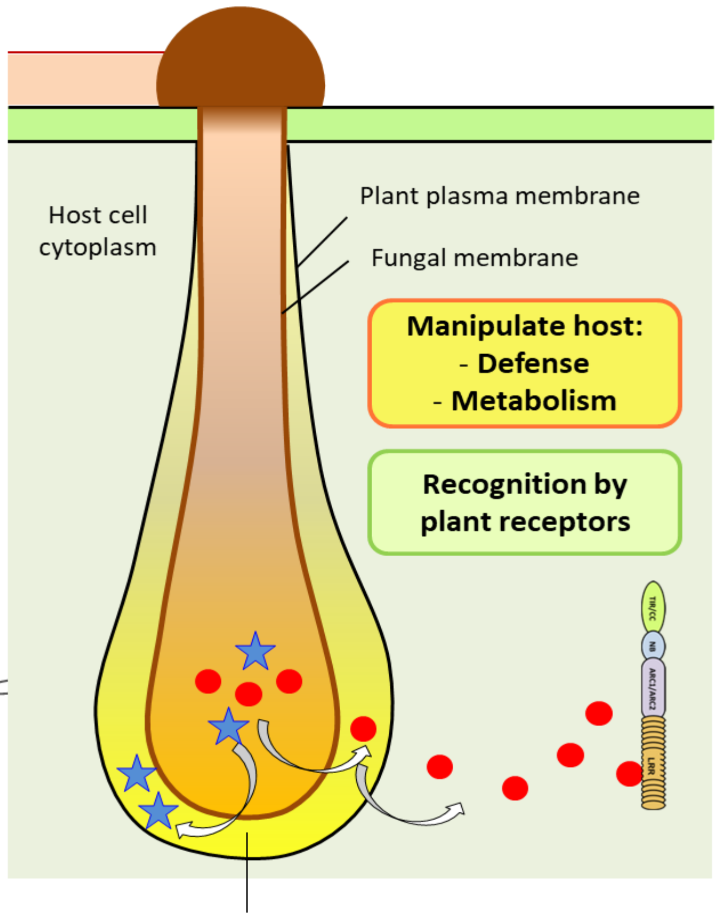
Biotrophy
2-4 days

Switch

Necrotrophy
5-6 days

Biotrophic invasion by M. oryzae





Manipulate host:
- **Defense**
- **Metabolism**

Recognition by plant receptors

EHIM Matrix: Extra-Invasive Hyphal Membrane Matrix

Extended fungus-host cell interface
→ **Secretion and translocation of effectors**

The regulation of gene virulence networks remains poorly characterized

BIP1 was identified by forward genetic

UMR CNRS-Bayer
Lyon, France

1000 hygromycin-resistant mutants REMI (Restriction Enzyme mediated Insertion)

↓

Screening for their pathogenesis reduction

↓

32 mutants with reduced pathogenicity

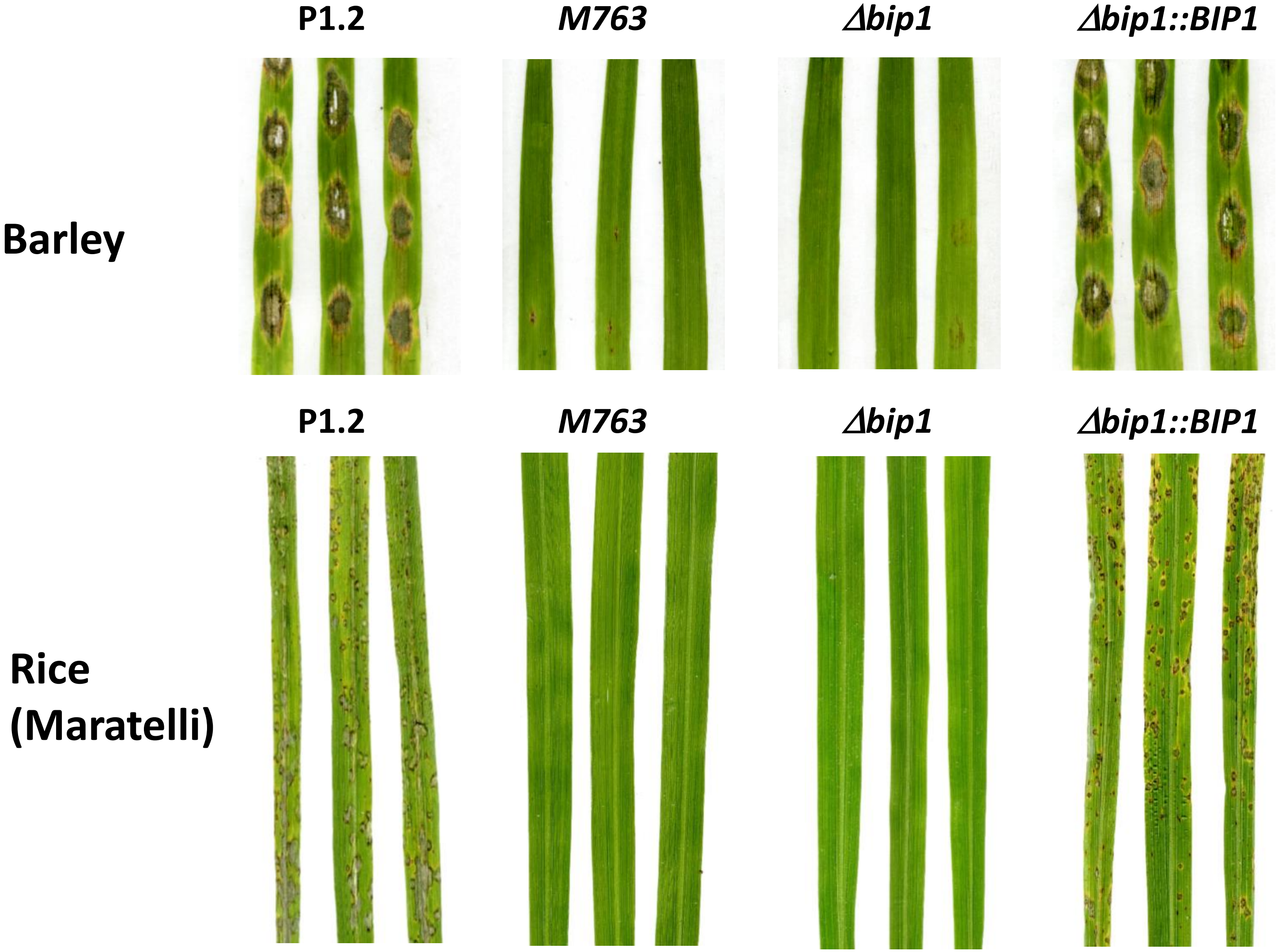
↓

Genetic analysis of 9 of these mutants

↓

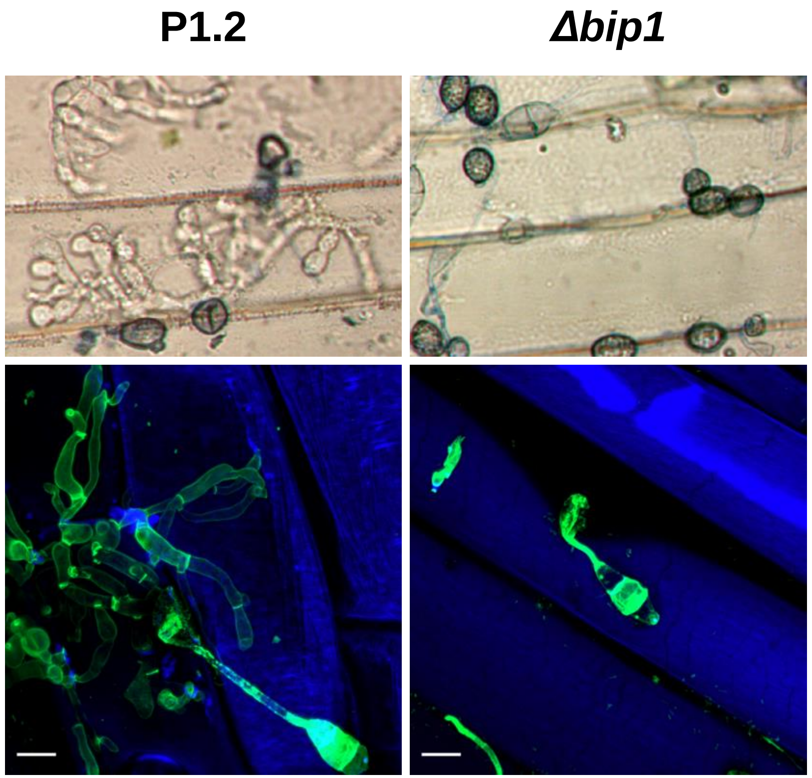
3 mutants due to plasmid insertion
Buf1
***Pls1* (Clergeot et al., 2001 PNAS)**
763=bip1

The M. oryzae insertion mutant M763 and Δbip1 are non-pathogenic

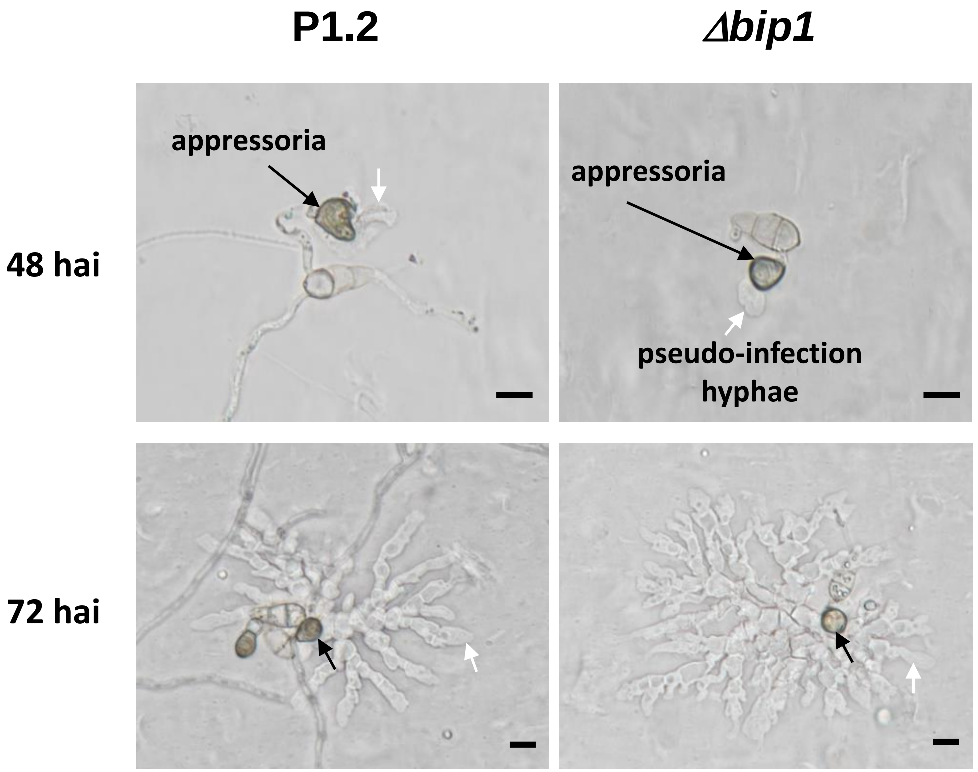


Lambou K., Tag A.*et al.*, 2024 PLOS Pathogens accepted

Bip1 is essential for the early establishment of the fungus in its host plant



Δbip1 can’t penetrate into host plant



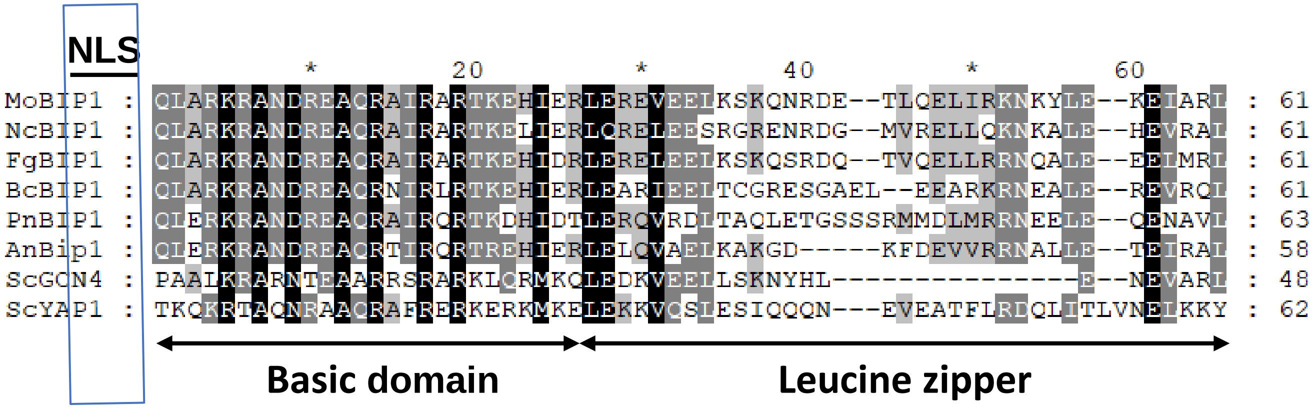
Δbip1 can penetrate into artificial cellophane membrane

Bip1 is not involved in the differentiation of penetration peg or primary infection hyphae but in the early establishment of the fungus in its host plant.

Lambou K., Tag A.*et al.*, 2024 PLOS Pathogens accepted

Bip1 encodes a B-ZIP transcription factor

B-ZIP domain of Bip1



```
                        *          20          *          40          *          60
MoBIP1 : QLARKRANDREAQRAIRARTKEHIERLEREVEELKSKQNRDE--TLQELIRKNKYLE--KEIARL : 61
NcBIP1 : QLARKRANDREAQRAIRARTKELIERLQRELEESRGRENRDG--MVRELLQKNKALE--HEVRAL : 61
FgBIP1 : QLARKRANDREAQRAIRARTKEHIDRLERELEELKSKQSRDQ--TVQELLRRNQALE--EEIMRL : 61
BcBIP1 : QLARKRANDREAQRNIRLRTKEHIERLEARIEELTCGRESGAEL--EEARKRNEALE--REVRQL : 61
PnBIP1 : QLERKRANDREAQRAIRQRTKDHIDTLERQVRDLTAQLETGSSSRMMDLMRRNEELE--QENAVL : 63
AnBip1 : QLERKRANDREAQRTIRQRTREHIERLELQVAELKAKGD------KFDEVVRRNALLE--TEIRAL : 58
ScGCN4 : PAALKRARNTEAARRSRARKLQRMKQLEDKVEELLSKNYHL---------------E--NEVARL : 48
ScYAP1 : TKQKRTAQNRAACRAFRERKERKMKELEKKVQSLESIQQQN---EVEATFLRDQLITLVNEIKKY : 62
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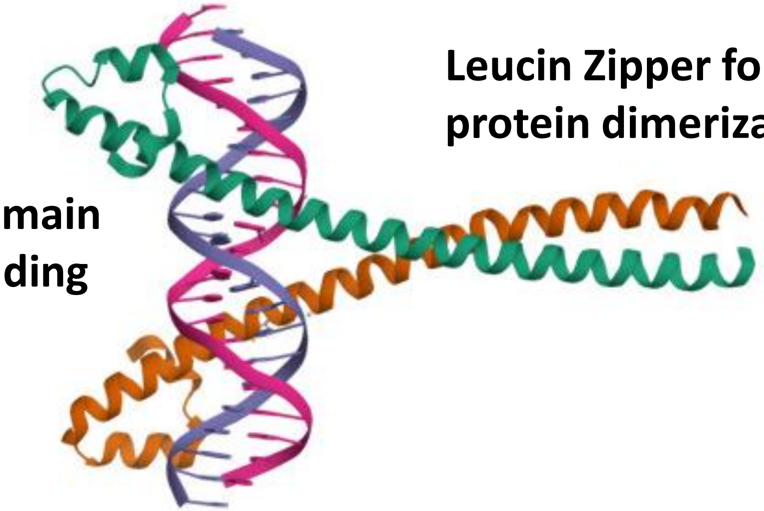
Lambou K., Tag A.*et al.*, 2024 PLOS Pathogens accepted



Leucin Zipper for protein dimerization

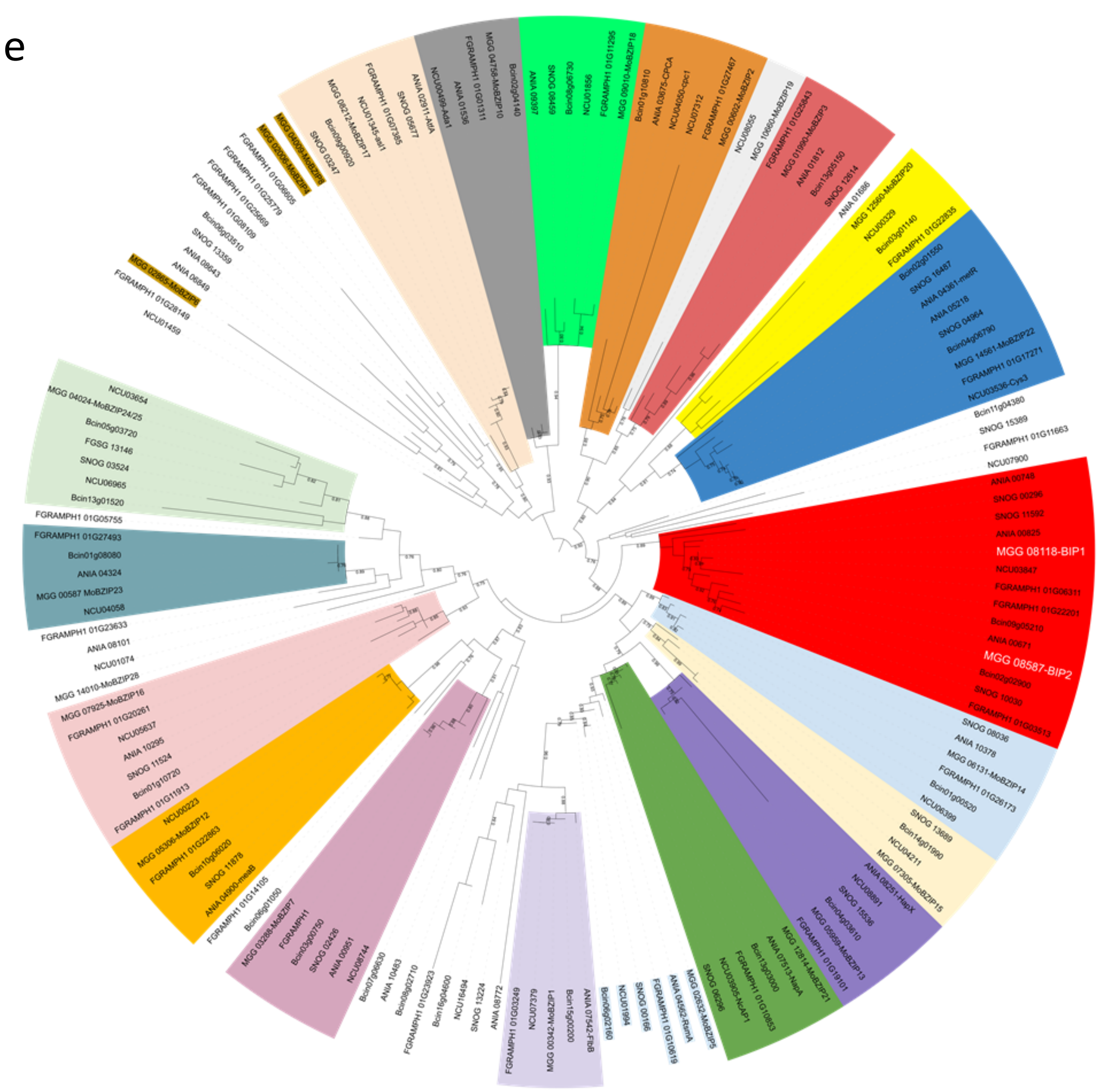
Basic domain DNA binding

Bip1 and Bip2 are paralogs and form a clade distinct from other fungal BZIPs

Phylogeny ofbZIP TF of M. oryzae
and 5 other Pezizomycotina

Colored ranges

- 🟥 BIP1 and BIP2 clade
- ⬜ MoBZIP1/MoFBLB clade
- 🟧 MoBZIP2/MoCPCA clade
- 🟥 MoBZIP3/IDI4 clade
- 🟪 MoBZIP7/MoZIF1 clade
- ⬛ MoBZIP10 clade
- 🟧 MoBZIP12/MoMEAB clade
- 🟪 MoBZIP13/MoHAPX clade
- ⬜ MoBZIP14 clade
- ⬜ MoBZIP15 clade
- ⬜ MoBZIP16 clade
- ⬜ MoBZIP17/MoATF1 clade
- 🟩 MoBZIP18/MoHAC1 clade
- ⬜ MoBZIP19 clade
- 🟨 MoBZIP20/MoT2-REP clade
- 🟩 MoBZIP21/MoAP1 clade
- 🟦 MoBZIP22/MoMETR clade
- 🟦 MoBZIP23 clade
- 🟩 MoBZIP24/25 clade



BIP1 and BIP2 clade

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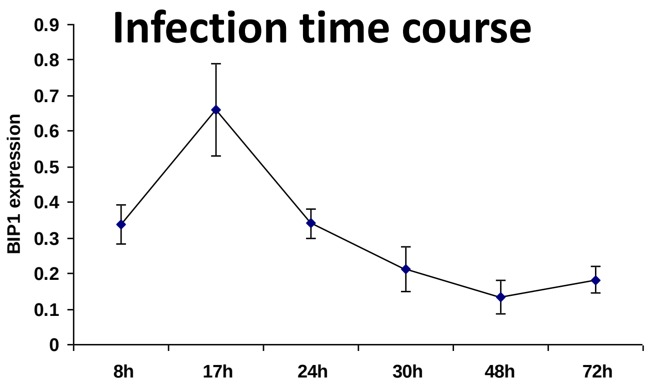
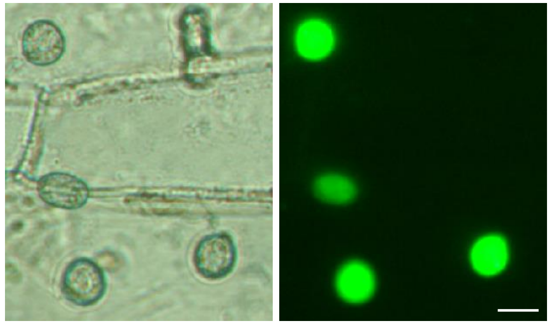
BIP1 expression

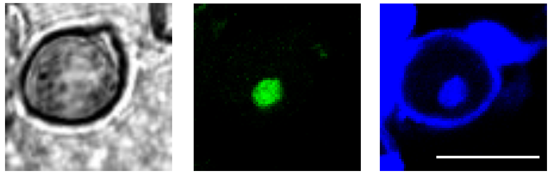
BIP1 mRNA
(qRT_PCR)



BIP1 promoter::3xeGFP



BIP1 promoter::BIP1-3XeGFP



BIP1 is specifically expressed in appressoria at an early stage of infection.

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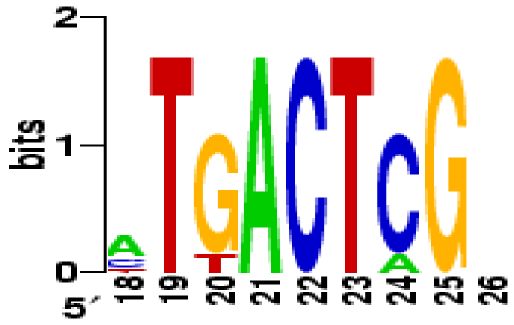
Δbip1 mutant: Transcriptomic analysis

Differential gene expression in appressoria (teflon) of WT *vs Δbip1* mutant

40 genes down-regulated in *Δbip1* appressoria

Shared binding site in promoters



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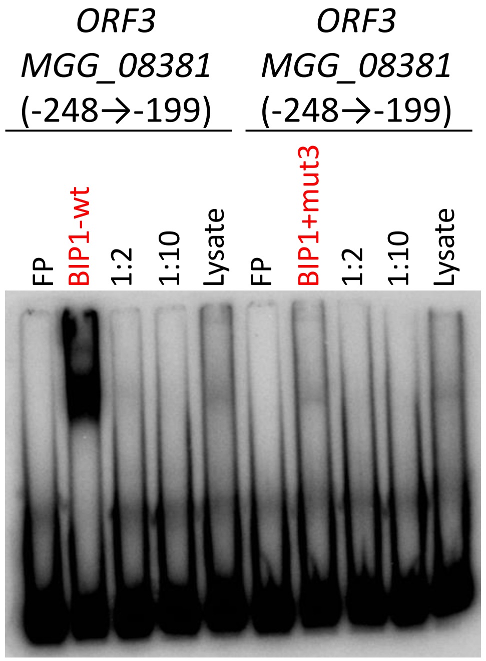
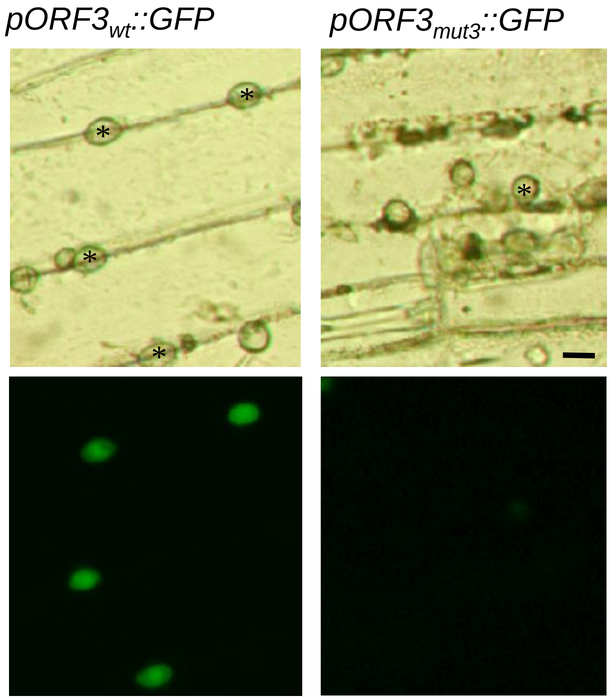
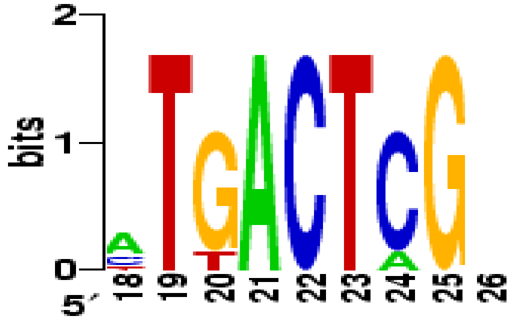
Binding of Bip1 to promoters of target genes

Target binding site validation using ORF3 from ACE1 secondary metabolism gene cluster

FP: free probe
Lysat Bip1 + WT labeled probe
Lysat Bip1 + mut3 labeled probe
1:2/1:10 Bip1 + unlabeled probe
Lysate wo Bip1







pORF3-wt GCAAAAGGTATTTTCGAGTCATGCTCCTAGTCATGGAATAAAAGATGGGA
pORF3-mut3 GCAAAAGGTATTTTTGGCGTAACCCTGTAGCCATTGAATAAAAGATGGGA

BIP1 binds to promoters of genes down-regulated in *Δbip1* appressoria
The BIP1 binding site in *ORF3* promoter is required for appressorium-specific expression

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Bip1 controls an appressorium-specific regulatory network (40 genes)

93% were over-expressed in mature WT appressoria

<u>40 genes down-regulated in *Δbip1* appressoria:</u>

Secreted proteins (40 %):

Small secreted proteins: including effectors BAS2, BAS3, AvrPi9 (EHIM and/or BIC) and SLP2 (LysM domain)

Secreted enzymes : Cutin and plant cell wall degradation

- Secondary metabolism (30 %) : mostly ACE1 cluster

- Signaling proteins (10 %): 3 GPCR and cAMP receptor-like protein

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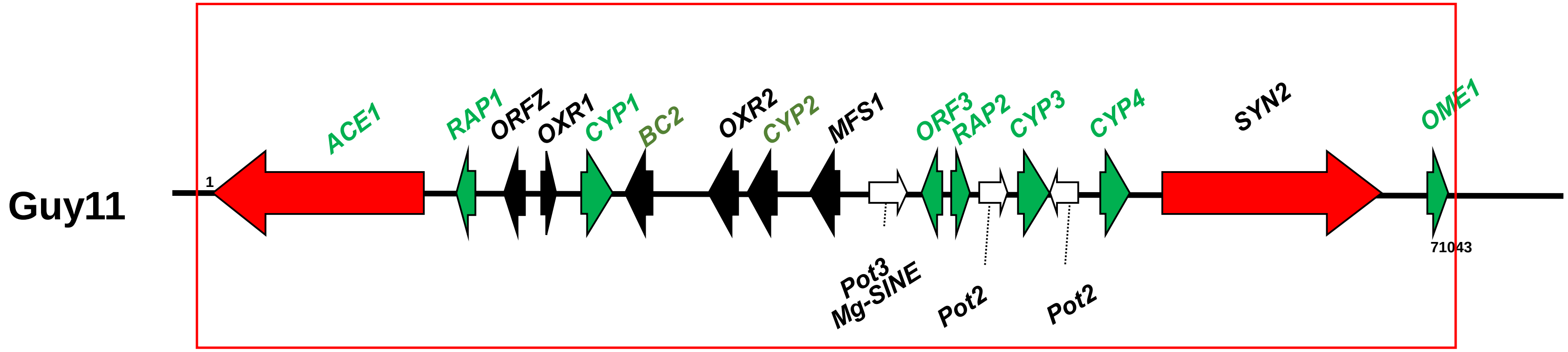
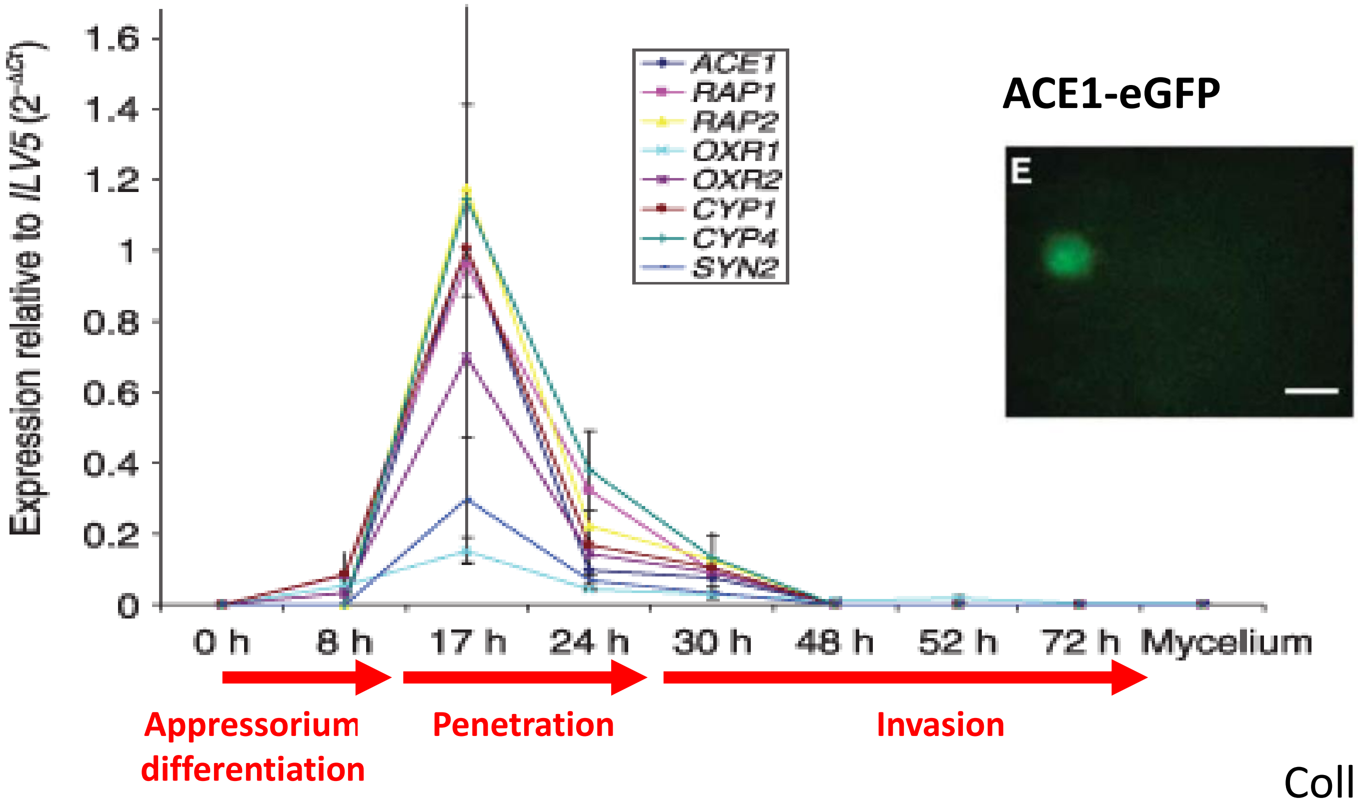
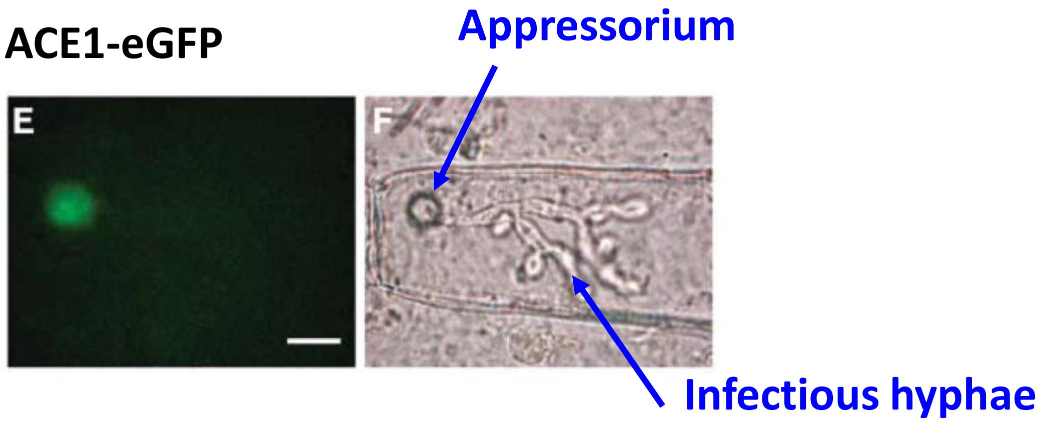
ACE1 cluster



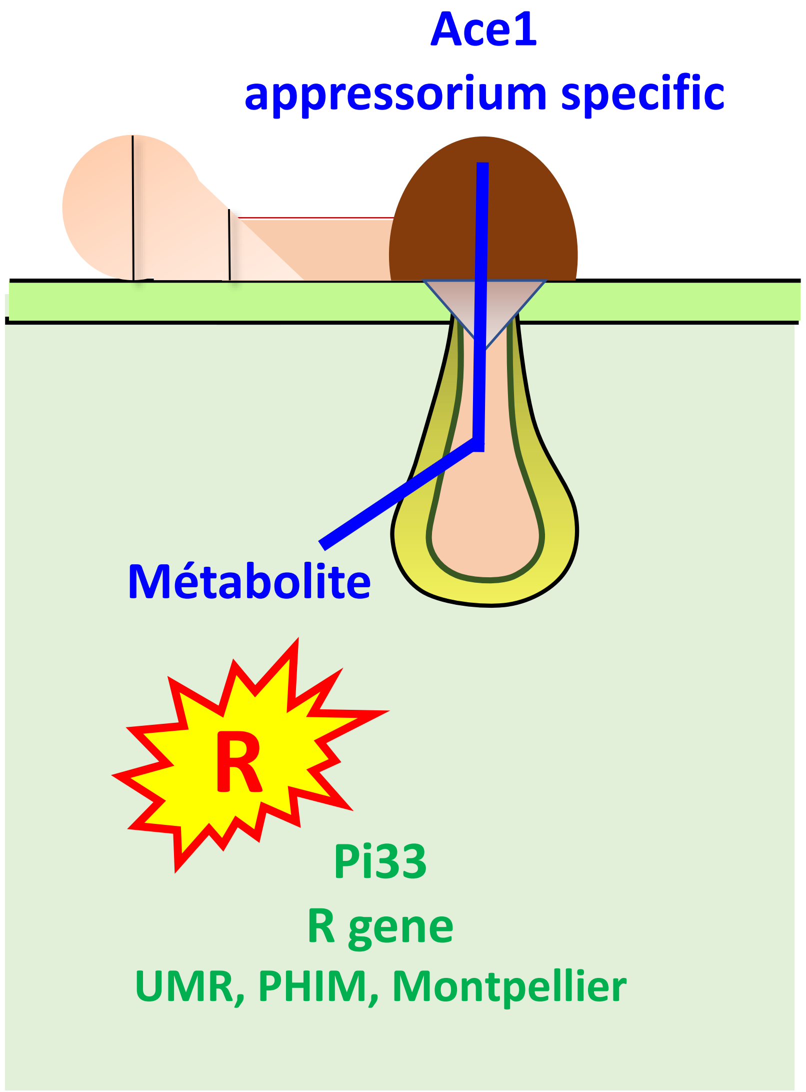




Appressorium specific expression

Collemare *et al.*, 2008 New phytologist

ACE1 cluster



Ace1
appressorium specific

Métabolite

Pi33
R gene
UMR, PHIM, Montpellier

Metabolite is probably similar to cytochalasin

Cytochalasin K

Inhibitor of plant actin assembly ?

Song *et al.*, 2015

Conclusions

- **Bip1 is *M. oryzae* bZIP transcription factor required for pathogenicity**
  (non-detected in previous genome-wide surveys of bZIP TFs in *M. oryzae*)
- **Bip1 and Bip2 : two novel clades of bZIP TFs in this major subdivision of ascomycetes**
- **Bip1 is essential for the early establishment of the fungus within plant cells.**
- **Bip1 is required for the expression of pathogenesis-related genes in appressoria (Ace1 cluster, effectors: Bas2, Bas3, AvrPi9, Slp2 and cuticle and plant cell wall degrading enzymes)**
- **Promoters of these *BIP1*-regulated genes shared a TGACTC GCN4/bZIP-like binding motif that bound *in vitro* to BIP1.**

Next Time

- **Role of Bip2 ?**
- **Role of Bip1 in overcoming plant defenses ?**
- **Régulation of ACE1 cluster (over-expression of BIP1)**
- **Bip1: tool for identification of final metabolite of ACE1 cluster**
- **Mutants KO of Bip1 targets genes (double mutant ACE1/SYN2)**
- **Regulation of Bip1 ?**

Thanks

The bZIP transcription factor BIP1 of the rice blast fungus is essential for infection and regulates a specific set of appressorium genes

Karine LAMBOU[¶*], **Andrew TAG[¶], Alexandre LASSAGNE, Jérôme COLLEMARE, Pierre-Henri CLERGEOT, Crystel BARBISAN, Philippe PERRET, Didier THARREAU, Joelle MILLAZO, Elia CHARTIER, Ronald P. DE VRIES, Judith HIRSCH, Jean-Benoit MOREL, Roland BEFFA, Thomas KROJ, Terry THOMAS and Marc-Henri LEBRUN**

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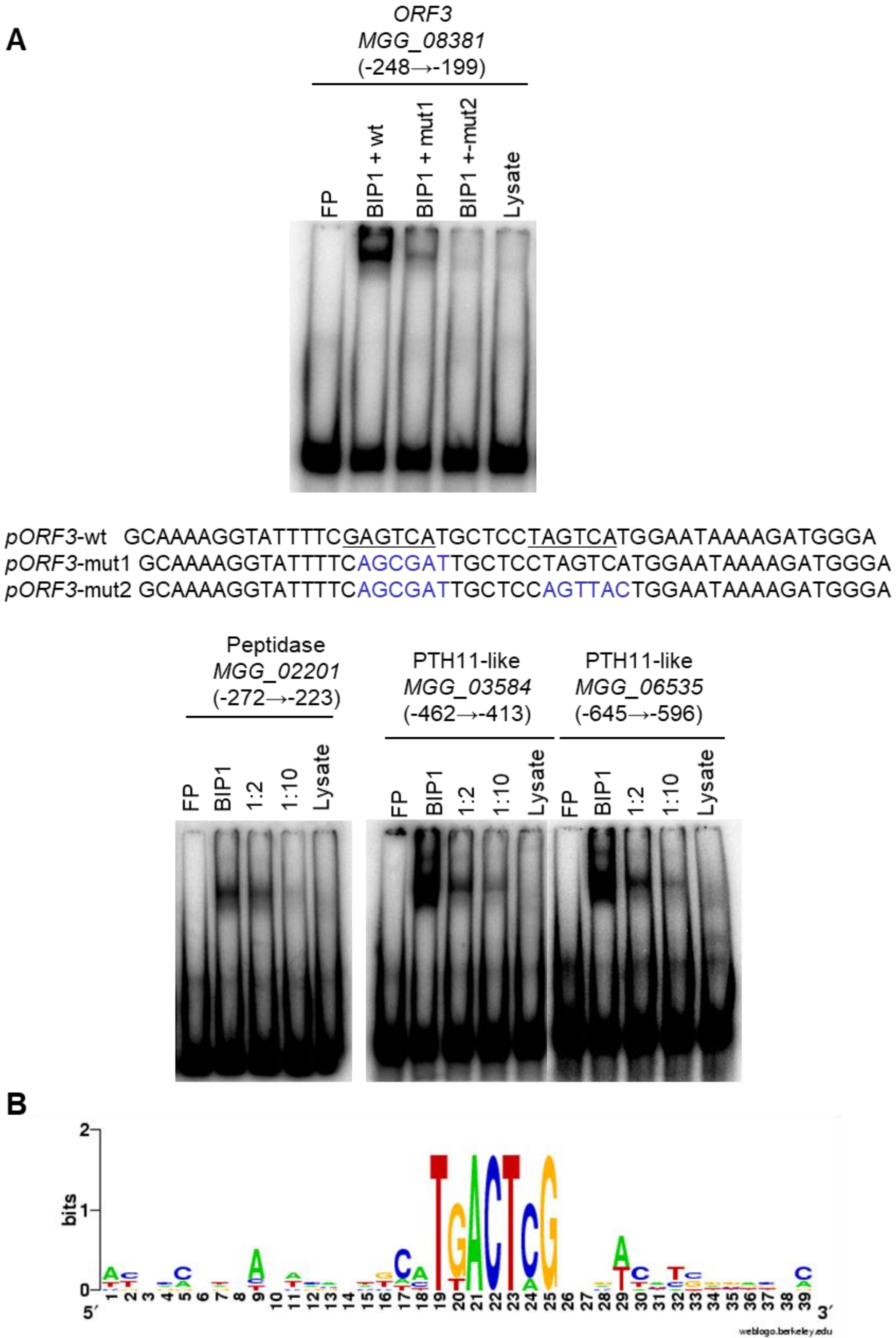
Diapo supplementaires

ene	Class	Function	Gene name	Gene cluster	Reference	µarray *Δbip1*/ wt	qPCR1 Ap *Δbip1*/ wt	qPCR2 WT Ap/ My	RNAsec WT* Ap/ Gc
_02420	Metabolism	Sugar 1,4 lactone oxidase				-1.6			nid
_02530	Metabolism	Quinate permease				-1.9			nid
_02559	Metabolism	Molybdenum cofactor sulphurase				-1.6			2.4
_03263	Metabolism	Betaine aldehyde dehydrogenase	*BADH2*		[43]	-1.5			2.8
_04240	Metabolism	FAD oxidoreductase				-1.5			4.4
_04738	Metabolism	Short-chain dehydrogenase				-1.6			5.1
_09681	Metabolism	Gluconolactonase				-2.4			4.3
_08236	Secondary Metabolism	Polyketide synthase				-2.2			6.3
_08377	Secondary Metabolism	O-Methyltransferase	*OME1*	*ACE1*	[36,44]	-2.1			6.6
_08378	Secondary Metabolism	Cytochrome P450	*CYP4*	*ACE1*	[36,44]	-2.8			6.6
_08379	Secondary Metabolism	Cytochrome P450	*CYP3*	*ACE1*	[36,44]	-2.4			7.3
_08380	Secondary Metabolism	Enoyl reductase	*RAP2*	*ACE1*	[36,44]	-3.8	-10.0	13.3	7.4
_08381	Secondary Metabolism	Diels-alderase	*ORF3*	*ACE1*	[36,44]	-4.1	-11.3	11.1	9.4
_08386	Secondary Metabolism	Zn finger transcription factor	*BC2*	*ACE1*	[36,44]	-1.8	-5	10.8	6.6
_08387	Secondary Metabolism	Cytochrome P450	*CYP1*	*ACE1*	[36,44]	-1.8			7.1
_08391	Secondary Metabolism	Enoyl reductase	*RAP1*	*ACE1*	[36,44]	-2.7			7.3
_11096	Secondary Metabolism	Thioesterase				-1.2			4.6
_13405	Secondary Metabolism	Terpene synthase				-1.3			9.7
_15928	Secondary Metabolism	Cytochrome P450	*CYP2*	*ACE1*	[36,44]	-1.6	-3.0	11.6	10.2
_02201	Secreted Enzyme	Peptidase A1				-4.1	-9.0	11.6	8.5
_03771	Secreted Enzyme	Feruloyl esterase	*FAEB*		[45]	-2.1			8.2
_05855	Secreted Enzyme	α/β Hydrolase				-1.6			4.1
_08480	Secreted Enzyme	α/β Hydrolase				-2.6			4.5
_11966	Secreted Enzyme	Cutinase				-1.6			7.8
_17153	Secreted Enzyme	Chitinase				-1.6			2.3
_00751	Secreted Protein	small secreted protein				-1.1			1.4
_03468	Secreted Protein	Lysm domain protein	*SLP2*		[39]	-2.8			5.1
_03504	Secreted Protein	small secreted protein				-1.6			1.9
_05638	Secreted Protein	small secreted protein				-2			8.8
_06666	Secreted Protein	small secreted protein				-2.3			2.2
_07934	Secreted Protein	small secreted protein				-1.8			8.0
_08428	Secreted Protein	small secreted protein				-2.8			7.0
_09693	Secreted Protein	Biotrophy assoc.protein 2	*BAS2*		[38]	-2.2			2.7
_11610	Secreted Protein	Biotrophy assoc.protein 3	*BAS3*		[38,40]	-3.1			nid
_12655	Secreted Protein	small secreted protein	*AVR-PI9*		[37]	-3.2			6.8
_17425	Secreted Protein	small secreted protein				-2.3			3.6
_02160	Signaling	GPCR PTH11 family, CFEM				-1.2	-1.1	3.4	2.2
_03526	Signaling	N6 Adenine DNA methylase				-1.7			3.2
_03584	Signaling	GPCR PTH11 family, CFEM				-3.3	-1.7	0.5	6.4
_06535	Signaling	GPCR PTH11 family				-2.9	-3.9	7.2	3.8
_10544	Signaling	GPCR cAMP Glucose receptor-like				-1.7			7
_00545	Unknown	Unknown				-2.7			3.7

Gene ID	Gene name in ACE1 cluster	Expression in wild type P1-2	Expression in ΔBIP1:hph	Fold change
MGG_12447	ACE1	$6.3\text{x}10^{-4} \pm 3.7\text{x}10^{-4}$	$8.8\text{x}10^{-6} \pm 4.4\text{x}10^{-6}$	0.01
MGG_08386	BC2	$1.3\text{x}10^{-5} \pm 5.2\text{x}10^{-6}$	$2.4\text{x}10^{-4} \pm 1.0\text{x}10^{-4}$	19
MGG_08387	CYP1	$1.2\text{x}10^{-3} \pm 3.7\text{x}10^{-4}$	$1.1\text{x}10^{-4} \pm 5.6\text{x}10^{-5}$	0.09
MGG_15928	CYP2	$1.3\text{x}10^{-4} \pm 1.8\text{x}10^{-5}$	$1.4\text{x}10^{-3} \pm 5.0\text{x}10^{-4}$	11
MGG_08379	CYP3	$1.8\text{x}10^{-4} \pm 5.3\text{x}10^{-4}$	$3.1\text{x}10^{-6} \pm 3.4\text{x}10^{-6}$	0.02
MGG_08378	CYP4	$1.4\text{x}10^{-3} \pm 2.1\text{x}10^{-4}$	$2.4\text{x}10^{-5} \pm 2.7\text{x}10^{-5}$	0.02
MGG_08377	OME1	$1.7\text{x}10^{-4} \pm 1.1\text{x}10^{-4}$	$6.7\text{x}10^{-5} \pm 4.1\text{x}10^{-5}$	0.40
MGG_08381	ORF3	$3.5\text{x}10^{-3} \pm 1.1\text{x}10^{-3}$	$1.6\text{x}10^{-4} \pm 1.6\text{x}10^{-4}$	0.05
MGG_08391	RAP1	$4.5\text{x}10^{-4} \pm 1.9\text{x}10^{-4}$	$1.4\text{x}10^{-4} \pm 1.6\text{x}10^{-4}$	0.30
MGG_08380	RAP2	$1.2\text{x}10^{-3} \pm 3.7\text{x}10^{-4}$	$6.7\text{x}10^{-5} \pm 4.1\text{x}10^{-5}$	0.05

A
ORF3
MGG_08381
(-248→-199)
FP
BIP1 + wt
BIP1 + mut1
BIP1 +-mut2
Lysate
pORF3-wt GCAAAAGGTATTTTCGAGTCATGCTCCTAGTCATGGAATAAAAGATGGGA
pORF3-mut1 GCAAAAGGTATTTTCAGCGATTGCTCCTAGTCATGGAATAAAAGATGGGA
pORF3-mut2 GCAAAAGGTATTTTCAGCGATTGCTCCAGTTACTGGAATAAAAGATGGGA
Peptidase
MGG_02201
(-272→-223)
PTH11-like
MGG_03584
(-462→-413)
PTH11-like
MGG_06535
(-645→-596)
FP
BIP1
1:2
1:10
Lysate
B
bits
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1
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
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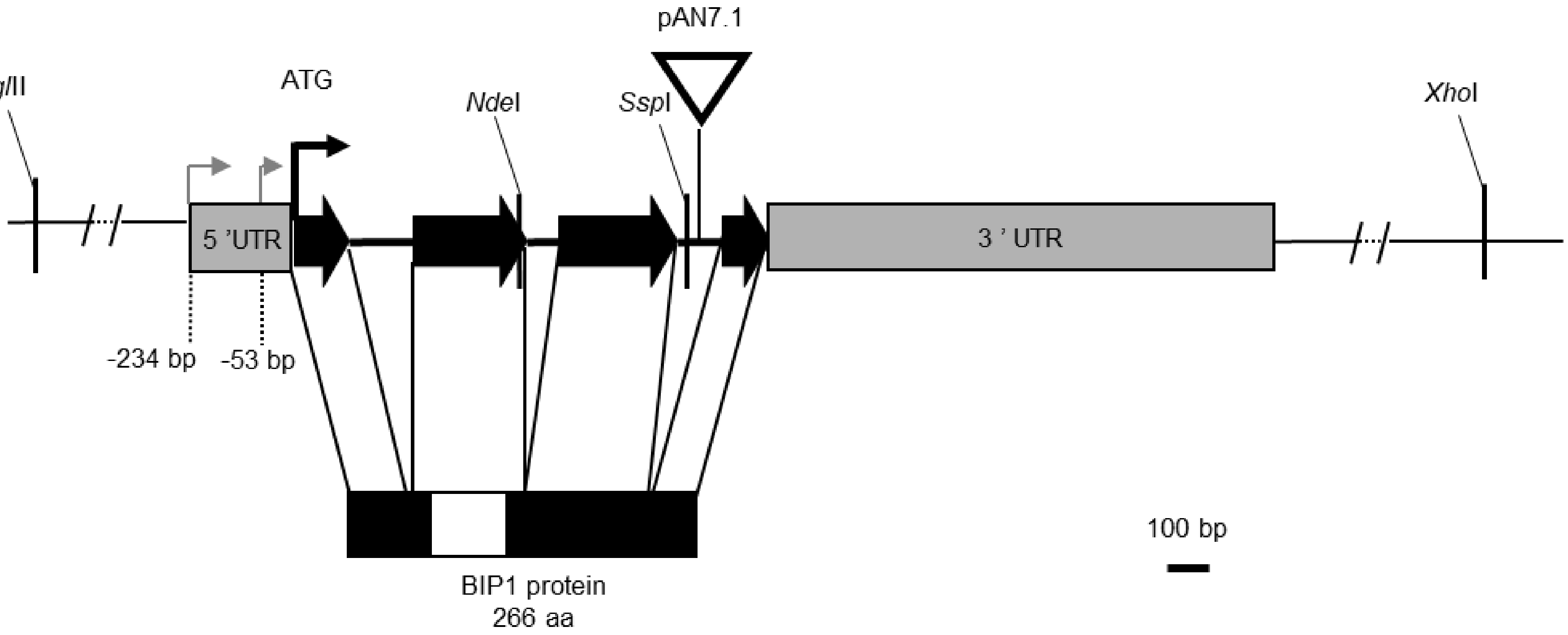
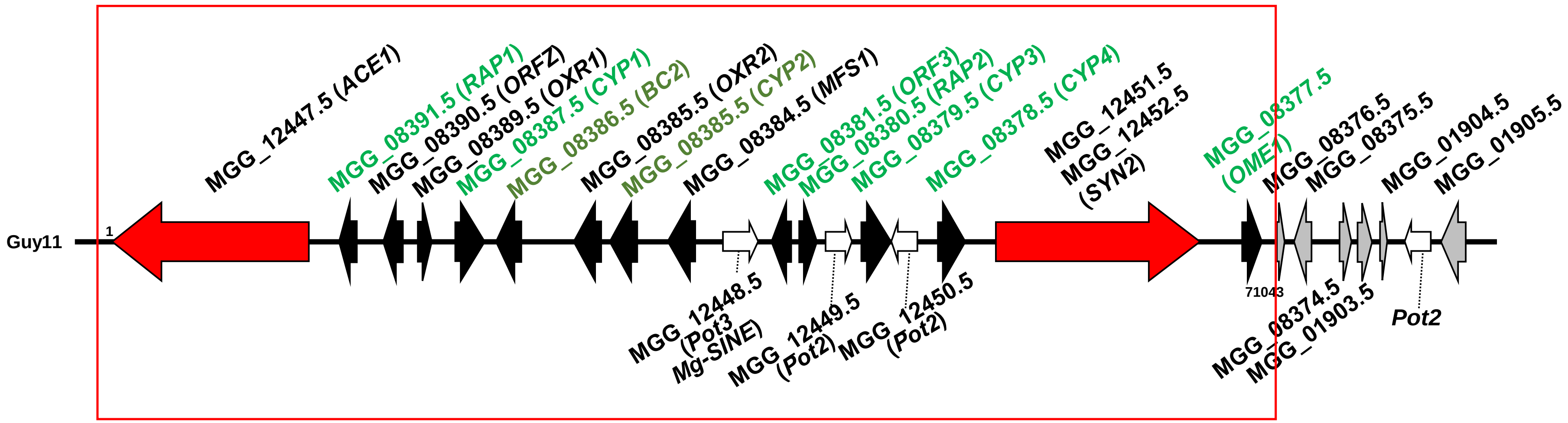

pAN7.1
*Bgl*II
ATG
*Nde*I
*Ssp*I
*Xho*I
5 'UTR
3 ' UTR
-234 bp
-53 bp
BIP1 protein
266 aa
100 bp

Figure S1



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