Genes controlling the infection process the septoria tritici blotch pathogen *Mycosphaerella graminicola*

Rahim Mehrabi

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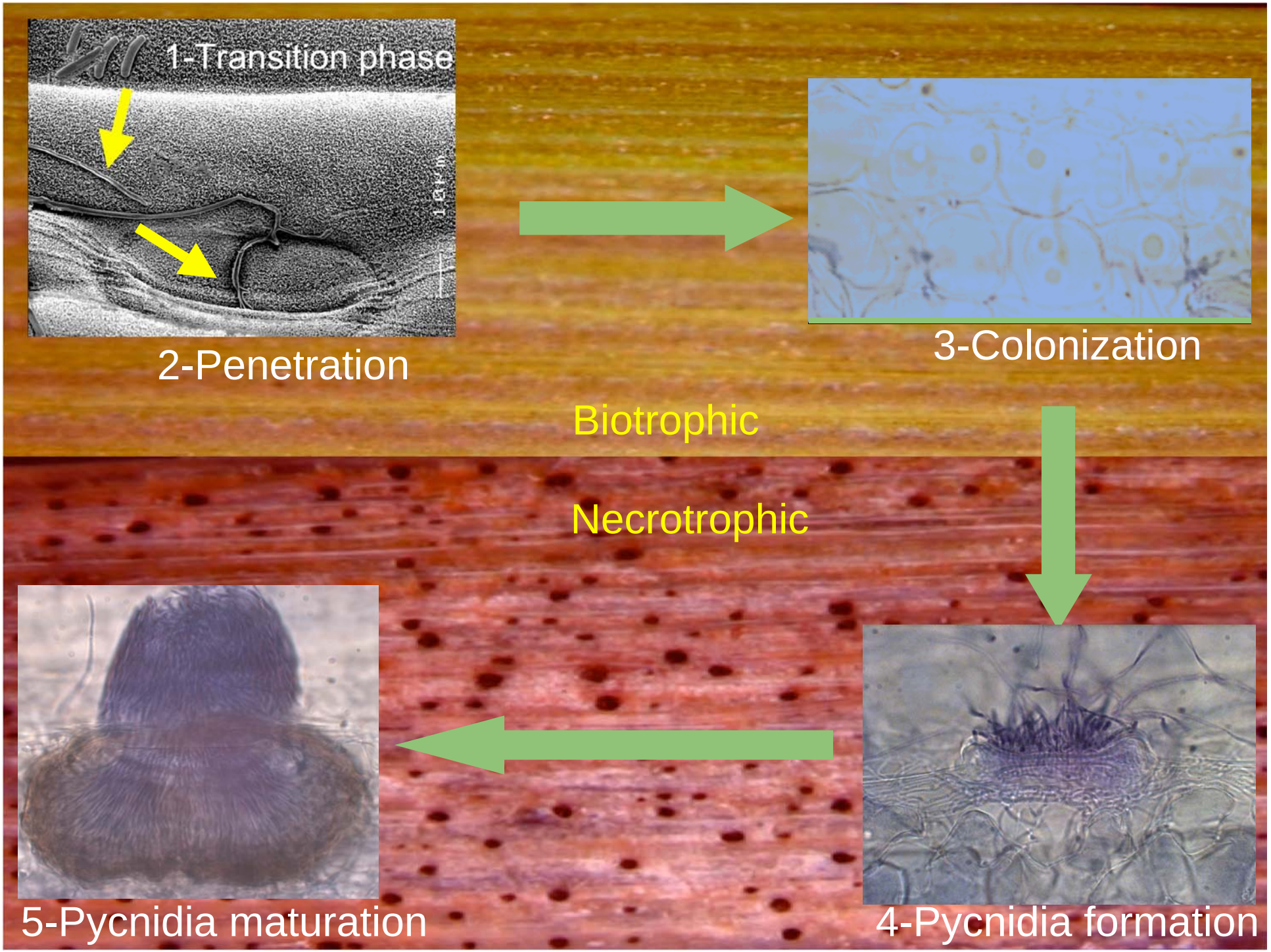
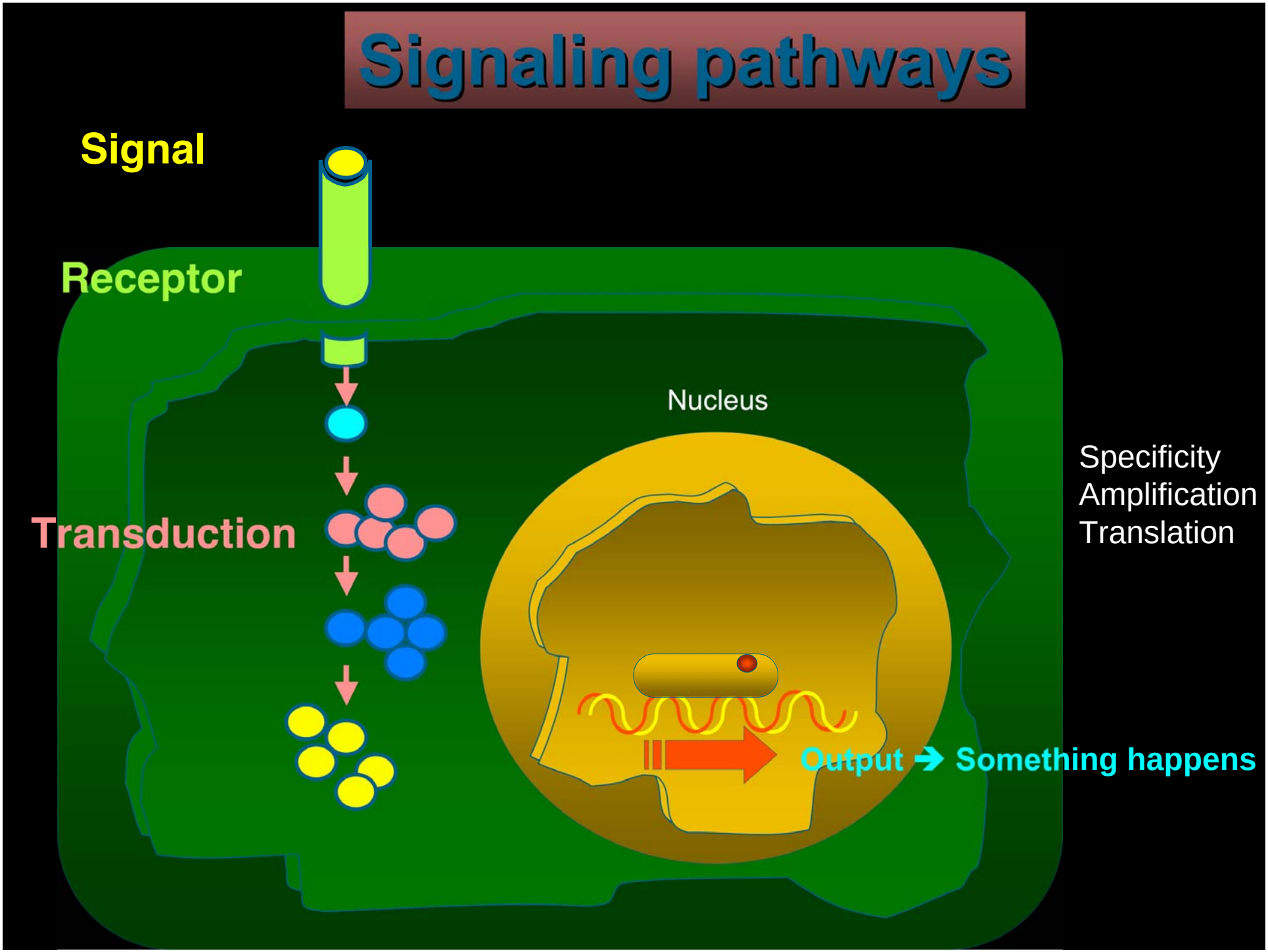
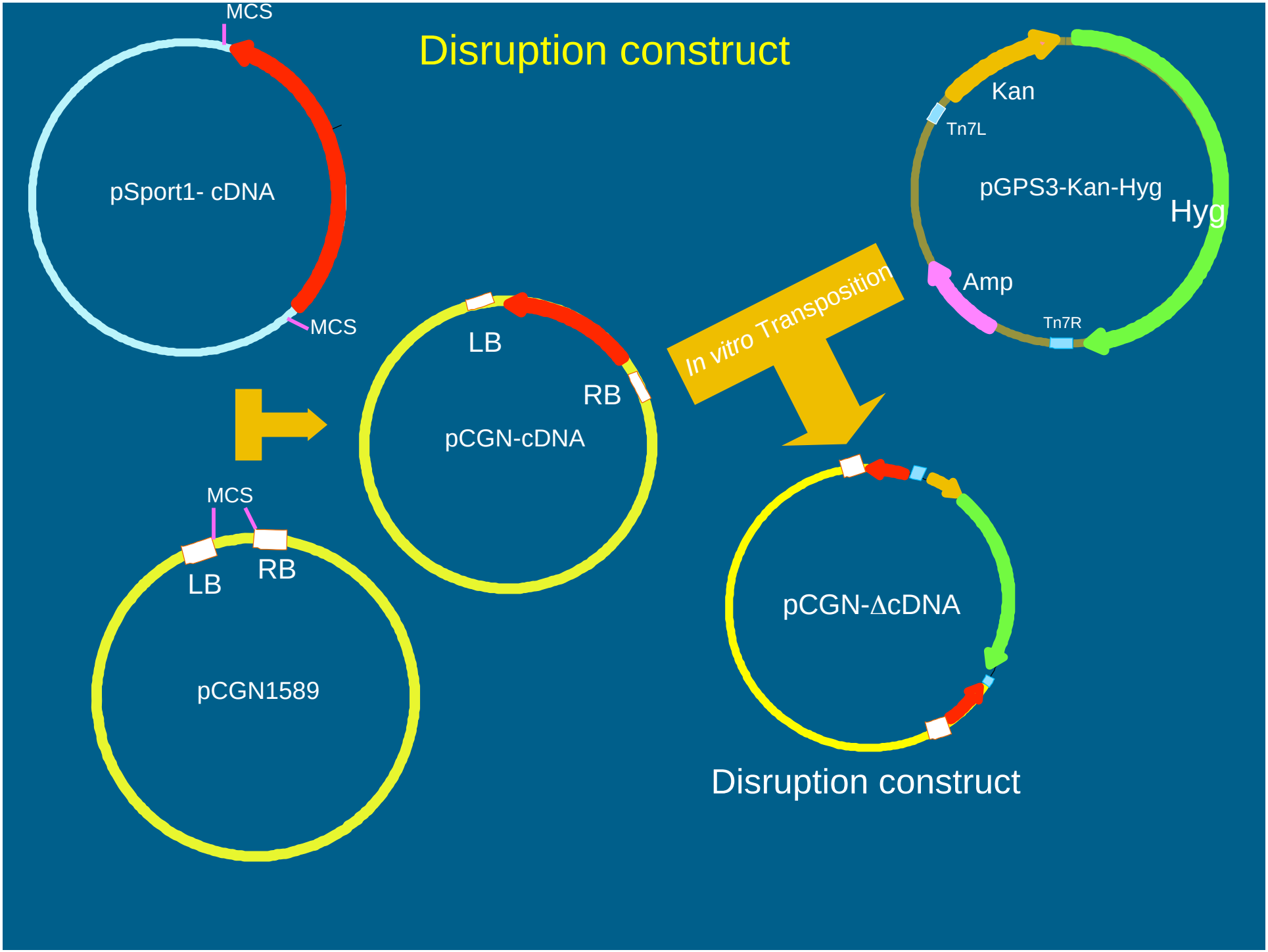

1-Transition phase
2-Penetration
3-Colonization
Biotrophic
Necrotrophic
4-Pycnidia formation
5-Pycnidia maturation

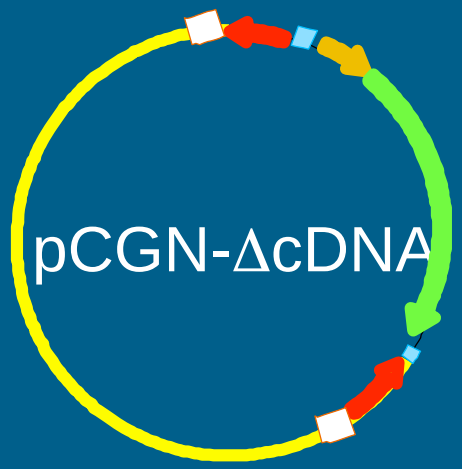

Signaling pathways
Signal
Receptor
Transduction
Nucleus
Specificity
Amplification
Translation
Output → Something happens

Methodology

- Identify targets
  - EST libraries
  - 30,000 ESTs (11000 unigenes)
  - >40 genes implicated in signal transduction pathways
- Targeted gene disruption
  - Disruption construct
  - *Agrobacterium tumefaciens*-mediated transformation
- Functional analyses


Disruption construct
MCS
pSport1- cDNA
MCS
LB
RB
pCGN-cDNA
MCS
LB
RB
pCGN1589
In vitro Transposition
Kan
Tn7L
pGPS3-Kan-Hyg
Hyg
Amp
Tn7R
pCGN-ΔcDNA
Disruption construct

Agrobacterium tumefaciens-mediated transformation



Disruption construct

Transformation

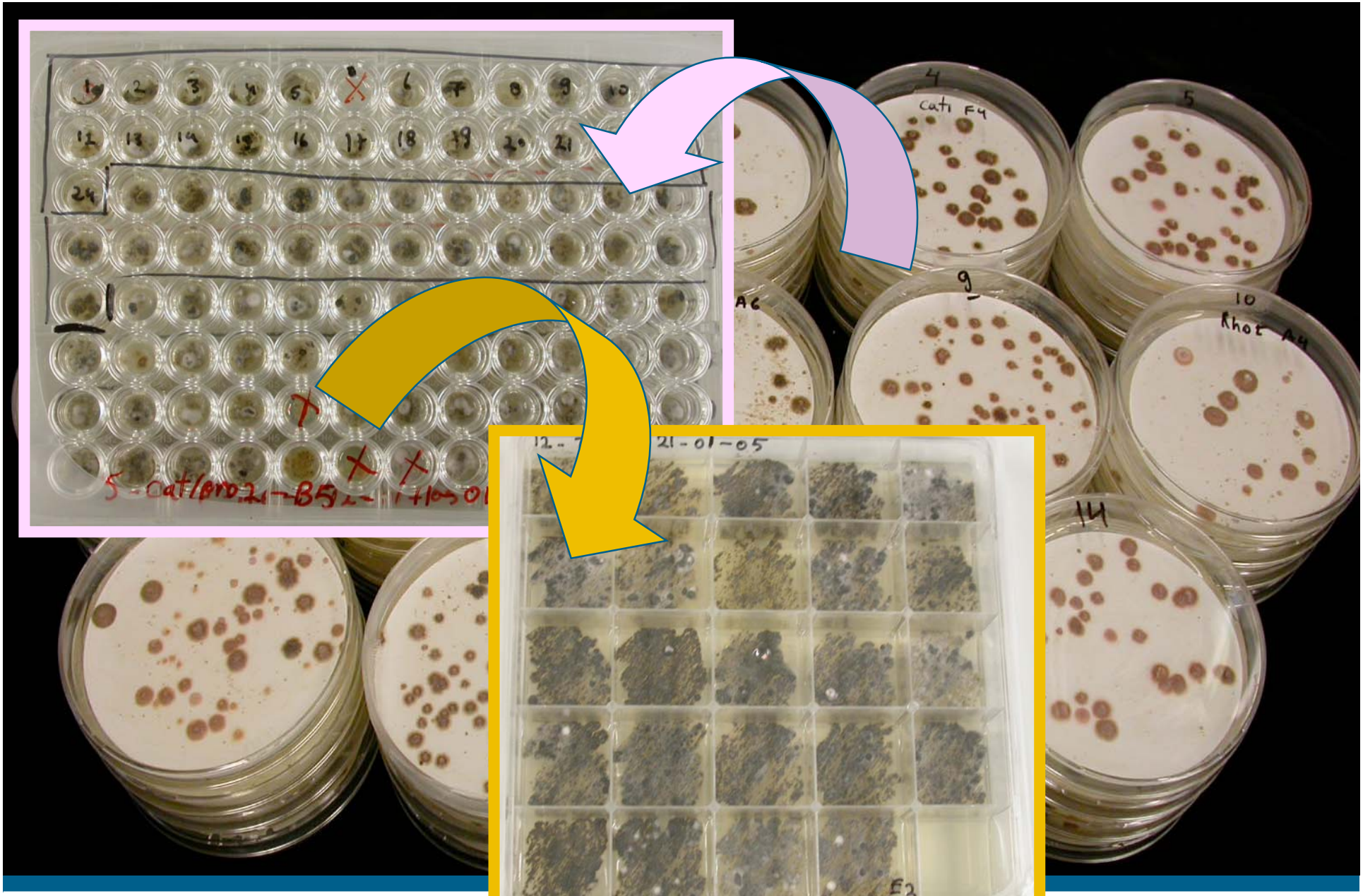
A. tumefaciens

Co-cultivation

M. graminicola

+

A. tumefaciens



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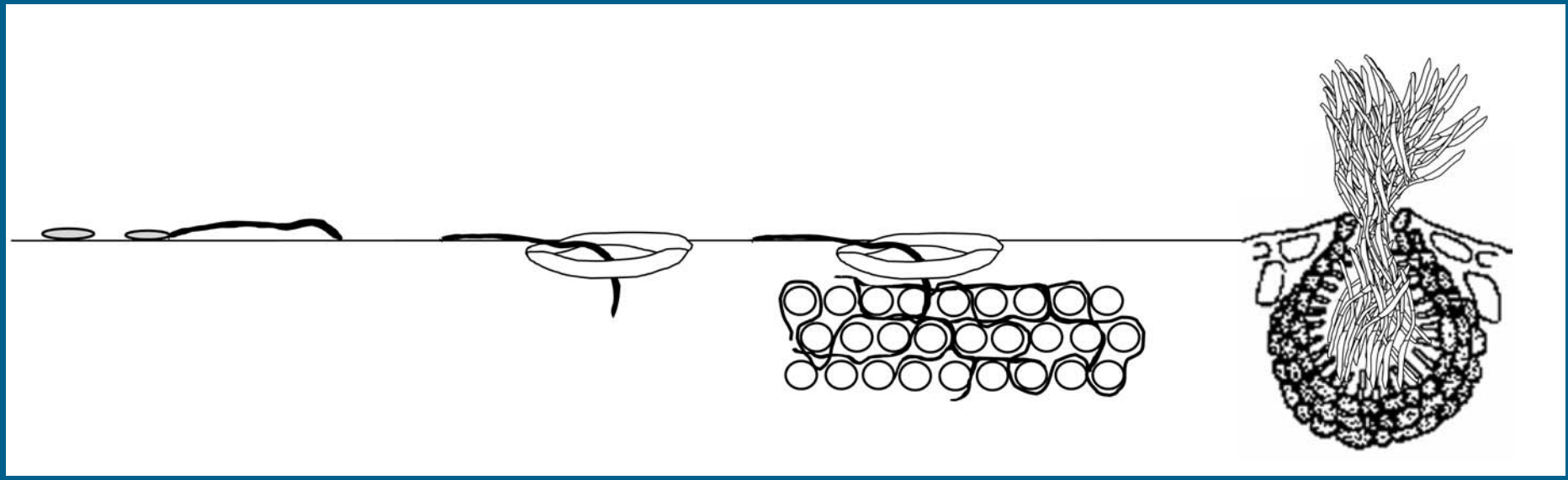
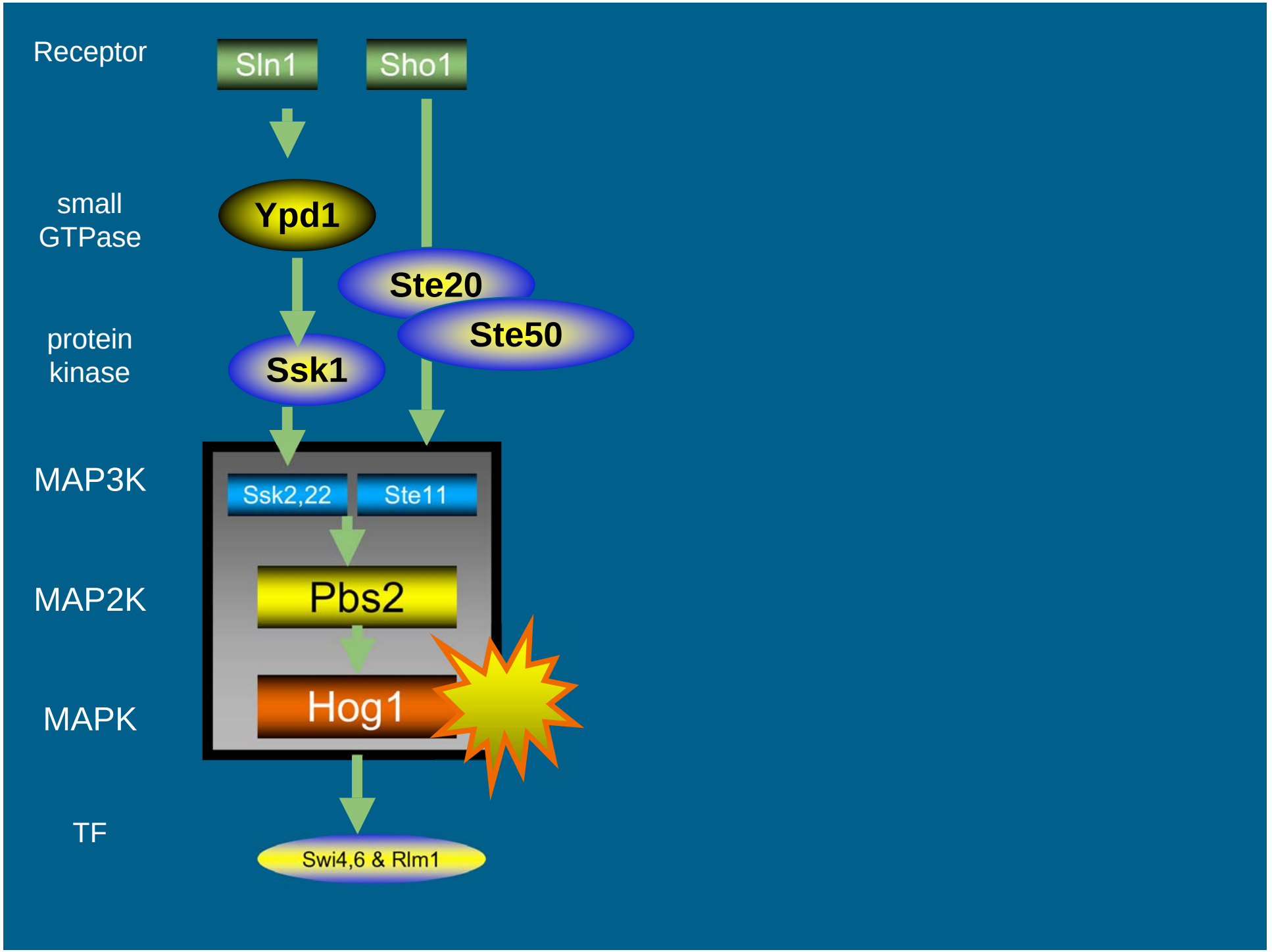
Infection Process

1- Transition **2- Penetration** **3- Colonization** **4- Fructification**




Receptor
Sln1
Sho1
small GTPase
Ypd1
Ste20
Ste50
protein kinase
Ssk1
MAP3K
Ssk2,22
Ste11
MAP2K
Pbs2
MAPK
Hog1
TF
Swi4,6 & Rlm1

Phenotype

IPO323 (WT) ΔMgHog1-E1 ΔMgHog1-2 ΔMgHog1-5

Osmosensitive

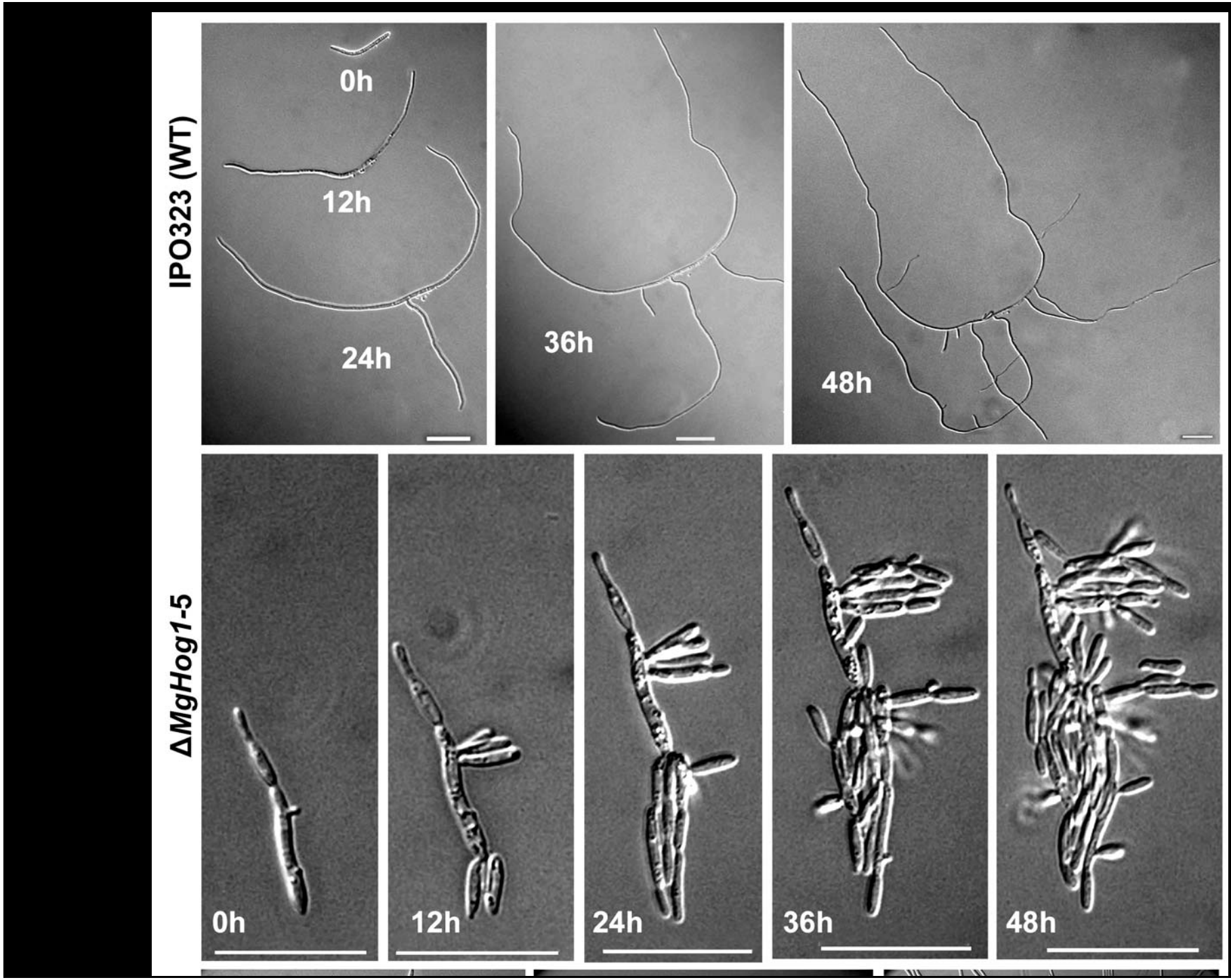
Resistant to fungicide

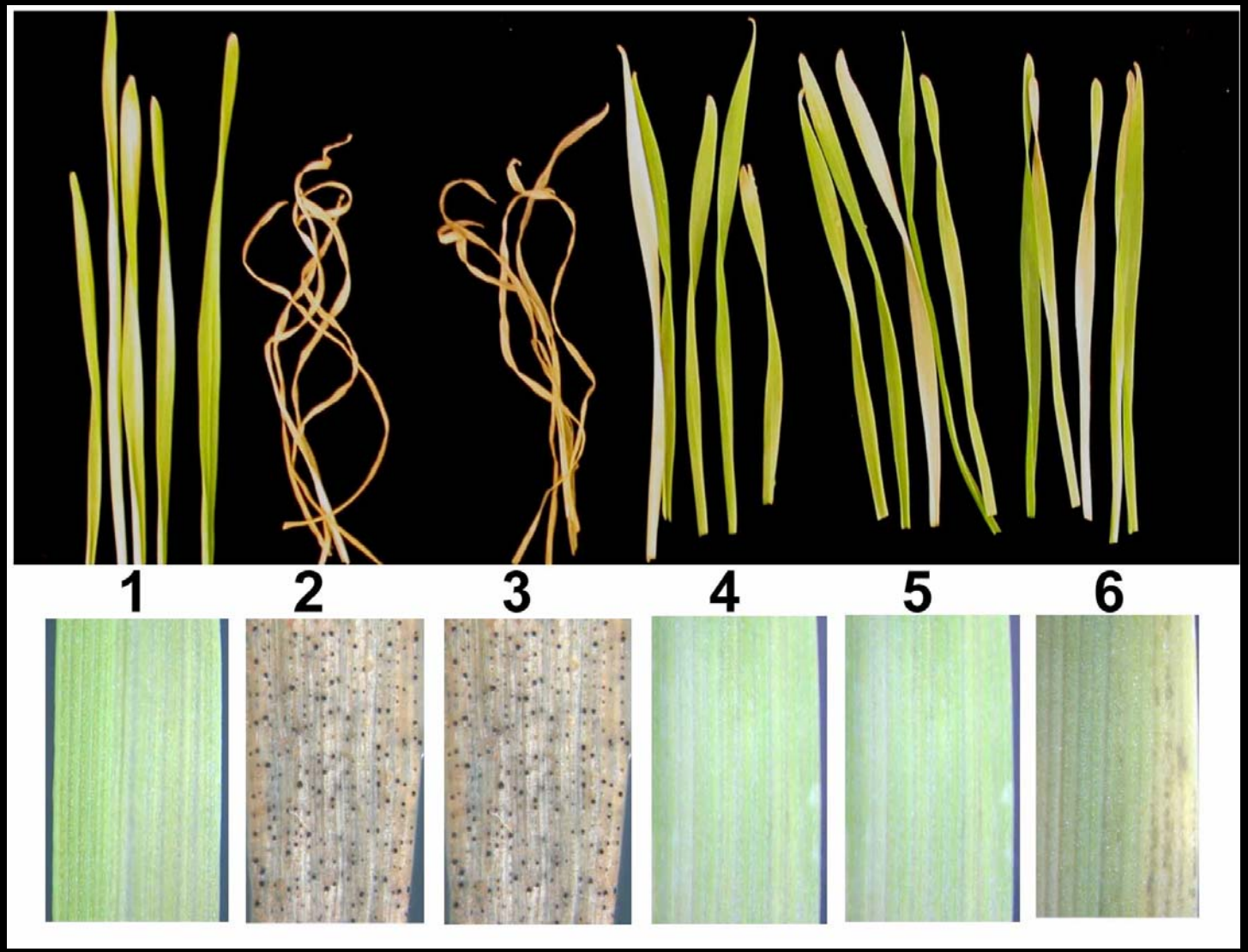
Fludioxonil (>30x)

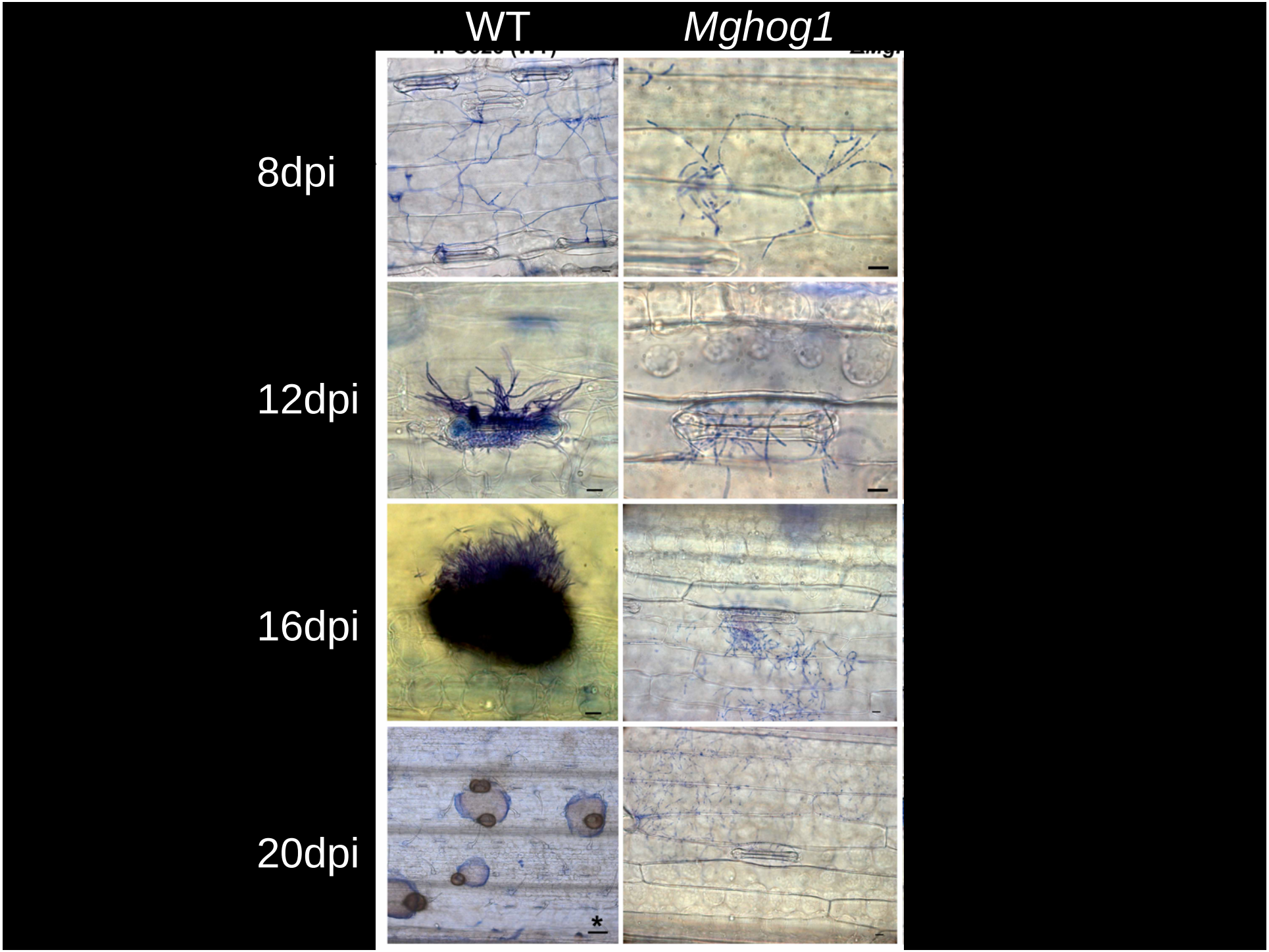
Fenpiclonil (> 30x)

Iprodione (>5x)

Non-melanized yeast like growth


IPO323 (WT)
0h
12h
24h
36h
48h
ΔMgHog1-5
0h
12h
24h
36h
48h


1
2
3
4
5
6
Control
WT
Ectopic
Mutants


WT
Mghog1
8dpi
12dpi
16dpi
20dpi

Conclusion (biological function of *MgHog1*)

- Involvement in osmo-stress growth
- Fungicide resistance through interference with the MgHOG1
- Regulation of dimorphic switch
- Virulence factor

✓

Dimorphic switch ➔ Penetration ➔ Colonization ➔ Fructification

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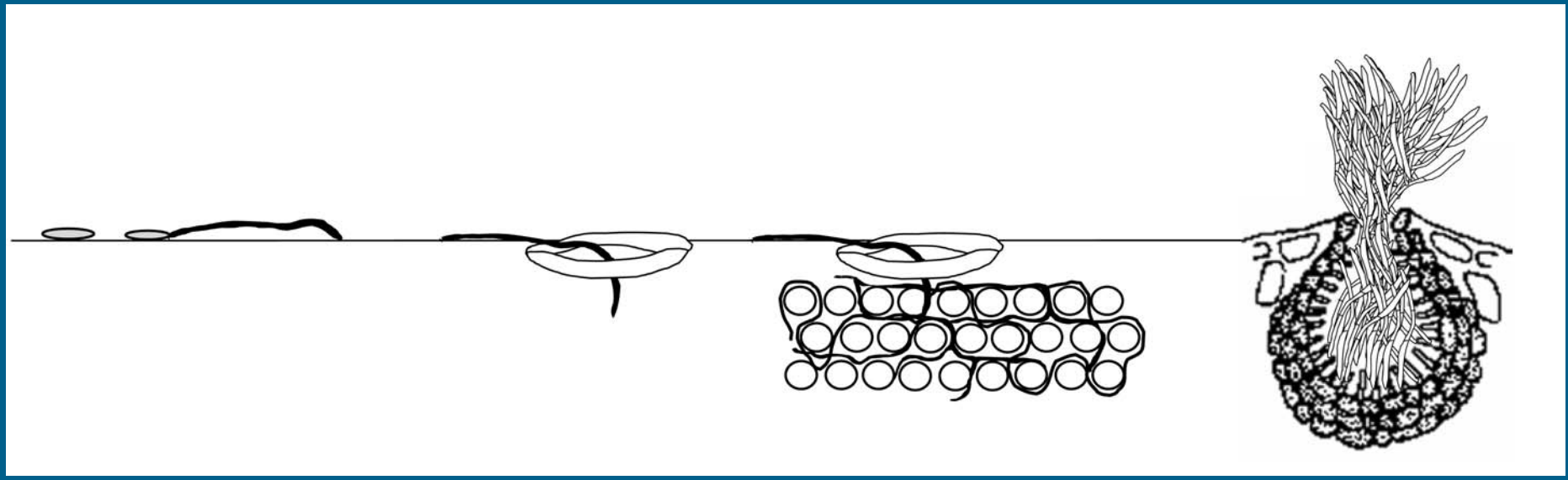
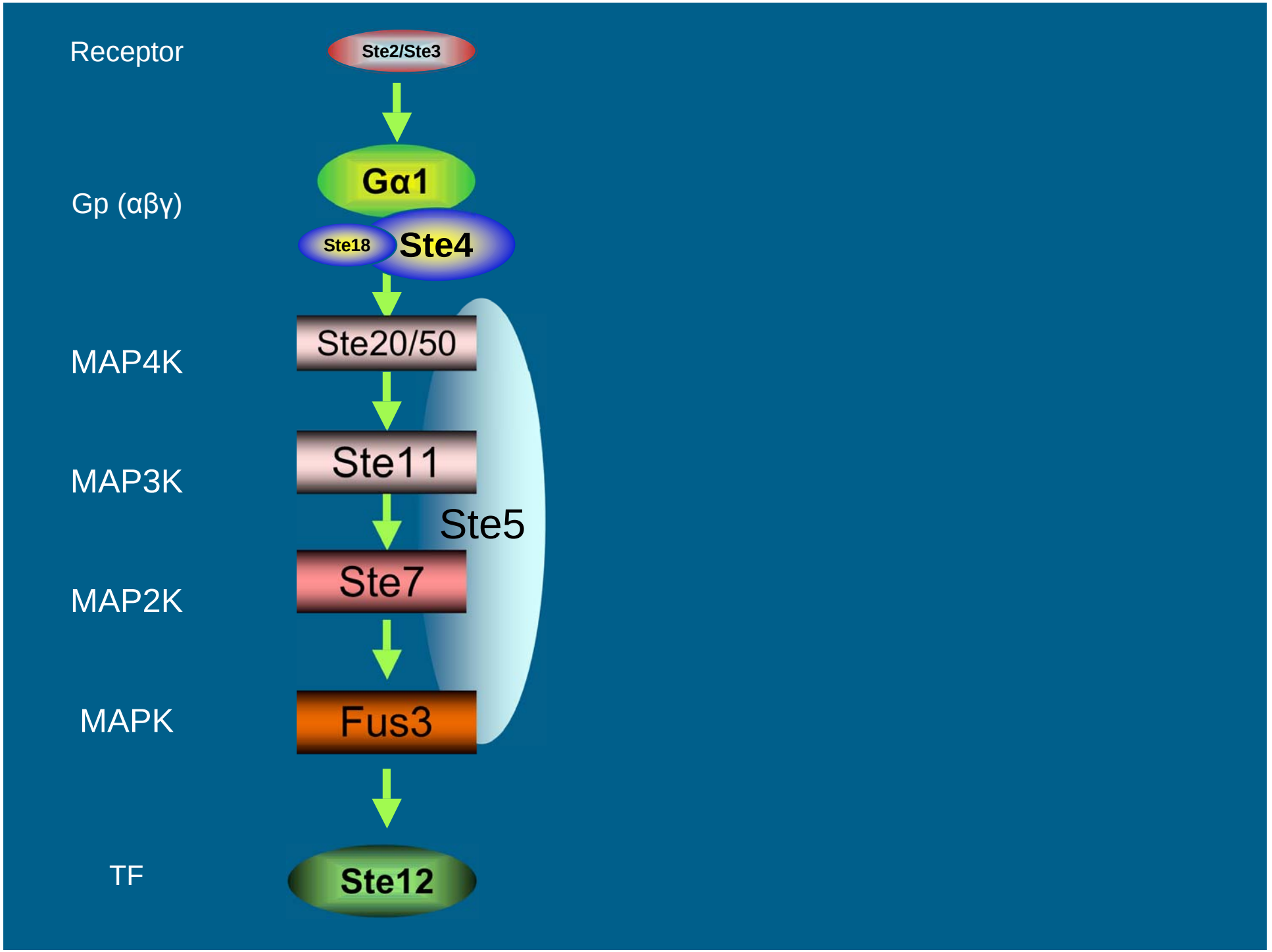
Mehrabi et al., 2006. MPMI (19)1262-1269

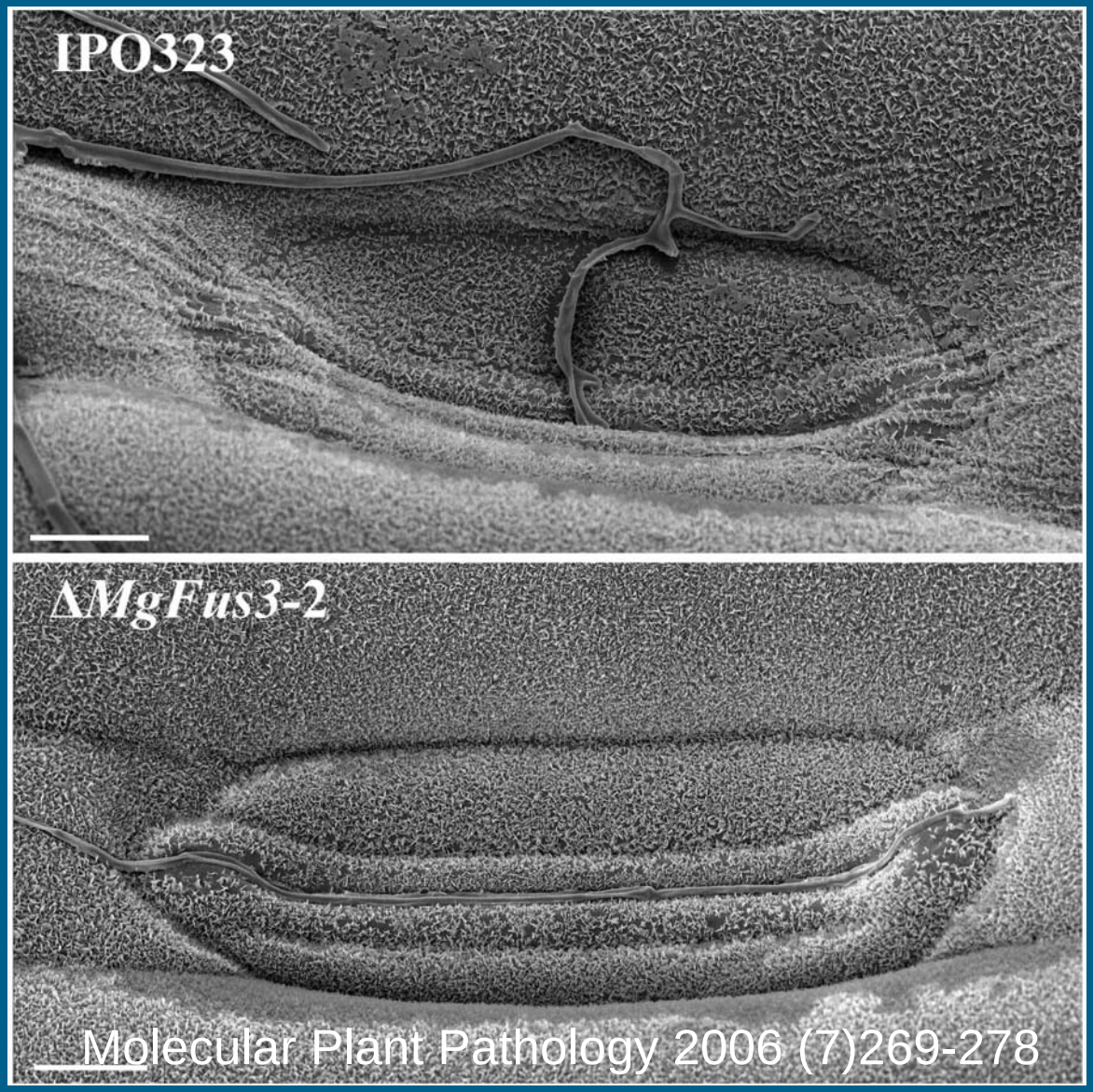
Infection Process

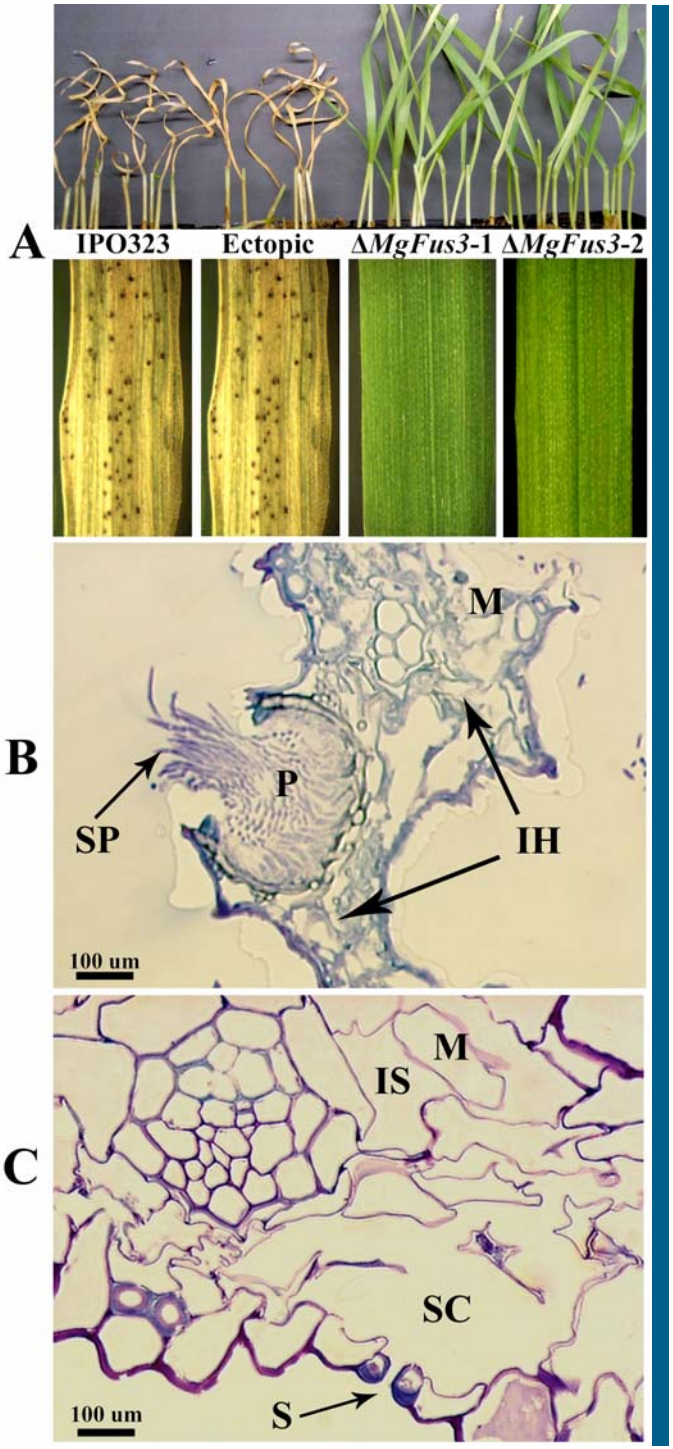
1- Transition **2- Penetration** **3- Colonization** **4- Fructification**




Receptor
Ste2/Ste3
Gp (αβγ)
Gα1
Ste18
Ste4
MAP4K
Ste20/50
MAP3K
Ste11
Ste5
MAP2K
Ste7
MAPK
Fus3
TF
Ste12

MgFus3 disruptants are unable to penetrate stomata





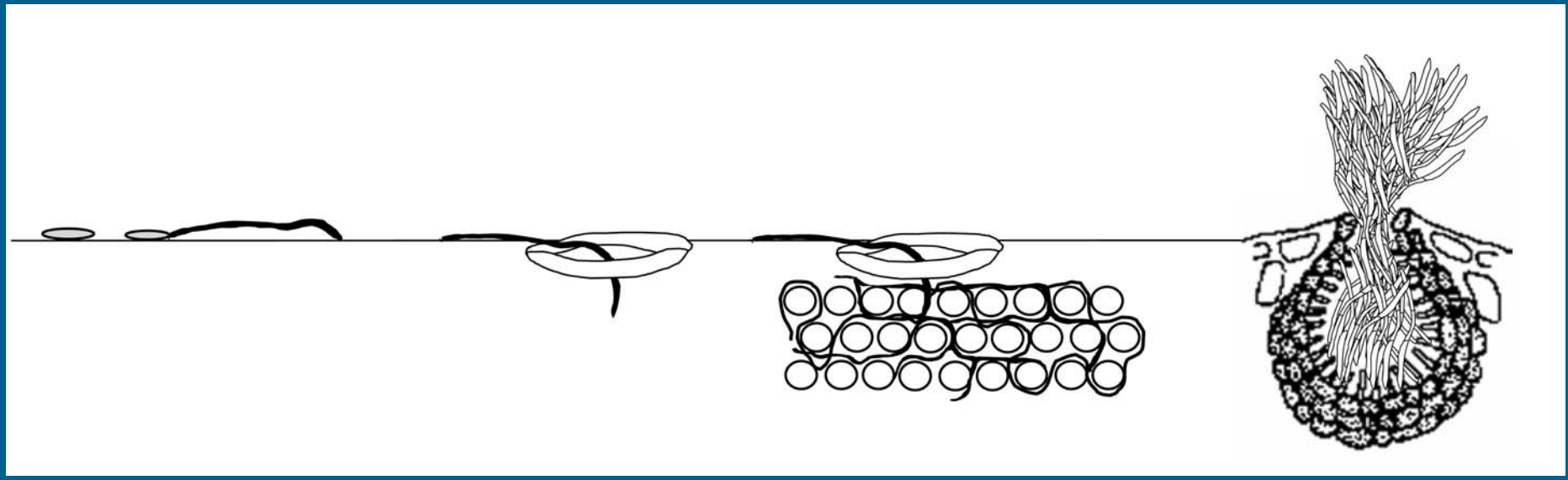
Conclusion (biological function of *MgFus3*)

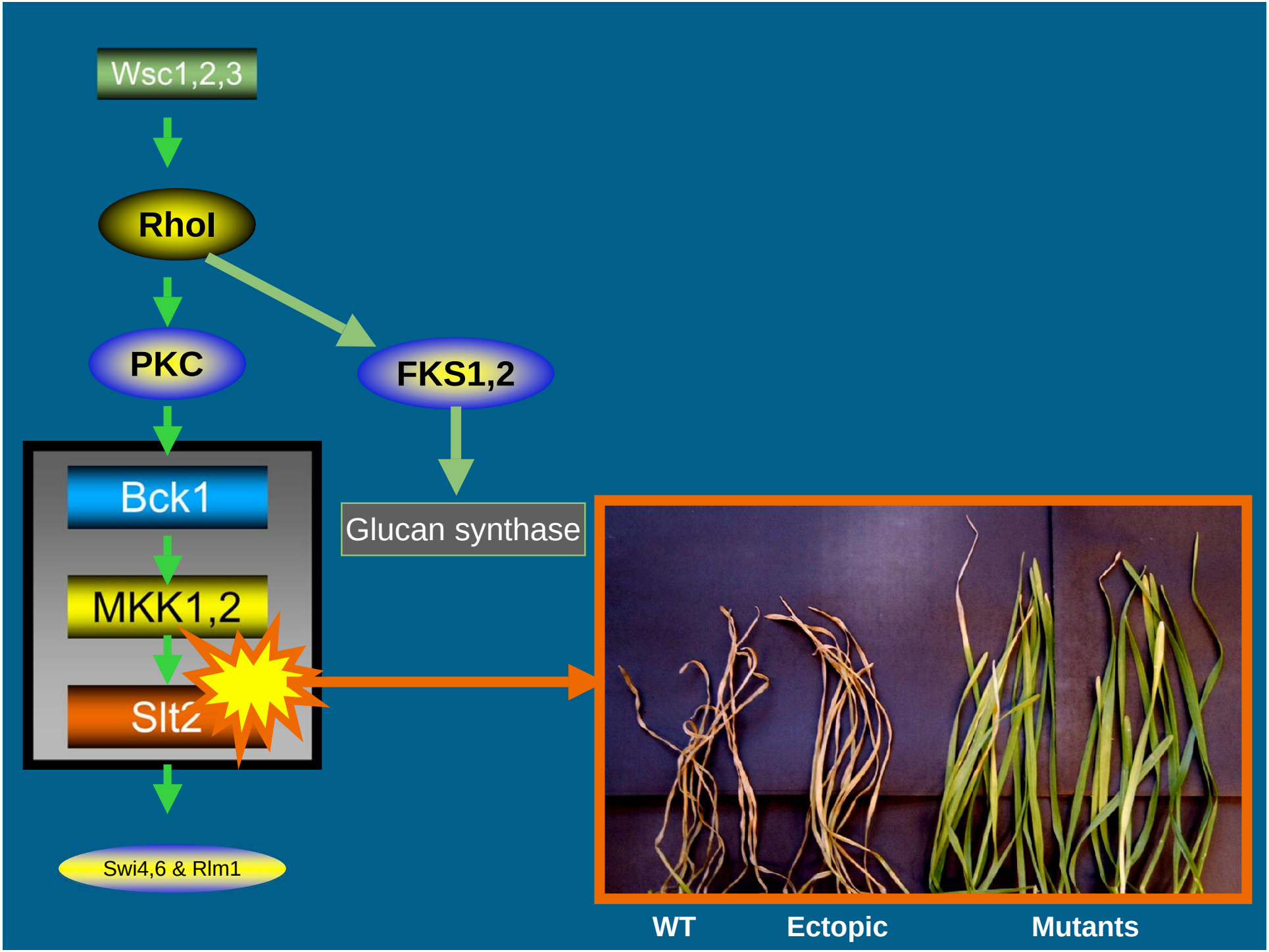
- No role in dimorphic switch
- Regulation of early stage of infection, perception of the host tissue, stomatal penetration
- Probably MgFUS3 have other downstream effector(s) rather than MgSTE12

Dimorphic switch ➔ Penetration ➔ Colonization ➔ Fructification

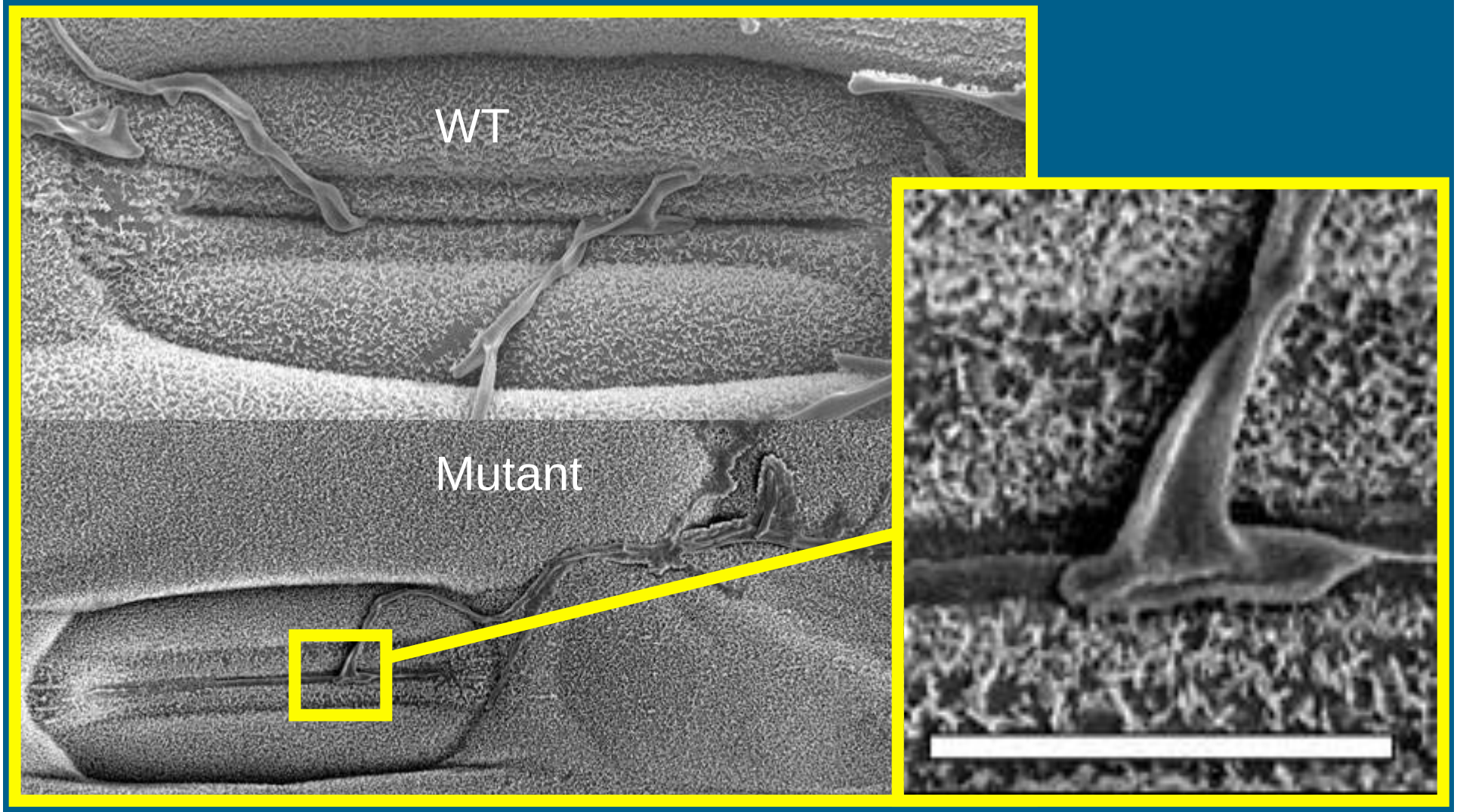
Infection Process

1- Transition **2- Penetration** **3- Colonization** **4- Fructification**

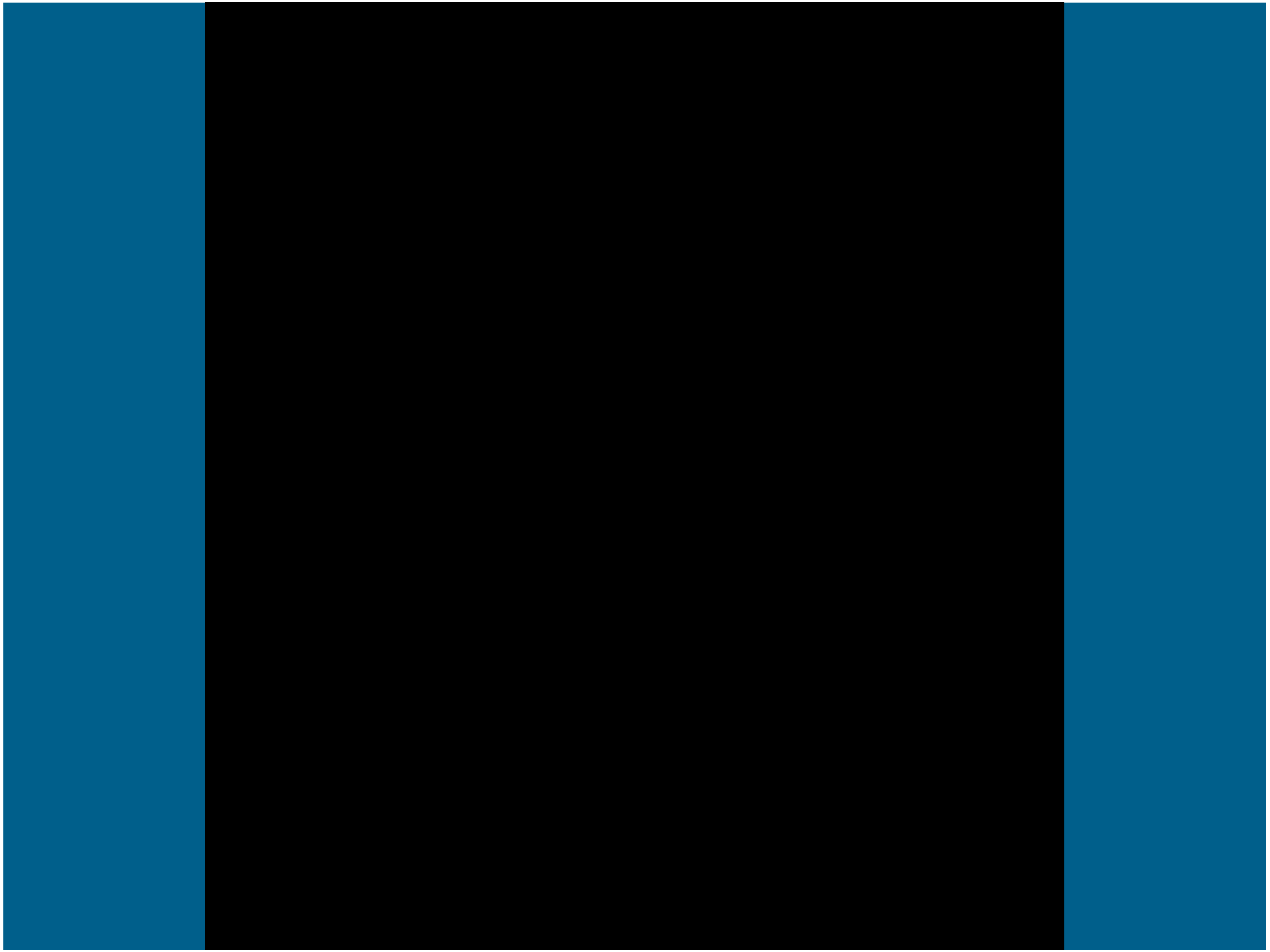



Wsc1,2,3
Rhol
PKC
FKS1,2
Bck1
MKK1,2
Slt2
Glucan synthase
Swi4,6 & Rlm1
WT
Ectopic
Mutants

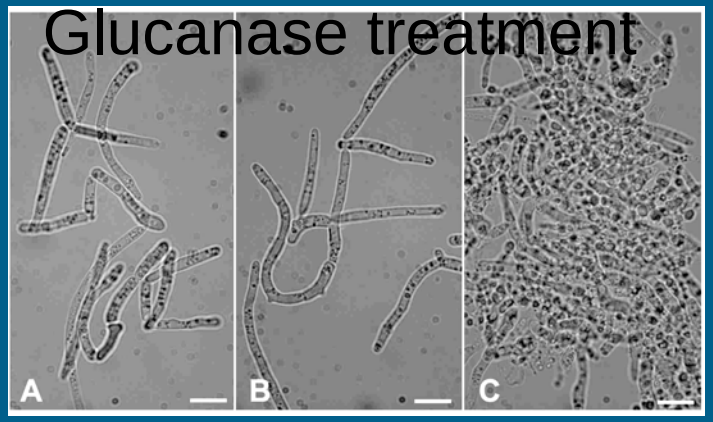
MgSlt2 mutants show attempts of penetration similar to the WT



Scanning electronic microscopy images of infected wheat leaves 72 h postinoculation



MgSlt2 is involved in cell wall strengthening and protects the cells from toxic compounds



Compounds	MIC (µg.ml^{-1})		Q value
	IPO323[a]	IPO323Δ*MgSlt2*[b]	
Antibiotics			
Cycloheximide	500	500	1
Fungicides			
Kresoxim-methyl	>0.25	>0.25	1
Fenpiclonil	2.5	2.5	1
Trifloxim	> 0.05	> 0.05	1
Miconazole	0.025	0.005	2
Bifonazole	0.1	< 0.025	>4
Imazalil	0.5	0.1	5
Cyproconazole	0.5	0.05	10
Plant metabolites			
Berberine	>500	>500	1
Camptothecin	>500	>500	1
Other			
Rhodamine 6G	25	25	1

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Conclusion (biological function of *MgSlt2*)

- Cell wall strengthening
- Dispensable for germination and penetration
- Required for colonization of mesophyll tissue
- The attenuated pathogenicity of *MgSlt2* mutants is probably due to an increased sensitivity to hitherto unknown plant defense compounds

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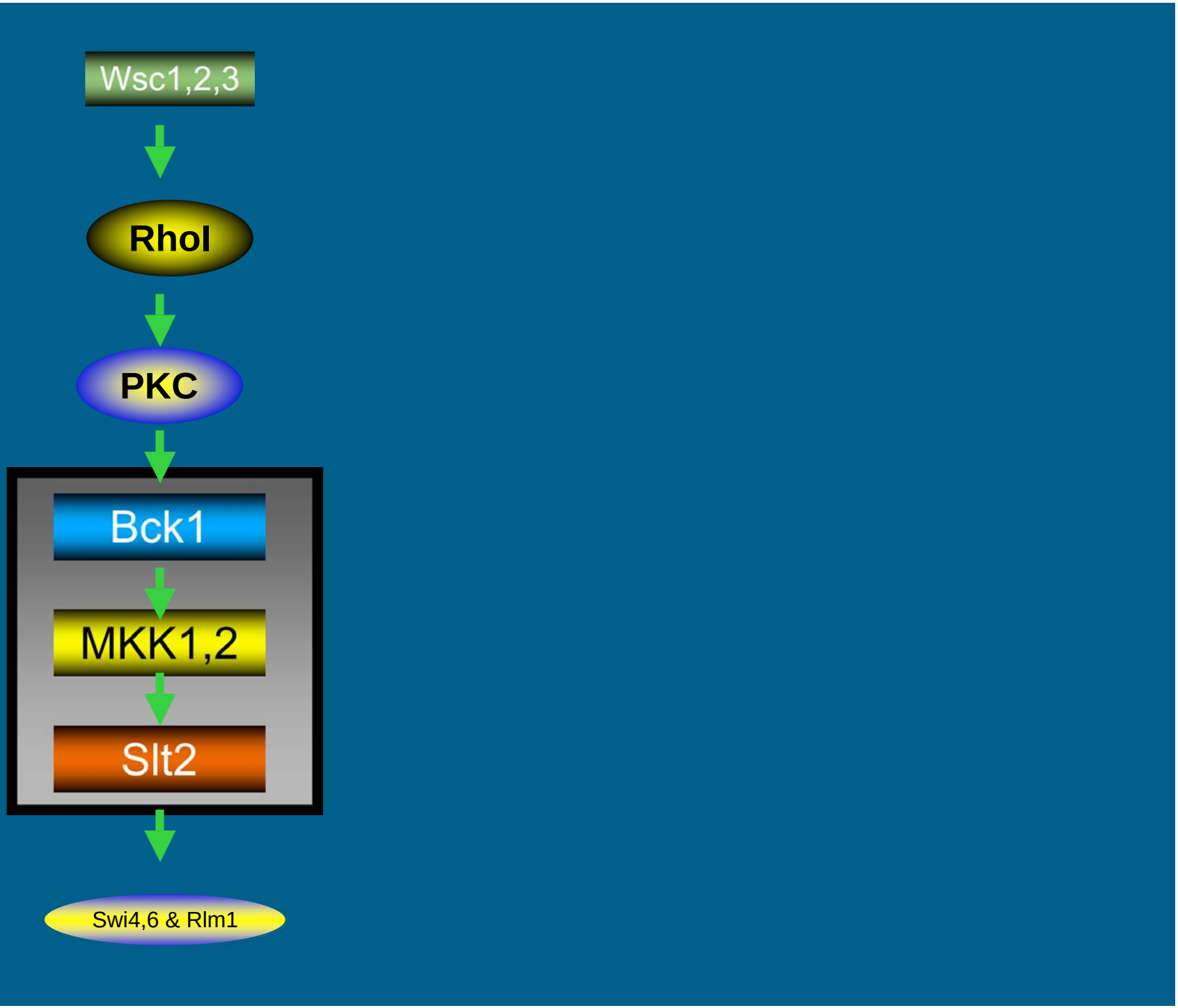
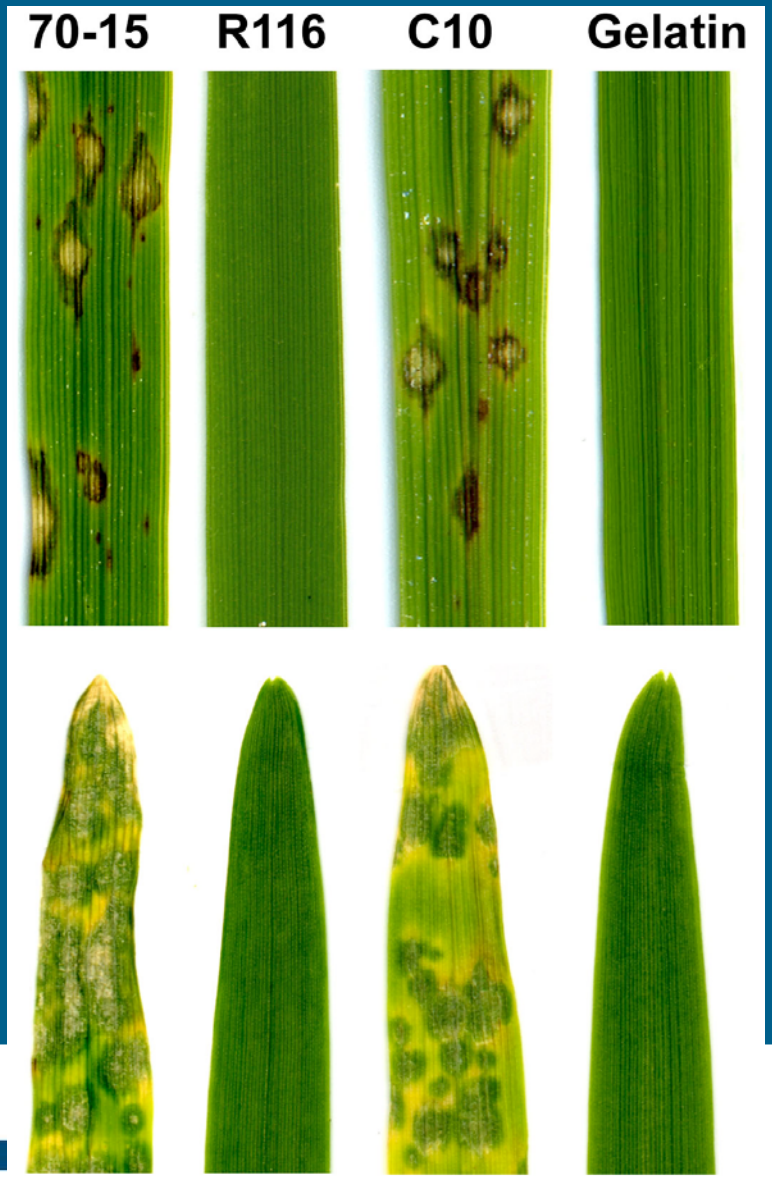
Mehrabi et al., 2006. MPMI (19)389-398


Wsc1,2,3
Rhol
PKC
Bck1
MKK1,2
Slt2
Swi4,6 & Rlm1

The *mig1* mutants of *Magnaporthe grisea* are non-pathogenic



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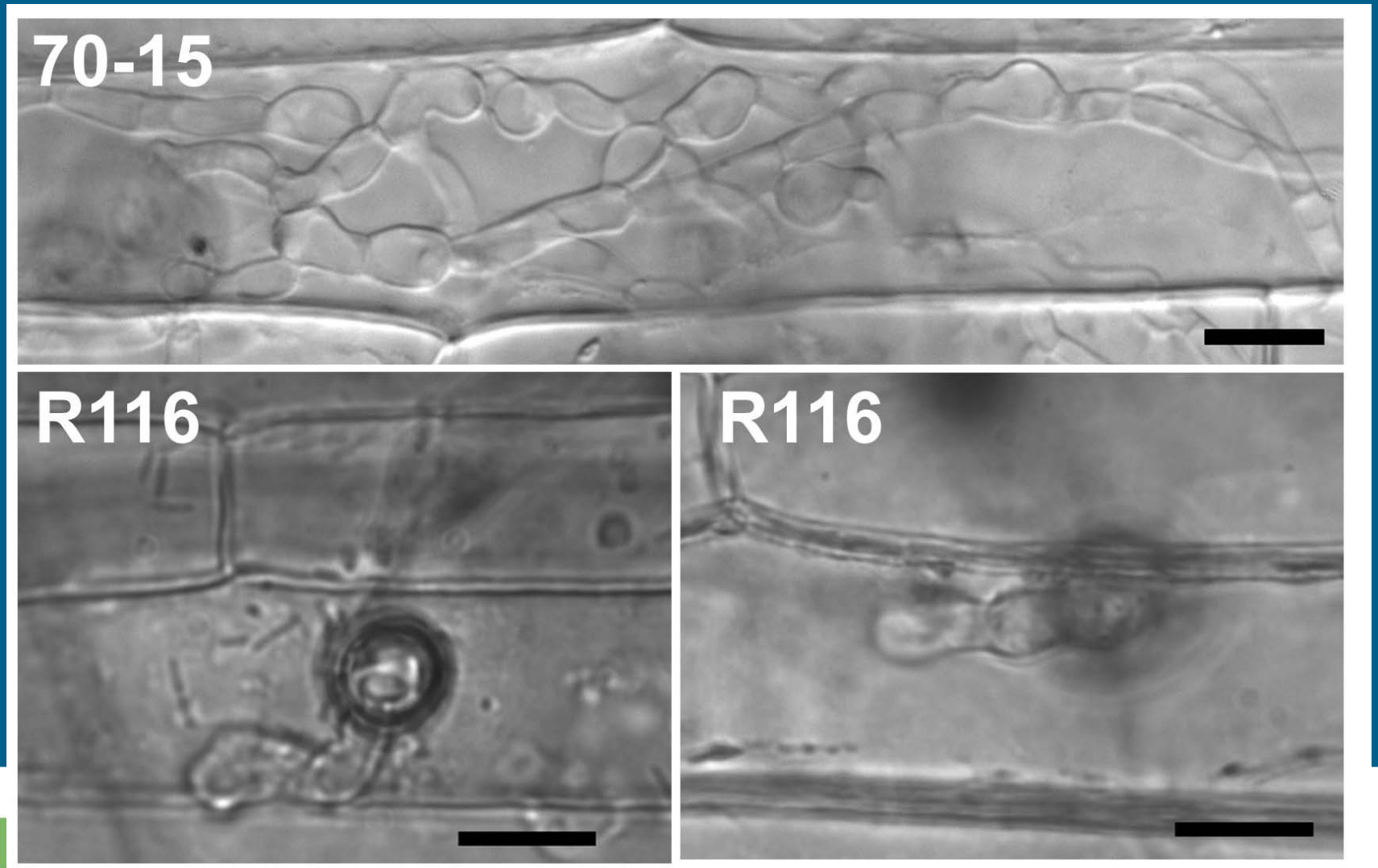
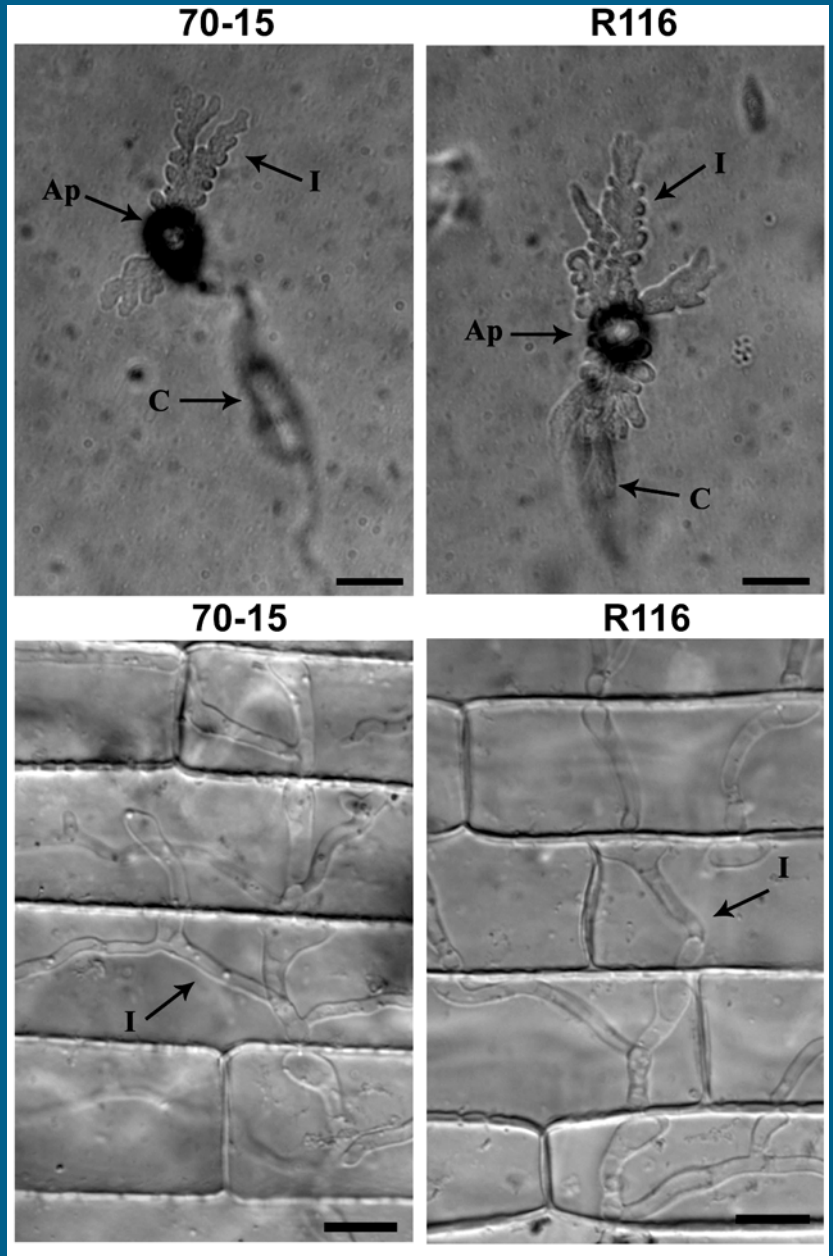
MIG1 is dispensable for appressorium formation but required for infectious growth



The *mig1* mutant might be defective in overcoming plant defense responses

Cellophane

Heat-killed rice epidermal cells

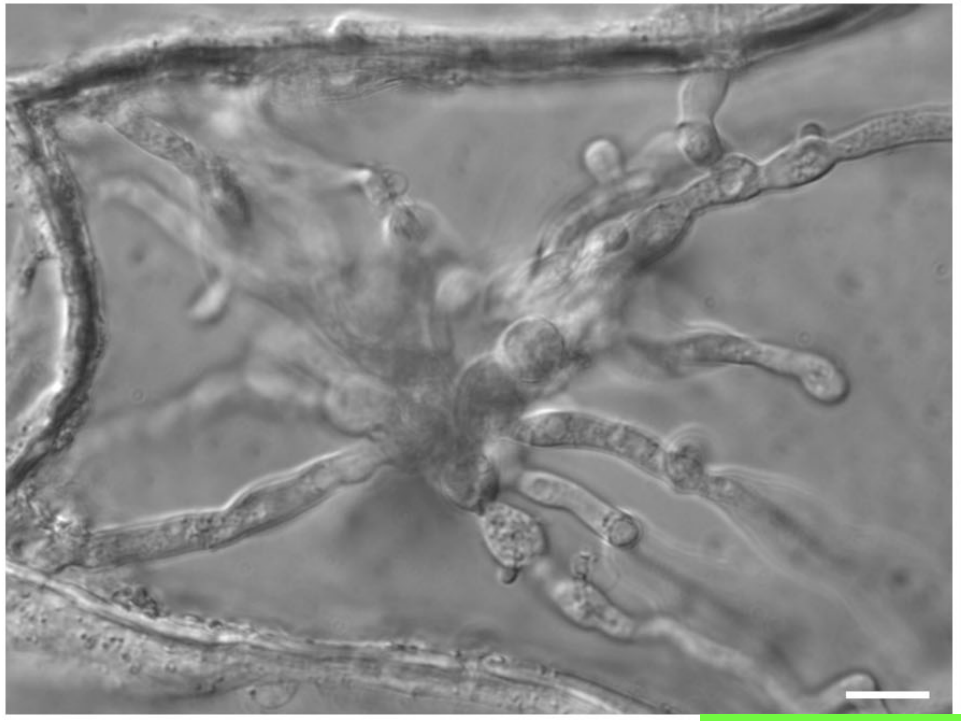


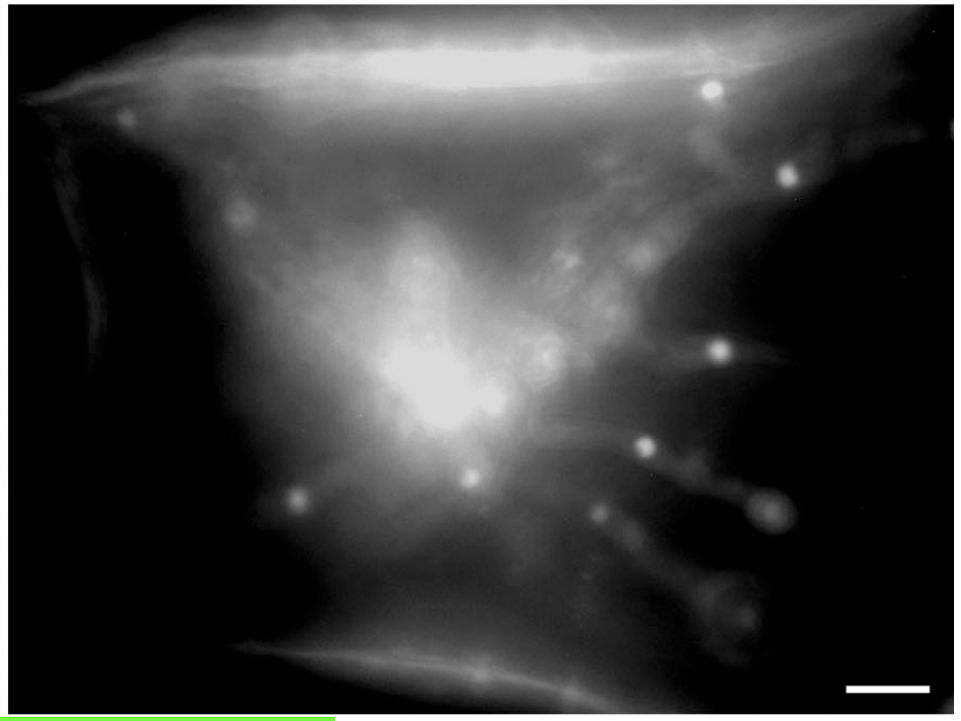
The Mig1-GFP fusion protein localizes to nucleus in infectious hyphae

DIC

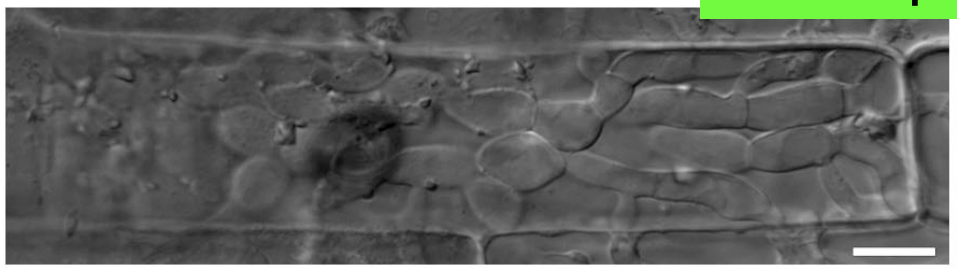
Onion epidermal cell

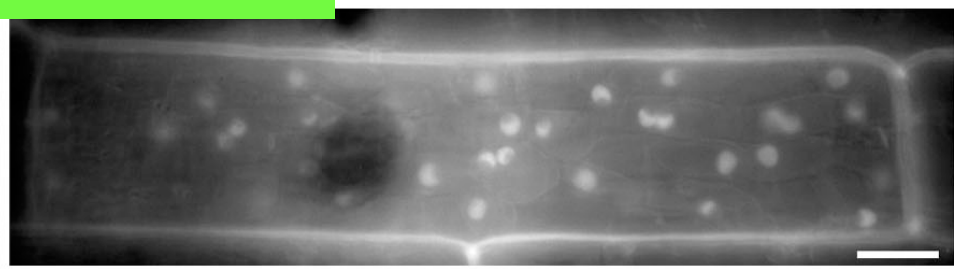
GFP



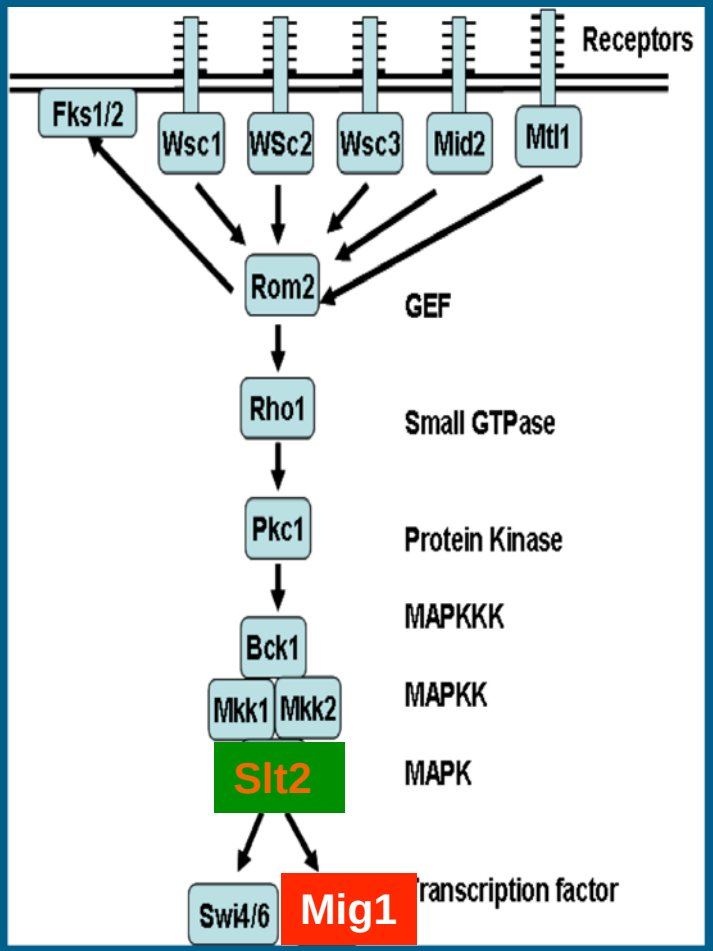


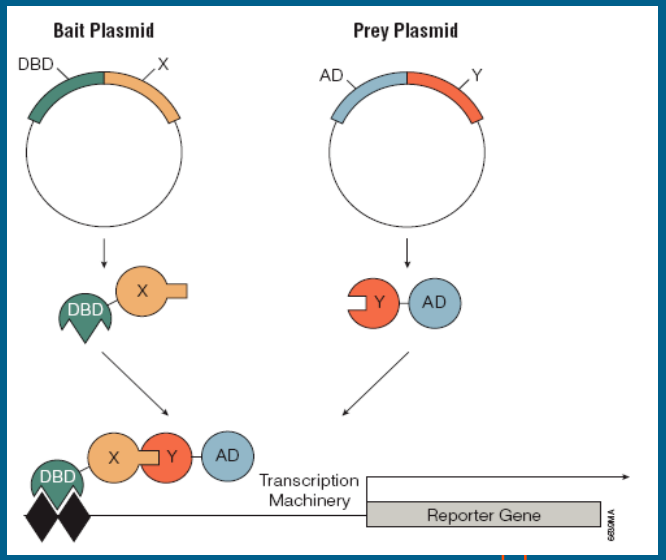
Rice Epidermal cell

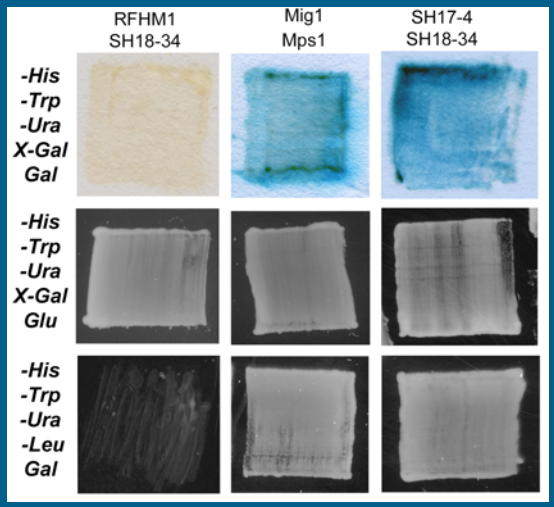




Mig1 is downstream of the Slt2 MAPK







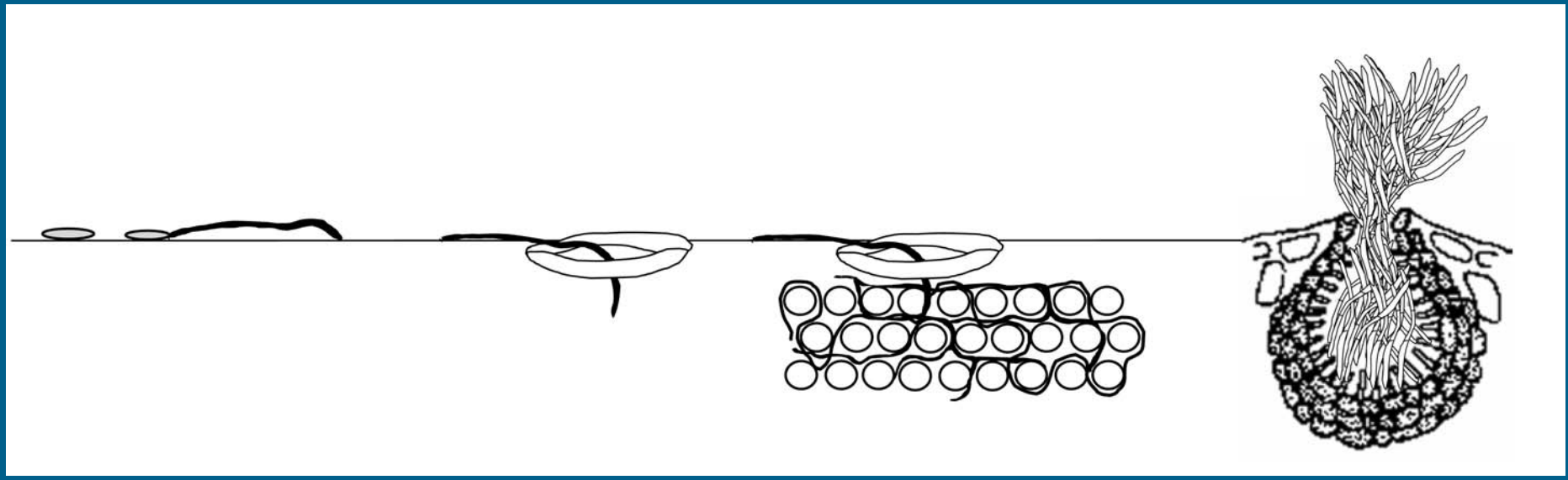
B-galactosidase

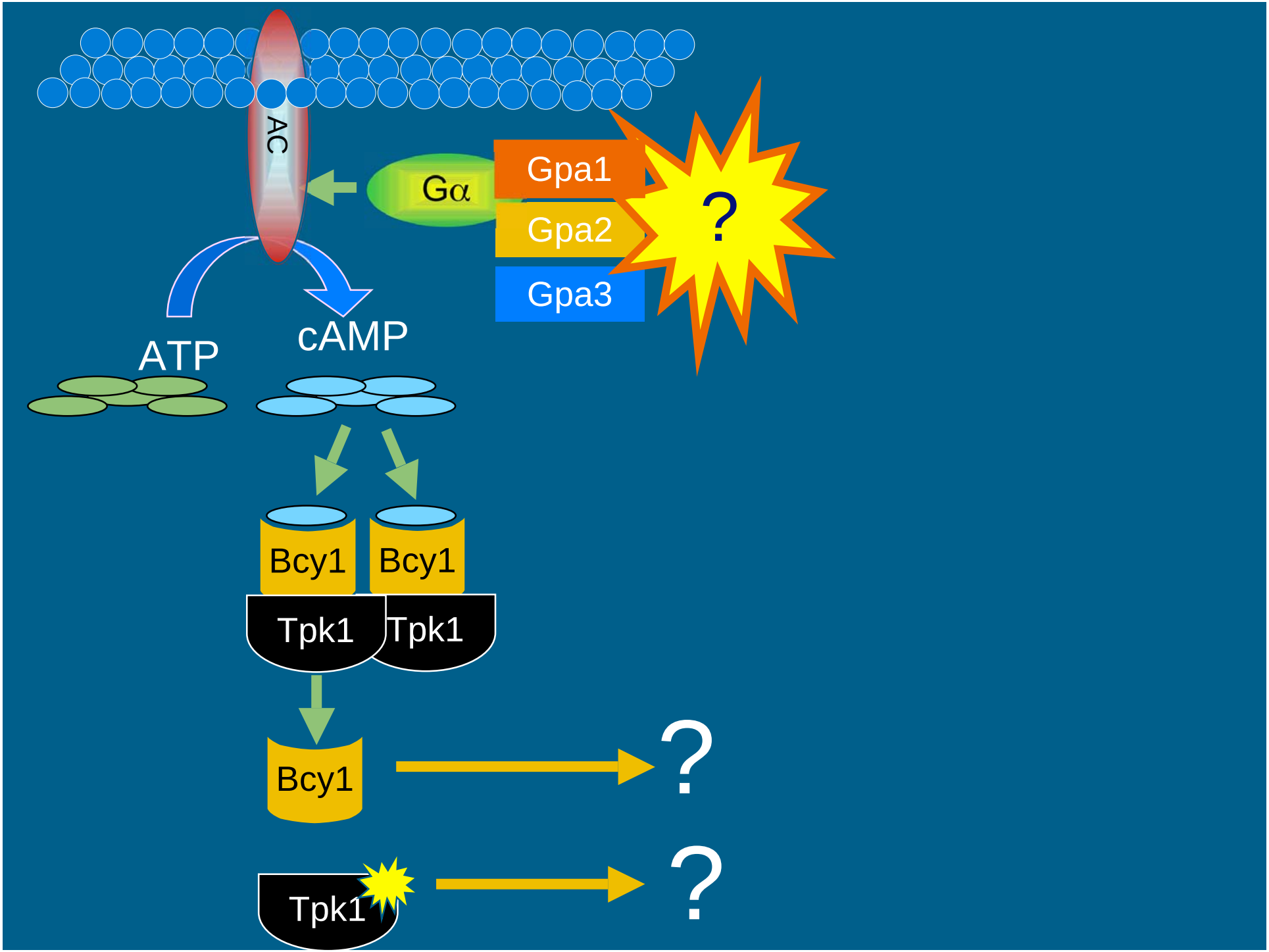
X-gal

Mehrabi et al., 2007. Eukaryotic cell. 7(5):791-799

Infection Process

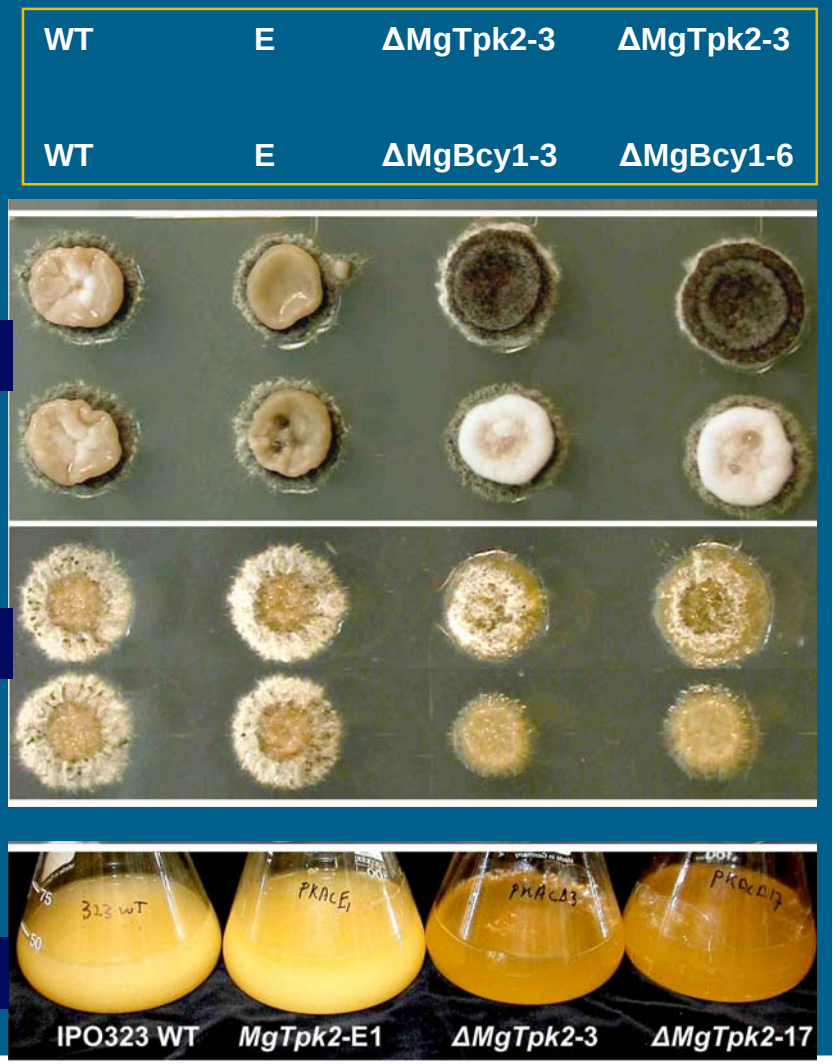
1- Transition **2- Penetration** **3- Colonization** **4- Fructification**




AC
Gα
Gpa1
Gpa2
Gpa3
?
ATP
cAMP
Bcy1
Bcy1
Tpk1
Tpk1
Bcy1
?
Tpk1
?

Morphological phenotype

- Mutation of *MgTpk2* facilitates melanization
- Mutation of *MgBcy1* hampers melanization
- Mutants of *MgBcy1* are osmosensitive
- *MgTpk2* mutants secrete dark pigment into YGB



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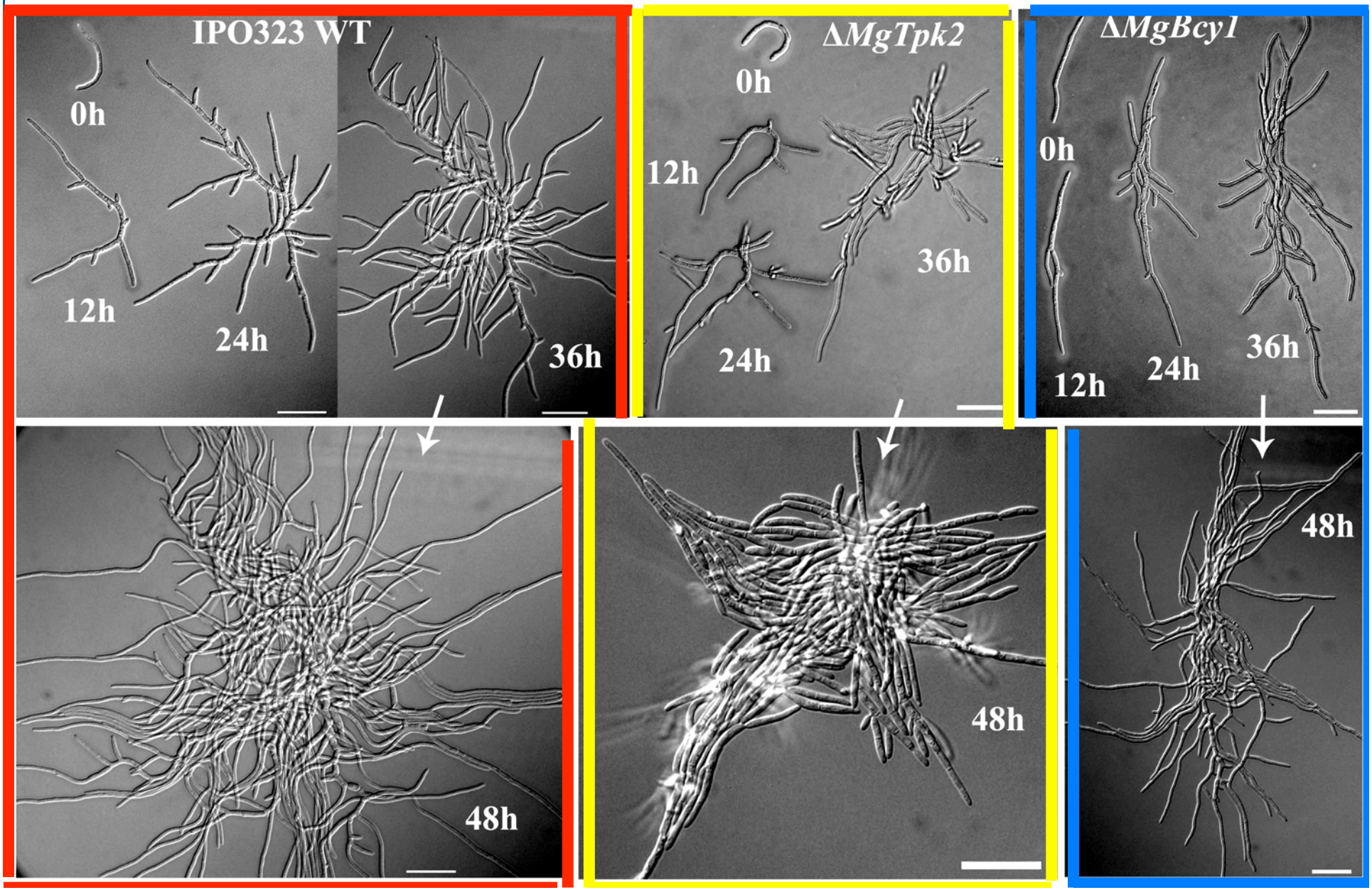
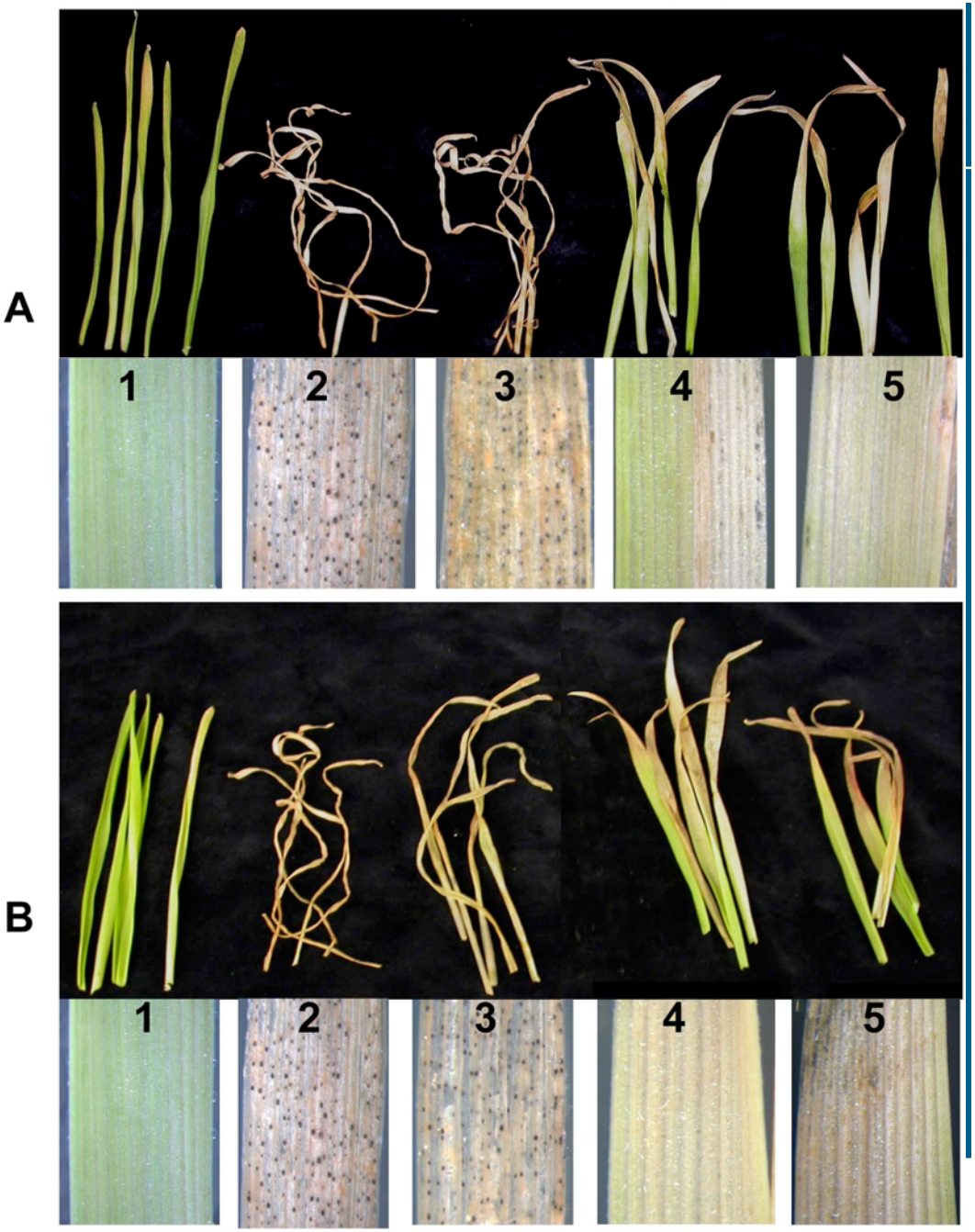
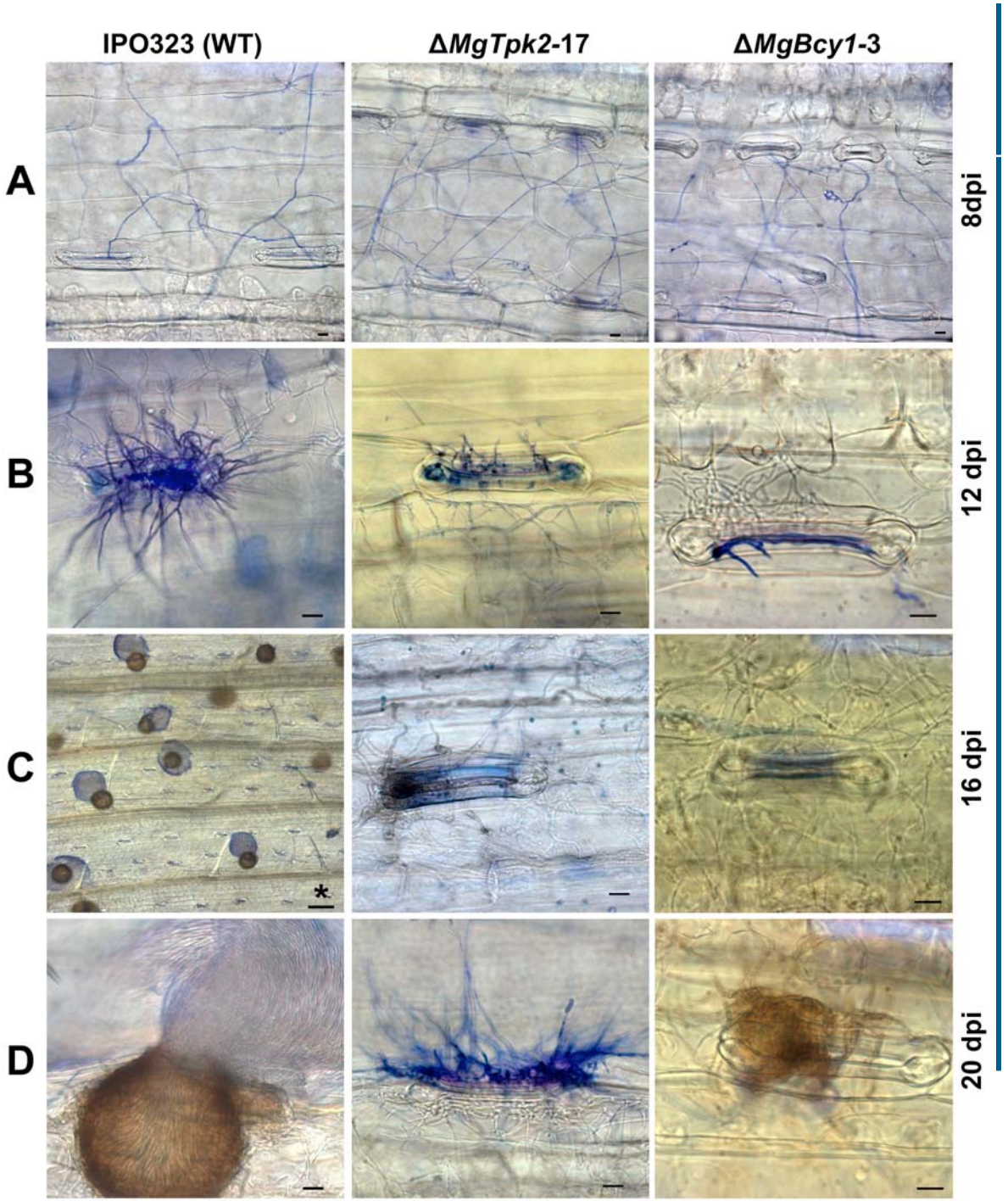
cAMP pathway regulates filamentation



- Proper regulation of cAMP pathway is required for virulence



- PKA subunits are involved in fructification



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Conclusion (Biological function of PKA subunits)

- No role in dimorphic switch (*in planta*)
- Dispensable for stomatal penetration and colonization
- Involvement in later stages of infection (pycnidia differentiation)
- High PKA activity positively regulates filamentation but negatively regulates melanization and osmo-regulation

X X X ✓

Dimorphic switch ➔ Penetration ➔ Colonization ➔ Fructification

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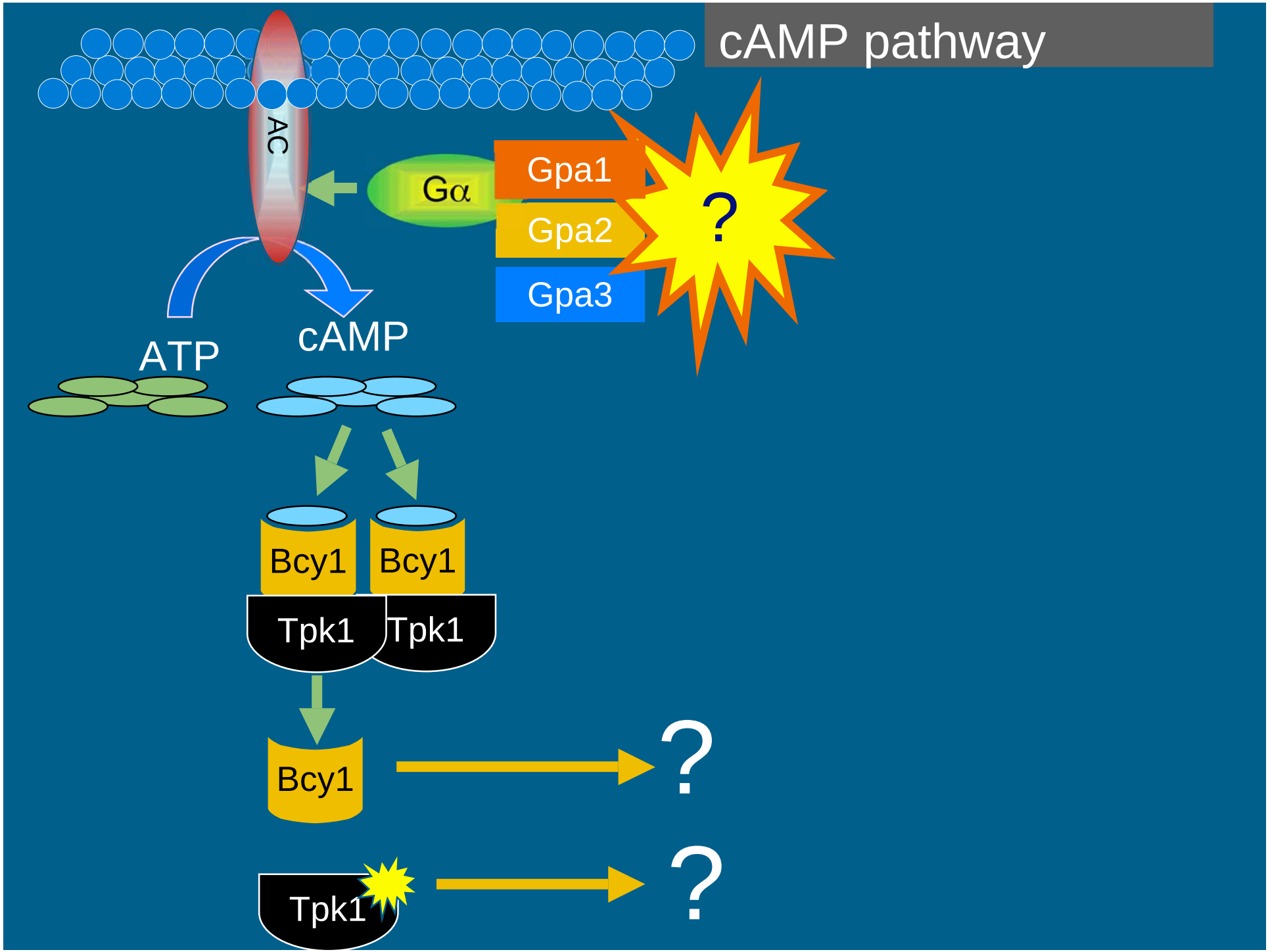
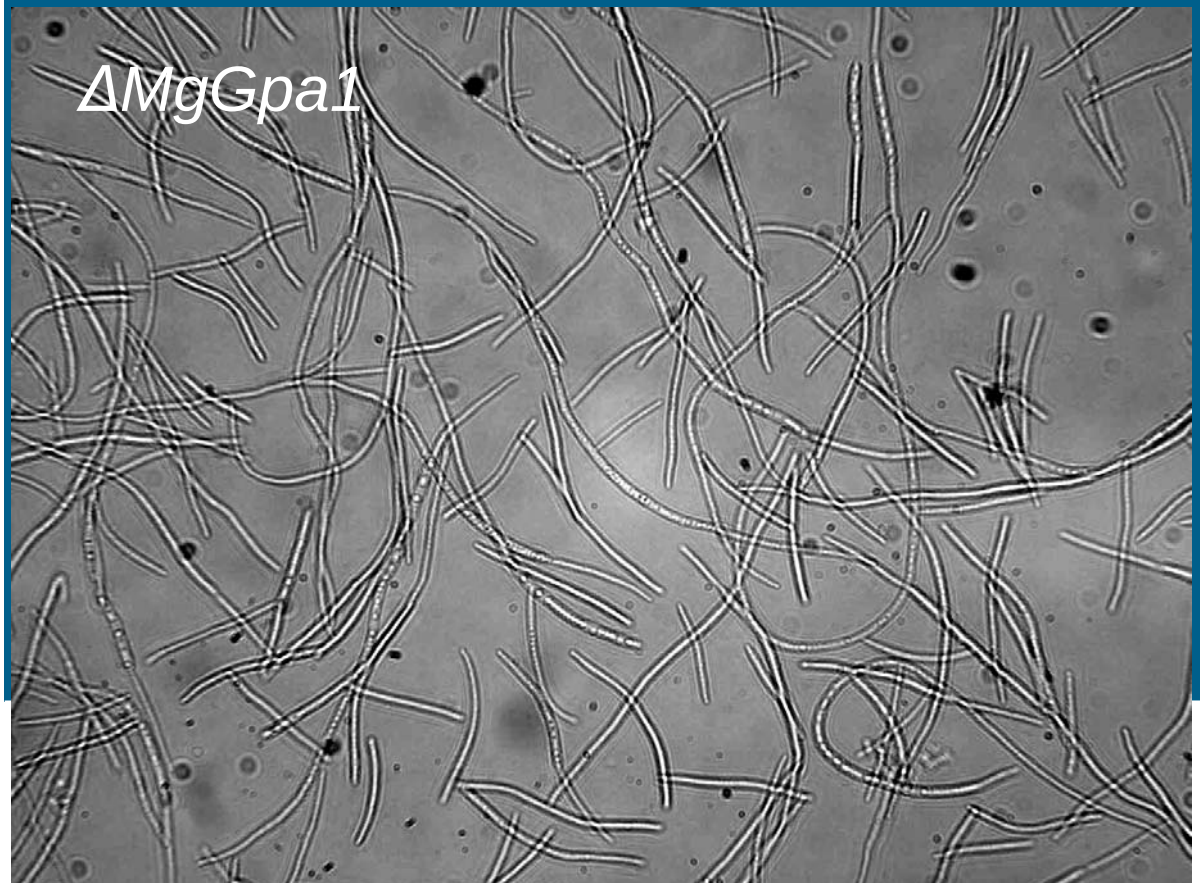
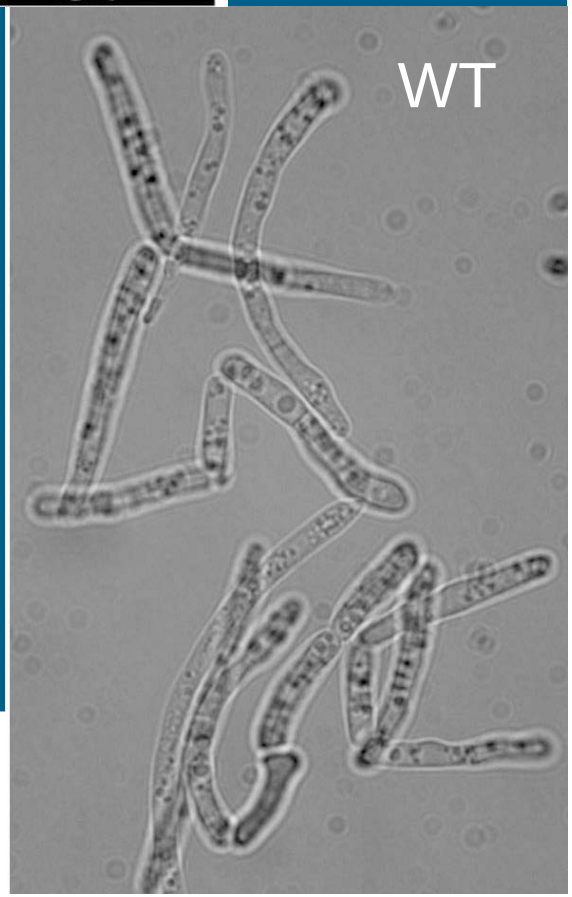
Mehrabi and Kema. 2006. MPP (7) 565-577

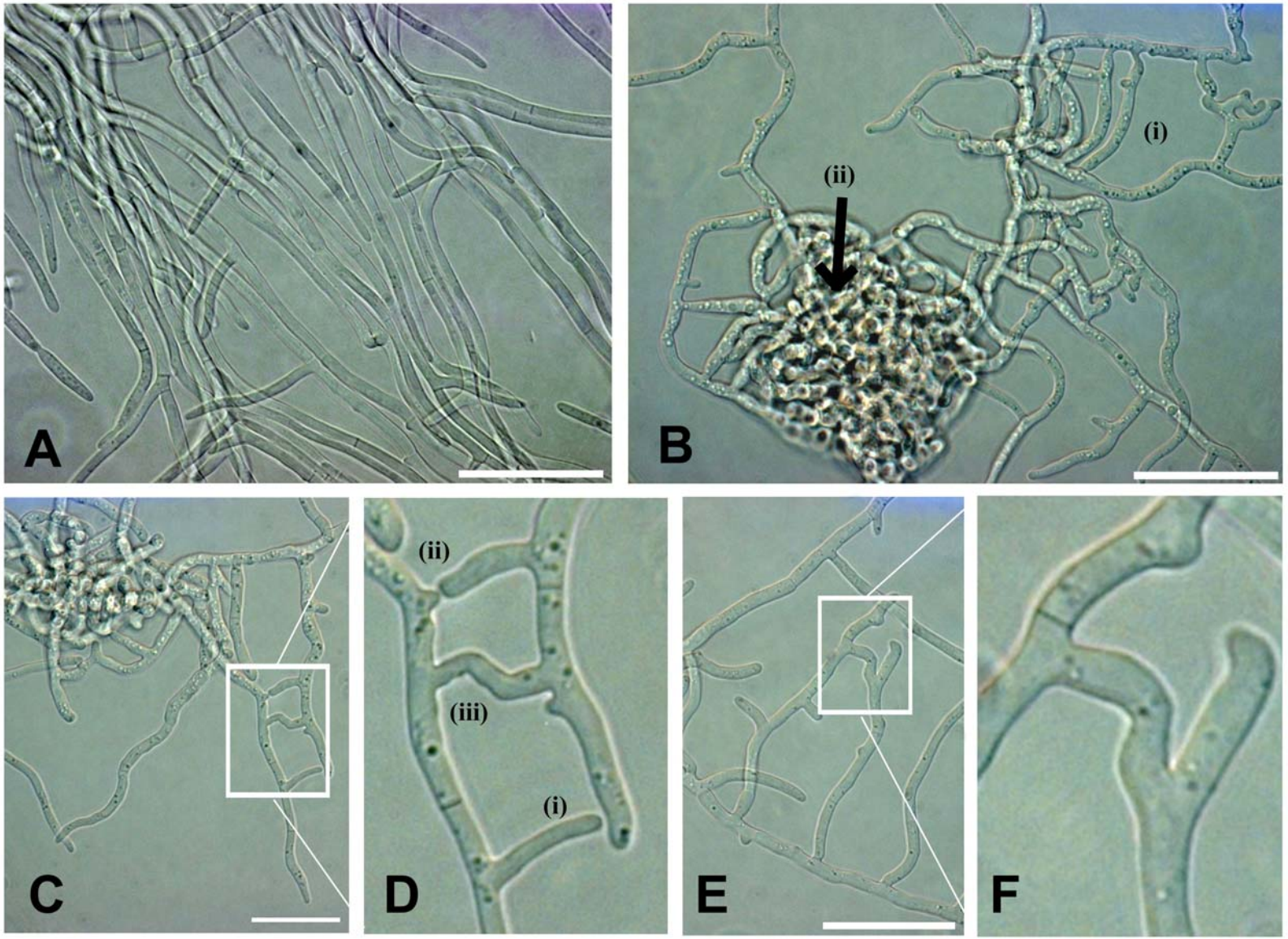

cAMP pathway
AC
Gα
Gpa1
Gpa2
Gpa3
?
ATP
cAMP
Bcy1
Bcy1
Tpk1
Tpk1
Bcy1
?
Tpk1
?

MgGpa1 negatively regulates filamentation





MgGpb1 negatively regulates cell fusion



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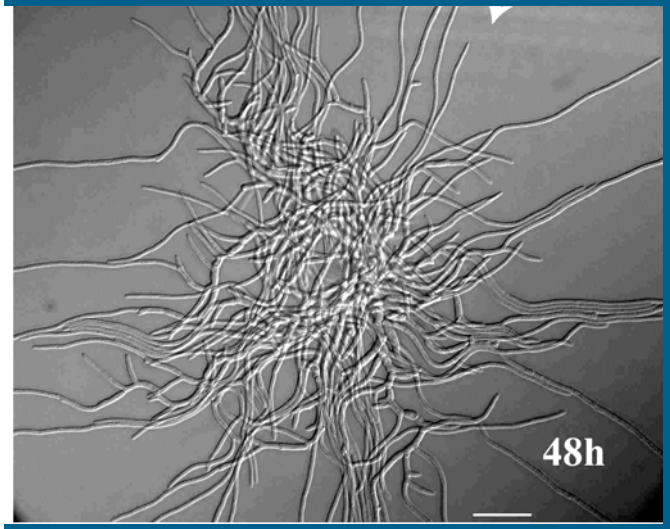
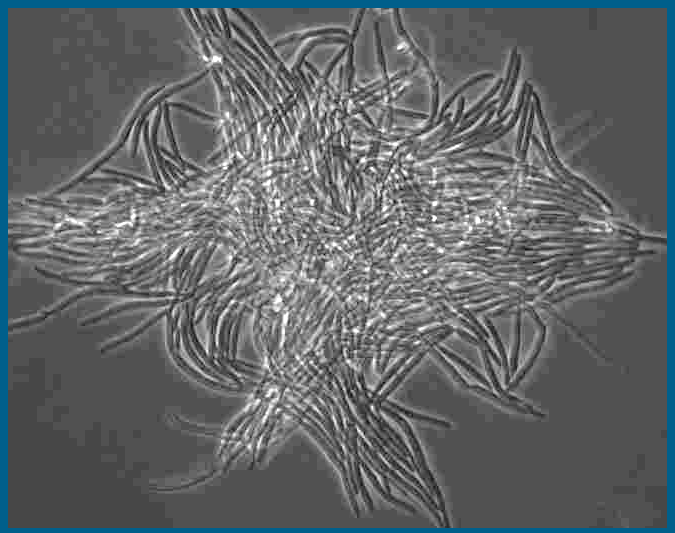
High phenotypic similarity of *MgTpk2* and *MgGpa3* mutants

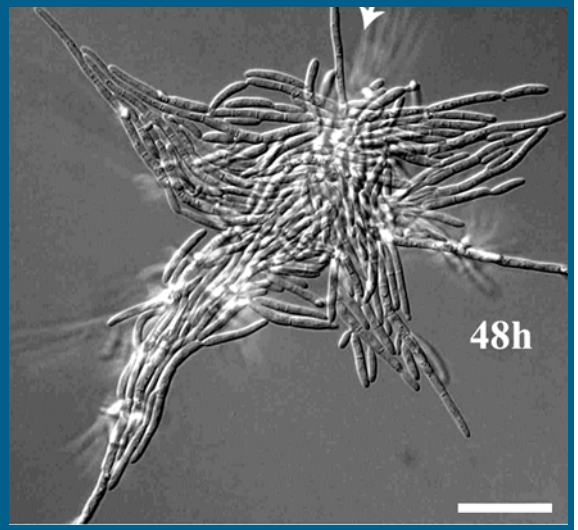
WT

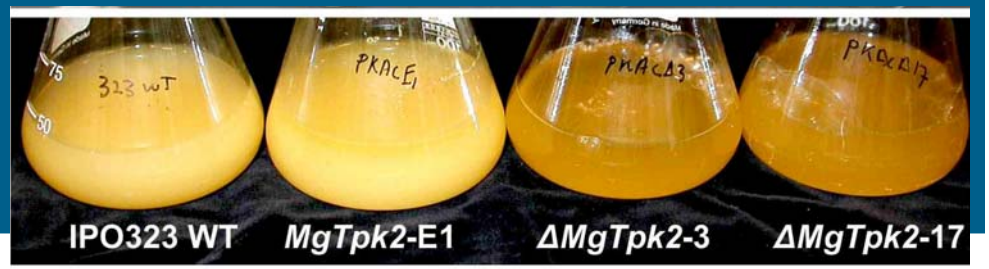


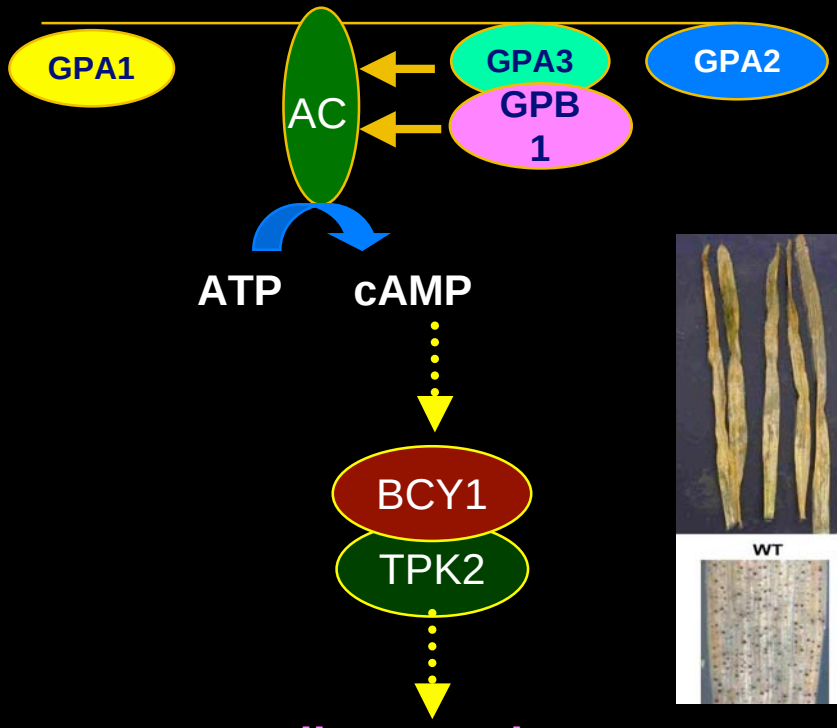
ΔMgGpa3

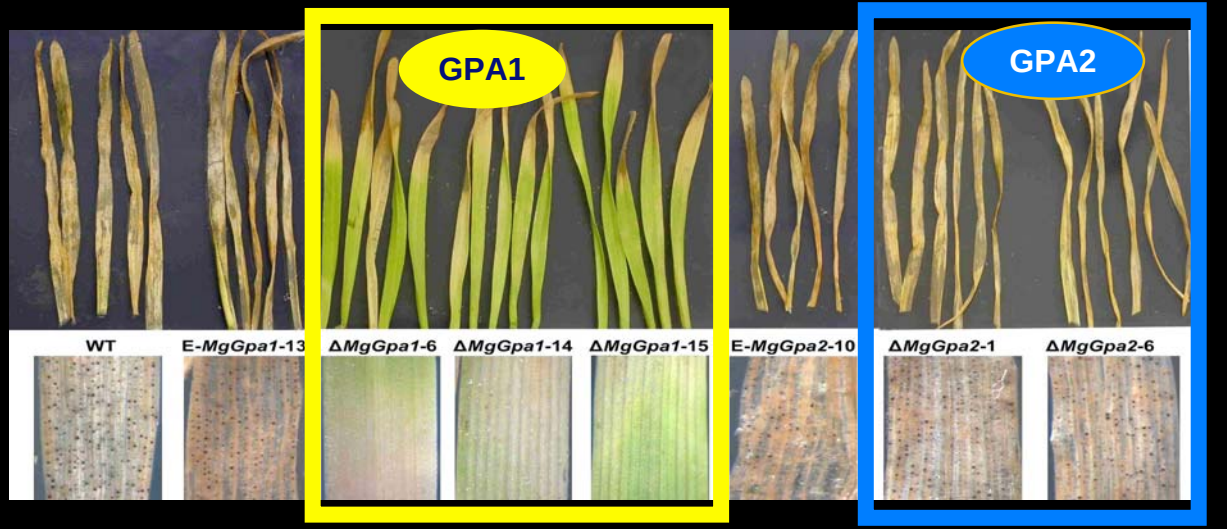


ΔMgTpk2



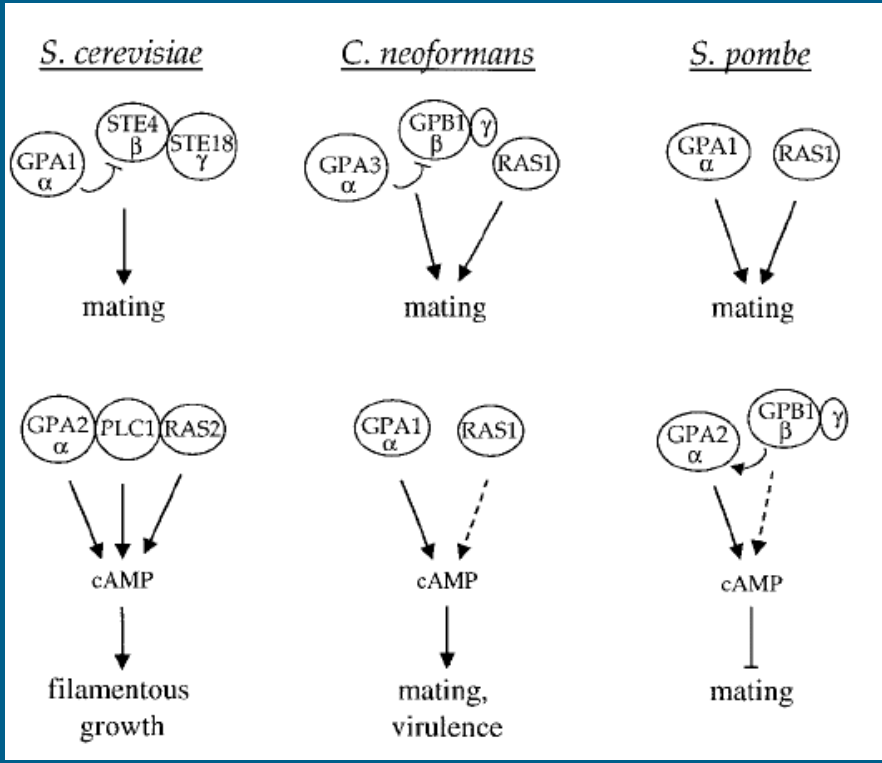


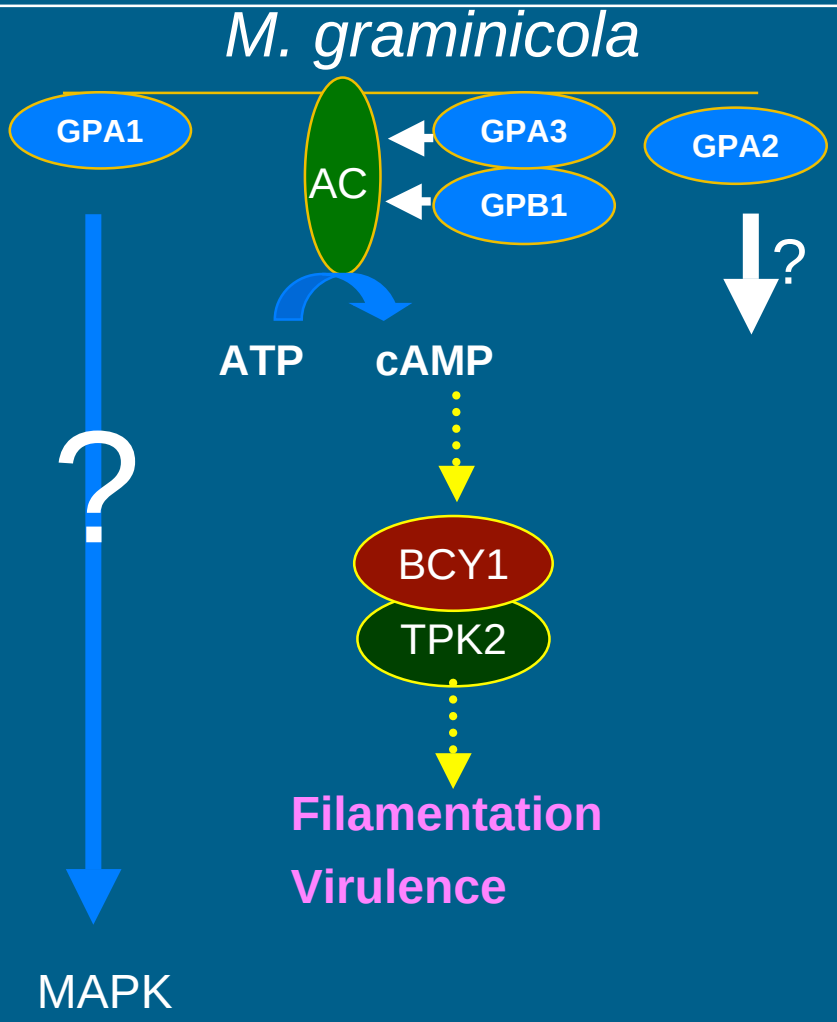

GPA1
AC
GPA3
GPB 1
GPA2
ATP
cAMP
BCY1
TPK2
Filamentation
Virulence

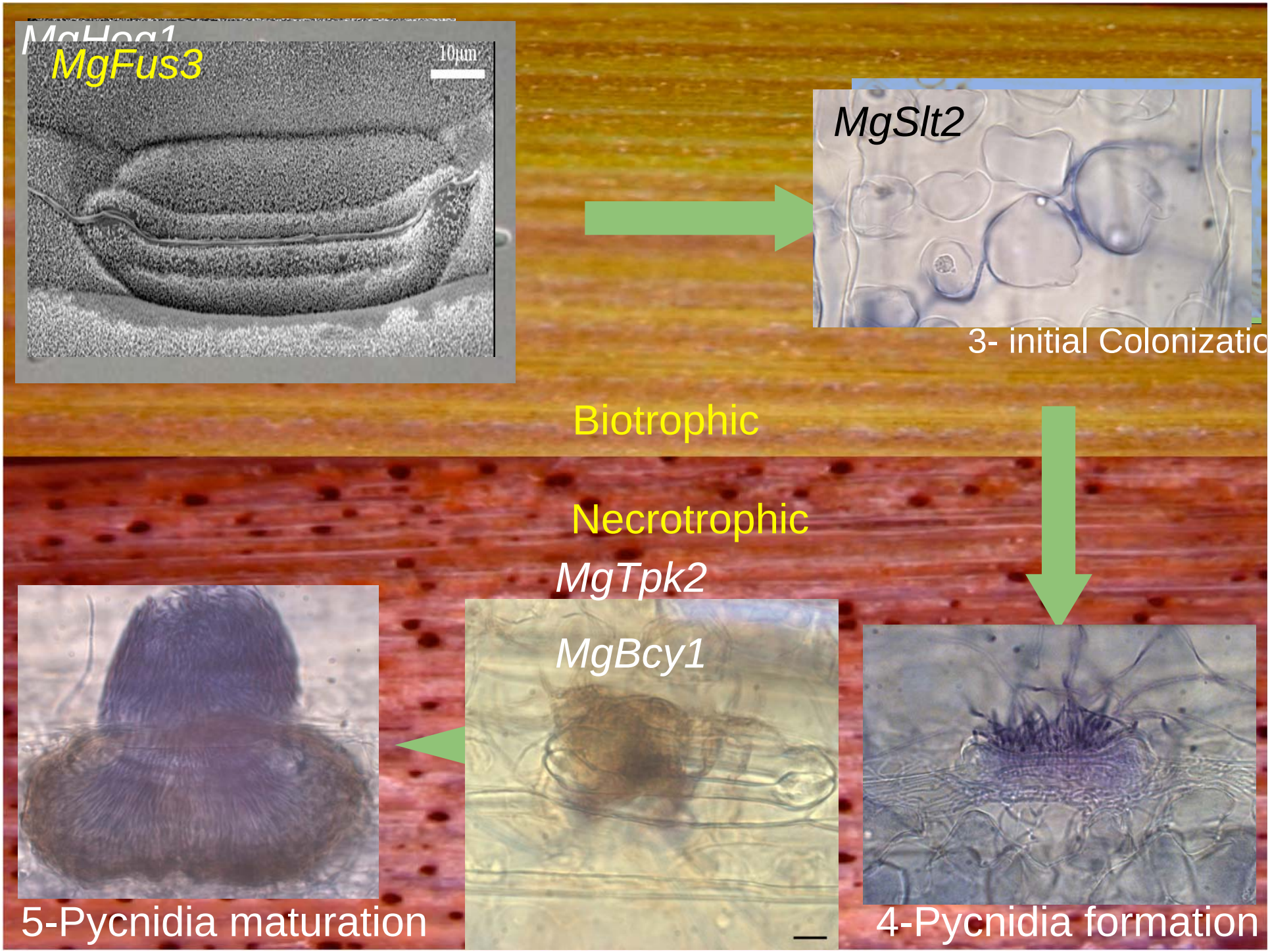

GPA1
GPA2
WT
E-MgGpa1-13
ΔMgGpa1-6
ΔMgGpa1-14
ΔMgGpa1-15
E-MgGpa2-10
ΔMgGpa2-1
ΔMgGpa2-6


GPA3
GPB 1
WT
E-MgGpa3-2
ΔMgGpa3-9
ΔMgGpa3-21
ΔMgGpa3-33
E-MgGpb1-1
ΔMgGpb1-3
ΔMgGpb1-22

Regulation of the cAMP pathway by G proteins






MgHog1
MgFus3
10µm
MgSlt2
3- initial Colonizatic
Biotrophic
Necrotrophic
MgTpk2
MgBcy1
5-Pycnidia maturation
4-Pycnidia formation

Thanks

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For quality of life

The reaction of Iranian tetraploid wheat accessions for resistance to septoria tritici blotch pathogen

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Septoria tritici blotch (STB), caused by the fungus *Mycosphaerella graminicola* (Fuckel) Schroeter, is a major disease of wheat worldwide. Identification of new sources of resistance to this disease is important for sustainable management of the disease. The diploid and tetraploid wheats are the parents of commercial hexaploid wheats and they have provided valuable sources of many new resistance genes. Since Iran is one of the wheat origins, it could be useful to study on Iranian wheat landraces in order to identify new sources of resistance to STB.

Materials and Methods

Leaf samples showing STB symptoms were collected from different wheat growing areas of Iran and 11 strains were isolated. The fully unfolded primary leaves of wheat accessions were inoculated with a mixture of spores adjusted to $2x10^6$/ml. Disease severity was evaluated at 21 days after inoculation (dai) using percentage of lesions covered by pycnidia.

Fig1. The wheat seedlings were inoculated in the greenhouse and kept under humid conditions (left). The symptoms developed by STB after 21 dai (right).

In this study we screened different tetraploid wheat accessions to STB. Some 95 accessions of *Triticum turgidum* ssp. *turgidum*, 4 accessions of *T. turgidum* ssp. *polynicum*, 7 accessions of *T. turgidum* ssp. *dicoccum*, 21 accessions of *T. turgidum* ssp. *turanicum* were evaluated to a mixture of 11 *Septoria tritici* isolates, which were collected from different wheat-growing areas of Iran.

Results

The studied accessions showed different levels of pycnidia formation ranging from 0 to 90% of leaf areas. We found several resistant accessions including 28, 3, 1, and 10 accessions of *T. turgidum* ssp. *turgidum*, *T. turgidum* ssp. *polynicum*, *T. turgidum* ssp. *dicoccum* and *T. turgidum* ssp. *turanicum*, respectively (Table 1 and Fig 2).

Table 1. The reaction of tetraploid wheats to STB. Disease severity below 5% is considered as resistance reaction.

Reaction[a]	Disease severity[b]	*Triticum turgidum* ssp.			
		turgidum	*turanicum*	*dicoccum*	*polynicum*
R	IS<5	28	10	1	3
MR	10>IS≥5	14	1	0	0
MS	30>IS≥10	22	3	4	0
S	IS≥30	31	7	2	1
total		95	21	7	4

a: R=Resistant, MR= Moderately Resistant, MS=Moderately Susceptible, S=Susceptible
b: Percentage of lesions covered by pycnidia.

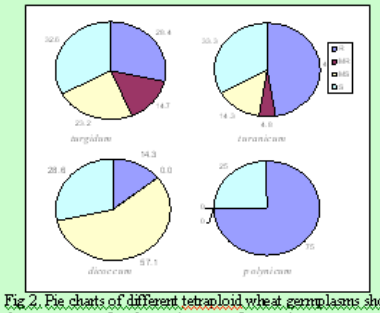

Fig 2. Pie charts of different tetraploid wheat germplasms showing the percentage of each reaction types from resistant to susceptible.

Conclusion

Our results suggested that the local landraces, which possess resistance are valuable sources and may contain novel resistance genes and therefore are useful in breeding efforts to improve STB resistance in wheat. The specific reaction of the resistant accessions to different isolates is underway.