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The role of *Epichloë festucae boiA*
and *boiB* in symbiotic maintenance of
endophyte-grass associations

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Abstract

The filamentous fungal endophyte *Epichloë festucae* and its plant host, perennial ryegrass, form a highly regulated mutualistic symbiosis. A key component in the regulation and maintenance of this symbiosis is the production of reactive oxygen species (ROS) by the NADPH oxidase (Nox) complex. Mutations in fungal Nox complex components lead to a disruption in the mutualistic association. In these mutants' hyphae are often defective in cell-cell fusion and undergo proliferative growth, while the infected plant host is severely stunted. The fungal NoxA complex consists of membrane bound NoxA and NoxD components and cytosolic subunits NoxR, RacA, BemA and Cdc24, which are recruited to the membrane in response to some as yet unidentified signal. This study investigates the hypothesis that BemA is recruited to the membrane via interaction with a BOI1-like protein, known to interact with Bem1 in yeast. *E. festucae* has two BOI1-like proteins, designated BoiA and BoiB. Phylogenetic analysis suggested the ancestral Boi gene was duplicated in Letiomyceta after the whole genome duplication (WGD) in yeast which resulted in the presence of BOI1 and BOI2. Previous transcriptomic studies have shown *boiA* is highly expressed *in planta* compared to in culture and is differentially expressed in mutants that disrupt the mutualistic interaction, suggesting an important role for *boiA* in symbiotic maintenance. Microscopy analysis showed that in comparison to wild-type, $\Delta boiA$ deletion mutants displayed loss of hyphal cell-cell fusion in culture, while *in planta* $\Delta boiA$ mutants exhibited multiple hyphae per intercellular space, intrahyphal hyphae, and hyphal breakage. The resulting $\Delta boiA$ infected plants were stunted compared to wild-type infected plants. In contrast, no phenotype was observed for $\Delta boiB$ mutants in culture, though *in planta* intrahyphal hyphae and hyphal breakage was observed. These results suggest that BoiA is required for hyphal cell fusion and regulation of the mutualistic association between *E. festucae* and perennial ryegrass. While hyphal breakage suggests a role in intercalary growth, the novel mechanism by which *E. festucae* keeps up with rapid host growth *in planta*. This study provides an in-depth analysis on the origin and function of *E. festucae* BoiA and BoiB in culture and *in planta*.

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Abbreviations

AD	Activation domain
AF	Alexafluor
Amp ^R	Ampicillin resistance
BD	Binding domain
bp	Base pair
BiFC	Bimolecular fluorescence complementation
CaCl ₂	Calcium chloride
CaCl ₂ .2H ₂ O	Calcium chloride dihydrate
CFW	Calcofluor white
cv	Cultivar
DIC	Differential interference contrast
DIG	Digoxigenin
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DO	Dropout
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
g	Gravity
GEF	Guanine nucleotide exchange factor
gDNA	Genomic DNA

GDP	Guanosine diphosphate
Gen ^R	Geneticin resistance
GTP	Guanosine triphosphate
Ham-9	Hyphal anastomosis mutant 9
HCl	Hydrochloric acid
Hepes	4-2-hydroxyethyl-1-piperazineethanesulfonic acid
<i>hph</i>	Hygromycin B phosphotransferase
H ₂ SO ₄	Sulfuric acid
Hyg ^R	Hygromycin resistance
JGI	Joint genome institute
kb	Kilobase
KCl	Potassium chloride
KO	Knock out
KOH	Potassium hydroxide
LiAc	Lithium acetate
LB	Luria bertani
LM	Light microscope
M	Molar
MAFFT	Multiple alignment using fast fourier transform
MgSO ₄	Magnesium sulfate
mL	Milliliter

Mm	Millimeter
mM	Millimolar
MMIC	Manawatu microscopy and imaging centre
MnCl ₂ .4H ₂ O	Manganese II chloride tetraydrate
mRFP	Monomeric red fluorescent protein
MSc	Master of Science
NCM	Nitrocellulose membrane
NaCl	Sodium chloride
NaHPO ₄	Sodium phosphate
NaH ₂ PO ₄	Sodium phosphate monohydrate
NaOH	Sodium hydroxide
NEB	New England Biolabs
ng	Nanogram
ng/μL	Nanogram per microliter
nm	Nanometer
Nox	NADPH oxidase complex
<i>nptII</i>	Neomycin phosphotransferase II
OD	Optical density
P	Proline rich domain
PB1	Phox and bem1
PBS	Phosphate buffered saline

PC	Plant cell
PCR	Polymerase chain reaction
PCW	Plant cell wall
PD	Potato dextrose
PEG	Polyethylene glycol
Pfam	Protein families database
PH	Pleckstrein homology
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
Pmol	Picomole
PX	Phox
qPCR	Quantitative PCR
RE	Restriction enzyme
RG	Regeneration
ROS	Reactive oxygen species
rpm	Revolutions per minute
SD	Synthetic dropout
SDS	Sodium dodecyl sulfate
SE	Standard error
SH3	Src homology domain 3
SOB	Super optimal broth
SOC	Super optimal broth with catabolite repression

Sp	Species
SSC	Saline sodium citrate
STRIPAK	Striatin interacting phosphatase and kinase complex
TB	Transformation buffer
TBE	Tris, Borate, EDTA buffer
TEM	Transmission electron microscopy
UV	Ultra-violet
V	Version
vol	Volume
v/v	Volume to volume
WGA	Wheat germ agglutinin
WGD	Whole genome duplication
WT	Wild-type
w/v	Weight to volume
YPD	Yeast extract peptone dextrose
YPDA	Yeast extract peptone dextrose adenine
μg	Micrograms
μg/mL	Micrograms per milliliter
μL	Microliter
μM	Micromolar
μm	Micrometer

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