

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**A conserved signalling network regulates *Epichloë festucae*
cell-cell fusion and the mutualistic symbiotic
interaction between *E. festucae* and *Lolium perenne*.**

**A thesis presented in partial fulfilment of the requirements
for the degree of**

**Doctor of Philosophy (PhD) in
Genetics**

**at Massey University, Manawatu,
New Zealand.**

Kimberly Anne Green

2016

Abstract

Epichloë festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with *Lolium perenne*. The NADPH oxidase complex components *noxA*, *noxR* and *racA*, the transcription factor *proA*, and the cell wall integrity (CWI) MAP kinases, *mkkA* and *mpkA*, are required for mutualistic *E. festucae*-*L. perenne* associations and cell-cell fusion. Homologues of these genes in *Neurospora crassa*, *Sordaria macrospora* and *Podospora anserina* are required for cell-cell fusion and sexual fruiting body maturation, thereby establishing a link between self signalling and hyphal network formation in the *E. festucae*-*L. perenne* symbiosis. In *Podospora anserina*, IDC2 and IDC3 are required for cell-cell fusion, crippled growth and fruiting body formation. In *S. macrospora* and *N. crassa*, components of the STRIPAK complex regulate cell-cell fusion and fruiting body formation. The aim of this project was to test if *E. festucae* homologues of IDC2 and IDC3, and the STRIPAK complex protein MOB3, named SymB, SymC and MobC, respectively, are also required for cell-cell fusion and plant symbiosis. Gel shift assays showed the promoters of *symB* and *symC* are targets for the transcription factor ProA. In culture, the frequency of cell-cell fusion of $\Delta mobC$ was reduced, but in $\Delta symB$ and $\Delta symC$ mutants, totally abolished. All three mutants hyperconidiated and formed intra-hyphal hyphae. Plants infected with these mutants were severely stunted and hyphae exhibited proliferative growth and increased colonisation of the intercellular spaces and vascular bundles. Expressoria formation, structures allowing colonisation of the leaf surface, was reduced in $\Delta mobC$, and abolished in $\Delta symB$ and $\Delta symC$ mutants. Microscopy analyses showed SymB-GFP and SymC-mRFP1 co-localise to the plasma membrane and septa. SymC also localised to highly dynamic punctate structures. Although $\Delta symB$ and $\Delta symC$ phenotypes are identical to $\Delta mpkA$, and the *E. festucae* pheromone response pathway scaffold $\Delta idcA$ mutants, MpkA and MpkB phosphorylation and cellular localisation was unchanged compared to wild-type. Using yeast-two-hybrid assays, an interaction between SymC and the STRIPAK complex associated protein GPI1 was demonstrated. Collectively these results show that MobC, SymB and SymC are required for *E. festucae* cell-cell fusion and host symbiosis. It is proposed that SymB and SymC interact to form a sensor complex at the cell wall which regulates cell-cell fusion in culture and hyphal network development *in planta*.

Acknowledgements

To my supervisor, Professor Barry Scott, thanks for investing the time to create this project and for always providing invaluable feedback on every aspect of my research. I have learnt so much from you and have received every possible opportunity that I could have imagined. To my co-supervisor, Dr Yvonne Becker, thanks for putting up with my constant supply of questions and teaching me absolutely everything. You have shaped the researcher I am today. I consider myself lucky to have learnt from someone who has an endless supply of patience and willingness to teach. Also thanks to you and your family, Matthias, Finja and Mia, for hosting me in Germany. I really appreciated my time spent there and I am fortunate to have met you. To my co-supervisor, Dr Helen Fitzimons, thanks for being an integral part of this project. Your comments, suggestions and knowledge have significantly enhanced the progression of this research.

To Dr Philippe Silar and Dr Hervé Lalucque, thank you for providing the sequences of IDC2 and IDC3. Without these, this project would not have been possible. To Dr Aiko Tanaka and Dr Daigo Takemoto, thank you for your inputs on the SymB and SymC manuscript. To Dr Stephan Seiler, Dr Anne Dettmann and Dr Yvonne Heilig, thank you for hosting me in Germany. To Ulrike Brandt, thank you for doing the *N. crassa* C-terminal Yeast-2-hybrid library screen work. To Dr André Fleißner, thank you for your invaluable discussions on the SymB and SymC manuscript. To the past and present MMIC members, Doug, Jianyu, Jordan, Niki and Matthew, thanks for always producing the highest level of quality help, sample preparation and advice. To Ann, Cynthia, Debbie and Paul, your administrative input has not gone unnoticed.

To Arvina, the heart, soul and magical hands of the laboratory, thank you for everything that you have done, not only for me but for every one else in the lab. Without you, the lab would be very empty (especially of chocolate) and probably fall apart. To the past lab members, Carla, Conni, Tetsuya, Leonie, Pip and Will, present lab members, Nazanin, Berit, Taryn, Dan, Yonathan and Alex, and honorary lab members, Asli, Benjamin, Ellie and Alyesha, thanks for the laughs, feedback and countless amounts of good advice that you have given me. I couldn't have asked for a better group of people to share this experience with. I wish each of you well in your own personal journeys to come.

To my family and friends, immediate and extended, you know who you are, the amount of support you have given me, and how much it has meant to me. Thanks for your unconditional love and patience.

This research was supported by a Massey University Doctoral Scholarship and a short term DAAD Research Scholarship.

Table of Contents

Abstract	i
Acknowledgements	ii
Table of Contents	iii
List of Figures	vii
List of Tables	xii
Abbreviations	xiii
1 Introduction	1
1.1 Plant-fungal interactions	2
1.2 <i>Epichloë festucae</i>: a model organism for studying mutualistic interactions	2
1.3 Life cycle of <i>E. festucae</i>	3
1.3.1 The asexual cycle	3
1.3.2 The sexual cycle	5
1.3.3 Formation of an epiphyllous hyphal network	5
1.4 Identification and characterisation of <i>E. festucae</i> mutants	5
1.4.1 The Nox complex	6
1.4.2 MAP kinase pathways	7
1.4.2.1 The Stress Activated MAPK pathway	8
1.4.2.2 The Cell Wall Integrity pathway	8
1.4.2.3 Pheromone Response MAPK pathway	11
1.4.3 The transcription factor ProA	11
1.4.4 Additional signalling pathways	12
1.5 Cell-cell fusion: required for mutualistic <i>E. festucae</i> associations?	12
1.6 <i>Sordaria macrospora</i>, <i>Neurospora crassa</i> and <i>Podospora anserina</i> sexual development and mutant screening	13
1.6.1 <i>Sordaria macrospora</i> life cycle and mutant screening	13
1.6.2 <i>Podospora anserina</i> and <i>Neurospora crassa</i> life cycles	15
1.6.3 <i>Neurospora crassa</i> and <i>Podospora anserina</i> mutant screening	15
1.7 Identification and characterisation of <i>pro</i>, <i>ham</i> and <i>IDC</i> mutants	17
1.7.1 The Nox complex	17
1.7.2 The transcription factor PRO1	18
1.7.3 Cell-cell fusion: a PR and CWI pathway ping-pong mechanism	18
1.7.3.1 MAK2 and the PR pathway	18
1.7.3.2 SO and the CWI pathway	19
1.7.3.3 PR and CWI signal integration: the STRIPAK complex	20

1.7.4 <i>Podospora</i> IDC2 and IDC3: a receptor complex for cell-cell fusion?.....	22
1.8 Cell-cell fusion a signalling network: candidate genes in <i>E. festucae</i>	23
1.9 Aims and Objectives	25
2 MobC manuscript	27
DRC16 form	28
Summary	29
Introduction	30
Results	31
<i>E. festucae</i> contains homologues of the STRIPAK complex	31
Culture phenotype of $\Delta mobC$	32
Symbiotic interaction phenotype of $\Delta mobC$	35
<i>mobC</i> has an accessory role in regulating <i>E. festucae</i> expressorium formation	38
Discussion	40
Experimental Procedures	42
Acknowledgements	45
References	46
Supporting Information	49
3 SymB and SymC manuscript	59
DRC16 form	60
Abstract	62
Introduction	62
Results	64
<i>E. festucae symB</i> and <i>symC</i> encode membrane-associated proteins	64
<i>symB</i> and <i>symC</i> promoters contain putative binding sites for the transcription	
factor ProA.....	65
Deletion of <i>symB</i> and <i>symC</i> genes	66
Culture phenotype of $\Delta symB$ and $\Delta symC$ strains	67
<i>In planta</i> phenotype of $\Delta symB$ and $\Delta symC$ strains	69
Expressorium formation in $\Delta symB$ and $\Delta symC$ strains	71
MpkA and MpkB phosphorylation and localisation in $\Delta symB$ and $\Delta symC$	
strains	74
SymB and SymC localisation.....	76
Discussion	78
Materials and Methods	83
Acknowledgements	88
Literature Cited	88

Supporting Information	97
4 SymC C-terminal analysis	119
4.1 Introduction	120
4.2 Materials and Methods	120
4.2.1 Strains and primers	120
4.2.2 Sterile conditions	123
4.2.3 <i>Saccharomyces cerevisiae</i> growth conditions and medium	123
4.2.3.1 YPD and YPDA medium	123
4.2.3.2 SD medium	124
4.2.4 <i>Escherichia coli</i> growth conditions and medium	124
4.2.4.1 Luria-Bertani (LB) medium	124
4.2.4.2 SOC medium	124
4.2.4.3 SOB medium	124
4.2.5 <i>Epichloë festucae</i> growth conditions and medium	124
4.2.5.1 Potato dextrose (PD) medium	124
4.2.5.2 Regeneration medium (RG)	125
4.2.5.3 Water agar medium	125
4.2.6 DNA and RNA isolation and quantification	125
4.2.6.1 Plasmid isolation	125
4.2.6.2 <i>Epichloë festucae</i> crude DNA extraction	125
4.2.6.3 <i>Epichloë festucae</i> RNA extraction and cDNA synthesis	125
4.2.7 DNA manipulation	126
4.2.7.1 <i>Taq</i> and <i>Q5</i> Polymerase PCR amplification	126
4.2.7.2 PCR product and Gel purification	126
4.2.7.3 Restriction Enzyme digests	126
4.2.8 Cloning	126
4.2.8.1 Gibson Assembly	126
4.2.8.2 Preparation and transformation of chemical competent <i>E. coli</i> cells	127
4.2.8.3 Invitrogen Clone Checker™ sytem	127
4.2.8.4 Sequencing	127
4.2.9 Generation of C-terminal deletion, expression and Yeast-2-hybrid constructs	127
4.2.9.1 <i>E. festucae</i> SymC C-terminal deletion and expression constructs	127
4.2.9.2 <i>N. crassa</i> Yeast-2-hybrid constructs	128
4.2.9.3 <i>E. festucae</i> Yeast-2-hybrid constructs	128

4.2.10 Fungal transformations.....	129
4.2.10.1 <i>E. festucae</i> protoplast preparation.....	129
4.2.10.2 <i>E. festucae</i> transformations and screening	130
4.2.10.3 <i>S. cerevisiae</i> single Yeast-2-hybrid transformations.....	130
4.2.10.4 <i>S. cerevisiae</i> library Yeast-2-hybrid transformation	130
4.2.11 Microscopy.....	131
4.2.12 Bioinformatics.....	131
4.2.12.1 Sequence acquisition.....	131
4.2.12.2 Protein alignment and domain predictions.....	131
4.3 Results	132
4.3.1 <i>E. festucae</i> SymC C-terminal domain deletion analysis	132
4.3.2 <i>Neurospora crassa</i> Yeast-2-hybrid screening.....	133
4.3.3 <i>E. festucae</i> Yeast-2-hybrid screening.....	140
4.4 Discussion	141
4.4.1 The C-terminal region of SymC is not required for cell-cell fusion	141
4.4.2 Identification of proteins that interact with the SymC C-terminus	142
4.4.2.1 NCU09375 (GpiA): <i>Sordaria macrospora</i> GPI1 homologue.....	142
4.4.2.2 NCU02668 (UthA): Septation protein Sun4 homologue.....	143
4.4.2.3 NCU03922 (ErgM): Erg-13 homologue	144
4.4.2.4 NCU09572 (Arp3): ARP2/3 complex subunit 3.....	145
5 Conclusions and Future work	146
5.1 Conclusions	147
5.2 Future work	149
5.2.1 Identification of upstream ProA signalling and further ProA targets	149
5.2.2 The involvement of SymB and SymC in a signalling pathway	149
5.2.3 Further characterisation of the STRIPAK complex	150
5.2.4 Expressoria formation in <i>E. festucae</i>	150
5.2.5 The ergosterol synthesis pathway in <i>E. festucae</i>	151
5.2.6 Cell-cell fusion: a culture phenotype for symbiosis mutant screening ...	151
6 References	152
7 Appendices	164

List of Figures

Introduction	
Figure 1.1 Life cycles of <i>E. festucae</i>	4
Figure 1.2 The Nox complexes	6
Figure 1.3 <i>S. cerevisiae</i> MAPK pathways	9
Figure 1.4 <i>E. festucae</i> F11 MAPK pathways	10
Figure 1.5 Life cycle of <i>Sordaria macrospora</i> and associated fruiting body mutants.	14
Figure 1.6 <i>Neurospora crassa</i> and <i>Podospora anserina</i> life cycles and mutant screening.	16
Figure 1.7 Oscillation of GFP labelled MAK-2 and dsRED labelled SO in homing <i>Neurospora crassa</i> tips.....	20
Figure 1.8 Diagram of the <i>Sordaria</i> STRIPAK complex components with their predicted interaction partners, transmembrane domains and cellular localisation patterns shown.....	22
Figure 1.9 Proposed signalling network for cell-cell fusion	25
MobC manuscript	
Fig.1 Culture phenotype of $\Delta mobC$ and wild-type strains	33
Fig.2 Conidiation and cell-cell fusion phenotype of $\Delta mobC$	34
Fig.3 Plant interaction phenotype of $\Delta mobC$	35
Fig.4 Transmission electron micrographs of <i>L. perenne</i> pseudostem cross sections infected with wild-type and $\Delta mobC$ strains.....	36
Fig.5 Confocal depth series images of aniline blue/WGA-AF488 stained <i>L. perenne</i> leaf sheaths infected with wild-type and $\Delta mobC$ strains.....	37
Fig.6 Confocal depth series images of wild-type and $\Delta mobC$ expressoria and sub- cuticular hyphae within <i>L. perenne</i> associations.	39
Fig.S1 <i>Epichloë festucae mobC</i> gene structure and amino acid sequence alignment.....	51
Fig.S2 <i>Epichloë festucae pro11</i> homologue gene structure and amino acid sequence alignment	52
Fig.S3 <i>Epichloë festucae pro22</i> homologue gene structure and amino acid sequence alignment	54
Fig.S4 <i>Epichloë festucae pro45</i> homologue gene structure and amino acid sequence alignment	55
Fig.S5 <i>mobC</i> deletion and complementation construct design, screening primers and Southern analysis	56
Fig.S6 Analysis of MpkA and MpkB phosphorylation in $\Delta mobC$	57

Fig.S7 Quantification of the whole plant phenotype of <i>Lolium perenne</i> inoculated with wild-type, $\Delta mobC$ and complemented $\Delta mobC/mobC$ strains	57
Fig.S8 Expressoria phenotype of $\Delta mobC$	58
SymB and SymC manuscript	
Fig.1 Electrophoretic mobility shift assays of <i>symB</i> and <i>symC</i> promoter fragments bound to ProA	66
Fig.2 Culture phenotype of wild-type, $\Delta symB$ and $\Delta symC$ strains.	68
Fig.3 Plant phenotype of <i>Lolium perenne</i> infected with wild-type, $\Delta symB$, $\Delta symC$ and complemented strains	69
Fig.4 <i>In planta</i> cellular phenotype of $\Delta symB$ and $\Delta symC$ mutants.....	70
Fig.5 <i>In planta</i> cellular phenotype of $\Delta symB$ and $\Delta symC$ mutants.....	72
Fig.6 Impaired development of expressoria in $\Delta symB$ and $\Delta symC$ mutants.	73
Fig.7 Analysis of MpkA and MpkB phosphorylation in culture.	74
Fig.8 Localisation of MpkA and MpkB in <i>E. festucae</i> cultures.	75
Fig.9 Localisation of SymB-eGFP in <i>E. festucae</i> $\Delta symB$	77
Fig.10 Localisation of SymC-mRFP1 in <i>E. festucae</i> $\Delta symC$	78
Fig. S1 <i>Epichloë festucae symB</i> gene structure, encoded protein domain structure and amino acid sequence alignment.....	101
Fig.S2 <i>Epichloë festucae symB</i> gene structure, encoded protein domain structure and amino acid sequence alignment.....	102
Fig.S3 Fold change of <i>symB</i> and <i>symC</i> expression <i>in planta</i> for $\Delta proA$, $\Delta noxA$ and $\Delta saka$ associations compared to wild-type	103
Fig.S4 Identification of ProA binding site in <i>symB</i> promoter.	104
Fig.S5 Identification of ProA binding site in <i>symC</i> promoter	105
Fig.S6 <i>symB</i> and <i>symC</i> deletion and complementation construct design, screening primers and Southern analysis.....	106
Fig.S7 Quantification and morphology of conidia recovered from $\Delta symB$, $\Delta symC$ and wild-type cultures	107
Fig.S8 Effect of cell wall stress agents and pH on growth of <i>E. festucae</i> wild-type (WT), $\Delta mpkA$, $\Delta symB$ and $\Delta symC$ strains.....	108
Fig.S9 Quantification of wild-type (WT), $\Delta symB$ and $\Delta symC$ hyphae colonising the intercellular spaces of host cells.....	109
Fig.S10 Localisation of MpkA-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphal tips	110
Fig.S11 Localisation of MpkA-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphae from a mid-section of the colony.....	111

Fig.S12 Localisation of MpkA-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphae from centre of the colony.....	112
Fig.S13 Localisation of MpkB-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphal tips	113
Fig.S14 Localisation of MpkB-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphae from a mid-section of the colony.....	114
Fig.S15 Localisation of MpkB-eGFP in <i>E. festucae</i> wild-type, $\Delta symB$ and $\Delta symC$ hyphae from centre of the colony.....	115
Fig.S16 Localisation of SymC-mRFP1 in <i>E. festucae</i> $\Delta symC$	116
Fig.S17 Localisation of SymB-eGFP and SymC-mRFP1 in <i>E. festucae</i> $\Delta symB$ and $\Delta symC$ and wild-type.....	117
SymC C-terminal analysis	
Figure 4.1 <i>E. festucae</i> SymC C-terminal domain deletion analysis	133
Figure 4.2 <i>Neurospora crassa</i> Yeast-2-hybrid analysis	134
Figure 4.3 <i>Epichloë festucae</i> <i>gpiA</i> gene structure and amino acid alignment	136
Figure 4.4 <i>Epichloë festucae</i> <i>uthA</i> gene structure and amino acid alignment	137
Figure 4.5 <i>Epichloë festucae</i> <i>ergM</i> gene structure and amino acid alignment	138
Figure 4.6 <i>Epichloë festucae</i> <i>arp3</i> gene structure and amino acid alignment	139
Figure 4.7 Yeast-2-hybrid interaction test using the <i>E.festucae</i> SymC C-terminal domain as bait against GpiA, UthA, ErgM and Arp3	140
Figure 4.8 Model summarising the proposed <i>SmGPI1</i> and <i>SmMOB3</i> interaction in WT and mutant strains.....	143
Conclusions and future work	
Figure 5.1 Summary of key findings.....	147
Appendices	
Figure 7.1 pRS426 vector	165
Figure 7.2 pSF15.15 vector	165
Figure 7.3 pII99 vector	166
Figure 7.4 pCR4-Topo®	166
Figure 7.5 pYR33 vector	167
Figure 7.6 pPN94 vector.....	167
Figure 7.7 pCE81.....	168
Figure 7.8 pMpkB-eGFP	168
Figure 7.9 pKG1 containing the <i>E. festucae</i> <i>symB</i> deletion construct.....	169
Figure 7.10 pKG2 containing the <i>E. festucae</i> <i>symC</i> deletion construct	169
Figure 7.11 pKG4 containing the <i>E. festucae</i> <i>mobC</i> deletion construct	170

Figure 7.12 pKG5 containing the first <i>E. festucae symB</i> complementation construct	170
Figure 7.13 pKG6 containing the <i>E. festucae symC</i> complementation construct	171
Figure 7.14 pKG7 containing the final <i>E. festucae symB</i> complementation construct	171
Figure 7.15 pKG8 containing the <i>E. festucae mobC</i> complementation construct	172
Figure 7.16 pKG9 containing the <i>E. festucae</i> half SymC C-terminal deletion construct	172
Figure 7.17 pKG10 containing the <i>E. festucae</i> full SymC C-terminal deletion construct	173
Figure 7.18 pKG11 containing the <i>E. festucae</i> truncated SymC C-terminal deletion construct	173
Figure 7.19 pKG12 containing the native <i>E. festucae symC::3GA::mRFP1</i> construct	174
Figure 7.20 pKG13 containing the native <i>E. festucae symB::3GA::GFP</i> construct	174
Figure 7.21 pKG14, pGBKT7 Yeast-2-Hybrid vector fused to <i>NCU00939</i> C-terminus	175
Figure 7.22 pKG19 containing the <i>E. festucae</i> truncated SymC C-terminal deletion construct fused to mRFP1	175
Figure 7.23 pKG20 containing the over expression <i>E. festucae symB::3GA::GFP</i> construct	176
Figure 7.24 pKG21 containing the over expression <i>E. festucae symC::3GA::mRFP1</i> construct	176
Figure 7.25 pKG23 containing the <i>E. festucae</i> full length SymC C-terminal deletion construct fused to mRFP1	177
Figure 7.26 pGADT7 Yeast-2-hybrid vector	177
Figure 7.27 pGBKT7 Yeast-2-hybrid vector	178
Figure 7.28 pKG24, pGBKT7 Yeast-2-hybrid vector fused to SymC C-terminus	178
Figure 7.29 pKG25, pGADT7 Yeast-2-hybrid vector fused to <i>E. festucae GpiA</i>	179
Figure 7.30 pKG26, pGADT7 Yeast-2-hybrid vector fused to <i>E. festucae Arp3</i>	179
Figure 7.31 pKG27, pGADT7 Yeast-2-hybrid vector fused to <i>E. festucae UthA</i>	180
Figure 7.32 pKG28, pGADT7 Yeast-2-hybrid vector fused to <i>E. festucae ErgM</i>	180
Figure 7.33 pGADT7-RHO-1 DA, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> RHO-1	181
Figure 7.34 pGADT7-RHO-1 DN, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> RHO-1	181
Figure 7.35 pGADT7-PKC-1, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> PCK-1	182
Figure 7.36 pGADT7-MIK-1 13kb N-term, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MIK-1	182

Figure 7.37 pGADT7-MIK-1 13kb C-term, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MIK-1	183
Figure 7.38 pGADT7-MEK-1, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MEK-1	183
Figure 7.39 pGADT7- MEK-1 N-term, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MEK-1	184
Figure 7.40 pGADT7- MEK-1 C-term, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MEK-1	184
Figure 7.41 pGADT7-MAK-1, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> MAK-1	185
Figure 7.42 pGADT7-BEM-1, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> BEM-1	185
Figure 7.43 pGADT7-NOR-1, pGADT7 Yeast-2-hybrid vector fused to <i>N. crassa</i> NOR-1	186

List of Tables

Introduction	
Table 1: Genes involved in <i>E. festucae</i> symbiosis and <i>S. macrospora</i> , <i>P. anserina</i> and <i>N. crassa</i> CAT, protoperithicium and CG formation.....	24
MobC Manuscript	
Supplementary Table 1 Biological Material.....	49
Supplementary Table 2 Primers used in this study.	50
Supplementary Table 3 Analysis of plant survival rates.	50
SymB and SymC Manuscript	
Supplementary Table 1 Biological Material.....	97
Supplementary Table 2 Primers used in this study.	99
Supplementary Table 3 Analysis of plant survival rates.	101
SymC C-terminal analysis table	
Table 4.1 Primers used in this study.....	120
Table 4.2 Strains used in this study.....	121
Table 4.3 Yeast-2-hybrid candidates that interact with <i>NCU00938</i> C-terminus.....	135

Abbreviations

aa	Amino acid
Amp	Ampicillin
Amp ^R	Ampicillin resistant
<i>Ao</i>	<i>Aspergillus oryzae</i>
<i>asc</i>	Ascogonia mutant
<i>Bc</i>	<i>Botrytis cinerea</i>
BLAST	Basic local alignment search tool
BLASTn	Nucleotide database search using a nucleotide query
BLASTp	Protein database search using a protein query
bp	Base pair(s)
CAT(s)	Conidial anastomosis germ tube(s)
cDNA	Complementary DNA
CFW	Calcofluor white
CG	Crippled growth
CIAP	Calf intestinal phosphatase
CLSM	Confocal Laser Scanning Microscopy
<i>Cp</i>	<i>Claviceps purpurea</i>
CW	Cell wall
CWI	Cell wall integrity
Cys	Cysteine
DIC	Differential interference contrast
DIG	Digoxigenin
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid

eGFP	Enhanced green fluorescent protein
EMSA	Electrophoretic mobility shift assay
FB	Fruiting body
<i>Fg</i>	<i>Fusarium graminearum</i>
<i>Fo</i>	<i>Fusarium oxysporum</i>
g	Gram
gDNA	Genomic DNA
GEF	Guanine nucleotide exchange factor
Gen	Geneticin
Gen ^R	Geneticin resistant
GFP	Green fluorescent protein
GPI	Glycosylphosphatidylinositol
h	Hour(s)
<i>ham</i>	Hyphal anastomosis mutant
H ₂ O ₂	Hydrogen peroxide
Hph	Hygromycin
Hyg ^R	Hygromycin resistant
<i>IDC</i>	Impaired development of crippled growth
kb	Kilobase(s)
KO	Knock-out
L	Litre
LB	Luria-Bertani broth
M	Molar
MAPK(K/K)	Mitogen activated protein kinase (kinase/kinase)
mg	Milligram
<i>Mg</i>	<i>Magnaporthe grisea</i>
μg	Microgram
min	Minute(s)
μL	Microlitre

mL	Millilitre
µm	Micrometre
µM	Micromolar
mm	Millimeter
mM	Millimolar
<i>Mo</i>	<i>Magnaporthe oryzae</i>
MOB	Monopolar spindle-one-binder
mRFP1	Monomeric red fluorescent protein
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
<i>Nc</i>	<i>Neurospora crassa</i>
NCBI	National Centre for Biotechnology Information
ng	Nanogram
Nox	NADPH oxidase
<i>Pa</i>	<i>Podospora anserina</i>
PB1	Protein binding domain 1
PC	Plant cell
PCR	Polymerase chain reaction
PD	Potato dextrose
PEG	Polyethylene glycol
<i>per</i>	Perithicia mutant
<i>pile</i>	Perithicia placement mutant
pg	Picogram
pmol	Picomole
PMSF	Phenylmethylsulfonyl fluoride
PP2A	Protein phosphatase 2A
PR	Pheromone response
<i>pro</i>	Protoperithicia mutant

RG	Regeneration
RNA	Ribonucleic acid
RNase	Ribonuclease
RNA-seq	Ribonuclease sequencing
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
SAK	Stress-activated kinase
SAM	Shoot apical meristem
<i>Sc</i>	<i>Saccharomyces cerevisiae</i>
SC	Synthetic complete
SD	Synthetic defined
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
STRIPAK	Striatin interacting phosphatase and kinase
<i>sym</i>	Symbiosis mutant
TBE	Tris-boric acid-EDTA
tBLASTn	Translated nucleotide database search using a protein query
T-DNA	Transfer-deoxyribonucleic acid
TEM	Transmission electron microscopy
TMD	Transmembrane domain
<i>Um</i>	<i>Ustilago maydis</i>
UV	Ultraviolet
V	Volts
v/v	Volume/volume ratio
WT	Wild-type

w/v	Weight/volume ratio
YE	Yeast extract
°C	Degrees Celsius

