

Towards Cloning the Self-Incompatibility Genes
from *Phalaris coerulescens*

by

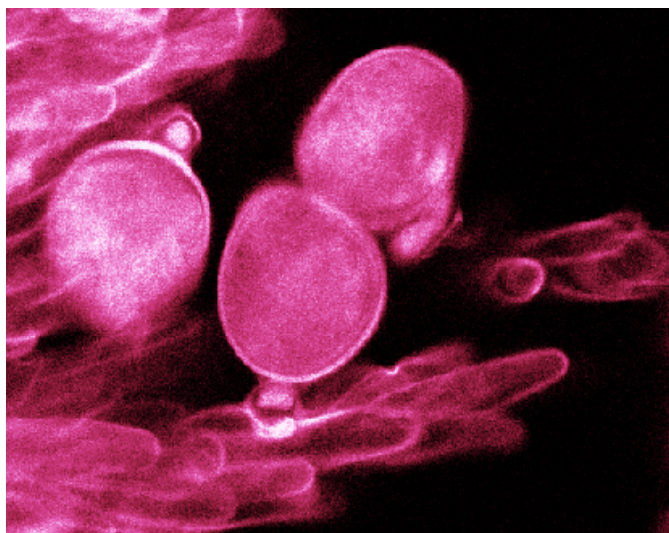
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October 2001



A confocal picture showing the interaction between *Phalaris* pollen and stigma

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Abstract

Self-incompatibility (SI) is an important genetic mechanism to prevent the inbreeding of flowering plants and also an excellent system for studying cell-cell recognition and signal transduction. During evolution, several SI systems have been evolved. A unique SI system widely spreads in the grasses. In the grasses, two unlinked, multi-allelic loci (*S* and *Z*) determine SI specificity. A putative self-incompatibility gene (*Bm2*) was previously cloned. In this study, the role of *Bm2* in self-incompatibility was investigated first. The cDNA homologues of *Bm2* were sequenced from two pollen-only mutants. The results indicated that *Bm2* is not the one of SI genes in *Phalaris*, but represents a subclass of thioredoxin *h*. Thus a map-based cloning strategy was then adopted to clone the SI genes from *Phalaris*. Fine linkage maps of the *S* and *Z* regions were constructed. RFLP probes from wheat, barley, oat and rye were screened and the *S* locus was delimited to 0.26 cM and the *Z* locus to 1.0 cM from one side using specially designed segregating populations. The *S* locus was located to the sub-centromere region of triticeae chromosome group 1 and the *Z* locus to the middle of the long arm of group 2. Finally, barley and rice bacterial artificial chromosome (BAC) clones corresponding to the *S* and *Z* region were identified to analyse the chromosome structures and to seek candidate SI genes. The abundant repetitive sequences in the identified barley BAC clones limit their usefulness. Identification of Rice BAC clones orthologous to the *S* and *Z* regions open the gate to use rice genome information to clone SI genes from the grasses. A positive rice clone (139.9 kb) orthologous to the *S* region contained 19 predicted genes. Several of these genes might be involved in pollen tube germination and pollen-stigma interaction, which are the major parts of SI reaction. A positive clone (118.9 kb) orthologous to the *Z* region gave 16 predicted genes. The predicted genes on the outmost ends of these clones could be used to construct contigs to cover the *S* and *Z* regions and delimit the *S* and *Z* loci in the grasses.

Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institute and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Xueyu Bian

October, 2001

Acknowledgements

I thank my supervisors, Prof. Peter Langridge and Dr Susan Barker for their care, encouragement and push, especially to Prof. Peter Langridge, who gave me the freedom to select my Ph. D. project. Many thanks are given to all the colleagues in the laboratory of Prof. Peter Langridge and in Department of Plant Science for their help not only with the lab work but in all areas of life.

Thanks are given to Dr X. Li for providing the original *Bm2* cDNA clone, its thioredoxin domain construct in pQE31 vector and rabbit polyclonal antiserum raised against the thioredoxin fusion protein; to Mr J. Juttner for providing some primers used for RT-PCR and two wild type cDNA sequences of the *S* linked thioredoxin; to Dr Chongmei Dong for her help in western analyses; to Dr A. Harvey for his help with functional prediction of the *S* linked thioredoxin; to Dr D. Hayman (Adelaide University, Australia) for providing the seeds of the *S* and *Z* tester populations and genotyping some of the potential recombinants, Mr Brendon King for his help with screening the barley BAC library.

The author would also like to thank Ms A. Friedrich, an honours student from Bonn University, Germany, who took part in the project of mapping of the *S* and *Z* loci. Ms Friedrich helped to extract 273 DNA samples of the *S* tester population, screened some of the RFLP probes used in this thesis and provided some Southern data for mapping, which are indicated by underline in the partial linkage groups of *S* and *Z*. Ms Friedrich participated in the screening of the BAC library with *Bm2* as well. Thanks are given to Ms J. Bai, a visiting scholar from Shanxi Agricultural Research Academy, China, who helped to confirm the AFLP primer combinations on the parents and recombinants.

I give my special thanks to the following scientists for their encouragement and kindness in provision of RFLP probes: Dr F.C.H. Franklin and Dr M. Gale from the United Kingdom; Dr P. Wehling from Germany, Dr K. Hatakeyama and Dr M. Kussaba from Japan and Dr E. Newbiggin from Australia.

Finally, I give thanks to my family, especially to my wife. Without their encouragement and love, this thesis could not be finished.

This work was supported by an Australian Overseas Postgraduate Research Scholarship and an Adelaide University Scholarship.

List of abbreviations

aa	amino acid
ATP	adenosine 5'-triphosphate
BAC:	bacterial artificial chromosome
BCIP:	5-bromo-4-chloro-3-indolyl phosphate
BLAST:	Basic Local Alignment Search Tool
bp:	base pair
BSA:	bovine serum albumin
°C:	degree centigrade
cDNA:	complementary deoxyribonucleic acid
CHEF:	contour-clamped homogeneous electric field
Ci:	Curie
Da:	Dalton
dATP:	2'-deoxyadenosine 5'- triphosphate
dCTP:	2'-deoxycytidine 5'- triphosphate
DEAE:	diethylaminoethyl
DEPC:	diethyl pyrocarbonate
dGTP:	2'-deoxyguanosine 5'- triphosphate
DMF:	dimethyl formamide
DMSO:	dimethyl sulfoxide
DNA:	deoxyribonucleic acid
dNTPs:	deoxyribonucleoside triphosphates
DTT:	dithiothreitol
dTTP:	2'-deoxythymidine 5'- triphosphate
EDTA:	ethylene diamine tetraacetate acid

EMBL:	European Molecular Biology Laboratory
g:	gram
HEPES:	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
IPTG:	isopropyl β -D-thiogalactopyranoside
kb:	kilobase
kda:	kiloDalton
M:	molar
MOPS:	3-(N-morpholino) propane-sulfonic acid
mRNA:	messenger ribonucleic acid
NBT:	nitroblue tetrazolium
OD ₆₀₀	optical density at 600 nm
ORF:	open reading frame
PAC:	P1-derived artificial chromosome
PAGE:	polyacrylamide gel electrophoresis
RFLP:	restriction fragment length polymorphism
RNA:	ribonucleic acid
RNase:	ribonuclease
rpm:	revolutions per minute
rRNA:	ribosomal ribonucleic acid
PFGE:	pulsed-field gel electrophoresis
PMSF:	phenylmethylsulfonyl fluoride
PVP:	polyvinyl-polyrrolidone
SDS:	sodium dodecyl sulfate
SDS-PAGE:	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Taq:	Thermus aquaticus DNA (polymerase)

Tris·Cl	tris(hydroxymethyl)aminomethane hydrochloride
UV:	ultraviolet
w/v:	weight /volume
X-gal:	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
YAC:	yeast artificial chromosome