A Genomics Approach to Investigate the Molecular Control of Meiosis in *Triticum aestivum*

by

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ABSTRACT

Meiosis is a cell division process central to the life cycle of all sexual eukaryotic organisms. Chromosome pairing, genetic recombination and subsequent nuclear division during meiosis produces four genetically distinct haploid gametes from a single diploid cell. Allohexaploid wheat (*Triticum aestivum*) behaves meiotically as a diploid, despite the existence in the genome of three closely related (homoeologous) genomes, A, B and D. Chromosome pairing during prophase I of meiosis in wheat is restricted to true homologous chromosomes, the result being the formation of 21 bivalents at meiotic metaphase I. The genetic control of chromosome pairing in wheat is under the control of several pairing homoeologous (*Ph*) genes, located predominantly on chromosome groups 3 and 5. The major suppressors of homoeologous pairing are *Ph1* and *Ph2*. Their cytogenetic effect has been intensively studied but at the molecular level little is known about their function. The isolation and characterisation of *Ph* genes from wheat would lead to greater understanding of chromosome pairing mechanisms in complex allopolyploids, and may enable development of effective strategies for alien gene introgression from related species to modern wheat cultivars.

In this study, several genomics-based approaches were adopted to explore the expressed portion of the wheat genome in order to identify and characterise genes that could function in the molecular processes regulating meiosis.

The first approach used comparative genetics to characterise the region deleted in the *ph2a* mutant (a deletion mutant at *Ph2*). The rice genomic region syntenous to that deleted in the *ph2a* mutant was identified through comparative mapping and used in searches of wheat databases to identify ESTs with significant similarity. Southern analysis confirmed a syntenous relationship in the wheat and rice genomic regions and defined precisely the position of the breakpoint in *ph2a*. What seems to be a terminal deletion on 3DS is estimated to be approximately 80 Mb in length. We can tentatively predict the identification of approximately 220 genes from the region deleted in *ph2a*. The putative role of identified candidate *Ph2* genes is discussed.

The second approach explored the validity of recent proposals suggesting the presence of a meiotic gene cluster in the region of *Ph2*. The transcriptional characteristics of genes linked to *Ph2* were investigated using data from wheat EST databases in combination with recently developed analysis software. The tissue-distribution of mRNAs derived from genes linked to *Ph2* is shown to resemble that of other large chromosomal regions in the wheat genome. It is concluded that the apparently high number of genes from the *Ph2* region expressed in wheat meiotic tissue is not indicative of a meiotic gene cluster in this region, but rather highlights the transcriptional complexity of meiotic anther tissue.

Finally, the meiotic expression pattern of approximately 1800 wheat genes was examined using cDNA microarrays. Two approaches were taken. Firstly, the applicability of microarrays to identify differentially expressed genes between wild-type anthers and anthers of three *Ph* mutant genotypes was investigated. These experiments failed to reveal significant down-regulation of genes in *Ph* mutant anthers compared to wild-type. Possible explanations are discussed. Secondly, the expression of all microarray clones was examined from pre-meiotic interphase through to the tetrad stage of meiosis. A number of candidate wheat genes involved in meiotic and anther developmental processes have been identified and are discussed.

Prior to this study, the methods available to identify wheat meiotic genes, in particular as candidates for *Ph2*, were limited. The recent development of genomics in plant biology provided an opportunity for a new approach towards gene discovery and genome structural analysis in relation to meiosis. This research illustrates the need for, and the effectiveness of a new approach to study meiosis, contributing to our knowledge of the structural and functional characteristics of genes linked to *Ph2*, and establishing a strong basis for further wheat meiotic gene characterisation.

DECLARATION

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

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

T. J. Sutton
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ABBREVIATIONS

aa	amino acid
aRNA	antisense ribonucleic acid
ATP	adenosine 5'-triphosphate
BAC	bacterial artificial chromosome
bp	base pair/s
BLAST	Basic Logical Alignment Search Tool
BSA	bovine serum albumin
$^{\circ}$C	degrees Celsius
CCV	Contig Constellation Viewer
cDNA	complementary deoxyribonucleic acid
cm	centimetre/s
cv	cultivar
Cy3	cyanine 3 dUTP
Cy5	cyanine 5 dUTP
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dTTP	2'-deoxythymidine 5'-triphosphate
dUTP	2'-deoxyuridine 5'-triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
DTT	diothiothreitol
EDTA	ethylenediaminetetraacetic acid
EMS	ethyl methane sulphonate
EST	expressed sequence tag
FISH	fluorescent *in situ* hybridisation
g	gram/s
x g	9.81 m/s^2
h	hour/s
IPTG	isopropyl-1-thio-b-D-galactosidase

IQR	interquartile range
ITEC	International Triticeae EST Cooperative
Kb	kilobase/s
kDa	kilodalton/s
L	litre/s
LB	Luria-Bertaini
M	molar
mA	milliampere
Mb	megabase/s
min	minute/s
mg	milligram/s
mL	millilitre/s
mM	millimolar
ng	nanogram/s
nm	nanometre/s
MOPS	3-(N-morpholino)propane-sulfonic acid
mRNA	messenger ribonucleic acid
OD_{260}	optical density at 260 nm
PAC	P1 artificial chromosome
PCR	polymerase chain reaction
poly(A)	polyadenylated
PVP	polyvinyl pyrollidone
RFLP	restriction-fragment length polymorphism
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcription-polymerase chain reaction
sarkosyl	N-lauroylsarcosine
SDS	sodium dodecyl sulphate
SSC	sodium chloride/sodium citrate
TAE	Tris/acetate/EDTA
Taq	*Thermus aquaticus*

TE	Tris/EDTA
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
U	units
μFD	microfarad/s
μg	microgram/s
μL	microlitre/s
UV	ultraviolet
V	volt/s
v/v	volume/volume
W	watt/s
w/v	weight/volume
x-gal	5-bromo-4-chloro-3-indolyl-b-D-galactosidase