CONTROLLED INTROGRESSION OF ALIEN CHROMATIN INTO WHEAT

by

Robert Max David Koehner, B. Agric. Sc. (Qld)

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Department of Agronomy,
Waite Agricultural Research Institute,
University of Adelaide,
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# Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Statement of originality</td>
<td>iv</td>
</tr>
<tr>
<td>Consent to photocopy and loan</td>
<td>v</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vi</td>
</tr>
<tr>
<td><strong>Chapter 1:</strong> GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td><strong>Chapter 2:</strong> REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Genomic structure of wheat and the concept of homoeology</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Genetic control of the suppression of glutensynthesis</td>
<td>5</td>
</tr>
<tr>
<td>2.3 Interaction of Phl with genes from other species</td>
<td>8</td>
</tr>
<tr>
<td>2.4 Mechanism and action of the Phl gene</td>
<td>11</td>
</tr>
<tr>
<td>2.5 Introgression of alien chromatin into wheat</td>
<td>16</td>
</tr>
<tr>
<td>2.6 Effect of alien chromatin on wheat phenotype</td>
<td>23</td>
</tr>
<tr>
<td><strong>Chapter 3:</strong> METHODOLOGY</td>
<td>27</td>
</tr>
<tr>
<td>3.1 Electrophoresis</td>
<td>27</td>
</tr>
<tr>
<td>3.1.1 Single dimensional SDS-PAGE</td>
<td>27</td>
</tr>
<tr>
<td>3.1.2 Two dimensional SDS-PAGE</td>
<td>28</td>
</tr>
<tr>
<td>3.1.3 Acid PAGE</td>
<td>29</td>
</tr>
<tr>
<td>3.1.4 Isoelectric focusing</td>
<td>30</td>
</tr>
<tr>
<td>3.1.5 Cellulose acetate gel electrophoresis</td>
<td>32</td>
</tr>
<tr>
<td>3.2 Cytology</td>
<td>33</td>
</tr>
<tr>
<td>3.2.1 Mitosis</td>
<td>33</td>
</tr>
<tr>
<td>3.2.2 Giemsa staining of root tip meristem</td>
<td>33</td>
</tr>
<tr>
<td>3.2.3 Meiosis</td>
<td>34</td>
</tr>
<tr>
<td>3.3 Stem root inoculation</td>
<td>34</td>
</tr>
</tbody>
</table>
Chapter 4: WHEAT-RYE RECOMBINATION
1. THE SHORT ARM OF RYE CHROMOSOME 1R

4.1 Introduction 36
4.2 Plant materials and methods 40

4.2.1 Plant materials 40
4.2.2 Marker loci used in the selection and characterisation of recombinants 40
4.2.3 Phenotypes of parental lines 41
4.2.4 Selection of a 1D/1DL-1RS translocation heterozygote, homozygous for ph1b 42
4.2.5 Selection of translocation heterozygotes 1D/1DL-1RS and 1B/1BL-1RS, nullisomic for 5B 43
4.2.6 Screening for wheat-rye recombination 43

4.3 Results 45

4.3.1 Identification of heterozygous 1DL-1RS, ph1bph1b plants, and analysis of testcross progeny 45
4.3.2 Further analysis of the four putative recombinant lines induced by ph1bph1b 50
4.3.3 Use of acid PAGE to detect nullisomy for chromosome 5B 56
4.3.4 Identification of 1D/1DL-1RS heterozygotes nullisomic for 5B, and screening for allo-syndetic recombination in their progeny 56
4.3.5 Identification of 1B/1BL-1RS heterozygotes nullisomic for 5B and screening their progeny for recombinants 66

4.4 Discussion 69

Chapter 5: WHEAT-RYE RECOMBINATION
2. THE LONG ARM OF RYE CHROMOSOME 1R

5.1 Introduction 77
5.2 Plant materials and methods 79

5.2.1 Plant materials 79
5.2.2 Production of populations for screening of wheat-rye recombination 79
5.2.3 Reduced SDS-PAGE phenotypes of parental lines 80
5.2.4 Marker characters used in screening for wheat-rye recombination 80
5.2.5 Recovery and verification of wheat-rye recombinants 81
5.3 Results 86
5.3.1 Selection of F2 plants homozygous 86
ph1b and heterozygous 1D:1DS-1RL 86
5.3.2 Glutenin phenotype and root tip telomere 88
number of the T and C populations
5.3.3 Confirmatory progeny tests of non-
parental plants 92
5.4 Discussion 99

Chapter 6: WHEAT-AEGILOPS RECOMBINATION: 103
CHROMOSOME 1U OF AEGILOPS UMBELLULATA

6.1 Introduction 103
6.2 Plant materials and methods 105
6.2.1 Plant materials 105
6.2.2 Production of populations for screening of 105
wheat - Aegilops allosynthetic recombination
6.2.3 Phenotypes of parental lines for 106
biochemical markers
6.2.4 Marker loci used in the detection 108
of wheat - Ae. umbellulata recombinants
6.2.5 Screening for wheat - Aegilops 108
recombination
6.3 Results 110
6.3.1 Selection of F2 individuals homozygous 110
ph1b and carrying endosperm protein markers
for both chromosomes 1U and 1B (or 1D)
6.3.2 Endosperm storage protein and glucose 114
phosphate isomerase phenotype of the T
and C populations
6.4 Discussion 129

Chapter 7: GENERAL DISCUSSION 135

BIBLIOGRAPHY 141
Abstract

Attempts to transfer alien genetic material to wheat have in the past involved addition or substitution of whole chromosomes or chromosome arms from an alien related species to the genome of wheat. Generally, such lines have suffered from loss of yield or quality compared to the normal wheat parent. The amount of alien chromatin present in these lines can be reduced by induction of meiotic recombination between alien and wheat chromosomes through suppression or deletion of the Phl gene on the long arm of wheat chromosome 5B. Although induced allosynthetic pairing has been frequently observed, few successful alien gene transfers have been achieved in this way. The possible mechanisms of action of Phl, the experience with alien introgression and the known effects of alien chromatin on wheat are reviewed.

The work reported in this thesis demonstrates for the first time that the chromosomes of wheat and cereal rye can be recombined by induction of homoeologous pairing by means of nullisomy for chromosome 5B and by the utilization of the phlb mutant. The frequency of recombination is low, but by developing rapid and reliable techniques using established biochemical and other markers, it was possible to screen for these recombinants relatively easily.

Two different wheat-rye translocation lines were used as starting points for the induction of allosynthetic recombination. The translocation chromosome involving the short arm of rye chromosome 1R carries a useful gene for resistance to stem rust, but lines with this rye segment translocated to the long arms of either 1D or 1B are characterised by dough quality defect. By testcrossing a homozygous phlb plant heterozygous for the 1DL-1RS translocation, one recombinant involving 1RS and 1DS was isolated out of 394 progeny, while progeny derived by self-fertilisation of nullisomic 5B plants heterozygous for the same translocation produced a further three wheat-rye recombinants in 531 progeny. The rye segment present in the 1BL-1RS translocation has also been recombined with wheat. The recombinants were selected on the basis of their
endosperm storage protein phenotype (two independent loci on 1DS, one on 1RS and on 1BS) and their reaction to stem rust. Recombinant lines were further characterized by analysis of phenotype for two isozymes, which have structural genes on the short arms of the homoeologous group 1 chromosomes. Twelve independent plants with an altered chromosome 1DS were also obtained; eleven of these possessed the proximal but not the distal endosperm storage protein locus, while one possessed the distal without the proximal locus. These lines will prove useful in the elucidation of the contribution of the gene products of these two protein loci to dough quality.

The long arm of rye chromosome 1R, which is marked both by a heterochromatic telomere and at the Glu-R1 locus, closely linked to the centromere, was induced to pair with wheat in a ph1bph1b background and 17 recombined chromosomes were recovered among 731 progeny derived by self-fertilisation. Due to self-sterility of some plants, some expected recombinants could not be subjected to a progeny test for verification and an estimate of the total number of recombinants obtained was made, giving a gametic recombination frequency of 1.4%. Control populations, where homoeologous pairing was suppressed, did not produce any confirmed recombinants.

Chromosome 1U from Aegilops umbellulata was also used in a study of wheat-allele recombination. It was expected that the allelosynthetic recombination frequency would be higher in this case than in rye, given that Aegilops and wheat are more closely related than wheat and rye. This chromosome was chosen as it possesses three easily scorable marker loci. Over a segment of the short arm of the Aegilops chromosome between the prolamin locus Glu-1U and a structural gene for the isozyme Opi-1U, a gametic recombination frequency of 8.0% was estimated within a population derived from a ph1bph1b parent, a third of the value for homologous recombination within wheat.

Some double homoeologous crossovers in the interval Glu-1U - Glu-1U were also recovered. When both the alien chromosome and a wheat homoeologue were present as monosomes, the rate of recombination was approximately double that recorded in
populations derived from a monosomic addition of chromosome 1U. No cross-overs were found in a control population, derived from a *Ph1b* parent.

Since codominant genetic markers allow the classification of both gametes in a single progeny, F2 populations were employed in most of this work, rather than the more conventional backcross techniques used in previous work with alien introgression. These populations are both simple to produce and are more efficient than test-cross populations as two gametes are screened simultaneously in a single individual. A comparison of the efficiency of induction of allozygotic recombination showed that SB nullisomy was at least as effective as the *ph1b* mutant. The availability of a gene soluble endosperm protein controlled by a gene on chromosome 5DL made selection of SB deficient plants simpler than those homozygous for *ph1b*, which required time-consuming cytological analysis and a progeny test to verify the identification.