ISOLATION, CHARACTERISATION AND QUALITY TESTING OF 1DS/1RS WHEAT-RYE RECOMBINANTS

DWI RATNA ANUGRAHWATI

Thesis submitted to the University of Adelaide
for the degree of Doctor of Philosophy

Discipline of Plant and Pest Science - School of Agriculture and Wine

The University of Adelaide

2006

TABLE OF CONTENTS

PREFACE *vi*
ABSTRACT *vii*
Declaration *ix*
Acknowledgement *x*

CHAPTER 1: GENERAL INTRODUCTION *1*

CHAPTER 2: REVIEW OF LITERATURE *6*
2.1. INTRODUCTION 6
2.2. TRANSFER OF ALIEN GENETIC MATERIAL INTO WHEAT 7
2.2.1. Transfer between homologous chromosomes 7
2.2.2. Transfer between homoeologous chromosomes 11
2.2.2.1. Transfer of whole chromosomes: Amphiploids, Addition and Substitution lines 12
2.2.2.2. Transfer of a chromosome arm or chromosome segment 14
2.3. UTILISATION OF GENETIC MARKERS IN ALIEN GENE TRANSFER **21**
2.4. SEED STORAGE PROTEINS AND THEIR ASSOCIATION WITH PHYSICAL DOUGH PROPERTIES **23**
2.4.1. Protein quantity 24
2.4.2. Protein composition 24
2.4.3. Other characteristics related to the quality of wheat flour 26
2.4.4. Measurement of physical properties of wheat flour and dough 28
2.5. TRANSFERS OF RYE CHROMOSOMES TO WHEAT AND THEIR CHARACTERISTICS **29**
2.5.1. Origin of translocation lines involving 1RS chromosome arm 30
2.5.2. Characteristics of 1RS-containing wheats 32
2.5.2.1. Disease resistance 32
2.5.2.2. Yield 32
2.5.2.3. Quality 33
2.5.3. Attempts to overcome the quality problems associated with 1RS 35
2.5.3.1. Utilisation of 1AL.1RS 36
2.5.3.2. Change of genetic background 36
2.5.3.3. Homoeologous recombination 39

CHAPTER 3: GENERAL METHODS *43*
3.1. PROTEIN MARKERS **43**
3.1.1. Extraction of endosperm protein 43
3.1.2. Electrophoresis 43

3.2.	**DNA-BASED MARKERS**	**44**
3.2.1.	Restriction Fragment Length Polymorphism	44
3.2.1.1.	Small scale DNA isolation	44
3.2.1.2.	Restriction Enzyme Digestion	45
3.2.1.3.	Gel Electrophoresis	46
3.2.1.4.	Southern Transfer	46
3.2.1.5.	Hybridization and Stringency Washing of Membranes	47
3.2.1.6.	Autoradiography	48
3.2.2.	Polymerase Chain Reaction (PCR)	48
3.2.3.	Squash Blot	49
3.3.	**QUALITY ASSESSMENT**	**49**
3.3.1.	Extensograph	50
3.3.2.	Size Exclusion High Performance Liquid Chromatography (SE-HPLC)	50
3.4.	**CYTOLOGY**	**51**
3.5.	**STEM RUST INOCULATION**	**51**
CHAPTER 4:	***ISOLATION AND CHARACTERISATION OF NEW 1DS.1RS RECOMBINANTS***	***53***
A.	**ISOLATION OF ADDITIONAL RECOMBINANTS**	**53**
4A.1.	**INTRODUCTION**	**53**
4A.2.	**MATERIAL AND METHODS**	**54**
4A.2.1.	Plant material and crossing procedure	54
4A.2.2	Marker loci used in the isolation of recombinant lines	56
4A.3.	**RESULTS AND DISCUSSION**	**57**
4A.3.1.	Criterion for detection of wheat-rye recombinants	57
4A.3.2.	Isolation of recombinants showing dissociation of protein markers	57
4A.3.3.	Confirmation of suspected recombinants from families 1, 2 and 3	60
4A.3.4.	Origin of dissociation phenotypes in Family 4	63
4A.3.5.	Further progeny test of dissociation phenotypes from Family 4	66
4A.3.6.	A model to explain this phenomenon	69
4A.4.	**CONCLUSION**	**71**
B.	**REDUCING THE LENGTH OF RYE CHROMATIN IN THE INTERSTITIAL 1DS.1RS RECOMBINANT (DRA-1)**	**72**
4B.1.	**INTRODUCTION**	**72**
4B.2.	**MATERIALS AND METHODS**	**74**
4B.2.1.	Plant material	74
4B.2.2.	Crossing procedure	76
4B.2.3.	Marker loci used for the isolation of recombinants	76
4B.2.4.	Identification of backcross plants heterozygous for 1D and homozygous for ph1b	78
4B.2.5.	Screening for new wheat-rye recombinants	79
4B.2.6.	Further analysis of non-parental phenotypes through progeny test	79

4B.3. RESULTS **81**
4B.3.1. Identification of Plants Heterozygous for Chromosome 1D and Homozygous ph1b 81
4B.3.2. Isolation of Recombinants 82
4B.3.3. Confirmation of Recombinants 86
4B.3.3.1. Putative recombinants T34-6 and T25-5 86
4B.3.3.2. Putative recombinant T6-1 87
4B.3.3.3. Putative recombinant T49-7 89
4B.3.4. Further analysis of putative recombinants T6-1 and T49-7 91
4B.3.4.1. Squash blot test for rye chromatin 91
4B.3.4.2. Restriction Fragment Length Polymorphism (RFLP) 92
4B.3.4.3. Polymerase Chain Reaction (PCR) 92

4B.4. DISCUSSION **96**

CHAPTER 5: YIELD AND QUALITY TESTING OF RECOMBINANT LINES ***99***

5.1. INTRODUCTION **99**

5.2. MATERIALS AND METHODS **102**
5.2.1. Seed Materials 102
5.2.2. Backcrossing and production of homozygous lines 105
5.2.3. Field Experiments 106
5.2.3.1. Yield and quality characteristics of primary recombinants (Experiment 91/1) 110
5.2.3.2. Comparison of the yield and quality characteristics of primary recombinants with their normal 1D sister lines (Experiment 91/2) 110
5.2.3.3. Comparison of the yield and quality characteristics of derived recombinants and their normal 1D sister lines (Experiment 93/1) 111
5.2.3.4. Yield and quality characteristics of primary versus derived recombinants (Experiment 93/2) 111
5.2.4. Quality assessment 112
5.2.5. Statistical analysis 115

5.3. RESULTS **116**
5.3.1. Experiment 91/1. Yield and quality parameters of primary recombinants and one derived recombinant 116
5.3.1.1. Analysis of variance from individual sites 116
5.3.1.2. Analysis of variance combined over sites 116
5.3.1.3. Comparison of individual lines for yield and extensograph parameters 118
5.3.1.4. Variation in SE-HPLC fractions 121
5.3.2. Experiment 91/2. Yield and quality parameters of primary recombinants versus their normal 1D sister lines and controls 126
5.3.2.1. Comparison of individual lines for yield and extensograph parameters 126
5.3.2.2. Variation in SE-HPLC fractions 130

5.3.3. Experiment 93/1. Yield and quality parameters of derived recombinants, their normal 1D sister lines, the recurrent parent Gabo and the translocation 1DL.1RS 133
5.3.3.1. Comparison of individual lines for yield and extensograph parameters 133
5.3.3.2. Variation in SE-HPLC fractions 137
5.3.4. Experiment 93/2. Yield and quality parameters of five primary recombinants, five derived recombinants and controls 140
5.3.4.1. Comparison of individual lines for yield and extensograph parameters 140
5.3.4.2. Variation in SE-HPLC fractions 144

5.4 DISCUSSION 147
5.4.1. Comparison involving primary recombinants 147
5.4.2 Comparison involving derived recombinant lines 149
5.4.3. Extensograph vs HPLC parameters as measures of flour quality 150

CHAPTER 6: GENERAL DISCUSSION 153
The role of secalin in quality 159
The critical test for the role of secalins in dough quality 159
A new line for wheat breeding programs 161

REFERENCES 162

PREFACE

The work presented in this thesis was conducted between 1992-1996. Review of literature, experimental work, all data analysis and first draft thesis were completed during that time. However, due to the restriction to extend the scholarship, this thesis could not be completed during that time and was planned to be finished in Indonesia.

In 2003, the opportunity to come back to Australia with another scholarship at Flinders University enabled some extra time for completion of this thesis. Efforts had been made to recover all the information from printed material and recovered discs. Review of literature in this thesis is not updated. New references are cited and discussed in general discussion where appropriate.

ABSTRACT

The incorporation of agriculturally useful genes into wheat from distantly related and uncultivated relatives has attracted much interest among wheat breeders over recent decades. Cereal rye, especially the short arm of rye chromosome 1R, is known as a useful source of genes for disease resistance and enhanced agronomic performances.

However, despite the numerous beneficial effects associated with these wheat-rye translocation lines, their inferior bread making quality has severely limited the use of this breeding material in some countries, including Australia. Several approaches have been used to eliminate or reduce these deleterious effects on the quality of wheat-rye translocation lines whilst still retaining the disease resistance character.

This research project had two overall objectives. The first study was to investigate the production of new wheat-rye recombinant lines carrying different lengths of rye chromosome in 1DL.1RS translocation lines. Secondly, this study attempted to elucidate the cause of the quality defect in 1DL.1RS wheat-rye recombinant lines by assessing the quality of each recombinant which carried different lengths of the rye 1RS chromosome segments.

The main objective was to produce recombinant lines with a smaller interstitial rye segment carrying the rye stem rust resistance gene and lacking as much other rye chromatin as possible. Following a previously published procedure, an experiment was carried out to select for recombination between the *Sec-1* and closely linked *SrR* loci by inducing a second round of homoeologous recombination in the derived

recombinant DRA-1 which already possessed all the known wheat storage proteins on chromosome arm 1DS.

The number of confirmed wheat-rye recombinants detected was low giving a frequency of 0.1%. Two new recombinants found from this study carry the least amount of rye chromatin resulted from splitting the rye segment of the derived recombinant DRA-1. Recombinant T6-1 (*Sec-1⁻SrR⁺*) will be the most critical recombinant, since it only carries the target gene for stem rust resistance without secalins. On the other hand, R49-7 (*Sec-1⁺SrR⁻*) is not expected to be of direct value in wheat breeding since it contains secalins and does not possess the stem rust resistance gene. However these recombinants are valuable sources for determining whether the secalin gene is a contributing factor towards the quality defect in translocation lines involving 1RS chromosome.

The availability of different types of 1DL.1RS wheat-rye recombinants with different lengths of rye segment has provided a valuable resource to elucidate the factors involved in the low quality, especially the low dough strength, in these wheat-rye recombinants. However, it has not given a simple answer as to the role of *Sec-1* gene in this problem.

In general, the dough quality parameters, especially dough strength of recombinant lines, were significantly better than the original translocation 1DL.1RS, but still less than the normal wheat cultivar Gabo.

In summary, many factors contribute to the bread-making quality of wheat-rye recombinants. The recombinant, which has all the wheat storage proteins with the shortest rye chromatin containing stem rust resistance gene without the inclusion of rye secalins will be the most useful, as has been produced in this study.

Declaration

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

I give consent for this thesis being made available for photocopy and loan.

Adelaide, 9 January 2006

Dwi Ratna Anugrahwati

Acknowledgement

I would like to express my sincere gratitude and appreciation to my supervisor Dr Ken Shepherd for his guidance throughout my study, his constructive analysis of the thesis and his so many help and warm welcome to my family. I am deeply indebted and would like to express my sincere appreciation to my supervisor Dr Ian Dundas whose invaluable help, support, stimulating suggestions and intense encouragement helped me during my research and especially in finishing this thesis.

I am very grateful to Dr Glenn McDonald for his help, understanding and support to the completion of this thesis. I also wish to extend my appreciation to Prof. Mansur Ma'shum and Dr Parman from University of Mataram for their constant encouragement and understanding.

I very much appreciate the practical help of people from Dr Peter Langridge Lab. for molecular work, Cereal Chemistry Lab. of SARDI for quality testing and Dr Colin Jenner for HPLC work. This study was previously funded by AusAID.

Special thanks go to the late Mr Peter Ellis for his kindness and assistance in performing glasshouse, field and cytological work. Many thanks go to all staff in cereal cytogenetics laboratory: Ms Nerida Hunt, Mr Steve Szarvas, Ms Naomi McMillan and Ms Dawn Verlin for their so many help and friendship during my time in the lab.

My deepest and sincere gratitude is due to my late mother and late father who formed part of my vision in life. "I still can feel your love, support and encouragement in every situation". This thesis is dedicated to the memory of them.

This thesis could not have been accomplished without the support of my husband, mas Zubaidi, and my children, Annis, Amalia and Arief who always with me giving warm encouragement and love, helps me to make at the end, the impossible, possible. I believe I owe my deepest thanks to all my family.

CHAPTER 1: GENERAL INTRODUCTION

The incorporation of agriculturally useful genes into wheat from distant and uncultivated relatives has attracted much interest over recent decades. These efforts have been aimed at broadening the genetic variability in wheat since the use of high-yielding pureline varieties has greatly decreased the variability among cultivated lines and in breeding populations (Feldman and Sears, 1981). The greatest value of genes introduced from distant relatives of wheat has been in the protection of the crop from devastation by pathogens.

The relatives of wheat in the genus *Triticum* and more widely the subtribe Triticinae, are known to carry many genes controlling valuable characters such as disease and pest resistance, drought tolerance and improved adaptability to nutrient deficient soils. The incorporation of these desirable genes into wheat has been possible through several strategies. The amount of alien chromatin transferred into wheat varies, ranging from the complete genome in amphiploids such as Triticale (*xTriticosecale* Wittmack) to the translocation of relatively small segments of chromosome arms (Sears, 1973; Friebe *et al.*, 1996).

The transfer of alien chromosome segments to wheat may succeed in the introduction of genes of value to the breeders (many examples listed by Friebe *et al.*, 1996), but unfortunately deleterious effects are also often transferred along with the target gene (McIntosh, 1991). Actually, there are very few cases where the incorporation of alien genetic material into wheat has resulted in a commercially successful cultivar (McIntosh, 1991). The three most successful examples of the commercial use of

introduced alien chromosome segments are (a) the 1BL.1RS#1 translocation containing rye (*Secale cereale* L.) chromatin carrying resistances against stem, leaf and stripe rusts and powdery mildew (Mettin *et al.,* 1973; Zeller, 1973), (b) the 6Ae#1L chromosome segment from decaploid *Agropyron elongatum* carrying stem rust resistance gene *Sr26* (Knott, 1961) and (c) the 3Ae#1L chromosome segment carrying the *Sr24/Lr24* stem and leaf rust resistance genes (Sears, 1973) also from decaploid *Agropyron elongatum.*

Cereal rye is known as a useful source of genes for disease resistance and enhanced agronomic performances principally under low nutrient conditions (Sharma and Gill, 1983). Of special interest is the short arm of rye chromosome 1R which carries genes for several agronomically desirable characters including resistance genes against leaf rust (*Lr26*), stripe rust (*Yr9*), powdery mildew (*Pm8*) and stem rust (*Sr31*), high yield performance and wide adaptability (Zeller, 1973; Rajaram *et al.,* 1983). According to Bartos (1993) the resistance provided by *Lr26*, *Yr9* and *Pm8* has broken down as has *Sr31* (CIMMYT, 1999) due to the occurrence of virulent pathotypes of these diseases. Another example of an agriculturally useful transfer of rye chromatin into wheat is the cultivar “Amigo” which carries the 1AL.1RS#2 chromosome with *Gb2* (greenbug), *Sr* (stem rust) and *Pm17* (powdery mildew) resistance genes.

Substitution of the short arm of chromosome 1R of rye for the short arm of group 1 chromosomes of wheat results in wheat-rye translocation lines has been used extensively in wheat breeding programs around the world. In 1988, about 50% of the CIMMYT advanced breeding lines carried the 1RS chromosome segment (Villareal *et al.*, 1991). Lines carrying the 1BL.1RS and the 1AL.1RS translocation which possess the rye arm from "Petkus" and "Insave F.A." rye respectively, have been used

in many wheat growing areas (Zeller and Hsam, 1983; Rajaram *et al.*, 1983 and Graybosch *et al.*, 1993a). Another type of translocation containing 1RS from Imperial rye was isolated at the Waite Institute about 30 years ago as a 1DL.1RS translocation (Shepherd, 1973). This has a stem rust resistance gene named "SrR", since it is unknown whether *SrR* is the same gene as *Sr31*. With the recently reported failure of resistance conferred by the *Sr31* gene in Africa (CIMMYT, 1999) it will now be possible to test whether the *SrR* and *Sr31* genes are identical, based on their reactions to that virulent strain of stem rust.

However, despite the numerous beneficial effects associated with these wheat-rye translocation lines, many of these cultivars show deleterious effects on dough quality characteristics such as poor mixing tolerance, decreased resistance and stickiness (Dhaliwal *et al.*, 1987; Graybosch, 1990; Martin and Stewart, 1990 and Pena *et al.*, 1990). This inferior bread making quality has severely limited the use of this breeding material containing the 1RS segment in some countries, including Australia. Several approaches are being used to eliminate or reduce these deleterious effects of wheat-rye translocation lines but still retaining the *SrR* gene. By inducing recombination between rye arm 1RS of the translocation line 1DL.1RS and the short arm of homoeologous wheat chromosome 1D, Koebner and Shepherd (1986) were able to isolate recombinants with shortened segments of 1RS chromatin. Two forms of recombinants isolated were of special interest because they appeared to have an approximately reciprocal structure, i.e. I-93 recombinant with a crossover proximal to the stem rust resistance gene and 82-180 recombinant which possessed all the rye arm except a small distal segment replaced by 1DS. These two recombinants were

intercrossed and a derived recombinant with interstitial segment was obtained (DRA-1), following the procedure originally proposed by Sears (1981).

After backcrossing several times with the locally adapted cultivar Gabo, these lines exhibited better dough properties than the original 1DL.1RS, but still less than the recurrent parent Gabo (K. Shepherd, personal communication). It has been theorized that the reduced dough quality of these Gabo lines can be attributed to either the presence of deleterious characteristics on the rye chromosome segment or the loss of genes on the wheat chromosome, which were lost during recombination between the wheat and rye chromosomes. On this basis the best alien genetic transfer would be the one that involves the least amount of alien chromatin transferred along with the target alien genes (Koebner and Shepherd, 1986). Therefore, only a small segment of rye chromosome containing the rust resistance gene needs to be transferred while as much as possible wheat segment needs to be retained. Hence, it is possible that the quality defect associated with these lines may be removed by inducing homoeologous recombination between the rye chromosome segment, thereby further reducing the size of the rye chromosome segment whilst still selecting for the stem rust resistance gene '*SrR*'.

The production of wheat-rye recombinants containing only a very small amount of rye chromosome carrying the stem rust resistance gene was one aim of this study. To test whether the *Sec-1* gene is the cause of the quality defect, or deleterious genes are dispersed along the whole rye 1RS chromosome segment, a series of recombinants with different length of rye chromosome produced earlier at the Waite Institute have been subjected to quality assessment compared to their normal sister lines. The 1DL.1RS recombinants produced in this study may provide further information on the

genetic control of dough quality in wheat-rye translocations, involving 1RS. They can be used further to test the hypothesis that the product from the secalin gene on rye chromosome 1RS may be the cause of quality defect in this translocation, or alternatively several deleterious genes affecting dough quality may be distributed along the rye chromosome.

CHAPTER 2: REVIEW OF LITERATURE

2.1. INTRODUCTION

Genetic variability is an important prerequisite in plant breeding, as it provides a source of useful genes used for plant improvement. However, in recent decades the replacement of the highly variable landraces by higher-yielding pure line varieties has led to the loss of potentially useful genes available to wheat breeders (Frankel, 1970). Because of the erosion of landrace materials, breeders are now trying to expand the gene pool available for wheat improvement by exploiting the genetic variability within the relatives of wheat including progenitors and even the more distantly related genera such as *Aegilops, Secale, Hordeum* and *Thinopyrum*. Bread wheat (*Triticum aestivum* L.) is an allopolyploid made up of three distinct but related genomes, referred to as A, B and D (Morris and Sears, 1967), which are known to have originated from progenitors within the family Gramineae. A consequence of the common ancestry of these three genomes is that hexaploid wheat can withstand chromosome manipulations due to the triplication of genetic material (Sears, 1966). The wild relatives of wheat would be of great potential value in broadening important genetic resources, since they are adapted to a wide range of environments and carry a large reservoir of useful genes including resistance to many diseases and insects (Knott, 1978; Feldman and Sears, 1981; Sharma and Gill, 1983; Friebe *et al.*, 1996).

Since many related species have desirable characters that would be useful if present in wheat and since wheat can be crossed with almost all members of the Triticeae, there has been much interest in incorporating some of the valuable characters from these

related species into wheat (reviewed by Sharma and Gill, 1983; Knott, 1987; Islam and Shepherd, 1991; Friebe *et al.*, 1996).

A number of techniques are now available for incorporating useful alien chromosome segments into wheat, and the amount of alien chromatin transferred is variable. Conventional methods of alien transfer begin with the production of wheat-alien hybrids through a simple crossing procedure. Inter-generic hybridisation has now been carried out between wheat and species in the genera *Aegilops, Agropyron, Secale, Hordeum* and several useful genes have been transferred from these wild relatives into cultivated wheat (reviewed by Sharma and Gill, 1983; Gale and Miller, 1987; Islam and Shepherd, 1991).

This review will first give an outline of several established techniques available for introducing alien genetic variation into wheat and then their contribution to wheat improvement will be discussed along with some special problems associated with these transfers. Finally, emphasis will be given to the incorporation of chromosome arm 1RS of rye, its contribution to wheat improvement and limitations to its use in wheat breeding programs including the possible factors responsible for its limited use so far.

2.2. TRANSFER OF ALIEN GENETIC MATERIAL INTO WHEAT

2.2.1. Transfer between homologous chromosomes

Transfer of useful genes to hexaploid wheat *(Triticum aestivum* AABBDD) from species having at least one homologous genome can be relatively simple. Providing

the gene is on a chromosome of the common genome, it could be transferred by homologous pairing and recombination following hybridisation, backcrossing and subsequent selection for 21 bivalent-forming plants carrying the desirable character (Knott, 1987; Islam and Shepherd, 1991).

A number of wild relatives with homologous genomes including the diploids *Triticum urartu* and *Triticum tauschii (Aegilops squarrosa)* (the donors of A and D genomes of cultivated wheats, respectively), tetraploid *Triticum turgidum* (the immediate progenitor of most tetraploid wheats) and several polyploid species of *Triticum* and *Aegilops,* having either the A or D genome, have been an important source of useful genes in hexaploid wheat breeding (Knott, 1987).

T. tauschii, the D genome progenitor, is considered an important source of valuable genetic material for breeding purposes since it is known to carry resistance to stem and leaf rust, Hessian fly and Greenbug (Kerber and Dyck, 1979). Several genes for disease resistance have been transferred from this species into the D genome of hexaploid wheat by using the amphiploid derived from crosses to *T. turgidum* (Kihara *et al.,* 1957). Such lines are called "synthetic" bread wheats. Genes conferring resistance to leaf rust, including *Lr21* on chromosome 1D for seedling resistance and *Lr22* on chromosome 2D for adult plant resistance have been transferred by Rowland and Kerber (1974). Joppa *et al.* (1980) used *T. turgidum* as a bridging species to form a synthetic hexaploid wheat in order to transfer resistance to Greenbug (*Schizaphis graminum* Rondani) from *T. tauschii.* Martin *et al.* (1982) used a similar approach to produce wheat germplasm resistant to Greenbug biotypes C and E and all known biotypes of Hessian fly *(Mayetiola destructor).*

Other diploid species *(T. monococcum)* (AA genomes) are also known to possess genes conferring resistance to stem rust *(Puccinia graminis* Pers. f. sp. *tritici).* By making direct hybridisation with hexaploid wheat or using tetraploid wheat as a bridging species, the gene for stem rust resistance *Sr22* located on chromosome 7A has been transferred to hexaploid wheat (Kerber and Dyck, 1973) and released in the Australian cultivar Schomburgk (Mackay, 1987). The resistance, however, showed reduced expression in progeny from some crosses with increased ploidy levels. Gerechter-Amitai *et al.* (1971) transferred stem rust resistance from *T. boeoticum* (AA genomes) to tetraploid durum wheat by a similar procedure.

Gene transfers from tetraploid to hexaploid wheat received much attention in early wheat breeding programs, since tetraploid wheat shares two genomes with hexaploid wheat and carries genes for resistance to many diseases, particularly the rusts. Genes transferred from these sources include *Sr11, Sr12, Sr13, Sr14* (Knott, 1962) and *Yr7* (McIntosh *et al.*, 1981).

More extensive work in transferring genes from species having partially homologous genomes with hexaploid wheat has been carried out with *T. timopheevii* Zhuk (AAGG) and *Ae. ventricosa* (DDUnUn). A number of attempts have been made to transfer genes controlling resistance to various diseases from *T. timopheevii* to hexaploid wheat. Allard and Shands (1954) transferred resistance to stem rust, leaf rust and powdery mildew. Since wheat has only limited variation in resistance to Eyespot *(Cercosporella herpotrichoides* Fron, Deighton), a resistance gene with a high level of resistance has been transferred to hexaploid wheat from *Ae. ventricosa* (Doussinault *et al.*, 1983).

Other *Aegilops* species such as *Ae. speltoides* (SS genomes) having at least one partially homologous genome with hexaploid wheat have also been used as a source of useful genes for wheat improvement. Dvorak (1977) transferred genes for leaf rust resistance from *Ae. speltoides* to the wheat cultivars Manitou and Nepawa. These transfers were made by hybridising these two species followed by successive backcrossing to hexaploid wheat with selection of the desired traits. Other useful genes transferred to wheat from *Ae. speltoides* are *Sr32* and *Sr39* (stem rust resistance) (McIntosh *et al.,* 1995) although neither of these genes has been released in a cultivar.

In most studies, gene transfer from species having at least one homologous genome with hexaploid wheat can be achieved either by direct hybridisation between the alien species and wheat followed by subsequent backcrosses to wheat or by using tetraploid wheat as a bridging species and then crossing the hybrid or its induced amphiploid with hexaploid wheat.

Direct hybridisation allows a rapid genetic transfer from species having homologous genomes. If the immediate progenitors such as *T. urartu* and *T. tauschii,* the diploid donors of the A and D genomes, and *T. turgidum*, the tetraploid progenitor of wheat, are used as a source of desirable genes to be transferred, direct hybridisation is usually possible since they have an almost complete homology and there will be very little adverse genetic interaction compared with polyploids with only one homologous genome.

Although many successful transfers of desirable genes have been made from species having a homologous genome with wheat, a frequent problem encountered in achieving this transfer is the low crossability, F_1 embryo abortion and the reduction or

even total loss of expression (due to inhibitory effects or other types of epistasis) of the desirable traits in hexaploid wheat (Knott, 1987).

2.2.2. Transfer between homoeologous chromosomes

Several genera more distantly related to wheat including *Secale, Hordeum*, and *Agropyron* are known to have genes controlling many useful characters such as disease, insect and stress resistance, but they do not have a genome which is homologous or partly homologous to the A, B or D genomes. These distant relatives provide a rich source of genetic variation for broadening the genetic resources for wheat improvement. However, the more distant their relationships, the more difficult it will be to make the transfer of genes into wheat (Islam and Shepherd, 1991).

Much attention has been given to developing methods for transferring useful genetic material into wheat from its distant relatives. However, due to the presence of genes *Kr1* and *Kr2* in some wheat cultivars that regulate crossability with distant relatives, it may be difficult to hybridise them and therefore to transfer alien genetic material (Snape *et al.*, 1979 Falk and Kasha, 1981). Riley and Chapman (1967) found that wheat cultivar Chinese Spring has the recessive alleles *kr1* and *kr2* at both crossability loci and therefore, this cultivar has frequently been used to produce the initial hybrids with alien species.

Over the last 35 years a range of methods for transferring valuable characters from alien species to wheat have been developed and they are outlined below.

2.2.2.1. *Transfer of whole chromosomes: Amphiploids, Addition and Substitution lines*

In most alien gene transfers, the first step is to produce suitable amphiploid stocks in order to introduce the alien chromosome carrying the desirable gene into a wheat background. Amphiploids are usually fertile and resulted from spontaneous or induced chromosome doubling of sterile interspecific or intergeneric hybrids (Gale and Miller, 1987; Knott, 1987).

However, plants possessing the combined genomes of wheat and alien species are quite different morphologically and physiologically from wheat and are usually cytologically unstable and low yielding. Moreover, since the alien species usually do not have a genome homologous to those in wheat, the amphiploid shows mostly bivalents and few if any multivalents at meiosis. Therefore, usually no crossing over will occur between the wheat and alien chromosomes in the amphiploid (Knott, 1987). The successful amphiploid Triticale (*X Triticosecale* Wittmack) derived from wheat-rye hybrids has been used widely in commercial agriculture (Gupta and Priyadarshan, 1982). Although these plants may carry and express the desirable alien gene, they are not wheat-like and represent a new species.

The amphiploid may be used to produce an addition line that carries only one alien chromosome pair carrying the gene controlling the desirable trait. This line can be produced by crossing the alien species with wheat, then backcrossing either the polyhaploid or derived amphiploid to the wheat parent. After selecting the desired character during a sequence of several backcrosses followed by selfing, the alien addition line can be selected. A set of addition lines produced in this way from an

amphiploid was first isolated by O'Mara (1940). Since then, many different protocols have been used to produce wheat-alien addition lines including *T. aestivum-Aegilops* (Kimber, 1967), *T. aestivum-Agropyron* (Dvorak and Knott, 1974) and *T. aestivum - H. vulgare* (Islam and Shepherd, 1981; 1990).

Addition lines are usually more wheat-like than the amphiploid and usually show good vigour but tend to be cytologically unstable (Knott, 1987). Therefore, they require cytological checking after each generation of selfing (Miller, 1984), due to the failure of the alien chromosomes to pair at meiosis, leading to their subsequent loss (Islam and Shepherd, 1991). Because of their instability and low yield, the addition lines have no direct use in commercial agriculture. However, they have proved very useful in gene mapping studies and they also form the starting point for further transfer of alien genetic material into wheat (Gale and Miller, 1987; Islam and Shepherd, 1991).

Substitution lines, which possess a pair of alien chromosomes substituted for a pair of homoeologous wheat chromosomes, have certain advantages over addition lines, because they are more stable and allow large-scale seed multiplication (Gale and Miller, 1987). Substitutions are produced by crossing a specific wheat monosomic line with the appropriate addition line, followed by selfing and selection for wheat-alien substitution (Sears, 1972).

The vigour and fertility of substitution lines depends on the degree of compensation by the alien chromosome for the replaced wheat chromosome (Knott, 1987). If an alien chromosome shows good compensation for the loss of the wheat chromosome pair, this provides evidence that the particular alien chromosome substituted is homoeologous to the deleted wheat chromosome. Since substitution combines the loss

of wheat chromatin and the addition of an alien chromosome, the phenotypic effects of these substitutions are sometimes more severe than found in alien additions (Riley and Kimber, 1966). Usually the substitution lines are agronomically inferior to the wheat recipient parents, but a 1R rye chromosome substituted for wheat 1B has been released as a commercial variety to exploit the disease resistance carried by 1R and the high degree of compensation shown by these chromosomes (Zeller, 1973).

2.2.2.2. Transfer of a chromosome arm or chromosome segment

The undesirable agronomic features often resulting from transferring a whole alien chromosome are likely to be lessened if only the arm or a portion of it carrying the desirable trait is transferred to wheat. The methods for doing this are described below.

2.2.2.2.1. Ionizing radiation

The first method tried to reduce the amount of undesirable alien chromatin transferred to wheat was based on using ionising radiation to induce chromosome breakage and rejoining. Chromosome breakages in wheat and alien chromosomes may rejoin spontaneously to produce wheat-alien translocation lines. The first successful attempt to transfer alien genetic material into wheat by using irradiation was made by Sears (1956), who irradiated a wheat-*Ae. umbellulata* addition line before meiosis and succeeded in transferring a segment of *Ae. umbellulata* Zhuk chromosome 6U carrying resistance to leaf rust (*Puccinia recondita* Rob. ex Desm.) to chromosome 6BL of wheat.

Later other workers used a similar approach to transfer rust resistance from *Agropyron* spp and rye to wheat by using various types of irradiation including X rays, Gamma rays and neutrons. Sharma and Knott (1966) used Sears' procedure to transfer leaf rust resistance from *Agropyron elongatum* to *Triticum* employing X rays and thermal neutrons as the radiation agents. Also resistance to wheat leaf rust (*Puccinia recondita*) and powdery mildew (*Erysiphe graminis* tritici March) have been transferred from rye to wheat following X irradiation (Driscoll and Jensen, 1964).

There are several problems associated with this procedure for inducing translocations. It requires a large amount of cytological work and the percentage of successful translocations obtained is low, since the chromosome break points induced by radiation are thought to be randomly distributed and the rejoining of the segments can involve non-homoeologous chromosomes, resulting in translocations which are deleterious. Only those translocations which have involved homoeologous chromosomes appear to have been useful in wheat breeding.

Another disadvantage of the use of ionizing radiation pointed out by Sears (1973) is that there is no assurance that the transferred alien segment is fully equivalent to the wheat segment replaced. Therefore, it may involve a deficiency of one segment and duplication of another segment of the two homoeologues.

Moreover, as also pointed out by Sears (1977) the most frequent outcome will be the replacement of a terminal rather than an interstitial segment of a wheat chromosome with a segment of the alien chromosome. In order to produce interstitial translocations where the desired alien segment replaces an equivalent segment of homoeologous chromosome, several simultaneous breakages and rejoinings are required. Another

disadvantage of radiation induced transfers is that they are likely to have a deleterious effect on the viability of gametes carrying them, resulting in reduced transmission of the chromosome carrying the translocation to progeny (Islam and Shepherd, 1991).

Despite these disadvantages, several successful transfers have been produced between alien and wheat chromosomes. The transfer of a segment of chromosome 6Ae#1L of *Agropyron elongatum* (Host) P.B. carrying the gene conferring resistance to stem rust (*Sr26)* to chromosome arm 6A of wheat represents the most successful translocation using ionizing radiation and it has been used extensively in Australian wheat breeding programs (McIntosh *et al*., 1995).

2.2.2.2.2. Robertsonian Translocations

The frequent rate of misdivision of centromeres in wheat univalents and some alien univalents in the same meiocyte at Anaphase I, provides an opportunity for the spontaneous rejoining of arms from the wheat and alien chromosomes, resulting presumably from fusion of centromeres (Sears, 1972). Therefore, the replacement of one arm of the wheat chromosome with the corresponding alien chromosome arm, represents another approach for the incorporation of desirable alien genetic material into wheat with less than a whole alien chromosome being added (Knott, 1971). Furthermore, the effect of alien genetic transfer in translocation lines is lessened both because the loss of wheat chromatin and the gain of alien chromatin are reduced compared to whole chromosome replacement (Koebner and Shepherd, 1988).

Many Robertsonian translocations involving wheat-rye chromosomes have been reported (Shepherd, 1973; Zeller, 1973; Mettin *et al*., 1973; Lawrence and Shepherd, 1981; Zeller and Hsam, 1983). Although some wheat-rye translocations have been

shown to reduce yield (Driscoll, 1981; Zeller and Hsam, 1983) or produce grain with a quality defect (Koebner *et al.*, 1984), some CIMMYT lines which contain the 1BL.1RS translocation have been associated with high yield over a range of environments (Rajaram *et al.*, 1983; Zeller and Hsam, 1983). However, this method of producing wheat-alien translocation chromosomes is less than ideal since a large segment of undesirable and possibly deleterious alien genetic material remains even with just one arm transferred.

2.2.2.2.3. Transfer using induced homoeologous pairing

The discovery by Okamoto (1957) and Riley and Chapman (1958) that the failure of pairing between wheat and related homoeologous chromosomes is due to the presence of the *Ph1* gene on chromosome arm 5BL has had a large impact on alien gene transfers. In the absence of chromosome 5B, pairing and recombination can be induced between homoeologous wheat chromosomes and between alien chromosomes and homoeologous wheat chromosomes (Riley, 1966).

This knowledge of the genetic control of pairing in wheat, has provided more effective methods for transferring genes from alien chromosomes and has largely eliminated the use of the radiation procedure, except where the alien chromosome is so dissimilar to any wheat chromosomes that homoeologous pairing does not occur or only very rarely.

There are three procedures that have been used to induce pairing between wheat and alien chromosomes: (1) production of nullisomy for chromosome 5B; (2) use of species carrying a suppressor of *Ph1* and (3) use of *ph* mutants.

The simplest way of producing wheat-alien genotypes nullisomic for 5B is to pollinate a wheat plant monosomic for chromosome 5B with pollen from the alien species (Sears, 1972). Homoeologous pairing should occur in the F_1 plants which are deficient for chromosome 5B and after backcrossing to wheat, recombinant chromosomes can be selected. Using this approach, Riley (1966) and Joshi and Singh (1978) transferred genetic material from *Ae. bicornis* (Forsk. Jaub. and Spach) and *S. cereale*, respectively, into wheat.

Another method developed by Sears (1966) to produce plants deficient for chromosome 5B was to use nullisomic 5B tetrasomic 5D stocks. By crossing monosomic 5B wheat with a wheat-alien substitution line, three quarters of the progeny should have 19"W + 1' 5B + 1'W + 1' alien. After pollinating these F_1 plants with nulli-5B tetra-5D, progeny selected which possess 1'W + 1' alien monosome, are trisomic 5D but lack 5B and show multivalent pairing, and the selected plants are crossed with wheat (or just allowed to self pollinate). Homoeologous crossing over in such plants should allow the desirable characters from the alien species to be transferred to a related wheat chromosome by recombination. Sears (1973) has used this procedure to transfer leaf rust *(Puccinia recondita)* resistance to wheat from wheat-*Agropyron* derivatives.

Although this method has proved to be valuable in achieving the transfer of alien segments carrying desirable genes to wheat chromosomes, one problem encountered with hybrids deficient for chromosome 5B is their low fertility resulting in few progeny seeds per plant. Therefore many critical plants need to be selected to produce a large enough population of progeny seeds for selecting the desired alien transfer (Sears, 1981; Koebner and Shepherd, 1986).

It has been found that certain lines of *Ae. speltoides* Tausch and *Ae. mutica* Boiss carry a dominant suppressor of the inhibitory effect of *Ph1* gene on chromosome pairing when they are crossed to wheat. Therefore, it is possible to make direct transfer of genetic material from these two *Aegilops* species to wheat by making F_1 hybrids and then backcrossing to wheat. Knott and Dvorak (1981) transferred leaf rust resistance from *Ae . speltoides* using this procedure and after backcrossing rust resistant progeny four or five times to wheat, four of the eleven lines selected had acceptable agronomic and quality characters.

Ae. speltoides and *Ae. mutica* can also be used indirectly to transfer genes from another alien species to wheat. In this case the first cross is made between the wheat-alien addition line carrying the desired gene and *Ae. speltoides* (or *Ae. mutica*). The hybrid which will allow homoeologous pairing at meiosis is then crossed and backcrossed several times to wheat followed by selection for recombinants carrying the desired character among progeny in each generation. Riley *et al.* (1968) have used this method to transfer a segment of *Ae. comosa (T. comosum)* chromosome carrying a gene for stripe rust resistance *(Yr 8)* to wheat.

The main problems with this approach are the low fertility of the initial F_1 and the unbalanced chromosome constitution of the early backcross generations. Also it is important that the *Aegilops sp.* used as a bridging species in the first cross does not carry the character transferred from the desirable species, since it is possible that both *Aegilops* and the second alien species transfer their genetic material (Knott, 1987).

In order to maintain satisfactory plant vigour and fertility, it is preferable to utilise *ph* mutants to induce homoelogous crossing over. Three *ph* mutants, *ph1b* and *ph2b*

which are deletions of *Ph1* on chromosome 5B and *Ph2* on chromosome 3D, respectively (Sears, 1977 Sears, 1982) and *ph2a* with the same effect as Sears' mutation (Wall *et al.*, 1971) are now available in wheat and can be used to induce homoeologous pairing.

The first step in utilising the *ph1b* mutant is to cross a wheat-alien substitution line with monosomic 5B to obtain approximately 75% of progeny which are triple monosomic (19"W + 1'W + 1' alien + 1'5B) having 5B, the alien chromosome and its homoeologue present as univalents. These triple monosomics are then crossed as females with the *ph1b* mutant line, to obtain progeny which are 19"W + 1'W + 1' alien + 1'5B *ph* and which will allow homoeologous pairing between the 1'W and 1' alien chromosomes at meiosis.

Sears (1981) transferred segments of chromosome 3Ae#1L (also called 3Ag) and 7Ae#1L (also called 7Ag) of *A. elongatum* carrying genes for rust resistance into wheat chromosome 3D and 7D, respectively, by induced homoeologous pairing. The problem encountered with the 7D-7Ae#1 transfer line was yellow flour colour carried by the *Agropyron* chromosome. Therefore, a smaller amount of *Agropyron* chromatin was required to obtain rust resistance without interference from genes affecting flour colour. Since the gene for leaf rust resistance is located away from the centromere, two procedures were proposed to achieve an interstitial transfer. If two types of translocation chromosomes are available, (i.e. proximal and distal crossover with each carrying the required alien gene) derived recombinants carrying the desired alien chromatin could be obtained by intercrossing these two transfers. Another way to produce an interstitial transfer is by inducing a second round of recombination between the transfer chromosome and a homoeologous wheat chromosome. This procedure

should be used if the exchanges are all proximal or distal to the critical gene (Sears, 1983).

Much effort has been given to incorporate characters from alien species that are simply inherited and controlled by a single gene such as disease and insect resistance, since these characters can be screened for relatively easily in the progeny. The introduction of alien characters that are more complex in inheritance such as tolerance to salinity or stress are more difficult to achieve since these characters are usually controlled by several genes on more than one chromosome. Furthermore, it is difficult to assess the potential of wild alien species for quantitative characters since they often have very different morphology and growth patterns from wheat (Gale and Miller, 1987). Nevertheless, Riley (1966) showed that it is possible to transfer genetic material from *Ae. bicornis* which affect both qualitative and quantitative characters in the recipient wheat.

2.3. UTILISATION OF GENETIC MARKERS IN ALIEN GENE TRANSFER

Recent improvement in the success of selecting alien genetic transfers into wheat has depended largely on the availability of markers present on wheat and alien chromosomes. Isozyme and protein (biochemical) markers were the first genetic markers to be used in identifying, characterising and selecting alien genetic material in alien-derived wheats (reviewed by Hart and Tuleen, 1983; Islam and Shepherd, 1991). These biochemical markers have been used to isolate and characterise the transferred

chromosomes (Hart *et al.*, 1980; Koebner and Shepherd, 1986; Koebner *et al.*, 1986; Islam and Shepherd, 1991).

Improved cytological methods for wheat chromosomes have also facilitated the identification of the transferred chromosomes (Lapitan *et al.,* 1986; Friebe *et al.*, 1991). Detection of the presence of entire chromosome, translocated arms and recombinant segments of rye were achieved by utilising cytological and biochemical markers (Friebe and Larter, 1988; Asiedu *et al.*, 1989; Schwarzacher *et al.*, 1992). Gupta and Shepherd (1992) used one-dimensional SDS-PAGE to analyse the grain protein composition of lines involving 1AL.1RS, 1BL.1RS and 1DL.1RS as well as cultivars with unknown pedigree. They found that protein electrophoresis revealing rye secalins provide a rapid and reliable technique for detecting wheats containing the 1RS chromosome arm. The isozyme glucose-6-phosphate-isomerase was used to distinguish 1BL.1RS and 1AL.1RS translocation lines (Hassett *et al.*, 1993). A monoclonal antibody specific for rye secalins has also been used to identify wheats carrying the 1RS rye chromosome arm (Howes *et al.*, 1989; Graybosch *et al.*, 1993b; Seo *et al.*, 1995).

The increased availability of genetic markers present in wheat and alien chromosomes has proved very useful to detect the presence of alien chromatin and facilitates the characterisation of the chromosome structure present in recombinants. The recent development of numerous DNA-based markers including RFLP and PCR markers is expected to facilitate even more the transfer of alien chromatin into wheat (Gale and Sharp, 1988). The advantage of these new markers is that their variation is abundant and they are highly polymorphic between wheat and alien species allowing more precise analysis of the structure of the chromosomes involved. RFLP analysis has

been used to characterise the introgressed alien chromatin (Sharp *et al.,* 1988; Rogowsky *et al.*, 1991, 1993). Detection of the presence of rye chromatin in the translocation lines 1BL.1RS and 1AL.1RS and visualisation of the rye chromosome segments in recombinants has recently been achieved using *in situ* hybridisation (Schwarzacher *et al.*, 1992; Islam-Faridi and Mujeeb-Kazi, 1995; Friebe *et al.*, 1996).

Attempts are also being made to introduce only a single gene of the alien species into wheat chromosomes by transformation. However, the exploitation of these methods in wheat will depend on being able to regenerate plants from protoplast culture of transformed cells (May and Appels, 1987). Recently several publications have reported success in producing fertile, transgenic wheat via biolistic or particle bombardment in transferring herbicide resistant genes (BAR) from *Streptomyces hygroscopis* (Vasil *et al.*, 1992 and Nehra *et al.*, 1994).

2.4. SEED STORAGE PROTEINS AND THEIR ASSOCIATION WITH PHYSICAL DOUGH PROPERTIES

The importance of understanding the molecular basis of bread-making quality of wheat flour has led to many investigations correlating various components of the grain and baking quality. Over the last 20 years, there have been many investigations aimed at a better understanding of these relationships.

The proteins in the wheat endosperm have long been known to be important in influencing the bread-making quality of wheat flour. These storage proteins are

believed to be responsible for the viscoelastic properties of a dough (Graveland and Henderson, 1987; Payne, 1987), which is a prerequisite for bread-making.

The bread-making quality of flour is a complex trait controlled by many genes, but it is widely accepted that the amount and composition of proteins in the flour have the most significant effects on its end-use quality. It is now well established that differences in bread-making quality between wheat cultivars may be due to qualitative and quantitative differences in the gluten fraction.

2.4.1. Protein quantity

Increase in flour protein content is generally associated with increased sedimentation volume (Axford *et al.*, 1979) and bread loaf volume (Bushuk *et al.*, 1969). Brunori *et al.* (1989) showed that dough strength is positively correlated with amount of protein in the flour. They suggested that high protein content tended to favour a balanced dough tenacity/extensibility ratio. In general, protein content is poorly heritable and largely influenced by environmental conditions such as type of soil, climate and nutrition levels.

2.4.2. Protein composition

Gluten protein fractions, especially the glutenins and gliadins which make up about 80% of the total seed proteins are of fundamental importance in determining some aspects of quality. However, it is not clear which of the two types of gluten proteins is the most important. There are several conflicting reports on whether glutenin or

gliadin is responsible for the various parameters of bread-making quality. While Wrigley *et al.* (1982), Branlard and Dardevet (1985a) have emphasized the role of gliadins, Payne *et al.* (1984), Moonen *et al.* (1982) and Lagudah *et al.* (1987) indicated that the glutenin component is the most important determinant of the quality of wheat flours for bread making. Variation in the fractionation procedures used gave different amounts of protein in the respective fractions, however, generally it is found that loaf volume depends more on the glutenin than the gliadin proteins (Orth and Bushuk, 1972; MacRitchie, 1978; Khan *et al.*, 1993).

Brunori *et al.* (1989) showed that a high glutenin-gliadin ratio correlated with good rheological properties. However, in their study Dexter and Matsuo (1977) suggested that differences in the chemical composition of the glutenin fraction or other chemical constituents might override the glutenin-gliadin ratio as the determining factor of flour quality.

In terms of physico-chemical properties, Graveland and Henderson (1987) pointed out that the strength of the gluten is affected by the size and the concentration of glutenin molecules. They suggested that the larger and more concentrated the glutenin molecules were, the more contact points occurred between molecules per unit volume of dough. Moreover, it was proposed that gliadin functions as a linker between glutenin molecules, thus it disrupts the direct contact between glutenin molecules resulting in a reduction of gluten strength. However, removing all gliadins is known to result in a crumbly flour. Therefore both glutenin and gliadin are important in maintaining the gluten consistency. The polymeric glutenin is considered as the most important component for dough elasticity and strength, while gliadin is assumed to contribute to gluten viscosity (Miflin *et al.*, 1983). This result agreed with the finding

of Branlard and Dardevet (1985b) that gliadin and glutenin interact to influence the expression of dough strength and extensibility.

2.4.3. Other characteristics related to the quality of wheat flour

Many attempts have been made to determine the genetical basis for variation in bread-making qualities between varieties. The electrophoretic patterns of seed endosperm proteins of wheat have been shown to be very useful for identifying different quality types. These proteins are strongly inherited and generally are not influenced by the environmental conditions. Variation in band patterns of gliadins and glutenins has been observed to correlate with differences in bread-making quality of wheat flours (Payne *et al.*, 1979; Wrigley *et al.*, 1982, Moonen *et al.*, 1982, Payne, 1987 and Gupta *et al.*, 1994). Different bands controlled by the *Glu-1* loci controlling high molecular weight (HMW) glutenin subunits have been associated with differences in bread-making quality, where bands 5+10 controlled by *Glu-D1* locus are more important than 2+12 for dough resistance (Payne *et al.*, 1979; Lawrence *et al.*, 1987 and Brunori *et al.*, 1989). Wrigley *et al.* (1982) showed an association between specific gliadin patterns and dough strength, in which gliadin band 19 corresponding to gliadin 45 in durum wheats in mobility was associated with strong dough properties (Wrigley *et al.*, 1982). Glutenins have been shown to be responsible for various parameters in quality characteristics. Rogers *et al.* (1989b) stated that about 30% of the variation in bread-making quality could be accounted for by variation in *Glu-1*-coded HMW glutenin subunits.

Low molecular weight (LMW) glutenin subunits which are coded by genes at the *Glu-3* loci are tightly linked with the *Gli-1* locus on the short arms of group 1 chromosomes of wheat (Payne *et al.*, 1984; Singh and Shepherd, 1988; Gupta and Shepherd, 1988). An association between these LMW subunits and bread-making quality has also been detected (Gupta *et* al., 1989).

Recently high performance liquid chromatography (HPLC) has been used to quantify the relative proportion of the main protein components of wheat endosperm by measuring the area under curve of the peaks. This procedure is now widely used in quality assessment because of its speed, semi-automation, quantitative capabilities, and small sample requirement (reviewed by Bietz, 1986). The reverse phase (Huebner and Bietz, 1987; Marchylo *et al.*, 1989) and size-exclusion (Dachkevitch and Autran, 1989; Lundh and MacRitchie, 1989) HPLC patterns of bread-wheat were used to predict bread-making quality of wheat. By using Size-Exclusion HPLC and sonication for extraction, Singh *et al.*, (1990a) and Singh *et al* (1990b) have been successful in completely extracting unreduced compounds of gluten flour and found that the relative quantity of glutenin was positively and significantly correlated with bread-making quality of many diverse wheat genotypes. This method has also been used to identify wheat lines containing the 1BL/1RS translocation (Lookhart *et al.*, 1991).

The advantage of these indirect tests for predicting bread-making quality of wheat flour is that they can be used in early generation screening, since small sample size or even a single kernel is sufficient for these tests.

2.4.4. Measurement of physical properties of wheat flour and dough

The rheological properties of wheat flour such as extensibility, resistance to stretching or mixing tolerance are generally measured by rheometers such as the farinograph, extensograph and mixograph.

The farinograph is generally used to measure the water absorption of flour to reach 500 Brabender Unit (BU) consistency during mixing prior to the extensograph test. The extensograph involves direct stretching of a dough until it breaks and provides measures of extensibility (E) and resistance to stretching (Rmax). The mixograph has been used routinely in USDA and some Australian laboratories to assess gluten strength (Finney and Shogren, 1972). It measures the rate of dough development time, resistance of the dough to mixing and the tolerance of the dough to extended mixing. Compared with the farinograph and extensograph, this instrument is less reliable due to the lack of temperature control during the testing procedure.

In general, these rheological tests provide a direct evaluation of the physical properties of dough. Controlled conditions are required to improve their reliability, since they are sensitive to variation in temperature. The most reliable and direct test for evaluating bread-making quality of wheat flour is the baking test. Bread loaf volume is related to the gluten strength and flour containing strong gluten will produce an extensive viscoelastic matrix during dough formation and give a loaf with large volume after baking.

2.5. TRANSFERS OF RYE CHROMOSOMES TO WHEAT AND THEIR CHARACTERISTICS

Cereal rye *(S. cereale)* offers much potential for increasing the genetic variability and germplasm resources for cultivated wheats. It has 7 pairs of chromosomes, and their homoeologous relationships with wheat chromosomes have been determined (Shepherd, 1973; Miller, 1984; Naranjo and Fernandez-Rueda, 1991).

The incorporation of several individual rye chromosomes or segments into wheat has been reported. For example the transfer of a segment of rye chromosome 2R carrying genes conferring resistance to powdery mildew *(Pm7)* and leaf rust *(Lr25)* onto 4BS[1] chromosome of wheat has been recorded in 4B.2R translocation Transec (Driscoll and Jensen, 1964). However, due to the deleterious effects of the translocation on seed size and grain yield, this transfer has not been successfully exploited in a cultivar (Driscoll, 1981). Friebe *et al.* (1990) found that 2BS.2RL translocation lines which carry a rye segment derived from "Chaupon" rye confer resistance to Hessian fly. This resistance was also found in a wheat-rye translocation involving chromosome 6RL from "Balbo" rye (Hatchett *et al.*, 1993. The genes conferring the ability of plants to tolerate Cu-deficient soils, which are located on the long arm of chromosome 5R in 5RL-4A and 5RL-5BS translocations, have been introduced into some Australian wheat cultivars (Graham *et al.*, 1987), but not used commercially because of quality problems. Resistance to cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) is controlled by a single major gene located on the long arm of rye chromosome 6R (Asiedu *et al.*, 1990). Attempts have been made to transfer this gene into wheat through

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[1]All references to chromosome 4A and 4B utilise the new nomenclature adopted at the 7th International Wheat Genetics Symposium (IWGS), Cambridge.

induced homoeologous recombination using Sears' *ph1b* mutant (Dundas *et al.*, 1988; 2001), but suitable wheat-rye recombinant chromosomes have not been identified so far.

2.5.1. Origin of translocation lines involving 1RS chromosome arm

Chromosome 1R of rye is one of the most extensively used sources of alien genetic material in bread wheat breeding. Substitution of the short arm of chromosome 1R of rye for the short arm of its homoeologous chromosomes of wheat by Robertsonian fusion has resulted in several wheat-rye translocation lines which have been used widely in wheat breeding programs around the world (Zeller and Hsam, 1983). Several European wheat cultivars carry translocations between chromosome 1BL of wheat and 1RS of "Petkus" rye. The wheat cultivars "Kavkaz" and "Avrora" ("Aurora") were the first ones detected carrying spontaneous intergeneric translocations derived from the rye cv. "Petkus" (Mettin *et al.*, 1973). They had been released as new wheat cultivars in the German Democratic Republic (German D. R.) in 1972. Since then, many other cultivars possessing 1BL.1RS have been produced mostly having Kavkaz or Aurora in their pedigree. This translocation has been reported to be present in numerous cultivars of wheat widely grown in many countries around the world including the USSR, Mexico, Pakistan, India, South Africa, European countries, and some African and South American countries (Mettin *et al.*, 1973 Zeller, 1973; Zeller and Hsam, 1983 and Lukaszewski, 1990). In Mexico, the successful high yielding and disease resistant CIMMYT cultivar "Veery" was also derived from Kavkaz (Merker, 1982; Rajaram *et al.*, 1983).

In the German D. R. appoximately 30% of the breeding lines were reported to possess the 1BL.1RS translocation in 1984 (Mettin and Bluthner, 1984), while in 1988, almost 50% of the CIMMYT advanced breeding lines carried this translocation (Villareal *et al.*, 1991). In Australia, the 1BL.1RS translocation has been incorporated into a few cultivars by backcrossing (Dhaliwal *et al.*, 1990), but because of quality problems the translocation has only been released in the biscuit wheat cv. Grebe.

Translocations 1DL.1RS and 1BL.1RS derived from "Imperial" rye were detected and produced at the Waite Institute in 1973 in a Chinese Spring background. They carry genes for resistance to all races of stem rust in Australia (Singh *et al.*, 1990c). These translocation chromosomes carrying the short arm of 1RS of Imperial rye resulted from centric fusion between 1RS and chromosome arms 1DL and 1BL of Chinese Spring wheat, respectively (Shepherd, 1973). Subsequently the translocations have been backcrossed to several locally adapted wheat cultivars including Warigal and Gabo. So far, this translocation has not been exploited in wheat breeding programs.

Besides these 1BL.1RS and 1DL.1RS translocations, a 1AL.1RS has been derived from rye cultivar "Insave" (Sebesta and Wood, 1978). Wheat cv. Amigo which was released in 1976 (Sebesta *et al.*, 1995) possesses a 1AL.1RS translocation derived from X-irradiated progeny of crosses between triticale Gaucho and advanced wheat breeding lines (Sears, 1972). The triticale Gaucho is derived from wheat cv. Chinese Spring X rye cv. Insave. This cultivar has been used in several hard red winter wheat breeding programs in USA (Sebesta and Wood, 1978; Graybosch *et al.*, 1993a).

2.5.2. Characteristics of 1RS-containing wheats

2.5.2.1. Disease resistance

The main reason for the utilisation of 1R in the form of substitution or translocation lines is the disease resistance they confer on wheat. The short arm of rye chromosome 1R from "Petkus" rye is known to carry genes *Pm8, Yr9, Lr26,* and *Sr31* controlling resistance to four major wheat diseases viz. powdery mildew *(Erysiphe graminis)*, stripe rust *(Puccinia striiformis)*, leaf rust *(Puccinia recondita)* and stem rust *(Puccinia graminis)*, respectively (Zeller, 1973; Zeller and Hsam, 1983). The translocation line cv. Amigo carries gene *Gb2* for resistance to greenbug (*Schizaphis graminum*) biotypes B and C and possess genes for resistance to powdery mildew, stem rust and leaf rust (Sebesta and Wood, 1978; Hollenhorst and Joppa, 1983). The *et al.* (1991) found that there were two different genes for resistance to *P. graminis* in Amigo; one is associated with the 1RS rye chromosome segment, and the other with an *Agropyron*-derived 3Ae#1 (3Ag) segment. Recently, GRS 1201 which is resistant to biotypes B, C, E, G and I of *S. graminum* derived from Insave rye was released (Porter *et al.*, 1993).

2.5.2.2. Yield

Lines carrying the 1BL.1RS and 1AL.1RS translocations have also been found to have broad adaptation (Zeller and Hsam, 1983), high yield performance and tolerance to environmental stress (Rajaram *et al.*, 1983). A positive effect of the 1RS translocations on yield found by several researchers is another reason for the utilisation of these translocation lines in breeding. Dhaliwal *et al.* (1987) compared the

agronomic characteristics of backcross derivatives of the 1BL.1RS lines and their sister lines and no significant effects were found on the grain yield and 1000-grain weight. Villareal *et al.* (1991) found that lines with the 1BL.1RS translocation had a higher above-ground biomass, produced more spikes per square metre and had higher 1000-grain weight, but lower harvest index compared to the normal genotypes of certain spring wheats. No significant difference was found for plant height, grain number per square metre or per spike and grain yield. Fenn *et al.* (1994) also found that 1000-kernel weight was higher in the cultivars containing this translocation. Schlegel and Meinel (1994) also found that the translocation line significantly outyielded the control plants, which was mainly due to increase in spikelet fertility. The 1BL.1RS translocation lines produced comparable grain yield, were also found to produce 4% higher kernel weight compared to homozygous 1B, but 5% less spikes per square metre (Moreno-Sevilla *et al.*, 1995b).

2.5.2.3. *Quality*

Despite the beneficial effects of the wheat-rye translocations involving 1RS rye chromosome arm, several reports have shown that lines carrying this translocation have reduced bread-making quality compared with normal wheat.

The disease-resistant European wheat cultivars carrying wheat-rye 1R(1B) substitution or 1BL.1RS translocations (Mettin *et al.*, 1973; Zeller, 1973) have been reported to have inferior flour quality including doughs with low resistance and stickiness, poor mixing tolerance and reduced sedimentation volume and poor crumb structure in the

baked bread (Zeller and Hsam, 1983; Moonen and Zeven, 1984; Graybosch *et al.*, 1990; Carver and Rayburn, 1995).

In an attempt to exploit the stem rust resistance transferred with 1RS of Imperial rye, Shepherd (1977) found that backcross derivatives involving 1DL.1RS recombinants in local cultivars have very low dough maximum resistance in the extensograph, preventing their release as cultivars. Similar features have been observed with 1BL.1RS-derived wheats in Australian wheat breeding programs, particularly dough stickiness after mixing (Dhaliwal *et al.*, 1987, 1988 Dhaliwal and MacRitchie, 1990). Martin and Stewart (1986) also reported pronounced dough stickiness and a lack of mixing tolerance in 1BL.1RS translocation lines derived from cv. Kavkaz parent. Australian material derived from cv. Amigo also showed intense dough stickiness (Martin and Stewart, 1990). They indicated that these problems have resulted in the very limited use of these translocations in Australia wheat breeding programs.

By comparing milling and baking quality characteristics of seven 1BL.1RS translocation wheats with six control wheats, Fenn *et al.* (1994) found that flour characteristics of several 1BL.1RS translocations were poorer than that of normal wheats as indicated by weaker dough strength, shorter dough extensibility, higher degree of stickiness, lower protein content and loaf volume, while no significant differences were found on flour yield and kernel hardness.

However, Rajaram *et al.* (1983) reported that some of the CIMMYT advanced breeding lines of bread wheats carrying the 1BL.1RS translocation showed high yield potential and good rust resistance under Mexican conditions. In particular, they reported that Veery 'S' lines besides, having high yield stability and wide adaptability,

have also shown satisfactory milling and baking characteristics. A similar result was reported by Pena *et al.* (1990) in comparing sister lines with and without 1BL.1RS translocations in CIMMYT trials. They found that variation in SDS sedimentation volume, alveograph characteristics, dough mixing and baking properties could not be attributed exclusively to the presence of the translocation in these lines. Graybosch *et al.* (1993a) also found that quality characteristics of 1BL.1RS derived lines were highly variable, thus no general conclusion could be drawn on the effect of this translocation on quality.

However, there are many reports of serious quality defects i.e. reduced dough strength, intolerance to overmixing, reduced loaf volume, poor crumb grain and stickiness associated with 1AL.1RS, 1BL.1RS as well as the 1DL.1RS translocation lines (Dhaliwal *et al.*, 1987, Gupta *et al*, 1991, Lee *et al.*, 1995, Seo *et al.*, 1995). The translocation line 1DL.1RS showed even worse dough quality than the translocation 1BL.1RS (Shepherd and Singh, 1984) in the same background genotype and 1AL.1RS was found by Graybosch *et al.* (1993a) to show the least deleterious effect compared with the other two translocation lines.

2.5.3. Attempts to overcome the quality problems associated with 1RS

The poor quality of the wheat-alien transfer lines is presumably due to the transfer of deleterious rye genes along with the useful genes in the transfer of 1RS chromosomes or the rye arm transferred cannot compensate for the loss of certain wheat genes on the wheat chromosome arm replaced (Zeller and Hsam, 1983; Islam and Shepherd, 1991).

Various approaches have been tried or suggested to overcome this problem and these are outlined below.

2.5.3.1. *Utilisation of 1AL.1RS*

The dough quality of the translocation lines varies depending on the particular wheat chromosome involved and the wheat genetic background. Shepherd and Singh (1984) found that in the Gabo background, the 1DL.1RS translocation showed much poorer dough quality than the 1BL.1RS translocation, while Graybosch *et al.* (1993a) found that the 1AL.1RS translocation was less deleterious than the 1BL.1RS translocation. This implies that either 1AS and 1BS cannot compensate for the negative effect of 1RS or that 1DS contains genes that are more important for quality (Bartos, 1993).

It appears that the utilisation of rye genes in form of a 1AL.1RS translocation is better than the other type of translocations, since it seems to have the least deleterious effects on dough quality as suggested by Graybosch *et al.* (1993a).

2.5.3.2. *Change of genetic background*

Another problem with the 1DL.1RS translocation was its origin in the cytogenetic stock Chinese Spring. Chinese Spring wheat is poorly adapted to field conditions because of its tallness, late maturity and tendency to shed grain from mature spikes and consequently it gives low yield and furthermore it has poor grain quality (Islam and Shepherd, 1991). Therefore the initial 1DL.1RS translocation in Chinese Spring was backcrossed into several locally adapted cultivars to give a more favourable genetic

background. However, in most cases, even after changing the background, the lines carrying the translocation had lower yield and quality than the recurrent parent (Islam and Shepherd, 1991)

Shepherd (Waite Institute, personal communication) proposed that an active search should be made for a genetic background which could better accommodate the alien segment and thus resolve the problems encountered in translocation lines. Over the last 15 years, he has made continuous attempts to improve the yield and quality characters in the translocation lines whilst retaining the rust resistance. The translocated chromosome was transferred into a number of different wheat backgrounds. However, he found that changing the genetic background had little effect on improving the quality, although it was useful in some cases for improving yield (K.W. Shepherd, unpublished)

Graybosch *et al.* (1990) suggested that the deleterious effects of the 1BL.1RS translocations on the end-use quality of wheat could be alleviated by using certain genetic backgrounds, since some of the 1RS-containing wheats tested showed better quality than the others, even though it was still regarded as being unacceptable for bread-making quality. Pena *et al.* (1990) reached a similar conclusion when they found that in certain families, the quality characteristics of 1BL.1RS was equal to those of its normal sister lines.

Recently, there have been many investigations aimed at finding the cause of quality defects associated with 1RS translocations. Using fractionation and reconstitution studies, Dhaliwal and MacRitchie (1990) assessed the contribution of various flour components to dough stickiness and mixing properties of wheat-rye translocation lines

compared with those of their normal recurrent parents. Reduction in the amount of water-soluble proteins in the reconstituted flours decreased the stickiness score. Also, dough stickiness was reduced by the addition of glutenin protein fraction. They concluded from this evidence that the weak and sticky dough properties of the translocation lines were due to a shift in the balance of the proportion of polymeric and monomeric proteins in the endosperm.

These results were supported by Lee *et al.* (1995). They tested the quality and biochemical properties of 373 wheat lines derived from seven 1BL.1RS lines. They found that 10% of the 1BL.1RS lines gave acceptable values for mixograph tolerance (=15 mm), while only 5% of the 1BL.1RS lines were equal or exceeded the mean value of mixograph tolerance in the non 1BL.1RS lines. The SDS sedimentation volume of 1BL.1RS lines showed 27% reduction compared to that of the non-1RS lines.

In general, significant quality defects were detected in 1BL.1RS lines compared to their normal sister lines in all populations. They pointed out that these detrimental quality effects of 1BL.1RS lines resulted from an alteration in the ratio of different types of protein in the flour, namely a decrease in aggregated form of glutenin, with a corresponding increase in non-aggregated gliadins and water-soluble proteins. Therefore, it was suggested that the quality of these translocation lines could be improved by finding an appropriate genetic background promoting the formation of polymeric glutenin and having a lower amount of water soluble proteins.

2.5.3.3. Homoeologous recombination

Using a similar approach to that developed by Sears (1972) for reducing the length of an *Agropyron* segment in wheat, Koebner and Shepherd (1986) tried to reduce the amount of rye chromatin that had been transferred to wheat by centromeric fusion of rye arm 1RS. They set out to induce homoeologous recombination between 1RS and 1DS using Sears' *ph1b* mutant. The rationale of this approach was to remove 1RS rye chromatin carrying deleterious genes and to replace it with much of the wheat chromatin, whilst retaining the rye segment carrying *SrR*.

2.5.3.3.1. Isolation of recombinants

By inducing recombination between rye arm 1RS of the translocation lines 1DL.1RS and 1BL.1RS and the short arm of homoeologous wheat chromosomes 1D and 1B using either a *ph1bph1b* or a nullisomic 5B background, Koebner and Shepherd (1986) were able to isolate recombinants from these translocations. The selection of recombinants in order to reduce the length of the rye segment in 1DL.1RS translocation lines was based on a dissociation between the seed storage protein markers TRI-D1 and GLI-D1 on 1DS wheat arm, GLI-B1 on 1BS and SEC-1 and stem rust resistance *(SrR)* on 1RS rye arm (Koebner, 1985). In the first experiment using *ph1b* they found 4 dissociations of endosperm proteins TRI-D1 and GLI-D1 out of 394 progenies tested with only 1 of them involving recombination between 1DS and rye 1RS segment indicated by finding complete linkage between GLI-D1 and SEC-1 in F2 progeny of cross with ditelo 1DL. In the second experiment using nullisomic 5B, 12 dissociations were observed out of 544 progenies tested, but only 2 of them were found to involve a rye segment, the ten other dissociations presumably resulted from

pairing between 1DS with one of its wheat homoeologues. Further tests showed that only one of the recombinants each from each of the experiments could be confirmed to be wheat-rye recombinants (82-180 and I-93). These two recombinants appeared to have approximately reciprocal structures, i.e. I-93 had a distal rye segment with the crossover point being proximal to the *Sec-1* and stem rust resistance loci and 82-180 had a proximal rye segment with the crossover point being distal to *Sec-1* and *SrR* loci. With the 1BL.1RS translocation, 3 putative recombinants out of 647 progenies were detected with dissociation between GLI-B1 and *SrR*, however these could not be confirmed in further tests (Shepherd, pers. comm).

A similar selection procedure was used by Rogowsky *et al.* (1991) to detect dissociation between TRI-D1 and GLI-D1 in 755 F3 and F4 progenies from the 1DL.1RS translocation in a Warigal background. Altogether 15 dissociations between the markers were observed and seven of them were confirmed as wheat-rye recombinants. The higher frequency of dissociation between TRI-D1 and GLI-D1 detected in this experiment compared to the earlier experiments of Koebner and Shepherd (1986) could be due to different genetic backgrounds or different growing conditions of the two experiments (Rogowsky *et al.*, 1991).

2.5.3.3.2. Characterisation of the recombinants

Initially, the selection and characterization of the wheat recombinant lines involving 1RS rye segment were based on the three seed endosperm protein markers (TRI-D1, GLI-D1 and SEC-1), two isozymes (leaf peroxidase and glucose phosphate isomerase) and two DNA markers (Nor-R1 and 5S RNA) (Koebner and Shepherd, 1986; Koebner *et al.*, 1986). Later, 22 RFLP and 3 PCR markers showing polymorphism between

wheat and rye short arms of group 1 were used to characterise all the nine primary recombinants produced, and at least four separate recombination breakpoints were identified (Rogowsky *et al.*, 1993). Recently, additional DNA markers utilising specific PCR primers for rye 1RS have been identified. They have distinguished four new recombination breakpoints, giving a total of 7 breakpoints identified for nine different recombinants (Langridge *et al.*, 1998).

2.5.3.3.3. Dough quality of the recombinants

Shepherd and Singh (1984) tested the quality of the original 1DL.1RS translocation in a Chinese Spring background and in successive backcrosses to Gabo. They found that the quality of the translocation line 1DL.1RS was markedly inferior compared to its normal sister lines over all stages of backcrossing with the maximum dough strength of 45% normal being reached after the third backcross, while in the same background 1BL.1RS reached 75% of normal dough strength.

After four backcrosses to Gabo, primary recombinant 82-180 produced by Koebner and Shepherd (1986) was included in a field trial and its quality was tested and compared to its parental lines. It gave higher extensograph resistance compared to the original translocation 1DL.1RS but it was still only 60 % of the recurrent parent, cv. Gabo (Koebner and Shepherd, 1988).

Following a procedure outlined by Sears (1981), the two primary recombinant lines produced by Koebner and Shepherd (1986) were intercrossed and a derived recombinant with an interstitial rye segment was obtained (DRA-1). This derived recombinant DRA-1 with one dose of Gabo, as well as 82-180 (BC4) and I-93 (BC2) were subjected to quality testing in 1989. Both recombinants 82-180 and I-93

produced dough resistance intermediate between that of Gabo and the original 1DL.1RS translocation, while DRA-1 showed poor dough strength similar to that of the translocation line. The latter result was thought to be due to the Chinese Spring background genotype because it had only been crossed once to Gabo.

In general, it is obvious that many useful genes occur in alien species, but despite this large number of potentially useful genes and the availability of techniques for transferring them into wheat, only a small number of the transfers have contributed significantly to agriculture (Islam and Shepherd, 1991; McIntosh, 1991). The major problem preventing the use of introgressed genetic material is the reduced yield and/or quality of the resulting lines.

CHAPTER 3: GENERAL METHODS

3.1. PROTEIN MARKERS

3.1.1. Extraction of endosperm protein

Unreduced Sodium Dodecyl Sulphate (SDS) - Polyacrylamide Gel Electrophoresis (PAGE).

The SDS-PAGE procedure (Lawrence and Shepherd 1981, as modified by Singh and Shepherd 1985) was used to monitor the presence of seed endosperm proteins TRI-D1, GLI-D1 and SEC-1 in unreduced total protein extracts. A small distal part of the brush end of the seed was cut with a clean scalpel blade and crushed with a hammer, taking care to avoid cross-contamination between samples. The protein was extracted by a two hour incubation at 60°C in 1.5 ml centrifuge tubes by addition of 100 µl sample buffer consisting of 0.06 M Tris (hydroxymethyl)-aminomethane (Sigma), 4% w/v SDS, 15% v/v glycerol and bromophenol blue, adjusted to pH 6.8 with concentrated HCl.

3.1.2. Electrophoresis

Prior to electrophoresis, the samples were briefly centrifuged at 12,000 rpm to obtain a clear supernatant. The gel technique is that of Lawrence and Shepherd (1981), as modified by Singh and Shepherd (1985).

The overlying stacking gel was made up of 3% w/v acrylamide (Sigma), 0.08% w/v bisacrylamide (bis) (Sigma), 0.1% w/v SDS in a 0.125 M Tris-HCl at pH. 6.8. The separating gel consisted of 10% w/v acrylamide, 0.08% w/v bis, 0.1% w/v SDS in 0.375 M Tris-HCl buffer at pH.8.8. The acrylamide solutions in both gels were polymerised by adding 2.5 μl/ml N,N,N,N'-tetramethyl-ethylenediamine (TEMED) (Sigma) and 0.25 mg/ml ammonium persulphate (APS) (Merck).

The electrode buffer for both upper and lower tanks (cathode and anode, respectively) contained 0.1% w/v SDS in 0.025 M Tris. The gels were electrophoresed at a constant current of 45 mA per gel for 1.5 to 2 hours in a refrigerated chamber at 4 to 6°C. Gels were stained overnight in a solution of 1% w/v coomassie brilliant blue R (Sigma) mixed with 40 parts of 6% w/v trichloroacetic acid (Sigma), and destained in tap water for several hours with gentle agitation.

3.2. DNA-BASED MARKERS

3.2.1. Restriction Fragment Length Polymorphism

3.2.1.1. Small scale DNA isolation

DNA isolations were made from approximately a 10 cm long segment of young, fully-expanded wheat leaves. Plants providing the leaf tissue were grown in the glasshouse at the Waite Campus, Urrbrae.

Leaves were folded and placed into 2.0 ml polypropylene Eppendorf tubes and frozen in liquid nitrogen. The leaf tissue was crushed using a plastic implement to a fine powder taking care that the tissue did not thaw during the crushing process. The leaf

tissue powder was then mixed with 600 μl extraction buffer (4% sarkosyl, 0.1 M Tris-HCl, 0.1 M NaCl, 10 mM EDTA, pH 8.5). An equal volume of phenol (Sigma)/chloroform/ isoamylalcohol (25:24:1) (equilibrated to pH 8.0) was added and the samples shaken for 20-30 seconds by hand. After separation of the phases by centrifugation at 12,000 rpm for 5-10 minutes, the upper aqueous phase was transferred into a new sterile centrifuge tube. A second phenol/chloroform/ isoamylalcohol extraction was performed. The final solution (approximately 500 μl buffer) was mixed with 50 μl 3 M Sodium Acetate pH 4.8 and 500 μl Isopropanol (propan-1-ol) to precipitate the DNA. After 7 minutes centrifugation at 12,000 rpm, the DNA was washed twice with 70% ethanol. The DNA pellet was dried briefly and resuspended in 40-50 μl R-40 (40mg/ml RNAse-A [Sigma] in 10 mM Tris-HCl, 1 mM EDTA, pH 8) at 37°C overnight. The R-40 was heated to 100°C in a water bath for 5 minutes just prior to use. Approximately 15 μg of DNA was obtained from each leaf sample.

3.2.1.2. Restriction Enzyme Digestion

All restriction enzymes were used according to the directions recommended by manufacturer (Promega). Two microlitres of enzyme (approximately 20 units) together with 1.2 μl of 10 x reaction buffer (supplied by manufacturer) were used in the reaction for digestion of 10 μl (approximately 3.5 μg) of the resuspended DNA. This reaction was incubated for 6 hours or left overnight at 37°C.

3.2.1.3. Gel Electrophoresis

Electrophoresis separation of DNA fragments was achieved by mixing the digested DNA with 2 µl ficoll dye (100 mM Tris-HCl, 200 mM Na_2EDTA, 0.25% bromophenol blue (Sigma), 30% ficoll MW 4000 (Pharmacia), 0.25% xylene cyanol FF (Sigma), pH.8 and running it on a 1% Agarose NA (Pharmacia) gel at 35 mA for 21 hours. After electrophoresis, the gel was immersed for 10 minutes in the water containing 10 µl/100 ml ethidium bromide (Sigma) (stock concentration 10mg/ml), washed briefly with nanopure water and then photographed under UV irradiation (312 nanometres).

3.2.1.4. Southern Transfer

This method was first introduced by Southern (1975) and is designed to transfer single stranded DNA fragments to a nylon or nitrocellulose membrane following electrophoresis. DNA fragments were denatured from double stranded to single stranded form by soaking the gels in an alkaline solution (1.5 M NaCl, 0.5 M NaOH) for 30 minutes. Gels were washed in a neutralising solution (1.5 M NaCl, 0.5 M Trizma-base, 0.001 M Na_2EDTA) for 20 minutes. DNA was transferred to a nylon membrane (hybond N+) (Amersham) by capillary blots using 20x SSC (3M NaCl, 0.3 M tri-sodium citrate) as the transfer solution. After completion of the overnight transfer, the membrane was rinsed briefly in 2x SSC to remove any adhering agarose gel particles. DNA on the membrane was then fixed to the nitrocellulose by covalent bonding by placing the membrane on Whatman #1 filter soaked with 0.4 M NaOH for

20 minutes. The membrane was rinsed in the neutralising solution for 5 minutes and washed briefly in 5x SSC.

3.2.1.5. Hybridization and Stringency Washing of Membranes

Hybridization of DNA probes to the restricted DNA fragments on the membranes was carried out overnight at 65°C in a "Hybaid" hybridization incubator. The membrane was initially placed in the hybridization bottle containing a pre-hybridization solution (1 ml nanopure water, 2 ml 5x HSB (3M NaCl, 100 mM PIPES [Sigma], 25 mM Na_2EDTA adjusted to pH 6.8), 3 ml Denhardt's III (2% bovine serum albumin (Sigma), 2% ficoll 400 (Pharmacia), 2% polyvinyl pyrolidone (Pharmacia), 10% SDS) and 3 ml of 25% dextran sulphate (Pharmacia) and 0.5 ml of autoclave denatured Salmon sperm DNA (Sigma) (5mg/ml solution boiled for 5 minutes then chilled in ice water).

DNA probes were labelled according to the random primer method (Sambrook *et al.*, 1989) by combining about 50 ng of denatured probe (PCR amplified insert of plasmid stocks obtained from Australian Triticeae Mapping Initiative) with 3 µl of denatured 9-mer random primers (approx. 0.3 ng of 0.1 µg/ml stock), 12.5 µl of an oligo-labelling mixture (0.20 µg each of dATP, dGTP and dTTP [Promega], 150 mM Tris-HCl pH 7.6, 150 mM NaCl, 30 mM $MgCl_2.6H_2O$, 300 µg/ml acetylated BSA), 4 µl of ^{32}P-labelled dCTP (Amersham, 10 mCi/ml) and 5 units of Klenow enzyme (Boehringer-Mannheim). The labelling mixture was incubated at 37°C for at least an hour and the labelled probed separated from the unincorporated radionucleotides by passing through a Sephadex G-100 (Pharmacia) column. The labelled probe was denatured by heating

to 100°C for 5 minutes and then chilling in iced water. The denatured probe was added to the bottles containing the pre-hybridization mixture and the membranes and left overnight at 65°C in the hybridization oven.

The membranes were then washed at 65°C for 20 minutes in each of four washing solutions containing 0.1% SDS and 2x SSC, 1x SSC, 0.5x SSC and 0.2x SSC, respectively.

3.2.1.6. Autoradiography

After drying on blotting paper and wrapping in a sealed plastic bag, the membranes were placed with a sheet of X-ray film (Fuji Super HR) in a light-proof cassette fitted with an intensifying screen. The cassette was kept at –80°C for 3-7 days and the film was subsequently developed.

3.2.2. Polymerase Chain Reaction (PCR)

The PCR were carried out in a 25 µl volume containing 1 µl DNA template (approximately 0.3 µg), 0.015 µg each of M13 Universal forward and reverse primers, 2 µM each of dATP, dCTP, dGTP and dTTP (from a stock concentration of 100 mM), 1.5 µl $MgCl_2$ and 1 unit of thermostable polymerase (Taq) (Integrated Sciences). The PCR was performed in a Programmable Thermal Controller (MJ Research) for amplification. The standard reaction of PCR program consisted of an initial denaturation step at 95°C for 2 minutes, 45 cycles of 2 min at 95°C, 2 min for primer annealing at 55°C and 2 min for strand extension at 72°C. An incubation at 72°C for 4

minutes allowed DNA strand extension to complete. The PCR products were fractionated by 2% agarose gels and bands were visualized after staining in an ethidium bromide solution (10 µl/100 ml of stock concentration of 10 mg/ml)) and irradiating with UV light at 312 nm.

3.2.3. Squash Blot

The procedure used was that described by Rogowsky *et al.* (1991). Three sheets of Whatman #1 filter paper soaked in 0.4 M NaOH were overlaid with a sheet of Amersham Hybond N+ nylon membrane previously soaked in 0.4 M NaOH. Excess NaOH was sponged away so that the Hybond was nearly dry. Green leaf tissue was directly squashed onto a nylon membrane with a metal plunger through 0.5 cm diameter holes of a plastic screen. Subsequently, the membrane was rinsed in 5x SSC and hybridized as described for the RFLP analysis.

3.3. QUALITY ASSESSMENT

All the quality assessments were done on white flour prepared in a Quadrumat Junior Mill. Wholemeal samples (10 g) prepared by grinding the grain in a hammer mill (Falling Number 3100) equipped with 0.8 mm screen, were used to measure grain moisture content by using Near Infra Red spectroscopy.

Cleaned sub-samples of grain from each plot (approximately 300 g) were tempered in a sealed plastic bag overnight with a measured amount of water to adjust their moisture content to 14% prior to milling on a Brabender Quadrumat Junior Mill equipped with a 1.0 mm screen.

3.3.1. Extensograph

Dough was prepared by mixing 50 g of flour in a Brabender Farinograph with the required amount of distilled water containing 1M NaCl at 30°C, to give final consistency of 500 Brabender Units after 5 minutes mixing. Seventy-five grams of this dough was formed into a ball, then rolled into a sausage shape and incubated in a dough fermentation cabinet for 45 minutes at 30°C. The dough was then stretched at a constant speed until it broke. The dough extensibility (E) was shown by the length of the extensogram (cm), while the dough strength/maximum resistance (Rmax.) was measured from the maximum height of the extensograph curve (Brabender unit/BU).

3.3.2. Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

Flour protein fractions were analysed by using SE-HPLC procedure described by Singh *et al.* (1990a, 1990b) and improved by Batey *et al.* (1991). Total unreduced proteins from 10 mg of flour samples were eluted in 0.5% (w/v) SDS in 0.05 M sodium phosphate buffer pH 6.9. Sonication was carried out directly in the protein extraction buffer using a Branson sonifier (Branson Sonic Power Company) with a 3 mm diameter microtip probe (using 1 ml extraction buffer in 1.5 ml eppendorf tubes). The sonication was done at power setting 5 (output 10 W) for 30 seconds. After sonication, samples were centrifuged at 12,000 rpm for 10 minutes and filtered through 0.45 μm Millex-13 HV filters.

SE-HPLC was carried out using a Waters HPLC system, using a Waters Protein Pak 300 column, a model 510 pump, a model 481 wave length detector and a model 710 B

WISP automated sample injector. Chromatography system model 840 was used for controlling the pump and for reprocessing of data from the detector. The eluting solvent used was 50% acetonitrile with 0.1% trifluoroacetic acid with a flow rate 0.5 ml/min (isocratic).

3.4. CYTOLOGY

The standard Feulgen method (Sharma and Sharma, 1980) was used to analyse the chromosome configurations at Metaphase I of meiosis in pollen mother cells. Individual anthers at the correct stage of meiosis were selected and fixed in 3 parts of absolute ethanol and 1 part of glacial acetic acid for 24 hours at 4°C. The anthers were then hydrolysed in 1N HCl at 60°C for 10 minutes and then stained with Feulgen reagents for approximately 45 minutes at room temperature before being squashed in 45% acetic acid for microscopic examination.

3.5. STEM RUST INOCULATION

Seeds were sown directly into soil in boxes in the glasshouse which accommodated up to 3 rows of 10 seedlings each. The seedlings at the 2-3 leaf stage were inoculated with stem rust race 343-1,2,3,5,6 virulent on both var. Chinese Spring and var. Gabo by painting each leaf with a mixture of urediospores in de-ionised water. The boxes were kept under high humidity overnight by putting the boxes in polythene bags containing about one litre of de-ionized water. Average temperatures in the glasshouse ranged from 25°C (day) to 15°C (night).

The rust pustules took between 14 and 21 days to develop and the plants were scored for their resistance or susceptibility.

CHAPTER 4: ISOLATION AND CHARACTERISATION OF NEW 1DS/1RS RECOMBINANTS

A. ISOLATION OF ADDITIONAL RECOMBINANTS

4A.1. INTRODUCTION

The wheat-rye translocation 1DL.1RS has been used to introduce stem rust resistance associated with the 1RS chromosome of "Imperial" rye into wheat (Shepherd, 1973). However, despite the beneficial effects of stem rust resistance associated with this translocation, cultivars carrying this translocation exhibit deleterious end-use quality characteristics such as poor dough mixing tolerance, decreased dough resistance to stretching and dough stickiness. This results in inferior bread-making quality making the dough from these wheat lines unacceptable for commercial exploitation. This quality defect has severely limited the utilisation of this source of stem rust resistance in Australian wheat breeding programs.

In order to eliminate or reduce these deleterious effects of the rye chromosome 1RS when transferred to wheat, several wheat-rye recombinants with smaller rye segments than the original wheat-rye translocation were produced at the Waite Institute by homoeologous recombination (Koebner and Shepherd, 1986). These recombinants showed improved quality compared to the original 1DL.1RS translocation lines, but this quality was still far inferior to that of the background wheat cultivar. We hypothesised that the quality defect could be eliminated or reduced by further reducing the size of the rye chromosome segment thereby either removing deleterious factors on

the rye chromosome (eg. secalins) or re-introducing quality-enhancing genes on the related wheat chromosome arm (1DS). Therefore an active search for additional wheat-rye recombinants was undertaken to test this possibility.

The aim of the experiment was to screen and isolate additional wheat-rye recombinants. Besides their potential use in wheat improvement, a series of new wheat-rye recombinants could find application in mapping activities with other genes. Such new recombinants carrying rye chromosome segments of differing sizes could be used for the localisation of other genetic markers (e.g. RFLP, PCR-based) and further characterize the gene order on the 1DS and 1RS chromosome arms (Rogowsky *et al.*, 1991). Ideally, a new recombinant with a molecular marker very closely linked to the stem rust resistance gene *SrR* could provide the basis for eventual cloning of that resistance gene using 'chromosome walking' techniques.

4A.2. MATERIAL AND METHODS

4A.2.1. Plant material and crossing procedure

The pedigree of the material tested is shown in Figure 4A.1. The starting material was a 1DL.1RS translocation line isolated in Chinese Spring background (Shepherd, 1973) and subsequently backcrossed to cultivar Halberd and then to cultivar Warigal [(1DL.1RS CS x Halberd^3) x Warigal ^{3}]. Following the strategy of Koebner (1985) this line was crossed to a stock of Sears' *ph1b* mutant in a Chinese Spring background (Sears, 1977) to induce homoeologous recombination. Due to the low number of seeds

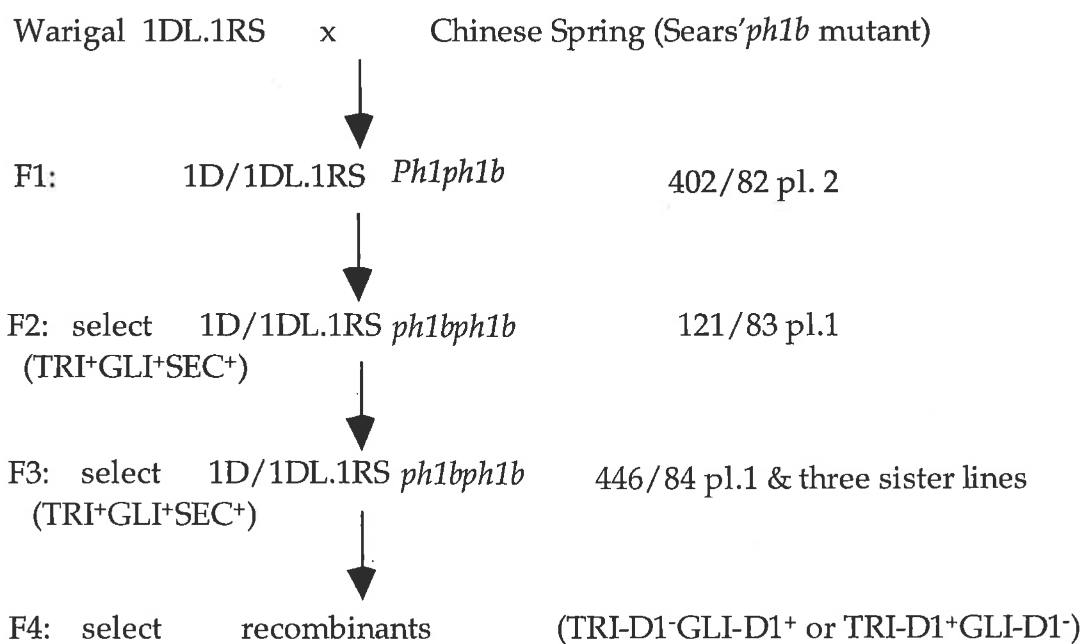

Figure 4A.1. Scheme for production of recombinants between 1DS and 1RS chromosome arms

produced in F_3 generation (progenies of 121/83 plant 1), F_3 plants deduced to be heterozygous for the 1DL.1RS translocation and homozygous for *ph1b* were allowed to self-fertilise and the F_4 progeny were screened to isolate recombinants.

4A.2.2 Marker loci used in the isolation of recombinant lines

The genetic markers used in the detection and isolation of the new recombinants in the current experiment were the same as used previously by Koebner and Shepherd (1986) to select the primary recombinants. These were the seed storage protein markers TRI-D1 and GLI-D1 (Singh and Shepherd, 1985), which are controlled by genes on the short arm of chromosome 1D of wheat. The TRI-D1 phenotype is controlled by the triticin gene *Tri-D1* which is located proximally on the chromosome 1DS arm.

The γ-gliadin storage protein gene *Gli-D1* is located distally on chromosome arm 1DS. Chromosome arm 1RS of rye is identified by the presence of the storage protein secalin SEC-1 (Shepherd and Jennings, 1971), controlled by *Sec-1*, and the gene for stem rust resistance (*SrR*) shown by Singh *et al.* (1990c) to be linked with *Sec-1* on the distal segment of rye 1RS.

The endosperm storage protein markers controlled by genes located on chromosomes 1DS and 1RS can all be visualised simultaneously by single dimension SDS-PAGE (Singh, 1985). The band TRI-1 is controlled by *Tri-D1* alone, while a second band TRI-2 is a hybrid molecule formed from the gene products of *Tri-D1* and *Tri-A1*, a homoeolocus of *Tri-D1* which is located on chromosome arm 1AS (Singh and Shepherd, 1985). Therefore the absence of the *Tri-D1* gene results in the loss of both

bands TRI-1 and TRI-2. The storage protein controlled by *Gli-D1* appears as a single band with greater mobility than the TRI-D1 band, while *Sec-1* of rye codes for a group of bands with higher mobility than GLI-D1. These patterns are shown in Figure 4A.2.

4A.3. RESULTS AND DISCUSSION

4A.3.1. Criterion for detection of wheat-rye recombinants

Recombination between the rye chromosome arm 1RS and homoeologous wheat chromosome 1DS would be expected to occur rarely and at random positions along the chromosomes. Occasionally, homoeologous recombination would be expected to occur between the loci *Tri-D1* and *Gli-D1*, which could be detected on protein gels by the breakage of linkage (dissociation) between the markers TRI-D1 and GLI-D1.

4A.3.2. Isolation of recombinants showing dissociation of protein markers

A total of 732 F4 progeny seeds from four F3 plants having putative genotype 1D/1DL.1RS *ph1bph1b* were screened with SDS-PAGE to detect additional recombinants. The different phenotypic patterns obtained for the progeny of these populations and the frequency of their occurrence are given in Table 4A.1.

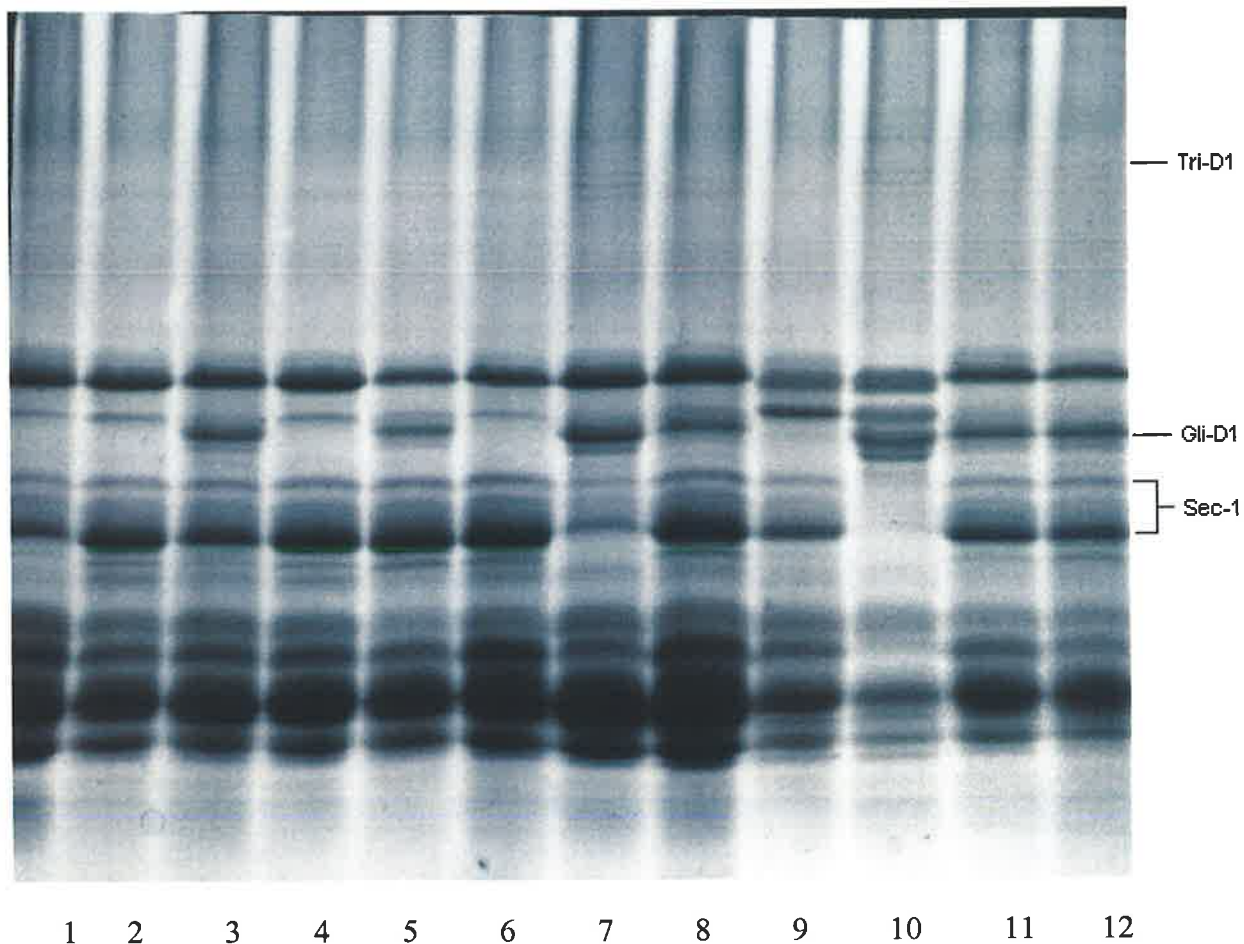

Figure 4A.2. Storage protein phenotype patterns obtained by SDS-PAGE of unreduced protein extracts of F_4 progenies 1D/1DL.1RS *ph1bph1b* and control seeds. Controls: 9) 1DL.1RS translocation (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$) and 10) Normal' Chinese Spring' wheat (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$), progenies screened: 1, 2, 4, 6 (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$), 3, 5, 7, 8, 11, 12 (Tri-D1$^+$Gli-D1$^+$Sec-1$^+$).

Using the presence of the SEC-1 marker as an indication of the 1RS chromosome segment, several suspected recombinants were isolated. The frequency of recovery of suspected recombinants of phenotypes "TRI-D1^{+}GLI-D1^{-}SEC-1^{+}" and "TRI-D1^{-} GLI-D1^{+}SEC-1^{+}" ranged from 0.6 to 6 % in Families 1 to 3. However, Family 4 showed a much higher than expected frequency of these suspected recombinant chromosomes (24.4%).

Table 4A.1. Endosperm protein phenotypes and their frequency in four *ph1bph1b* 1D/1DL.1RS F4 families of translocation lines in a Warigal background. Suspected recombinant phenotypes are progenies with dissociation between TRI-D1 and Gli-D1.

Endosperm Protein Phenotype			Observed Frequency Family No.			
TRI-D1	GLI-D1	SEC-1	1	2	3	4*
+	+	-	28	52	50	0
-	-	+	42	30	46	48
+	+	+	56	84	100	122
+	-	+	3	1	5	29
-	+	+	5	0	5	26
+	-	-	0	0	0	0
-	+	-	0	0	0	0
Total screened			134	167	206	225

+ : protein present - : protein absent

*This family came from 446/84 plant 1.

In Families 1 to 3, most of the progeny showed parental phenotype combinations "TRI-D1^{+} GLI-D1^{+} SEC-1^{-}", "TRI-D1^{-} GLI-D1^{-} SEC-1^{+}" and "TRI-D1^{+} GLI-D1^{+}

SEC-1^{+}", representing the transmission of either a normal 1D chromosome or a translocation 1DL.1RS from heterozygous parents. These parental phenotypes could reflect either the presence of unchanged parental chromosomes or the presence of 1 parental chromosome and a recombinant chromosome masked by the presence of the parental-type chromosome. Family 4 was unusual in that it did not show any SEC-1^{-} progeny suggesting that the parent plant must have been homozygous for *Sec-1* (Figure 4A.3).

Recombinant phenotypes ‘TRI-D1^{+}GLI-D1^{-}SEC-1^{-}‘ and ‘TRI-D1^{-}GLI-D1^{+}SEC-1^{-}‘ which would reflect the presence of the products of wheat-wheat recombination were not detected. However, these types of wheat-wheat recombinants could still have been produced in these families but their occurrence could be masked by the presence of an unaltered parental chromosome.

4A.3.3. Confirmation of suspected recombinants from families 1, 2 and 3

A total of 19 seeds with recombinant phenotypes were detected in Families 1, 2 and 3 (excluding Family 4). In order to confirm their recombinant status, the embryo portions of each grain were planted but only 11 of the 19 embryos germinated. The resulting plants were crossed to Gabo ditelo 1DL and the endosperm protein phenotype from 12 hybrid seeds from each cross was determined in gels to detect their segregation for TRI-D1, GLI-D1 and SEC-1 markers.

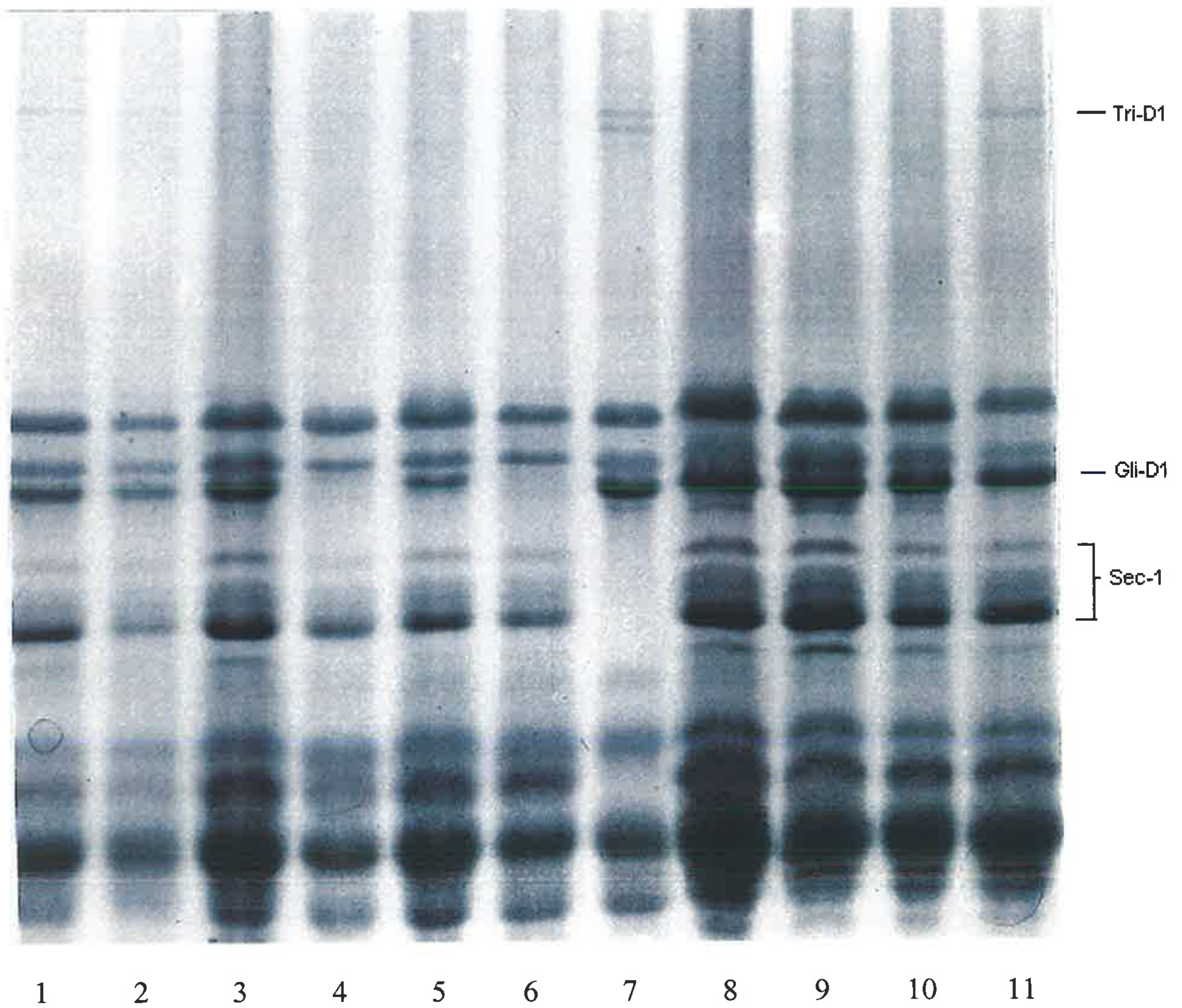

Figure 4A.3. Storage protein phenotype patterns obtained by SDS-PAGE of unreduced protein extracts of Family 4 progenies and control seeds. Controls: (lane 7) Normal' Chinese Spring' wheat (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$) and (6) 1DL.1RS translocation (Tri-D1$^-$Gli-D1$^-$ Sec-1$^+$) and suspected recombinants in lanes 5, 8, 9 (Tri-D1$^-$ Gli-D1$^+$Sec-1$^+$) and in lane 4 (Tri-D1$^+$Gli-D1$^-$Sec-1$^+$)

In six of the putative recombinants, the wheat chromosome 1D markers segregated independently of the rye chromosome 1R markers in these hybrid seeds and hence were considered not to be wheat-rye recombinants. It is assumed that they arose from recombination between homoeologous wheat chromosomes, and therefore represent wheat-wheat recombinants. Progeny from four of the five remaining plants, showed complete linkage between 1D and 1R markers, indicating likely wheat-rye recombinants. The phenotype of the progeny from the fifth plant indicated that it did not have a recombinant phenotype and had been misclassified in the original screening.

These four additional suspected wheat-rye recombinants were not characterised further, because it was thought at that time that Family 4 might be a more valuable source of crossover products considering the high frequency of putative recombinants produced. A large variety of crossover products could be useful for understanding the basis of quality problem of translocation lines.

Further investigation of Family 4

Family 4 gave a very high percentage of progeny plants with dissociation phenotypes (suspected recombinants). Among 225 progeny seeds examined, 55 showed dissociation phenotypes (24.4%) with 29 plants being "TRI-D1^{+}GLI-D1^{-}SEC-1^{+}" and 26 plants being "TRI-D1^{-}GLI-D1^{+}SEC-1^{+}" (Table 4A.2). It was of some interest to find whether the high frequency of dissociation phenotypes was due to recombination or some other cause.

Table 4A.2. Summary of dissociation phenotypes detected in Family No. 4

Endosperm Protein Phenotype			Observed Frequency
TRI-D1	GLI-D1	SEC-1	
+	-	+	29
-	+	+	26
Total dissociation phenotypes			55
Total seed screened			225

+ : protein present - : protein absent

4A.3.4. Origin of dissociation phenotypes in Family 4

There were two unexpected features observed in the progenies of Family 4. Firstly there was a very high rate of dissociation phenotypes detected and secondly all F_4 progeny were SEC-1^+. The first check was to find whether any of these 55 plants with dissociation phenotypes carried wheat-rye recombinant chromosomes and this is normally determined by crossing them to Gabo ditelo 1DL and observing whether the TRI-$D1^-$ or GLI-$D1^-$ markers were co-segregated with SEC-1^+ in the progeny. However, given the likelihood that the parent plant was homozygous for *Sec-1*, there would be no segregation for *Sec-1* in this cross and a further generation of selfing would be required with the F_1 plants which were now heterozygous for *Sec-1*. Only 30 out of the 55 embryos showing dissociation phenotypes germinated and the resulting plants (named DP-1 to DP-30) were crossed with Gabo ditelo1DL. From 5 to 16 F_1 progeny seeds from each of the 30 families were screened in gels to record their segregation for protein marker genes.

The 13 plants with TRI-D1$^-$GLI-D1$^+$SEC-1$^+$ dissociation phenotype (Table 4A.3), gave F_1 progeny which segregated for "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^-$SEC-1$^+$" (11 families) and 2 families (DP-1 and DP-8) showed no segregation (all progeny had phenotype TRI-D1$^-$GLI-D1$^+$SEC-1$^+$). However, family DP 6 also produced 2 F_1 progeny with the unexpected phenotypes TRI-D1$^-$GLI-D1$^-$SEC-1$^-$ and TRI-D1$^-$GLI-D1$^+$SEC-1$^-$. Note that all 135 F_1 progeny except these two had the SEC-1$^+$ phenotype indicating again that the Family 4 parent-plant (Table 4A.1) was most likely homozygous for *Sec-1*.

Table 4A.3. Endosperm protein phenotype of F_1 progeny from crosses between plants having phenotype TRI$^-$GLI$^+$SEC$^+$ and Gabo ditelo 1DL (TRI$^-$ GLI$^-$ SEC$^-$)

Dissociation phenotype (-++) plant no	Endosperm Protein Phenotype of F_1 progeny					
	-++	--+	+-+	+++	-+-	---
DP 1	10	0	0	0	0	0
DP 2	6	4	0	0	0	0
DP 3	4	8	0	0	0	0
DP 4	3	7	0	0	0	0
DP 5	5	5	0	0	0	0
DP 6	4	6	0	0	1	1
DP 7	4	6	0	0	0	0
DP 8	13	0	0	0	0	0
DP 9	3	5	0	0	0	0
DP 10	3	7	0	0	0	0
DP 11	3	7	0	0	0	0
DP 12	5	5	0	0	0	0
DP 13	5	7	0	0	0	0

These results are consistent with the selected dissociation progeny plants being homozygous for *Sec-1* and heterozygous for *Gli-D1* except for DP-1 and DP-8 which were probably homozygous for *Gli-D1* as well as *Sec-1*. The unusual phenotypes in the progeny of DP-6 could be due to rare aneuploidy (---) or to segregation for *Sec-1* (-+-).

The 17 plants with dissociation phenotype TRI-D1$^+$GLI-D1$^-$SEC-1$^+$ (Table 4A.4) gave F_1 progeny which segregated for the protein phenotypes "TRI-D1$^+$GLI-D1$^-$SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^-$SEC-1$^+$" (15 families) and two families (DP-14 and DP-29) which showed no segregation (all plants TRI-D1$^+$GLI-D1$^-$SEC-1$^+$, except 1 showed TRI-D1$^+$GLI-D1$^-$SEC-1$^-$ in DP-29). However, there were also a few phenotypes TRI-D1$^+$GLI-D1$^-$SEC-1$^-$ and TRI-D1$^-$GLI-D1$^-$SEC-1$^-$ in 7 of the families. These segregation patterns suggest that plant DP-14 is homozygous for *Sec-1* and *Tri-D1* (no segregation of either loci) and plants DP-16, DP-17, DP-19, DP-24, DP-26 and DP-27 are homozygous for *Sec-1* and heterozygous for *Tri-D1*.

The presence of SEC-1$^-$ and GLI-D1$^-$ phenotypes in progeny of DP-15, DP-18, DP-25, DP-28 and DP-30 suggest that these particular plants are heterozygous for both *Sec-1* and *Tri-D1* and DP-29 homozygous for *Tri-D1* and heterozygous for *Sec-1*. Note presence of TRI-D1$^+$GLI-D1$^-$SEC-1$^-$ individuals in progeny of these 6 DP plants (all heterozygous for *Sec-1*) indicates that these plants at least do not contain wheat-rye recombinant chromosomes.

Table 4A.4. Endosperm protein phenotype of F_1 progenies from crosses between plants having phenotype $TRI^+GLI^-SEC^+$ and Gabo ditelo 1DL.

Dissociation phenotype (+-+) plant no	Endosperm Protein Phenotype of F_1 progeny					
	-++	--+	+-+	+--	++-	---
DP 14	0	0	11	0	0	0
DP 15	0	3	6	1	0	0
DP 16	0	7	3	0	0	0
DP 17	0	4	1	0	0	0
DP 18	0	1	1	4	0	0
DP 19	0	7	4	0	0	1
DP 20	0	2	6	0	0	0
DP 21	0	6	6	0	0	0
DP 22	0	5	5	0	0	0
DP 23	0	11	5	0	0	0
DP 24	0	3	7	0	0	0
DP 25	0	4	2	3	0	1
DP 26	0	5	5	0	0	0
DP 27	0	6	3	0	0	1
DP 28	0	3	6	1	0	0
DP 29	0	0	11	1	0	0
DP 30	0	4	5	1	0	0

4A.3.5. Further progeny test of dissociation phenotypes from Family 4

In order to investigate the nature of Family 4 further, F_1 seeds showing dissociation phenotypes (now heterozygous for *Sec-1*) were analysed, to find whether the dissociation phenotype co-segregated with SEC-1^+ and was therefore linked to it by a recombination event. Two F_1 progeny seeds with dissociation phenotypes (TRI-$D1^-$ GLI-$D1^+$SEC-1^+ and TRI-$D1^+$GLI-$D1^-$SEC-1^+) from each cross between the

individual original dissociation phenotypes and Gabo ditelo-1DL (from Table 4A.3 and 4A.4) were planted and allowed to self fertilise. However, only 23 of them could be further tested, due to germination problems. There were F_2 progeny from 7 F_1 plants of the type TRI-D1$^-$GLI-D1$^+$SEC-1$^+$ and 16 F_1 plants with the phenotype TRI-D1$^+$GLI-D1$^-$SEC-1$^+$.

With the TRI-D1$^-$GLI-D1$^+$SEC-1$^+$ dissociation types, all progenies showed some plants with the marker pattern TRI-D1$^-$GLI-D1$^+$SEC-1$^-$ indicating that *Gli-D1* did not co-segregate with *Sec-1* in these plants. With the TRI-D1$^+$GLI-D1$^-$SEC-1$^+$ dissociation types, 15 of the 16 BCF_2 progenies gave some plants with marker pattern TRI-D1$^+$GLI-D1$^-$SEC-1$^-$ or TRI-D1$^-$GLI-D1$^-$SEC-1$^+$ indicating that *Tri-D1* did not co-segregate with *Sec-1* in these plants. Therefore, the dissociation phenotypes analysed involved wheat-wheat recombination and not wheat-rye recombination. F_2 of family DP-26 showed no segregation indicating either wheat-rye recombinant or chance failure to segregate in a small F_2 family (Table 4A.5).

It seems clear from these results that the parent plant of family 4 (446/84 plant 1 in Figure 4A.1) must have contained wheat-wheat recombinants, in which 1DS chromosome arm had recombined with its homoeologous 1AS or 1BS chromosome arms in an earlier generation. The high frequency of dissociation observed would then result from segregation of these translocated chromosomes in the selfed progeny.

Table 4A.5. Endosperm protein phenotypes (TRI-D1/GLI-D1/SEC-1) of F_2BC_1 progenies obtained from selfing of selected F_1BC_1 seeds.

F_1 backcross	Phenotype	Endosperm Protein Phenotype (F_2BC seeds)					
		+-+	--+	-++	+--	-+-	---
F_1 DP4 x ditelo 1DL	-++	0	1	8	0	1	1
F_1 DP5 x ditelo 1DL	-++	0	0	6	0	2	0
F_1 DP6 x ditelo 1DL	-++	0	2	0	0	7	1
F_1 DP8 x ditelo 1DL	-++	0	3	6	0	2	1
F_1 DP8 x ditelo 1DL	-++	0	0	9	0	3	0
F_1 DP9 x ditelo 1DL	-++	0	0	3	0	4	4
F_1 DP10 x ditelo 1DL	-++	0	1	6	0	2	1
F_1 DP14 x ditelo 1DL	+-+	5	1	0	4	0	2
F_1 DP14 x ditelo 1DL	+-+	7	2	0	3	0	0
F_1 DP15 x ditelo 1DL	+-+	7	5	0	0	0	0
F_1 DP17 x ditelo 1DL	+-+	5	2	0	3	0	2
F_1 DP19 x ditelo 1DL	+-+	7	2	2	3	0	0
F_1 DP19 x ditelo 1DL	+-+	1	2	0	1	0	2
F_1 DP20 x ditelo 1DL	+-+	6	4	0	2	0	0
F_1 DP20 x ditelo 1DL	+-+	5	1	0	0	0	0
F_1 DP21 x ditelo 1DL	+-+	7	2	0	1	0	2
F_1 DP21 x ditelo 1DL	+-+	6	4	0	2	0	0
F_1 DP22 x ditelo 1DL	+-+	7	2	0	2	0	1
F_1 DP22 x ditelo 1DL	+-+	9	1	0	2	0	0
F_1 DP26 x ditelo 1DL	+-+	10	0	0	0	0	1
F_1 DP27 x ditelo 1DL	+-+	4	3	0	1	0	0
F_1 DP28 x ditelo 1DL	+-+	10	0	0	1	0	0
F_1 DP29 x ditelo 1DL	+-+	8	1	0	4	0	0

It is possible that the line of Chinese Spring wheat carrying Sears' *ph1b* mutant, used in these crosses, might have carried one or more wheat-wheat recombinant chromosomes as remnants from previous meiotic cycles of homoeologous recombination. Also in this project, the plants were screened in F4 generation and had undergone two cycles of meiosis in *ph1b ph1b* condition increasing the chances of accumulating wheat-wheat translocations.

4A.3.6. A model to explain this phenomenon

Table 4A.1 clearly shows the isolation of large numbers of TR1-D1^{+}GLI-D1^{-} and TR1-D1^{-}GLI-D1^{+} plants in Family 4, all with SEC-1 present. As mentioned previously, this family did not segregate for secalin (Table 4A.1). A model was proposed to explain the nature of Family 4 (Figure 4A.4) based on homozygosity for the 1DL.1RS translocation and the presence of two recombinants chromosomes involving the short arm of group 1 chromosomes. Segregation of these two recombinant chromosomes would give the high percentage of dissociation phenotypes in F_2.

In this model, chromosome arm 1DL is present in 3 doses allowing the formation of trivalents at meiosis. Hence, it is difficult to predict expected ratios accurately. The presence of trivalents would provide an explanation of why several of the progeny plants were heterozygous for *Sec-1*, even though the parent plant appeared to be homozygous. It should be noted that the 1DS recombinant chromosome shown in this model could instead bear a terminal deletion of normal 1D and still be Tri-D1^{+}Gli-D1^{-}.

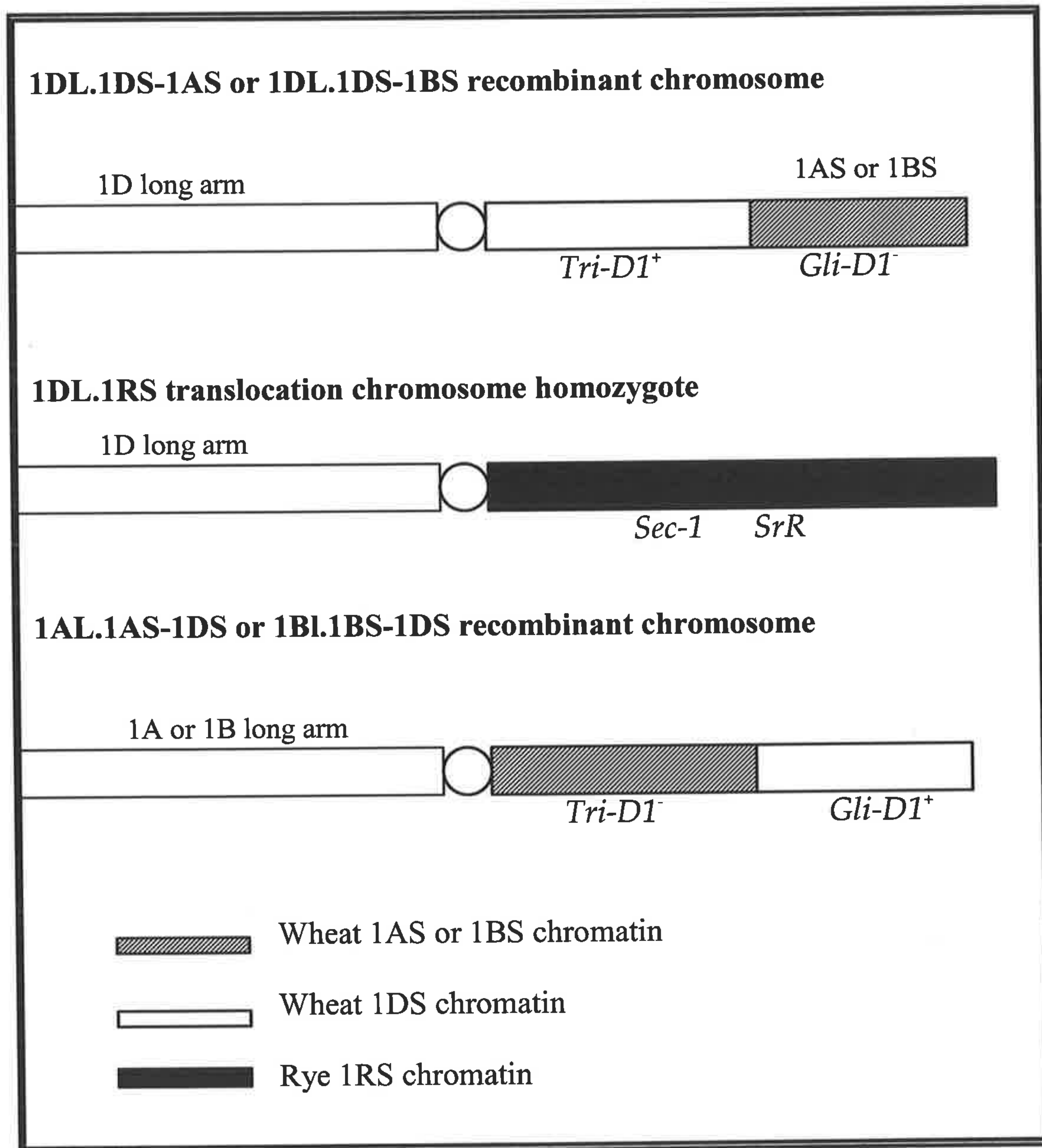

Figure 4A.4. Proposed model of chromosome structure of parent plant of family 4 (446/84 plant 1) showing homozygosity for 1DL.1RS and two prior wheat-wheat chromosomal interchanges

4A.4. CONCLUSION

The conclusion which can be drawn from this study is that caution should be used in allowing two generations of selfing in the presence of *ph1bph1b* before selecting for recombinants between alien chromosomes and wheat. Also, it is inefficient to select for wheat-rye recombinants by using dissociation of wheat markers as selection criteria. A better procedure would be to select for dissociation of markers on the alien chromosome itself. In the present case, however, there were only two known markers on the 1RS chromosome, namely, *Sec-1* and *SrR* and they are known to be closely linked (Singh *et al.*, 1990c) limiting their use as selective markers.

B. REDUCING THE LENGTH OF RYE CHROMATIN IN THE INTERSTITIAL 1DS/1RS RECOMBINANT (DRA-1)

4B.1. INTRODUCTION

Wheat-rye recombinants with a shorter rye segment were sought in an attempt to produce wheat lines having the stem rust resistance gene from rye without the associated inferior end-use quality characteristics of the complete arm translocation.

At the Waite Institute, previous attempts have been made to overcome the deleterious effects of the 1DL.1RS translocation on flour quality by reducing the length of rye segment in the translocation line by using induced homoeologous recombination. Koebner and Shepherd (1986) were able to isolate recombinants containing a smaller rye segment than the original translocation lines, namely recombinants 82-180 with a distal rye segment and I-93 with a crossover proximal to the stem rust resistance gene. These two different types of recombinant were intercrossed to produce a new derived recombinant with an interstitial rye segment even smaller than the segments in the primary recombinants. After backcrossing to the locally adapted cultivar, Gabo, the initial field experiment showed that the quality of the recombinants was improved over that of the original 1DL.1RS translocation, but still less than that of normal recurrent parent, Gabo (Shepherd and Singh, 1984; Shepherd *et al.*, 1990).

It is still unclear whether the quality defect associated with the wheat-rye translocation resulted from the introduction of rye chromatin or alternatively from the loss of critical wheat genes. If it is due to the loss of wheat genes, recombinants which have regained most of the 1DS segment and in particular the known seed storage protein genes, will

be less affected by the dough quality problem. Alternatively if the deleterious genes occur dispersed along the rye arm, recombinants with the smallest rye segment carrying the rye stem rust resistance will be the most useful. Similarly, if the presence of the secalin gene(s) is the cause of the quality problem in the translocation line as suggested by Zeller and Hsam (1983), a recombinant which has the shortest rye segment containing the stem rust resistance gene without the inclusion of the secalin gene(s) is likely to be free of the quality problems encountered in 1RS-containing wheats.

One aim of the current research project was to produce such recombinant lines with a smaller interstitial rye segment carrying the rye stem rust resistance gene and lacking as much other rye chromatin as possible. Since it is known that the map distance between these two loci is 7.0 cM (Singh *et al.,* 1990c) in rye, it was thought that it would be possible to break the linkage between these genes by homoeologous recombination, even if rarely. Therefore, an experiment was carried out to select for recombination between the *Sec-1* and closely linked *SrR* loci by inducing a second round of homoeologous recombination in the derived recombinant DRA-1 which already possesses all the known wheat storage protein genes on chromosome arm 1DS.

4B.2. MATERIALS AND METHODS

4B.2.1. Plant material

The following genotypes were utilised in this program with seeds supplied by Dr K.W. Shepherd:

- Derived recombinant DRA-1 in Gabo background produced by Koebner and Shepherd (1986). This recombinant was produced by intercrossing primary recombinants I-93 and 82-180 and selecting progenies which were TRI-D1$^+$ SEC-1$^+$ SrR$^+$ GLI-D1$^+$. The origin and structure of DRA-1 is presented in Figure 4B.1.
- Sears (1977) *ph1b* mutant in Chinese Spring wheat.
- Gabo ditelosomic 1DL obtained by backcrossing Chinese Spring ditelo 1DL to Gabo
- *Aegilops variabilis* Eig var. *peregrina (Triticum peregrinum* Hack. in J. Fraser)
- 1DL.1RS in Gabo background
- Triple translocation 1AL.1RS 1BL.1RS 1DL.1RS (Gupta and Shepherd, 1992)

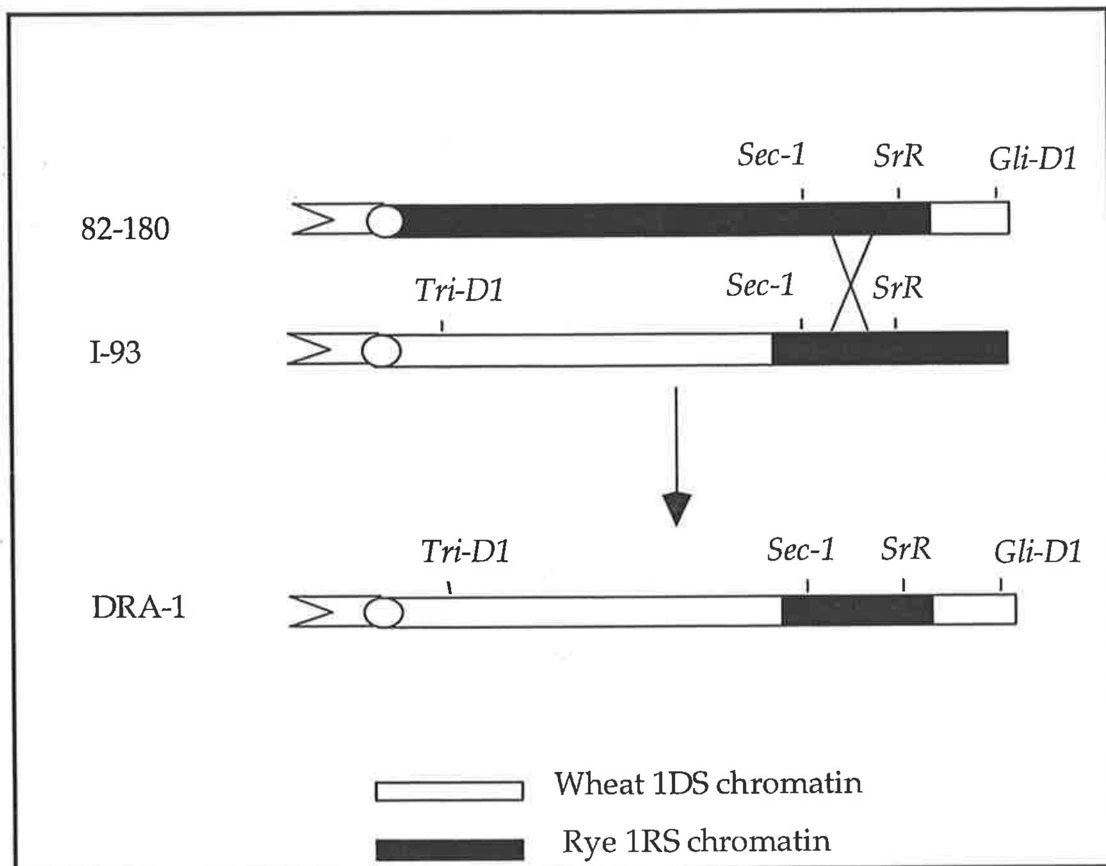

Figure 4B.1. Schematic outline of the origin of derived recombinant DRA-1 carrying an interstitial rye segment (Koebner and Shepherd, 1986).

4B.2.2. Crossing procedure

Recombinant DRA-1 (Koebner and Shepherd, 1986) was first crossed to Sears' *ph1b* mutant in a Chinese Spring background, to produce the F_1 plants. Since normal wheat chromosome 1D does not carry any alternative co-dominant alleles to rye marker genes *Sec-1* and *SrR*, it was not possible to select among F_2 progeny directly for plants heterozygous for normal chromosome 1D and chromosome 1D carrying the DRA-1 rye segment. Instead, the F_1 plants were backcrossed to Sears' *ph1b* mutant in order to isolate plants homozygous for *ph1b* and heterozygous for 1D as shown in Figure 4B.2.

4B.2.3. Marker loci used for the isolation of recombinants

Unreduced SDS-PAGE screening was used to detect any recombinants among F_2 progeny from the selected BC_1 plants with genotype 1D/DRA-1 *ph1bph1b*. Recombinants were detected by selecting for dissociation between two markers on 1RS namely endosperm protein SEC-1 determined by unreduced SDS PAGE, and the stem rust resistance gene *SrR,* determined by rust testing.

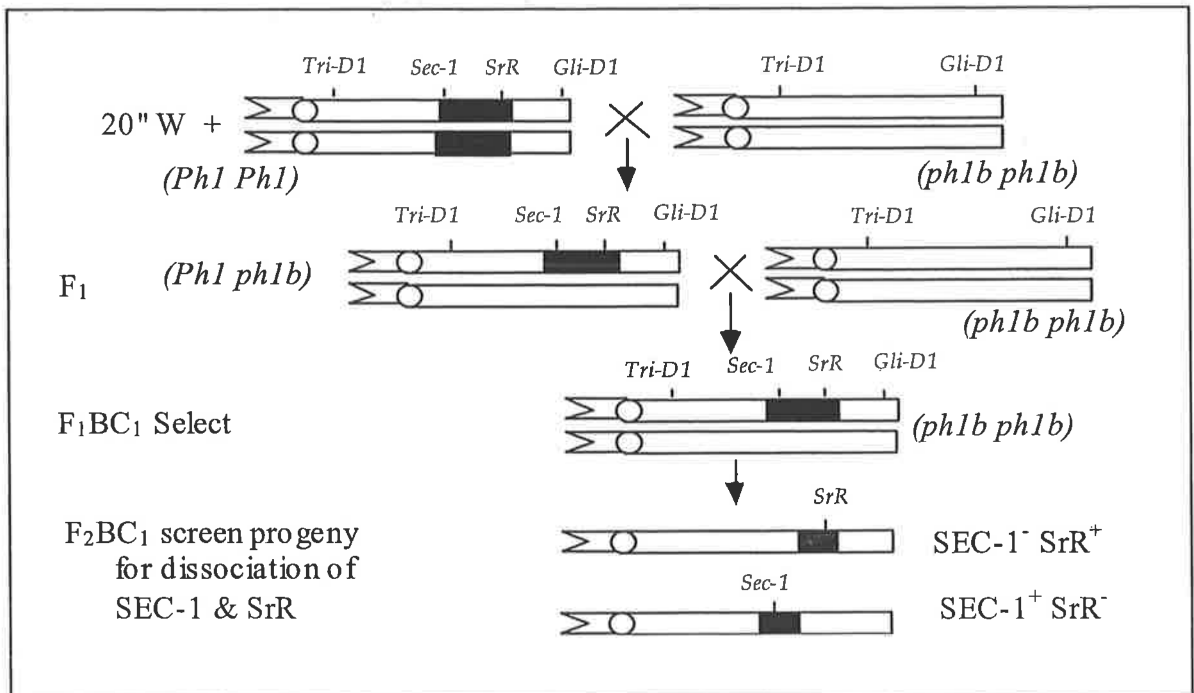

Figure 4B.2. Crossing and selection procedures used to select new recombinants from derived recombinant DRA-1.

4B.2.4. Identification of backcross plants heterozygous for 1D and homozygous for ph1b

Plants heterozygous for the normal chromosome 1D and 1DS/1RS recombinant chromosome (DRA-1) in F_1BC_1 progeny were obtained by selecting progeny seeds, which had all the storage proteins TRI-D1, GLI-D1 and SEC-1 in SDS PAGE (Chapter 3). The selected heterozygous seeds were grown singly in large pots (30 cm diameter) in the glasshouse at the Waite Institute to identify those plants, which were also homozygous for *ph1b*.

To identify homozygous *ph1bph1b* plants, anthers with pollen mother cells (PMCs) at metaphase I of meiosis were identified and fixed in 3:1 Ethanol: Glacial acetic acid. Squash preparations were made using the standard Feulgen procedure (Chapter 3.) and their chromosome pairing behaviour was observed. Also, to confirm the homozygosity for *ph1b*, one spike of each F_1BC_1 plant was pollinated by *Ae. variabilis*, an indicator species used by Sears (1977) to identify the presence of *ph1b*. The remaining spikes were allowed to self-fertilise. At least six F_1 progeny seeds from the *Ae. variabilis* cross on each F_1BC_1 plants were later grown in pots. At heading, the chromosomal pairing behaviour was observed in each of the plants using standard Feulgen method and the presence of a high degree of homoeologous pairing (more than ten bivalents in five cells observed) at metaphase I in all six progeny was taken as evidence for homozygosity for *ph1b* in the original F_1BC_1 plant.

4B.2.5. Screening for new wheat-rye recombinants

The individual progeny seeds from selected F_1BC_1 plants, which were heterozygous for 1D and the DRA-1 rye segment and homozygous for *ph1b* were analysed for the presence or absence of the protein bands TRI-1, GLI-D1 and SEC-1 using the protein extraction procedures and unreduced SDS-PAGE described earlier (Chapter 3.). The main aim was to search for progeny, which showed evidence of dissociation between *Sec-1* and *SrR*. However, the presence or absence of TRI-D1 and GLI-D1 was also monitored to indicate whether dissociation might have occurred because of part chromosome deletions.

After analysis of the protein extracts from the brush end of the grain, the corresponding embryo halves were sown in boxes in ordered positions and seedlings at the 3-leaf-stage, along with controls, were inoculated with stem rust strain 343-1,2,3,5,6 to determine their rust reaction (Chapter 3). The reaction types shown by the experimental and control seedlings are illustrated in Figure 4B.3.

4B.2.6. Further analysis of non-parental phenotypes through progeny test

Individuals lacking the *Sec-1* locus were expected to lack the stem rust resistance gene, whereas individuals having the *Sec-1* locus were expected to show stem rust resistance (*SrR*) as well, unless the rye chromosome segment had been recombined with wheat. All suspected recombinants with a non-parental phenotype (SEC-1^- SrR^+ or SEC-1^+ SrR^-) were transplanted into pots in the glasshouse, and they were allowed to self-fertilise.

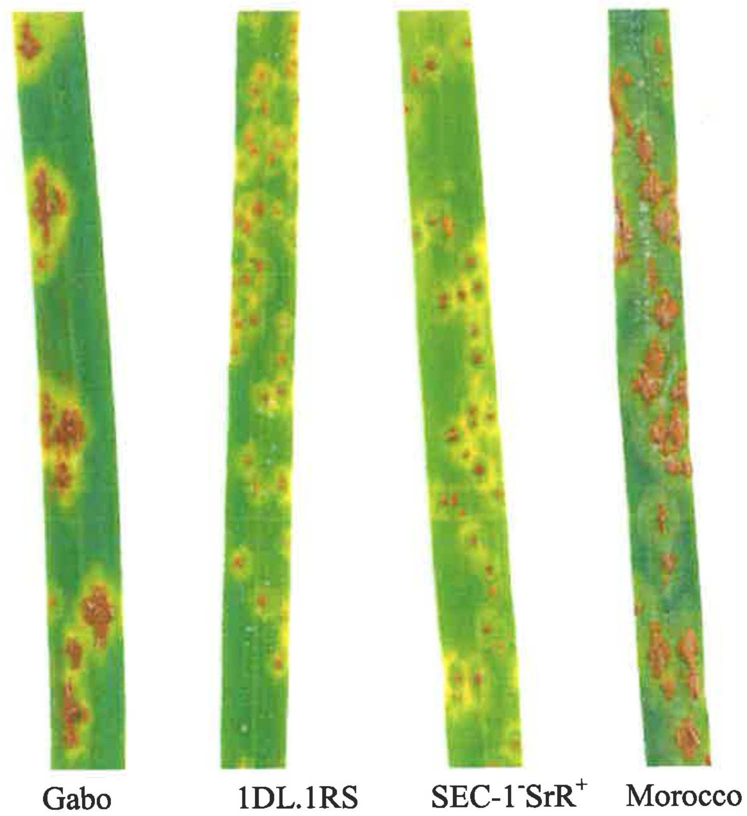

Figure 4B.3. Stem rust reaction (strain 343-1,2,3,5,6) of Gabo, Gabo 1DL.1RS, putative recombinant T6-1 (SEC-1^{-}SrR^{+}) and Morocco, a highly susceptible cultivar.

In order to confirm the recombinant status of the selected plants, progeny testing was carried out using the selfed seeds of the putative recombinants, to determine whether the dissociation of the endosperm protein marker SEC-1 (unreduced SDS PAGE) from the stem rust resistance was also shown in the progeny. In a further test of their authenticity, one spike of the suspected recombinants was crossed to Gabo ditelo 1DL and another to Gabo and derived progeny seed were later analysed in order to confirm their phenotypes.

4B.3. RESULTS

4B.3.1. Identification of Plants Heterozygous for Chromosome 1D and Homozygous ph1b

In a total of 45 F_1BC_1 progeny seeds analysed, thirteen of them contained the secalin marker. These seeds were grown and analysed further to select plants which were homozygous for *ph1b*. Homozygous *ph1b* plants were expected to show multivalents at metaphase I of meiosis (Sears, 1977), but because of the possibility of translocations occurring during the maintenance of the *ph1b* stock, this criterion alone is not sufficient for diagnosis (Koebner and Shepherd, 1986). Therefore, the progeny from the cross between suspected *ph1b* homozygotes and *Ae. variabilis* were grown for meiotic pairing analysis. The meiotic chromosome pairing of six progeny plants from each cross was investigated cytologically. Whenever any progeny plant showed a low frequency of pairing (less than three rod bivalents in five cells observed), it was considered that it was derived from a heterozygous *Ph1-* rather than a homozygous

ph1bph1b plant. If all six progeny plants tested showed a high frequency of pairing (about 10-12 rod bivalents, the corresponding parent was classified as homozygous for *ph1b*. Four of the F_1BC_1 plants were classified as being heterozygous for 1D and homozygous for *ph1b*.

Recently the locus recognized by 5B long arm RFLP probe PSR128 has been found to be located in the same segment of chromosome as shown to be deleted in the *ph1b* mutant (Foote *et al.*, 1997). Therefore, probe PSR 128 can be used to select plants of *ph1bph1b* genotype since plants homozygous for *ph1b* will not produce a 5BL RFLP band when DNA from that plant, which has been digested with *Dra*I, *Eco*RI or *Hin*dIII, is hybridized with this particular probe. Use of PSR128 confirmed that the four plants selected were indeed homozygous for *ph1b* (Figure 4B.4).

4B.3.2. Isolation of Recombinants

The parental chromosomes (normal 1D and the recombinant DRA-1 chromosomes) were expected to pair homologously in most PMCs and only rarely to show homoeologous recombination between the rye segment and 1DS. Thus the majority of progeny were expected to carry parental type combinations, namely, normal 1D and DRA-1 chromosomes. Seedlings were classified as parental when they retained the phenotypic combination SEC-1 plus stem rust resistance (TRI-D1$^+$ SEC-1$^+$ SrR$^+$ GLI-D1$^+$) or lacked both of these phenotypes (TRI-D1$^+$ SEC-1$^-$ SrR$^-$ GLI-D1$^+$). Any plants which showed dissociation between *Sec-1* and *SrR* (SEC-1$^+$ SrR$^-$ or SEC-1$^-$ SrR$^+$) were selected as putative recombinants.

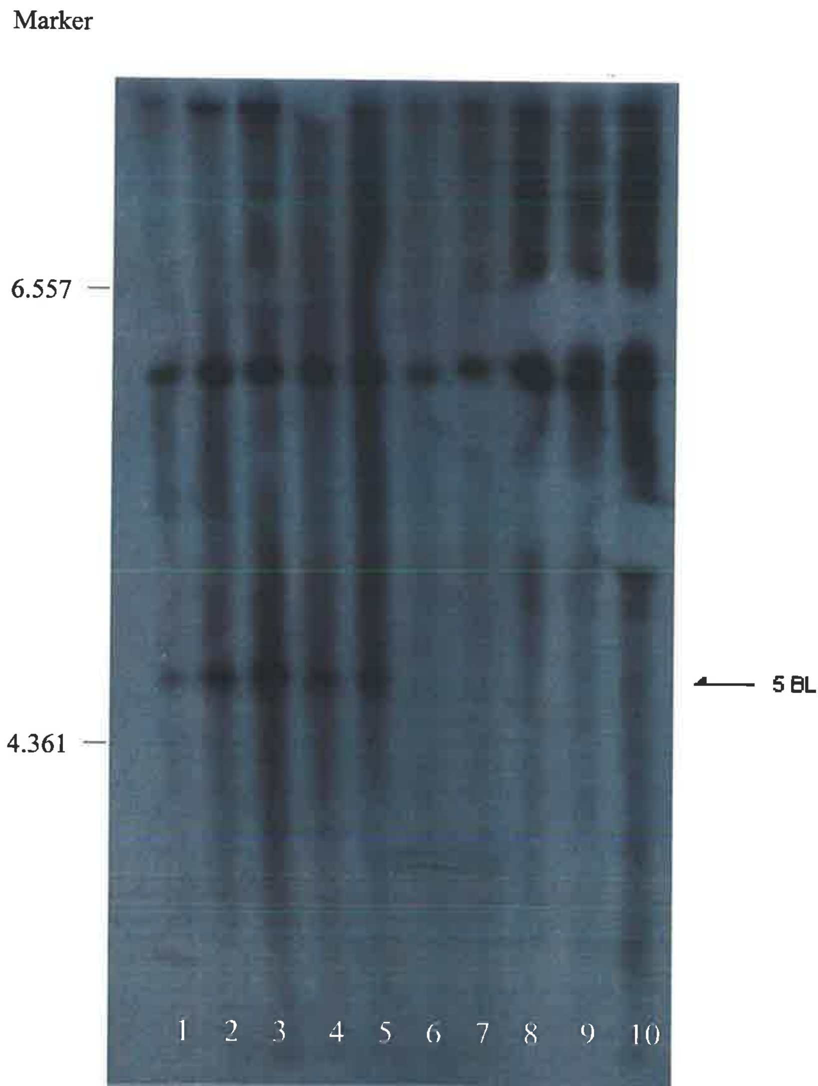

Figure 4B.4. Confirmation of F_1BC_1 plants homozygous for *ph1b* using IPSR 128 probe. Control plants: Normal Chinese Spring in lane 5 and CS *ph1b ph1b* in 6. Lanes 7, 8, 9, and 10 do not possess the chromosome 5 BL encoded marker (arrowed) and are confirmed to be homozygous for *ph1b*. Genomic DNA digested with Dra I.

A total of 1889 progeny seeds were screened for seed protein phenotype and stem rust resistance reaction. Assignment of endosperm protein phenotype by unreduced SDS-PAGE to score for the presence of TRI-D1, GLI-D1 and SEC-1 presented no difficulty. The phenotypic patterns observed are illustrated in Figure 4B.5, and the frequency of their occurrence is given in Table 4B.1. The parental 1D phenotype and the DRA-1 phenotype were transmitted with an approximate frequency ratio 1:3 as expected for the dominant marker genes *Sec-1* and *SrR*.

The phenotypes of the progenies screened for recombinants are shown in Figure 4B.5. Six suspected recombinants were detected as listed in Table 4B.1. To confirm their recombinant status, progeny testing was carried out to check their segregation for the endosperm protein marker SEC-1 and for their reaction to stem rust.

Table 4B.1. Endosperm protein and rust reaction phenotypes in F_2 progeny of four F_1BC_1plants heterozygous for 1D and homozygous for *ph1b*.

Endosperm Protein Phenotype	Rust Reaction	Frequency in Family				Classification
SEC-1	SrR	1	2	3	4	
+	+	216	346	470	330	parental
-	-	82	117	191	131	parental
+	-	0	1	0	1	recombinant
-	+	2	2	0	0	recombinant
Total screened		300	466	661	462	1889

+: marker present -: marker absent

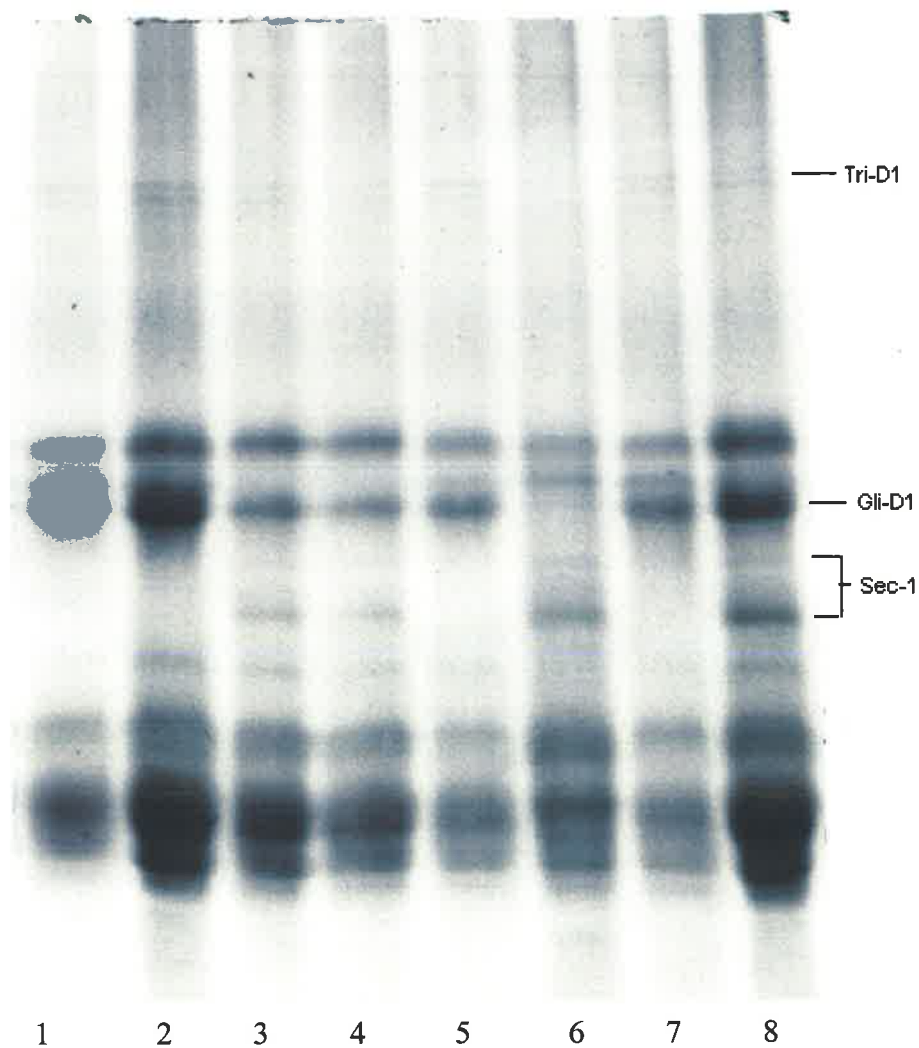

Figure 4B.5. Storage protein phenotype patterns obtained by SDS-PAGE of unreduced protein extracts of F_2 back cross progenies, showing positive for all 1D markers (Tri-D1$^+$Gli-D1$^+$) and segregation of Sec-1. Control plants: Normal 'Chinese Spring' wheat (*Tri-D1$^+$Gli-D1$^+$Sec-1$^-$*) in lane 5 and 1DL.1RS translocation (*Tri-D1$^-$Gli-D1$^-$Sec-1$^+$*) in lane 6.

4B.3.3. Confirmation of Recombinants

The six suspected recombinants showing dissociation between the secalin and stem rust resistance gene markers were grown to maturity and allowed to self-fertilise in order to give progeny seed for further testing. Two of the putative recombinant plants were completely sterile and it was not possible to carry out progeny tests in these plants. Only four of suspected recombinant plants gave sufficient seed for progeny testing (Table 4B.2).

Table 4B.2. Phenotype of putative recombinants and their progenies after selfing

Putative recombinant			Phenotype of Progeny			
Plant	Phenotype		SEC-1		SrR	
No.	SEC-1	SrR	-	+	-	+
T6-1	-	+	25	0	9	16
T25-5	-	+	9	9		
T26-13	+	-	sterile			
T34-6	-	+	3	8		
T37-26	-	+	sterile			
T49-7	+	-	6	14	20	0

4B.3.3.1. Putative recombinants T34-6 and T25-5

Plants T34-6 and T25-5 were classified as having phenotype SEC-1$^-$ SrR$^+$, but in both cases this phenotype could not be confirmed in the progeny test, since each progeny group contained some seeds with secalins (Table 4B.2). It appears that these plants were misclassified for protein phenotype in the screening test, since otherwise it would be expected that all the progeny seed would be negative for secalin.

4B.3.3.2. Putative recombinant T6-1

None of the 25 progeny seeds obtained by self fertilisation of suspected recombinant T6-1, which was classified as having phenotype SEC-1$^-$ SrR$^+$, showed the presence of any secalin bands. Sixteen of the progeny plants were resistant to stem rust and nine were susceptible.

Two of the rust resistant progeny plants (F_3#1 and F_3#2) were each crossed with Gabo ditelo 1DL as male parent. The progeny seeds were analysed for their seed protein phenotype and also for their stem rust reaction (Figure 4B.6). None of the 14 progeny seeds analysed from F_3#1 possessed the secalin band, and they segregated for resistant and susceptible plants in a 1:1 ratio. The progeny from the cross of F_3#2 and ditelo 1DL, were also all negative for secalins but in this case they did not segregate for stem rust reaction and were all resistant.

Ten progeny seeds from the cross between F_3#3 and Gabo (normal cultivar), were all negative for secalin and segregated for reaction to stem rust giving 6 resistant and 4 susceptible. Two of the rust resistant progeny were allowed to self fertilise, and a total of 26 progeny from the two plants were screened with SDS PAGE and all of them were negative for secalin. When tested with stem rust, both showed segregation with seventeen being resistant and nine susceptible plants. Thus all of these progeny tests were consistent in showing the absence of secalin band and segregation or homozygosity for stem rust reaction.

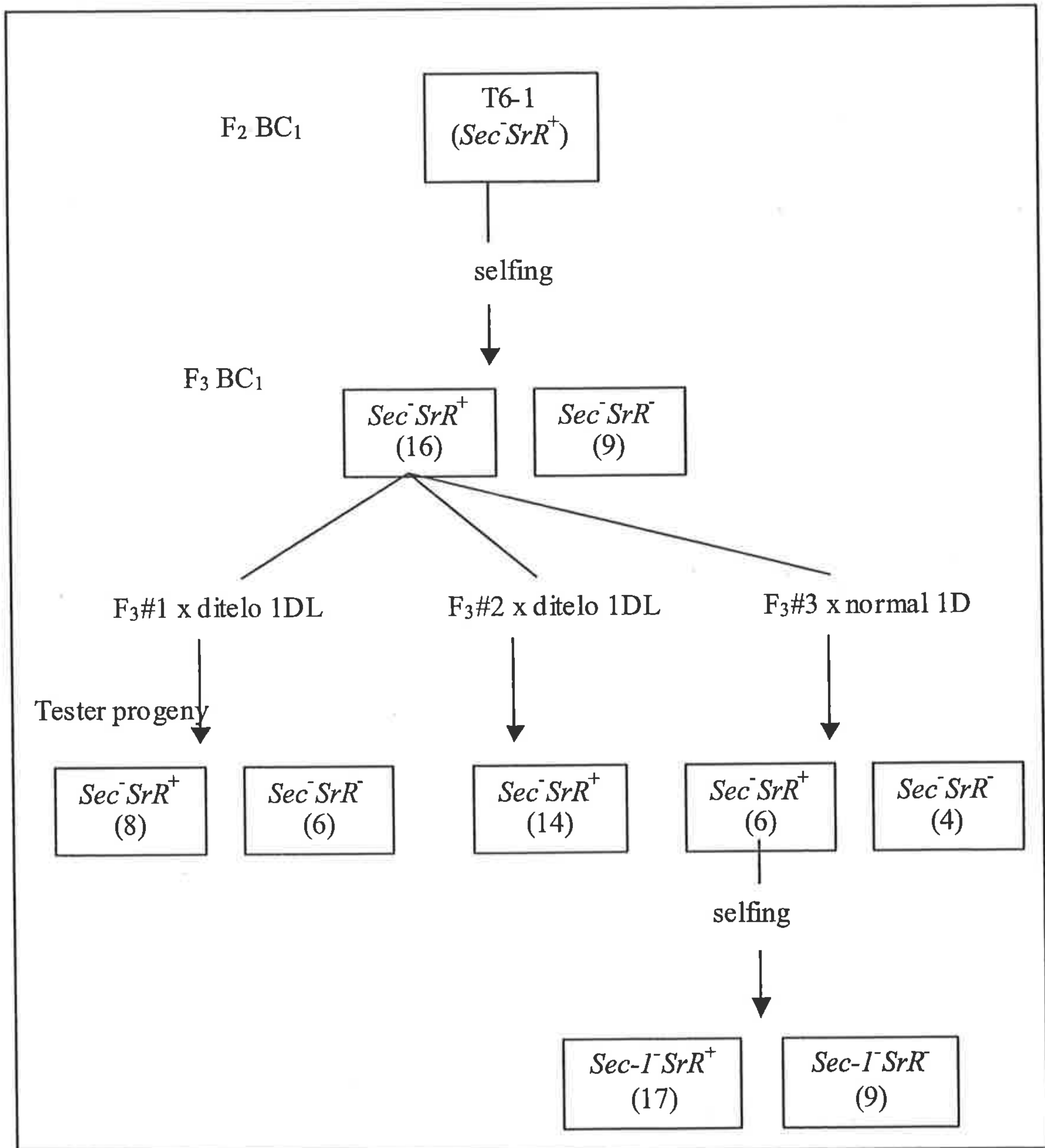

Figure 4B.6. Progeny testing of putative recombinant T6-1 (*Sec⁻ SrR⁺*)

4B.3.3.3. Putative recombinant T49-7

Putative recombinant T49-7, which was positive for rye secalins but susceptible to stem rust, was allowed to self-fertilise to produce seeds for progeny testing to confirm its recombinant status. Fourteen of the 20 seeds were positive for secalins and the other six seeds were negative. All of the seedlings grown from these seeds were susceptible to stem rust as expected from the initial classification of T49-7 as being susceptible to stem rust. One progeny plant with phenotype SEC-1^{+} SrR^{-} (F_3#1) was crossed to Gabo ditelo 1DL, and the F_1 progeny seed were tested for their secalin phenotype. Three of the 7 progeny seeds were positive for secalin and the remaining four were negative, and all progeny were susceptible to stem rust.

Progeny seeds from the cross of recombinant F_3#2 with Gabo (normal cultivar) were also tested by SDS-PAGE and out of the three tested, two were positive for secalin and one was negative. All of these plants were susceptible to stem rust. In a further test, the two progenies positive for secalin were allowed to self fertilise and the progeny were rust tested and screened for secalins. All 26 progeny were susceptible to stem rust and segregated for the presence of secalins, with four being negative and the other 22 being positive (Figure 4B.7).

These progeny tests confirmed that the initial classification of the phenotypes of putative recombinants T6-1 and T49-7 was correct. However, it was necessary to obtain further information to confirm that the recombinants still contained a segment of rye chromatin.

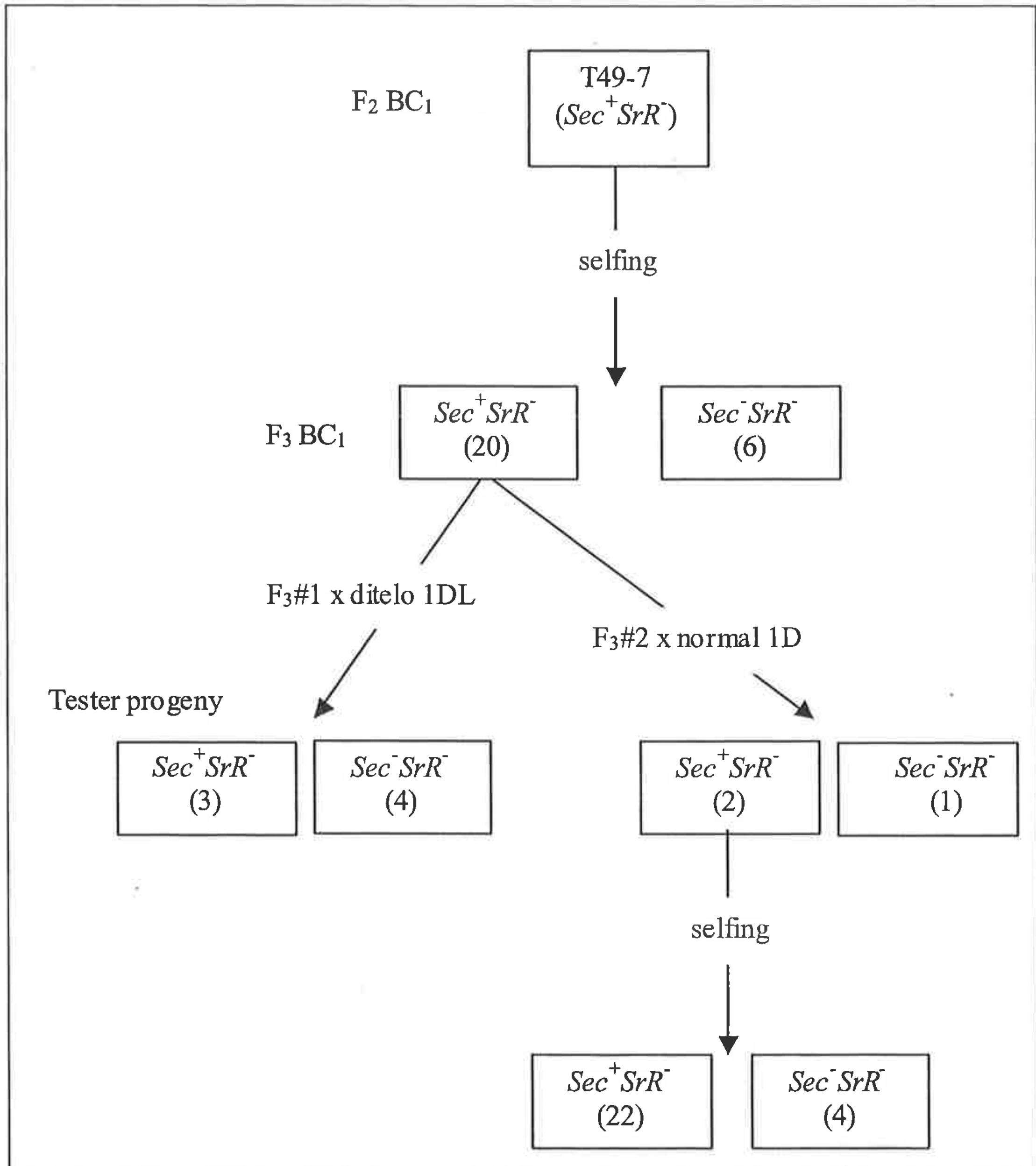

Figure 4B.7. Progeny testing of putative recombinant T49-7 (*Sec*$^+$ *SrR*$^-$)

4B.3.4. Further analysis of putative recombinants T6-1 and T49-7

Although seed protein markers and stem rust resistance had been used to screen the wheat-rye recombinants to detect dissociation between the *Sec-1* and the stem rust resistance characters, and the phenotypes had been confirmed in progeny tests using these same markers, it was necessary to obtain more critical diagnostic information on the structure of these suspected recombinants. For example with the SEC-1$^-$ SrR$^+$ phenotype, the possibility that the new phenotype was not associated with recombination of the rye segment, but was due to rare stray pollination from another wheat plant carrying a rust resistance allele effective against the tested rust strain, needed to be explored. The first requirement was to show that the new recombinants do in fact carry rye chromatin and then it was necessary to determine whether it is located interstitially in chromosome 1DS. Molecular markers are most suited for obtaining evidence for the presence of rye chromatin, since they are abundant and widely distributed along the rye chromosomes.

4B.3.4.1. Squash blot test for rye chromatin

The first test for the presence of rye chromatin was to apply the squash-blot procedure developed by Guidet *et al.* (1990) to detect the presence of interspersed rye-specific R173 family using pAW 173 from rye as the probe. This probe tests for the presence of a family of rye repetitive DNA dispersed widely on the rye genome and present in wheat in low copy number. All the previously isolated primary and derived wheat-rye recombinants along with wheat controls including cultivars Gabo, Warigal and Chinese Spring and Imperial rye and the original wheat-rye translocation 1DL.1RS,

were tested along with the putative new recombinants. Imperial rye showed the strongest signal and the 1DL.1RS translocation also showed a strong signal. Moderate signals were shown by all the recombinants isolated previously such as I-93 and 82-180. The two new recombinants showed very weak signal using this method just like the normal wheat controls. However this was an equivocal result because these recombinants are expected to have just a small segment of rye chromatin and therefore to show a lower signal than DRA-1, and perhaps not recognizably more than wheat (Figure 4B.8).

4B.3.4.2. Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) probes were also used to detect the presence of any rye chromatin in those recombinants. Several RFLP probes KSU18, IH69, KSU19, KSUG9 which have been located to the short arm of chromosome 1R in DRA-1 (Rogowsky *et al.*, 1991), were tried combined with various restriction enzymes. However, no rye bands characteristic of 1DL.1RS translocation were detected in these assays with recombinants T6-1 and T49-7.

4B.3.4.3. Polymerase Chain Reaction (PCR)

The establishment of standard conditions for Polymerase Chain Reaction (PCR) analysis, allowed this technique to be used more easily and quickly. Several primers for DNA sequences known to be located in the rye segment of DRA-1 were used in this work. These primers were obtained by a procedure, which targeted λ clones carrying low copy number DNA sequences in the rye segment of DRA-1.

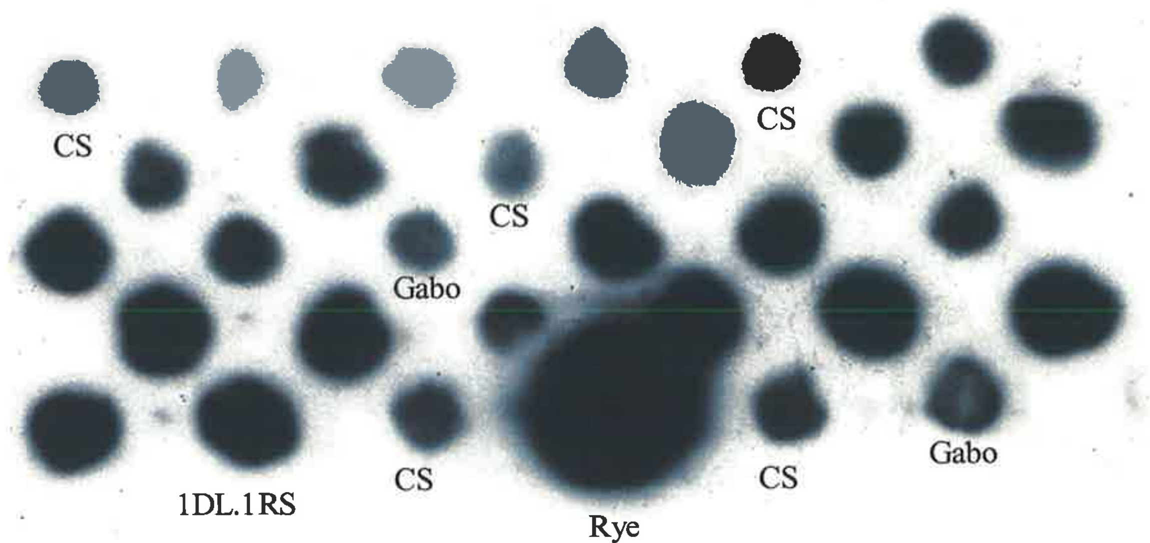

Figure 4B.8. Squash blot using pAW173 rye specific probe to detect the presence of interspersed rye chromatin, from top row left to right to the bottom row: Chinese Spring (CS), three progenies of new putative recombinants *Sec⁻SrR⁺*, CS, three progenies of new putative recombinants *Sec⁺SrR⁻*, CS, derived recombinants: DRA-1; DW-12; DW-13; DW-14; DW-18; Gabo, Derived recombinants: DW-92; DW-93; DW-94; Primary recombinants: I-93; 82-180, Warigal, Primary recombinants: WR-1; WR-2; WR-3; WR-4; CS 1DL.1RS, CS, Imperial Rye, CS, Gabo

When identified, these clones were subcloned and sequenced to produce the primers (Langridge *et al.*, 1998). Primers no 2.5, 7.4, and 5.3 produced in this way were kindly provided by Dr. P. Langridge and their sequences are presented in Table 4B.3.

Table 4B.3. Sequences of nucleotide bases for each of the three primer sets used to characterise new wheat-rye recombinant chromosomes derived from line DRA-1.

Primer Set	**Sequence**
2.5	
Forward	5' GAA TCC CAT TGT TCA GCA AGT 3'
Reverse	5' TAG CAC TCC AGC AGA CTC CAC 3'
5.3	
Forward	5' GAT TAC TGC TCA AAA 3'
Reverse	5' TGC CTA CAT TCT ATG 3'
7.4	
Forward	5' GTT TGC CGA AAT ACT CAC CTC 3'
Reverse	5' CGC CCA CGG TTG AAC TAA AAA 3'

PCR analysis (as described in Chapter 3) was performed on plants of the following types, (a) four F_2 progeny plants (showing phenotype SEC-1$^-$ SrR$^+$) from the cross between recombinant T6-1 and Gabo, (b) three progeny plants (showing phenotype SEC-1$^+$ SrR$^-$) from selfing of putative recombinant T49-7, (c) Chinese Spring (CS) (negative control plant) and (d) CS 1DL.1RS (positive control plant) (Figure 4B.9).

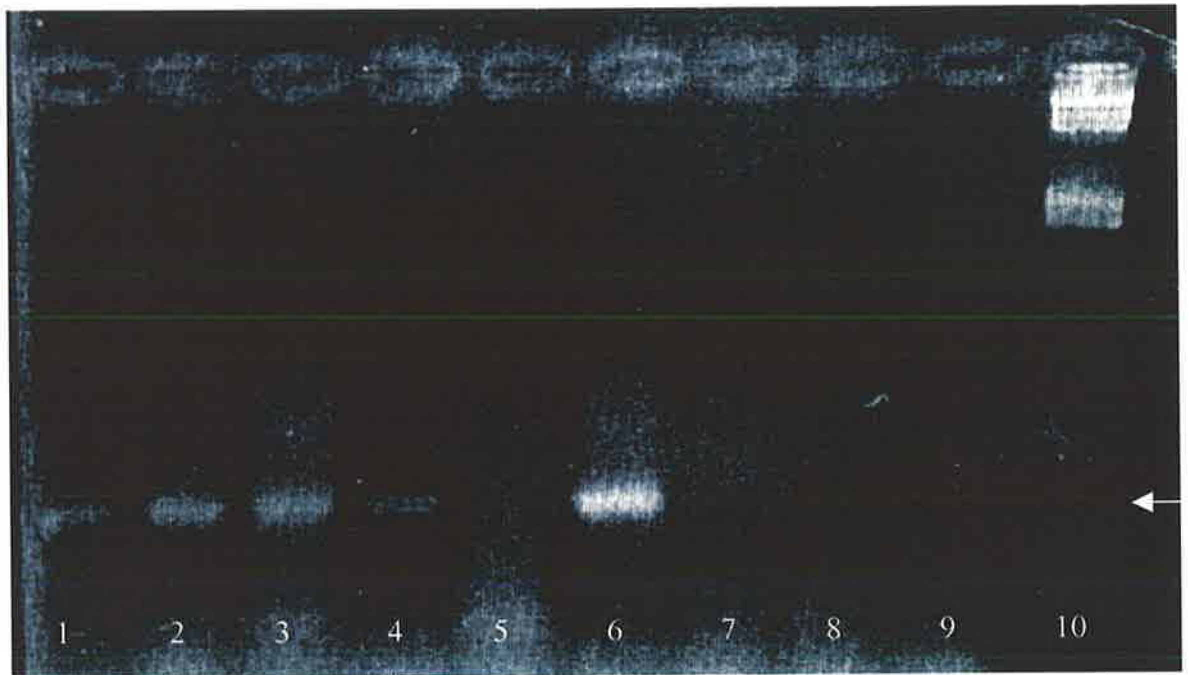

Figure 4B.9. PCR products using primer 2.5 on DNA extracts of putative recombinants and controls to search for rye specific bands in the recombinants. Lane 1-4: progenies of T6-1 *(Sec⁻SrR⁺)*; 5: Chinese spring (control); 6: 1DL.1RS (control); 7-9: progenies of T49-7 *(Sec⁺SrR⁻)*; 10: marker. Line DRA-1 (not shown) showed an identical pattern to 1DL.1RS. Note rye 2.5 band present in T6-1 progeny but not T49-7. Arrow shows primer 2.5 band.

Using this approach, no rye-specific bands were detected in either recombinants with PCR primer 7.4. All four progeny of putative recombinant T6-1 which were SEC-1$^-$ SrR$^+$ and the control 1DL.1RS produced a rye-specific band with the primer 2.5 providing clear evidence of the presence of part of the rye segment from DRA-1 in this recombinant. On the other hand, no band was detected for the progeny of recombinant T49-7 with the phenotype SEC-1$^+$ SrR$^-$ nor for the normal wheat control line (CS).

The control 1DL.1RS recombinant plant and the triple translocation showed a rye specific band with primers 5.3, but no bands were produced with DNA from the progeny of T6-1 nor Chinese Spring and Gabo control (Figure 4B.10).

4B.4. DISCUSSION

Utilisation of Chinese Spring Sears' *ph1b* mutant has resulted in induced recombination between the genes for rye endosperm secalins and a gene for stem rust resistance. Two recombinant families were identified and confirmed over two consecutive generations, in which the first recombinant family showed a resistant reaction to stem rust in the absence of secalin bands, while in the other family the presence of secalins was associated with a susceptible reaction to stem rust. The results indicate that the linkage between the genes for stem rust resistance and the gene controlling rye secalins estimated to be 7.0 cM apart (Singh *et al.*, 1990c) has been broken by homoeologous recombination.

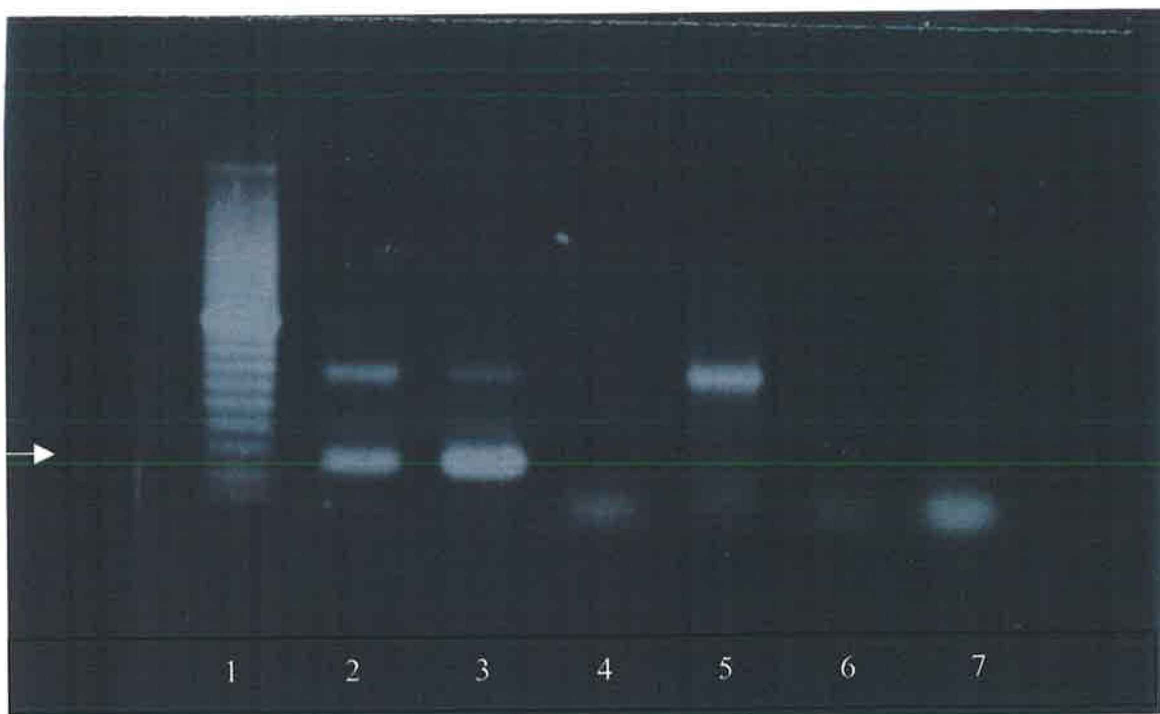

Figure 4B.10. PCR products using primer 5.3 on DNA extracts of putative recombinants and controls: 1) 1000bp marker, 2) 1DL.1RS, 3) Triple translocation, 4) Chinese Spring, 5) Gabo, 6) *Sec⁻SrR⁺*, 7) No DNA. Line DRA-1 (not shown) showed an identical pattern to 1DL.1RS. Note: absence of rye 5.3 band in *Sec⁻SrR⁺* recombinant. Arrow shows primer 5.3 band.

The number of confirmed recombinants detected in this study was low giving a frequency of only 0.1%. Koebner (1985) in his work found no evidence for breaking the linkage between *Sec-1* and *SrR* the gene for stem rust resistance among 528 of the progeny derived from nullisomic 5B parents and 221 of the control progeny involving 1DL.1RS.

The new 1DS-1RS recombinants produced in this study carry the least amount of rye chromosome so far obtained in recombination experiments with translocation material involving 1DL.1RS. If the defect of the quality in 1DL.1RS material is only due to the loss of wheat genes, then these recombinants may not suffer from the dough quality problem, since they possess all the known 1DS seed storage protein genes and have regained a large amount of the wheat chromatin.

These wheat-rye recombinants also could be used to test the hypothesis that the rye secalin seed storage proteins may themselves contribute to the quality problem in translocation lines involving 1RS chromosome (Zeller *et al.*, 1982). Recombinant T6-1 ($Sec\text{-}1^{-}SrR^{+}$) will be the most critical recombinant in this respect, since it only carries the target gene for stem rust resistance without secalins. On the other hand T49-7 ($Sec\text{-}1^{+}SrR^{-}$) is not expected to be of direct value in wheat breeding since it contains secalins and does not possess the stem rust resistance gene. However this material is expected to be useful in testing whether the secalin gene is a contributing factor towards the quality defect in translocation lines involving 1RS chromosome.

CHAPTER 5: YIELD AND QUALITY TESTING OF RECOMBINANT LINES

5.1. INTRODUCTION

As outlined earlier (Chapters 2 and 4), wheat-rye translocations involving the transfer of the short arm of rye chromosome 1 (1RS) to the long arm of group 1 chromosomes of wheat have attracted the attention of wheat breeders because they have the potential to enhance agronomic performance in terms of yield and disease resistance. However, the utilisation of these 1RS translocation lines has been restricted because of deleterious effects of the translocation on the quality of end use products.

There are several reports that 1RS-containing wheats have severe dough quality defects, especially low dough resistance, poor mixing tolerance and stickiness (Martin and Stewart, 1986; Dhaliwal *et al.,* 1987; Pena *et al.*, 1990). These characteristics of 1RS translocations have severely limited their use as breeding material in Australia, where quality of grain is of great importance for the export trade.

There have been many investigations aimed at finding the possible cause of the quality defects associated with these 1RS translocations. It has been argued that the deleterious effects on quality can be overcome by incorporating the translocation into a more appropriate genetic background (Graybosch *et al.*, 1990; Lee *et al.*, 1995). Much research has been carried out recently on assessing the quality and biochemical attributes of the 1RS translocation in different genetic backgrounds to deduce the possible cause of these quality problems. (Pena *et al.*, 1990; Fenn *et al.*, 1994; Lee *et al.*, 1995; Seo *et al.*, 1995; Graybosch *et al.*, 1999).

Studies on the molecular weight distribution of the seed endosperm proteins have indicated that there is a relative decrease of the polymeric glutenin aggregates and an increase of monomeric gliadins and salt-water soluble protein in 1RS-derived lines compared to normal sister lines (Dhaliwal and MacRitchie, 1990; Graybosch *et al.*, 1993a).

Other workers have argued that the inferior quality of translocation lines is due to the transfer of too much other unidentified rye chromatin which carries deleterious genes along with the target genes, or alternatively that there are critical genes on the missing wheat chromosome arm that are not compensated by the translocation (Koebner and Shepherd, 1988). These possibilities have prompted attempts to reduce the length of rye chromatin in the translocation lines. This approach has been explored most extensively with the 1DL.1RS translocation line isolated at the Waite Institute and many primary recombinant lines with different length of rye chromatin have been produced by homoeologous recombination (Koebner and Shepherd, 1986; Rogowsky *et al.*, 1991).

Subsequently, particular primary recombinants with overlapping rye segments were intercrossed and derived recombinants with an interstitial rye segment were obtained from among their progeny. For example, the recombinant DRA-1 was derived by intercrossing primary recombinants 82-180 (proximal rye) and I-93 (distal rye) and selecting progeny that carry only the overlapping rye segment. This recombinant carries all the known 1DS wheat storage protein genes (Figure 4B.1). In a preliminary field experiment, this interstitial rye recombinant DRA-1 showed better dough quality than the primary recombinants at the same level of backcrossing (Shepherd *et al.*, 1994) and subsequently several other derived recombinants were produced from the

other primary recombinants isolated at the Waite Institute. These derived rye recombinants with an interstitial rye segment were produced in the same way as DRA-1 by intercrossing different combinations of distal and proximal rye recombinants with different breakpoints.

The amount and type of seed proteins present in wheat flour is known to be very important in determining the quality of dough (see review by Bushuk *et al.*, 1969). Therefore, it was expected that a recombinant line that carries all of the normal wheat storage protein loci might have improved quality compared with translocation lines lacking some of the protein components.

In the first part of the present study, all of the 1DL.1RS primary recombinants available with different lengths of rye chromatin were grown in field experiments and then assessed for yield and quality to determine whether elimination of segments of rye chromatin, resulted in improved agronomic characteristics.

In a follow-up study, the derived recombinants were examined in similar experiments to determine whether further reduction in the size of the rye segment resulted in a greater improvement in quality. There was particular interest in the performance of the interstitial derived recombinants because they possess only a relatively small segment of rye chromatin and all of the normal wheat storage protein genes. In this work, it was hoped to gain a clearer idea of whether it is the restoration of all wheat storage protein genes or the loss of some deleterious genes carried by rye chromosome arm 1RS that is important for restoring quality, or alternatively whether both genetic factors affect the quality of 1RS derived lines.

5.2. MATERIALS AND METHODS

5.2.1. Seed Materials

The seed materials used in these field experiments included all of the 1DL.1RS wheat-rye recombinant lines produced from previous studies, including:

(a) Four primary wheat-rye (WR) recombinant lines with a proximal rye segment: WR-2, WR-3, WR-4 (Rogowsky *et al.*, 1993) and 82-180 (Koebner and Shepherd, 1986). All of these wheat-rye recombinants have been shown to have the TRI-D1$^-$ GLI-D1$^+$ SEC-1$^+$ phenotype.

(b) Four primary recombinants with a distal rye segment: WR-1, WD-1, WD-2 (Rogowsky *et al.*, 1993), I-93 (Koebner and Shepherd, 1986). Recombinants WR-1 and I-93 have the TRI-D1$^+$ GLI-D1$^-$ SEC-1$^+$ phenotype, whereas WD-1 and WD-2 are TRI-D1$^+$ GLI-D1$^-$ SEC-1$^-$.

(c) Five derived (D) recombinants: DRA-1 (Koebner and Shepherd, 1986), DW-12, DW-14, DW-18, DW-92 (Shepherd, unpublished) were selected from intercrosses between distal and proximal recombinants with an overlapping rye segment, as described below. The same procedure used to isolate derived recombinant DRA-1 (Koebner and Shepherd, 1986) shown in Figure 4B.1 was used to isolate the other derived recombinants. Proximal and distal recombinants with overlapping rye segments were intercrossed in all combinations to allow homologous recombination to occur between the overlapping rye segment present in both recombinants.

The derived recombinants with an interstitial rye segment were selected by electrophoretic screening among the progeny. DW-12, DW-14 and DW-18 were

derived by intercrossing distal recombinant WR-1 and proximal recombinants WR-2, WR-4 and 82-180, respectively, while DW-92 was derived by crossing distal recombinant I-93 and proximal recombinant WR-2. The derived recombinants carried all the wheat endosperm storage protein loci of 1DS and *Sec-1* as well, but contained a smaller rye segment compared to the two primary recombinants.

(d) The original wheat-rye translocation 1DL.1RS, carrying the entire short arm of chromosome 1 of Imperial rye in a Gabo background was used as a control. Initially, the 1DL.1RS translocation was isolated in Chinese Spring wheat background (Shepherd, 1973), but since Chinese Spring also has many undesirable agronomic characters, the translocation was transferred to Gabo, a cultivar with more acceptable quality characteristics, by serial backcrossing.

(e) The normal cultivar Gabo. This cultivar has been chosen as the recurrent parent, since it was the bread-making quality standard for Australian wheats over a long period. The chromotypes of the lines used in this study are shown in Figure 5.1.

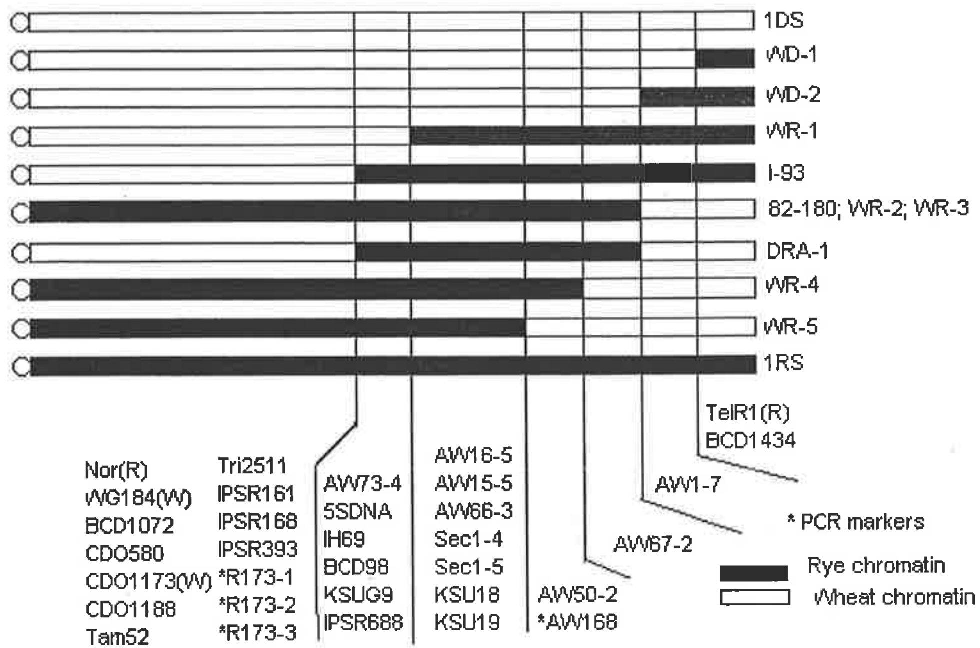

Figure 5.1. Chromotypes deduced from mapping of RFLP and PCR markers on wheat-rye recombinants (adapted from Rogowsky *et al.*, 1993)

5.2.2. Backcrossing and production of homozygous lines

The original recombinants were obtained in a mixed genetic background including the Chinese Spring genes introduced by crossing to Sears' *ph1b* mutant. Therefore before quality testing, it was necessary to backcross them to a cultivar with acceptable bread making quality such as Gabo. The designation, phenotype of recombinant lines and number of backcrosses to Gabo are shown in Table 5.1. All recombinants except WD-1 and WD-2 carried *Sec-1,* therefore with these two exceptions, the secalin phenotype was used as a marker for the presence of the recombinant chromosome in each backcross generation. After a variable number of backcrossess, heterozygous plants of each recombinant line were selected and allowed to self fertilise. The recombinant chromosomes in WD-1 and WD-2 were detected during backcrossing by a DNA marker specific for rye telomeres (Rogowsky *et al.*, 1993).

Progeny homozygous for the recombinant chromosome or homozygous normal for the 1D wheat chromosome were selected by determining their SDS-PAGE patterns of seed protein. At least sixteen seeds from each line were screened in unreduced SDS-PAGE gels (Chapter 3). Seeds lacking rye secalins were selected as the homozygous normal 1D genotypes. For the proximal rye recombinants, progeny with phenotype TRI-D1^{-} GLI-D1^{+} SEC-1^{+} were selected as the homozygous recombinant sister lines, while for distal rye recombinants, progeny with phenotype TRI-D1^{+} GLI-D1^{-} SEC-1^{+} were selected as the equivalent homozygous recombinant lines.

For the derived recombinants with phenotype TRI-D1^{+} GLI-D1^{+} SEC-1^{+}, homozygous normal progeny were selected after backcrossing in a similar way to that for the primary recombinants, that is by selecting progeny which lacked secalins. However,

in order to obtain the homozygous recombinant sister lines, it was necessary to progeny test the SEC-1$^+$ plants to distinguish between homozygous and heterozygous SEC-1$^+$ types. Progeny with phenotype TRI-D1$^+$ GLI-D1$^+$ SEC-1$^+$ were selected from the selfed progeny of backcross plants and at least 12 progenies from individual selected plants were then tested. Progeny lines which did not produce any SEC-1$^-$ progeny were assumed to be homozygous for the secalin locus.

Prior to planting in field experiments, the selected sister lines, (homozygous normal 1D and homozygous recombinants) were grown in rows in a bird-proof enclosure at the Waite Institute over summer for seed multiplication.

5.2.3. Field Experiments

Yield performance and quality characteristics including protein content, dough strength and extensibility, as well as SE-HPLC protein fractions of the primary and derived recombinants were tested in four field experiments in years 1991 and 1993 at three sites in the cereal-growing areas of South Australia. The sites chosen were Clinton, Sandergrove and Roseworthy (Figure 5.2). Relevant characteristics of the experimental sites are shown in Table 5.2.

Table 5.1. Designation and characteristics of 1RS recombinants from 1DL.1RS translocation

Type	Rye segments	Designation	Phenotype	No. of backcrosses	Parent combination
Primary recombinant	proximal	82-180	Tri-D1$^-$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	4	
		WR-2	Tri-D1$^-$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	2	
		WR-3	Tri-D1$^-$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	2	
		WR-4	Tri-D1$^-$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	3	
	distal	I-93	Tri-D1$^+$ Gli-D1$^-$ Sec-1$^+$ SrR$^+$	4	
		WR-1	Tri-D1$^+$ Gli-D1$^-$ Sec-1$^+$ SrR$^+$	3	
		WD-1	Tri-D1$^+$ Gli-D1$^-$ Sec-1$^-$ SrR$^-$ Tel-R1$^+$	2	
		WD-2	Tri-D1$^+$ Gli-D1$^-$ Sec-1$^-$ SrR$^-$ Tel-R1$^+$	2	
Derived recombinant	interstitial	DRA-1	Tri-D1$^+$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	4	I-93 x 82-180
		DW-12	Tri-D1$^+$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	3	WR-1 x WR-2
		DW-14	Tri-D1$^+$ Gli-D1$^+$ Sec-1$^+$SrR$^+$	3	WR-1 x WR-4
		DW-18	Tri-D1$^+$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	3	WR-1 x 82-180
		DW-92	Tri-D1$^+$ Gli-D1$^+$ Sec-1$^+$ SrR$^+$	3	I-93 x WR-2

Figure 5.2. Average annual rainfall map of Central District of South Australia

C= Clinton; R= Roseworthy; S= Sandergrove

Table 5.2. Characteristics of Experimental sites

Site characteristic	Experimental sites		
	Clinton	Roseworthy	Sandergrove
Soil type	Loamy mallee	Mallee brown soil	sandy loam over clay
Normal district sowing date	Early June	Early June	Early June
Long term average annual rainfall	375.0 mm	443.0 mm	493.0 mm

Each experimental plot consisted of four rows 4 m long and 15 cm apart, with 30 cm space between adjacent plots, and they were sown with a plot seeder at commercial densities. Cultivars with distinctive appearance such as brown glumes and awned head were used as grid control plots sown in asymmetric patterns to check that the planting had been carried out correctly. However, these grid plots were not included in the statistical analysis of the data.

Grain from the four field experiments were analysed to determine the yield and quality of recombinants and controls.

The grain used for quality assessment of the primary recombinants (Experiment 91/1 and Experiment 91/2) were kindly provided by Dr. KW Shepherd who had grown these genotypes in field experiments carried out in 1991. Field experiments 93/1 and 93/2 provided further grain for quality assessment of primary and derived recombinants and the trials were conducted in 1993 as part of the present study.

5.2.3.1. Yield and quality characteristics of primary recombinants (Experiment 91/1)

The aim of the experiment was to compare yield performance and quality characteristics between primary recombinants with different lengths and position of rye chromatin, along with the control recurrent parent Gabo and the translocation line 1DL.1RS. This experiment included three separate homozygous lines derived from each of primary recombinants WR-1, WR-2, WR-3, WR-4, WD-1, WD-2, I-93 and one homozygous line of 82-180, backcrossed to Gabo. Two entries of the recurrent parent Gabo and the original Gabo 1DL.1RS translocation and one entry of the derived recombinant DRA-1 were also included. These lines were sown in a randomized complete block design with 27 entries and 2 replicates. This experiment was conducted at Clinton in 1991.

5.2.3.2. Comparison of the yield and quality characteristics of primary recombinants with their normal 1D sister lines (Experiment 91/2)

The aim of the experiment was to obtain a more sensitive test of whether the recombinant chromosome had a deleterious effect on yield or quality. For each primary recombinant, a comparison was made of the yield and quality of sister lines with and without the recombinant chromosome along with control lines.

The experiment was made up of 7 sub-blocks, corresponding to each of the recombinant types (WR-1, WR-2, WR-3, WR-4, WD-1, WD-2 and DRA-1). Each sub-block included just one of the recombinants and consisted of eight 4-row plots made up of 3 homozygous recombinant lines and 3 homozygous normal 1D sister lines, the recurrent parent Gabo and the Gabo 1DL.1RS translocation line as controls.

The experiment was sown in 1991 at 2 country sites, Clinton and Roseworthy, South Australia, with two replicates, however, due to time limitations quality assessment was only carried out on one replicate of each site. For convenience, the data obtained from these two sites were analysed as two replicates of a single experiment in the statistical analysis.

5.2.3.3. Comparison of the yield and quality characteristics of derived recombinants and their normal 1D sister lines (Experiment 93/1)

The aim of the experiment was to compare yield performance and quality characteristics of the derived recombinants with their normal sister lines. For each derived recombinant, a comparison was made of the yield and quality of sister lines with and without the recombinant chromosome along with control lines.

Four entries of each derived recombinant line (DW-12, DW-14, DW-18 and DW-92), along with four normal 1D sister lines and 2 entries of each the recurrent parent Gabo and the Gabo 1DL.1RS translocation line were included in this experiment. These 36 entries were included in a completely randomized block design with two replicates. They were grown at Sandergrove in 1993.

5.2.3.4. Yield and quality characteristics of primary versus derived recombinants (Experiment 93/2)

The aim of the experiment was to compare yield performance and quality characteristics of derived recombinants with those of their parental primary

recombinants, in order to determine whether the reduced length of rye chromatin leads to improved agronomic and quality performance of the derived recombinant lines.

Two homozygous lines of each of the derived recombinants DRA-1, DW-12, DW-14, DW-18 and DW-92, and one of each of the primary recombinant lines 82-180, I-93, WR-1, WR-2, WR-4 and the recurrent parent Gabo and the original translocation line 1DL.1RS were completely randomized within a block (17 entries). They were sown in field plots at Sandergrove in 1993 using a completely randomized block design (CRBD) with three replicates.

In all of these field experiments, the agronomic practices used (seeding times and rates, fertiliser, pesticide and herbicide application) were those normally applied to the plots in wheat breeders' trials, which in turn reflected farming practice in the area.

5.2.4. Quality assessment

After harvest, seed material was cleaned using a Carter Dockage tester and weighed to provide the yield data. Sub-samples (250-300 g) of grain from each plot were tempered to 14% moisture content and milled in a Quadrumat Junior Mill prior to quality assessment. The protein content of the flour was measured by NIR (Near Infra Red Reflectance).

Dough extensibility (E) was measured from the length of the extensogram (cm), while dough strength (Rmax.) was determined from the maximum height of the extensograph curve (Brabender Unit/BU) (Figure 5.3). Procedures used for the Extensograph and the Size-Exclusion High Performance Liquid Chromatography tests were described earlier in Chapter 3.

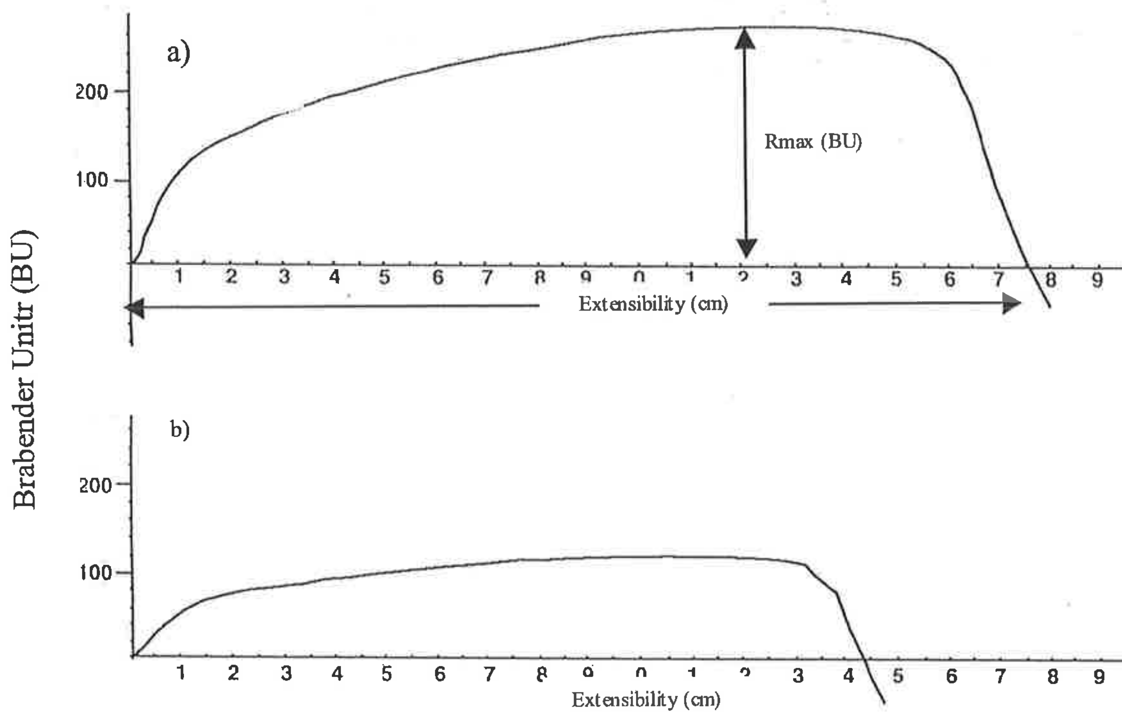

Figure 5.3. Profile of extensograph parameters showing dough strength/Rmax (BU) and extensibility (cm) (a) Gabo and (b) 1DL.1RS translocation.

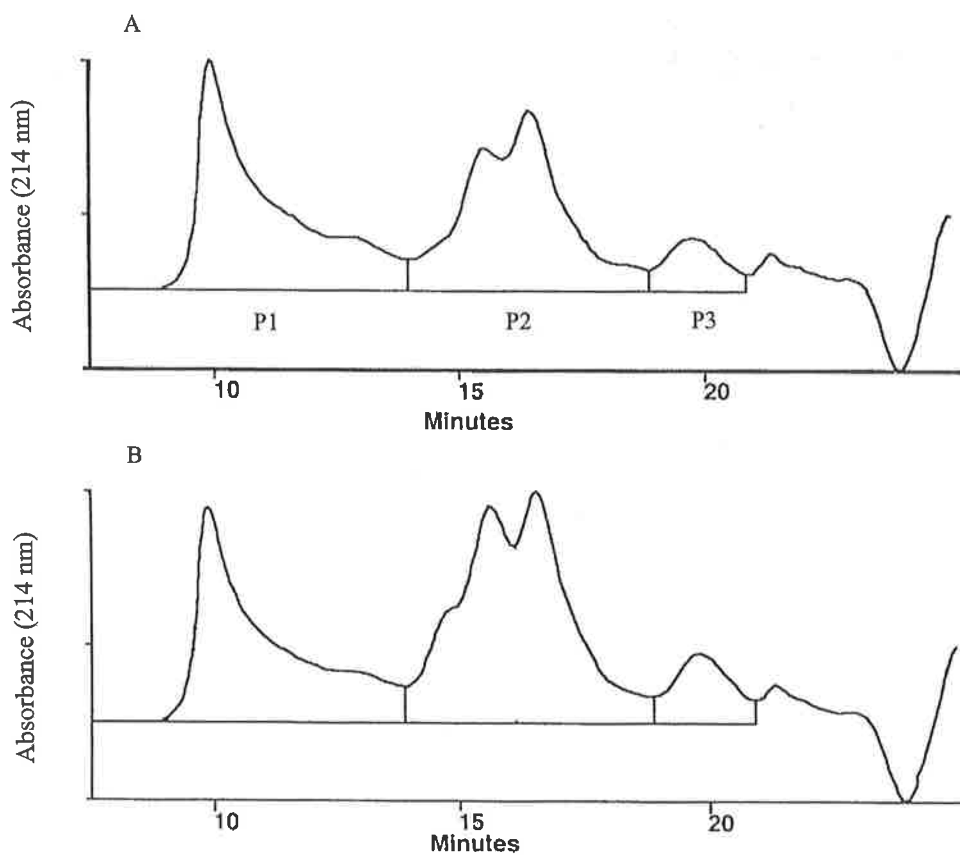

Figure 5.4. SE-HPLC profile of relative proportion of three mean peaks: polymeric proteins (P1), monomeric gliadins (P2), and salt-water soluble proteins (P3) of Gabo (A) and 1DL.1RS translocation (B).

Size-exclusion high performance liquid chromatography (SE-HPLC) was used to quantify the relative proportion of the main protein components of wheat endosperm by measuring the relative area under the peaks (Figure 5.4). Three main peaks were observed in SE-HPLC profiles: Peak 1 (P1) with the molecular weight >100 kD contains polymeric proteins, peak 2 (P2) which is between 25-100 kD contains mostly monomeric gliadins, while peak 3 (P3) with the molecular weight <25 kD contains albumin and globulin or salt-water soluble proteins (Singh *et al.*, 1991; Batey *et al.*, 1991; Lee *et al.*, 1995). It should be noted that since these peaks are reported as percentages of the total proteins, it follows that any decrease (or increase) in P1 will automatically be accompanied by an increase (or decrease) in at least one of the other fractions (P2, P3).

5.2.5. Statistical analysis

Analysis of Variance was performed by Genstat 5 statistical program. Means were compared using least significant difference (LSD). Pair-wise comparisons between genotypes for tests of significance were done by using the Scheffe Test for the experiments with unequal numbers of replicates of genotypes tested.

5.3. RESULTS

5.3.1. Experiment 91/1. Yield and quality parameters of primary recombinants and one derived recombinant

5.3.1.1. Analysis of variance from individual sites

Analysis of variance was carried out separately for each of the two sites in order to test the homogeneity of their performance and to test the variability of recombinant lines. The residual mean squares for individual sites are required to test for homogeneity before carrying out a combined analysis over all sites.

5.3.1.2. Analysis of variance combined over sites

Since the results from both sites were quite homogeneous, analysis of variance combined over sites was carried out to observe the yield and quality of recombinant lines over sites (Table 5.3 and Table 5.4).

The site effects on the yield and quality of recombinant lines were all highly significant except for Rmax. The genotype effects were not significant for yield, but significant for all quality parameters tested including HPLC parameters. No significant genotype X environment interactions were found except for the HPLC parameters Peak 1 and Peak 2 at the 5% level, indicating that the recombinant lines performed similarly at both sites for most characters. Therefore, the results were combined and average value for all parameters are shown in Tables 5.5 and 5.6.

Table 5.3. F value and level of significance from Analysis of Variance for grain yield (Y), flour yield (FY), protein content (PC), dough strength (Rmax), and extensibility (E) of Experiment 91/1.

Source of Variation	Y	FY	PC	Rmax	E
Genotype	1.73 ns	42.88 ***	6.86 ***	12.78 ***	5.36 ***
Site	41.57 ***	20.05 ***	40.92 ***	1.82 ns	26.00 ***
Site*Genotype	0.63 ns	1.64 ns	1.28 ns	0.97 ns	1.25 ns

*** significant at P= 0.001
ns non significant

Table 5.4. F value and level of significance from Analysis of Variance for HPLC parameters peak 1, peak 2, peak 3, P1/P2, and P1/P3 of experiment 91/1.

Source of Variation	P1	P2	P3	P1/P2	P1/P3
Genotype	65.71 ***	47.69 ***	68.36 ***	53.04 ***	86.90 ***
Site	26.34 ***	23.07 ***	1.13 ns	24.46 ***	0.58 ns
Site*Genotype	2.40 *	2.52 *	1.13 ns	2.83 **	1.07 ns

* significant at P= 0.05
** significant at P= 0.01
*** significant at P= 0.001
ns non significant

5.3.1.3. *Comparison of individual lines for yield and extensograph parameters*

There were no significant differences in yield between any of the lines (Table 5.3), indicating that the whole 1RS translocation or smaller segments of it did not significantly lower yield.

In this experiment, there was a significant effect of genotype on the flour yield, but only between recombinant line I-93 and the other recombinant lines and controls. It was detected that recombinant I-93 which had a very low flour yield (49.3 g) produced a very crumbly flour with the flour tending to stick to the bran, resulting in low flour yield.

Dough strength as measured by maximum resistance to stretching is one of the important parameters for dough quality. As shown in Table 5.5 and Figure 5.5, the dough strength of all the recombinant lines was significantly higher than that of the original 1DL.1RS translocation line, but several of them still showed significantly less strength than Gabo. The dough strengths of I-93 and distal recombinants WD-1, WD-2 and derived recombinant DRA-1 were not significantly different from each other and were similar to that of the recurrent parent Gabo (Figure 5.5).

There was variation in dough extensibility of the rye-containing lines ranging from 15.3 to 18.9 cm. The extensibility of Gabo and all of the proximal recombinants except 82-180 was significantly higher than that of 1DL.1RS, while none of the distal recombinants showed significant difference to 1DL.1RS, except WD-1 which has the shortest rye segment on the distal part of the chromosome.

Unexpectedly, the distal recombinant I-93 which lacked *Gli-D1/Glu-D3* and contained more rye chromatin than the other distal recombinants WR-1, WD-1 and WD-2 also had comparable dough strength and extensibility to the recurrent parent Gabo (Table 5.5 and Figure 5.5).

Table 5.5. Mean values of yield (Y), protein content (PC), flour yield (FY), dough strength (Rmax) and extensibility (E) of primary recombinants, derived recombinant DRA-1 and controls (Gabo and Gabo 1DL.1RS) (Exp. 91/1).

Lines	Y (g)	PC (%)	FY (%)	Rmax (BU)	E (cm)
Control					
Gabo	858.3	12.45	64.33	281.3	18.6
1DL.1RS	788.8	14.18	65.75	137.0	15.3
Proximal recombinant					
82-180	802.3	12.45	65.89	216.8	16.2
WR-2	735.9	14.01	64.45	251.8	18.7
WR-3	731.1	14.53	65.24	224.8	18.8
WR-4	751.2	14.07	65.09	233.5	18.5
Distal recombinant					
I-93	830.2	13.18	49.34	275.5	17.4
WR-1	703.3	13.40	65.76	229.7	15.8
WD-1	802.5	14.24	63.94	282.7	17.9
WD-2	815.3	13.55	64.60	289.4	16.7
Derived recombinant					
DRA-1	810.7	13.53	65.69	285.4	18.9
LSD	105.3	0.8	4.2	26.4	1.7

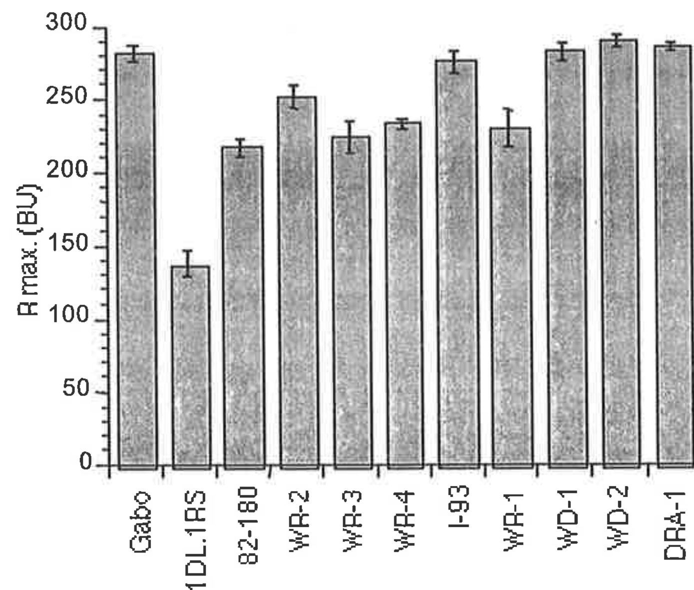

Figure 5.5. Mean value of dough strength (Rmax) for controls (recurrent parent Gabo and Gabo 1DL.1RS translocation line) and proximal primary (82-180, WR-2, WR-3, WR-4), distal primary (I-93, WR-1, WD-1, WD-2) recombinants, derived recombinant DRA-1 in Experiment 91/1. Error bars shown represent standard error of the means.

5.3.1.4. *Variation in SE-HPLC fractions*

There were significant differences in the protein fractions of the lines tested when measured by SE-HPLC (Table 5.4). The relative amount of polymeric protein/ Peak1 (P1) in Gabo was significantly higher than that of 1DL.1RS translocation (Table 5.6). In general, the relative amount of P1 in the primary recombinants was between the value of 1DL.1RS and normal Gabo. The distal recombinants had significantly less P1 component compared to the proximal recombinants, except no significant difference was found between WD-2 and 82-180. DRA-1 was similar to normal Gabo in all HPLC parameters (Table 5.6 and Figure 5.6).

Table 5.6. Mean value of HPLC parameters (polymeric protein P1, monomeric protein P2, salt-water soluble protein P3 and their ratios) of primary recombinants, derived recombinant DRA-1 and controls (Gabo and Gabo 1DL.1RS) (exp. 91/1).

Lines	P1 (%)	P2 (%)	P3 (%)	P1/P2	P1/P3
Control					
Gabo	43.92	48.12	7.97	0.92	5.52
1DL.1RS	35.48	55.41	9.12	0.64	3.89
Proximal recombinant					
82-180	41.12	51.01	7.88	0.81	5.22
WR-2	41.74	49.78	8.48	0.84	4.93
WR-3	41.96	49.93	8.12	0.84	5.18
WR-4	42.03	50.24	7.74	0.84	5.43
Distal recombinant					
I-93	37.78	52.41	9.80	0.72	3.86
WR-1	37.50	53.26	9.24	0.70	4.06
WD-1	40.02	50.60	9.39	0.79	4.27
WD-2	40.88	50.01	9.11	0.82	4.49
Derived recombinant					
DRA-1	43.39	48.73	7.88	0.89	5.51
LSD	0.7	0.7	0.3	-	-

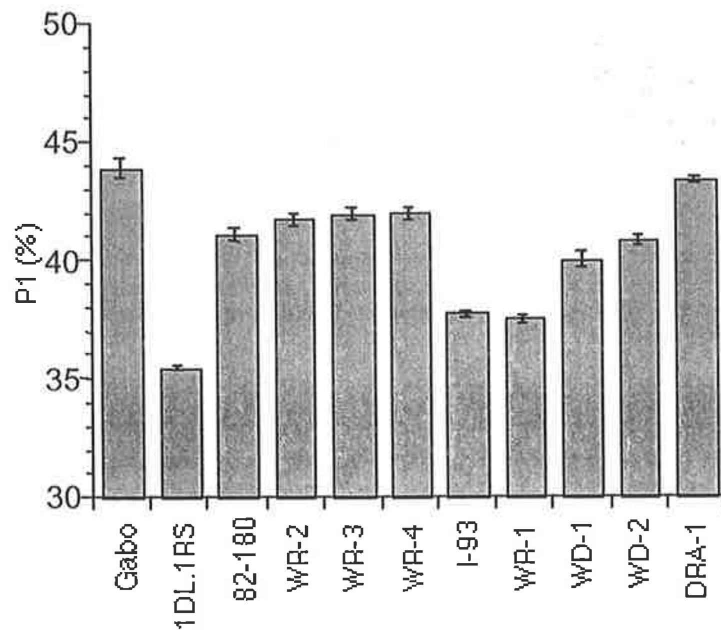

Figure 5.6. Mean values of Peak 1 (polymeric proteins) for controls (recurrent parent Gabo and Gabo 1DL.1RS translocation line), proximal primary (82-180, WR-2, WR-3, WR-4), distal primary (I-93, WR-1, WD-1, WD-2) recombinants and derived recombinant DRA-1 in experiment 91/1. Error bars represent standard error of the means.

In contrast with the relative amounts of P1, the translocation line 1DL.1RS had a significantly higher amount of Peak 2 compared to all recombinant lines and Gabo. All primary recombinant lines had significantly lower amount of total P2 than the translocation 1DL.1RS, but significantly greater than Gabo. The data indicated that distal recombinants tend to have higher P2 than proximal recombinants, but many of them were not significantly different (Table 5.6 and Figure 5.7).

Peak 3, which consists mostly of water-soluble proteins, also showed significant differences. The translocation line 1DL.1RS had a significantly higher amount of P3 compared to Gabo and the proximal recombinants. The distal recombinants contained significantly higher amount of P3 compared to the proximal recombinants and there was no difference between the value of distal recombinants and the 1DL.1RS translocation (Table 5.6 and Figure 5.8). Accordingly, P1/P2 and P1/P3 ratio of proximal recombinants were higher than those of distal recombinants. In addition, the value of HPLC parameters of proximal recombinants was similar to Gabo and significantly different to 1DL.1RS.

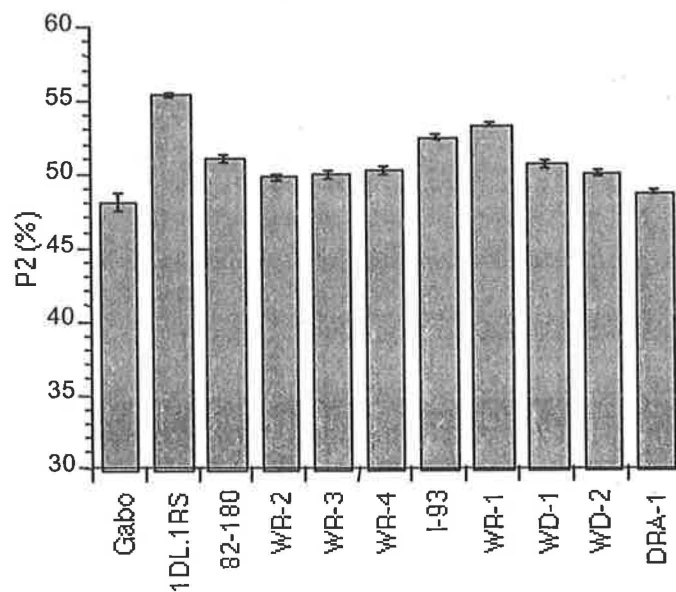

Figure 5.7. Mean values of Peak 2 (monomeric proteins) for controls (recurrent parent Gabo and Gabo 1DL.1RS translocation line), proximal primary (82-180, WR-2, WR-3, WR-4), distal primary (I-93, WR-1, WD-1, WD-2) recombinants and derived recombinant DRA-1 in experiment 91/1. Error bars represent standard error of the means.

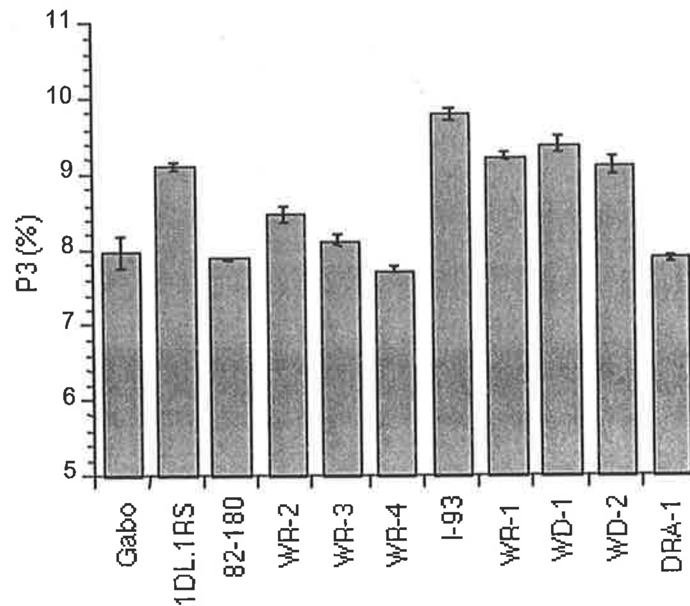

Figure 5.8. Mean values of Peak 3 (salt-water soluble proteins) for controls (recurrent parent Gabo and Gabo 1DL.1RS translocation line), proximal primary (82-180, WR-2, WR-3, WR-4), distal primary (I-93, WR-1, WD-1, WD-2) recombinants and derived recombinant DRA-1 in experiment 91/1. Error bars represent standard error of the means.

5.3.2. Experiment 91/2. Yield and quality parameters of primary recombinants versus their normal 1D sister lines and controls

This experiment was designed to obtain more critical comparisons of the agronomic characteristics of each recombinant line and their respective normal sister lines in the equivalent genetic background. As described earlier, eight plots made up of 3 isolates of a particular homozygous recombinant line, 3 isolates of homozygous normal 1D sister lines and 2 controls (the recurrent parent Gabo and the Gabo 1DL.RS translocation line) were completely randomized within sub-blocks. The statistical analysis used was for a split plot design with comparisons restricted to entries within each sub-block. That is, comparisons were made between the sister lines with and without recombinant chromosomes, and the adjacent controls.

5.3.2.1. Comparison of individual lines for yield and extensograph parameters

Although yield of most genotypes carrying recombinant 1DS/1RS chromosome was lower than that of Gabo and the respective normal sister lines, none of the differences were significant. A similar result was also found with flour yield (FY). No significant difference was also found between the yield of recombinant lines and the translocation 1DL.1RS. In general, the grain protein content of the recombinant lines was higher than that of normal sister lines, but the differences were not significant, except for WR-4 (Table 5.7).

Comparisons of dough strength in control cultivars showed that Gabo gave significantly higher Rmax than the original translocation in the Gabo background in all sub-blocks. All recombinants also gave significantly greater Rmax compared to the

original 1DL.1RS translocation. Several primary recombinant lines (WR-2, WR-4 and WR-1) showed significantly less Rmax compared to their respective normal sister lines and the control cultivar Gabo. The WR-3 recombinant line also gave a lower Rmax value than sister lines but the difference was not significant. The WD-1 and WD-2 lines showed Rmax values comparable to Gabo and no significant difference to their sister lines. In this experiment, yield and extensibility of DRA-1 were similar to those of its sister lines, but its dough strength was significantly lower. (Table 5.7 and Figure 5.9).

Although Gabo tended to be more extensible than the translocation 1DL.1RS, differences were not significant in most of the sub-blocks. The extensibilities of all the recombinant lines were not significantly different from those of their normal sister lines, except WR-1 showed significantly less extensibility than its normal sister line.

Table 5.7. Mean values of yield (Y), protein content (PC), flour yield (FY), dough strength (Rmax) and extensibility (E) for 7 recombinant lines, their respective normal sister lines (N1D) and the average of control entries (Gabo and Gabo 1DL.1RS) grown at Clinton and Roseworthy in 1991 (Exp. 91/2).

Lines	Y (g)	PC (%)	FY (%)	Rmax (BU)	E (cm)
Gabo	762.9	12.29	64.90	287.9	16.59
1DL.1RS	630.1	13.65	64.03	131.8	14.07
WR-2 N1D	608.0 ns	12.73 ns	65.04 ns	312.2 **	17.8 ns
WR-2 Rec.	617.2	13.70	64.20	241.8	17.3
WR-3 N1D	571.0 ns	14.00 ns	65.69 ns	242.6 ns	17.3 ns
WR-3 Rec.	571.5	14.63	62.77	182.5	16.7
WR-4 N1D	599.3 ns	13.67 **	62.82 ns	303.5 **	19.8 ns
WR-4 Rec.	537.2	15.37	62.31	242.2	17.8
WR-1 N1D	704.2 ns	12.92 ns	64.10 ns	282.5 **	19.8 **
WR-1 Rec.	645.8	13.67	66.40	205.2	15.9
WD-1 N1D	624.3 ns	13.40 ns	62.50 ns	300.2 ns	17.6 ns
WD-1 Rec.	561.7	14.70	62.90	276.3	15.9
WD-2 N1D	868.8 ns	12.57 ns	66.60 ns	303.7 ns	17.5 ns
WD-2 Rec.	798.7	12.90	66.70	292.5	15.5
DRA-1 N1D	657.0 ns	13.00 ns	61.63 ns	313.0 **	18.8 ns
DRA-1 Rec.	606.0	13.67	65.02	264.7	18.4

Comparison between recombinant and normal sister lines
** significant at P=0.01
ns non significant

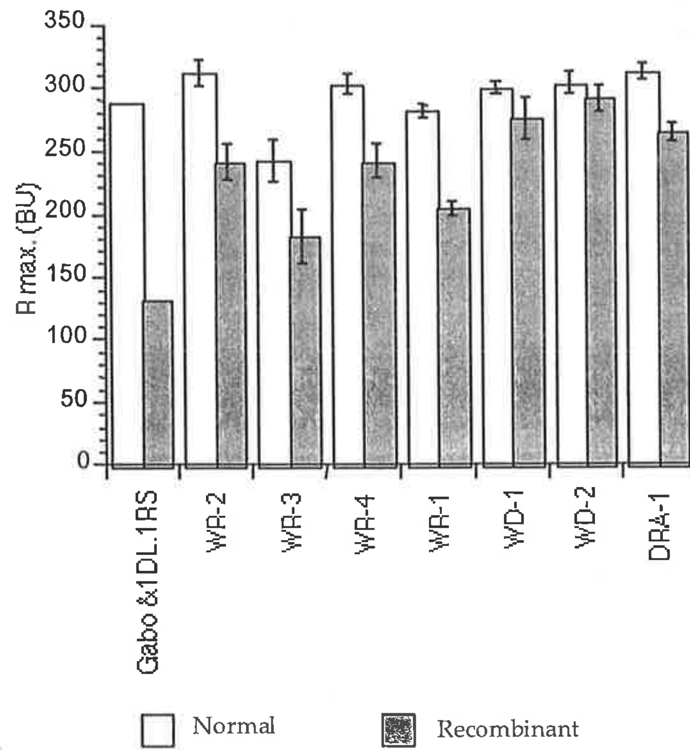

Figure 5.9. Mean values of dough strength (Rmax.) of the average of controls (cv. Gabo and Gabo 1DL.1RS translocation line), proximal (WR-2, WR-3, WR-4) and distal (WR-1, WD-1, WD-2) recombinants, derived recombinant DRA-1 and their normal sister lines. Error bars represent standard error of the means.

Direct comparison cannot be made between recombinant lines because the experiment was not randomized with respect to the different recombinant lines but rather was designed to make more precise comparisons between each recombinant line and its normal sister lines. However, if an index of dough resistance is calculated, based on ratio of Rmax value in recombinants compared to normal sister lines, legitimate comparisons can be made between the recombinants, but there is no information on whether these differences are statistically significant (Table 5.8).

Table 5.8. Ratio of dough resistance of recombinant lines compared to their respective sister lines

WR-2	WR-3	WR-4	WR-1	WD-1	WD-2	DRA-1
0.77	0.75	0.80	0.73	0.92	0.96	0.85

5.3.2.2. *Variation in SE-HPLC fractions*

The recombinant lines had significantly lower amounts of polymeric protein (Peak 1), significantly higher amounts of monomeric protein (Peak 2) and slightly, but significantly higher amounts of salt-water soluble protein (Peak 3) compared to those of their normal sister lines, except for WR-4 which had a similar amount of P3 compared to its normal sister line (Table 5.9).

The relative amount of P1 in recombinant lines was significantly higher than that of the translocation 1DL.1RS, but significantly lower than that of normal Gabo (Figure 5.10). Meanwhile, the amount of P2 in recombinant lines was significantly higher than in Gabo, but less (not significantly) than in the translocation 1DL.1RS in all sub-

blocks. The amount of P3 showed similar differences for all recombinants except for WR-4 which had similar levels of P3 in both groups of sister lines.

Table 5.9. Mean values of HPLC parameters (polymeric protein P1, monomeric protein P2, salt-water soluble protein P3 and their ratios) of 7 recombinant lines, their respective normal sister lines (N1D) and the average of control entries (Gabo and Gabo 1DL.1RS) (exp. 91/2).

Lines	P1 (%)	P2 (%)	P3 (%)	P1/P2	P1/P3
Gabo	44.02	48.43	7.55	0.91	5.84
1DL.1RS	35.35	55.76	8.89	0.63	3.98
WR-2 N1D	44.57***	47.80***	7.62*	0.93	5.85
WR-2 Rec.	41.57	50.27	8.16	0.83	5.10
WR-3 N1D	43.50**	48.71***	7.68*	0.89	5.59
WR-3 Rec.	41.17	50.60	8.23	0.82	5.02
WR-4 N1D	44.74***	47.88***	7.38^{ns}	0.93	6.07
WR-4 Rec.	41.22	51.45	7.33	0.80	5.63
WR-1 N1D	45.47***	46.56***	7.97**	0.98	5.70
WR-1 Rec.	37.35	53.68	8.97	0.70	4.17
WD-1 N1D	44.34***	48.00***	7.66**	0.93	5.79
WD-1 Rec.	39.72	51.36	8.92	0.78	4.47
WD-2 N1D	45.67***	46.60***	7.74**	0.98	5.92
WD-2 Rec.	40.81	50.18	9.01	0.81	4.54
DRA-1 N1D	45.44***	46.92***	7.64*	0.97	5.95
DRA-1 Rec.	43.05	49.25	7.71	0.88	5.58

Comparison between recombinant and sister lines

*** significant at P=0.001

** significant at P=0.01

ns non significant

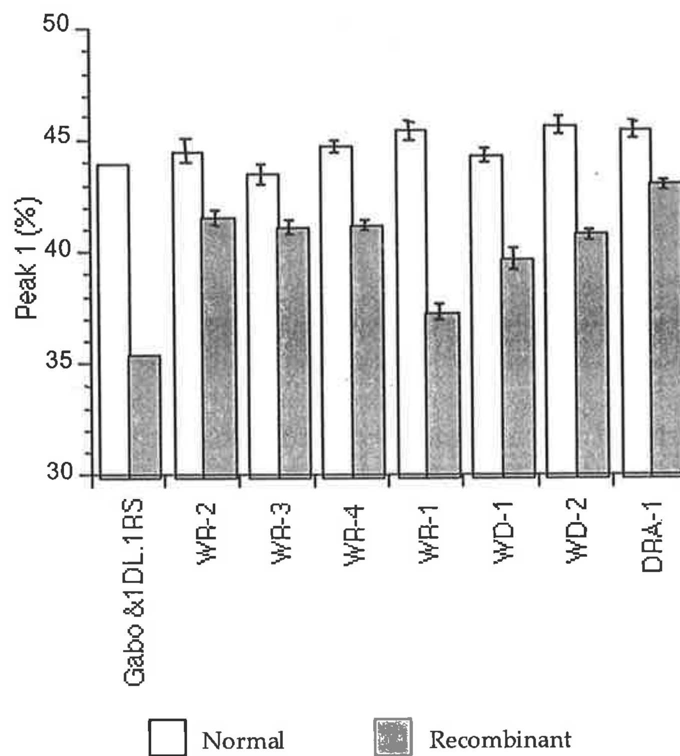

Figure 5.10. Mean values of Peak 1 of the average of controls (Gabo and Gabo 1DL.1RS translocation line), proximal (WR-2, WR-3, WR-4) and distal (WR-1, WD-1, WD-2) recombinants and derived recombinant DRA-1. Error bars represent standard error of the means.

Table 5.10. Ratio of relative amounts of each SE-HPLC fraction in recombinant lines versus their respective sister lines

Lines	Peak 1	Peak 2	Peak 3
WR-2	0.93	1.05	1.07
WR-3	0.95	1.04	1.07
WR-4	0.92	1.07	0.99
WR-1	0.82	1.15	1.13
WD-1	0.90	1.07	1.16
WD-2	0.89	1.08	1.16
DRA-1	0.95	1.05	1.01

Comparison of the ratio of SE-HPLC fractions of recombinant lines to their respective sister lines indicated that P1 of proximal recombinants tended to be higher than that of distal recombinants. In contrast, P3 was higher in distal recombinants than in proximal recombinants, while the highest value of P2 was shown by WR-1 (Table 5.10).

5.3.3. Experiment 93/1. Yield and quality parameters of derived recombinants, their normal 1D sister lines, the recurrent parent Gabo and the translocation 1DL.1RS

5.3.3.1. Comparison of individual lines for yield and extensograph parameters

Analysis of variance showed that there were significant genotypic differences for most of the characters measured including grain yield (GY), flour yield (FY), dough

strength (Rmax) and all the HPLC parameters (P1, P2 and P3), whereas no differences were found for flour protein content (PC) and dough extensibility (E) (Table 5.11).

Table 5.11. Mean squares and level of significance for genotypes from analysis of variance for the characters of yield (Y), protein content (PC), flour yield (FY), dough strength (Rmax) and extensibility (E) (Exp. 93/1).

Source of Variation	df	Y	PC	FY	Rmax	E
Genotype	9	31894***	1.35 ^{ns}	13.13 ***	6003.2***	1.41 ^{ns}
Residual	61	8781	1.37	2.92	358.9	1.55

*** significant at P=0.001
ns non significant

Table 5.12. Mean squares and level of significance for genotypes from analysis of variance of protein components from HPLC (Peak 1, Peak 2, Peak 3, ratio Peak 1 to Peak 2, ratio Peak 1 to Peak 3) (Exp. 93/1).

Source of Variation	df	P1	P2	P3	P1/P2	P1/P3
Genotype	9	97.60 **	77.62 ***	3.26 ***	0.245 ***	1.84 ***
Residual	61	0.85	2.62	1.02	0.005	0.14

*** significant at P=0.001
** significant at P=0.01

There were no significant differences between the derived recombinants, their normal sister lines and controls for grain and flour yield and protein content, except between lines DW-18 recombinant and 1DL.1RS for grain and flour yield (Table 5.13).

Table 5.13. Mean values of yield (Y) and quality parameters protein content (PC), flour yield (FY), dough strength (Rmax) and extensibility (E) of four derived recombinants, their normal 1D sister lines (N1D) and controls (Gabo and Gabo 1DL.1RS) grown at Sandergrove in 1993 (Exp. 93/1).

Lines	Entries	Y (g)	PC (%)	FY (%)	Rmax (BU)	E (cm)
Controls						
Gabo	4	722.7	12.10	65.66	214.3	14.8
1DL.1RS	4	501.9	12.65	63.68	110.8	12.5
Derived recombinant						
DW12-N1D	8	692.6	11.21	64.80	213.4	14.2
DW12-Rec	8	585.2	11.63	66.14	172.4	13.8
DW14-N1D	8	635.3	11.47	66.70	211.0	13.9
DW14-Rec	8	603.4	11.58	65.47	193.4	13.8
DW18-N1D	8	707.1	11.19	67.15	227.6	14.2
DW18-Rec	8	751.4	11.54	68.77	206.3	14.1
DW92-N1D	8	656.6	11.75	65.10	218.8	14.0
DW92-Rec	8	631.0	10.86	66.24	179.1	13.8
Mean		653.0	11.51	66.11	198.3	13.9
Scheffe	4 vs 4 entr.	283.6	3.55	5.17	57.3	3.8
Test	8 vs 8 entr	200.5	2.51	3.65	40.5	2.7
	4 vs 8 entr	245.6	3.07	4.48	49.7	3.3
cv.		14.4	10.2	2.6	9.6	8.9

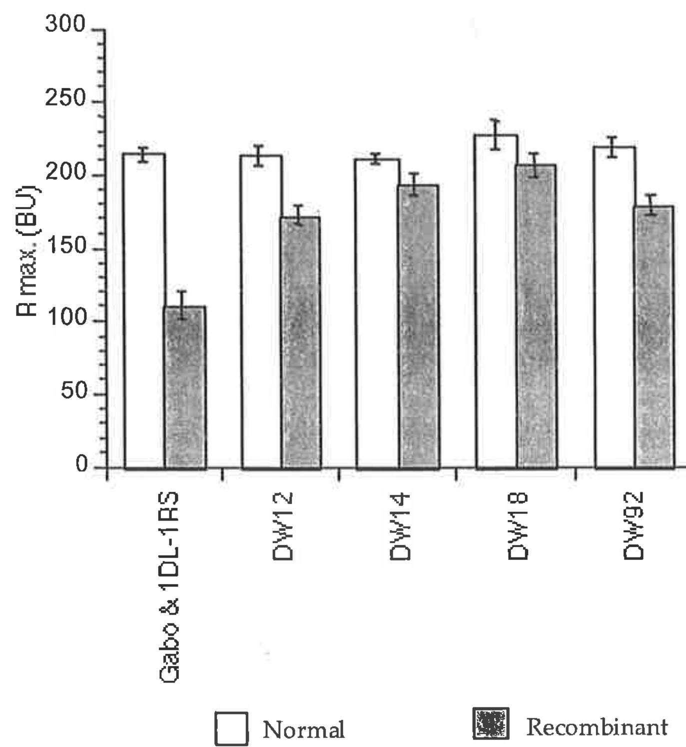

Figure 5.11. Mean values for dough strength (Rmax.) of derived recombinant lines vs. their normal sister lines. The error bars represent the standard error of the means.

In general, the dough strength of all the derived recombinant lines was less than that of their normal sister lines, however, it was not significant statistically except with DW-12. The dough strength of all the derived recombinants was significantly higher than that of 1DL.1RS, and more similar to the recurrent parent Gabo (Table 5.13 and Figure 5.11). In respect to dough extensibility, there were no significant differences between all lines tested.

5.3.3.2. Variation in SE-HPLC fractions

All the derived recombinants had significantly less polymeric P1 fraction and significantly more monomeric P2 compared to their normal sister lines and the control cultivar Gabo. On the other hand, they all had significantly higher P1 and significantly lower P2 compared to the translocation line Gabo 1DL.1RS. In fact, their P1 and P2 values were in between the two control lines Gabo and 1DL.1RS, while no significant difference was found for P3 between all lines tested (Table 5.14 and Figure 5.12 for P1).

Table 5.14. Mean values of HPLC parameters (polymeric protein P1, monomeric protein P2, salt-water soluble protein P3 and their ratios of four derived recombinant, their normal 1D sister lines (N1D) and controls (Gabo and Gabo 1DL.1RS) grown at Sandergrove in 1993 (Exp. 93/1).

Lines		Entries	P1 (%)	P2 (%)	P3 (%)	P1/P2	P1/P3
Control							
Gabo		4	51.22	38.23	10.56	1.35	4.90
1DL.1RS		4	39.68	47.74	12.58	0.83	3.20
Derived recombinant							
DW12-N1D		8	51.55	36.95	11.51	1.40	4.53
DW12-Rec		8	45.38	42.18	12.44	1.08	3.67
DW14-N1D		8	51.73	36.93	11.35	1.40	4.58
DW14-Rec		8	46.47	42.18	11.35	1.11	4.13
DW18-N1D		8	51.76	36.99	11.25	1.40	4.64
DW18-Rec		8	45.82	43.08	11.10	1.07	4.18
DW92-N1D		8	51.47	37.54	10.99	1.38	4.75
DW92-Rec		8	45.87	41.47	12.66	1.11	3.64
Mean			48.39	40.02	11.59	1.23	4.24
Scheffe	4 vs. 4 entr.		2.79	4.90	3.06	0.21	1.13
Test	8 vs. 8 entr..		1.97	3.46	2.16	0.15	0.80
	4 vs. 8 entr.		2.42	4.24	2.65	0.19	0.98
cv.			1.9	4	8.7	5.7	8.8

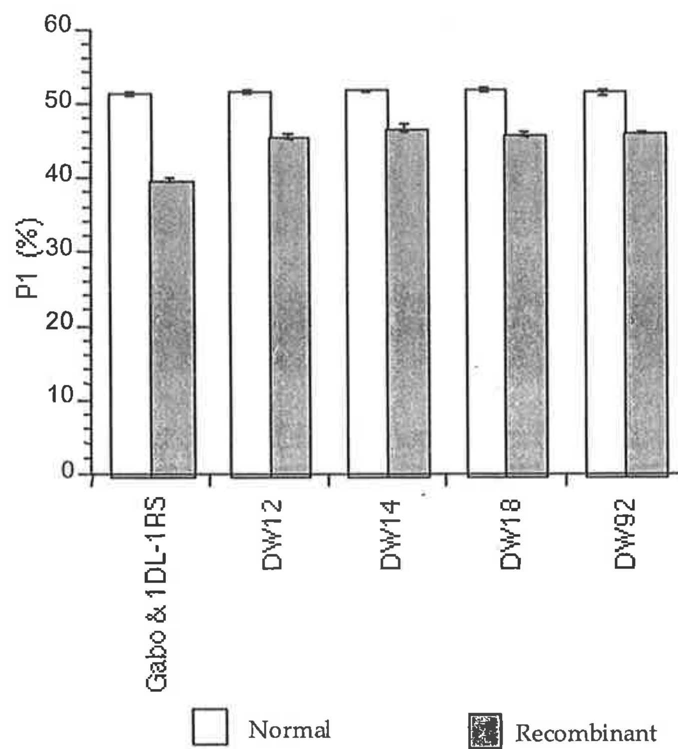

Figure 5.12. Mean values for Peak 1 (SE-HPLC) of derived recombinant lines vs. their normal sister lines. The error bars represent the standard error of the means.

5.3.4. Experiment 93/2. Yield and quality parameters of five primary recombinants, five derived recombinants and controls

5.3.4.1. Comparison of individual lines for yield and extensograph parameters

Analysis of variance showed that there were significant genotype effects for all characters measured in the 12 different genotypes tested (Table 5.15).

Table 5.15. Mean squares and significance levels from analysis of variance for yield (Y), protein content (PC), flour yield (FY), dough strength (Rmax) and extensibility (E) of lines grown at Sandergrove in 1993 (Exp. 93/2).

Source of Variation	df	Y	PC	FY	Rmax	E
Genotype	11	527200***	1.4542***	9.185***	6041.3***	7.0211***
Residual	37	2535	0.1150	2.068	246.3	0.6338

*** significant at P=0.001

The yields of the translocation and most of the recombinants were less than the Gabo control parent, except for derived recombinants DW 18 and DW 92 which yielded slightly more than Gabo. However, because of the variability in yield between replicates, none of these differences were statistically significant, except recombinants WR-2, WR-1 and DRA-1 gave significant lower yields than Gabo. There were some significant differences in the protein content of flour of three lines, but these values were highly negatively correlated with the yield differences. The grain protein content in this experiment was lower than that from experiment 93/1 at the same site but the reason for this is not known.

As in the earlier experiments, most of the primary recombinant lines showed significantly higher dough strength than 1DL.1RS. However in this experiment, some of the primary recombinants (WR-2, WR-4 and I-93) gave higher dough strength than Gabo, but none of these differences were significant and each of these lines had a higher grain protein content than Gabo which would be expected to increase their Rmax values. The only derived recombinant which gave higher dough strength than its parental primary recombinant was DRA-1, but the increase was significant only compared with its proximal recombinant parent 82-180 (Table 5.16 and Figure 5.13). Unexpectedly, all the other derived recombinants gave lower Rmax values than at least one of its parental primary recombinants.

There was no significant difference between the extensibility of the translocation line 1DL.1RS and normal Gabo. The dough extensibility of WR-2 and WR-4 as well as DRA-1 was significantly higher than that of most of the other recombinants and part of this could be due to the higher grain protein content of these lines.

Table 5.16. Mean values of grain yield, protein content, flour yield, dough strength (Rmax) and extensibility of 5 primary recombinants, 5 derived recombinant lines and controls (Gabo and Gabo 1DL.1RS) grown at Sandergrove in 1993 (Exp. 93/2).

Lines	Entries	Yield (g)	Protein (%)	F Yield (%)	Rmax. (BU)	Extensibility (cm)
Control						
Gabo	3	736.2	9.47	64.78	217.7	12.47
1DL.1RS	3	577.8	10.83	63.61	114.3	12.63
Proximal recombinant						
82-180	3	720.9	9.67	64.12	151.7	13.67
WR-2	3	484.6	10.90	63.49	252.7	15.13
WR-4	3	548.2	10.50	62.71	236.0	16.03
Distal recombinant						
I-93	3	686.5	10.43	64.44	216.0	13.43
WR-1	3	521.0	10.13	65.59	189.3	12.87
Derived recombinant						
DRA-1	6	549.4	10.30	65.13	252.7	15.43
DW-12	6	601.9	9.47	65.23	175.3	12.68
DW-14	6	663.2	9.47	66.73	203.2	13.18
DW-18	6	805.4	9.15	67.24	182.5	11.88
DW-92	6	802.8	9.48	67.15	186.7	13.82
Scheffe	3 vs. 3 entr.	195.7	1.32	5.59	61.0	3.09
Test	6 vs. 6 entr.	138.4	0.93	3.95	43.1	2.19
	3 vs. 6 entr.	169.5	1.14	4.84	52.8	2.68
cv.		7.7	3.40	2.20	7.9	5.90

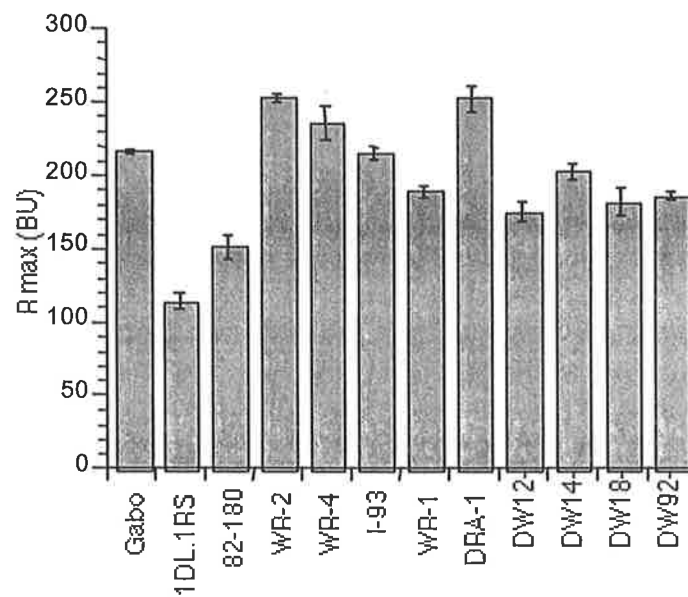

Figure 5.13. Mean values for dough strength (Rmax.) of derived recombinant lines, their parental primary recombinants and controls. Error bars represent the standard error of the means.

5.3.4.2. Variation in SE-HPLC fractions

Analysis of variance showed that there were significant genotype effects for all the HPLC parameters (Table 5.17).

Table 5.17. Mean squares and significance levels for HPLC parameters (Peak 1, Peak 2, Peak 3, ratio Peak 1 to Peak 2, ratio Peak 1 to Peak 3) (Exp. 93/2).

Source of Variation	df	P1	P2	P3	P1/P2	P1/P3
Genotype	11	27.794***	20.106***	2.976***	0.0642***	6.120***
Residual	37	0.154	0.597	0.295	0.0010	0.832

*** significant at P=0.001

As mentioned before, the derived recombinant lines resulted from intercrosses between two types of parents ie. a distal rye recombinant (I-93 and WR-1), and a proximal rye recombinant (82-180, WR-2 and WR-4). In general, comparison of the quality parameters of the four new derived recombinants and their parental lines showed that P1 of derived recombinants gave values similar to that of their proximal recombinant parents rather than the distal parent. No significant differences were found with respect to P2 and P3 in any of the lines (Table 5.18 and Figure 5.14 for P1).

Among the derived recombinant lines tested, DRA-1 showed the highest relative amount of P1, however, it should be noted that valid comparison could not be made

between DRA-1 and the other derived recombinant lines, since DRA-1 had been backcrossed to Gabo four times and the others only three times (Table 5.1).

Table 5.18. Mean values of HPLC parameters of 5 primary recombinants, 5 derived recombinants and control lines (Gabo and Gabo 1DL.1RS) grown at Sandergrove in 1993 (Exp. 93/2).

Lines	Entries	Peak 1 (%)	Peak 2 (%)	Peak 3 (%)	P1/P2	P1/P3
Control						
Gabo	3	51.89	35.18	12.93	1.47	4.01
1DL.1RS	3	39.94	45.91	14.15	0.87	2.82
Proximal recombinant						
82-180	3	46.07	41.17	12.76	1.12	3.61
WR-2	3	47.47	39.98	12.56	1.19	3.79
WR-4	3	47.62	40.31	12.07	1.18	3.96
Distal recombinant						
I-93	3	43.37	42.31	14.32	1.03	3.03
WR-1	3	43.60	41.71	14.70	1.05	2.97
Derived recombinant						
DRA-1	6	48.17	39.41	12.42	1.22	3.89
DW-12	6	46.55	38.89	14.56	1.20	3.20
DW-14	6	47.77	38.87	13.36	1.23	3.58
DW-18	6	47.00	39.82	13.19	1.18	3.57
DW-92	6	46.50	40.13	13.37	1.16	3.48
Scheffe	3 vs. 3 entr.	1.53	3.00	2.11	0.12	0.58
Test	6 vs. 6 entr.	1.08	2.12	1.49	0.09	0.41
	3 vs. 6 entr.	1.32	2.60	1.83	0.11	0.50
cv.		0.80	1.90	4.10	2.60	4.30

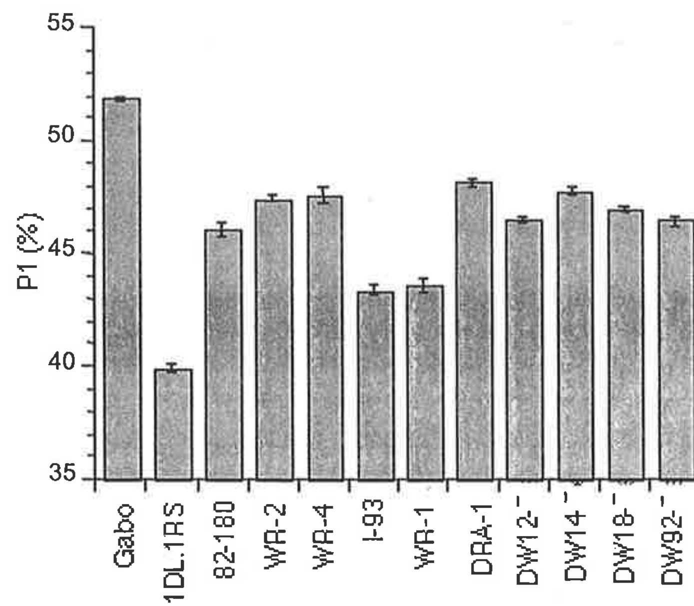

Figure 5.14. Mean values for Peak 1 (SE-HPLC) of controls (Gabo and Gabo 1DL.1RS), derived recombinant lines and their primary recombinant parents. Error bars represent the standard error of the means.

5.4 DISCUSSION

5.4.1. Comparison involving primary recombinants

The quality parameters, especially the dough strength of the recombinant lines showed considerable improvement compared to the original translocation 1DL.1RS, with some approaching the value for the recurrent parent cultivar Gabo. In this experiment, it was found that recombinants with the least amount of rye chromatin i.e. DRA-1, WD-1 and WD-2 showed dough strength values similar to Gabo.

In the second experiment in this chapter, comparisons were made between the recombinant lines and their normal sister lines giving a more critical test of the effect of the recombinant chromosomes on agronomic and quality characters, since they all have a similar genetic background. The yield of the recombinant lines was similar to that of their normal sister lines, moreover there was no significant difference between the yield of the translocation and the normal Gabo. In contrast, Moreno-Sevilla *et al.* (1995a) and Schlegel and Meinel (1994), found that the 1BL.1RS (Petkus rye) translocation line significantly outyielded the control normal plants. Schlegel and Meinel (1994) found that the high grain yield of the 1RS-containing wheats resulted from the higher number of fertile spikelets. Shepherd (unpublished) also found that the translocation line 1BL.1RS (Imperial rye) could produce equivalent yield to the normal lines.

In general, the dough quality parameters (i.e. dough strength and extensibility) of recombinant lines were still less than that of normal lines even though they were better than the translocation 1DL.1RS. Most of the recombinants showed significantly less

dough strength than their normal sister lines, except WD-1 and WD-2. These 2 lines had a comparable dough strength to the normal sister lines and closest value to Gabo.

The good quality parameters of WD-1 and WD-2 are probably due to the fact that they do not possess any rye secalin. DRA-1, although it contains all of the wheat important genes affecting quality, it also carries *Sec-1*, so that the balance of the quantitative amount of protein component is disrupted.

Polymeric proteins (the percent area of peak 1) are an important component of dough elasticity and strength (Miflin *et al.*, 1983). The amount of polymeric protein fraction in the recombinant lines was significantly lower than that of normal lines, with the distal rye recombinants being more severely affected. All of the distal recombinant lines possess the telomeric portion of rye 1RS and lack the low molecular weight glutenin locus *Glu-D3*, which would be expected to result in a decrease of the total polymeric proteins. Two of them (WR-1 and I-93) also carry the *Sec-1* locus, which would also contribute to this effect. Singh *et al.* (1990b) considered that the positive effect of LMW glutenin on dough quality was mainly due to the quantitative effect of increasing the total polymeric protein. Ceoloni *et al.* (1996) and Pogna *et al.* (1996) have also reported a positive effect on bread-making quality when LMW glutenin subunits encoded by the Glu-D3 locus are translocated into durum genotypes. Both HMW and LMW glutenins are known to have an effect to the dough strength (Lukow, 2000).

Monomeric or gliadin proteins are thought to contribute to gluten viscosity (Miflin *et al.*, 1983) and they have a favourable influence on bread volume and dough extensibility (Orth and Bushuk, 1972). The relative proportion of P2 in recombinant lines is significantly higher than that of their sister lines and Gabo, even though they

are significantly lower than 1DL.1RS translocation. P2 of proximal recombinants WR-1 and I-93 was significantly higher than that of WD-1 and WD-2 indicating that the *Sec-1* locus may contribute to higher value of P2.

The percent area of Peak 3 was increased in the recombinant lines. The increase in Peak 3 was especially obvious in the distal rye recombinants (Figure 5.8). This was supported by Sybenga *et al.* (1990) who reported that the genes encoding gamma and omega secalin are tightly clustered at the telomeric region of chromosome 1RS.

It was noted that WR-1 and I-93 showed the biggest disturbance to the molecular weight distribution of endosperm proteins giving lower P1 and higher P2 and P3 than the other recombinants. However, whereas WR-1 gave the lowest Rmax of any of the distal recombinants, I-93 gave high Rmax values (Figure 5.5). This difference in behaviour of these two recombinants may be due to the unusual flour properties (low yield and crumbly texture) encountered with I-93 in this experiment.

5.4.2 Comparison involving derived recombinant lines

In general, as found with the primary recombinant lines, all the derived recombinant lines studied here had significantly better dough strength than the original translocation 1DL.1RS but usually still less than Gabo (Table 5.16) and their sister lines (Table 5.13) even though the difference was not significant, except for DW-12 (Table 5.13). No significant difference was found in the dough strength of the new derived recombinants (DW-12, DW-14, DW-18 and DW-92) as might be expected, since they all have similar characteristics in carrying *Sec-1* and all wheat seed storage protein loci, with only small differences in the length of rye segment. However, it was

surprising that except for DRA-1, none of the derived recombinants gave higher Rmax values than either of their primary recombinant parents.

The difference in the value of Rmax. could be related to the changes in the proportion of flour protein components as measured by HPLC. In all derived recombinant lines, it is obvious that compared to 1DL.1RS there is a large increase in the relative amount of P1 present, and the increase in P2 and P3 is less than in the translocation 1DL.1RS. These results confirm previous published papers (Dhaliwal *et al.*, 1988 Dhaliwal and MacRithie, 1990; Graybosch *et al.*, 1990, 1993a) that the inferior quality of 1RS derived lines is associated with low polymeric protein concentration (P1) and higher levels of salt-water soluble proteins (P3).

Since all the derived recombinant lines studied here possess all wheat protein genes i.e. *Glu-D3*, *Gli-D1* and *Tri-D1*, they were expected to have the normal ratios of flour protein fractions and to be similar to the normal wheat cultivar. However, although the differences in dough strength of the derived recombinant lines and the recurrent parent Gabo were only significant with DW-12, they all exhibited lower dough strength and significantly lower P1 values compared to Gabo, and this may be due to the presence of the *Sec-1* locus.

5.4.3. Extensograph vs HPLC parameters as measures of flour quality

In this study, two different measures of the quality of recombinant lines were used, namely Extensograph and SE-HPLC. It is of interest to compare the results obtained from these two measures. Both measures gave consistent results across all experiments in that all recombinants in terms of quality are more like wheat control

than original wheat rye translocation. The Rmax values of the recombinant lines are much improved and their relative P1 values are higher than the original 1DL.1RS translocation.

Across all of the field experiments, the measurement of the relative proportion of protein fractions using SE-HPLC gave consistent and more precise results in dealing with small differences in 1RS-containing wheats than Rmax. Consistently it was shown from the data that there is a decrease in the relative proportion of P1 in all the recombinants, with a concomitant increase in the P2 and P3 fractions. The values of the SE-HPLC parameters of recombinant lines were also consistently in between the normal cultivar Gabo and the translocation 1DL.1RS, with the proximal recombinants showing higher P1 and lower P2 and P3 compared to the distal recombinants.

On the other hand, although the dough strength as measured by Rmax of all recombinant lines was found to be consistently higher than that of the translocation 1DL.1RS, some of the Rmax values for individual recombinants were not consistent across experiments. While the effect of wheat-rye recombination on SE-HPLC parameters is evident, their Rmax values varied between experiments. This is particularly shown with the common recombinants in experiments 91/1 and 93/2. Whereas the relative P1 values were remarkably similar in both experiments (Figures 5.6 and 5.14), their Rmax values showed less correspondence (Figures 5.5 and 5.13) particularly with recombinants WR-2 and WR-4 which gave a significantly lower Rmax value than Gabo in 91/1 but more than Gabo in 93/2, although the latter difference was not significant.

The HPLC values for molecular weight distribution appear to reflect differences in the seed storage protein loci present as suggested by earlier worker (Gupta *et al.*, 1989;

Singh *et al.*, 1990a, 1990b). Marked differences were detected between distal recombinants (*Sec-1*$^{+}$, *Gli-D1/Glu-D3*$^{-}$) and proximal recombinants (*Sec-1*$^{+}$, *Gli-D1/Glu-D3*$^{+}$). The absence of *Gli-D1/Glu-D3* in WD-1 and WD-2 resulted in lower P1, higher P2 and P3 fractions, but had little effect on Rmax.

It is well known that many factors affect the strength of wheat doughs as measured by extensograph. Therefore SE-HPLC measures of the distribution of polymeric versus non-polymeric proteins may provide a better indicator of the differences between the recombinant lines in respect of their bread-making quality potential.

Gupta (1996) found that there is higher correlation between dough strength and the proportion of unextractable polymeric proteins rather than the total polymeric proteins. Unfortunately this parameter was not measured in the current study and the amount of unextractable protein may have contributed to the lower correlation between HPLC and extensograph parameters.

CHAPTER 6: GENERAL DISCUSSION

The aim of this study was three-fold, namely to:

(1) Investigate in detail the nature of the quality problem associated with bread wheat lines carrying rye chromosome arm 1RS in a 1DL.1RS translocation,

(2) Attempt to elucidate the cause of the quality defect by producing variant wheat lines which carried different amount of the rye 1RS chromosome segment carrying stem rust resistance, and

(3) Determine whether the quality defect had been removed from the rye chromosome segment following production of new recombinants involving chromosomes 1DS and 1RS and thereby make breeders' lines carrying the *SrR* gene suitable for agriculture.

An active search for additional wheat-rye recombinants has been undertaken in the Cereal Cytogenetics Laboratory, University of Adelaide over decades to test the possibility that the quality defect could be eliminated or reduced by further reducing the size of the rye chromosome segment thereby either removing deleterious factors on the rye chromosome (eg. secalins) or re-introducing quality-enhancing genes on the related wheat chromosome arm (1DS).

Following the strategy of Koebner (1985), induction of homoeologous recombination was made by crossing the wheat-rye 1DL.1RS translocation to a stock of Sears' *ph1b* mutant in a Chinese Spring background (Sears, 1977), using storage protein markers TRI-D1 and GLI-D1 (Singh and Shepherd, 1985), on the short arm of chromosome 1D

of wheat to select for recombinants. Chromosome arm 1RS of rye is identified by the presence of the storage protein secalin, controlled by *Sec-1* (Shepherd and Jennings, 1971) and the gene for stem rust resistance (*SrR*) shown by Singh *et al.* (1990c) to be linked with *Sec-1* on the distal segment of rye 1RS.

A total of 732 F4 progeny seeds from four F3 plants having putative genotype 1D/1DL.1RS *ph1bph1b* were screened with SDS-PAGE to detect additional recombinants. The frequency of recovery of suspected recombinants of phenotypes "TRI-D1$^+$GLI-D1$^-$SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" ranged from 0.6 to 6 % in 3 families observed.

Family 4 gave a very high percentage of progeny plants (24.4%) with dissociation phenotypes (suspected recombinants). However from extensive progeny testing, it was deduced that family 4 parent plant must have contained two wheat-wheat recombinant chromosomes, in which 1DS chromosome arm had recombined with its homoeologous 1AS or 1BS chromosome arms in an earlier generation. Therefore these lines were not relevant to the present project.

Wheat-rye recombinants with a shorter rye segment were sought in an attempt to produce wheat lines having the stem rust resistance gene from rye without the associated inferior end-use quality characteristics of the complete arm translocation.

As suggested by Zeller and Hsam (1983), a recombinant which has the shortest rye segment containing the stem rust resistance gene without the inclusion of the secalin gene(s) is likely to be free of the quality problems encountered in 1RS-containing wheats.

Chinese Spring Sears' *ph1b* mutant was utilised for inducing a second round of homoeologous recombination between the genes for rye endosperm secalins *Sec-1* and closely linked *SrR* locus conferring stem rust resistance, in the derived recombinant DRA-1, which contained an interstitial rye segment and already possessed all the known wheat storage protein genes on chromosome arm 1DS. Two recombinant plants were identified among 1889 plants tested and confirmed over two consecutive generations, in which the first recombinant family (*Sec-1*$^-$ *SrR*$^+$) showed a resistant reaction to stem rust in the absence of secalin bands, while in the other family (*Sec-1*$^+$ *SrR*$^-$), the presence of secalins was associated with a susceptible reaction to stem rust. The presence of a rye segment in *Sec-1*$^-$*SrR*$^+$ recombinant was confirmed using a PCR marker known to be located in the rye segment of DRA-1.

Recently, the two recombinants produced in this study (*Sec-1*$^-$ *SrR*$^+$ and *Sec*$^+$ *SrR*$^-$) and derived recombinant DRA-1 were provided to Mago *et al.* (2002) to develop DNA markers specific for the Sr+ and Sec-1+ segments of 1RS. They identified several different markers specific for these rye segments in these two recombinants, thus confirming their structure and were able to locate 16 RFLP markers in this region (Mago *et al.*, 2002). This work also demonstrated the value of the recombinants with short segments of rye chromatin for the location of markers to specific segments of rye 1RS.

The number of confirmed second-round homoeologous recombinants detected in this study was low giving a frequency of 0.1%, consistent with the tight linkage found between these loci by homologous recombination (Singh *et al.*, 1990c). Koebner (1985) in his work found no evidence for breaking the linkage between *Sec-1* and *SrR*

the gene for stem rust resistance among 528 of the progeny derived from nullisomic 5B parents and 221 of the control progeny involving 1DL.1RS.

The new 1DS-1RS recombinants produced in this study carry the least amount of rye chromosome so far obtained in recombination experiments with translocation material involving 1DL.1RS. If the defect of the quality in 1DL.1RS material is only due to the loss of wheat genes, then these recombinants should not suffer from the dough quality problem, since they possess all the known 1DS seed storage protein genes and have regained a large amount of the wheat chromatin.

These wheat-rye recombinants also could be used to test the hypothesis that the rye secalin seed storage proteins may themselves be the cause of the quality problem in translocation lines involving 1RS chromosome (Zeller *et al.*, 1982).

In a similar approach, Lukaszewski (2000; 2001) used cytological screening following several cycles of homoeologous recombinations between the chromosome arms 1RS of Petkus rye in 1BL.1RS and 1DL.1RS translocations and its homoeologous wheat arms 1AS, 1BS and 1DS in cultivar Pavon, to detect a whole range of primary, secondary and tertiary recombinants. A large number of breakpoints with different position and various lengths of 1RS rye segments were produced with the overall recombination frequency of 0.4% in 1BL.1RS and 86% of them were recombined with 1BS, while the overall recombination frequency of 0.2% was detected in 1DL.1RS and 67% of them were recombined with 1DS. Recombinants lacking the *Sec-1* locus were also produced in his studies.

The quality defects associated with 1RS chromosome arm have been extensively described and include reduced dough-mixing time, low dough extensibility and low

resistance to extension. Sticky dough is another characteristic of 1RS containing lines, but despite many efforts to develop better methods of measuring the differences in 1RS derived-wheats, this characteristic has proved difficult to quantify in the laboratory (Dhaliwal *et al.*, 1990; Martin and Stewart, 1990). These characteristics have hindered the commercial exploitation of wheat carrying this chromosome in Australia.

Several strategies have been used to alleviate the quality defects associated with 1RS chromosomes. Several previous studies reported that by backcrossing to wheat cultivars displaying acceptable quality, the defects associated with the translocation lines could be minimised. Graybosch *et al.* (1999) found that a change in genetic background of 1AL.1RS and 1BL.1RS lines markedly increased their quality attributes. A number of 1BL.1RS recombinants in different genetic background produced comparable end use quality to the non-1BL.1RS sister lines (Pena *et al.*, 1990; Lee *et al.*, 1995). However, this observation was not confirmed in the present study. The original 1DL.1RS translocation line in a Chinese Spring background was backcrossed four times into Gabo. This is an Australian cultivar highly successful in agricultural production, albeit several decades ago, and the 1DL.1RS translocation line still showed severely diminished quality attributes. This could be related to the fact that 1D chromosome contributes more to the dough quality compared to 1A and 1B chromosomes (Shepherd and Singh, 1984; Graybosch *et al.*, 1993a).

Chromosome engineering was employed to attempt to minimise the quality defect of the 1DL.1RS translocation lines. Several recombinants have been isolated in previous studies (Koebner *et al.*, 1986; Rogowsky *et al.*, 1991). The overall results of previous and current studies with these lines are:

i) There was an improvement in dough strength and extensibility of primary recombinants compared to the original 1DL.1RS translocation, however their quality attributes were still far less than the normal recurrent parent Gabo i.e. reducing the size of the rye 1RS arm, and retaining either proximal or distal segments, has a positive effect on quality. This suggests that multiple factors exist on the rye and /or wheat chromosomes which influence quality. It also supports the previously proposed hypothesis that reducing the amount of rye chromatin and re-introducing as much wheat chromatin as possible can help restore quality.

ii) Proximal rye recombinants which possess wheat protein loci *Gli-D1* and *Glu-D3,* but have had the segment carrying the triplet proteins replaced by rye chromatin, showed better quality in many cases. This suggests that the presence of the distal part of the 1DS chromosome is essential for high quality, since it contains two important genes for endosperm proteins, i.e. *Gli-D1* and *Glu-D3*. This is true even if both types of recombinants (proximal and distal recombinants) carry a rye segment containing the *Sec-1* gene.

iii) The dough strength of the derived recombinant DRA-1, which possesses all wheat storage protein and has an interstitial segment of rye 1RS, is far better than the original 1DL.1RS translocation and all the primary recombinants. It is clear that gaining all the wheat storage protein responsible for wheat quality (including the triplet protein loci) is beneficial. However, the quality of DRA-1 is still less than that of Gabo as shown by the measurement made on sister lines (experiment 93/2). This is most likely due to the presence of deleterious factors on the rye chromosome segment, possibly the secalin gene.

The role of secalin in quality

The possibility that secalin, or at least the rye chromosome segment carrying that locus, has a detrimental effect on quality is supported by results of experiments with recombinants WD-1 and WD-2, which possess a small distal part of 1RS chromosome of rye. These lines showed increased dough strength comparable to derived recombinant DRA-1, despite the absence of the wheat seed storage protein loci *Gli-D1/Glu-D3*. These particular recombinants carry a small distal part of 1RS rye segment and do not possess the secalin gene. It provides a clue that the presence of *Sec-1* in the rye segment in the wheat-rye recombinants is also important in the contribution to low dough quality, especially Rmax.

It has been argued previously by other workers that *Sec-1* is responsible for the deleterious effect of wheat containing rye segment. Recently, Kumlay *et al.* (2003) surveyed a wide range of wheat rye recombinants and concluded that the introduction of *Sec-1* into wheat is primarily responsible for the quality defect of translocation lines, not the removal of corresponding wheat loci. Studies on the effect of different *Sec-1* alleles in 1RS lines did not reveal any differences in their effects on dough quality (Graybosch *et al.*, 1999; Kumlay *et* al., 2003).

The critical test for the role of secalins in dough quality

It has been established so far that the presence of wheat proteins on 1DS is important for dough quality. In addition, it has been indicated that the presence of rye secalins may be detrimental to quality. Until this current study, no 1R wheat-rye translocation lines existed which had retained a small segment of rye chromosome carrying the rye

secalin locus along with all known wheat protein genes to allow a direct comparison of wheats with and without the secalin locus.

The current research project has produced two unique lines which can now enable this critical test to be done. These two lines were produced from the shortest known interstitial recombinant, DRA-1, which carries the *SrR* stem rust resistance gene, *Sec-1* rye locus and all known wheat proteins. The two new interstitial recombinants have the phenotypes SEC-1$^-$ SrR$^+$ and SEC-1$^+$ SrR$^-$. Comparison of the quality attributes of these two lines was expected to give a direct indication of the role of the secalin gene on dough properties.

Because of the limitations of time, it was not possible to complete the necessary backcrossing and field testing within the current project. Subsequently, completion of these aspects of the study was undertaken by Dr K.W. Shepherd and technical staff of the Cereal Genetics Laboratory, University of Adelaide.

After backcrossing at least three times to the normal cultivar Gabo, these two types of recombinants were recently subjected to quality testing in a four-replicate field experiment. Data from this experiment indicated that recombinant type 1 (*Sec-1*$^-$ *SrR*$^+$) produced no significant difference in Rmax (189 ± 9.7 BU) compared to the normal cultivar Gabo (195 ± 4.6 BU), its recurrent parent. On the other hand, recombinant type 2 (*Sec-1*$^+$ *SrR*$^-$) showed no significant difference in Rmax (159 ± 2.4 BU) compared to derived recombinant DRA-1 (169 ± 3.3 BU), but this was significantly lower than both Gabo and the reciprocal new recombinant *Sec-1*$^-$ *SrR*$^+$ (K.W. Shepherd, unpublished results).

It is clear from these results that the secalin gene, or perhaps some other undescribed gene closely linked to it, plays an important role in reducing the Rmax, an important

parameter affecting bread-making quality. Also, the recombinants should also contain all the wheat genes for quality, since it was found in this present study that WD-1 and WD-2 with a short rye segment replacing the *Gli-D1/Glu-D3* loci on the distal part of wheat 1DS, even though it does not contain secalin gene, its Rmax. is still a little lower than that of the normal cultivar Gabo and comparable to DRA-1.

A new line for wheat breeding programs

The new wheat-rye recombinant *Sec-1*$^{-}$ *SrR*$^{+}$ appears to be the best recombinant and is a valuable resource for wheat breeding programs, since it contains the stem rust resistance gene without the inclusion of the quality defect accompanying the transferred rye segment. Hence, it is now available to wheat breeding programs in Australia for inclusion in their crossing programs.

The question remains as to the relationship between the *SrR* gene from Imperial rye and the *Sr31* gene from Petkus rye. It was reported earlier that a virulent strain of stem rust (Ug99) had arisen in Uganda, Africa which is capable of overcoming the resistance of *Sr31* gene from rye. Unpublished observations by Dr Jim Kolmer (University of Minnesota, USA) have indicated that wheat lines carrying the *SrR* gene are resistant to the Ugandan stem rust pathotype. This indicates that the gene *SrR* is different from *Sr31* and subsequently still may have a valuable role to play in protecting the Australian wheat crop.

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