STUDIES ON RESISTANCE TO BIOTIC AND ABIOTIC STRESSES IN WHEAT

Bosco Chemayek

M.Sc. (Crop Science)

A thesis submitted in fulfilment of the requirements for the degree of

THE DOCTOR OF PHILOSOPHY

Faculty of Agriculture and Environment
Plant Breeding Institute, Cobbitty
The University of Sydney
March 2016
Certificate of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge, it contains no material previously published or written previously by any other person, except where due references are made in the text.

Bosco Chemayek
ACKNOWLEDGEMENTS

I extend my profound gratitude and indebtedness to my supervisor Assoc. Prof. Harbans Bariana for his most invaluable and inspiring guidance, constructive criticism and generosity throughout my research work and preparation of this thesis. Your mentorship will always remain in my heart. I am deeply grateful to my co-supervisor Dr. Urmil Bansal for her incredible guidance and edifying training and suggestions during the course of the research. I am truly indebted to her detailed training and assistance in conducting the molecular research and for her constant advises and counselling during the course of my studentship. My deepest gratitude goes to associate-supervisor Assoc. Prof. Margaret Barbour for professional assistance and implausible training, guidance and support in conducting the physiology experiments. My thoughtful thanks also go to Dr. Peng Zhang for her professional help and guidance in molecular cytology.

I also extend my gratefulness to National Agricultural Research Organisation of Uganda for awarding me the scholarship and Grains Research and Development Corporation (GRDC) for funding part of my research. I am forever grateful to Dr. William Wagoire for believing in me and for strongly supporting my bid for further studies. I cherish your professional and parental guidance during the time we worked together and during the course of my PhD study.

My special thanks to Emeritus Prof. Robert McIntosh for giving valuable time to discuss my results and suggesting valuable information during the study. My sincere appreciation goes to Profs. Robert Park and Peter Sharp for their advice when I was applying for my PhD program at University of Sydney. I express my cordial thanks to my fellow students and friends (Shahnoosh, Vallence, Mesfin, Huma, Naeela, Pakeerathan, Vanessa, Huda, Peace, Peter, Rohayu, Rouja, Jiapeng, Paul, Irum, Tewanda, Mahbub, Mandeep and Mumta) for encouraging and supporting me during my study. I am also thankful to Drs. Hanif Miah and Muhammad Gill for their most valuable suggestions during field and greenhouse experiments. I would also like to express my gratitude to Matthew Williams, Gary Standen, Keshab Kandel, Sami Hoxha, James Hull and Kate Vincent for their constant help throughout my study. I gratefully thank Ms Kate Rudd, Mr James Bell and Pradhan Dayaram for all the administrative support and valuable assistance.

My great thanks and appreciation goes to my parents Mr and Mrs Towett for bringing me to this world and raising me up to be the man I am today. I forward my wholehearted gratefulness to the most special person my beloved wife Ms. Godia Kwaga and children Vanessa, David and Jonan for praying for me and being patient and understanding during my absence and for forfeiting your right to have all my love and attention during the period of this work.

Lastly to the Almighty God who has given me good health, endurance and perseverance throughout this period of PhD studies.

Bosco Chemayek
DEDICATION

To my wife Ms. Godia Kwaga and children Vanessa, David and Jonan
Summary

This investigation was focused on the assessment of genetic diversity for resistance to stem rust and stripe rust in an international wheat nursery, genetic characterisation of adult plant stripe rust resistance in Australian wheat cultivar Sentinel, understanding of genetic relationship between two stem rust resistance genes (Sr36 and Sr39) located on chromosome 2B and assessment of genetic diversity for physiological traits among a set of wheat landraces.

Ten seedling stem rust resistance genes (Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31 and Sr38) and seven stripe rust resistance genes (Yr3, Yr4, Yr6, Yr9, Yr17, Yr27 and Yr34) were postulated either singly or in combinations in an international wheat nursery. Genotypes carrying uncharacterised resistance for stem rust and stripe rust against the Australian rust flora were identified for genetic analysis.

Three consistent QTL (QYr.sun-1BL, QYr.sun-2AS and QYr.sun-3BS) were demonstrated to condition high level of adult plant stripe rust resistance in Sentinel. QYr.sun-1BL, QYr.sun-2AS and QYr.sun-3BS explained on an average 18.0%, 15.6% and 10.6% variation in stripe rust response, respectively. Additive nature of three QTL to condition high level of stripe rust resistance was demonstrated through comparison of recombinant inbred lines (RILs) carrying these QTL in all different combinations. Detailed characterisation of these loci will be performed.
Stem rust tests on F$_3$ populations involving $Sr39$ on a large and a shortened
$Aegilops speltoides$ translocation with $Sr36$ on a $Triticum timopheevi$ segment showed
complete repulsion linkage. The molecular cytogenetic analysis however indicated that
these can be recombined using large F$_2$ population.

Significant variation for water-use efficiency related physiological traits was
observed among wheat landraces. Genotypes with low and high mesophyll conductance,
stomatal conductance and other physiological attributes will be useful in designing
crosses to achieve high water-use efficiency in future wheat cultivars.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>I</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>II</td>
</tr>
<tr>
<td>Summary</td>
<td>III</td>
</tr>
<tr>
<td>CHAPTER 1</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 2</td>
<td>5</td>
</tr>
<tr>
<td>Literature review</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Wheat</td>
<td>5</td>
</tr>
<tr>
<td>2.2 Rust diseases of wheat</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Control of rust diseases of wheat</td>
<td>8</td>
</tr>
<tr>
<td>2.4 Assessment of genetic diversity for rust resistance in wheat</td>
<td>8</td>
</tr>
<tr>
<td>2.5 Genetic analysis for rust resistance in wheat</td>
<td>12</td>
</tr>
<tr>
<td>2.5.1 All stage resistance</td>
<td>13</td>
</tr>
<tr>
<td>2.5.2 Adult plant resistance</td>
<td>14</td>
</tr>
<tr>
<td>2.5.3 Genomic location of rust resistance in wheat</td>
<td>15</td>
</tr>
<tr>
<td>2.5.3.1 Bulk segregant analysis</td>
<td>15</td>
</tr>
<tr>
<td>2.5.3.2 QTL Mapping</td>
<td>16</td>
</tr>
<tr>
<td>2.5.3.3 Identification of markers linked with resistance genes</td>
<td>16</td>
</tr>
<tr>
<td>2.6 Genetic linkage of rust resistance genes in wheat</td>
<td>17</td>
</tr>
<tr>
<td>2.7 Abiotic constraints to wheat production</td>
<td>18</td>
</tr>
<tr>
<td>2.7.1 Drought</td>
<td>19</td>
</tr>
<tr>
<td>2.7.2 Water Use Efficiency</td>
<td>20</td>
</tr>
<tr>
<td>2.7.3 Stomatal conductance</td>
<td>21</td>
</tr>
<tr>
<td>2.7.4 Mesophyll conductance</td>
<td>22</td>
</tr>
<tr>
<td>2.7.4.1 Respone of gm to variations in environmental conditions</td>
<td>23</td>
</tr>
<tr>
<td>2.7.4.2 Genetic control of mesophyll conductance</td>
<td>24</td>
</tr>
<tr>
<td>CHAPTER 3</td>
<td>25</td>
</tr>
<tr>
<td>Assessment of genetic diversity for stem rust and stripe rust resistance in an international wheat nursery</td>
<td>25</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>25</td>
</tr>
</tbody>
</table>
3.2 Materials and methods
  3.2.1 Host materials
  3.2.2 Pathogen materials
  3.2.3 Greenhouse tests
  3.2.4 Field screening
  3.2.5 DNA extraction and quantification
  3.2.6 Molecular marker genotyping

3.3 Results
  3.3.1 Postulation of resistance genes
    3.3.1.1 Stem rust
    3.3.1.2 Stripe rust
  3.3.2 Field screening
  3.3.3 Molecular marker genotyping

3.4 Discussion

CHAPTER 4
Molecular mapping of adult plant stripe rust resistance in Australian wheat cultivar Sentinel
  4.1 Introduction
  4.2 Materials and methods
    4.2.1 Plant Materials
    4.2.2 Pathogen material
    4.2.3 Stripe rust assessments
      4.2.3.1 Field tests
      4.2.3.2 Greenhouse tests
    4.2.4 Molecular mapping
      4.2.4.1 DNA extraction
      4.2.4.2 Detection of stripe APR genes \textit{Yr18} and \textit{Yr29}
    4.2.5 Statistical Analysis
  4.3 Results
    4.3.1 Stripe rust response assessments
4.3.1.1 Field 59
4.3.1.2 Stripe rust response in the greenhouse 61
4.3.2 Detection of APR genes *Yr18* and *Yr29* 62
4.3.3 Linkage map construction 63
4.3.4 QTL analysis 63
4.3.5 Contribution of Sentinel alleles 66
4.3.6 Additive effects of QTL combinations 67

4.4 Discussion 69

CHAPTER 5 73
Genetic relationship between wheat stem rust resistance genes *Sr36* and *Sr39* 73
5.1 Introduction 73
5.2 Materials and Methods 75
  5.2.1 Plant material 75
  5.2.2 Pathogen material 75
  5.2.3 Stem rust screening 76
  5.2.4 Molecular marker analysis 76
    5.2.4.1 DNA isolation and quantification 76
    5.2.4.2 PCR amplification and electrophoresis 76
  5.2.5 Molecular cytogenetic analysis 78
  5.2.6 Statistical analyses 79
5.3 Results 79
  5.3.1 Stem rust response tests 79
  5.3.2 Marker and cytological analysis 82
  5.3.3 Over-transmission of *Sr36* 83
  5.3.4 Validation of marker *rwgs28* 85
5.4 Discussion 87

CHAPTER 6 91
Genetic variation in mesophyll conductance and response to sustained drought stress in selected bread wheat (*Triticum aestivum* L.) landraces 91
6.1 Introduction 91
6.2 Materials and Methods 94
6.2.1 Plant Materials and Growth conditions 94
6.2.1.1 Experiment 1 94
6.2.1.2 Experiment 2 95
6.2.2 Leaf gas exchange and on-line carbon isotope discrimination measurements 95
6.2.3 Estimation of maximum carboxylation rate ($V_{cmax}$) and electron transport rate ($J_{max}$) 97
6.2.4 Estimation of mesophyll limitations and stomatal limitations 97
6.2.5 Measurement of leaf water potential ($\psi_{leaf}$) 97
6.2.6 Stable isotope analysis of leaf organic material 98
6.2.7 Statistical Analysis 98
6.3 Results 99
6.3.1 Experiment 1 99
6.3.2 Experiment 2 104
6.3.2.1 Leaf water potential response to drought 104
6.3.2.2 Leaf gas exchange responses to drought 105
6.3.2.3 Genotypic variation in maximum carboxylation rate ($V_{cmax}$), maximum electron transport ($J_{max}$), mesophyll limitations ($L_m$) and stomatal limitations ($L_s$) to photosynthesis 108
6.3.2.4 Stable isotope analysis of leaf organic material 110
6.4 Discussion 110

CHAPTER 7 116
Conclusions 116
REFERENCES 120
Appendix 1 171
Appendix 2 173
List of tables

Table 3.1  Pedigree information, postulated genes and molecular marker data for 95 entries of an international wheat screening 29
Table 3.2  List of Pgt and Pst pathotypes used and their virulence spectrum 37
Table 3.3  Seedling stem rust resistance variation in the CIMMYT international wheat screening nursery 42
Table 3.4  Seedling stripe rust resistance variation in the CIMMYT international wheat screening nursery 45
Table 4.1  Frequency distribution of Sentinel/Nyb RIL population when tested against Pst pathotype 134E16A+Yr17+Yr27 at the 4th leaf stage 61
Table 4.2  Stripe rust QTL detected in Sentinel/Nyb3 RIL population 64
Table 4.3  Comparison of mean stripe rust severities of genotypes carrying positive and negative alleles for each QTL 66
Table 4.4  Mean stripe rust severities of Sentinel/Nyb3 RILs carrying different QTL combinations 68
Table 5.1  Infections types produced by parental genotypes and a susceptible control when infected with Sr36 virulent and avirulent pathotypes 80
Table 5.2  Distribution of F₃ families when tested with Pgt pathotype 98-1,2,3,5,6,7 82
Table 5.3  Frequency distribution of F₃ families with respect to genotypic status for closely linked markers 83
Table 5.4  Genotypic constitution of single plants derived from five F₃ heterozygous families 84
Table 5.5  Amplicon sizes produced by the different cultivars when genotyped with marker rwgs28 86
Table 6.1  The effect of drought stress conditions on maximum carboxylation rate (Vₘₐₓ), electron transport rate (Jₘₐₓ), stomatal 109
## List of Figures

| Fig. 3.1 | a) Seedling stem rust and b) seedling stripe rust response variations observed among the entries tested in this international wheat nursery. |
| Fig. 3.2 | Field stem rust response variation during seasons 2012 and 2013. |
| Fig. 3.3 | Field stripe rust response variation during two seasons 2012 and 2013. |
| Fig. 4.1 | Stripe rust responses on P1-Sentinel and P2-Nyb3 in (a) field and (b) greenhouse. |
| Fig. 4.2 | Frequency distribution of Sentinel/Nyb3 RILs with respect to adult plant stripe rust response variation (LDN – Lansdowne, K – Karalee). |
| Fig. 4.3 | Stripe rust QTL detected on chromosomes (a) 1BL, (b) 2AS and (c) 3BS of Sentinel/Nyb3 RIL population. |
| Fig. 4.4 | Adult plant stripe rust severity of Sentinel/Nyb3 RILs carrying different QTL combinations. |
| Fig. 5.1 | Infection types expressed by (L-R) Cook, RWG1 and Morocco when tested with (a) Pgt pathotype 98-1,2,3,5,6,7. (b) Pgt 34-1,2,3,4,5,6,7. |
| Fig. 5.2 | STS marker rwgs28 profiled on both the parents and F3 lines from RWG1/Cook cross. |
| Fig. 5.3 | Genomic hybridization of parental lines and F3 heterozygous families left to right; RWG1 carrying Sr39 translocation; Cook carrying Sr36 translocation; heterozygous progeny 1 and heterozygous progeny 2 having one copy each of Ae. speltoides and T. timopheevii segment. |
| Fig. 6.1 | Genetic variation in A) photosynthesis rate (A), B) stomatal conductance (g_s), C) intrinsic water use efficiency (A/g_s), and D) mesophyll conductance (g_m) observed among 41 wheat landraces and 11 commercial genotypes evaluated under non-limiting conditions. Aridity index values of the origin countries and regions from where the landraces were collected were available only for the first 20 genotypes. Values are mean ± standard error, n = 6. |
| Fig. 6.2 | Relationship between mesophyll conductance (g_m) and A) Photosynthesis rate (A), B) stomatal conductance (g_s), and C) intrinsic water use efficiency (A/g_s) for 41 wheat landraces and 11 commercial genotypes. |
genotypes evaluated under non-limiting conditions. Values are mean ±
standard error, n = 6. Lines are least squared linear regressions with
fitted parameters indicated

**Fig. 6.3** Relationship between carbon isotope discrimination ($\Delta^{13}$C$_{leaf}$) and
A) sub-stomatal CO$_2$ concentration to atmospheric CO$_2$
concentration (C$_i$/C$_a$), B) chloroplast CO$_2$ concentration to
atmospheric CO$_2$ concentration (C$_c$/C$_a$) for all 52 wheat
genotypes under non-limiting conditions and C) C$_i$/C$_a$, D) C$_c$/C$_a$
for the five selected wheat genotypes tested under drought stress.
Red symbols denote drought stressed
plants, while black symbols denote well-watered plants. Values are
means, ± standard error, n = 6 for A) and B) and n = 3 for C) and D)

**Fig. 6.4** Relationships between leaf mass per unit area (LMA) and mesophyll
conductance ($g_m$) for A) all 52 wheat genotypes under non-limiting
conditions, B) and five selected wheat genotypes under drought stress
(Red colour symbols-drought stressed, black colour symbols-well
watered). Relationship between leaf Nitrogen per unit area ($N_a$) and
photosynthesis rate ($A$) for C) 52 wheat genotypes under non-limiting
conditions, D) five selected wheat genotypes screened under drought
stress. E) relationship between leaf Nitrogen per unit area and
mesophyll conductance for 52 wheat genotypes under non-limiting
conditions, F) relationship between leaf Nitrogen per unit area and
mesophyll conductance for five selected wheat genotypes evaluated
under drought stress. Values are means, ± standard error, n = 6 for A),
C) and D) and n = 3 for B), D) and F)

**Fig. 6.5** Changes in A) leaf water potential ($\Psi_{leaf}$), B) photosynthetic rate ($A$),
C) stomatal conductance ($g_s$), D) mesophyll conductance ($g_m$) and E)
intrinsic WUE ($A/g_s$) in response to 14 days induced sustained drought
stress for the five selected wheat genotypes. Values are mean ±
standard error, n = 3, except for panel A, in which values are for a
single plant. Asterisks indicate significant differences for genotypes of
the matching colour for the individual day.
CHAPTER 1

Introduction

Wheat (*Triticum aestivum*) is one of the most important agricultural commodities and it is second to rice as a source of calories and first as a source of protein. It provides 25% of calories and 20% protein to more than 5 billion people (Braun et al. 2010; Hawkesford et al. 2013). Wheat is currently grown on approximately 217 million hectares worldwide, with an average yield of around 3 t/ha, but there is considerable variation between countries. Annual global wheat production is estimated at 729 million metric tons, with China being the highest producer (35%). Africa and Australia each contribute about 3% of the global wheat production (FAOSTAT 2016). Demand for wheat is projected to increase to 60% by 2050, at the same time climate change-induced temperature increases and drought stress are expected to reduce wheat production by 29% (Rosegrant et al. 1995; Singh et al. 2011). This scenario is compounded by stagnating yields, increasing irrigation and fertilizer costs (Hawkesford et al. 2013) and new virulent and aggressive pathogen strains such as race Ug99 of the stem rust fungus (Singh et al. 2011).

Global Wheat production is significantly hampered by both biotic and abiotic stresses. The most important biotic stresses of wheat are the three rust diseases; stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) (Roelfs et al. 1992). Historically, stem rust has been a major problem in Africa, the Middle East, Asia, Australia, New Zealand, Europe, South and
North America (Singh et al. 2011). Leaf rust and stripe rust epidemics have been frequent worldwide in recent years (Bolton et al. 2008; Chen et al. 2014; Wellings 2011). Deployment of resistance genes to rust diseases in adapted wheat cultivars is the best option and can be achieved through the use of all stage resistance (ASR) conditioned generally by major genes or adult plant resistance (APR) controlled often by minor genes (Njau et al. 2010; Singh et al. 2011). This is underpinned by continuous identification and characterisation of new sources of rust resistance to counter the rapidly evolving pathogens (Njau et al. 2010).

The deployment of ASR genes singly in commercial cultivars is discouraged due to their proneness to be defeated by emergence of virulent pathotypes. Pyramiding of two or more ASR genes and/or use of multiple APR genes is recommended for stable resistance against variable plant pathogens (Wang et al. 2001; Garzon et al. 2008; Bariana et al. 2010, Singh et al. 2011). Development of new rust resistant varieties with multiple resistance genes is often limited by selection of combinations in conventional breeding programs. Advancements in high throughput genotyping platforms have made several rust resistance-linked markers available for marker-assisted selection (Bariana et al. 2007a; Bernardo et al. 2013). These markers allow quick and accurate identification of genotypes carrying combinations of rust resistance genes present in breeding material. In addition, selection can also be performed for other marker-tagged traits.

In addition to rust diseases of wheat, drought is the single largest abiotic stress factor that significantly reduces grain quality and yield of wheat (Budak et al. 2013; Kiliç and Yağbasanlar 2010). It is estimated that more than 70% of fresh water globally
is used for agricultural production (Morison et al. 2008). There is increasing scarcity of fresh water emanating from increased evapo-transpiration, limited precipitation and the population increase. Water scarcity is a key global constraint to agricultural productivity and is expected to get worse with increasing effects of climate change and variability (Budak et al. 2013; Flexas et al. 2015). This highlights the need for drought resilient and water use efficient genotypes that can maximize output per unit of water used. Diffusional limitations of CO$_2$ through the stoma and from intercellular airspaces to the sites of carboxylation significantly reduce the rate of CO$_2$ assimilation especially under drought stress (Warren 2008; Flexas et al. 2013). High stomatal and mesophyll conductance increases photosynthetic rate and yield (Fischer et al. 1998; Barbour et al. 2010), but decreases water use efficiency. Manipulation of internal CO$_2$ concentrations by increasing mesophyll conductance and decreasing stomatal conductance is seen as a better way of increasing photosynthetic rate and water use efficiency (Barbour et al. 2010; Flexas et al. 2008, 2013). Studies have also shown that maintaining high photosynthetic rate while improving WUE under drought stress requires increased mesophyll conductance (Flexas et al. 2015).

This investigation was planned to identify and characterize diverse sources of rust resistance in wheat and to characterize variation in mesophyll conductance and its influence on water use efficiency (WUE) under well-watered and drought stress conditions. The key objectives of this study are:
1. To assess genetic diversity for rust resistance among an international wheat nursery

2. To conduct genetic analysis of adult plant resistance to stripe rust in wheat cultivar Sentinel

3. To understand the genetic relationship between stem rust resistance genes Sr39 and Sr36 located on chromosome 2B of wheat

4. To assess the genetic variation in mesophyll conductance and its influence on WUE in selected wheat landraces from the Watkins collection
CHAPTER 2

Literature review

2.1 Wheat

Common wheat (*Triticum aestivum*) is among the most important agricultural commodities supplying over 20% of the world’s food (Braun et al. 2010). It is an important crop as human food, animal feed and for industrial uses including starch and starch-derived products, alcohol and bio-fuel production (Mergoum et al. 2009; Hawkesford et al. 2013; Tyagi et al. 2014). Wheat is second to rice as a source of calories in the diets of populations from both developed and developing countries (Braun et al. 2010; 2011; Khan et al. 2013). The comparatively high protein content of wheat grain makes it the most important source of human nutrition, and its demand is expected to increase by 60% by 2050 (Braun 2011; Rosegrant et al. 1995; Hawkesford et al. 2013; Weigand 2011).

Sustainable wheat production is continuously threatened by a number of biotic stresses (pests and diseases) and abiotic stresses (drought and extreme temperatures) (Maqsood et al. 2012; Khan et al. 2013). This is compounded by the increasing effects of climate change (Rosegrant et al. 1995; Singh et al. 2011a). The three rust diseases (stem rust caused by *Puccinia graminis* f. sp. *tritici*; stripe rust caused by *P. striiformis* f. sp. *tritici* and leaf rust caused by *P. triticina*) are the most devastating biotic stresses of wheat worldwide (Singh et al. 2011a; Khan et al. 2013; Randhawa et al. 2013). Development of high yielding wheat varieties with resistance/tolerance to both biotic
and abiotic stresses will help to meet the increasing demand for wheat and wheat products (Braun et al. 2010; Khan et al. 2013).

2.2 Rust diseases of wheat

Rust diseases are among the most important diseases of wheat worldwide causing significant yield and quality losses (Roelfs et al. 1992; McIntosh et al. 1995). This is due to their capacity to produce large number of spores which are widely distributed by wind, capacity to evolve to new races with acquisition of virulence for genes deployed in wheat cultivars, ability to move long distances, and potential to develop rapidly under optimal environmental conditions (Bariana et al. 2007a; Khan et al. 2013; Roelfs et al. 1992). All three rust pathogens undergo mutation and once virulence is present it is readily selected on corresponding previously resistant genotypes leading to rust epidemics (Bansal et al. 2011). Stem rust develops well under hot and humid conditions, whereas stripe rust prefers a cool climate and leaf rust is adapted to a relatively wide range of conditions (Bariana et al. 2007a). Stem rust can cause up to 100% yield loss depending on environmental conditions, susceptibility of the cultivar and time of disease onset during the growing season (Roelfs et al. 1992; McIntosh et al. 1995; Todorovska et al. 2009; Singh et al. 2011b).

Stem rust, also known as black rust, has historically devastated wheat crops globally (Roelfs et al. 1992; Singh et al. 2002; Leonard and Szabo, 2005). In the mid-20th century stem rust epidemics occurred in Europe and many other countries including Australia, China and India (Leonard and Szabo 2005; Todorovska et al. 2009). It has been controlled for the past five decades by use of resistant wheat cultivars. Potentail
stem rust control cost was estimated to be A$478 m annually in Australia (Murray and Brennan 2009). A new stem rust race, Ug99, with wide range of virulence on most of the deployed resistance genes was reported in Uganda in 1999 (Pretorius et al. 2000). It was later detected in Kenya, Ethiopia, Yemen, Middle East and South Asia (Singh et al. 2006). Yield losses attributed to Ug99 were estimated at US$3 billion (Todorovska et al. 2009). Ug99, designated TTKSK based on the North American nomenclature (Wanyera et al. 2006), has rendered over 90% of global cultivated wheat cultivars susceptible (Singh et al. 2006; 2011a). Leaf rust (brown rust) occurs more regularly in wider regions of the world and can cause yield losses exceeding 50% (Bolton et al. 2008; Todorovska et al. 2009; Vanzetti et al. 2011; Khan et al. 2013).

Stripe rust (yellow rust) can cause up to 100% yield loss on susceptible cultivars, especially if the disease occurs early in the growing season and weather conditions are favorable (Roelfs et al. 1992; Chen 2005; Wellings 2007; 2011; Bariana et al. 2010). Yield losses due to stripe rust epidemics in US in 2003 were estimated at about 2.4 million tons (Høvmøller et al. 2011), while in 2009, epidemics reached record high levels in northern Africa and central and western Asia, where more than 90% of important wheat varieties were susceptible to the disease (Ezzahiri et al. 2009; Mboup et al. 2009; Sharma et al. 2009). Stripe rust, historically known to occur in temperate areas with cool, humid summers (Chen et al. 2014; Roelf et al. 1992), or in high altitude warm areas with cool nights, is now causing devastating epidemics in warmer areas where the disease was previously considered absent (Høvmøller et al. 2010). This is because of the pathogen adaptation to warmer temperatures (Høvmøller et al. 2011; Milus et al. 2009).
2.3 Control of rust diseases of wheat

Farmers employ a number of control strategies to manage rust diseases of wheat ranging from cultural practices, fungicide application and planting of resistant cultivars (Roelfs et al. 1992; Loughman et al. 2005). The decision to apply fungicides and the timing of fungicide application is based on response of the wheat cultivar planted, growth stage, and weather conditions (Loughman et al. 2005; Wanyera et al. 2009; 2010). However, fungicide application in most countries, particularly in the developing world where wheat is a subsistence crop is an unrealistic solution (Priyamvada and Tiwari 2011). The most economical and environmentally friendly control strategy is to release rust resistant wheat cultivars. Breeding for resistance to rust diseases in wheat requires a constant inflow of diverse sources of resistance as the rapidly evolving rust pathogens acquire virulence for genes deployed in commercial cultivars (Singh et al. 2011; Lowe et al. 2011).

2.4 Assessment of genetic diversity for rust resistance in wheat

Achievement of durable control of wheat rust diseases requires constant surveillance of pathogen populations, identification and deployment of diverse sources of resistance (Kolmer et al. 2007; Bariana et al. 2007a; Belayneh et al. 2012). Postulation of genes is the most practical method of detecting probable resistance genes in a given set of germplasm and facilitates maintenance of genetic diversity in breeding programs (Kolmer 1996; Sawhney 1994; Singh et al. 2014). It involves seedling tests on host lines to be studied using an array of pathotypes differing in virulence with respect to the genetically characterized resistance genes (Singh et al. 2001). This approach is based on
the gene-for-gene interaction, where the infection types produced by a pathotype on test genotypes is compared to the infection types (ITs) produced by the same isolates on lines carrying the known resistance gene (Pathan and Park 2007; McIntosh et al. 1995). High infection type (IT) on the test genotype indicates absence of effective resistance against the test isolate, whereas a low IT indicates the presence of at least one resistance gene in the cultivar (Kolmer 1996). Postulation of resistance genes has been reported to be effective only when the pathotypes used are well characterized and carry diverse combinations of virulence and avirulence genes (Kolmer 2003; Pathan and Park 2007; Belayneh et al. 2012).

Pathan and Park (2007) reported the presence of stem rust resistance genes $S_r7b$, $S_r8a$, $S_r8b$, $S_r9b$, $S_r9g$, $S_r11$, $S_r15$, $S_r17$, $S_r29$, $S_r31$, $S_r31$, $S_r36$ and $S_r38$ in European wheat cultivars. Singh et al. (2008) postulated $S_r5$, $S_r8a$, $S_r9g$, $S_r12$, $S_r30$, $S_r31$, $S_r36$ and $S_r38$ in wheat cultivars from the United Kingdom. Stem rust resistance genes $S_r5$, $S_r7a$, $S_r7b$, $S_r8a$, $S_r9e$, $S_r11$, $S_r21$, $S_r27$, $S_r29$, $S_r30$ and $S_r37$ were postulated to be in Ethiopian durum and wheat cultivars and breeding lines either singly or in combinations (Admassu et al. 2012). Toor et al. (2013) postulated stem rust resistance genes $S_r8a$, $S_r8b$, $S_r9e$, $S_r9g$, $S_r12$, $S_r13$, $S_r17$ and $S_r23$ in tetraploid landraces. Eight stem rust resistance genes ($S_r2$, $S_r9d$, $S_r9e$, $S_r9g$, $S_r12$, $S_r13$, $S_r14$ and $S_r17$) were detected in tetraploid wheat by Roelfs (1988). Spanic et al. (2015) reported presence of four stem rust resistance genes ($S_r8a$, $S_r31$, $S_r36$ and $S_r38$) in Croatian wheat cultivars.

Winzeler et al. (2000) attributed seedling leaf rust resistance in 72 winter wheat lines from Europe to genes $L_r1$, $L_r3a$, $L_r3ka$, $L_r10$, $L_r14a$, $L_r17b$, $L_r20$ and $L_r26$. In the
same study, they noted that gene *Lr37* was more effective at the adult plant stage. Singh et al. (2001) postulated *Lr13* (57%), *Lr26* (22%), *Lr37* (20%), *Lr10* (17%), *Lr17b* (10%), *Lr1* (7%), *Lr3a* (6%) and *Lr20* (4%) in a set of 70 wheat cultivars from the United Kingdom. Seedling resistance genes *Lr1, Lr2a, Lr9, Lr10, Lr11, Lr18* and *Lr26* were detected in 35 soft red winter wheat cultivars and 17 breeding lines from southern USA (Kolmer 2003). Leaf rust resistance genes *Lr1, Lr3, Lr10, Lr16, Lr17, Lr18* and *Lr23* were detected in wheat cultivars grown in Egypt (McVey et al. 2004), whereas resistance genes *Lr1, Lr3a, Lr3ka, Lr10, Lr13, Lr14a, Lr17b, Lr20, Lr26* and *Lr37* were reported in European wheat cultivars (Pathan and Park 2006). Eleven different *Lr* genes *Lr1, Lr3a, Lr3ka, Lr9, Lr10, Lr16, Lr17, Lr19, Lr24, Lr26, Lr41* and APR genes *Lr34* and *Lr35* were postulated in 66 bread wheat cultivars from Argentina (Vanzetti et al. 2011). Spanic et al. (2015) reported six leaf rust resistance genes (*Lr2a, Lr3, Lr10, Lr14a, Lr17* and *Lr26*) among Croatian wheats. Similarly, a number of leaf rust resistance gene postulation studies have been performed on CIMMYT elite and breeding germplasm. Examples include a study by Singh and Rajaram (1992) that showed presence of genes *Lr3, Lr10, Lr13, Lr26* and APR gene *Lr34* in cv. Frontana and three other CIMMYT wheats. In another study, Singh (1993) reported presence of genes *Lr1, Lr3, Lr13, Lr16, Lr17, Lr23, Lr26* and *Lr34* in 26 lines from CIMMYT spring wheat germplasm. Dadkhodaie et al. (2011) postulated seedling leaf resistance genes *Lr3a, Lr13, Lr16, Lr19, Lr23, Lr24, Lr26, Lr27+Lr31* in 109 lines of the 35th international bread wheat screening nursery (IBWSN).

Stripe rust resistance genes *Yr3, Yr6, Yr7* and *Yr9* were reported in CIMMYT wheat germplasm (Dubin et al. 1989). *Yr6, Yr7, Yr9, Yr17* and *Yr27* were postulated
either singly or in combinations in the 1st Australian Special Nursery (ASN), 22nd semi-arid wheat screening nursery (SAWSN) and 12th high temperature wheat yield trial (HTWYT). Perwaiz and Johnson (1986) tested 26 wheat cultivars from Pakistan with 18 British *P. striiformis* f. sp. *tritici* (Pst) pathotypes and postulated *Yr2*, *Yr6*, *Yr7*, and/or *Yr9*. Bartos et al. (1987) postulated *Yr1*, *Yr2*, *Yr3a+Yr4a*, *Yr9*, and *Yr32* (*YrCV*) in 17 Czechoslovakian and two Russian wheat cultivars following tests with 18 Pst pathotypes. Sharma et al. (1995) inoculated seedlings of 38 wild emmer derivatives and 53 advanced wheat lines from Nepal with 18 Pst pathotypes and found 28 wild emmer derivatives were resistant to all pathotypes and five resistance genes (*Yr2*, *Yr6*, *Yr7*, *Yr9*, and *YrA*) were postulated in Nepalese wheat cultivars and advanced lines. In China, Wang et al. (1994a; 1994b) identified eight resistance genes (*Yr1*, *Yr2*, *Yr3*, *Yr7*, *Yr9*, *Yr10*, *YrSu*, and *YrSD*) in 59 Chinese wheat cultivars using 20 Pst pathotypes. Niu et al. (2000) screened 50 Chinese wheat cultivars using 26 pathotypes and noted the high frequency of *Yr9*. Resistance genes *Yr2*, *Yr3a*, *Yr4a*, *Yr6*, *Yr7*, *Yr9*, *Yr26*, *Yr27* and *YrSD* were postulated either singly or in combinations in 72 Chinese wheat lines (Li et al. 2006). The resistance genes *Yr9* and *Yr26* were found to be frequent in Chinese wheat. Zeng et al. (2014) reported *Yr5*, *Yr9*, *Yr17*, *Yr18* and *Yr26* in a set of 330 Chinese cultivars and 164 advanced breeding lines and the most frequent gene was *Yr9*. Singh et al. (2008) postulated stripe rust resistance genes *Yr1*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr27*, *YrHVII* either singly or in combinations in wheat cultivars from UK, while Pathan et al. (2008) reported 11 seedling resistance genes *Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr27*, *Yr32*, *YrHVII* and *YrSPW* including a range of unidentified seedling resistances. Qamar et al. (2008) showed that a high proportion of Australian common wheat cultivars carry *Yr17* either singly or in combination with *Yr7* and that *Yr17* expresses better at higher
temperature. Furthermore, Dawit et al (2012) postulated stripe rust resistance genes $Yr2$, $Yr3a$, $Yr4a$, $Yr6$, $Yr7$, $Yr8$, $Yr9$, $Yr27$, $Yr32$ and $YrSU$ singly or in various combinations in Ethiopian wheat germplasm. It was also observed that genes $Yr2$, $Yr6$, $Yr7$, $Yr8$, $Yr9$, $Yr27$ and $Yr32$ were not providing adequate protection in Ethiopia.

2.5 Genetic analysis of rust resistance in wheat

Success of a breeding program for disease resistance relies on availability of well characterized genetically diverse sources of rust resistance and their strategic deployment in adapted cultivars (Bansal et al. 2011). Resistance to wheat rust diseases is categorized into two types; qualitative and quantitative (Bariana 2003). Qualitative resistance, also referred to as seedling resistance/all stage resistance, is controlled by genes that condition complete resistance and often referred to as major genes. Whereas quantitative resistance is conditioned by the small effect minor genes (Singh et al. 2000; Parlevliet 2002; Bariana 2003; Chen 2005; Clair 2010; Kou and Wang 2010; Lowe et al. 2011) and it is also called adult plant resistance.

Currently, there are more than 205 formally named rust resistance genes in the “wheat gene symbol catalogue 2014”. These includes about 74 leaf rust, 58 stem rust and 75 stripe rust resistance genes (McIntosh et al. 2013; 2014). The majority of these genes have been defeated by pathotypes of the respective rust pathogen. Rust pathogens evolve to produce new pathotypes virulent on already deployed resistance genes. Examples include the detection of Pgt pathotype Ug99 in Uganda in 1999 (Pretorius et al. 2000), a new pathotype of $P. triticina$ with specific virulence to durum wheat in

The understanding of genetic basis of resistance in target germplasm is important to ensure diversity for resistance. Genetic analysis estimates the number of resistance genes and provides information about their modes of inheritance. It involves bi-parental crosses between resistant and susceptible genotypes to generate segregating populations. F₂, F₃, backcross (BC) families, double haploids (DH) and/or recombinant inbred lines (RIL) have been commonly used for inheritance and mapping studies (Bariana 2003).

2.5.1 All stage resistance

All stage resistance (Chen 2005) is often based on genes that are effective at the seedling stage and remain effective at all stages of plant growth (Zadoks 1961). This type of resistance is generally controlled by single genes (Priyamvada and Tiwari 2011). ASR is prone to succumb to new pathotypes through acquisition of corresponding virulence for the target resistance gene (McIntosh and Brown 1997; Randhawa et al. 2014). Durability of ASR can be achieved through their deployment in combinations (Bariana et al. 2007a; Bernardo et al. 2013).

In the last five years alone, a number of ASR genes have been characterized and formally named. These include three stem rust resistance genes Sr52 (Qi et al. 2011), Sr53 (Liu et al. 2011) and Sr54 (Ghazvini et al. 2013); four leaf rust resistance genes Lr65 (Mohler et al. 2012), Lr71 (Singh et al. 2012), Lr72 (Herrera-Foessel et al. 2014.)
and \textit{Lr73} (Park et al. 2014) and about 10 stripe rust resistance genes \textit{Yr50} (Liu et al. 2013), \textit{Yr51} (Randhawa et al. 2014), \textit{Yr52} (Ren et al. 2012), \textit{Yr53} (Xu et al. 2013), \textit{Yr55} (Bansal and Bariana unpublished), \textit{Yr57} (Randhawa et al. 2015), \textit{Yr58} (Chhetri 2015), \textit{Yr60} (Herrera-Foessel et al. 2015), \textit{Yr61} (Zhou et al. 2014a) and \textit{Yr63} (Bansal and Bariana unpublished).

\textbf{2.5.2 Adult plant resistance}

Adult plant resistance (APR) is generally effective at the adult plant stages and is often detected in the field experiments (Roelfs et al. 1992; Hovmøller et al. 2011). Commercially acceptable levels of APR are conditioned by combinations of more than two APR genes of additive nature (Singh and Rajaram 2002). Stem rust APR genes \textit{Sr55} (Herrera-Foessel et al. 2014), \textit{Sr56} and \textit{Sr57} (Bansal et al. 2014), \textit{Sr58} (Singh et al. 2013) and leaf rust APR genes \textit{Lr67} (Herrera-Foessel et al. 2011a, 2014), \textit{Lr68} (Herrera-Foessel et al. 2012), \textit{Lr74} (UK Bansal pers. comm.) were formally named in recent years. In addition, five stripe rust APR genes \textit{Yr46} (Herrera-Foessel et al. 2011a, 2014), \textit{Yr48} (Lowe et al. 2011), \textit{Yr54} (Basnet et al. 2014), \textit{Yr56} (Bariana and Bansal unpublished), \textit{Yr59} (Zhou et al. 2014b) and \textit{Yr62} (Lu et al. 2014) have been designated in the last five years (McIntosh et al. 2014). The development of closely linked markers during the detailed characterization of resistance loci enables their pyramiding in future wheat cultivars.
2.5.3 Genomic location of rust resistance in wheat

Technological advancements in cost effective next-generation high throughput genotyping platforms (e.g. DArTseq, 90K wheat intinium SNP chip) has facilitated characterisation and mapping of numerous rust resistance loci in wheat (McIntosh et al. 2013; Rosewarne et al. 2013, Yu et al. 2014; Li et al. 2014). Following phenotypic evaluations, genomic location of resistance genes can be performed using one of the following approaches.

2.5.3.1 Bulked segregant analysis

Bulked-segregant analysis (BSA; Michelmore et al. 1991) and also called selective pooling (Darvasi and Soller 1994) is used to identify chromosomal locations of resistance genes through marker-trait associations. BSA involves molecular comparison of two pooled DNA samples from the phenotypic extremes of a segregating population derived from a single cross (Michelmore et al. 1991; Collard et al. 2005). Markers that show polymorphisms between the two pools enable identification of chromosomal location of the gene under study through the marker-trait association. Trait-linked markers are then amplified on the entire mapping population to observe the extent of recombination. This method has been successfully used in chromosomal location of markers closely linked with stripe rust resistance genes Yr4 (Bansal et al. 2010), Yr47 (Bansal et al. 2011), Yr5l (Randhawa et al. 2014) and Yr57 (Randhawa et al. 2015). Leaf rust resistance gene Lr72 was mapped to the distal end of 7BS through bulk segregant analysis (Herrera-Foessel et al. 2014.)
2.5.3.2 QTL Mapping

APR to diseases are controlled by several genes of small effect known as quantitative traits (Collard et al. 2005; Takagi et al. 2013). The regions within the genome associated with these traits are known as quantitative trait loci (QTL; Tanksley 1993). The discovery of molecular markers and availability of powerful biometric methods led to considerable progress in QTL mapping (Collard et al. 2005). QTL mapping is based on the principle that genes and markers segregate via chromosome recombination during meiosis thus allowing their analysis in the progeny (Paterson 1996). Recent reviews of quantitative trait loci (QTL) for rust resistance in wheat included 141 QTL for stem rust (Yu et al. 2014), 140 QTL for resistance to stripe rust (Rosewarne et al. 2013) and about 80 QTL for APR to leaf rust (Li et al. 2014).

2.5.3.3 Identification of markers linked with resistance genes

Molecular markers have been developed for several stem rust resistance genes and these include: \textit{Sr2} (Spilmeyer et al. 2003; Hayden et al. 2004), \textit{Sr6} (Tsilo et al. 2009), \textit{Sr9a} (Tsilo et al. 2007), \textit{Sr22} (Khan et al. 2005; Periyannan et al. 2011), \textit{Sr24} and \textit{Sr26} (Mago et al. 2005), \textit{Sr25} and \textit{Sr26} (Liu et al. 2010), \textit{Sr28} (Bansal et al. 2012; Rouse et al. 2012), \textit{Sr31/Yr9} (Mago et al. 2002; Das et al. 2006), \textit{Sr32} (Dundas et al. 2007; Mago et al. 2013), \textit{Sr35} (Babiker et al. 2009; Zhang et al. 2010; Saintenac et al. 2013), \textit{Sr36} (Bariana et al. 2007a; Tsilo et al. 2008), \textit{Sr38/Yr17} (Helguera et al. 2003), \textit{Sr39} (Gold et al. 1999; Mago et al. 2009; Niu et al. 2011), \textit{Sr40} (Wu et al. 2009), \textit{Sr44} (Liu et al. 2013), \textit{Sr45} (Periyannan et al. 2014), \textit{Sr49} (Bansal et al. 2015), \textit{Sr50} (Anugrahwati et al. 2008), \textit{Sr51} (Liu et al. 2011), \textit{Sr52} (Qi et al. 2011) and \textit{Sr53} (Liu et al. 2011), \textit{SrCad}
Markers linked with APR genes \textit{Lr67/Yr46/Sr55} (Forrest et al. 2014), \textit{Sr56} (Bansal et al. 2014b), \textit{Lr34/Yr18/Sr57} (Lagudah et al. 2006; 2009) and \textit{Lr46/Yr29/Sr58} (ES Lagudah unpublished) have also been identified.

Markers linked with stripe rust resistance genes cover \textit{Yr1} (Randhawa 2015), \textit{Yr5} (Sun et al. 2002; Yan et al. 2003; Chen et al. 2003b), \textit{Yr9} (Shi et al. 2001), \textit{Yr10} (Frick et al. 1998; Shao et al. 2001; Bariana et al. 2002; Smith et al. 2002; Wang et al. 2002), \textit{Yr15} (Chagué et al. 1999; Peng et al. 2000; Ramírez-González et al. 2015; Mandoulakani et al. 2015; Yaniv et al. 2015), \textit{Yr17} (Robert et al. 1999; Seah et al. 2001; Helguera et al. 2003), \textit{Yr18} (Lagudah et al. 2006; 2009), \textit{Yr24} (Zakeri et al. 2003), \textit{Yr26} (Ma et al. 2001), \textit{Yr28} (Singh et al. 2000), \textit{Yr32} (Eriksen et al. 2004), \textit{Yr34} (Bariana et al. 2006), \textit{Yr36} (Uauy et al. 2005), \textit{Yr45} (Li et al. 2011), \textit{Yr51} (Randhawa et al. 2014), \textit{Yr52} (Ren et al. 2012), \textit{Yr57} (Randhawa et al. 2015), \textit{Yr58} (Chhetri 2015), \textit{Yr59} (Zhou et al. 2014b), \textit{Yr60} (Herrera-Foessel et al. 2015), \textit{YrH52} (Peng et al. 2000) and \textit{Yrns-B1} (Börner et al. 2000). These markers are useful in pyramiding rust resistance genes and/or marker based detection of target genes in germplasm collections.

2.6 Genetic linkage of rust resistance genes in wheat

The detailed understanding of the genetic relationship between genes located on the same chromosomes arms is important for their deployment in combinations (Bariana et al. 2007a). Resistance genes that exhibit close repulsion linkage are hard to combine in a single genotype. Large population sizes are required to select rare recombinants. Both
coupling and repulsion linkages for rust resistance in wheat have been reported (McIntosh et al. 2013). Bansal et al (2008) reported repulsion linkage between \(Lr48\) and \(Lr13\) (13.7 cM) in chromosome 2BS using the leaf rust phenotypic data. This linkage is loose and hence these genes can be combined. Zakeri et al. (2003) reported 3% recombination (repulsion) between stripe rust resistance genes \(Yr15\) and \(Yr24\) in chromosome 1B of wheat. These authors recombinated these two genes in a single genotype. Singh et al. (2001) reported close repulsion linkage between \(Lr17b\) and \(Lr37\) in chromosome 2AS. Example of coupling linkage, primarily on translocated segments include; \(Yr35\) and \(Lr53\) (Marais et al. 2005b). Linkage analysis showed recombination frequency of 3% between the genes (Dadkhodaie et al. 2011). Other rust resistance genes linked in coupling are \(Sr31/Yr9/Lr26, Sr38/Yr17/Lr37, Yr29/Lr46, Yr18/Lr34 Sr24/Lr24, Sr15/Lr20, Sr25/Lr19\) (McIntosh et al. 1995, 2013, 2014). Several other examples are listed in McIntosh et al. (2013, 2014).

2.7 Abiotic constraints to wheat production

While a major emphasis of this study was on understanding genetics of resistance to biotic stresses, an attempt was made to understand genetic variation for abiotic stress among a set of landraces. The major abiotic stresses limiting crop productivity globally include drought, extreme temperatures, acidity, alkalinity and declining soil fertility (Hawkesford et al. 2013; Rosegrant et al. 1995). Low water availability is the most important environmental constraint to plant growth globally (Flexas et al. 2009), and global climate change is expected to amplify the effects of water scarcity on agricultural productivity across vast amounts of land (Chaves et al. 2008). With the current effects of
climate change and variability, climate change-induced droughts and temperature increases are expected to reduce wheat production by over 29% (Rosegrant et al. 1995).

2.7.1 Drought

Drought limits wheat production in more than 50% of the wheat cropped area causing yield losses between 15-60% depending on the region and growth stage of the crop (Pfeiffer et al. 2005, Mergoum et al. 2009). More than 70% of the world’s allocable water is used for irrigation (Morison et al. 2008). The higher grain yields achieved post green revolution were partly due to the release of semi-dwarf genotypes that could respond well to increased use of pesticides, fertilizers and irrigation (Tilman et al. 2002). However, to achieve a 30% increase in yields of the current genotypes will require 100% increase in fresh water for irrigation (Rockström et al. 2007). On the other hand, increasing frequency of droughts coupled with increasing water scarcity continues to make agriculture an increasingly daunting task, especially among resource-poor smallholder farmers (Chen et al. 2011; IPCC 2013; Kharrou et al. 2011). This scenario will be exacerbated by the increasing effects of climate change and variability (IPCC 2007). Drought stress limits plant growth by limiting photosynthesis and thus reducing the plant carbon balance (Flexas et al. 2009). Reduced diffusion of CO$_2$ from the atmosphere to the stroma in the chloroplast is the main limitation to photosynthesis under water deficit stress (Chaves et al. 2008; Erismann et al. 2008; Flexas et al. 2004; 2009; Grassi and Magnani 2005; Peeva and Cornic 2009). Improvement in photosynthetic rate and water use efficiency (WUE) under water limited conditions is critical for increased production of food for the growing population (Flexas et al. 2013; 2015).
2.7.2 Water use efficiency

Water use efficiency (WUE), defined as amount of carbon gained per unit of water used (Bacon 2004; Flexas et al. 2015), is an important physiological trait involved in plant response to drought stress (Tanner and Sinclair 1983; Chen et al. 2003; Condon et al. 2004; Xue et al. 2006). It describes the compromise between fixation of CO$_2$ and water loss that occur in plants when water evaporates from leaves tissues whenever stomata open for diffusion of CO$_2$ (Bramley et al. 2013). Improving the ratio of CO$_2$ assimilation rate to transpiration rate at the leaf level is one means of selecting plants for water limited environments (Barbour et al. 2010; Bramley et al. 2013).

Due to the increasing scarcity of water, breeding for water-use efficient genotypes has been a key component in most breeding programs (Condon et al. 2004). Carbon isotope discrimination as recorded in plant organic matter ($\Delta^{13}C_p$) has emerged as an important tool in selecting for improved water-use efficiency since the discovery of a negative relationship between $\Delta^{13}C_p$ and leaf intrinsic water use efficiency (WUE$_i$, or photosynthetic rate divided by stomatal conductance) (Farquhar and Richards 1984; Condon et al. 1990; Acevedo 1993; Rebetzke et al. 2002; Richards et al. 2002). C$_3$ plants discriminate against the heavier stable carbon isotope ($^{13}CO_2$) during diffusion through stomata and during carboxylation (Farquhar and Richards 1984), so that $\Delta^{13}C_p$ provides an integrative record of the relative supply of and demand for CO$_2$ (Condon et al. 1990). Selection for both high and low $\Delta^{13}C_p$ has been included in the development and release of two Australian wheat cultivars ‘Drysdale’ and ‘Rees’ (Condon et al. 2004; Rebetzke et al. 2002).
Diffusion of CO\(_2\) from the atmosphere to the intercellular airspaces through the stomata (stomata conductance) and from the intercellular airspaces to the sites of carboxylation (mesophyll conductance) are key determinants of net photosynthesis (\(A_N\)) (Flexas et al. 2008; 2013, Warren 2008). Increasing stomatal and mesophyll conductance to CO\(_2\) increases net photosynthetic rate but reduces WUE (Warren 2008). Based on numerous studies it has been suggested that one way to improve WUE and increase \(A_N\) in C\(_3\) plants is by improving mesophyll conductance to CO\(_2\) (\(g_m\)) while maintaining stomatal conductance (Barbour et al. 2010; Flexas et al. 2013).

### 2.7.3 Stomatal conductance

Stomatal conductance describes the conductance to diffusion of either water vapour (\(g_{sw}\)) or CO\(_2\) (\(g_{sc}\)) through the leaf stomata to the intercellular airspaces of the leaf. Conductance to diffusion of water vapour (\(g_{sw}\)) is influenced by the density, size, and degree of opening of the stomata. The more open stomata allow greater conductance, and consequently high photosynthesis and transpiration rates (Pietragalla and Pask 2012; Cowan 1965; Yu et al. 2004). Periods of drought cause a reduction in leaf water potential and stomatal closure (El-Sharkawy 2007). The rapid closure of leaf stomata due to moisture deficit and the resulting decline in transpiration lessens the decrease in leaf water potential and soil water depletion, thus protecting leaf tissues from turgor loss and desiccation (Cock et al. 1985; El-Sharkawy 2003). Although stomatal closure can improve crop water use efficiency, it also leads to reductions in potential photosynthesis and in turn total biomass and grain yield (Liang et al. 2002). Stomatal closure affects
photosynthesis by reducing intercellular CO₂ concentration, and thereby carboxylation in chloroplasts (Yu et al. 2001).

2.7.4 *Mesophyll conductance*

Mesophyll conductance ($g_m$), the diffusion of CO₂ from intercellular airspaces to the sites of carboxylation, is a significant and variable limitation to photosynthesis and intrinsic water use efficiency ($WUE_i$) (Flexas et al. 2008; Barbour et al. 2010). It refers to the diffusional conductance to CO₂ from the mesophyll airspaces, through the mesophyll cell walls, the plasma membrane, the cytoplasm, and across the chloroplast envelope into the stroma (Flexas et al. 2008; Kaldenhoff 2012). Low $g_m$ restricts diffusion of CO₂ to the chloroplasts, which in turn reduces photosynthesis and $WUE$ (Flexas et al. 2008; Barbour et al. 2010). Recent studies have indicated that having plants with high mesophyll conductance and low stomatal conductance would improve photosynthesis as well as minimizing water loss (Barbour et al. 2010).

Genotypic variation in mesophyll conductance has been reported in a number of different plant species. Barbour et al. (2010) reported variation in $g_m$ ranging from 0.05-0.6 mol m⁻² s⁻¹ bar⁻¹ in six barley genotypes. Significant genotypic variation in $g_m$ has also been reported in wheat (six genotypes, Evans and Vellen 1996; eleven genotypes, Jahan et al. 2014) with $g_m$ values ranging from 0.5-1.0 mol m⁻² s⁻¹ bar⁻¹. Gu et al. (2012a) observed variation in $g_m$ between 0.03-0.1 mol m⁻² s⁻¹ bar⁻¹ among thirteen rice genotypes. A review by Flexas et al. (2008) showed that significant variation exists within single functional groups, genus or species. He reported high $g_m$ values within
herbaceous plants, with \( g_m \) values >1 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in cotton (Brugnoli et al. 1998) and sunflower (Laisk and Loreto 1996). Low \( g_m \) of 0.08 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) was found in Loosstrife (Lysimachia minoricensis) (Galmés et al. 2007). Woody deciduous angiosperms had \( g_m \) values ranging from > 1 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in Betula pendula (Laisk et al. 2005) to < 0.06 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in Acer palmatum (Hanba et al. 2002, 2003). In the same review by Flexas et al. (2008), large variability within genus is reported. For example 0.02-0.42 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in citrus, 0.04-0.50 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in Populus and 0.07-0.30 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in Quercus. Others include Abies (0.02-0.13 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)), Acer (0.02-0.09 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)), Alnus (0.10-0.17 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)), Beta (0.18-0.34 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)), Eucalyptus (0.11-0.19 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)) and Pinus (0.04-0.17 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\)). Large variability in \( g_m \) is also found in a wide range of cultivars such as 0.16-0.39 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in bean, 0.3-1.8 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in cotton, 0.07-0.30 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in grapevines, 0.08-0.35 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in olives and 0.09-0.50 mol m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) in tobacco, Flexas et al. (2008).

2.7.4.1 Response of \( g_m \) to variations in environmental conditions

In addition to being highly variable among species and genotypes, \( g_m \) has also been reported to respond to changes in environmental conditions (Flexas et al. 2008). Reduction of \( g_m \) under drought has been observed in several studies (Flexas et al. 2002; 2006; Grassi and Magnani, 2005; Misson et al. 2010; Roupsard et al. 1996). Studies by Ferrio et al. (2012) and Théroux-Rancourt et al. (2014) observed delayed response of \( g_m \) compared to \( g_s \) as drought developed. In addition, a number of studies have reported increase in \( g_m \) with increasing light intensity (Cano et al. 2011, Douthe et al. 2011,
The response of $g_m$ to CO$_2$ concentration has been variable, with some studies reporting a decline in $g_m$ with an increase in CO$_2$ (Bunce 2010, Douthe et al. 2011, Flexas et al. 2007), while others observed no response of $g_m$ to changes in CO$_2$ levels (Tazoe et al. 2009, Von Caemmerer and Evans 1991). A high variability in responses of $g_m$ to leaf temperature has also been reported (Bernacchi et al. 2002; Evans and von Caemmerer 2013; Scafaro et al. 2011; Warren and Dreyer 2006; Yamori et al. 2006; von Caemmerer and Evans 2015).

### 2.7.4.2 Genetic control of mesophyll conductance

While a number of studies have reported variation in $g_m$ between species and genotypes of a single species, and its significant limitation to photosynthesis and $WUE$ (Flexas et al. 2008; 2013; Douthe et al. 2011; Evans & von Caemmerer 2013), less has been done to understand the genetic control of $g_m$. A recent study by Barbour et al. (2016) reported the first $g_m$ QTL, responsible for 9% of the variation observed in $g_m$ and Gu et al. (2012b) found association of variation in $g_s$ and $g_m$ with a previously mapped QTL for photosynthesis in rice. These two studies demonstrate that characterisation of chromosomal regions conditioning genotypic variations in $g_m$ and their deployment in modern varieties to enhance $WUE$ is possible.
CHAPTER 3

Assessment of genetic diversity for stem rust and stripe rust resistance in an international wheat nursery

3.1 Introduction

Rust diseases of wheat are among the most important production constraints in all wheat growing regions of the world (McIntosh et al. 1995; Roelfs et al. 1992). Stem rust caused by Puccinia graminis f. sp. tritici (Pgt) and stripe rust caused by P. striiformis f. sp. tritici (Pst) can cause up to 100% yield losses on susceptible cultivars (Roelfs et al. 1992; McIntosh et al. 1995; Chen 2005; Bansal et al. 2011). Frequent emergence and rapid spread of more virulent and aggressive races of stem rust (Pretorius et al. 2000; Nazari et al. 2009) and stripe rust (Chen 2005; Wellings 2007; FAO 2014) continue to pose a serious threat to global food security.

Various control options are available to minimize losses caused by rust pathogens. Fungicides have been shown to effectively control stem rust (Wanyera et al. 2009; Tadesse et al. 2010; Macharia et al. 2013) and stripe rust (Wellings 2007; Murray and Brennan 2009). The use of fungicides in Australia reduced losses from stripe rust by A$359 million annually (Murray and Brennan 2009). In China about 6 million hectares of wheat are treated with fungicides (Zhensheng et al. 2010). This is an expensive method of rust control; especially for small scale farmers. Host plant resistance is the most effective, economical and eco-friendly method of controlling wheat rust diseases (Bariana et al. 2007a; Maqsood et al. 2008; Vanzetti et al. 2011). Long term success in breeding for triple rust resistance is influenced by knowledge of pathotypic evolution,
availability of genetically diverse sources of resistance, and the access to high throughput screening methodologies (Bariana et al. 2007a; Singh et al. 2011).

Knowledge of the genetic basis of resistance in wheat cultivars and advanced breeding material form the basis of successful breeding for disease resistance (Bariana 2003; Bariana et al. 2007a; Admassu et al. 2012). Host resistance is categorized into two types; all stage resistance (ASR) and adult plant resistance (APR) (Bariana 2003; Chen 2005; Bariana et al. 2007a; Kou and Wang 2010). ASR is controlled by genes with major effects and it is often short lived as it is prone to be matched by evolution of virulence in pathogen populations. Durability of this resistance can be achieved by pyramiding more than two genes in new cultivars (Bariana et al. 2007a; Bernardo et al. 2013). On the other hand, APR is controlled by genes of small effects and expresses at the post seedling stages. A combination of more than two APR genes is essential to achieve acceptable level of resistance (Bariana and McIntosh 1995, Singh et al. 2000). Deployment of combinations of 4-5 APR genes confers ‘near-immune’ resistance and lasts for a longer time (Singh and Rajaram 2002; Singh et al. 2011). Hence achievement of durable control of wheat rust diseases requires identification, characterization and deployment of combinations of diverse sources of resistance (Kolmer et al. 2007; Bariana et al. 2007a, 2007b; Admassu et al. 2012).

Advances in molecular marker technology and increasing availability of gene-linked or gene-specific markers ensure efficient pyramiding of rust resistance genes (Kolmer et al. 2013). Molecular markers have been developed for several stem rust and
stripe rust resistance genes (http://maswheat.ucdavis.edu/Index.htm). These markers can be used for marker based prediction/postulation of target genes in germplasm collections.

Tests with an array of pathotypes differing in virulence genes offer the most efficient way to determine the genetic diversity for resistance to a target disease among a set of germplasm (Singh et al. 2014). It is based on the gene-for-gene concept in the case of rust diseases. Resistance genes are postulated by comparing infection types (ITs) produced by an array of pathotypes on genotypes under consideration with ITs produced by genotypes carrying known resistance gene(s) (Pathan and Park 2007; Singh et al. 2008; Singh et al. 2014). This methodology has been widely used to postulate resistance genes to stem rust, leaf rust and stripe rust in wheat. It requires well characterized pathotypes with diverse combinations of virulence and avirulence genes and such resources are available in several laboratories (Kolmer 2003; Pathan and Park 2007; Admassu et al. 2012; Singh et al. 2014). Field testing of seedling susceptible genotypes enables identification of genotypes carrying APR.

Wheat cultivars derived from the International Maize and Wheat Improvement Center (CIMMYT) germplasm are grown all over the wheat growing regions of the world through continuous exchange of material with national research programs (Singh and Rajaram 2002; Ortiz et al. 2008; Pretorius et al. 2015). CIMMYT workers incorporate diverse rust resistance genes into elite germplasm. The rust resistant lines with good agronomic traits are compiled into screening nurseries and distributed annually for rust screening in many wheat growing countries. Although wheat lines distributed globally by CIMMYT are selected based on their resistance to the three rust
diseases (Singh et al. 2008), screening of germplasm against the local rust flora is essential. This study was planned to test an international wheat screening nursery against several Australian Pgt and Pst pathotypes in the greenhouse to understand genetic diversity for stem rust and stripe rust resistance genes and it was also screened under field conditions against commercially important pathotypes to observe adult plant responses.

3.2 Materials and methods

3.2.1 Host materials

A set of 95 lines from a CIMMYT C21SAWYT-AUS wheat screening nursery were tested in the greenhouse to postulate stem rust and stripe rust resistance genes. Pedigree details are listed in Table 3.1. Stem rust and stripe rust differential sets with known resistance genes were included as controls (McIntosh et al. 1995).

3.2.2 Pathogen materials

Wheat genotypes were tested with seven Australian Pgt pathotypes: 34-1,2,3,4,5,6,7 (103); 34-1,2,3,6,7,(8),9 (205); 34-1,2,3,5,7,8,9 (206); 343-1,2,3,5,6,(8),9 (465); 98-1,2,(3),(5),6 (279); 34-1,2,7+Sr38 (565); 34-2,4,5,7,11 (99) and five Pst pathotypes: 134 E16A+Yr17+ (599); 134 E16A+Yr17+Yr27 (617); 110 E143A+ (444); 108 E141A+ (420), 104 E137A+ (414). The avirulence/virulence formulae of different pathotypes used are presented in Table 3.2.
Table 3.1 Pedigree information, postulated genes and molecular marker data for 95 entries of an international wheat screening

<table>
<thead>
<tr>
<th>QCode</th>
<th>Pedigrees</th>
<th>Genes postulated</th>
<th>Yr4</th>
<th>Lr24/Sr24</th>
<th>Lr26/Yr9/Sr31</th>
<th>Lr34/Yr18</th>
<th>Sr2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/SERI<em>3//RL6010/4</em>YR/3/PASTOR/4/BAV92</td>
<td>Yr3?</td>
<td>Sr12, Sr8a, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PARURAQ</td>
<td>Yr3?</td>
<td>Sr8a, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PARURAQ</td>
<td>Yr?</td>
<td>Sr8a, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4:ZWW12</td>
<td>NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/SERI<em>3//RL6010/4</em>YR/3/PASTOR/4/BAV92</td>
<td>Yr27</td>
<td>Sr30, Sr23, Sr8a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5:ZWW12</td>
<td>NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/PARURAQ</td>
<td>Yr27</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8:ZWW12</td>
<td>KA/NAC//TRCH/5/ESDA//ALTA R 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX</td>
<td>Yr17, Yr27</td>
<td>Sr30, Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9:ZWW12</td>
<td>KA/NAC//TRCH/5/ESDA//ALTA R 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX</td>
<td>Yr17</td>
<td>Sr12, Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10:ZWW12</td>
<td>KA/NAC//TRCH/5/ESDA//ALTA R 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX</td>
<td>Yr17</td>
<td>Sr8a, Sr17, Sr30, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11:ZWW12</td>
<td>KA/NAC//TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92</td>
<td>Yr17</td>
<td>Sr8a, Sr12, Sr17, Sr30, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12:ZWW12</td>
<td>KA/NAC//TRCH/3/MUU</td>
<td>NIL</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13:ZWW12</td>
<td>KA/NAC//TRCH/3/PARURAQ</td>
<td>Yr?</td>
<td>Sr8a, Sr12, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15:ZWW12</td>
<td>KA/NAC//TRCH/5/SERI<em>3//RL6010/4</em>YR/3/PASTOR/4/BAV92</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QCode</td>
<td>Pedigrees</td>
<td>Genes postulated</td>
<td>Yr4</td>
<td>Lr24/Sr24</td>
<td>Lr26/Yr9/Sr31</td>
<td>Lr34/Yr18</td>
<td>Lr37/Yr17/Sr38</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td>-----</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>16:ZWW12</td>
<td>KA/NAC/TRCH/3/PAURAQ</td>
<td>Yr27</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17:ZWW12</td>
<td>KA/NAC/TRCH/3/PAURAQ</td>
<td>Yr3</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19:ZWW12</td>
<td>1447/PASTOR//KRICKAUFF/3/PAURAQ</td>
<td>Yr17, Yr27</td>
<td>Sr24, Sr38</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20:ZWW12</td>
<td>ANNUELLO/3/KA/NAC/TRCH</td>
<td>Yr3?</td>
<td>Sr8a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21:ZWW12</td>
<td>VEE/LIRA//BOW/3/BCN/4/KAUZ/5/DANPHE #1</td>
<td>Yr3?</td>
<td>Sr8b, Sr17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23:ZWW12</td>
<td>WBLL1/PAURAQ</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24:ZWW12</td>
<td>WBLL1/PAURAQ</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>25:ZWW12</td>
<td>ASTREB/CHONTE</td>
<td>NIL</td>
<td>Sr8a, Sr17</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26:ZWW12</td>
<td>MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE</td>
<td>Yr17</td>
<td>Sr12, Sr17</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28:ZWW12</td>
<td>MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE</td>
<td>Yr17</td>
<td>Sr17, Sr30</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>29:ZWW12</td>
<td>MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ</td>
<td>Yr6</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30:ZWW12</td>
<td>MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ</td>
<td>Yr17</td>
<td>Sr8a, Sr30</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>31:ZWW12</td>
<td>METSO/ER2000/5/2<em>SERI</em>3//RL 6010/4*YR/3/PASTOR/4/BAV92</td>
<td>Yr17</td>
<td>Sr17, Sr30</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>32:ZWW12</td>
<td>METSO/ER2000/5/2<em>SERI</em>3//RL 6010/4*YR/3/PASTOR/4/BAV92</td>
<td>Yr17</td>
<td>Sr8a, Sr17</td>
<td>Sr30</td>
<td>Sr30</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>33:ZWW12</td>
<td>AGT</td>
<td>Yr17</td>
<td>Sr24, Sr38</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>34:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/ATTILA*2/PBW65</td>
<td>Yr?</td>
<td>Sr8b, Sr9b, Sr12+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>35:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92</td>
<td>Yr3, Yr17</td>
<td>Sr8a, Sr17, Sr30</td>
<td>Sr30</td>
<td>Sr30</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>QCode</td>
<td>Pedigrees</td>
<td>Genes postulated</td>
<td>Yr4</td>
<td>Lr24/Sr24</td>
<td>Lr26/Yr9/Sr31</td>
<td>Lr34/Yr18</td>
<td>Lr37/Yr17/Sr38</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>------------------</td>
<td>-----</td>
<td>-----------</td>
<td>---------------</td>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>36:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4 /VEE#5/5/KAUZ/6/FRET2/7/PATUS //MILAN/KAUZ/3/BAV92</td>
<td>Yr17</td>
<td>Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>37:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4 /VEE#5/5/KAUZ/6/FRET2/7/PATUS //MILAN/KAUZ/3/BAV92</td>
<td>Yr6</td>
<td>Sr9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>39:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4 /VEE#5/5/KAUZ/6/FRET2/7/DATE //MILAN/KAUZ/3/BAV92</td>
<td>Yr27</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>\</td>
</tr>
<tr>
<td>42:ZWW12</td>
<td>WORRAKATTA/2*PASTOR/DANPEHE #1</td>
<td>Yr3?</td>
<td>Sr24</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>\</td>
</tr>
<tr>
<td>43:ZWW12</td>
<td>WORRAKATTA/2*PASTOR/DANPEHE #1</td>
<td>NIL</td>
<td>Sr23, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>44:ZWW12</td>
<td>KRICHAUFF/2*PASTOR//CHO /NTE</td>
<td>Yr3?</td>
<td>Sr24</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45:ZWW12</td>
<td>SUNCO.6/FRAME//PASTOR/3/DANPEHE #1</td>
<td>Yr3?</td>
<td>Sr30+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>46:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPEHE #1</td>
<td>NIL</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPEHE #1</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>49:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPEHE #1</td>
<td>NIL</td>
<td>Sr30+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>51:ZWW12</td>
<td>BERKUT/MUU//DANPEHE #1</td>
<td>NIL</td>
<td>Sr17, Sr8b, Sr9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>\</td>
</tr>
<tr>
<td>52:ZWW12</td>
<td>AGT YOUNG<em>2/5/TUI//2</em>SUNCO/SA 1166/3/TUI/4/FINSI</td>
<td>Yr6</td>
<td>Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>53:ZWW12</td>
<td>AGT YOUNG<em>2/5/TUI//2</em>SUNCO/SA 1166/3/TUI/4/FINSI</td>
<td>NIL</td>
<td>Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>54:ZWW12</td>
<td>AGT YOUNG<em>2/5/TUI//2</em>SUNCO/SA 1166/3/TUI/4/FINSI</td>
<td>Yr17</td>
<td>Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>56:ZWW12</td>
<td>MON/IMU//ALD/PVN/3/BORL95/5/OASIS/2*BORL95/5/EMB16/CBRD//CBRD</td>
<td>Yr17</td>
<td>Sr31, Sr38</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QCode</td>
<td>Pedigrees</td>
<td>Genes postulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57:ZWW12</td>
<td>1447/PASTOR//KRICHAUFF/5/2 <em>SERI</em>3/RL60104/4*YR/3/PAST OR/4/BAV92</td>
<td>Yr17</td>
<td>Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>58:ZWW12</td>
<td>1447/PASTOR//KRICHAUFF/5/2 <em>SERI</em>3/RL60104/4*YR/3/PAST OR/4/BAV92</td>
<td>Yr17</td>
<td>Sr8a, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>59:ZWW12</td>
<td>TUI/2*SUNCO/SA1166/3/TUI/4/FINSI/5/SOKOLL/6/KA/NAC/T RCH</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>61:ZWW12</td>
<td>ITP50/3/KA/NAC//TRCH</td>
<td>Yr?</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>\</td>
</tr>
<tr>
<td>62:ZWW12</td>
<td>EMB16/CBRD//CBRD/3/SUNCO.6/FRAME//PASTOR/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92</td>
<td>Yr9, Yr17</td>
<td>Sr24, Sr31, Sr38</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>63:ZWW12</td>
<td>C80.1/3<em>BATAVIA//2</em>WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92</td>
<td>Yr4, Yr9, Yr17</td>
<td>Sr31, Sr38</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>\</td>
</tr>
<tr>
<td>64:ZWW12</td>
<td>C80.1/3<em>BATAVIA//2</em>WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92</td>
<td>Yr3, Yr9, Yr17</td>
<td>Sr31, Sr38</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>67:ZWW12</td>
<td>KA/NAC//TRCH/3/SLVS/ATTIL A//WBLL1/4/KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>68:ZWW12</td>
<td>KA/NAC//TRCH/3/SLVS/ATTIL A//WBLL1/4/KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>69:ZWW12</td>
<td>KA/NAC//TRCH/3/SLVS/ATTIL A//WBLL1/4/KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>70:ZWW12</td>
<td>KA/NAC//TRCH/3/SLVS/ATTIL A//WBLL1/4/KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>77:ZWW12</td>
<td>SUNCO/2*PASTOR/3/SLVS/ATTIL A//WBLL1/4/KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>78:ZWW12</td>
<td>SLVS/3/CROC_1/AE.SQUARRSA (224)//OPATA/5/VEE/LIRA//BO W/3/BCN/4/KAUZ/6/2*KA/NAC//TRCH</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QCode</td>
<td>Pedigrees</td>
<td>Genes postulated</td>
<td>Yr4</td>
<td>Lr24/Sr24</td>
<td>Lr26/Yr9/Sr31</td>
<td>Lr34/Yr18</td>
<td>Lr37/Yr17/Sr38</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----------</td>
<td>---------------</td>
<td>------------</td>
<td>----------------</td>
</tr>
<tr>
<td>85:ZWW12</td>
<td>CNDO/R143//ENTE/MEXI_2/3/A EGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2<em>JANZ/6/SERI/3//RL6010/4</em>YR/3/PAST OR/4/BAV92/7/VORB</td>
<td>Yr3?, Yr6 Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>87:ZWW12</td>
<td>CNDO/R143//ENTE/MEXI_2/3/A EGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2<em>JANZ/8/CAL/NH/567.71/3/SERI/4/CAL/NH/567.71/5/2</em>KAUZ/6/WH576/7/WH 542</td>
<td>Yr27+ Sr9b or Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88:ZWW12</td>
<td>CNDO/R143//ENTE/MEXI_2/3/A EGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/VORB</td>
<td>Yr27 Sr8a, Sr12, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>97:ZWW12</td>
<td>WORRAKATTA/2*PASTOR//PA RUS/PASTOR/3/SOKOLL</td>
<td>Yr17 Sr8a, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Null</td>
</tr>
<tr>
<td>98:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/VORB</td>
<td>Yr6 Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR/MILAN/KAUZ/3/BAV92</td>
<td>Yr17 Sr38+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>101:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR/MILAN/KAUZ/3/BAV92</td>
<td>Yr6 Sr8a, Sr12, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>102:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR/MILAN/KAUZ/3/BAV92</td>
<td>NIL Sr30+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>107:ZWW12</td>
<td>TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR/MILAN/KAUZ/3/BAV92</td>
<td>Yr? Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>108:ZWW12</td>
<td>NS-732/HER/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/CHONTE</td>
<td>Yr27 Sr8a, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>109:ZWW12</td>
<td>KA/NAC/TRCH/3/VORB</td>
<td>Yr6 Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>\</td>
<td>-</td>
</tr>
<tr>
<td>111:ZWW12</td>
<td>KA/NAC/TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92</td>
<td>Yr3?+ Sr8b+ ??, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>QCode</td>
<td>Pedigrees</td>
<td>Genes postulated</td>
<td>Yr4</td>
<td>Lr24/Sr24</td>
<td>Lr26/Yr9/Sr31</td>
<td>Lr34/Yr18</td>
<td>Lr37/Yr17/Sr38</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>------------------</td>
<td>-----</td>
<td>-----------</td>
<td>---------------</td>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>112:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr34?+</td>
<td>Sr8b, Sr12, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>113:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr3?</td>
<td>Sr8b, Sr12, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>114:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr34?</td>
<td>Sr8b, Sr9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>115:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr3</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>116:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr34?</td>
<td>Sr8b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>117:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr34?</td>
<td>Sr8b, Sr9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>118:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr?</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>119:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>Yr3?</td>
<td>Sr9b, Sr8b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120:ZWW12</td>
<td>KA/NAC//TRCH/3/DANPHE #1</td>
<td>NIL</td>
<td>Sr8b, Sr9b, Sr12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>121:ZWW12</td>
<td>KA/NAC//TRCH/3/PARUAQ</td>
<td>NIL</td>
<td>Sr9b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>122:ZWW12</td>
<td>KA/NAC//TRCH/5/SERI<em>3//RL6010/4</em>YR/3/PASTOR/4/BAV92</td>
<td>NIL</td>
<td>Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>127:ZWW12</td>
<td>KA/NAC//TRCH/3/KINDE</td>
<td>Yr17</td>
<td>Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>128:ZWW12</td>
<td>MILAN/KAUZ//Dharwar DRY/3/BAV92/4/CHONTE</td>
<td>Yr17</td>
<td>Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>129:ZWW12</td>
<td>MILAN/KAUZ//Dharwar DRY/3/BAV92/4/CHONTE</td>
<td>NIL</td>
<td>Sr24+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>130:ZWW12</td>
<td>WORRAKATTA/2*PASTOR//MUU/3/DANPHE #1</td>
<td>Yr17</td>
<td>Sr30, Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>131:ZWW12</td>
<td>MON/IMU//ALD/PVN/3/BORL95/4/OASIS/2<em>BORL95/5/2</em>SKAUZ/BAV92</td>
<td>Yr3?</td>
<td>Sr8a, Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>132:ZWW12</td>
<td>BERKUT/MUU//MUU</td>
<td>NIL</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>133:ZWW12</td>
<td>BERKUT/MUU//DANPHE #1</td>
<td>NIL</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>136:ZWW12</td>
<td>EMB16/CBRD/CBRD/4/BETTY/3/CHEN/AE.SQ//2<em>OPATA PASTOR//HXL753/2</em>BAU/23/PAU/WEAVER/KIRITATI</td>
<td>Yr27</td>
<td>Sr17, Sr30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>137:ZWW12</td>
<td>CNDO/R143//ENTE/MEXI_2/3/EGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/SKAUZ/BAV92</td>
<td>NIL</td>
<td>Sr17, Sr24</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>138:ZWW12</td>
<td>CNDO/R143//ENTE/MEXI_2/3/EGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/SKAUZ/BAV92</td>
<td>Yr17</td>
<td>Sr8a, Sr9b, Sr17, Sr38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>140:ZWW12</td>
<td>METSO/ER2000/3/PASTOR//HX</td>
<td>Yr17</td>
<td>Sr38+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

34
<table>
<thead>
<tr>
<th>QCode</th>
<th>Pedigrees</th>
<th>Genes postulated</th>
<th>Yr4</th>
<th>Lr24/Sr24</th>
<th>Lr26/Yr9/Sr31</th>
<th>Lr34/Yr18</th>
<th>Lr37/Yr17/Sr38</th>
<th>Sr2</th>
</tr>
</thead>
<tbody>
<tr>
<td>141:ZWW12</td>
<td>METSO/ER2000/3/PASTOR/HX</td>
<td>Yr17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L7573/2*BAU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>142:ZWW12</td>
<td>METSO/ER2000/3/PASTOR/HX</td>
<td>Yr17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L7573/2*BAU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>145:ZWW12</td>
<td>METSO/ER2000/3/PBW343*2/KU</td>
<td>Yr27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Null</td>
</tr>
<tr>
<td></td>
<td>KUNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- = absence, + = present, \ = missing data and Null = no amplification, ? = not sure
3.2.3 Greenhouse tests

Experimental materials were sown in 9 cm pots filled with a mixture of pine bark and river sand in a ratio of 2:1. An initial dose of 10 g of water soluble fertilizer Aquasol® dissolved in 10 l of tap water was applied to the filled pots before sowing. Four entries were sown per pot with six seeds each per clump. Seven-day old seedlings were fertilized with Urea at the same dose as Aquasol®. Ten to 12-day old seedlings were inoculated with urediniospores of the different Pgt and Pst pathotypes suspended in light mineral oil Isopar-L® using a hydrocarbon pressure pack. Stem rust inoculated seedlings were humidified on water filled steel trays covered with plastic hoods under natural light at 18-20°C for 48 hours, while stripe rust inoculated seedlings were incubated in the dark at 9±2°C for 24 hours. Following incubation, seedlings were moved to microclimate rooms maintained at 25±2°C (stem rust) and at 17±2°C (stripe rust). Two sets were planted for screening against Pgt pathotype 34-2,4,5,7,11. One set each was incubated at 25±2°C and 20±2°C post inoculation to postulate temperature-sensitive stem rust resistance genes Sr6 and Sr12.

Seedling stem rust assessments were made 14 days after inoculation, using the 0-4 scale described by Stakman et al. (1962) with modifications by McIntosh et al. (1995). Stripe rust responses were scored 15 days post inoculation using 0-4 scale as described in Bariana and McIntosh (1993).
### Table 3.2 List of Pgt and Pst pathotypes used and their avulence/virulence spectrum

<table>
<thead>
<tr>
<th>PBIC</th>
<th>Pathotype</th>
<th>PBIA</th>
<th>Avirulence</th>
<th>Virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stem Rust</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>34-1,2,3,4,5,6,7</td>
<td>74-L-1</td>
<td>Sr8b,9e,13,24,27,30,32,33,35,37,38,39,40,45,46,48,49</td>
<td>Sr5,6,8a,9b,9g,11,12,15,17,36</td>
</tr>
<tr>
<td>205</td>
<td>34-1,2,3,6,7,(8),9</td>
<td>76-L-7</td>
<td>Sr8b,9e,13,17,24,27,32,33,35,36,37,38,39,40,45,46,48,49</td>
<td>Sr5,6,8a,9b,9g,11,12,15,30,Agi</td>
</tr>
<tr>
<td>206</td>
<td>34-1,2,3,5,7,8,9</td>
<td>76-L-8</td>
<td>Sr8a,8b,9e,13,24,27,32,33,35,36,37,38,39,40,45,46,48,49</td>
<td>Sr5,9b,9g,11,12,15,17,30,Agi</td>
</tr>
<tr>
<td>465</td>
<td>343-1,2,3,5,6,8,9</td>
<td>890005</td>
<td>Sr8b,9e,9g,13,15,24,27,32,33,35,36,37,38,39,40,45,46,48,49</td>
<td>Sr5,6,8a,9b,11,12,17,(30),Agi</td>
</tr>
<tr>
<td>279</td>
<td>98-1,2,(3),(5),6</td>
<td>780129</td>
<td>Sr8b,9e,13,15,24,27,30,32,33,35,36,37,38,39,40,45,46,48,49</td>
<td>Sr5,6,8a,(9b),9g,11,12,(17)</td>
</tr>
<tr>
<td>565</td>
<td>34-1,2,7+Sr38</td>
<td>10130</td>
<td>Sr8a,8b,9b,9e,12,13,17,24,27,30,32,33,35,36,37,39,40,45,46,49</td>
<td>Sr5,6,7b,9g,11,15,38,48</td>
</tr>
<tr>
<td>99</td>
<td>34-2,4,5,7,11</td>
<td>640231</td>
<td>Sr6,8a,9b,9e,12,13,24,27,30,32,33,35,37,38,39,40,45,46,48,49</td>
<td>Sr5,7b,9g,11,15,17,36</td>
</tr>
<tr>
<td><strong>Stripe Rust</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>599</td>
<td>134 E16A+</td>
<td>61639</td>
<td>Yr1,3,4,5,10,15,24,27,32,33,34,47,SD,Su,ND,Sp</td>
<td>Yr2,6,7,8,9,17,A</td>
</tr>
<tr>
<td>617</td>
<td>134 E16A+</td>
<td>101975</td>
<td>Yr1,3,4,5,10,15,24,32,33,34,47,SD,Su,ND,Sp</td>
<td>Yr2,6,7,8,9,17,A,27</td>
</tr>
<tr>
<td>444</td>
<td>110 E143A+</td>
<td>861725</td>
<td>Yr1,5,8,9,10,15,17,24,27,32,33,47,Sp,</td>
<td>Yr2,3,4,6,7,SD,Su,ND,A,34</td>
</tr>
<tr>
<td>420</td>
<td>108 E141A+</td>
<td>831917</td>
<td>Yr1,5,7,8,9,10,15,17,24,27,32,33,47,Sp</td>
<td>Yr2,3,4,6,A,SD,Su,ND,34</td>
</tr>
<tr>
<td>414</td>
<td>104 E137A+</td>
<td>821552</td>
<td>Yr1,5,6,7,8,9,10,27,32,33,47,Sp</td>
<td>Yr2,3,4,4A,34</td>
</tr>
</tbody>
</table>

PBIC: Plant Breeding Institute culture number assigned in the cereal rust collection
PBIA: Plant Breeding Institute accession number assigned in the cereal rust collection
3.2.4 Field screening

The whole nursery was tested against commercially important Pgt and Pst pathotypes in the field at the Plant Breeding Institute field sites Karalee during the 2012 and 2013 cropping seasons. Individual entries were sown as hill plots and each block of 35 entries was surrounded by stem rust and stripe rust susceptible infector mixture to create rust epidemics. Adult plant responses were assessed using the 1-9 scale described in Bariana et al. (2007b).

3.2.5 DNA extraction and quantification

Leaf samples of 2 cm length from eight leaves per entry were collected in 2 ml tubes and dried on silica gels for 3 days. DNA was extracted from 95 wheat entries following procedures described by Bansal et al. (2014). DNA was quantified using a nanodrop ND-100 spectrophotometer and dilutions of 30 ng/µl of genomic DNA were made using deionized water.

3.2.6 Molecular marker genotyping

The entire wheat nursery was genotyped with gene-linked markers to detect the presence of stem resistance genes Sr2, Sr24, Sr31, Sr38 and stripe resistance genes Yr4, Yr9, Yr17 and Yr18. Hartog (Sr2), Janz (Sr24, Yr18), Sunlin (Sr26), AvS/6*Yr9 (Sr31/Yr9), Cook (Sr36), Trident (Sr38/Yr17) and Rubric (Yr4) were included as positive controls for respective markers.
PCR amplifications were performed in 10 µl reaction volumes containing 60 ng/µl of genomic DNA from each entry and respective controls, 0.2 mM dNTPs, 1× PCR buffer containing 1.5 mM MgCl₂ (Bioline), 0.5 µM of each primer (forward and reverse) and 0.02 U Immolase Taq DNA polymerase (Bioline). PCR reactions were performed in T100™ thermal cycler (BioRad USA) machines using published PCR conditions/profiles for the different primers. PCR products and restriction enzyme digests (csSr2) were separated on 2% agarose gels stained with gel red, and visualised under UV light. PCR amplification for M₁₃-labelled barc75 was carried out in a total reaction volume of 10 µl containing 30 ng/µl of genomic DNA, 1×MgCl₂ buffer, 0.75× dNTPs, 0.4×1.25 µM forward primer labelled with M₁₃, 0.4×5 µM Reverse primer, 0.1×0.50 µM M₁₃-tailed primer labelled with IRDye 700 or 800 and 0.04 µl×0.02 U Immolase Taq DNA polymerase (Bioline). The PCR reactions were carried out in BioRad machine and PCR products were separated on 6.5% Polyacrylamide gel using electrophoresis apparatus LICOR-4300 DNA analyser system (Li-COR Bio-science USA).

3.3 Results

3.3.1 Postulation of resistance genes

Resistance genes were postulated by comparing IT patterns of the different Pgt and Pst pathotypes on test genotypes with those of differential lines with known resistance genes. A high IT on a test genotype demonstrated the lack of resistance gene for which that pathotype was avirulent. Genotypes that show low ITs with all pathotypes are likely to carry either a gene effective against all pathotypes or combinations of genes with
compensating pathotypic specificities. The various rust responses observed among the
wheat screening nursery tested in this study is illustrated in Fig. 3.1.

![Fig. 3.1](image)

**Fig. 3.1** a) Seedling stem rust and b) seedling stripe rust response variation observed
among the entries tested in the international wheat nursery

### 3.3.1.1 Stem rust

Seedling stem rust resistance genes *Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31* and *Sr38* were postulated (Table 3.1 and Table 3.3). Majority of these genes were
present in combinations and a few lines carried *Sr8a* (1), *Sr8b* (1), *Sr9b* (2), *Sr24* (2) and
*Sr30* (17) singly. Thirty entries carried *Sr8a* in combinations with one to three genes
including *Sr9b, Sr12, Sr17, Sr23, Sr30* and *Sr38*. Similarly *Sr8b* was postulated in nine
entries in combination with *Sr9b, Sr12, Sr17* and *Sr30*. *Sr30, Sr38, Sr17* and *Sr8a* were
the most predominant stem rust resistances genes detected in this nursery. *Sr24* was
postulated in seven entries and *Sr31* in four lines.
3.3.1.2 Stripe rust

Stripe rust multipathotype testing results are summarized in Table 3.1 and 3.4. Screening with five different Pst pathotypes detected seven seedling stripe rust resistance genes (\(Yr3, Yr4, Yr6, Yr9, Yr17, Yr27\) and \(Yr34\)) either singly or in combinations. \(Yr3\) was present singly in 11 entries and in combination with \(Yr6\) in one line (85:ZWW12). \(Yr3\) in combination with an additional gene was postulated in entry 35:ZWW12 and 111:ZWW12. \(Yr3\) in combination with \(Yr9\) and \(Yr17\) was detected in entry 64:ZWW12. Stripe rust resistance gene \(Yr4\) in combination with \(Yr9\) and \(Yr17\) were postulated in entry 63:ZWW12. \(Yr6\) was detected singly in six entries (Table 3.4). \(Yr9\) and \(Yr17\) were postulated in one line (62:ZWW12). Additionally, \(Yr17\) was present singly in 24 entries and in combinations with \(Yr27\) in two lines. Likewise, \(Yr27\) was detected singly in eight entries and in combination with additional unknown resistance in one line (87:ZWW12). On the other hand, \(Yr34\) was postulated singly in three lines and in combination with extra unknown resistance in one entry. Seven entries carried seedling resistance genes that could not be postulated by an array of Pst pathotypes used. Twenty six entries were susceptible at seedling stage to all the Pst isolates used, suggesting that they did not carry any seedling stripe rust resistance genes. ASR genes \(Yr17\) (32\%) followed by \(Yr3\) (16\%) and \(Yr27\) (12\%) were the most frequent resistance genes detected. \(Yr4\) (1\%), \(Yr9\) (3\%) and \(Yr34\) (4\%) were the least frequent genes detected. The postulation of \(Yr3\) and \(Yr34\) should be treated with caution. It is important to confirm the presence of these genes once diagnostic markers are available.
Table 3.3 Seedling stem rust resistance variation in the CIMMYT international wheat screening nursery

<table>
<thead>
<tr>
<th>Resistance genes detected</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr8a</td>
<td>1 (20:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr9b, Sr17, Sr38</td>
<td>1 (138:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr9g, Sr12, Sr17, Sr30, Sr38</td>
<td>1 (11:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr12, Sr17, Sr30, unknown</td>
<td>1 (13:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr12, Sr30</td>
<td>2 (88:ZWW12, 101:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr12, Sr17, Sr30</td>
<td>1 (1:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr17, Sr30, Sr38</td>
<td>5 (8:ZWW12, 10:ZWW12, 32:ZWW12, 35:ZWW12, 97:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr17, Sr30</td>
<td>4 (2:ZWW12, 3:ZWW12, 25:ZWW12, 131:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr17, Sr38</td>
<td>5 (52:ZWW12, 53:ZWW12, 54:ZWW12, 57:ZWW12, 58:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr23, Sr30</td>
<td>1 (4:ZWW12)</td>
</tr>
<tr>
<td>Sr8a, Sr30, Sr38</td>
<td>1 (30:ZWW12)</td>
</tr>
<tr>
<td>Sr8b</td>
<td>1 (116:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr9b</td>
<td>2 (117:ZWW12, 119:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr9b, Sr12</td>
<td>1 (120:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr9b, Sr12+</td>
<td>1 (34:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr9b, Sr17</td>
<td>1 (51:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr12, Sr30</td>
<td>2 (112:ZWW12, 113:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr12</td>
<td>1 (114:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr17</td>
<td>1 (21:ZWW12)</td>
</tr>
<tr>
<td>Sr8b, Sr30, unknown</td>
<td>1 (111:ZWW12)</td>
</tr>
<tr>
<td>Sr9b</td>
<td>2 (37:ZWW12, 122:ZWW12)</td>
</tr>
<tr>
<td>Sr9b, Sr30</td>
<td>1 (87:ZWW12)</td>
</tr>
<tr>
<td>Sr12, Sr17, Sr30, Sr38</td>
<td>1 (26:ZWW12)</td>
</tr>
<tr>
<td>Sr12, Sr36, Sr38</td>
<td>1 (9:ZWW12)</td>
</tr>
<tr>
<td>Sr17, Sr24</td>
<td>1 (137:ZWW12)</td>
</tr>
<tr>
<td>Sr17, Sr30</td>
<td>5 (12:ZWW12, 39:ZWW12, 46:ZWW12, 132:ZWW12, 136:ZWW12)</td>
</tr>
<tr>
<td>Sr17, Sr30, unknown</td>
<td>1 (134:ZWW12)</td>
</tr>
<tr>
<td>Sr17, Sr30, Sr38</td>
<td>2 (28:ZWW12, 31:ZWW12)</td>
</tr>
<tr>
<td>Resistance genes detected</td>
<td>Frequency</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Sr17+</td>
<td>1 (18:ZWW12)</td>
</tr>
<tr>
<td>Sr23, Sr30</td>
<td>1 (43:ZWW12)</td>
</tr>
<tr>
<td>Sr24</td>
<td>2 (42:ZWW12, 44:ZWW12)</td>
</tr>
<tr>
<td>Sr24+</td>
<td>1 (129:ZWW12)</td>
</tr>
<tr>
<td>Sr24, Sr31, Sr38</td>
<td>1 (62:ZWW12)</td>
</tr>
<tr>
<td>Sr24, Sr38</td>
<td>2 (19:ZWW12, 33:ZWW12)</td>
</tr>
<tr>
<td>Sr26, Sr38</td>
<td>1 (55:ZWW12)</td>
</tr>
<tr>
<td>Sr30, unknown</td>
<td>3 (45:ZWW12, 49:ZWW12, 102:ZWW12)</td>
</tr>
<tr>
<td>Sr30, Sr38, unknown</td>
<td>1 (130:ZWW12)</td>
</tr>
<tr>
<td>Sr31, Sr38</td>
<td>3 (56:ZWW12, 63:ZWW12, 64:ZWW12)</td>
</tr>
<tr>
<td>Sr38, unknown</td>
<td>7 (36:ZWW12, 100:ZWW12, 127:ZWW12, 128:ZWW12, 140:ZWW12, 141:ZWW12, 14:ZWW12)</td>
</tr>
</tbody>
</table>
3.3.2 Field screening

All entries produced adult plant stem rust responses 2 to 3 under field conditions in two consecutive years 2012 and 2013 (Fig. 3.2). A high proportion of entries in 2012 (85%) and 2013 (93%) displayed adult plant stripe responses 2 to 4, while the remaining 15% in 2012 and 7% in 2013 exhibited adult plant stripe rust responses varying between 5 and 6 (Fig. 3.3). None of the entries were susceptible to stem rust or stripe rust in the field. Out of the 26 entries that did not carry any stripe rust seedling resistance genes i.e were susceptible to all the Pst pathotypes used in greenhouse, only two entries (12:ZWW12, 49:ZWW12) had field score ‘6’, three entries (23:ZWW12, 53:ZWW12, 129:ZWW12) were scored 4 and the remaining 21 entries were scored 2 to 3 in the field, suggesting that these entries carry more than one APR gene.
Table 3.4 Seedling stripe rust resistance variation in the CIMMYT international wheat screening nursery

<table>
<thead>
<tr>
<th>Resistance genes postulated</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yr3, unknown</td>
<td>2 (35:ZWW12, 111:ZWW12)</td>
</tr>
<tr>
<td>Yr3, Yr6</td>
<td>1 (85:ZWW12)</td>
</tr>
<tr>
<td>Yr3, Yr9, Yr17</td>
<td>1 (64:ZWW12)</td>
</tr>
<tr>
<td>Yr4, Yr9, Yr17</td>
<td>1 (63:ZWW12)</td>
</tr>
<tr>
<td>Yr9, unknown</td>
<td>1 (18:ZWW12)</td>
</tr>
<tr>
<td>Yr9, Yr17</td>
<td>1 (62:ZWW12)</td>
</tr>
<tr>
<td>Yr17, Yr27</td>
<td>2 (8:ZWW12, 19:ZWW12)</td>
</tr>
<tr>
<td>Yr27, unknown</td>
<td>1 (87:ZWW12)</td>
</tr>
<tr>
<td>Yr34</td>
<td>3 (114:ZWW12, 116:ZWW12, 117:ZWW12)</td>
</tr>
<tr>
<td>Yr34, unknown</td>
<td>1 (112:ZWW12)</td>
</tr>
</tbody>
</table>
Fig. 3.2 Field stem rust response variation among the international nursery during 2012 and 2013 cropping seasons

Fig. 3.3 Field stripe rust response variation among the international nursery during 2012 and 2013 cropping seasons
3.3.3 Molecular marker genotyping

Linked molecular markers can be used to detect APR genes and to confirm the postulation of ASR genes. CAPS marker csSr2 detected APR gene Sr2 in four entries (Hope allele; 172bp, 112bp and 53bp), whereas 80 entries amplified the susceptible Marquis allele (225bp and 112bp) after digestion with the restriction enzyme BspHI. Eleven entries did not amplify any product. Out of the 95 entries genotyped with the dominant STS marker Sr24#12, only nine entries and the positive control Janz produced a 500bp amplicon associated with Sr24, whereas no amplification was observed in the remaining 86 entries. The marker iag95 confirmed the postulation of Sr31/Yr9/Lr26 in three entries, while the marker Ventriup + LN2 validated the presence of Sr38/Yr17/Lr37 in 32 entries. One genotype was confirmed to carry Yr4 when genotyped with linked SSR marker barc75. Based on the marker csLV34, 22 entries were observed to carry Yr18/Lr34/Sr57 (Table 3.1). Of the 22 entries confirmed to carry Yr18, three entries (24:ZWW12, 53:ZWW12 and 132:ZWW12) were susceptible at the seedling stage.

3.4 Discussion

Strategic deployment of rust resistance genes depends on a better understanding of the genetic diversity among donor sources (Bariana et al. 2007a). The main objective of this study was to assess the genetic diversity for stem rust and stripe rust resistance in an international wheat screening nursery.

Multipathotype evaluations identified stem rust ASR genes Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31 and Sr38 and stripe rust resistance genes Yr3, Yr4,
Yr6, Yr9, Yr17, Yr27 and Yr34 either singly or in combinations. Unfortunately, most of these genes are not effective individually against a multitude of Pgt and Pst pathotypes worldwide (Singh et al. 2008). Postulation of the aforementioned stem rust and stripe rust seedling resistance genes is not surprising because these seem to be fixed in breeding populations due to their widespread use. For example; Singh et al. (2008) postulated eight stem rust resistance genes (Sr5, Sr8a, Sr9g, Sr12, Sr30, Sr31, Sr36 and Sr38) and seven stripe rust resistance genes (Yr1, Yr6, Yr7, Yr9, Yr17, Yr27, YrHVII) either singly or in combinations in wheat cultivars from the United Kingdom. Admassu et al. (2012) reported 11 stem rust resistance genes (Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30 and Sr37) either singly or in combinations in durum and bread wheat cultivars and breeding lines from Ethiopia. Spanic et al. (2015) reported four stem rust resistance genes (Sr8a, Sr31, Sr36, Sr38) in Croatian wheat cultivars. Kolmer et al. (2007) when comparing the frequency of stem rust resistance genes in United States winter wheat and spring wheats found that resistance genes Sr2, Sr6, Sr17, Sr24, Sr31, Sr36 and SrTmp are common in winter wheats, while genes Sr6, Sr9b, Sr11 and Sr17 are more frequent in spring wheats. This study found Sr8a, Sr12, Sr17, Sr30 and Sr38 to be more common in CIMMYT spring wheat. Postulation of common genes in different studies is attributed to the use of CIMMYT germplasm directly or as parents in many countries (Ortiz et al. 2007; 2008; Pretorius et al. 2015). It is estimated that about 70-80% of spring wheat cultivars released in the developing world are CIMMYT lines or lines derived from CIMMYT parents (Wang et al. 2003; Ortiz et al. 2007)

Sr30 was the most frequent stem rust seedling resistance gene. Although a Pgt pathotype virulent on Sr30 was reported in Eastern Australia by Park and Wellings
(1992), it is still effective against commercially important Pgt pathotypes in Australia (Bariana et al. 2007b). Sr30 virulence was also reported in many other countries including Spain, Ethiopia, Turkey, Pakistan and South American countries (Huerta-Espino 1992). Sr30 is still a common gene among CIMMYT and Australian germplasm (McIntosh et al. 1995, Bariana et al. 2007b). Other stem rust resistance genes detected in high frequency were Sr38 (34%), Sr8a (33%), Sr17 (33%) and Sr12 (13%). Virulence for Sr38 was first detected in Western Australia in 2001 (Park 2008); however, this gene is still being used in breeding programs because of its linkage with cereal cyst nematode gene Cre5 (Jahier et al. 2001). Resistance genes Sr31, Sr24 and Sr23 were detected at a very low frequency in this nursery. The virulence in Ug99 and its variants on these genes has possibly been responsible for this trend (Singh et al. 2015).

The most predominant seedling stripe rust resistance genes detected were Yr17 (34%), Yr3 (16%) and Yr27 (12%). Pathan (2003) reported presence of Yr17/Lr37/Sr38 (VPM) cluster in many European wheats. VPM segment has been widely deployed in commercial cultivars in many parts of the world, including Australia (Park 2008). The popularity of this useful translocation has declined due to reported virulence for all the three rust resistance genes (Singh et al. 2008). Virulence for Yr17 was first detected in eastern Australia in 1999 and was thought to have originated from an existing pathotype via mutation (Wellings 2007) and by 2006 a pathotype with combined virulence for Yr17, Yr6, Yr7 and YrA was identified (Wellings 2007).

The second highly frequent stripe rust resistance gene in this study was Yr3 (16%). Yr3 was also postulated in CIMMYT wheat germplasm by Dubin et al. (1989).
Yr3 was not an important gene for Australia until the detection of WA pathotype in 2002, which carried virulence for Yr6, Yr7, Yr8, Yr9, and YrA and avirulence for Yr3 and Yr4 (Wellings et al. 2003; Wellings and Kandel 2004). The effectiveness of the 1BL.1RS (Lr26/Yr9/Sr31) translocation in protecting wheat against stem rust for over 30 years before the detection of Ug99 in 1999 (Pretorius et al. 2000), led to high frequency of these three genes in most wheats globally (Singh et al. 2007). High proportions of Yr9 have been reported in Chinese wheat cultivars (Zeng et al. 2014). Pathan et al. (2008) also reported a high frequency of Yr9 in European wheats. Singh et al. (2014) postulated the stripe rust resistance gene Yr9 (1BL.1RS rye-derived) in 58% of the 12th HTWYT, 17% of the 22nd SAWSN and 2% entries of the 1st ASN. In the contrary, the Yr9-rye translocation was detected in only 3% of the entries in the nursery screened in the present study. The declining frequency of Yr9 in CIMMYT germplasm could be due to the reported virulence for Sr31 and Yr9 located on the IBL-1RS translocation (Pretorius et al. 2000; Wellings et al. 2003). Seedling stripe rust resistance gene Yr27 was present in a number of CIMMYT wheats including Ciano 79, Nacozari 76, Crow, Tesia 79, Opata 85, Bacanora 88, Bakhtawar, WH542, Atrak, Memof, PBW343, MH97, Chamaran, Kousa, and Shirudi (Wellings 1992). However, the outbreak of Yr27 virulent pathotype in 2010-2013 caused significant yield losses in Afghanistan, Azerbaijan, Ethiopia, Iran, Iraq, Kenya, Morocco, Syria, Turkey, Uzbekistan (Singh et al. 2012; FAO 2014). The ineffectiveness of this widely deployed resistance gene posed a serious threat to food security and livelihoods of resource-poor farmers and their communities.

Yr34 was mapped on chromosome 5AL of wheat genotype WAWHT2046 (Bariana et al. 2006). It is effective against 134 E16A+ and its variants. It was only
present in 4% of the entries. Seven entries carried resistance that could not be postulated by the array of Pst pathotypes used. These entries either carry a new gene that is effective against all pathotypes used in this study or combinations of genes. Twenty seven percent of entries did not carry any seedling resistance genes for stripe rust, but exhibited resistant to moderately resistant type of responses in the field during the 2012 and 2013 crop seasons indicating the presence of APR genes. The information presented in this study is useful for wheat breeders to devise strategies for achieving durable rust control.
CHAPTER 4

Molecular mapping of adult plant stripe rust resistance in Australian wheat cultivar Sentinel

4.1 Introduction

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is one of the most devastating diseases of wheat globally (Wellings 2011; Rosewarne et al. 2013). It infects leaf tissue and significantly reduces grain yield and quality in susceptible cultivars (Chen 2005). Yield losses are severe if infection occurs at early stages of growth (Bariana et al. 2010). Stripe rust was first detected in eastern Australian in 1979 and was believed to be a foreign incursion from Europe due to its similarity in pathogenicity with races prevalent in Europe at that time (O’Brien et al. 1980). Wheat growing areas of Western Australia remained free from stripe rust for more than two decades and a new exotic pathotype 134 E16A+ with virulence for *Yr6, Yr7, Yr8, Yr9* and *YrA* and avirulence for *Yr3* and *Yr4* was detected in 2002 (Wellings 2003). It spread to all wheat growing regions in Australia in 2003 and caused epidemics in Eastern Australia (Wellings 2007). The pathotype 134 E16A+ acquired virulence for stripe rust resistance genes *Yr10, Yr17, Yr24, Yr27, YrJ* and *YrT* through stepwise mutations (Wellings 2007; Bariana et al. 2007a; Bansal et al. 2014).

The new pathotypes of Pst have also caused significant wheat yield losses in other parts of the world in recent years. In 2009-10, the outbreak of a new pathotype of Pst with virulence for *Yr27* caused significant yield losses in Azerbaijan, Ethiopia, Iran, Iraq, Kenya, Morocco, Syria, Turkey and Uzbekistan threatening the food security and
livelihood of resource-poor farmers (FAO 2014). There has been significant use of chemicals to control stripe rust epidemics (Murray and Brennan 2009). Genetic resistance however is the most economical and environmentally safe means of stripe rust control and hence deployment of combinations of stripe rust resistance genes in new wheat cultivars is the best strategy to mitigate potential yield losses (Line and Chen 1995; Bariana et al. 2007a, 2010; Chen 2007).

Resistance to stripe rust is categorized into two groups; all stage resistance (ASR) conditioned by major genes and adult plant resistance (APR) conditioned by minor genes (Bariana 2003; Chen 2005). Seventy six stripe rust resistance genes have been formally named and there are 40 temporarily designated genes or QTL (Chen 2005; McIntosh et al. 1995; 2013; 2014; Ren et al. 2012). Seventeen of the 76 named resistance genes (\textit{Yr11}, \textit{Yr12}, \textit{Yr13}, \textit{Yr14}, \textit{Yr16}, \textit{Yr18}, \textit{Yr29}, \textit{Yr30}, \textit{Yr36}, \textit{Yr39}, \textit{Yr46}, \textit{Yr48}, \textit{Yr52}, \textit{Yr58}, \textit{Yr68}, \textit{Yr71} and \textit{Yr75}) confer APR or high temperature adult plant (HTAP) resistance, whereas the rest confer ASR (Chen et al. 2014; Herrera-Foessel et al. 2011b; Lowe et al. 2011; McIntosh et al. 2013, 2014; Ren et al. 2012; Singh 1992; Singh et al. 2001; Uauy et al. 2005; William et al. 2003). The ease of transfer of ASR genes into commercial cultivars made them a popular choice among wheat breeders; however, new pathotypes often overcome this type of resistance in due course. Wheat breeders have now shifted focus to breed cultivars with APR genes. To achieve acceptable levels of APR, combinations of two or more genes is essential (Bariana and McIntosh 1995; Singh et al. 2000). This highlights the need to identify, characterise and map more APR genes.
Cytogenetic techniques were successfully employed to determine genomic locations of many ASR genes for stripe rust in wheat (Bariana et al. 2013); however, their role in determining chromosomal locations of APR genes have been limited due to poor expression of APR genes in the heterozygous state. Modern strategies based on the construction of genetic linkage maps using molecular markers have been more successful in detecting genomic locations of APR genes. Quantitative trait loci (QTL) mapping is used to identify chromosome regions that control APR (Bansal et al. 2014). Recent advances in high throughput (next-generation) sequencing platforms such as genotyping by sequencing (GBS) has led to affordable options for whole genome sequencing using large number of markers (Elshire et al. 2011; Yan et al. 2009).


Australian common wheat cultivar Sentinel has remained resistant to stripe rust under field conditions since its release in 2005 and the genetic basis of resistance was unknown. This study focused on molecular mapping of APR to stripe rust in Sentinel.
4.2 Materials and methods

4.2.1 Plant Materials

Sentinel was crossed with a stripe rust susceptible genotype Nyabing 3 (Nyb3) and a set of 117 recombinant inbred lines (RILs) were developed. Susceptible cultivars Morocco and Nyb3 were included as spreaders during field testing.

4.2.2 Pathogen material

Pst pathotype 134 E16A+Yr17+Yr27+ was used for creating stripe rust epidemics in the field and for greenhouse studies.

4.2.3 Stripe rust assessments

4.2.3.1 Field tests

The Sentinel/Nyb3 F6 RIL population (117) was grown in the field as 10 seed hill plots at the Plant Breeding Institute (PBI), Cobbitty in 2014 and 2015 crop seasons. The parents Sentinel and Nyb3 were included as controls. Each block of seventy hill plots was surrounded by a mixture of susceptible spreader (Morocco and Nyb3) to create stripe rust epidemic. The RIL population was evaluated at one experimental site (Lansdowne = LDN) during the 2014 crop season and at two sites (Karalee = K and LDN) in the 2015 crop season. The 2014 experiment was sown in the first week of July, whereas the 2015 experiments were planted in the first week of June at the K site and second week of June at the LDN site. The experimental area was irrigated to create favourable conditions for stripe rust development. The experiments were artificially
inoculated using Pst pathotype 134 E16A+Yr17+Yr27 suspended in light mineral oil (Isopar L®) and misted over the whole experiment using an ultra-low volume Micron sprayer. Further inoculation was done by brushing rusty potted seedlings on RILs and dropping them between susceptible spreader rows. Adult plant stripe rust response assessments were performed using a 1-9 scale (1 = very resistant, and 9 = very susceptible) described in Bariana et al. (2007b). The 1-9 scale based scores were converted into percent severities for comparison of genotypes carrying different number of QTL.

4.2.3.2 Greenhouse tests

The Sentinel/Nyb3 F6 RIL population and both parents were tested at the 2-leaf, 3-leaf and 4-leaf stages with pathotype 134 E16A+Yr17+Yr27+ at two temperatures regimes (18±2°C and 21±2°C) to observe expression of resistance under the greenhouse conditions. Stripe rust assessments were made using a 0-4 scale described in McIntosh et al. (1995).

4.2.4 Molecular mapping

4.2.4.1 DNA extraction

Leaf tissue of about 2.5 cm was picked from eight seedlings from each of the F6 RILs and put in well-labelled 2 ml eppendorf tubes and dried on silica gel for 3 days. DNA was isolated using CTAB method as described in Bansal et al. (2014). DNA samples were quantified using a Nanodrop ND-1000 spectrophotometer and 30 ng/µl genomic DNA dilutions were made.
4.2.4.2 Detection of stripe rust APR genes *Yr18* and *Yr29*

*Yr18*-linked marker *csLV34* (Lagudah et al. 2006) and *Yr18* gene-specific markers *cssfr1*, *cssfr2*, *cssfr3*, *cssfr4*, *cssfr5* and *cssfr6* (Lagudah et al. 2009) were used to detect the presence of *Yr18* in parents Sentinel and Nyb3 and *Lr34*-carrying cultivar Janz was included as positive control. PCR amplifications were performed in 10 µl reaction volumes containing 30 ng of genomic DNA, 0.2 mM dNTPs, 1x PCR buffer containing MgCl₂ (Bioline), 0.5 µM of each forward primer and reverse primer and 0.02 U Immolase Taq DNA polymerase (Bioline). PCR products were separated in 2% agarose gel.

*Yr29*-linked SNP marker *Lr46_SNPIG22* was employed to detect the presence of these genes in the parents. Lalbahadur+*Lr46* and Lalbahadur were included as positive and negative controls, respectively. PCR reactions were performed in 8 µl reaction volume containing 3 µl of 30 ng/µl genomic DNA, 4 µl of 2x KASP mix (KBioscience), 0.11 µl assay mix (containing 12 µM each allele-specific forward primer and 30 µM of reverse primer) and 0.89 µl of autoclaved double distilled water. PCR conditions used included 94°C for 15 minutes, 10 touchdown cycles of 94°C for 20 seconds, 65-57°C for 60 seconds (dropping 0.8°C per cycle); and 26-35 cycles of 94°C for 20 seconds and 57°C for 60 seconds. Reading was taken by fluorescence detection of the reactions at 40°C for 30 seconds, and the data were analysed using CFX Manager 3.1 software (Biorad).
4.2.4.3 Linkage map construction and QTL analysis

DNA of 92 Sentinel/Nyb3 RILs and the two parents were sent to Diversity Arrays Pty. Ltd., Canberra, Australia for DArTseq genotyping. A total of 16,815 DArTseq markers were used to genotype Sentinel/Nyb3 RIL population and scored ‘1’ for presence and ‘0’ for absence of the target marker. Chi-squared ($\chi^2$) analysis was performed to test for segregation distortion of markers from the expected ratio of 1:1 and markers with Chi-squared values greater than 4 were excluded from the linkage map. Monomorphic markers, redundant markers and markers with more than 10% missing data were also excluded from the linkage map. Sentinel/Nyb3 RILs linkage groupings were created using ‘MapDisto’ software version 1.7.5 for MS windows 2007 and linkage groups were selected based on a LOD score of 3. MapManager QTXb20 (Manly et al. 2001) was used for construction of the linkage map. Composite interval mapping (CIM) was performed using QTL Cartographer. Analysis was carried out with 1,000 permutations and 2 cM step to detect stripe rust resistance QTL (Bansal et al. 2014).

4.2.5 Statistical Analysis

The minimum number of genes segregating for stripe rust resistance in the field experiments was estimated using the Wright’s method (Wright 1968) adjusted for the level of inbreeding in the original formula (Cockerham 1983). The equation used is as below:

$$n = (GR)^2 / 4.27 \times \sigma_g^2$$

Where $n =$ minimum number of effective genes, GR = genotypic range and $\sigma_g^2 =$ genetic variance of RILs.
Chi Squared (χ²) analysis was performed to determine the goodness of fit of the observed segregation ratios at 4-leaf stage and to check segregation distortion of markers. Critical differences (CD) were calculated using the formula: CD=Standard error x t value at P=0.05.

4.3 Results

4.3.1 Stripe rust response assessments

4.3.1.1 Field

The resistant parent Sentinel produced adult plant stripe rust response ‘2’, whereas the susceptible parent Nyb3 was scored ‘9’ in the field, when tested with Pst pathotype 134 E16A+Yr17+Yr27 (Fig. 4.1a). Adult plant stripe rust responses of Sentinel/Nyb3 RILs varied from ‘2’ to ‘9’ in all experiments and showed normal distribution with both parents falling at the tail ends of the curve (Fig. 4.2). Stripe rust response data of Sentinel/Nyb3 RIL population was subjected to estimation of number of resistance loci involved using the modified Wright’s method. The involvement of two to three loci in controlling stripe rust response variation among Sentinel/Nyb3 RILs was estimated.
**Fig. 4.1** Stripe rust responses on P1-Sentinel and P2-Nyb3 in (a) field and (b) greenhouse.

**Fig. 4.2** Frequency distribution of Sentinel/Nyb3 RILs with respect to adult plant stripe rust response variation (LDN – Lansdowne, K – Karalee).
4.3.1.2 Stripe rust response in the greenhouse

Sentinel expressed high level of resistance in the field and in order to discount the possibility of involvement of any seedling resistance, Sentinel and Nyb3 were tested at the 2-leaf stage under greenhouse conditions. Both cultivars produced susceptible responses [infection type (IT) 3+]. These results demonstrated the absence of seedling resistance in both genotypes. Both parents were then tested at the 2-leaf, 3-leaf and 4-leaf stages and post-inoculation incubations were performed at two temperature regimes (17±2°C and 21±2°C). Both Sentinel and Nyb3 produced IT3+ at the post-inoculation temperature 17±2°C and at all growth stages. In the 21±2°C post-inoculation experiment, Nyb3 produced susceptible IT3+ at all growth stages. In contrast, Sentinel exhibited susceptible responses (IT3+) at 2-leaf and 3-leaf growth stages and it displayed IT23c at the 4-leaf stage (Fig 4.1b). The entire RIL population was tested at the 4-leaf stage with 21±2°C as the post-inoculation temperature and it showed monogenic segregation [58 homozygous resistant (HR; ‘IT23c’):59 homozygous susceptible (HS; ‘IT3+’), $\chi^2_{1:1} = 0.01$, non-significant at $P = 0.05$ and 1 d.f.] (Table 4.1). The resistance locus was tentatively named YrSen.

**Table 4.1** Frequency distribution of Sentinel/Nyb RIL population when tested against Pst pathotype 134E16A+Yr17+Yr27 at the 4th leaf stage

<table>
<thead>
<tr>
<th>Category</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2_{1:1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous Resistant (HR)</td>
<td>58</td>
<td>58.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Homozygous Susceptible (HS)</td>
<td>59</td>
<td>58.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>117</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Table value of $\chi^2$ at $P=0.05$ and 1 d.f. = 3.84
4.3.2 Detection of APR genes *Yr18* and *Yr29*

APR genes *Yr18* and *Yr29* are widespread in wheat germplasm. In order to check whether Sentinel carries any of these genes, linked markers were used. The *Yr18*-linked STS marker *csLV34* amplified a 150bp PCR product in the positive control Janz and a 229bp product in Sentinel and Nyb3. Six *Yr18* gene-specific markers (*cssfr1*-*cssfr6*) were also amplified using Sentinel and Nyb3 DNA to further confirm the absence of *Yr18* in Sentinel. The dominant marker *cssfr1* resulted in amplification of a 517bp amplicon in Janz and no amplicon in Sentinel and Nyb3, while *cssfr2* amplified 523bp amplicon in Sentinel and Nyb3 and no amplicon was recorded in Janz. The co-dominant marker *cssfr3* produced two bands (150bp/517bp) in Janz and a single band (229bp) in Sentinel and Nyb3. Another such marker *cssfr4* amplified 150bp PCR product in Janz and two amplicons (229bp/523 bp) in Sentinel and Nyb3. The third co-dominant marker *cssfr5* resulted in amplification of 751bp product in Janz and a 523 bp amplicon in both Sentinel and Nyb3. CAPS marker *cssfr6* produced three amplicons (63bp/135bp/451bp) in positive control Janz and two (63bp/589bp) in Sentinel and Nyb3, following digestion with the restriction enzyme *Fnu4HI*. These results demonstrated the absence of *Yr18* in both Sentinel and Nyb3. The *Yr29*-linked SNP marker *Lr46_SNPIG22* amplified allele ‘A’ in Sentinel and the positive control Lalbaughadur+*Lr46* and the alternate allele ‘G’ in Nyb3 and Lalbahadur. The entire RIL population was tested with *Lr46_SNPIG22* and monogenic segregation (68 ‘A’: 49 ‘G’; \( \chi^2_{1:1} = 3.09 \), non-significant at \( P = 0.05 \) and 1 d.f.) was noted.
4.3.3 Linkage map construction

The RIL population was subjected to whole genome profiling with 16,815 DArTseq markers. A total of 4,891 DArTseq markers exhibited segregation distortion, 1,502 had more than 10% missing data and 2,079 were monomorphous between parental genotypes and therefore were not included in the linkage map. Of the remaining 8,343 markers, chromosome locations were known only for 3,605, whereas 4,738 markers were not assigned to any chromosomes and were labeled UK (unknown). Redundant markers were filtered out from the 8,343 polymorphic markers and only 3,369 markers were suitable for linkage map construction.

A set of 3,364 polymorphic DArTseq markers were mapped to 35 discrete linkage groups and 5 markers remained unlinked. The linkage map covered 7,141.1 cM with an average marker density of one marker per 2.12 cM. Marker density was highest for the B genome and covered 2673.2 cM. The A genome map included 2,790.3 cM and the coverage of D genome was low with 1,677.6 cM. There was an average marker density of one marker per 1.92 cM and 2.02 cM in A and B genomes respectively and one marker per 2.83 cM in the D genome. YrSen was also included in the linkage map.

4.3.4 QTL analysis

Composite interval mapping (CIM) was used to scan the Sentinel/Nyb3 RIL population genome to detect quantitative trait loci (QTL) associated with stripe rust resistance. CIM analyses detected three consistent QTL on chromosomes 1B, 2A and 3B. All QTL were contributed by Sentinel (see graphs below QTL g) and were temporarily designated *QYr.sun-1BL*, *QYr.sun-2AS* and *QYr.sun-3BS*. QTL *QYr.sun-1BL*, *QYr.sun-2AS* and
QYr.sun-3BS explained on an average 18%, 15.3% and 10.6% of phenotypic variation in adult plant stripe rust response among the Sentinel/Nyb3 RIL population, respectively (Table 4.2 and Fig. 4.3).

QYr.sun-1BL explained 13-26% of variation in stripe rust response and peaked at the DArTseq marker 4406454 and it was flanked by markers 1317149 and 1107928. Marker 4539050 mapped closest to QYr.sun-2AS and contributed 11-22% towards phenotypic variation. Markers 1095379 and 1104226 defined the QTL interval. QTL QYr.sun-3BS corresponded to YrSen and markers 4409093 and 1012045 flanked it (Table 4.2 and Fig. 4.3). This QTL explained 6 to 13% phenotypic variation in stripe rust response in Sentinel/Nyb3 RIL population.

**Table 4.2** Stripe rust resistance QTL detected in Sentinel/Nyb3 RIL population

<table>
<thead>
<tr>
<th>QTL</th>
<th>Season/Site</th>
<th>Peak marker</th>
<th>Flanking markers</th>
<th>LOD</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>QYr.sun-1BL</td>
<td>2014 LDN</td>
<td>4406454</td>
<td>1317149-1107928</td>
<td>10.45</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>4406454</td>
<td>1317149-1107928</td>
<td>6.69</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>4406454</td>
<td>1317149-1107928</td>
<td>6.18</td>
<td>15</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>QYr.sun-2AS</td>
<td>2014 LDN</td>
<td>4539050</td>
<td>1095379-1104226</td>
<td>4.54</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>4539050</td>
<td>1095379-1104226</td>
<td>9.54</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>4539050</td>
<td>1095379-1104226</td>
<td>4.54</td>
<td>13</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>QYr.sun-3BS</td>
<td>2014 LDN</td>
<td>YrSen</td>
<td>4409093-1012045</td>
<td>2.73</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>YrSen</td>
<td>4409093-1012045</td>
<td>6.17</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>YrSen</td>
<td>4409093-1012045</td>
<td>4.47</td>
<td>12.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>10.6</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.3 Stripe rust resistance QTL detected on chromosomes (a) 1BL, (b) 2AS and (c) 3BS of Sentinel/Nyb3 RIL population.

The bottom contours showed contribution by Sentinel.
4.3.5 Contribution of Sentinel alleles

To assess the individual contribution of each QTL towards adult plant stripe rust severity reduction, rust response data collected on a 1-9 scale were converted to percent rust severity (0-100) as described in Bariana et al. (2007b). Comparisons of mean stripe rust responses of RILs carrying alternative alleles at the peak marker locus were made to confirm the phenotypic contributions of each QTL (Table 4.3). Detected QTL were considered real, when the mean rust response of RILs carrying the positive allele was less than the mean of those possessing the alternate allele. Contributions of peak markers 4406454 (QYr.sun-1BL), 4539050 (QYr.sun-2AS) and YrSen (QYr.sun-3BS) are listed in Table 4.3. QYr.sun-1BL, QYr.sun-2AS and QYr.sun-3BS reduced stripe rust severities by 23-42%, 27-33% and 25-50%, respectively.

Table 4.3 Comparison of mean stripe rust severities of genotypes carrying Sentinel and Nyb3 alleles for each QTL

<table>
<thead>
<tr>
<th>QTL</th>
<th>Experiment</th>
<th>Chromosome</th>
<th>Marker</th>
<th>Sentinel allele</th>
<th>Nyb3 allele</th>
<th>Severity reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QYr.sun-1BL</td>
<td>2014 LDN</td>
<td>1BL</td>
<td>4406454</td>
<td>34.6</td>
<td>77.2</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>1BL</td>
<td>4406454</td>
<td>56.1</td>
<td>79.4</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>1BL</td>
<td>4406454</td>
<td>45.0</td>
<td>75.3</td>
<td>30.3</td>
</tr>
<tr>
<td>QYr.sun-2AS</td>
<td>2014 LDN</td>
<td>2AS</td>
<td>4539050</td>
<td>44.1</td>
<td>77.2</td>
<td>33.1</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>2AS</td>
<td>4539050</td>
<td>48.0</td>
<td>79.4</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>2AS</td>
<td>4539050</td>
<td>47.9</td>
<td>75.3</td>
<td>27.4</td>
</tr>
<tr>
<td>QYr.sun-3BS</td>
<td>2014 LDN</td>
<td>3BS</td>
<td>YrSen</td>
<td>26.7</td>
<td>77.2</td>
<td>50.6</td>
</tr>
<tr>
<td></td>
<td>2015 K</td>
<td>3BS</td>
<td>YrSen</td>
<td>49.2</td>
<td>79.4</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>2015 LDN</td>
<td>3BS</td>
<td>YrSen</td>
<td>50.0</td>
<td>75.3</td>
<td>25.3</td>
</tr>
</tbody>
</table>
4.3.6 Additive effects of QTL combinations

Comparison of mean percent stripe rust severities of individual QTL and different QTL combinations across sites and years are presented in Table 4.4 and Fig. 4.4. Critical difference (CD) was calculated for each experiment to see whether the stripe rust severities displayed in RILs with different QTL combinations were significantly different. RILs carrying single QTL were observed to have higher disease severities compared to RILs with combinations of two or three QTL. In 2014 crop season at the LDN site, RILs carrying $QYr\text{-}sun\text{-}3BS$ exhibited significantly lower stripe rust severity (27%) compared to $QYr\text{-}sun\text{-}2AS$ (44%). $QYr\text{-}sun\text{-}2AS$ and $QYr\text{-}sun\text{-}1BL$ did not differ significantly. Stripe rust severities of RILs with single QTL did not differ significantly in two other experiments. Of three dual QTL combinations, RILs carrying $QYr\text{-}sun\text{-}1BL+QYr\text{-}sun\text{-}2AS$ produced significantly lower stripe rust severity (11.7%) in 2014 than that exhibited by $QYr\text{-}sun\text{-}2AS+QYr\text{-}sun\text{-}3BS$ possessing RILs (28.5%). RILs with the third dual combination $QYr\text{-}sun\text{-}1BL+QYr\text{-}sun\text{-}3BS$ showed stripe rust severity significantly lower than those with $QYr\text{-}sun\text{-}2AS+QYr\text{-}sun\text{-}3BS$. In the remaining two experiments severities exhibited by three different dual combinations of QTL did not differ significantly. Mean rust severity of RILs carrying the Sentinel alleles for all three QTL produced significantly lower stripe rust severities than single and dual QTL carrying RILs (Table 4.4, Fig 4.4).
Table 4.4 Mean stripe rust severities of Sentinel/Nyb3 RILs carrying different QTL combinations

<table>
<thead>
<tr>
<th>Loci</th>
<th>Rust Severity (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014</td>
<td>2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDN</td>
<td>K</td>
<td>LDN</td>
</tr>
<tr>
<td>QYr.sun-1BL</td>
<td>34.58</td>
<td>56.07</td>
<td>45.00</td>
</tr>
<tr>
<td>QYr.sun-2AS</td>
<td>44.11</td>
<td>48.04</td>
<td>47.91</td>
</tr>
<tr>
<td>QYr.sun-3BS</td>
<td>26.67</td>
<td>49.17</td>
<td>50.00</td>
</tr>
<tr>
<td>QYr.sun-1BL + QYr.sun-2AS</td>
<td>11.67</td>
<td>34.50</td>
<td>28.75</td>
</tr>
<tr>
<td>QYr.sun-1BL + QYr.sun-3BS</td>
<td>17.06</td>
<td>34.38</td>
<td>30.74</td>
</tr>
<tr>
<td>QYr.sun-2AS + QYr.sun-3BS</td>
<td>28.50</td>
<td>28.75</td>
<td>26.25</td>
</tr>
<tr>
<td>QYr.sun-1BL + QYr.sun-2AS + QYr.sun-3BS</td>
<td>6.72</td>
<td>12.94</td>
<td>13.53</td>
</tr>
<tr>
<td>Critical Difference (CD)</td>
<td>9.68±4.98</td>
<td>10.77±5.54</td>
<td>9.88±5.08</td>
</tr>
</tbody>
</table>

Fig. 4.4 Adult plant stripe rust severity of Sentinel/Nyb3 RILs carrying different QTL combinations
4.4 Discussion

Common wheat cultivar Sentinel displayed high level of resistance to stripe rust since its release in 2005. The continuous distribution of stripe rust response variation among Sentinel/Nyb3 RIL population, when tested against the Pst pathotype 134E16A+Yr17+Yr27, suggested quantitative inheritance of resistance. The involvement of two to three genes in conditioning APR to stripe rust in Sentinel was estimated. This estimation was confirmed with the detection of three consistent QTL, one each on chromosomes 1B, 2A and 3B of Sentinel.

The $Q_{Yr.sun-1BL}$ peaked at the marker 4406454 and mapped in the same genomic region as the APR gene $Yr29$. Presence of $Yr29$ in Sentinel was confirmed using a closely linked SNP marker $Lr46\_SNP1G22$. The total length of chromosome 1B is 259 cM and the location of marker 4406454 on the Sentinel/Nyb3 map is 129 cM. Based on this location $Q_{Yr.sun-1BL}$ appears to map on the long arm of chromosome 1B. The location of APR gene $Yr29$ on the long arm of chromosome 1B has been reported in a number of studies (Bansal et al. 2014; Bariana et al. 2001, 2010; Herrera-Foessel et al. 2011b; Jagger et al. 2011; Lillemo et al. 2008; Melichar et al. 2008; Rosewarne et al. 2008, 2012; William et al. 2006; Zwart et al. 2010). In the present study $Q_{Yr.sun-1BL}$ explained 13-26% stripe rust variation at LOD score range of 6.2-10.5. The wide range of LOD scores (2.8-23) and phenotypic variation explanations (4.5-65%) attributed to the $Yr29$ locus in several studies has been highlighted in a review by Rosewarne et al. (2013). The $Q_{Yr.sun-1BL}$ from this study was concluded to be $Yr29$. 
The second QTL, \textit{QYr.sun-2AS}, peaked at the marker 4539050 located on the short arm of chromosome 2A. Studies by Agenbag et al. (2012), Bansal et al. 2014, Bariana et al. (2010), Boukhatem et al. (2002), Chhuneja et al. (2008), Dedryver et al. (2009), Hao et al. (2011), Mallard et al. (2005), Singh et al. (2014) and Vazquez et al. (2012) reported QTL in chromosome 2A. Most of these QTL mapped in 2AL based on the location of associated markers, except markers associated with the stripe rust resistance QTL reported by Bansal et al. (2014) which mapped on chromosome 2AS in a durum wheat mapping population. The QTL reported by Bansal et al. (2014) was later shown to express resistance at the 4-leaf stage and was formally designated \textit{Yr56} (Bansal and Bariana unpublished results). \textit{QYr.sun-2AS} from this study does not express resistance at the 4-leaf stage and hence is likely to be different from \textit{Yr56}.

The third QTL, \textit{QYr.sun-3BS}, detected in this study peaked at the stripe rust resistance locus, \textit{YrSen}, which was characterised at the 4-leaf stage at 21±2°C and was flanked by DArTseq markers 4409093 and 1012045. Several studies reported a QTL on the short arm of chromosome 3B (Börner et al. 2000; Singh et al. 2000; Suenaga et al. 2003; William et al. 2006; Lillemo et al. 2008; Dedryver et al. 2009; Bariana et al. 2010; Hao et al. 2011; Lowe et al. 2011; Yang et al. 2013). The QTL reported in these studies corresponded to the APR gene \textit{Yr30} on chromosome 3BS and DArT marker \textit{wPt-6802} was the closet marker (William et al. 2006; Bariana et al. 2010). Marker \textit{wPt-6802} is located at position 72.9 cM and one of the markers (1012045) that flank \textit{YrSen} is mapped at 36.4 cM. These two markers are located 36.5 cM apart indicating that \textit{Yr30} and \textit{YrSen} are different.
The short arm of chromosome 3B also carries all stage stripe rust resistance gene \textit{Yr57}. This gene is located at the distal end of chromosome 3BS and is flanked by markers \textit{gwm389} at 2.0 cM proximally and \textit{BS00062676} at genetic distance of 2.3 cM distally (Randhawa et al. 2015). The post seedling expression of \textit{QYr.sun-3BS} differentiates it from \textit{Yr57}, which produces near immune responses at the 2-leaf stage. Stripe rust resistance gene \textit{Yr58} was also mapped on the distal end of chromosome 3BS (Chhetri 2015). \textit{Yr58} can be detected at the 4-leaf stage at 21±2°C. The detection of \textit{Yr58} and \textit{YrSen} under similar conditions in the greenhouse suggested that these genes may represent the same locus. \textit{Yr58 (QYr.sun-3BS)} however explained 34 to 59% variation in stripe rust response in W195/BT-Schomburgk RIL population and in contrast \textit{QYr.sun-3BS (YrSen)} contributed 6 to 12% to phenotypic variation. Based on these results and effectiveness of \textit{QYr.sun-3BS (YrSen)} against Pst pathotype 110 E143A+, \textit{YrSen} is likely to be different from \textit{Yr58}. APRs that express early in the crop growth cycle are expected to offer greater protection compared to those that express late at the flag leaf stage (Chen et al. 2014). The expression of \textit{QYr.sun-3BS} at the 4-leaf stage highlights the importance of this locus in early management of stripe rust.

The stripe rust resistance QTL detected in Sentinel/Nyb3 RILs exhibited lower stripe rust severity. RILs with combinations of two QTLs had intermediate stripe rust severities while RILs carrying single QTL had relatively higher disease severities. Several studies have reported such results (Bansal et al. 2014; Bariana and McIntosh 1995; Qamar 2010; Singh et al. 2013; Singh et al. 2000; Singh and Rajaram 1992; Venkata et al. 2006). The maintenance of high level of adult plant stripe rust resistance
by Sentinel since 2005 is indicative of durability of $Q{Yr}.sun-1BL$, $Q{Yr}.sun-2AS$ and $Q{Yr}.sun-3BS$. 
CHAPTER 5

Genetic relationship between wheat stem rust resistance genes Sr36 and Sr39

5.1 Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is among the most devastating biotic constraints to the global wheat production. Stem rust epidemics caused severe yield losses in Asia (Joshi and Palmer 1973; Nagarajan and Joshi 1975), Australia (Rees 1972; Watson 1981; Park 2007), USA (Stakman and Harrar 1957; Leonard 2001; Leonard and Szabo 2005; Lopez-Vera et al. 2014) and Europe (Zadoks 1963, 2008). It was however been successfully controlled in the past 50 years through deployment of host resistance (Singh et al. 2008; Lopez-Vera et al. 2014). Breeding for stem rust resistance was estimated to save the Australian wheat industry A$124 million annually (Brennan and Murray 1988). Emergence of a highly virulent stem rust race Ug99 in Uganda in 1998 (Pretorius et al. 2000) and its migration to other countries presented a major threat to global wheat production. The race Ug99 designated as TTKSK (Wanyera et al. 2006) is virulent on most of the widely deployed resistance genes and rendered 90% of global wheat cultivars susceptible (Singh et al. 2011a; Olivera et al. 2012). Control of stem rust has often been achieved through use of race-specific resistance genes that result in hypersensitive response to inhibit growth of the invading pathogen. Sustainability of this approach is dependent upon availability of diverse sources of resistance for pyramiding in adapted wheat cultivars (Bariana et al. 2001).
Wild relatives of wheat are important sources of rust resistance and more than 20 stem rust resistance genes have been transferred into common wheat (Jin et al. 2007; Lui et al. 2011; McIntosh et al. 2013; Qi et al. 2011; Singh et al. 2006). Most of these genes have not been widely deployed due to the negative traits associated with the large alien translocations on which these genes are located (Dundas et al. 2007; Jin et al. 2014).

Stem rust resistance gene Sr39 was originally transferred together-with leaf rust resistance gene Lr35 from Ae. speltoides onto chromosome 2B of wheat cultivar Marquis (Kerber and Dyck 1990). C-banding studies showed that the translocated segment spans over both arms and the centromere is probably derived from Ae. speltoides (Friebe et al. 1996). In an attempt to facilitate deployment of Sr39 in commercial wheat cultivars, the alien translocation segment was shortened and linked molecular markers were developed to enable pyramiding of this gene with other genes in modern cultivars (Niu et al. 2011). The Australian prime hard wheat cultivar Cook carries stem rust resistance gene Sr36 on a large Triticum timopheevi translocation on chromosome 2BS (Bariana et al. 2001). Despite the large translocation, Sr36 has been deployed in commercial wheat cultivars globally (Bariana et al. 2001; Jin et al. 2009; Sai Prasad et al. 2014). Sr39 is effective against all Australian Pgt pathotypes and all variants of Ug99, whereas Sr36 is ineffective against a variant of Ug99 and some Australian Pgt pathotypes. It is however effective against currently predominating Pgt pathotypes in Australia (RF Park personal communication). This investigation was planned to determine the genetic relationship between Sr36 and Sr39 (both in the original translocation and in the shortened segment) with the aim of combining the two.
genes in a single genotype and secondly to validate \( Sr39 \)-linked molecular markers designed for the shortened \( Ae. \) speltoides segment for marker assisted selection.

5.2 Materials and Methods

5.2.1 Plant material

Wheat genotypes RL6082 and RWG1, carrying \( Sr39 \) on a large translocation and small \( Ae. \) speltoides segments, respectively, were crossed with Cook. One hundred sixty nine and 91 F\(_3\) lines were generated from the RWG1/Cook and RL6082/Cook crosses, respectively. Chinese spring (CS) and nine \( Sr36 \)-carrying Australian cultivars (Baxter, Cook, Lang, Sunbri, Sunco, Sunvale, Wylie, Yarralinka, Young) and twenty two \( Sr36 \)-lacking cultivars (Calingiri, Carnamah, Derrimut, DiamondBird, Espada, EGA Bellaroi, EGA BonnieRock, EGA Gregory, EmuRock, Forest, Giles, Gladius, Hyperno, Magenta, Merlin, Orion, Schomburgk, Scout, Spitfire, Tasman, Ventura, Wyalkatchem) were used for validation of \( Sr39 \)-linked marker \( rwgs28 \).

5.2.2 Pathogen material

Pgt pathotypes 98-1,2,(3),(5),6,7 (culture 580) and 34-1,2,3,4,5,6,7 (culture 103) were used to test F\(_3\) families. Pathotype 98-1,2,(3),(5),6,7 is avirulent on both \( Sr36 \) and \( Sr39 \) and was used to identify genotypes that carry either of the two genes since it produces different ITs. Whereas the Pathotype 34-1,2,3,4,5,6,7 virulent on \( Sr36 \) and avirulent on \( Sr39 \) was used to confirm lines carrying \( Sr36 \).
5.2.3 Stem rust screening

Twenty to 25 seeds of each line were sown in 9 cm pots filled with a mixture of pine bark and river sand in the 2:1 ratio and kept in rust free microclimate rooms. First dose of water soluble fertiliser Aquasol® at 10 grams : 10 litres of tap water for 100 pots was applied before sowing and a dose of nitrogenous fertiliser Urea was applied to seven-day old seedlings at the same rate. Ten to 12-day old seedlings were inoculated using Pgt urediniospores suspended in light mineral oil Isopar-L® by using a hydrocarbon pressure pack. Stem rust inoculated seedlings were incubated in water filled steel trays covered with plastic hoods under natural light at 18-20°C for 48hrs before transferring to microclimate rooms maintained at 25±2°C. Seedling stem rust assessments were made 14 days after inoculation using the 0-4 scale described in McIntosh et al. (1995).

5.2.4 Molecular marker analysis

5.2.4.1 DNA isolation and quantification

Genomic DNA was isolated from leaf tissue from a bulk of 10 seedlings per F₃ line including the parents RWG1 and Cook following a CTAB protocol described in Bansal et al. (2014a). Genomic DNA was quantified using a nanodrop ND-100 spectrophotometer and dilutions of 30 ng/µl were made.

5.2.4.2 PCR amplification and electrophoresis

PCRs for agarose based SSR markers Sr39#22r (Mago et al. 2009) and stm773-2 (Bariana et al. 2007a) were performed in 96 well plates with 10 µl reaction volumes containing 30 ng/µl of genomic DNA, 0.2 mM dNTPs, 1x Immolase PCR buffer
(Bioline) containing 1.5 mM MgCl₂, 0.5 µM each of forward and reverse primer and 0.04 U Immolase Taq DNA polymerase (Bioline). The PCR program for Sr39#22r consisted of initial denaturation at 95°C for 10 minutes, followed by 30 cycles of denaturation at 92°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 40 seconds and final extension of 72°C for 10 minutes and 12°C indefinitely. The PCR program for stm773-2 comprised of a touchdown program with an initial denaturation of 95°C for 10 minutes, followed 92°C for 30 s, 59°C (with 1°C drop down with every cycle) for 30 s and 72°C for 30 s for seven cycles and 35 cycles of denaturation at 92°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 72°C for 30 seconds and final extension at 72°C. The PCR products were separated on 2% agarose gel and stained with gel-red.

PCRs for polyacrylamide based STS markers rwgs27, rwgs28 and rwgs29, and the SSR markers gwm319 were each carried out in a total reaction volume of 10 µl containing 60 ng/µl genomic DNA, 1X Immolase PCR buffer containing 1.5 mM MgCl₂, 125 nM dNTPs, 50 nM forward primer with M13 tail, 100 nM reverse primer, 50 nM infrared 700 or 800-labelled M13 primer and 0.04 U Immolase DNA polymerase (Bioline). PCR amplification were carried out in a T100™ thermal cycler (BioRad USA) using a touchdown profile comprising of initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 92°C for 35 seconds, annealing 60°C for 40 seconds and final extension at 72°C for 50 seconds. Two µl of the PCR product was ran on 2% agarose gel electrophoresis to check for amplification. For each PCR product, 30 µl of loading dye (0.5% Fuschin dye+100% formamide liquid + 0.5 M EDTA pH 8.0)
was added and the total product denatured for 5 minutes at 95°C and quickly chilled on ice for 5 minutes. The denatured PCR products were separated on 8% Polyacrylamide gel using electrophoresis apparatus LICOR-4300 DNA analyser system (Li-COR Bioscience USA) as described in Bansal et al. (2014b).

5.2.5 Molecular cytogenetic analysis
The parental lines (Cook and RWG1) and the progeny of a heterozygote were characterized by genomic in situ hybridization (GISH) following the procedure of Zhang et al. (2001). Roots were collected separately from individual germinating seeds. Total genomic DNA from *Triticum timopheevii* (C06.77; Cytogenetic stock accession, Plant Breeding Institute, University of Sydney) was labelled with Biotin-16-dUTP (Roche Diagnostic Australia, Castle Hill, NSW, Australia) using nick translation. Unlabeled total genomic DNA of wheat was used as blocker. The probe to blocker ratio was ~1:80. Signals were detected with fluorescein-avidin DN (Vector Laboratories, Burlingame, CA). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen Life Science, Carlsbad, CA) and pseudocolored red. Slides were analyzed with a Zeiss Axio Imager epifluorescence microscope. Images were captured with a Retiga EXi CCD (charge-coupled device) camera (QImaging, Surrey, BC, Canada) operated with Image-Pro Plus version 7.0 software (Media Cybernetics Inc., Bethesda, MD) and processed with Photoshop version CS6 software (Adobe Systems, San Jose, CA).
5.2.6 Statistical analyses

Chi-squared ($\chi^2$) analyses were performed to determine goodness of fit of the observed segregation to the expected genetic ratios.

5.3 Results

5.3.1 Stem rust response tests

Parents Cook and RWG1 produced infection types (ITs) 0; and 2-, respectively, against Pgt pathotype 98-1,2,3,5,6,7 (Table 5.1; Fig. 5.1a). In contrast, Cook was susceptible (IT3+) and RWG1 was resistant (IT2=) against Pgt pathotype 34-1, 2,3,4,5,6,7 (Table 5.1; Fig. 5.1b). RL6082, carrying a large Sr39 translocation from Ae. speltoides, produced similar infection types to those expressed by RWG1 against the two Pgt pathotypes (Table 5.1). The susceptible cultivar Morocco displayed IT3+ against both Pgt pathotypes.
Fig. 5.1 Infection types expressed by (L-R) Cook, RWG1 and Morocco when tested with (a) Pgt pathotype 98-1,2,3,5,6,7. (b) Pgt pathotype 34-1,2,3,4,5,6,7

Table 5.1 Infections types produced by parental genotypes and a susceptible control when infected with Sr36 virulent and avirulent pathotypes

<table>
<thead>
<tr>
<th>Genotype/Pathotype</th>
<th>98-1,2,3,5,6,7</th>
<th>34-1,2,3,4,5,6,7</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL6082 (Original Sr39 translocation)</td>
<td>2-</td>
<td>2=</td>
</tr>
<tr>
<td>RWG1 (Shortened Sr39 translocation)</td>
<td>2-</td>
<td>2=</td>
</tr>
<tr>
<td>Cook (Sr36)</td>
<td>0;</td>
<td>3+</td>
</tr>
<tr>
<td>Morocco</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>
Stem rust response variation among F₃ families is summarised in Table 5.2. The RL6082/Cook and RWG1/Cook-derived populations, respectively, segregated into three classes; 25 (Sr39Sr39sr36sr36; IT2-) : 53 (Sr39sr39Sr36sr36; IT0; and IT3+) : 13 (sr39sr39Sr36Sr36; IT0;) and 23 (Sr39Sr39sr36sr36; IT2-) : 78 (Sr39sr39Sr36sr36; IT0; and IT2-) : 68 (sr39sr39Sr36Sr36; IT0, when tested with the Sr36 and Sr39 avirulent Pgt pathotype 98-1,2,(3),(5),6,7 (Table 5.2). No line was scored non-segregating susceptible. Chi-squared analysis of data presented in Table 5.2 showed highly significant deviations from segregation at two independent loci in both populations. These results indicated complete repulsion linkage between Sr39 and Sr36. The number of families carrying Sr36 in homozygous state was half the number of those homozygous for Sr39 in RL6082/Cook population. The trend was opposite in RWG1/Cook population. Both F₃ populations were tested with Sr36-virulent pathotype (34-1,2,3,4,5,6,7) and scored as non-segregating resistant (IT2=), segregating (IT2= and IT3+) and non-segregating susceptible (IT3+). All lines that showed IT;0 with the previous pathotype displayed IT3+, those which displayed IT 2= to 2- remained the same and all the lines that were previously segregating (IT0; and IT2-) produced IT3+ and IT2= to 2-, when tested against pathotype 34-1,2,3,4,5,6,7. Although stem rust response segregation in RL6082/Cook population was a close fit to monogenic inheritance for Sr39 (25 Sr39Sr39:53 Sr39sr39; χ² = 5.6, non-significant at P = 0.05 and 2 d.f.), the number of Sr39-carrying lines was 50% less than the expected. RWG1/Cook population deviated significantly from the 1:2:1 segregation ratio (23 Sr39Sr39:78 Sr39sr39:68 sr39sr39; χ² = 25, significant at P = 0.01 and 2 d.f). These results indicated over-transmission of Sr39 and Sr36, respectively, among RL6082/Cook and RWG1/Cook populations.
**Table 5.2** Distribution of F₃ families when tested with Pgt pathotype 98-1,2,3,5,6,7

<table>
<thead>
<tr>
<th></th>
<th>Sr36Sr36</th>
<th>Sr36sr36</th>
<th>sr36sr36</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL6082/Cookᵃ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr39Sr39</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sr39sr39</td>
<td>0</td>
<td>53</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>sr39sr39</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>53</td>
<td>25</td>
<td>91</td>
</tr>
<tr>
<td>RWG1/Cookᵇ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr39Sr39</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Sr39sr39</td>
<td>0</td>
<td>78</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>sr39sr39</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>78</td>
<td>23</td>
<td>169</td>
</tr>
</tbody>
</table>

ᵃ  $\chi^2$ Sr39 vs Sr36 = 172.5, significant at $P=0.01$ and 8 d.f.  $\chi^2$ Sr39 vs sr39 = 5.6 non-significant at $P=0.01$ and 2 d.f.  $\chi^2$, Sr36 vs sr36 = 5.6, non-significant at $P=0.01$ and 2 d.f.

ᵇ  $\chi^2$ Sr39 vs Sr36 = 461.3, significant at $P=0.01$ and 8 d.f.  $\chi^2$ Sr39 vs sr39 = 25, significant at $P=0.01$ and 2 d.f.  $\chi^2$, Sr36 vs sr36 = 25, significant at $P=0.01$ and 2 d.f.

5.3.2 Marker and cytological analysis

Sr39-linked STS markers *rwgs28* (Niu et al. 2011) amplified different sized amplicons in parents (Fig. 5.2). Marker *rwgs28* amplified 430bp/440bp/446bp products in Cook and 440bp/446bp/449bp in RWG1. It amplified the RWG1 allele in 23 lines that were *Sr39Sr39sr36sr36* genotypically and Cook allele in 67 *sr39sr39Sr36Sr36* genotypes, while 68 doubly heterozygous (*Sr39sr39Sr36sr36*) lines carried both alleles (Fig. 5.2 and Table 5.3). Some lines had DNA degradation issues and hence results are not presented. These results indicated cosegregation of *rwgs28* with the *Sr39*-carrying translocation. The *Sr39*-linked dominant marker *Sr39#22* did not amplify any product when DNA of Cook was used, but it amplified 840bp amplicon in RWG1. This marker resulted in amplification of the 840bp fragment in 95 lines and no amplification in 74 lines (Table 5.3). Similar to the phenotypic data, molecular markers most tightly linked
to Sr36 (Stm773.2) and Sr39 (rwgs28 and Sr39#22) also showed segregation distortion in this population (Table 5.2 and Table 5.3).

**Fig. 5.2** STS marker rwgs28 profiled on both parents and F3 lines from the RWG1/Cook cross. R = Sr39Sr39, S = sr39sr39, H = Sr39sr39 F3 lines, P1 = RWG1 (carrying Sr39 translocation), P2 = Cook (carrying Sr36 translocation), CS = Chinese Spring (lacking both Sr39 and Sr36). CS was included to determine the specificity of rwgs28 marker in detecting the shortened Sr39 Ae. speltoides segment (449bp and 446bp products were hard to separate, photoshop was used fine tune images)

**Table 5.3** Frequency distribution of RWG1/Cook F3 families with respect to genotypic status for closely linked markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cook allele</th>
<th>Heterozygotes</th>
<th>RWG1 allele</th>
<th>$\chi^2_{1:2:1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stm773.2</td>
<td>70</td>
<td>71</td>
<td>22</td>
<td>30.98**</td>
</tr>
<tr>
<td>Gwm319</td>
<td>89</td>
<td>54</td>
<td>23</td>
<td>72.75**</td>
</tr>
<tr>
<td>Rwgs28</td>
<td>67</td>
<td>68</td>
<td>23</td>
<td>27.57**</td>
</tr>
<tr>
<td>Sr39#22</td>
<td>74</td>
<td>0</td>
<td>95</td>
<td>dominant marker</td>
</tr>
</tbody>
</table>

**Significant at P = 0.01 and 2 d.f. (Table value = 9.21)

5.3.3 Over-transmission of Sr36

To confirm the over transmission of Sr36 linked alleles for different markers, 16 seeds each from five heterozygous F3 families (#9, #10, #11, #17 and #26) were sown and inoculated at the two-leaf stage with Pgt pathotype 98-1,2,3,5,6,7 (Table 5.4). DNA was
isolated from all the 16 plants individually in each family and genotyped with
codominant \textit{Sr36}-linked marker \textit{stm773.2} and \textit{Sr39}-linked dominant marker \textit{Sr39#22} (Table 5.4). Marker \textit{stm773.2} amplified either 160bp or both 160bp and 190bp
amplicons in plants that expressed IT 0; and 190bp allele in plants with IT 2= in all families. The presence of \textit{Sr39} was confirmed by amplification of 840bp product in
plant producing IT2= using the dominant marker \textit{Sr39#22}. The over transmission was
noted in all families. Marker \textit{Sr39#22} was used for the ease of its separation using
agarose gels.

\textbf{Table 5.4} Genotypic constitution of single plants derived from five F$_3$ heterozygous families

<table>
<thead>
<tr>
<th>Family</th>
<th>Phenotype</th>
<th>\textit{Stm773.2}</th>
<th>\textit{Sr39#22}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>160bp</td>
<td>160/190bp</td>
</tr>
<tr>
<td>#9</td>
<td>0;</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0;</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2=</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#10</td>
<td>00;0;</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>00;</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2=</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#11</td>
<td>00;0;</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0;</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0;2=</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#17</td>
<td>0;</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0;</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2=-2=</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#26</td>
<td>00;0;</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>00;0;</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>00;1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

84
Genomic in situ-hybridization (GISH) analysis of the parents Cook and RWG1 confirmed the presence of a large *T. timopheevii* translocation in Cook spanning across the centromere of chromosome 2B and a shortened *Ae. speltoides* segment in RWG1 at the distal end of chromosome 2BS (Fig. 5.3). Samples from a heterozygous F₃ line shown to be heterozygous using markers linked with these genes were included in the GISH analysis and showed presence of both *T. timopheevii* and *Ae. speltoides* segments similar to that of Cook and RWG1 (Table 5.4 and Fig. 5.3).

**Fig. 5.3** Genomic hybridization of parental lines and F₃ heterozygous families left to right; RWG1 carrying *Sr39* translocation; Cook carrying *Sr36* translocation; heterozygous progeny 1 and heterozygous progeny 2 having one copy each of *Ae. speltoides* and *T. timopheevii* segment. The arrows show the position of the alien translocation segments.

5.3.4 Validation of marker *rwgs28*

Marker *rwgs28* showed close linkage with the shortened *Sr39* segment and negatively validated in 32 Australian cultivars. This marker did not amplify the resistance-linked allele in any of the Australian cultivars used for validation (Table 5.5). Nine *Sr36*-carrying Australian cultivars (Baxter, Cook, Lang, Yarralinka, Sunbri, Sunco, Sunvale,
Wylie, Young) and nine Sr36-lacking cultivars (Chinese spring, Gladius, Merlin, Orion, Schomburgk, Scout, Spitfire, Tasman, Wyalkatchem) amplified Cook allele (430bp/440bp/446bp). In addition, five Sr36-lacking cultivars (Calingiri, Espada, Carnamah, Derrimut and DiamondBird) produced three amplicons (433bp/440bp/446bp) different from Cook and RWG1 alleles. The remaining nine Sr36-lacking cultivars (Forest, Giles, Ventura, EGA Bellaroi, Hyperno, EGA BonnieRock, Magenta, EGA Gregory and EmuRock) amplified a different allele (430bp/438bp/446bp/450bp). None of the cultivars amplified the Sr39-specific allele implying that rwgs28 is diagnostic for the Ae. speltoides segment (Table 5.5).

**Table 5.5** Amplicon sizes produced by the different cultivars when genotyped with marker *rwgs28*

<table>
<thead>
<tr>
<th>No.</th>
<th>Cultivar</th>
<th>Amplicon (bp)</th>
<th>No.</th>
<th>Cultivar</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cook</td>
<td>430/440/446</td>
<td>18</td>
<td>Derrimut</td>
<td>433/440/446</td>
</tr>
<tr>
<td>2</td>
<td>Baxter</td>
<td>430/440/446</td>
<td>19</td>
<td>Giles</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>3</td>
<td>Lang</td>
<td>430/440/446</td>
<td>20</td>
<td>Diamond bird</td>
<td>433/440/446</td>
</tr>
<tr>
<td>4</td>
<td>Schomburgk</td>
<td>430/440/446</td>
<td>21</td>
<td>Gladius</td>
<td>430/440/446</td>
</tr>
<tr>
<td>5</td>
<td>Yarralinka</td>
<td>430/440/446</td>
<td>22</td>
<td>Ventura</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>6</td>
<td>Sunbri</td>
<td>430/440/446</td>
<td>23</td>
<td>EGA Bellaroi</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>7</td>
<td>Sunco</td>
<td>430/440/446</td>
<td>24</td>
<td>Hyperno</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>8</td>
<td>Sunvalle</td>
<td>430/440/446</td>
<td>25</td>
<td>Whyalkatchem,</td>
<td>430/440/446</td>
</tr>
<tr>
<td>9</td>
<td>Tasman</td>
<td>430/440/446</td>
<td>26</td>
<td>EGA BonnieRock</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>10</td>
<td>Wylie</td>
<td>430/440/446</td>
<td>27</td>
<td>Magenta</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>11</td>
<td>Young</td>
<td>430/440/446</td>
<td>28</td>
<td>EGA Gregory</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>12</td>
<td>Calingiri</td>
<td>433/440/446</td>
<td>29</td>
<td>Merlin</td>
<td>430/440/446</td>
</tr>
<tr>
<td>13</td>
<td>Espada</td>
<td>433/440/446</td>
<td>30</td>
<td>Emurock</td>
<td>430/438/446/450</td>
</tr>
<tr>
<td>14</td>
<td>Scout</td>
<td>430/440/446</td>
<td>31</td>
<td>Orion</td>
<td>430/440/446</td>
</tr>
<tr>
<td>15</td>
<td>Carnamah</td>
<td>433/440/446</td>
<td>32</td>
<td>CS</td>
<td>430/440/446</td>
</tr>
<tr>
<td>16</td>
<td>Forest</td>
<td>430/438/446/450</td>
<td>33</td>
<td>C90.1*(Sr39)</td>
<td>440/446/449</td>
</tr>
<tr>
<td>17</td>
<td>Spitfire</td>
<td>430/440/446</td>
<td>34</td>
<td>RWG1*(Sr39)</td>
<td>440/446/449</td>
</tr>
</tbody>
</table>

*aThe 449 bp amplicon is diagnostic of Sr39*
5.4 Discussion

Stem rust control was achieved in Australia through the release of rust resistant cultivars. Majority of these cultivars carried single ASR genes and or combinations of genes. The resistance has often been overcome by new Pgt pathotypes believed to arise mainly through introduction or mutations (Bariana et al. 2007a; Park 2007; Singh et al. 2008). Wild relatives of hexaploid wheat have been exploited as a valuable source of rust resistance genes (McIntosh et al. 1995; Zaharieva et al. 2001). Several stem rust resistance genes have been transferred to wheat from wild relatives and deployed in commercial wheat cultivars (McIntosh et al. 1995). Stem rust resistance gene Sr39 is effective against Ug99 and its derivatives, but it was not used in breeding programs due to linkage drag associated with the large Ae. speltoides segment (Singh et al. 2006; Dundas et al. 2007; Yu et al. 2010). This study used the original large translocation (RL6082) and the most reduced segment (RWG1) to determine genetic association of Sr39 with Sr36. Sr36 and Sr39 were observed to be tightly linked in repulsion in both crosses. The lack of recombination between these two genes may be attributed to the absence of recombination between the genomes G (Sr36) and S (Sr39). McIntosh and Arts (1996) associated the reduced recombination between chromosome 2A genes Pm4a derived from T. turgidum and Yr1 from common wheat to the divergent origins of the two genomes. A rare recombinant (Combination III) carrying Sr9e and Sr36 was identified by McIntosh and Luig (1973). This recombinant appears to be a product of an allosyndetic event and resulted in the deletion of black point resistance carried on this segment (HS Bariana personal observation). The marker and GISH analysis in this study provided no evidence of over-lapping between T. timopheevii and Ae. speltoides.
segments in this population. The GISH results further confirmed that *T. timopheevii* translocation harboring *Sr36* in Cook is still too large spanning across both arms of 2B. Flemmig (2012) reported that the alien *T. timopheevii* translocation carrying *Sr36* covers about 80% of chromosome 2B. It is therefore logical to think that the presence of such a large alien translocation could be hindering recombination between the two genes. It appears that the distance between the two translocations is too small to allow recombination to occur.

Most alien chromosomes introgressed into wheat backgrounds are associated with segregation distortion due to the presence of segregation distortion (*Sd*) and gametocidal (*Gc*) factors (Ceoloni et al. 1996; Marais et al. 2010; Niu et al. 2011). Fifteen wheat segments carrying segregation distortion loci of which one each was on 2BL and 2BS were reported by Xue et al. (2008). Paillard et al. (2003) observed greater segregation distortion in chromosome 2B than any other chromosome. The presence of *Sd* genes in chromosome 2B partly explains the high segregation distortion observed in numerous studies involving this chromosome. Previous studies by Niu et al. (2011) and Klindworth et al. (2012) reported significant segregation distortions that resulted in over transmission of the large translocations carrying *Sr39* and reduced transmission of the small *Ae. speltoides* segments. In the current study over-transmission of *Sr39* (original large segment) over the *Sr36* was observed. In contrast, preferential transmission of *Sr36* was observed in the second cross involving the shortened *Sr39*-carrying *Ae. speltoides* segment suggesting that a genetic determinant of meiotic drive is deleted in the shortened *Ae. speltoides* segment. Numerous studies have reported preferential transmission of *Sr36* and *Pm6* genes, both transferred from the short arm of
chromosome 2G of *T. timopheevi*, to chromosome 2B of wheat (Allard and Shands 1954; Brown-Guedira et al. 2003; Tsilo et al. 2008). Evidence of preferential transmission of chromosome 2G was also reported by Brown-Guedira et al. (1998). Both *Sr36* and *Sr40* were transferred from chromosome 2G of *T. timopheevii*; however, Wu et al. (2009) observed preferential transmission of the 2G segment harboring *Sr36* and reduced transmission of the *Sr40*-containing segment. Similar trends were observed by Bariana et al. (2001) suggesting that over transmission of *Sr36*-carrying *T. timopheevii* segment is a common phenomenon.

Durable stem rust resistance can be achieved by pyramiding two or more stem rust resistance genes into adapted commercial cultivars as opposed to deployment of single resistance genes in new cultivars (Bariana et al. 2007a). Success of gene pyramiding depends on the availability of diagnostic molecular markers for target genes. Niu et al. (2011) reported several markers that could detect the shortened *Ae. speltoides* chromatin carrying *Sr39*, but this study demonstrated that markers *Sr39#22r* (Mago et al. 2009) and STS marker *rwgs28* were diagnostic for the shortened *Ae. speltoides* chromatin in the RWG1/Cook-derived population. *Sr39#22r* is a dominant marker and could not differentiate heterozygous plants from homozygotes, which is in agreement with previous studies (Bernado et al. 2013; Mago et al. 2009; Niu et al. 2011). Marker *rwgs27* did not show complete association with *Sr39* and *rwgs29* was not polymorphic between RWG1 and Cook (Data not presented). The monomorphism of marker *rwgs29* observed in this study was consistent with reports by Bernado et al. (2013). Bernardo et al. (2013) reported that *rwgs27* amplified *Sr32*-specific allele, while marker *Sr39#22r* produces *Sr40*-specific amplicon similar to that of *Sr39*-specific allele.
This makes these markers unsuitable for selection of \( Sr39 \). Niu et al. (2011) used a cross between \( Ae. \) speltoides translocation (\( Sr39 \)) and Chinese spring in their study, while in this study we used a cross between the \( Ae. \) speltoides translocation (\( Sr39 \)) and the \( T. \) timopheevi translocation (\( Sr36 \)). The negative validation of STS marker \( rwgs28 \) on 32 Australian cultivars showed that this marker clearly detects the small \( Ae. \) speltoides segment carrying \( Sr39 \) and thus suitable for pyramiding of this gene into modern cultivars. The amplification of Cook allele in some \( Sr36 \)-lacking wheat cultivars suggested that two translocations do not overlap and hence can be combined using large \( F_2 \) populations.
CHAPTER 6

Genetic variation in mesophyll conductance and response to sustained drought stress in selected bread wheat (*Triticum aestivum* L.) landraces

6.1 Introduction

Adverse environmental factors, especially water scarcity, represent the most severe constraint to agriculture, accounting for more than 70 percent of potential yield losses worldwide (Chaves et al. 2008; Flexas et al. 2004; Koohafkan and Stewart 2008). As climate change-induced droughts continues to ravage the world’s arable land, making agriculture an increasingly daunting task (Chaves et al. 2008; Luterbacher et al. 2004; Schar et al. 2004), the anticipated impacts of drought and increased temperatures will have far reaching consequences for millions of people entirely dependent on agriculture for food and employment. Drought stress affects plant productivity and growth by limiting photosynthesis (Galle et al. 2007, Haldimann et al. 2008). Numerous studies on various crops have highlighted stomatal conductance and mesophyll conductance ($g_m$) as the key factors limiting CO$_2$ assimilation during moisture stress (e.g. Flexas et al. 2002, 2009; Galle et al. 2007, 2008).

Wheat is the most widely grown cereal crop and is extensively grown in dryland regions under both irrigated and non-irrigated conditions (Koohafkan and Stewart, 2012). Climate change-induced droughts and temperature increases are expected to reduce wheat production by over 29% globally (Rosegrant et al. 1995; Singh et al. 2011a). The scenario is compounded by stagnating yields (Hawkesford et al. 2013),
increasing irrigation costs and scarcity of water for irrigation due to competition from other sectors. Agriculture is the largest consumer of water in the world with more than 80% of the world’s fresh water being consumed by irrigated agriculture (Condon et al. 2004; Koohafkan and Stewart 2008). As climate change makes wheat environments less favorable, development of water-use efficient (WUE) and drought resilient wheat genotypes to offset these impacts is of utmost importance. It has been reported that regulation of $g_s$ and $g_m$ may be an efficient means for optimizing the relationship between water loss and carbon uptake in plants (Barbour et al. 2010; Hommel et al. 2014).

Mesophyll conductance is defined as the diffusive conductance of CO$_2$ between the intercellular air spaces and the sites of carboxylation in chloroplasts (Buckley and Warren 2013). It is a significant and variable limitation to photosynthesis and also influences leaf water-use efficiency (Barbour et al. 2010; Flexas et al. 2008 2013; Niinemets et al. 2009a,b; Sun et al. 2014). Significant genotypic variation in $g_m$ has been identified in cereal crops, including barley (six genotypes, Barbour et al. 2010), wheat (six genotypes, Evans and Vellen, 1996; eleven genotypes, Jahan et al. 2014) and rice (thirteen genotypes, Gu et al. 2012a). Genetic control of photosynthetic traits like $g_s$, transpiration efficiency ($TE$) and chlorophyll fluorescence have been reported in cereals such as rice (Adachi et al. 2011; Gu et al. 2012b; Teng et al. 2004) and wheat (Czyczyło-Mysza et al. 2013; Wang et al. 2015; Zhang et al.2010). However, only a few studies on understanding the genetic control of $g_m$ had been conducted until recently when Barbour et al. (2016) reported a QTL on chromosome 2A of wheat that explained 9% of the variation in mesophyll conductance (Gu et al. 2012).
Studies have shown that \( g_m \) is often affected by variations in environmental conditions such as drought stress (Delfine et al. 1998; Galmés et al. 2007; Niinemets et al. 2009a, b), temperature (Bernacchi et al. 2002; Scafaro et al. 2011) and availability of nutrients (Warren 2004). Drought stress significantly reduces mesophyll conductance in a number of plant species including herbaceous plants like sugar beet (Monti et al. 2006), tobacco (Galle et al. 2009, 2010), tomatoes and beans (Warren 2008) and tree species such as Eucalyptus (Warren 2008), Oak (Galle et al. 2007, 2011), rockrose (Galle et al. 2011), \textit{Olea europaea} var. \textit{sylvestris}, \textit{Rhamnus alaternus} and \textit{Cneorum tricoccon} (Varone et al. 2012), \textit{Populus tremula} (Rancourt et al. 2014; Tosens et al. 2012). Despite detailed studies on these species, the response of \( g_m \) to drought stress in cereals is not clearly understood and some studies report no response of \( g_m \) to drought (e.g. in \textit{Capsicum annuum}; Monti et al. 2006). It has also been illustrated that \( g_m \) strongly reduces during the first days (1-3 days) of moisture deficit but quickly restores to control levels in the fourth day (Galle et al. 2009; Pou et al. 2012). The value of \( g_m \) is also highly influenced by leaf traits such as leaf dry matter per unit area (Flexas et al. 2008; Galmés et al. 2011).

There is increasing evidence of a correlation between \( g_m \) and \( g_s \) (Barbour et al. 2010; Douthe et al. 2011; Warren 2008; Yin et al. 2009). Both \( g_m \) and \( g_s \) follow the same pattern of declining responses to short-term increases in CO\(_2\) partial pressure and increase with increasing irradiance. In fact for plants to maintain high photosynthetic rate while minimizing water loss under water limiting conditions, it would be ideal to have plants with low \( g_s \) but with increased \( g_m \) so as to maintain high CO\(_2\) concentration at the sites of carboxylation (\( C_c \)) and therefore high \( A \) (Galmés et al. 2011; Flexas et al. 2010).
2010). The current study was designed to; 1). Examine genotypic variation in $g_m$ among wheat landraces collected by Arthur E. Watkins in the 1920’s and establish the influence of $g_m$ and $g_s$ on photosynthetic rate and $WUE_i$ under non-limiting conditions. 2). Determine the responses of $g_m$, gas exchange parameters, leaf carbon isotope discrimination and leaf water potential to progressive drought stress.

6.2 Materials and Methods

6.2.1 Plant Materials and Growth conditions

6.2.1.1 Experiment 1

Forty one common wheat (*Triticum aestivum* L.) landraces collected by British botanist Arthur E. Watkins in the 1920’s were selected to represent diverse environments of the 33 countries from which they were collected. An additional nine cultivars from Australia, India, Mexico and Uganda were included as checks. Three seeds per genotype in three replicates were space planted in 8L pots filled with commercial potting compost mixed with slow release fertilizer osmocote® and grown in a controlled-environment growth room at the Centre for Carbon, Water and Food, University of Sydney. After four weeks the seedlings were thinned to two plants per pot and a light dose of water soluble fertilizer aquasol® (10 grams:10 litres of tap water for 100 pots) was applied. The growth room was maintained at 25°C during the 14 hour light period and 17°C during the 10 hour dark period. Relative humidity was controlled at 75% at all times and light-period photosynthetic photon flux density of 800μmol m$^{-2}$ s$^{-1}$ at the top of the plants. All pots received adequate water throughout the experiment.
6.2.1.2 Experiment 2

In the second experiment three landraces and two commercial genotypes selected based on results from the first experiment were sown in 8L pots replicated 20 times. The plants were grown in the same controlled-environment room using the same procedures and growth conditions as stated for Experiment I with the exception of thinning to one plant per pot three weeks after germination. All pots were well-watered for the first six weeks and thereafter ten pots of each genotype remained well-watered, while the second half of the replicate pots were subjected to a slowly-developing drought by withholding water until temporary wilting point (8 days) and thereafter water availability was gravimetrically maintained by weighing pots daily and adding water to replace water transpired and evaporated.

6.2.2 Leaf gas exchange and on-line carbon isotope discrimination measurements

Measurements of leaf gas exchange and carbon isotope discrimination ($\Delta^{13}$C) were conducted six weeks after planting using a portable photosynthesis system (Li6400xt, Li-Cor Inc., Lincoln, NE, USA) coupled with an online tunable diode laser absorption spectrometer (TDLAS, model TGA100A, Campbell Scientific, Logan, UT, USA) to give $A$, $g_s$, $WUE_i$ and instantaneous carbon isotope discrimination ($\Delta^{13}$C$_{obs}$) (Barbour et al. 2007, 2010) under non-limiting conditions for Experiment I. Two individual plants per pot for the three replicate pots per genotype were used for the measurements. In Experiment II, the same parameters as stated for Experiment I were measured daily for 13 days under both well-watered and moisture deficit conditions using the same apparatus. Mid-sections of 2-4 youngest fully expanded leaves were placed side-by-side in a 2 cm x 6 cm Li6400 leaf chamber fitted with red-green-blue light (Li6400-18; Li-
Cor Inc.). The leaf temperature was controlled at 25°C, and the leaf chamber controlled at RH of 70-80% and a CO₂ concentration of 400 µmol mol⁻¹ for all the measurements. The flow rate was set at 500 µmol s⁻¹ and irradiance at 1300 µmol m⁻² s⁻¹ (Jahan et al. 2014). All the plants used for measurements were at vegetative stage.

Mesophyll conductance was estimated as previously described (Evans et al. 1986; Barbour et al. 2010) and including a ternary correction (Farquhar and Cernusak 2012). We assumed that fractionation during dissolution and diffusion of CO₂ in water was 1.8‰, during carboxylation 29‰ (Roeske and O’Leary 1984), fractionation associated with photorespiration was 16.2‰ (Evans and von Caemmerer 2013), during diffusion through the boundary layer 2.9‰, fractionation during day respiration 3‰ (Tcherkez et al. 2010). Day respiration itself was 0.8 µmol m⁻² s⁻¹ at a leaf temperature of 25°C (Jahan et al. 2014) and the compensation point of photosynthesis in the absence of day respiration was 37.3 µmol mol⁻¹ (Bernacchi et al. 2001). Measurements of δ¹³C of the CO₂ in the growth room (δ¹³C_CO₂) were made using a Picarro ¹³CO₂ laser analyser (G1101-I, Picarro, CA, USA). Average δ¹³C_CO₂ was calculated for each day during the light period and 7-day running averages calculated from daily averages. The running average over the 7 days prior to gas exchange measurement was used in gₘ calculations and for Δ¹³C calculation of leaf tissue samples (Δ¹³C_leaf). The Picarro laser was calibrated as described by Thurgood et al. (2014). The δ¹³C_CO₂ values were used to calculate total fractionation during day respiration, following Wingate et al. (2007).
6.2.3 Estimation of maximum carboxylation rate ($V_{cmax}$) and electron transport rate ($J_{max}$)

Photosynthetic CO$_2$ response curves were measured at the start of the treatments, on day eight and at the end of the experiment after 14 days from the start of treatments. Photosynthetic response to CO$_2$ concentration between 0 and 1200 ppm was measured for six replicate plants per genotype using a Li6400xt fitted with a standard 2 cm x 3 cm leaf chamber and a blue-red light source set at 1500 µmol m$^{-2}$ s$^{-1}$. Response curves were measured starting at ambient CO$_2$ concentration, decreasing to 0 ppm, and then increasing from ambient to 1200 ppm. Curves were used to fit maximum carboxylation rate ($V_{cmax}$) and electron transport rate ($J_{max}$) using the spreadsheet developed by Sharkey et al. (2007), except that $g_m$ was entered as a known value for each plant, rather than being fitted.

6.2.4 Estimation of mesophyll limitations and stomatal limitations

Photosynthetic limitation analysis described by Farquhar and Sharkey (1982) was used to estimate stomatal ($L_s$) and mesophyll ($L_m$) limitations to photosynthesis at an ambient CO$_2$ concentration of 400 µmol mol$^{-1}$ using the $A/C_i$ curves and $g_m$ measurements, as outlined in Warren et al. (2003).

6.2.5 Measurement of leaf water potential ($\psi_{leaf}$)

The $\psi_{leaf}$ was measured daily at midday from the start of treatment for 14 days using a Scholander-style pressure bomb (model 115, Soil Moisture Equipment, Santa Barbara, CA, USA). The youngest fully expanded leaf per plant per genotype was wrapped in plastic film before cutting to minimize transpiration. The cut leaf was quickly fitted in
the pressure boom chamber, pressure applied and readings taken immediately as the first meniscus of water appeared on the cut end of the leaf. Leaves were chosen from replicate plants that were not used for gas exchange and \( g_m \) measurement.

6.2.6 Stable isotope analysis of leaf organic material
The youngest fully expanded leaf per plant per genotype was collected for analysis of nitrogen concentration and stable carbon isotope composition at the end of gas exchange measurements. Ten cm long leaf samples were excised from each plant using a pair of scissors and the width of the cut ends measured using a digital caliper before oven drying for two weeks at 70°C. Dry weights of the leaf tissues were taken for leaf mass per unit area measurements before grinding the tissue to a fine powder in a Retsch MM300 Mixer mill. 0.85 mg of dry powder was analysed by isotopic mass spectrometry (Delta V Advantage coupled to a Conflo IV and a FlashHT in dual-reactor setup; Thermo Fisher Scientific, Bremen, Germany). Carbon isotope compositions were converted to isotope discriminations using a 7-day running average of measured \( \delta^{13}C_{CO2} \).

6.2.7 Statistical Analysis
All statistical procedures were conducted using GENSTAT 16\(^{\text{th}}\) edition in Windows (VSN International Ltd. [www.vsni.co.uk](http://www.vsni.co.uk)). Analysis of variance (ANOVA) was performed to test the main effects and interactions, against appropriate error terms. Differences were considered significant at \( P \leq 0.05 \). Means were compared using Fishers Protected Least Significant Difference (LSD) test at \( P \leq 0.05 \). Graphical presentation of
data was performed using data analysis and graphing software SigmaPlot for Windows, version 11.0, Systat Software, Inc. San Jose, CA.

6.3 Results

6.3.1 Experiment 1

Significant genotypic variation ($P=0.001$) was observed for $g_m$ (values ranged between 0.5 and 1.4 mol m$^{-2}$ s$^{-1}$), $g_s$ (0.3 to 0.8 mol m$^{-2}$ s$^{-1}$), $A$ (24 to 30 µmol m$^{-2}$ s$^{-1}$), and $A/g_s$ (40 to 70 µmol mol$^{-1}$) (Fig. 6.1A-D). There was a stronger positive correlation between $g_m$ and $g_s$ ($r^2=0.23$, $P=0.0004$, Fig. 6.2B) than $g_m$ and $A$ ($r^2=0.12$, $P=0.013$, Fig. 6.2A). In addition, a negative correlation ($r^2=0.20$, $P=0.001$) was observed between $g_m$ and $A/g_s$ (Fig. 6.2C).
Fig. 6.1 Genetic variation in A) photosynthesis rate ($A$), B) stomatal conductance ($g_s$), C) intrinsic water use efficiency ($A/g_s$), D) mesophyll conductance ($g_m$) observed among 41 wheat landraces and 11 commercial genotypes evaluated under non-limiting conditions. Aridity index values of the origin countries and regions from where the landraces were collected were available only for the first 20 genotypes. Values are mean ± standard error, n = 6
Fig. 6.2 Relationship between mesophyll conductance ($g_m$) and A) Photosynthesis rate (A), B) stomatal conductance ($g_s$), and C) intrinsic water use efficiency ($A/g_s$) for 41 wheat landraces and 11 commercial genotypes evaluated under non-limiting conditions. Values are mean ± standard error, $n = 6$. Lines are least squared linear regressions with fitted parameters indicated.

The carbon isotope composition of leaf tissue ($\Delta^{13}C_{\text{leaf}}$) was significantly different between genotypes and was positively related to instantaneous measurements of both the ratio of intercellular to atmospheric CO$_2$ partial pressures ($C_i/C_a$) and the ratio of chloroplastic to atmospheric CO$_2$ partial pressures ($C_c/C_a$) (Fig. 6.3A-C). Variation in $C_c/C_a$ explained slightly more of the observed variation in $\Delta^{13}C_{\text{leaf}}$ than did $C_i/C_a$ (Fig. 6.3D).

There was no relationship between LMA and $g_m$ among all the fifty genotypes under none-stressing moisture conditions (Figure 6.4A). Similarly no relationship between $N_a$ and $g_m$ was observed among the fifty two genotypes evaluated under none-
limiting conditions (Figure 6.4E). Similar results were observed for $N_a$ and $A$ (Fig. 6.4C).

**Fig. 6.3** Relationship between carbon isotope discrimination ($\Delta^{13}C_{\text{leaf}}$) and A) substomatal CO$_2$ concentration to atmospheric CO$_2$ concentration ($C_i/C_a$), B) chloroplast CO$_2$ concentration to atmospheric CO$_2$ concentration ($C_c/C_a$) for all 52 wheat genotypes under non-limiting conditions and C) $C_i/C_a$, D) $C_c/C_a$ for the five selected wheat genotypes tested under drought stress. Red symbols denote drought stressed plants, while black symbols denote well-watered plants. Values are means, ± standard error, $n = 6$ for A) and B) and $n = 3$ for C) and D)
Fig. 6.4 Relationships between leaf mass per unit area (LMA) and mesophyll conductance \( (g_m) \) for A) all 52 wheat genotypes under non-limiting conditions, B) and five selected wheat genotypes under drought stress (Red colour symbols-drought stressed, black colour symbols-well watered). Relationship between leaf Nitrogen per unit area \( (N_a) \) and photosynthesis rate \( (A) \) for C) 52 wheat genotypes under non-limiting conditions, D) five selected wheat genotypes screened under drought stress. E) relationships between leaf Nitrogen per unit area and mesophyll conductance for 52 wheat genotypes under non-limiting conditions, F) relationship between leaf Nitrogen per unit area and mesophyll conductance for five selected wheat genotypes evaluated under drought stress. Values are means, ± standard error, \( n = 6 \) for A), C) and D) and \( n = 3 \) for B), D) and F)
Data on aridity index/classes of the original location where these genotypes were collected from was retrieved from the online database http://figs.icarda.net/, but we were able to get information for only 20 genotypes out of 41 used in the study (Supplementary Table 1). There was no indication that the aridity of the location of origin significantly influenced any of the gas exchange parameters or Δ\textsuperscript{13}C\textsubscript{leaf} (Fig. 6.1).

Based on this experiment three landraces with contrasting results were selected for detailed studies on response of A, \(g_m\) and \(g_s\) to drought stress; genotype Aus28230 (\(g_m=1.05, \ g_s=0.51, \ A=27.29, \) and \(A/g_s=53.51\)), Aus28112 (\(g_m=0.97, \ g_s=0.39, \ A=22.69, \) and \(A/g_s=58.18\)) and Aus28119 (\(g_m=0.72, \ g_s=0.32, \ A=21.92, \) and \(A/g_s=68.50\)). Two commercial cultivars Sunpict (\(g_m=1.13, \ g_s=0.63, \ A=27.45, \) and \(A/g_s=43.57\)) and Yitpi (\(g_m=0.99, \ g_s=0.48, \ A=26.97, \) and \(A/g_s=56.19\)) were included as controls.

6.3.2 Experiment 2

6.3.2.1 Leaf water potential response to drought

Leaf water potential (\(\Psi_{leaf}\)) varied between -0.68 and -0.98 MPa in well-watered plants (Fig. 6.5A). Upon cessation of watering, \(\Psi_{leaf}\) gradually decreased and was significantly lower than well-watered plants from seven days after the start of the treatment. Sunpict maintained higher \(\Psi_{leaf}\) than the other four genotypes. At the end of the drought period (13\textsuperscript{th} day), all genotypes showed values between -1.69 MPa in Aus28230 and -1.99 MPa in Yitpi.
6.3.2.2 Leaf gas exchange responses to drought

Drought also reduced photosynthetic rate (A) in all genotypes, although the day on which a significant reduction in A was observed varied between genotypes. A significant treatment effect on A was observed on day 3 in Sunpict (P≤0.001), day 5 in Aus28230 (P≤0.05), day 7 in Aus28112 (P≤0.001) and Yitpi (P≤0.05), while variation between treatments in Aus28119 (P≤0.05) for A was only observed on day 9. Similarly, the largest reduction in A due to drought was observed for the cultivar Sunpict (33% reduction; from 35 µmol m\(^{-2}\) s\(^{-1}\) under normal watering to 12 µmol m\(^{-2}\) s\(^{-1}\) under drought stress), while the smallest reduction in A was observed for Aus28119 (12%). The commercial genotype Yitpi had the highest overall A, at 30 µmol m\(^{-2}\) s\(^{-1}\) under drought and up to 40 µmol m\(^{-2}\) s\(^{-1}\) under well-watered conditions (Figure 6.5B).

Drought lowered stomatal conductance (g\(_s\)) in a similar way in all the genotypes, but Aus28119 responded early to stress with a significant difference (P≤0.05) between treatments observed on day three of the stress, while Sunpict (P≤0.001) and Aus28230 (P≤0.05) showed significant differences on day 4. All genotypes had a highly significant reduction in g\(_s\) by day 5 (Fig. 6.5C). There was a significant genotype by treatment interaction (P < 0.01) with the greatest reduction in g\(_s\) in response to drought observed for Sunpict (61%) and lowest reduction for Yitpi (41%).

The response of g\(_m\) to drought was less clear, although overall there was a significant reduction in g\(_m\) due to the drought treatment (P< 0.001). Significant reductions in g\(_m\) between well-watered and drought plants were observed on day 7 in Sunpict, on day 8 in Yitpi, while in Aus28112 and Aus28119 a significant reduction in
$g_m$ was observed on days 10 and 11, respectively. However, these differences were small and often for only a single day, with no significant genotype, time of measurement, genotype by treatment or genotype by time interactions observed using ANOVA. Nonetheless, drought stressed Sunpict plants generally had significantly lower $g_m$ values starting from the seventh day of drought stress until end of the experiment, while Aus28230 had significantly lower $g_m$ values during the last two days of the experiment. Genotype Yitpi and Aus28112 maintained a high $g_m$ throughout the drought period (Fig. 6.5D).

As expected, drought stress caused a significant increase in $WUE_i$ in all five genotypes with the highest increases in Sunpict andAus28119 (99%) and the smallest in Yitpi (47%) (Fig. 6.5E, Supplementary Table 2). Significant drought effects on $WUE_i$ were observed first in genotype Aus28119 (on day 3) with Sunpict and Aus28230 responding on day 4, while all five genotypes recorded a significant response to the stress on day 5 (Fig. 6.5E). There was no significant overall difference in $WUE_i$ between genotypes. However, there were highly significant ($P \leq 0.001$) treatment and time effects, treatment by time interactions, genotype by treatment interactions ($P \leq 0.01$) and all factor interactions ($P \leq 0.05$). That is, the timing and magnitude of the response to drought differed between genotypes.
* Value significant at $P \leq 0.05$; ** Value significant at $P \leq 0.01$; *** Value significant at $P \leq 0.001$

**Fig. 6.5** Changes in A) leaf water potential ($\Psi_{\text{leaf}}$), B) photosynthetic rate (A), C) stomatal conductance ($g_s$), D) mesophyll conductance ($g_m$) and E) intrinsic WUE ($A/g_s$) in response to 14 days induced sustained drought stress for the five selected wheat genotypes. Values are mean ± standard error, $n = 3$, except for panel A, in which values are for a single plant. Asterisks indicate significant differences for genotypes of the matching colour for the individual day.
6.3.2.3 Genotypic variation in maximum carboxylation rate ($V_{cmax}$), maximum electron transport ($J_{max}$), mesophyll limitations ($L_m$) and stomatal limitations ($L_s$) to photosynthesis

There were no significant drought effects on either $V_{cmax}$ or $J_{max}$ and no significant differences among genotypes (Table 6.1). There was a general decline in both $V_{cmax}$ and $J_{max}$ over time in both well-watered and drought-stressed plants, presumably related to plant aging (the youngest fully expanded leaves were measured in all cases).

Drought significantly increased both stomatal ($L_s$) and mesophyll limitations ($L_m$) to photosynthesis (Table 6.1), but genotypes did not differ significantly in either $L_m$ or $L_s$. There were however highly significant treatment and time effects on $L_m$ and $L_s$ (Table 6.1). Severe drought increased $L_m$ from 0.02 to 0.05±0.01 and $L_s$ from 0.1 to 0.2±0.01 with a high increase in $L_s$ of 65% registered in Aus28119 (Table 6.1). $L_s$ was at least triple than $L_m$ in all plants.
**Table 6.1** The effect of drought stress conditions on maximum carboxylation rate ($V_{\text{max}}$), electron transport rate ($J_{\text{max}}$), stomatal limitations to photosynthesis ($L_s$) and mesophyll limitations to photosynthesis ($L_m$)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Day after Trt</th>
<th>$V_{\text{max}}$</th>
<th>$J_{\text{max}}$</th>
<th>$L_m$</th>
<th>$L_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Watered</td>
<td>Drought</td>
<td>%red.</td>
<td>Watered</td>
<td>Drought</td>
</tr>
<tr>
<td>Aus28112</td>
<td>Day 0</td>
<td>125±10</td>
<td>187±15</td>
<td>0.03±0.00</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>151±9</td>
<td>186±12</td>
<td>0.06±0.01</td>
<td>0.10±0.00</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>130±7</td>
<td>177±12</td>
<td>0.05±0.01</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Aus28119</td>
<td>Day 0</td>
<td>152±14</td>
<td>152±9</td>
<td>0.03±0.00</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>146±14</td>
<td>197±21</td>
<td>0.04±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>106±6</td>
<td>198±12</td>
<td>0.05±0.01</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Aus28230</td>
<td>Day 0</td>
<td>136±3</td>
<td>196±8</td>
<td>0.04±0.00</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>156±26</td>
<td>202±6</td>
<td>0.05±0.01</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>127±13</td>
<td>172±29</td>
<td>0.05±0.01</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>Yitpi</td>
<td>Day 0</td>
<td>173±18</td>
<td>229±3</td>
<td>0.04±0.00</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>135±5</td>
<td>203±34</td>
<td>0.05±0.01</td>
<td>0.09±0.00</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>133±7</td>
<td>183±11</td>
<td>0.04±0.00</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Sunpict</td>
<td>Day 0</td>
<td>132±6</td>
<td>206±10</td>
<td>0.04±0.00</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>150±8</td>
<td>170±24</td>
<td>0.05±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>141±8</td>
<td>169±18</td>
<td>0.05±0.01</td>
<td>0.10±0.01</td>
</tr>
</tbody>
</table>

% red: Percentage reduction under drought stress; % incr: Percentage an increase under drought stress. The values are ±standard error
6.3.2.4 Stable isotope analysis of leaf organic material

Stable carbon isotope discrimination of leaf tissue ($\Delta^{13}$C$_{\text{leaf}}$) decreased under reduced water supply, with highest reductions in Aus28230 (from 23.7‰ under well-watered conditions to 19.4‰ under drought conditions), while the smallest reduction was observed in Sunpict (from 23.1‰ under well-watered conditions to 21.1‰ under drought stress). Positive relationships between $\Delta^{13}$C$_{\text{leaf}}$ and both $C_i/C_a$ and $C_c/C_a$ were observed, with 83% and 78% of variation in $\Delta^{13}$C$_{\text{leaf}}$ explained by variation in $C_i/C_a$ and $C_c/C_a$, respectively (Fig. 6.3C and D).

LMA was highest for drought-stressed plants than in well-watered plants (Fig. 6.4B). The increase in $g_m$ was accompanied with increase in LMA for all the genotypes in the drought experiment, except Yitpi which had a higher $g_m$ in well-watered plants than in drought-stressed plants (Fig. 6.4B). Drought stress increased $N_a$ in all the five genotypes, with $g_m$ decreasing with $N_a$ increase (Fig. 6.4F). A similar trend was observed for $N_a$ and $A$ (Fig. 6.4D).

6.4 Discussion

It is now well established that genotypic variability in mesophyll conductance exists within cereal crops and that this variability contributes to leaf intrinsic water-use efficiency (Centritto et al. 2009; Barbour et al. 2010; Gu et al. 2012a; Giuliani et al. 2013; Jahan et al. 2014). Further, recent work indicates a level of genetic control of $g_m$ in common wheat (Barbour et al. 2016), with a region on chromosome 2A responsible
for a small but significant level of variation in $g_m$ among a doubled haploid mapping population.

In this study we report significant genotypic variation in leaf gas exchange parameters, including mesophyll conductance and leaf intrinsic water-use efficiency, among common wheat landraces. We also show that when wheat plants are exposed to moderate water stress ($\Psi_{\text{leaf}}$ -1.7 to -2.0 MPa) $g_m$ is not strongly affected, although there was genotypic variability in the timing and degree of response for other gas exchange parameters. Significant differences in the degree of response of $A/g_s$ among landraces suggest that valuable variation may exist to provide a good resource for genetic improvement of water-use efficiency and productivity.

Consistent with earlier studies on cereals, a positive relationship was found between $g_m$ and photosynthetic rate among landraces. However, the correlation was not strong, with only 12% of variation in $g_m$ explained by variation in $A$. This is similar to the recent work on common wheat cultivars by Jahan et al. (2014), but much lower than relationships reported in rice (89%; Centritto et al. 2009) and barley (77%; Barbour et al. 2010). A positive relationship was also found between $g_m$ and stomatal conductance, with double the explanatory power at 22% and similar to previous studies (e.g. Centritto et al. 2009; Jahan et al. 2014). The stronger relationship between $g_s$ and $g_m$ than between $A$ and $g_m$ also is evident from the observation that $g_m$ is significantly but negatively correlated with $A/g_s$. That is, among the common wheat landraces and
cultivars studied here, stomatal conductance has a stronger influence on leaf intrinsic water-use efficiency than does photosynthetic rate.

Under conditions of unlimited water, a plant with very high $A$ and high harvest index will be the most productive. The two cultivars, Yitpi and Sunpict, both have high photosynthetic rates, likely due to inadvertent selection during breeding (Barbour et al. 2010). Improving WUE for water-limited conditions through increased $g_m$ will require decoupling of $g_s$ and $g_m$, with a low $g_s$ and high $g_m$ when water availability is low to ensure maximum chloroplastic CO$_2$ concentration and so high photosynthetic rate, while minimizing water loss (Barbour et al. 2010). There is clear evidence of the uncoupling of $g_m$ from $g_s$ in response to drought for the genotypes studied here, with large reductions in $g_s$ and a limited response of $g_m$. Overall, drought stress reduced $g_m$, but significant drought effects were observed for individual genotypes only on specific days and the landrace AUS28230 never recorded a day on which significant differences in $g_m$ were observed between drought-stressed and well-watered plants. In contrast, drought stress lowered $g_m$ in a number of other species (Delfine et al. 2001; Monti et al. 2006; Warren 2008; Galle et al. 2009; Tosens et al. 2012). Working on rice at flowering and grain filling, Gu et al. (2012a, b) also found that moderate drought did not consistently affect $g_m$ for all of the 11 genotypes studied (i.e. lower $g_m$ under drought for four genotypes at flowering and three genotypes at grain-filling, but only one genotype had lower $g_m$ under drought at both measurement times). Perhaps the leaf anatomy, hydraulic properties or aquaporin activity in cereal leaves (or some combination of these properties) allows $g_m$ to remain high even under water stress. Further studies to understand the responses under more extreme water stress may be informative.
Also consistent with previous work on other species, moderate drought did not significantly impair photosynthetic biochemistry (Centritto et al. 2003; Grassi and Magnani 2005; Galmes et al. 2007; Gu et al. 2012a,b), with no significant effects on maximum carboxylation rate or electron transport rate. However, there was an overall decline in both $V_{c_{\text{max}}}$ and $J_{\text{max}}$ from week 6 to week 8 after emergence. Declines in leaf gas exchange of wheat as plants age are common, with Rawson et al. (1983) reporting a decline in $A$ for successive wheat leaves after the emergence of the seventh leaf.

Of the five genotypes investigated under water stress in this experiment, the landrace AUS28230 had the highest leaf intrinsic water-use efficiency under drought, with relatively high $g_m$ and $A$, and relatively low $g_s$. The cultivar Sunpict was the most sensitive to water stress, with significantly lower $A$ as early as the third day of water being withheld and significantly lower stomatal conductance by day 4. Sunpict also had the highest proportional reductions in both $A$ and $g_s$. By contrast, the landrace AUS28119 was the last to record a significant reduction in photosynthetic rate caused by drought, nine days after the start of the drought treatment, and had the smallest overall reduction in $A$.

Photosynthetic carbon isotope discrimination, as recorded in leaf tissue ($\Delta^{13}C_i$), was significantly different between landraces and decreased for plants under drought stress as expected (Farquhar et al. 1984). Theory predicts that $\Delta^{13}C_i$ will be more closely related to the ratio of chloroplastic to ambient CO$_2$ concentration ($C_c/C_a$) than to the ratio of intercellular to ambient concentration ($C_i/C_a$) if $g_m$ varies considerably.
between leaves (Farquhar and Richards 1984; Barbour et al. 2010). However, $\Delta^{13}C_1$ is an integrative measure, recording leaf gas exchange over the period that the tissue was formed. This makes for a useful integrative proxy for $A/g_s$, but means that it is difficult to compare spot measurements of leaf gas exchange to $\Delta^{13}C_1$. In this study we found that variation in $C_d/C_a$ explained the same amount of variation in $\Delta^{13}C_1$ as $C_l/C_a$ in the first experiment using 52 genotypes ($r^2$ was 0.38 in both cases). Variation in $C_l/C_a$ and $C_d/C_a$ were expanded in the second experiment when drought was imposed, and this resulted in a wider range in $\Delta^{13}C_1$ values and closer correlations between $\Delta^{13}C_1$ and $C_d/C_a$ and $C_l/C_a$. Contrary to theory, there was a slightly closer relationship between $C_l/C_a$ and $\Delta^{13}C_1$ than between $C_d/C_a$ and $\Delta^{13}C_1$ in the second experiment ($r^2 = 0.83$ compared to $r^2 = 0.77$). This is probably due to differences between the environmental conditions under which gas exchange measurements were made (1300 $\mu$mol m$^{-2}$ s$^{-1}$ PAR) compared to growth conditions (800 $\mu$mol m$^{-2}$ s$^{-1}$). Genotypes of rice are known to differ in the responsiveness of $g_m$ to light (Gu et al. 2012a), and it seems likely that wheat genotypes also differ. For instance, Tazoe et al. (2009) reported no significant effect of light on $g_m$ in the cultivar Yecora, but our recent measurements suggest that $g_m$ increases with increasing light in the cultivars Tasman, Scout and Vorobey (Barbour, Jahan and Holloway-Phillips, unpublished data).

The results from our study showed a higher LMA for drought-stressed leaves than in leaves from well-watered plants and the increase in LMA led to an increased $N_a$. These imply that drought-stressed plants increased leaf tissue thickness to minimize water loss. Another probable explanation could that the cell turgor was not high enough
to expand cells of drought stressed plants to the same size as those under well-watered conditions, since smaller cells lead to higher biomass per unit leaf area. These results are not surprising since it has been demonstrated in literature that LMA adjusts to long term environmental conditions (Westoby et al. 2002; Poorter et al. 2009). Our results did not agree with what was reported in previous studies where $g_m$ decreased with increasing LMA across species (Flexas et al. 2008; Galmés et al. 2011; Gu et al. 2012b; Niinemets et al. 2009b), suggesting that LMA should not be used as a proxy for $g_m$ within a single species.

Based on findings from this study, we conclude that there is significant genetic variation in $g_m$, $g_s$, $A$ and $WUE_i$ among wheat landraces from the Watkins collection of 1930s. It’s further concluded that genotypes responded differently to drought stress, and that drought stress reduced $g_s$, $A$, and leaf water potential, and caused an increase in $WUE_i$ in all the five genotypes used, while $g_m$ was less affected by low soil moisture availability. Stomatal conductance was more sensitive to drought stress and dropped within the third day of moisture deficit stress whereas $A$ and $\psi_{leaf}$ responded seven days after moisture stress was imposed, implying that the reduction in stomatal conductance maintained high $\psi_{leaf}$ and $A$ during first seven to nine days of drought stress. The wide genetic variation in wheat landraces is a good resource for improving water efficiency in wheat and the non-responsive of $g_m$ to drought stress implies that wheat breeders can now select for WUE wheat genotypes without concern about the influence of moderate drought stress on $g_m$. 

115
CHAPTER 7

Conclusions

This investigation covered assessment of genetic diversity for resistance to stem rust and stripe rust in an international wheat nursery, genetic analysis of adult plant stripe rust resistance in Australian wheat cultivar Sentinel, genetic association of stem rust resistance genes in chromosome 2B and assessment of genetic diversity for physiological traits among a set of wheat landraces.

Ninety five entries of an international CIMMYT wheat screening nursery were tested against seven Australian Pgt pathotypes and five Pst pathotypes. Ten seedling stem rust resistance genes (Sr8a, Sr8b, Sr9b, Sr12, Sr17, Sr23, Sr24, Sr30, Sr31 and Sr38) and seven stripe rust resistance genes (Yr3, Yr4, Yr6, Yr9, Yr17, Yr27 and Yr34) were postulated either singly or in combinations. Sr30, Sr38 and Yr17 were the most common stem rust and stripe rust resistance genes. Postulations of Sr24, Sr31/Yr9, Sr38/Yr17 and Yr4 were confirmed using linked molecular markers. Presence of APR genes Sr2 and Yr18 were detected using linked molecular markers cssr2 and csLV34, respectively. Genotypes carrying uncharacterised stem rust and stripe rust resistance genes were identified for genetic analysis.

Stripe rust is among the top five diseases of wheat in Australia. Emergence of new virulent pathotypes continues to render resistance genes ineffective. Deployment of combinations of rust resistance genes is essential to achieve durable control of stripe rust. Australian common wheat cultivar Sentinel was observed to be highly resistant to
stripe rust under field conditions and was susceptible at the seedling stage to Pst pathotype 134 E16A+Yr17+Yr27. One hundred and seventeen (117) Sentinel/Nyabing 3 (Nyb3) derived recombinant inbred lines (RILs) were phenotyped at three sites in two consecutive crop seasons. A linkage map of the Sentinel/Nyb3 RIL population comprising 1730 DArTseq markers was used to determine the chromosomal locations of adult plant stripe rust resistance possessed by Sentinel. Composite interval mapping (CIM) detected three consistent quantitative trait loci (QTL) on chromosomes 1B, 2A and 3B. These QTL were designated $Q_{Yr.sun-1BL}$, $Q_{Yr.sun-2AS}$ and $Q_{Yr.sun-3BS}$. All the three QTL were contributed by Sentinel. $Q_{Yr.sun-1BL}$, $Q_{Yr.sun-2AS}$, $Q_{Yr.sun-3BS}$ explained on an average 18%, 15.6% and 10.6% variation in stripe rust response respectively. RILs carrying single QTL produced relative higher stripe rust severities ranging from 27-56%, while RILs carrying combinations of two QTL produced intermediate stripe rust severities (11.7-34.5%). RILs carrying combination of all three QTL produced the lowest stripe rust severity (6.7-13.5%). $Q_{Yr.sun-1B}$ corresponded to previously characterised gene Yr29 and $Q_{Yr.sun-2AS}$ appears to be a new locus. The QTL $Q_{Yr.sun-3BS}$ was expressed at the 4-leaf stage at 21±2°C and based on monogenic segregation it was tentatively names YrSen. Detailed characterisation of these loci will be required for formal naming.

Stem rust resistance genes Sr39 (RL6082) and Sr36 (Cook) were transferred from Aegilops speltoides and Triticum timopheevi to chromosome 2B of wheat. Sr39 and Sr36 showed complete repulsion linkage. The GISH analysis showed that the shortened Aegilops speltoides translocation does not overlap with the Triticum timopheevi translocation carrying Sr36 suggesting that these two genes can be recombined using
large F₂ population. Since meiotic pairing has been reported to start at the telomere, it may be prohibiting recombination.

Climate change-induced drought and temperature increases are expected to reduce wheat production by over 29% globally (Rosegrant et al. 1995; Singh et al. 2011a). Mesophyll conductance (gm) and leaf intrinsic water use efficiency (WUEi) have been reported as appropriate traits for selecting water use efficient genotypes. This study quantified genetic variation in gm and its influence on photosynthetic rate (A) and WUEi in wheat landraces under non-limiting water conditions and under developing water stress. Stomatal conductance (gs) was highly sensitive to drought, with a reduction of up to 1/3 on the third day of water stress while leaf water potential (ψleaf) and A remained unchanged until seven days after the water stress was imposed, and gm displayed no significant response. The genotypes differed in the timing and magnitude of the effects of drought on leaf gas exchange. Both maximum carboxylation rate (Vcmax) and electron transport rate (Jmax) declined as plants aged, but there were no genotype or drought effects. The wide genetic variation in wheat landraces is a good resource for improving water use efficiency in wheat.

Overall this study reported assessment of genetic diversity for resistance to stem rust and stripe rust among an international wheat nursery against the Australian rust flora. Genetic analysis of adult plant stripe rust resistance enabled identification of two new QTL for stripe rust resistance. Stem rust tests on progenies from two Sr39 and Sr36 carrying crosses and molecular cytogenetic analysis indicated that Sr36 can be combined
with the shortened segment $Sr39$. Landrace genotypes showing variation in water-use efficiency related physiological traits were identified for future studies.
REFERENCES


Allard RW, Shands RG (1954) Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from Triticum timopheevi. Phytopathol 44:266-274


Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat XV. Location of rust resistance genes in VPM1 and their genetic linkage with other resistance genes in chromosome 2A. Genome 36:476-482


Dadkhodaie NA, Karaoglou H, Wellings CR, Park RF (20011) Mapping genes Lr53 and Yr35 on the short arm of chromosome 6B of common wheat with microsatellite markers and studies of their association with Lr36. Crop Sci 49:871-879


Darvasi A, Soller M (1994) Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus. Genetics 138:1365-1373


Evans JR, Vellen L (1996) Wheat cultivars differ in transpiration efficiency and CO₂ diffusion inside their leaves. In Crop Research in Asia: Achievements and


Herrera-Foessel SA, Lagudah ES, Huerta-Espino J, Hayden MJ, Bariana HS, Singh D, Singh RP (2011a) New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor Appl Genet 122:239-249


resistance to Ug99 stem rust in Canadian wheat cultivars ‘Peace’ and ‘AC Cadillac’. Theor Appl Genet 122:143-149


Joshi LM, Palmer LT (1973) Epidemiology of stem, leaf and stripe rusts of wheat in northern India. Plant Dis Rept 57:8-12

Kerber ER, Dyck PL (1990) Transfer to hexaploid wheat of linked genes for adult-plant
leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops*
*speltoides* × *Triticum monococcum*. Genome 33:530-537

Khan HM, Bukhari A, Dar ZA, Rizvi SM (2013) Status and strategies in breeding for

Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet
111:846-850

L, Jarlan L (2011) Water use efficiency and yield of winter wheat under

components and some quality traits of durum wheat (*Triticum turgidum* ssp.

Introgression and characterization of a goat grass gene for a high level of
resistance to Ug99 stem rust in tetraploid wheat. G3 2:665-673

34:435-455

Kolmer JA (2003) Postulation of leaf rust resistance genes in selected soft red winter
wheat. Crop Sci 43:1266-1274

Agirc Res 58:631-638


located on chromosome 1RS of rye using wheat-rye translocation lines. Theor Appl Genet 104:1317-1324


McIntosh RA, Arts CJ (1996) Genetic linkage of the Yr1 and Pm4 genes for stripe rust and powdery mildew resistance in wheat. Euphytica 89:401-403


Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc National Academic of Science USA, pp 88:9828-9832

Milus EA, Kristensen K, Hovmoller MS (2009) Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp *tritici* causing stripe rust of wheat. Phytopathol 99:89-94


Perwaiz MS, Johnson R (1986) Genes for resistance to yellow rust in seedlings of wheat cultivars from Pakistan tested with British isolates of *Puccinia striiformis*. Plant Breed 97:289-296


rust resistance gene (Sr52) effective against race Ug99 from *Dasypyrum villosum* into bread wheat. Theor Appl Genet 123: 159-167


Singh D, Park RF, McIntosh RA (2001) Postulation of leaf (brown) rust resistance genes in 70 wheat cultivars grown in the United Kingdom. Euphytica 120:205-218

Singh D, Park RF, McIntosh RA, Bariana HS (2008) Characterisation of stem rust and stripe rust seedling resistance genes in selected wheat cultivars from the United Kingdom. J Plant Pathol 90 (3):553-562


Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. Phytopathol 105:872-884


appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. Plant, Cell and Environ 32:448-464


Zakari A, McIntosh RA, Hovmoller MS, Wellings CR, Shariflou MR, Hayden M, Bariana HS (2003) Recombination of *Yr15* and *Yr24* in chromosome 1BS. In


## Appendix 1

### Supplementary Table 1

Wheat landraces used in the experiment including countries where they were collected and the Aridity indices, where available.

<table>
<thead>
<tr>
<th>Accession Number</th>
<th>Country Collected from</th>
<th>Rainfall Max. Temperature</th>
<th>Min. Temperature</th>
<th>Potential evapotranspiration</th>
<th>Aridity index</th>
<th>Aridity Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aus26438</td>
<td>Cyprus</td>
<td>977.52</td>
<td>20.58</td>
<td>11.39</td>
<td>914.49</td>
<td>1.069</td>
</tr>
<tr>
<td>Aus26491</td>
<td>Greece</td>
<td>346.09</td>
<td>25.85</td>
<td>10.64</td>
<td>1348.3</td>
<td>0.257</td>
</tr>
<tr>
<td>Aus26526</td>
<td>Morocco</td>
<td>256.49</td>
<td>30.84</td>
<td>16.15</td>
<td>2060.7</td>
<td>0.124</td>
</tr>
<tr>
<td>Aus26667</td>
<td>Tunisia</td>
<td>693.6</td>
<td>23.27</td>
<td>11.55</td>
<td>1195.4</td>
<td>0.58</td>
</tr>
<tr>
<td>Aus27279</td>
<td>Afghanistan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27294</td>
<td>Algeria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27315</td>
<td>Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27355</td>
<td>Myanmar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27356</td>
<td>Myanmar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27362</td>
<td>Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27374</td>
<td>China</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27382</td>
<td>China</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27419</td>
<td>China</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27471</td>
<td>Yugoslavia</td>
<td>922.65</td>
<td>16.33</td>
<td>6.13</td>
<td>698.96</td>
<td>1.32</td>
</tr>
<tr>
<td>Aus27835</td>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27852</td>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27856</td>
<td>Pakistan</td>
<td>79.51</td>
<td>30.03</td>
<td>13.03</td>
<td>1774.2</td>
<td>0.045</td>
</tr>
<tr>
<td>Aus27858</td>
<td>Pakistan</td>
<td>79.86</td>
<td>30.36</td>
<td>13.03</td>
<td>1797.3</td>
<td>0.044</td>
</tr>
</tbody>
</table>

171
<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
<th>Area</th>
<th>Population</th>
<th>GDP</th>
<th>GDP PPP</th>
<th>Climate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aus27873</td>
<td>Russian Federation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus27878</td>
<td>Italy</td>
<td>921.53</td>
<td>17.7</td>
<td>8.79</td>
<td>759.77</td>
<td>Humid (0.75 - 1)</td>
</tr>
<tr>
<td>Aus27894</td>
<td>Spain</td>
<td>431.24</td>
<td>18.25</td>
<td>6.8</td>
<td>1316.4</td>
<td>Semi-arid (0.2 - 0.5)</td>
</tr>
<tr>
<td>Aus27899</td>
<td>Morocco</td>
<td>634.74</td>
<td>24.8</td>
<td>12.89</td>
<td>1377.6</td>
<td>Semi-arid (0.2 - 0.5)</td>
</tr>
<tr>
<td>Aus27940</td>
<td>Poland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus28009</td>
<td>Egypt</td>
<td>55.98</td>
<td>28.16</td>
<td>15.28</td>
<td>1645.5</td>
<td>Arid (0.03 - 0.2)</td>
</tr>
<tr>
<td>Aus28015</td>
<td>Union of Soviet Socialist</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Republics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus28082</td>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus28096</td>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus28112</td>
<td>Iraq</td>
<td>444.15</td>
<td>28</td>
<td>12.41</td>
<td>1593.9</td>
<td>Semi-arid (0.2 - 0.5)</td>
</tr>
<tr>
<td>Aus28119</td>
<td>Morocco</td>
<td>372.49</td>
<td>22.3</td>
<td>13.38</td>
<td>1200.9</td>
<td>Semi-arid (0.2 - 0.5)</td>
</tr>
<tr>
<td>Aus28124</td>
<td>Morocco</td>
<td>709.9</td>
<td>24.19</td>
<td>12.49</td>
<td>1335.5</td>
<td>Sub-humid (0.5 - 0.75)</td>
</tr>
<tr>
<td>Aus28125</td>
<td>Morocco</td>
<td>709.9</td>
<td>24.19</td>
<td>12.49</td>
<td>1335.5</td>
<td>Sub-humid (0.5 - 0.75)</td>
</tr>
<tr>
<td>Aus28127</td>
<td>Morocco</td>
<td>821.42</td>
<td>22.04</td>
<td>8.17</td>
<td>1326.3</td>
<td>Sub-humid (0.5 - 0.75)</td>
</tr>
<tr>
<td>Aus28177</td>
<td>Iran</td>
<td>335.44</td>
<td>32.28</td>
<td>16.87</td>
<td>1982.8</td>
<td>Arid (0.03 - 0.2)</td>
</tr>
<tr>
<td>Aus28183</td>
<td>Iran</td>
<td>1462.1</td>
<td>20.55</td>
<td>10.87</td>
<td>963.01</td>
<td>Humid (0.75 - 1)</td>
</tr>
<tr>
<td>Aus28230</td>
<td>Turkey</td>
<td>611.77</td>
<td>22.63</td>
<td>10.82</td>
<td>1134</td>
<td>Sub-humid (0.5 - 0.75)</td>
</tr>
<tr>
<td>Aus28245</td>
<td>Bulgaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aus28254</td>
<td>Greece</td>
<td>898.71</td>
<td>21.23</td>
<td>12.88</td>
<td>1006.5</td>
<td>Humid (0.75 - 1)</td>
</tr>
</tbody>
</table>
Appendix 2

Supplementary Table 2 Response of selected wheat genotypes under well-watered and drought-stress conditions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Photosynthesis (A)</th>
<th>Stomatal conductance ($g_s$)</th>
<th>Mesophyll conductance ($g_m$)</th>
<th>WUE (A/$g_s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>watered</td>
<td>drought</td>
<td>%red.</td>
<td>watered</td>
</tr>
<tr>
<td>aus28112</td>
<td>30.11</td>
<td>23.44</td>
<td>22</td>
<td>0.60</td>
</tr>
<tr>
<td>aus28119</td>
<td>28.61</td>
<td>25.21</td>
<td>12</td>
<td>0.60</td>
</tr>
<tr>
<td>aus28230</td>
<td>31.49</td>
<td>24.69</td>
<td>22</td>
<td>0.57</td>
</tr>
<tr>
<td>Sunpict</td>
<td>32.88</td>
<td>22.13</td>
<td>33</td>
<td>0.67</td>
</tr>
<tr>
<td>Yitpi</td>
<td>32.64</td>
<td>27.27</td>
<td>33</td>
<td>0.60</td>
</tr>
<tr>
<td>LSD</td>
<td>2.05</td>
<td>2.48</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CV%</td>
<td>15</td>
<td>23</td>
<td>30</td>
<td>56</td>
</tr>
</tbody>
</table>

ns : non-significant
% red: Percentage reduction under drought stress
% incr: an increase in WUE under drought stress
LSD : Least significant difference;
CV (%) : Coefficient of variation