The Faculty of Landscape Planning, Horticulture and Agricultural Sciences
Självständigt arbete vid LTJ-faculteten, SLU, Alnarp
Master thesis in Biology, Plant Breeding Programme
Alnarp, 2012

Isolation and evaluation of different wheat-rye translocation lines obtained from a disease resistant double translocation line with 1BL/1RS and 2RL/2BS

Mahbubjon Rahmatov

SLU, Swedish University of Agricultural Sciences
The Faculty of Landscape Planning, Horticulture and Agricultural Sciences
The Department of Plant Breeding and Biotechnology

Author	**Rahmatov Mahbubjon**
Title in English	**Isolation and evaluation of different wheat-rye translocation lines obtained from a disease resistant double translocation line with 1BL/1RS and 2RL/2BS**
Title in Swedish	**Isolering och utvärdering av olika vete-råg translokerade linjer som erhållits från en sjukdomsresistent dubbeltranslokerad linje innehållande 1BL/1RS och 2RL/2BS**
Keywords	**C-banding, coleoptile colour, resistance, Simple Sequence Repeats, stem rust, translocation, wheat-rye, yellow rust**
Supervisor	**Professor Eva Johansson, Swedish University of Agricultural Sciences, Department of Agrosystems**
Co-supervisors	**Professor Hafiz Muminjanov, Tajik Agrarian University** **Dr. Larisa Gustavsson, Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology**
Examiner	**Professor Tomas Bryngelsson**
Programme	**Plant Breeding**
Course title	**Degree Project for Msc Thesis in Plant Breeding**
Course code	**IN0813**
Subject	**Biology**
Credits	**60 HEC**
Level	**A2E**

Självständigt arbete vid LTJ-faculteten, SLU
Alnarp, 2012

Dedication to the memory of the late Professor Arnulf Merker and my Grandmother

ACKNOWLEDGEMENT

I am very thankful to Allah the Almighty who enabled me to carry out this study. I would like to express my sincere appreciation and deep gratitude to my supervisors Professors Eva Johansson, [Arnulf Merker], Hafiz Muminjanov and Dr. Larisa Gustavsson, for their support, scientific advice and this opportunity. To Professor Eva Johansson for the excellent suggestions, corrections of the thesis, comments during write-up of the thesis and for fruitful discussions which are greatly acknowledged. I am very grateful to Dr. Mogens Hovmøller from Global Rust Reference Center, Aarhus University based in Flakkebjerg research station in Slagelse for his kind help and teaching seedling resistance test on yellow rust. I am thankful to Dr. Nazari Kumarse and pathology department in ICARDA for their teaching and explaining regarding ICARDA works for wheat rust diseases. I am also thankful to CIMMYT-Kenya and Kenyan Agricultural Research Institute for kindly helping and conducting the adult plant resistance test of my materials to stem rust disease race Ug99 and also for inviting to the training course in Kenya. My deepest thanks to Arne Hede for his excellent teaching and explaining the wheat breeding in the field, and I am sure that we have had a great selection for further developing of new wheat varieties in Tajikistan. I am very grateful to Rutger Persson for his kind support in Tajikistan and in Sweden, also for fruitful collaboration with Svalöf Consulting and Sida Seed Project in Tajikistan. I also greatly acknowledge Dr. Bernd Pett for collaboration and teaching me about plant diseases and weeds during the field trip in Tajikistan.

I have got my MSc scholarship from the project supported by Sida, in cooperation on higher education between Tajik Agrarian University and Swedish University of Agricultural Sciences. I would like to acknowledge Professors [Arnulf Merker] and Usmon Mahmadyorov for their contribution and support in getting this opportunity.

I express deep thanks to Ann-Sofie Fält as my Swedish mother for her invaluable technical assistance in the laboratory and kind explanation about lab techniques, also for taking care and harvesting of my plants in the greenhouse. I also appreciate the help of Ann-Charlotte Strömdahl during molecular works. I am thankful to Dr. Mulatu Dida Geleta for useful and valuable

discussions. In particular, I am grateful to Professor Heneen Waheeb for kindly advising and explaining during cytological analysis.
I am very thankful to Tomas Bryngelsson and Carina Larsson for their excellent support for my studies at SLU. Special thanks to all teachers, namely Li-Hua Zhu, Sten Stymne, Anders Carlson, Agnese Kolodinska-Brantestam, Helena Persson, Annelie Ahlman, Jan Eric Mattsson, Jan-Eric Englund and other teachers for excellent lectures, laboratory practical work, for sharing their knowledge and experience on plant breeding and biotechnology. I also want to thanks Jonas Hansson, for his technical support.

Thanks also to Helen Lindgren, Maria Luisa Prieto-Linde, Anna Zborowska, for their kind cooperation and assistance during of my study. I feel great pleasure to express my appreciation to my friends: Carlos Henry Loaisiga, Bahrom Huseinov, Dickson Ng'uni, Sergey Hegay, Marufkul Makhkamov, Therese Bengtsson, Svetlana Leonova, Anders Smolka, Mehboob Alam, Ali Hafeez Malik, Birjan Usubaliev, Rui Guan, Therese Bengston, Firuz Odilbekov, Masoud Ahmadi Afzadi, Mbaki Muzila, Ida Lager, Mohammed Elsafi, Leonardo Crespo, Maksat Amanov, Rui Guan, Staffan Andersson, Tiny Motlhaodi, Isabel Herrera, Busisiwe Nsibande, and all other people for their cooperation and discussion during that time.

Finally, I would like to express my deep love and thanks to my family, my Mom, Daddy, sister, brothers, my wife Makhfirat and children's Muhayokhon and Mahmudjon and other family members who prayed for me and also for their constant emotional support.

ABSTRACT

Wheat-rye translocations involving 1RS and 2RL of rye are the most useful sources of genes for disease resistance in wheat breeding. Rye genes are known to control resistance to biotic and abiotic stresses. Wheat-rye translocations have been widely used by breeders all over the world because genes located on translocated chromosome arms or fragments from the rye genome can determine a number of useful traits in wheat, such as high yield, wide adaptation, diseases and pest resistance.

The wheat-rye translocation lines used in this study were derived from a cross between the Swedish bread wheat variety Topper and the line KR99-139 being homozygous for the two different wheat-rye translocations 1BL/1RS and 2RL/2BS. BC_1F_1 materials were obtained through one back-cross with either the line KR99-139 or the variety Topper. Thereafter, BC_1F_2 and BC_1F_3 were obtained by once and twice selfing. In the obtained material, it was thereafter possible to define four different possible homozygous translocation combinations. Thus, lines containing both 1RS and 2RL translocations, containing only 1RS or 2RL and without any translocation were identified.

For identification of the four possible homozygous wheat-rye translocation lines mentioned above, three different methods were used. First, lines of different types were characterized and isolated based on a phenotypical marker, i.e. if the plant showed red or green coleoptile colour. Plants with homozygous presence of 2RL were known to develop red coleoptile, as a gene for red coleoptile has been verified to be present at 2RL in these lines. The analyses of coleoptile colours were done in the BC_1F_2 (obtained from selfed BC_1F_1 lines determined by molecular markers at BAZ, Germany to be 1RS– –/2RL+–) and BC_1F_3 (obtained from the BC_1F_2 lines having a red coleoptile) wheat-rye translocation lines, where the variety Topper had been used for backcrossing. Moreover, the BC_1F_2 (obtained from selfed BC_1F_1 lines determined by molecular markers at BAZ, Germany to be 1RS++/2RL+–) wheat-rye translocation lines for which the KR99-139 line was used for backcrossing, was selfed, and analyses of coleoptile colours were done in the BC_1F_3 (on a representative sample of all combinations of presence and absence of 2RL). The results from the coleoptiles colour analyses generally showed that it was possible to distinguish lines having 2RL++ (red coleoptiles) and 2RL– – (green coleoptiles).

Plants having 2RL+– were sometimes classified as having green and sometimes as having red coleoptiles. Therefore, if coleoptiles colour is going to be used for selection of lines with presence/absence of 2RL in homozygous form, at least two generations have to be analyzed and lines not segregating in either of the analyses can be judged as being homozygous as related to their coleoptiles colour. For identification of lines with presence of heterozygous 1RS+– and homozygous 2RL++ rye chromosome the Giemsa C-banding technique was used. The Giemsa C-banding techniques on the BC_1F_3 segregating population generally resulted in well-defined sharp, distinct bands in the wheat-rye translocation lines and both the rye chromosome arms, 1RS and 2RL were identified. Additionally, five microsatellite (SSR) markers SCM9, SCM39, SCM43, SCM69 and SCM75 were used for verification of the presence of 1RS and 2RL. Among the five SSR markers, SCM9 and SCM75 resulted in reliable amplification of expected products, 220 bp and 191 bp respectively. The line KR99-139 containing both 1RS and 2RL showed correct amplification products with both mentioned primers while the bread wheat variety Topper without any rye chromosome showed no amplification with both SSR primers pairs.

Resistance towards yellow rust and stem rust were evaluated through seedling resistance test in the greenhouse (Global Rust Reference Center, Denmark) to *Puccinia striiformis*, and adult plant resistance to *Puccinia graminis*, race Ug99 (TTKSK) in Njoro, Kenya. For the seedling resistance test, pathogenicity of 17 races/isolates of yellow rust was used. The BC_1F_3 which carries combination of 1RS++/2RL++, 1RS++/2RL+– and the KR99-139 were found to be highly resistant to some races/isolates whether the variety Topper was fully susceptible to all races/isolates. The results showed *Yr9* to be one possible gene that could be responsible for the obtained yellow rust resistance. However, the results were not that clear so than not other possible genes could also be an alternative. For adult plant resistance towards Ug99, a total of 28 of the BC_1F_3 wheat-rye translocation lines and their parents were evaluated in the field of Njoro, Kenya. The results indicated that out of the 30 tested lines 20 were susceptible, 8 moderately susceptible to susceptible and in 2 lines the resistance to Ug99 was identified. The two BC_1F_3 wheat-rye translocation lines that were found to be resistant towards Ug99 were both being homozygous for 1RS++ and heterozygous for 2RL+–. Thus, these results indicated presence of several genes/QTLs controlling resistance indicating possible epistatic effects of the genes involved. The lines identified as resistant will be utilized in combination with Tajik germplasm

to develop a mapping population for determining the underlying basis of resistance. To summarize results from the research outlined in this thesis indicate that wheat-rye translocation lines and used methods can be highly relevant for wheat breeding programs and further research.

Keywords: C-banding, coleoptile colour, resistance, Simple Sequence Repeats, stem rust, translocation, wheat-rye, yellow rust

LIST OF ABBREVIATIONS

APR – Adult Plant Resistance

BC – Backcross

CIMMYT – International Maize and Wheat Improvement Center

CWANA – Central West Asia and North Africa

DNA – Deoxyribonucleic Acid

FAO – Food and Agriculture Organization

GRRC – Global Rust Reference Center

HCL – Hydrochloric Acid

ICARDA – International Center Agricultural Research on Dry Areas

IWWIP – International Winter Wheat Improvement Program

OECD – Organization for Economic Cooperation and Development

OSU – Oklahoma State University

PCR – Polymerase Chain Reaction

SSC – Saline Sodium Citrate

SLU – Swedish University of Agricultural Sciences

SSR – Simple Sequence Repeat

USDA – United States Department of Agriculture

CONTENTS

Acknowledgement…….. 4

Abstract…….. 6

List of Abbreviations…… 9

CHAPTER I…….. 12

INTRODUCTION…… 12

1. Wheat in Tajikistan…… 14

1.1. Wheat cultivation in Tajikistan…… 15

1.2. Wheat diseases…… 17

1.3. Breeding strategy of wheat in Tajikistan; History and at present…… 20

2. Wheat taxonomy and origin…….. 23

3. Genome of bread wheat……. 24

4. The rye genome in wheat breeding and sources of resistance genes……. 26

5. Breeding methodology…… 28

6. Backcrossing method…….. 29

7. Single seed descent method…… 29

8. The crossing strategy of Topper x KR99-139 and backcrossing of KR99-139 x F_1 and Topper x F_1……... 30

9. Research aims of the study……... 32

CHAPTER II…… 33

APPLICATION OF DIFFERENT TECHNIQUES: SELECTION OF RED AND GREEN COLEOPTILES, GIEMSA C-BANDING AND SIMPLE SEQUENCE REPEATS (SSR) MARKERS, FOR IDENTIFICATION AND ISOLATION OF WHEAT LINES WITH DIFFERENT COMBINATIONS OF RYE TRANSLOCATIONS (1RS AND 2RL)…… 33

INTRODUCTION……. 33

MATERIALS AND METHODS……. 35

Plant Materials…….. 35

Determination of red and green coleoptiles…… 35

Cytological analysis with Giemsa C-banding techniques…….. 36
Pretreatment…….. 36
Preparation of the slides……... 37
Acid and Basic treatment……. 37
Staining…….. 37
Mounting…… 37
DNA Extraction……. 37
RESULTS…….. 40
Screening of homozygous presence of 2RL through the use of red/green coleoptile selection in BC_1F_2 and BC_1F_3 wheat-rye translocation segregating population…… 40
Identification of 1RS and 2RL with Giemsa C-banding techniques……. 43
Verification of 1RS and 2RL with SSR markers……. 45
DISSCUSSION……. 46

CHAPTER III……. 48
YELLOW RUST SEEDLING RESISTANCE AND ADULT PLANT RESISTANCE TO STEM RUST IN THE WHEAT-RYE TRANSLOCATION LINES AND THEIR PARENTS……. 48
INTRODUCTION…….. 48
MATERIAL AND METHODS…… 49
Plant materials……. 49
Seedling resistance test to yellow rust……. 50
Adult plant resistance to stem rust……. 51
RESULTS…….. 54
Seedling resistance test for yellow rust in BC_1F_3 and their parents……. 54
Adult plant resistance to stem rust Ug99 race……... 55
DISCUSSION…… 57
CONCLUSIONS…… 59
FUTURE RECOMMENDATIONS……. 60
REFERENCES…….. 61

CHAPTER I

INTRODUCTION

Wheat is one of the major and most important staple food crops of the world, grown in over 225 million hectares of land, thereby occupying the largest amount of the crop acreage worldwide (Pagesse, 2001; USDA, 2009). Totally, wheat is producing 683 million metric tons with an average yield of 3 t/ha (USDA, 2009). Therefore, wheat is the principal cereal grain crop used for food consumption in most developed and developing countries of the world. Thus, wheat is feeding a high proportion of the world population, also contributing to a high amount of the total food calories and protein required for human nutrition (Pagesse, 2001). Actually, wheat is the main source of calories for a large proportion of people worldwide (USDA, 2009). Wheat growing was part of the evolution that converted men from being hunters and food gatherers to become farmers. Cultivation of wheat is as old as the ancient civilizations of Babylonia, Egypt, Greece and Rome (Pagesse, 2001). Wheat belongs to the genus *Triticum* that comprises about 500 species. The two main commercial types of wheat is durum (*Triticum durum* 2 = 4x = 28) and common/bread (*Triticum aestivum* 2 = 6x = 42) wheat, the latter being the more widely grown. Globally 95% of wheat production is bread wheat (*Triticum aestivum*), while the tetraploid durum wheat (*Triticum durum*) is 5% (Peng, et al., 2011).

With the continuous growth of the world population, the demands for food production will continually be expanding. The demand for wheat is expected to increase faster than the demand of any other major crop such as rice and maize. To keep pace with the anticipated growth of the human population, the predicted demand of wheat production for the year 2018 is projected to reach 722 million tons. However, production of coarse grains is projected to reach 1 284 million tons by 2018, despite the fact that the yield growth is expected to be lower than the yield growth observed during the last ten years. If this scenario will be fulfilled, coarse grain production will be more than 16% above that of 2008 (OECD-FAO, 2009). In the future, the nutritional composition of the world wheat supply will become even more critical due to the fact that the world demand for wheat as a calorie and protein source will continue to grow simultaneously, as the world wheat stocks might continue to decrease as has happened recently. The world population which currently stands at well over 7 billion people is expected to reach 9 billion by 2050 (OECD-FAO, 2009).

Wheat yield is influenced by a large number of different abiotic and biotic factors. The biotic factors, mostly fungal pathogens, are the major cause of yield loss in wheat production and attacks from fungal pathogens often also decrease bread-making quality. Dependent on the specific environment for wheat production, the presence of specific pathogens as well as the extent of damage from these pathogens may vary. The most important fungal diseases are yellow, leaf and stem rusts, powdery mildew and tan spot (Duveiller et al., 2005; Reynolds and Borlaug, 2006). The wheat gene pool contains a large variety of resistant germplasm against specific pathogens and improving disease resistance is a highly relevant goal in many breeding programs. Naturally occurring resistance, particularly complete resistance against biotrophic pathogens, is frequently based on single, race specific resistance genes which can be durable, but are frequently broken by new, virulent pathogen races (Dvorak et al. 1998). Introgression of resistance genes can be made possible by transfer of genes from other related crops into the wheat genome. A large number of translocation lines (that have exchanged genetic material with another species) containing alien genes for resistance to various abiotic and biotic stresses have been developed (Hysing et al., 2007).

The majority of alien chromosome segments included in the wheat genome, conferring disease and pest resistance to wheat, have been derived from species of the genus *Aegilops*, two species of perennial wheat grasses, and from rye (Friebe, 1996). Specifically, the 1RS and 2RL rye chromosomes have long been utilized by wheat breeders around the world as sources of genes for resistance to diseases and pests (Lee et al., 1996; Rabinovich, 1998).

Future wheat breeding programs will need to focus on a number of traits in wheat that will ensure superior varieties with high and stable yield potential in commercial production. Examples of traits that will ensure superior varieties include: redesigning the entire wheat plant to ensure efficient management of water and drought in dry areas, superior systems of uptake and translocation of nutrients, adaption to conservation tillage practices, durable and multiple disease resistance, as well as discovery and assembly of hybrid vigor (Rajaram, 2001). Traditional plant breeding tools are still useful to perform the needed breeding. However, the traditional methods have to be combined and used in association with modern plant breeding techniques such as double haploid lines production and marker assisted selection (William et al., 2007).

WHEAT IN TAJIKISTAN

Wheat is one of the most significant crops in Tajikistan and it is critically important as a food supply. Wheat contributes substantially to the national food security by providing about 65% of the calories to the people in Tajikistan (Muminjanov, 2003). The gross grain production has grown considerably since 1995 and the increasing growth trend remains. According to the Ministry of Agriculture the annual production of grain in 2009 was about 1 million tons, including 760 thousand tons of wheat (Figure 1). The increase in grain production has been achieved by extension of the acreage of the cereal crops as well as increased yield. However the yield has remained relatively low because of lack of improved varieties, poor quality seed, lack of inputs (fertilizer, chemicals etc.), presence of diseases, pests and weeds, poor crop management and lack of modern field equipment (Muminjanov et al., 2008).

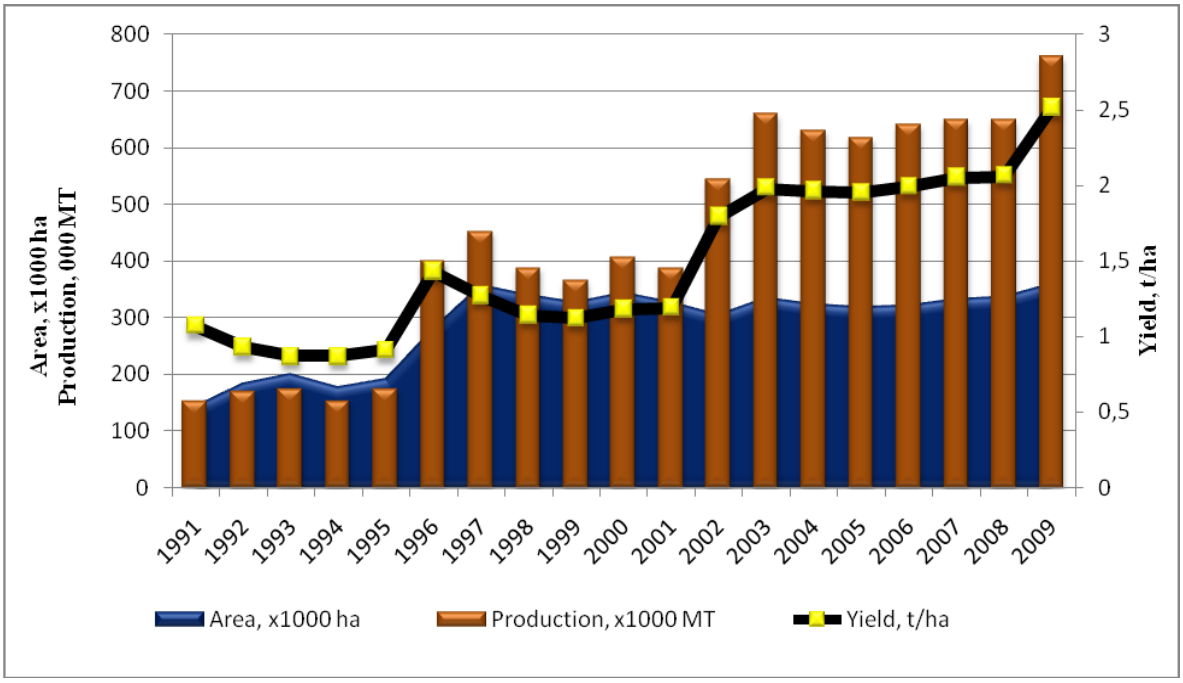

Figure 1. Trends on wheat production in Tajikistan 1991-2009 (The Statistical Collection, 2009)

Any further expansion of arable land is increasingly difficult due to limited land resources and lack of water for irrigation. Therefore, for further increase in grain production an increase in crop production per unit area is required. Thus, improvement and introduction of new high yielding varieties resistant to diseases, pests and unfavourable conditions (drought, high temperature etc.) is needed together with a more intensive utilization of irrigated land for the cultivation of a second crop during the summer time.

WHEAT CULTIVATION IN TAJIKISTAN

Wheat (*Triticum aestivum*) is one of the most important cultivated crops in Tajikistan, occupying 45% of the crop acreage. Today, wheat is grown on more land area, over 350 thousand ha, than any other commercial crop and continues to be the most important food grain source for the population (The Statistical Collection, 2009). In order to meet the rapid population increase in the country, wheat grain production must increase with an annual rate of 2%, without any additional area of land becoming available for this crop. In order to meet this challenge, a new level of understanding of the structure and function of the wheat breeding and cultivation is required, together with development of the local wheat breeding program (Muminjanov, 2003; 2008).

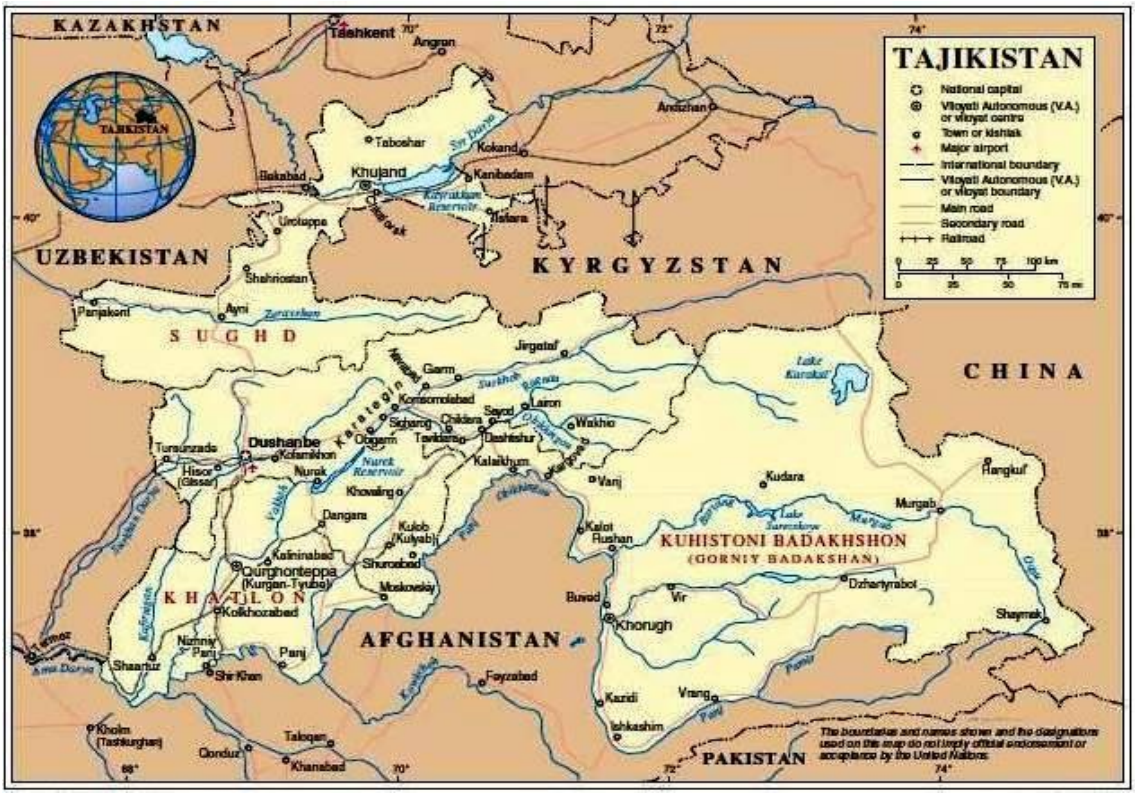

Figure 2. Map of Tajikistan with different regions (Source: www.untj.org)

In an agrarian country, with limited land resources, use of each hectare of land for food production is considered as the sacred duty. Therefore, after the country became independent the agricultural policy of the Government has been to achieve food security in general and of grain independence in particular. Grain-crops are grown almost all over the country and are considered to be the basic food crops (Muminjanov, 2003; Pett and Muminjanov, 2004). However, the greatest part of the grain

production area in Tajikistan is located in Khatlon, which stands for 55-60% of the total production in the country. The contribution from the Sughd region is 20-22% of the wheat production, while Central of Tajikistan produces 17-19% and Badakhshan 2-4% (Figure 2). The area sown by wheat has increased in Dehkan farms and households while the sowing area has decreased in State farms (Figure 3) (The Statistical Collection, 2009).

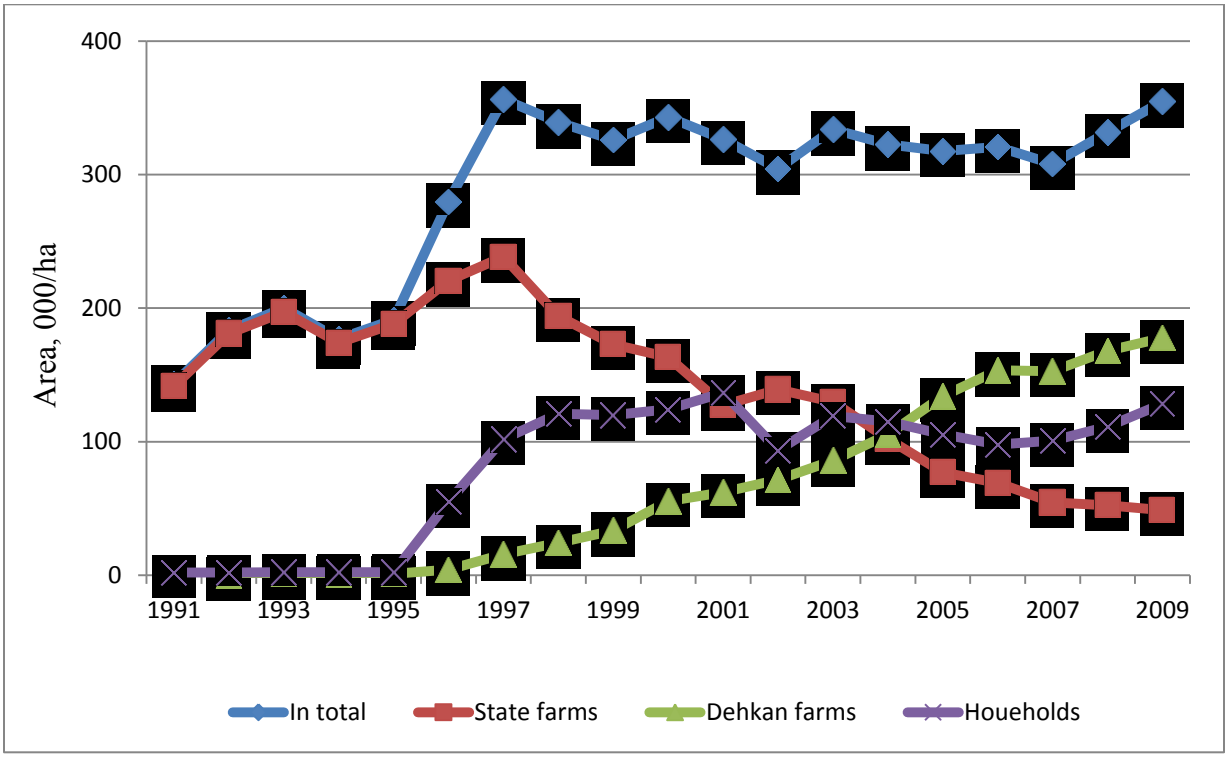

Figure 3. Sowing areas of wheat in Tajikistan during 1991-2009 (Statistic Yearbook, 2009)

Cultivation of grain-crops is considered to be the main condition to sustain food security of the households in Tajikistan. Wheat is grown in valley areas both by irrigation and on rain fed field. The yield from the part of lands allocated for wheat production under rain fed conditions is mainly attributed to the level of precipitation. Frequently droughts and hot winds reduce productivity of the wheat strongly in rain fed field regions. For example in 2000 and 2001 drought caused a huge damage to grain grown in Tajikistan (Muminjanov, 2003; 2008).

WHEAT DISEASES

Bread wheat is sensitive towards different pathogens and pests. Wheat diseases such as yellow rust, leaf rust, stem rust, tan spot, powdery mildew, common bunt, loose smut etc. reduce grain quality and final yield seriously, resulting in a decreased financial return to the farmers (Saari and Prescot, 1985; Ensermu et al. 1998).

Wheat rusts are important foliar diseases of wheat worldwide, causing extensive losses and damage to the wheat produced in the world (Singh et al., 1992). There are three types of wheat rusts, namely yellow rust (*Puccinia striiformis*), stem rust (*Puccinia graminis*) and leaf rust (*Puccinia triticina*). The rust diseases all produce similar disease symptoms on their host plants and mostly have similar optimal conditions for infection (Marsalis and Goldberg, 2006). Other common foliar diseases are powdery mildew and tan spot. Powdery mildew is caused by the fungus *Blumeria graminis* (Murray et al., 1998), and tan spot is caused by the fungus *Pyrenophora tritici-repentis* (*Drechslera tritici-repentis*). Tan spot also occurs in many other species of native and cultivated grasses (Ali and Francl, 2003). Loose smut caused by *Ustilago tritici* and common bunt caused by *Tilletia tritici* are examples of fungal seed borne diseases in wheat. Loose smut is one of the most distinct and evident wheat diseases. It appears throughout the wheat growing areas in the world. The yield losses from loose smut depend usually on the incidence of infected spikes in the field (Knox et al., 2002). Common bunt is also known as stinking smut. The symptom of common bunt cannot be obviously detected until the wheat spikes are fully mature and the common symptom of the disease is stunted growth of the infected plant together with a smell of fish (Curtis et al., 2002).

Rusts, in particular yellow rust, has become a serious disease, significantly decreasing grain yield in Tajikistan and many other countries around the world. Infections of yellow rust have been observed in all zones of Tajikistan both under irrigation and rainfed conditions (Rahmatov et al., 2008; 2010). The increase in yellow rust infection of wheat growing in the Central Asian countries has a direct relationship with the expansion of the wheat growing area in the region although existence of natural geographic barriers might limit the outbreaks. However, severe epidemics of yellow rust occur, in particular during years with high amount of precipitation and at such conditions the pathogen then damages wheat everywhere (Pett and Muminjanov, 2004). Yellow rust epidemics totally destroy the wheat plants and may lead to a decrease in wheat yield with up to 60-70% (Chen,

2005). Yellow rust has been a severe disease in Tajikistan during several years during the period 2003-2010, damaging varieties and advanced lines in the breeding nurseries (Figure 4). A decrease in the frequency of yellow rust was observed during the period 2003-2009 and might be explained by the development of more resistant wheat lines and varieties as compared to wheat grown during former years or due to dry of climatic condition. However in 2010 the level of yellow rust disease in wheat was again increased, probably due to weather conditions together with the spreading of a new race of the pathogen (Rahmatov 2011a). The severity of the yellow rust in 2010 resulted in that 70% of the breeding and advanced lines were damaged in three locations.

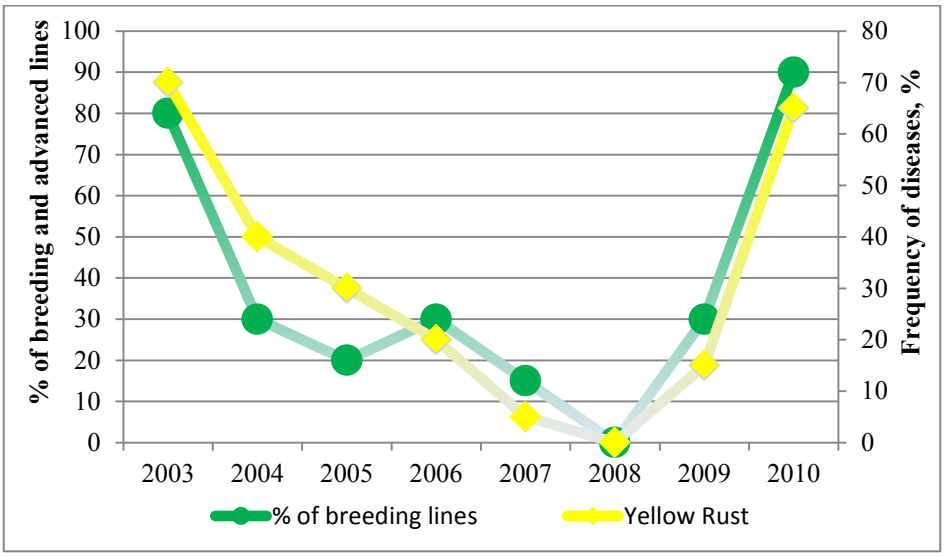

Figure 4. Epidemiology of yellow rust during 2003-2010 in breeding and advanced lines over three locations (Hissar, Sharora and Isfara) in Tajikistan

In 1998, severe stem rust infections were identified on wheat in Uganda, and a new race was observed and designated as Ug99 (Pretorius et al., 2000). The race Ug99 was subsequently detected in Kenya and Ethiopia in 2005 (Wanyera et al., 2006). It is predicted that the race Ug99 will migrate to North Africa, Middle East, Asia, and beyond. Thus, Ug99 is a huge challenge for wheat breeders, scientists and policy makers. There will be a need to identify resistant wheat material and genetic resources for Ug99, to develop new commercial wheat varieties, and replace most of the wheat varieties at present susceptible to Ug99 with new resistant varieties. For this reason, a number of widely grown varieties and advanced lines from the Tajik breeding program were sent to Kenya to be tested for stem rust against race Ug99. The results showed that 85 of the tested varieties and

advanced breeding lines were susceptible (S) to Ug99, 10 of the sent varieties and lines were moderately susceptible (MS), and only 5 of the advanced lines were moderately resistance (MR). None of the sent lines was resistant (R) to stem rust against race Ug 99 (Figure 5) (Rahmatov et al., 2011).

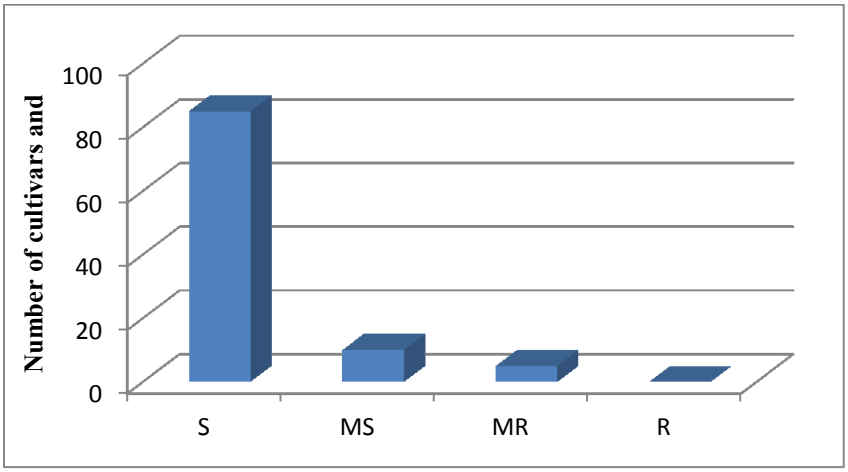

Figure 5. Resistance and susceptibility in a number of Tajik varieties and advanced lines being tested in Kenya against stem rust, race Ug99

During the time period (2003-2010) tan spot has been investigated in breeding and advanced lines in Tajikistan. Within this period, an increase of tan spot has been observed in all regions. Figure 6 shows the results of the survey related to presence of tan spot in breeding nurseries in three locations of Tajikistan.

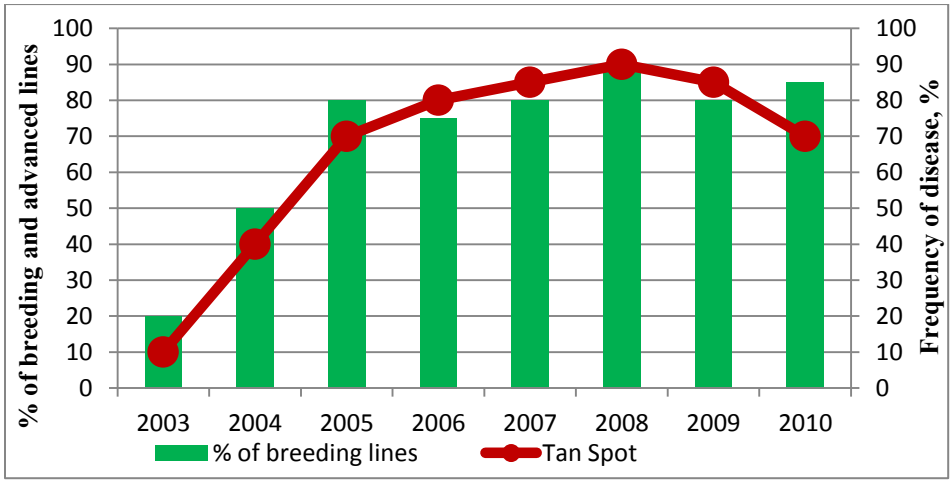

Figure 6. Severity of tan spot during 2003-2010 in breeding and advanced lines over three locations (Hissar, Sharora and Isfara) in Tajikistan

BREEDING STRATEGY OF WHEAT IN TAJIKISTAN; HISTORY AND AT PRESENT

Tajikistan has been described as one of the centers of origin and diversity of cereals (Vavilov, 1935). A number of wheat landraces are still being grown by small scale farmers in the mountain regions at altitudes up to 3000 meter above sea level. During the Soviet Union time, the main breeding resources went into cotton breeding and as a consequence wheat breeding was not well developed. During the period from 1955 to 1990 Tajik wheat breeders have submitted thirty-four wheat lines to official registration trials of which fifteen has been released (Muminjanov, 2003). However, due to a number of abiotic and biotic constraints, combined with lack of certified seed and modern crop production technologies, the wheat grain yield in Tajikistan is very low averaging 1,8-2,0 t/ha. The main objective of the National wheat breeding program is to obtain new wheat varieties with high grain yield, resistance to diseases and with good baking quality.

During later years, regional and international collaboration has been established within the National wheat breeding program. The objectives of these collaborations are to strengthen the national breeding program by germplasm exchange, research development, and information sharing. Linkage with the Turkey-CIMMYT-ICARDA International Winter Wheat Improvement Program (IWWIP) located in Turkey has been of high importance, contributing to the distribution of winter and facultative wheat to Tajikistan. Recently a joint breeding program has been established with Oklahoma State University (OSU) in which segregating F_2 nurseries from Oklahoma State University breeding program are distributed to Tajikistan for further selection. Advanced wheat lines selected by the public and private breeding sector are annually tested in Tajikistan in multi-location yield trials (Rahmatov et al., 2008; 2009). These yield trials are carried out in two private farms and one public Institution in Tajikistan (Figure 7);

- North – Seed Production Farm “Chilgazi” of Isfara district;
- Central – Scientific Research Farming Institute in Hissar district;
- Central – Seed Production Farm “L. Murodov” of Hissar district.

The annual testing was initiated through the National Wheat Breeding and Seed Multiplication Program within the GTZ/CIMMYT program, and has since then continued through the Sida funded Seed Industry Development Project. A number of advanced lines and varieties have been identified as high yielding, rust resistant and with good bread making quality. The best of those lines are

submitted for official variety testing and release in Tajikistan (Rahmatov et al., 2008; 2009; Eshonova et al., 2010).

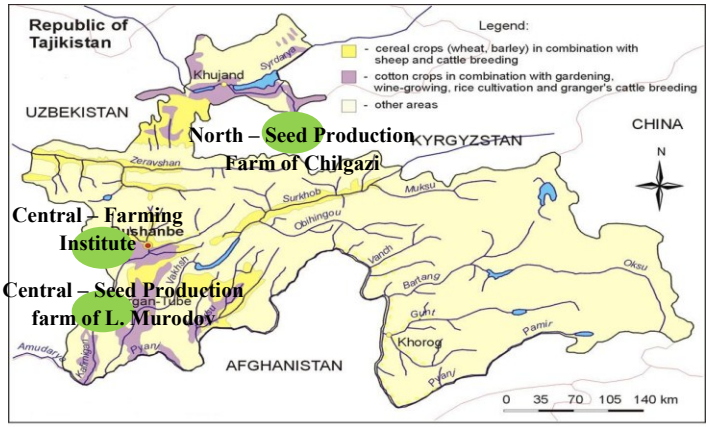

Figure 7. Map of Tajikistan with locations for breeding trials (Source: www.untj.org)

The wheat breeding in Tajikistan has progressed rapidly since the collaboration with international centers and donors was started. The breeding and selection strategies applied in the Tajik wheat breeding program are described in figure 8. The main focus of the selections in the wheat breeding program is to improve diseases and pest's resistance, yield, early maturation, quality and agronomic performance.

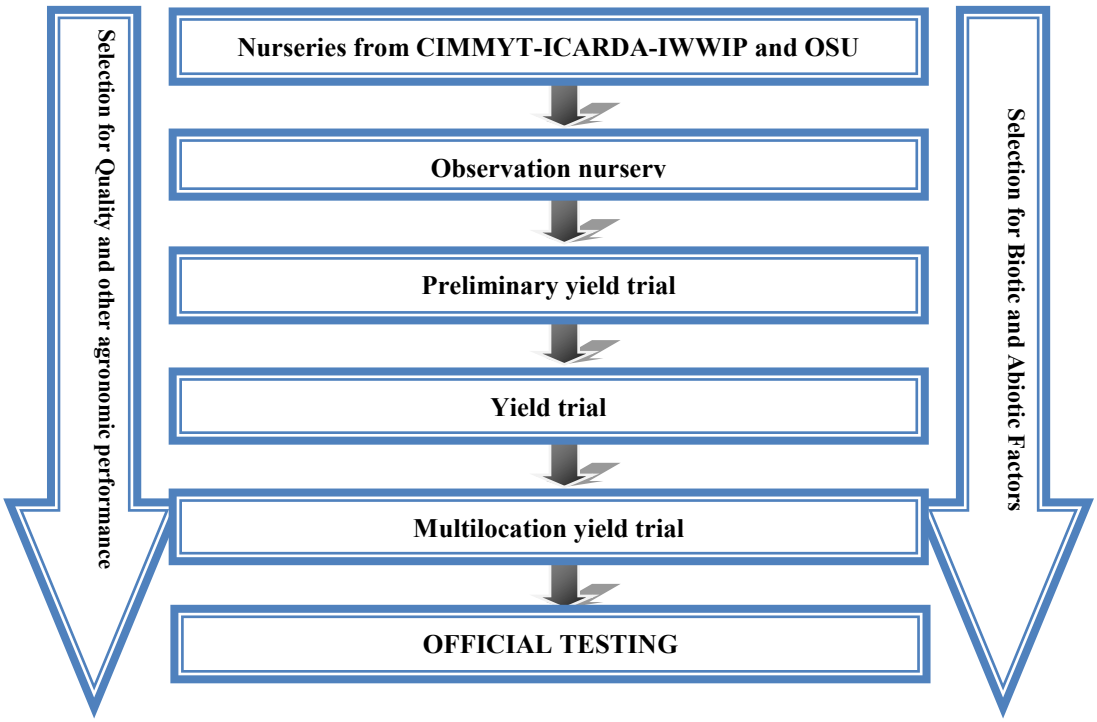

Figure 8. Breeding methods and selections within the Tajik breeding programs

A number of varieties have already been released and are at present grown by Tajik farmers. In 2007 two varieties, Alex and Norman, were released in Tajikistan and in 2008 further two varieties, Ormon and Somoni, were released. The additional eight varieties that are presented in Table 1 are at present (2010) tested in the state committee variety testing and will probably be further released. All the varieties presented in Table 1 originates from the CIMMYT and IWWIP breeding program germplasm and are further selected as described in figure 8. Results from multi-location yield trials in 2008-2009 led to recommendations to submit the following lines for official variety testing trials; CHEN\AEGILOPS/SQUARROSA(TAUS)/ /BCN/3/BAV92, SOROCA, VORONA SN079, SW89.5181/KAUZ and SHARK/F4105W2.1 (Table 1), (Pett et al., 2005; Eshonova et al, 2006; 2010; Rahmatov et al., 2008; 2010; 2011a; 2011b).

Table 1. List of bread wheat from International centers being officially tested and released in Tajikistan

Variety	Name of origin Nurseries	Cross	Origin	Growth habit as developed
Alex	1WWEEERYT	PYN/BAU	Mexico	Winter/Facultative
Sadokat	Special nursery	JUP/BJY//URES	Mexico	Spring/Facultative
Norman	5FAWWON	ORF1.158/FDL//BLO/3/SHI4414/CROW	Syria	Winter/Facultative
Ormon	8FAWWON	NWT/3/TAST/SPRW//TAW12399.75	Syria	Winter/Facultative
Tacikar	5FAWWON	TAST/SPRW//ZAR	Syria	Winter/Facultative
Iqbol	n.a.	RSK/CA8055//CHAM6	Turkey	Winter/Facultative
Oriyon	n.a.	ND/VG9144//KAL/BB/3/YACO/4/VEE#5	Mexico	Spring/Facultative
Somoni	n.a.	n.a	Turkey	Winter/Facultative
Isfara	ESWYT 25	VORONA/CNO79//KAUZ/3/MILAN	Mexico	Spring/Facultative
Sarvar	ESWYT 25	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92	Mexico	Spring/Facultative
Yusufi	ESWYT 25	SOROCA	Mexico	Spring/Facultative
Vahdat	ESWYT 25	SW89.5181/KAUZ	Mexico	Spring/Facultative

For the national wheat breeding program also a number of lines will be received from different nurseries of the CIMMYT-ICARDA-IWWIP programs for breeding purposes. These nurseries obtained from International centers, are to be tested in different agro ecological areas of Tajikistan.

WHEAT TAXONOMY AND ORIGIN

Wheat belongs to the grass family *Poaceae,* which contains more than 10 000 species, and includes also other cultivated cereals as barley, maize, oats and rice as well as related wild grass species (Evans and Peacock 1981). Wheat is further classified into the genus *Triticum.* The genus *Triticum* includes both diploid (2x=14), tetraploid (4x=28) and hexaploid (6x=42) species. Thus, all the three taxonomic groups have the basic chromosome numbers x = 7 (Curtis et al., 2002). The most commonly grown wheat is the hexaploid bread wheat (AABBDD). Dinkel wheat *Triticum spelta* is an older form of hexaploid wheat containing the same genomes as bread wheat (AABBDD). The second most widely grown species of wheat is the tetraploid durum wheat *Triticum durum* (AABB) that has developed from emmer wheat *Triticum dicoccoides* (AABB) (Peng et al., 2011). Also other tetraploid and hexaploid wheat species exist that is cultivated although to a limited extent. The einkorn is a diploid (*Triticum monococcum* L., 2x = 14) wheat, still cultivated although wild form exist as well. Bread wheat (2 = 6x = 42, AABBDD) contains two genomes homologous with the A and B genomes of durum wheat. The D genome in bread wheat most likely originates from *Aegilops tauschii*. Thus hexaploid wheat is a hybrid between a tetraploid wheat (AABB) and *Aegilops tauschii* also known as *Aegilops squarrosa* (DD) (McFadden and Sears, 1946; Boyko et al., 1999).

Wheat was one of the first domesticated food crops and has become one of the largest staple foods of the present day human population (Mergoum et al., 2009). Archaeological and botanical studies of wild and cultivated species of einkorn (*Triticum monococcum*), *Triticum turgidum spp. dicoccum* and its relatives have pointed out the Fertile Crescent to be the birth place of cultivated wheat about 8000 to 10000 years ago (Gill and Friebe, 2001; Mujeeb-Kazi and Villareal, 2002). Tetraploid emmer wheat was probably formed following a spontaneous hybridization event and went on to grow as a successful new species of a wild cereal that spread throughout the Near East long before people began to collect or cultivate its seed. Wild emmer wheat was certainly growing profusely alongside the diploid einkorn wheat in the Jordan Valley and in Syria more than 23 000 years ago (Van Zeist and Casparie, 1968; Hillman and Davies, 1990). It is likely that wild emmer

would have been made into a crude paste and eaten as a form of porridge before the development of baking techniques that made bread making possible (Harlan,. 1967). Thus, starting 8 000 to 10 000 years ago, the wild forms of both the tetraploid wheats, *Triticum turgidum ssp. dicoccoides* and *Triticum timopheevii ssp. armeniacum,* were widely grown in the Fertile Crescent (Gill and Friebe, 2001; Feldman, 2001). The tetraploid hulled wheat, *Triticum turgidum ssp. dicoccum*, was another of the ancient cultivated wheat's. The remains of cultivated emmer (*Triticum turgidum* ssp. *dicoccum*) have been discovered at several archaeological sites in Syria dating to 7500 BC (Zohary, 1986; 1999). Among the diploid wheat, einkorn is still cultivated to a limited extent, and its wild form, *Triticum aegilopoides*, is widely distributed in the Middle East (Johnson, 1976). The *Triticum dicoccoides* has been found exclusively in Israel, Syria, and Lebanon, while *Triticum armeniacum* is dominantly found in Azerbaijan and Armenia, and yet both overlap in Turkey, northern Iraq and possibly Iran (Gill and Friebe, 2001; Feldman, 1995, 2001).

GENOME OF BREAD WHEAT

Bread wheat (*Triticum aestivum*) is hexaploid (2 = 6x = 42, AABBDD), meaning that it contains three closely related homologous sets of chromosomes the A, B and D genomes. The size of the genome is 16,700 Mb/1C with about 90% repetitive DNA (Li, 2004). Bread wheat evolved from hybridization and combination of three different diploid species belonging to the *Triticum* and *Aegilops* species. The first step of hybridization was between *Triticum urartu* (AA genome) and *Aegilops speltoides* or a closely related species (BB genome). The specific identity of the B genome donor remains to be an issue of discussion for wheat cytogeneticists (Kerby et al., 1988). The result of this hybridization was tetraploid wheat, *Triticum turgidum* (AABB). Several distinct groups of this species exist, of which, *Triticum turgidum* var. *dicoccoides*, is believed to be most primitive type. One derivative of *Triticum turgidum* var. *dicoccoides* is *Triticum turgidum* var. *dicocccon*, which is the most likely progenitor of hexaploid bread wheat as a result of spontaneously hybridization with *Aegilops tauschii* (DD genome) (Feldman et al., 1995). This suggests that bread wheat is an allohexaploid (having two or more complete sets of chromosomes derived from different species) (2 = 6x = 42) plant and comprises three sub-genomes (Waines and Barnhart, 1992) and that it originated by the hybridization of the emmer group (AABB) and *Triticum tauschii* (DD) (Figure 9).

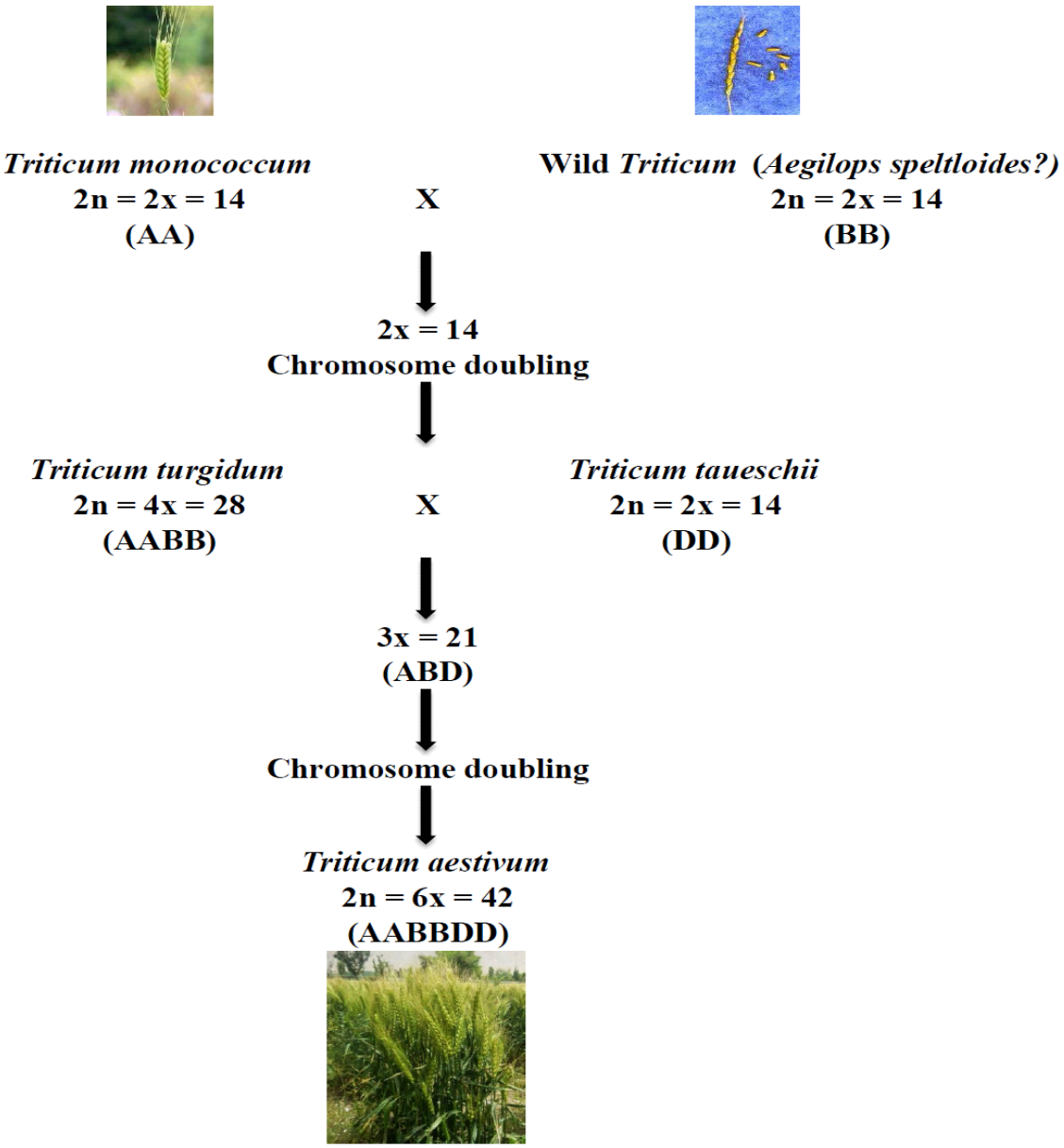

Figure 9. Evolution and hybridization of hexaploid wheat

The A genome progenitor *Triticum uratu,* has been identified to be the same also for a series of other species, all closely related to bread wheat, e.g. *Triticum beoticum, Triticum monococcum* and *Triticum thaoudar* (Kimber et al., 1987).

THE RYE GENOME IN WHEAT BREEDING AND SOURCES OF RESISTANCE GENES

Since Biffen (1905) has showed Mendelian inheritance with crossing of susceptible and resistant bread wheat cultivar to yellow rust. All individuals of the F_1 were susceptible to yellow rust, and in F_2 population 3 susceptible : 1 resistant (3:1) yellow rust plants were found. New sources of resistance have continuously been found and utilized in breeding. For internationally common diseases as the rusts, powdery mildew etc. a high number of resistance genes have been found. For many of the genes encoding resistance against diseases, a number of alleles have been detected. Several resistance genes have been transferred to wheat from related genera like *Aegilops*, *Thinopyrum* and *Secale* (Friebe et al., 1996). Continued wheat breeding towards resistance against diseases is the most effective and economically sufficient method if compared with other methods of plant protection. The main difficulties in resistance breeding arise from the occurrence of physiological races of the different pathogens. The numbers of races present for each of the diseases and for each crop are very high. The pathogens are furthermore evolving rapidly. Therefore it is necessary to maintain a permanent control of the variability in the hosts as well as in the parasite. According to Van der Plank (1968) there are two types of resistance: Vertical/race-specific resistance and horizontal/race non-specific resistance. Vertical/race-specific resistance can provide full protection against a disease, and is usually effective against some races of the parasites and ineffective against others. Vertical/race-specific resistance can provide a strong pressure on the parasite population, resulting in accumulation of virulent races, or it can create opportunities for new races to evolve with new virulence genes. Horizontal resistance/race non-specific is weaker and independent of the racial diversity of the parasite. Horizontal/race non-specific resistance reduces the effect of the infection. The disease on varieties/lines with this type of resistance develops slowly and the yield reduction is not significant. Horizontal/race non-specific resistance does not provide full protection of the host, but it is more durable. Although criticized these concepts are still practical and are frequently used among resistance geneticists and breeders (Nelson, 1978; Harris and Frederiksen, 1984).

Chromosome translocation is caused by interchange of parts between non-homologous chromosomes (different chromosomes of a haploid chromosome set). Alien translocations means transfers of individual chromosome parts from one species to another. This type of translocations has been shown to transfer successful traits in between crops, mainly in polyploid crops. Wheat-rye

translocations have been widely used by breeders all over the world, due to the reason that genes located on rye chromosome arms translocated to wheat or on fragments of those arms can improve a number of useful traits in wheat such as yield, wide adaptation, and disease and insect resistance (Rabinovich, 1998). There are many examples in wheat where rye transfers have resulted in transfer of disease and pest resistance genes (Friebe et al. 1996). The wheat-rye translocations involving 1RS and 2RL of rye are the most successfully used alien resources for wheat improvement. The first report on wheat-rye substitution of spontaneous 5R (5A) are routinely found in nature by Katterman (1937) and O'Mara (1946), and later the 1R (1B) substitution and 1BL/1RS; 1AL/1RS translocations were described in several widely grown wheat cultivars from all over the world (Zeller 1972; Zeller 1973; Schlegel and Korzun, 1997). These lines (1R/1B substitution, 1BL/1RS and 1AL/1RS translocation) have been further developed and are currently bases of the world's highest yielding wheat varieties. In particular, the wide distribution of promising commercially valuable wheat varieties resistant to biotic and abiotic environmental factors is determined by the presence of the rye chromosome 1R, or its arm 1RS, in the wheat genome. More than 5 million hectares of the sown area are occupied by wheat varieties carrying the translocated chromosomes 1BL/1RS. (Villareal et al., 1998). However, in 2011, about 1.050 varieties carry the 1RS.1BL translocation, about 100 varieties the 1RS.1AL translocation, and about 30 varieties a 1R (1B) substitution (http://www.rye-gene-map.de/rye-introgression/html/entry_a-d.html).

Wheat-rye translocations continue to play a significant role in international wheat breeding programs and especially 1BL/1RS translocation chromosomes are present in germplasm produced in the USA (Lukaszewski, 1990), Eastern Europe and Mexico (Villareal et al., 1997). The 1RS segment of this translocation carries race-specific genes for resistance to yellow rust *Yr*9 (*Puccinia striiformis*), stem rust *Sr*31 (*Puccinia graminis*), leaf rust *Lr*26 (*Puccinia recondita*), and powdery mildew *Pm*8 and *Pm*17 (*Blumeria graminus*) (McIntosh, 1983). The 1RS segment has also been reported to improve adaptation towards the environment through stress tolerance, acid soil, drought, salinity tolerance, and yield potential in bread wheat (Merker, 1982).

Wheat–rye translocation lines in the form of 2RL/2BS have been developed for improvement of disease and pest resistance, and performance in unfavorable crop production environments. Further, 2RL rye chromatin has been used to introduce agronomically useful traits into bread wheat (Friebe,

1996). The long arm of the rye chromosome, 2RL, has been shown to contribute positively to yield of wheat, as well as towards high resistance to powdery mildew (Hysing et al. 2007). Also, chromosome 2RL carries genes for resistance to tan spot and Hessian fly as well as leaf rust and stem rust (Lee et al., 1996)

In wheat breeding, including rye chromosome substitutions and translocations for improved resistance and performance of wheat, triticale is a suitable donor of rye chromatin. When triticale (genome AABBRR) is crossed with wheat (genome AABBDD), the F_1 obtain the genome AABBDR. When the F_1 is then backcrossed with bread wheat, rye chromosome substitutions and translocations can be isolated in later generations (Merker 1984).

BREEDING METHODOLOGY

The most efficient breeding strategy to use in breeding for improved resistance is to combine genes for specific resistance with those for non-specific resistances (Van der Plank, 1968; Watson, 1970; Clifford, 1974). The role of the specific resistance genes is to delay the start of the epidemic, while the role of the non-specific resistance genes is to retard the rate of the increase of the disease (Van der Plank, 1968). By the use of hybridization and selection procedures, it might be possible to combine a high level of non-specific resistance with high yield and quality characteristics (Hooker, 1967; Lupton and Johnson, 1970). The presence of genes for specific resistance could complicate selection for non-specific resistance. However the effect of such genes could be removed by selecting only in lines lacking useful resistance genes, or by utilizing races that are virulent for resistance genes. Moreover, derived lines should be tested with the widest possible range of pathogen races/isolates in both the greenhouse (seedling resistance test) and field (adult plant resistance test) (Hooker, 1967; Watson, 1970). Specific resistance genes can be added to the established lines by the use of backcross procedures (Watson, 1970). Beside the above described breeding strategy, there are several breeding methodologies in wheat breeding for new varieties resistant against biotic and abiotic stresses. In this study, both backcrossing and single seed descent methods have been applied in order to create wheat-rye translocation lines.

Backcrossing method

Backcrossing is a strategy widely used in wheat breeding programs to transfer desirable alleles, for specific traits from a donor parent to a recurrent parent (adapted cultivar in targeted region) (Briggs and Allard, 1953). The backcrossing method has been used effectively as a short–term breeding strategy to incorporate dominant genes for control of devastating pathogens, such as those causing stem rust, in otherwise highly productive and adapted varieties (Green and Campbell, 1970). Backcrossing in wheat breeding provides a precise way of improving varieties with excellency in a large number of attributes simultaneously being deficient in a few characteristics. The use of backcrossing in a wheat breeding program is useful if a variety has superior characteristics, for most traits although being susceptible to e.g. new diseases. Within this thesis work the susceptible parent (recurrent parent variety Topper) was crossed with a resistant parent (donor parent KR99-139 line). The procedures of crossing and selection need to differ in relation to whether the disease trait is dominantly or recessively inherited. If the disease resistant gene is dominant, all offspring would be heterozygous and resistant to the disease after the first cross. If the disease resistant gene is recessive, then after the first cross all F_1 would be heterozygous and not resistant to the disease. The obtained F_1 in the present study were backcrossed to the both the recurrent (Topper) and the donor (KR99-139) parent. The progenies of the BC_1F_1 (Topper x F_1) would segregate for 25% 1RS+– and 2RL+–, 25% 1RS+– and 2RL– –, 25% 1RS– – and 2RL+–, 25% 1RS– – and 2RL– –. The BC_1F_1 (KR99-139 x F_1) would segregate for 25% 1RS++ and 2RL++, 25% 1RS++ and 2RL+–, 25% 1RS+– and 2RL++, 25% 1RS+– and 2RL+–. Backcrossing was used just once, since the desired chromosomes (1RS and 2RL) were confirmed with molecular marker and segregation pattern in the BC_1F_1.

Single seed descent method

Single seed descent (SSD) is a modification of the bulk population system in wheat breeding. The main advantage of SSD is in the acceleration of selection and the production of new breeding lines (Borojevic, 1990; Poehlman et al., 1995). Once a cross is made, single seeds of each plant are randomly sampled in subsequent generations. As only single seeds are used, the segregating generations can be grown in the greenhouse allowing 2-3 generations per year. This method is used when a population of genotypes is sampled and at least one seed is saved from each genotype in successive generations of breeding until homozygosity is reached (Stoskopf et al, 1993; Mitchell et

al., 1992). The clear advantage of this method is the smaller nursery space needed compared to the pedigree method of breeding while trying to reach homozygosity faster with minimum resources. Also time is one of the most important issues in the wheat breeding process, and therefore the breeders have to take time into consideration when selecting whether to use single seed descent as the breeding method.

The crossing strategy of Topper x KR99-139 and backcrossing of KR99-139 x F_1 and Topper x F_1

The winter wheat variety Topper (without any wheat-rye translocations) was crossed with the double translocation line KR99-139 (with homozygous presence of the 1RS/1BL and 2RL/2BS wheat-rye translocations). In this study the variety Topper was the recurrent parent and the KR99-139 line was the donor parent, and the backcross breeding method was applied. The obtained heterozygous F_1 population was backcrossed with the variety Topper and the KR99-139 line, respectively using both these as a female parent in order to transfer main genes into the progenies. In the obtained BC_1F_1, molecular marker was used to verify the presence of the wheat-rye translocations. On the results of the molecular marker analysis, the offspring was separated into four groups (see page 26). The backcrossed progenies of (KR99-139 x F1 and Topper x F1) were selfed until the BC_1F_3 generation (Figure 10). The BC_1F_1 material was developed within a separate cooperation between SLU and BAZ in Germany.

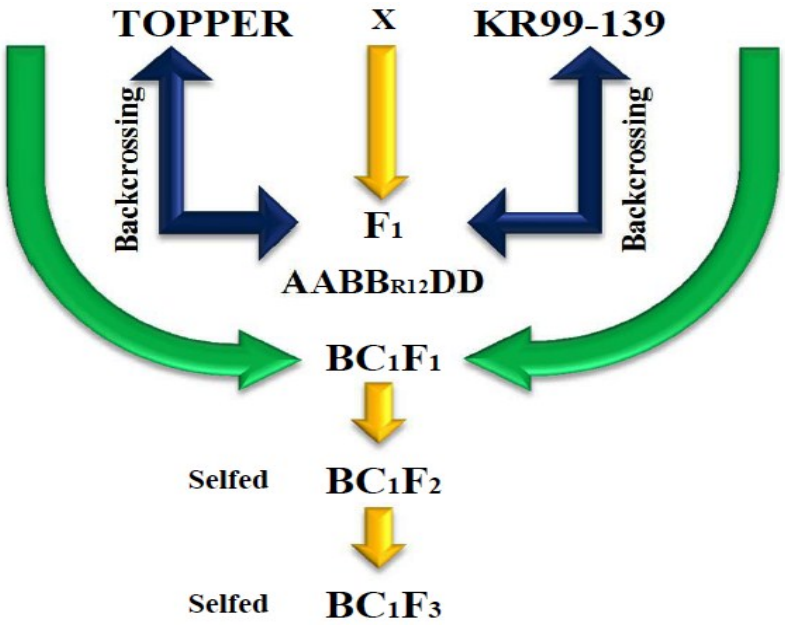

Figure 10. The scheme of crossing and backcrossing of the two wheat variety parents in the present investigations

Topper x F_1

In the material obtained through backcrossing to the variety Topper, the translocations are not present or present in heterozygous condition in the BC_1F_1. The obtained materials of BC_1F_1 wheat-rye translocation lines were divided for four groups based on segregating pattern and molecular analysis in BAZ in Germany as following:

1. Single plants with 1RS+–/2RL+–; 37 plants
2. Single plants with 1RS+–/2RL– –; 40 plants
3. Single plants with 1RS– –/2RL+–; 41 plants
4. Single plants with 1RS– –/2RL– –; 40 plants

KR99-139 x F_1

The obtained F_1 between Topper x KR99-139 were also backcrossed to KR99-139. In this material, the translocations are present in the BC_1F_1 in homozygous or heterozygous conditions. The BC_1F_1 wheat-rye translocation lines were divided for four groups based on segregating pattern and molecular analysis in BAZ in Germany as following:

1. The single plants with 1RS ++ and 2RL ++; 24 plants
2. The single plants with 1RS ++ and 2RL+–; 20 plants
3. The single plants with 1RS+– and 2RL ++; 15 plants
4. The single plants with 1RS+– and 2RL +–; 30 plants

(++ designates of homozygous presence and –+ heterozygous presence)

RESEARCH AIMS OF THE STUDY

The overall aims of the study were to obtain wheat lines resistant to prevalent diseases, pests and to abiotic factors by the use of wheat-rye translocation lines. The more long-term goals were to use these lines in wheat improvement and breeding programs. Therefore, the line KR99-139, developed by late Professor Arnulf Merker and previously described as containing both the 1BL/1RS and 2RL/2BS translocations, carrying disease resistance genes from rye was used for crossings and back-crossings.

The objectives of this study were as follows:

- To isolate different lines from the cross between KR99-139 and Topper containing no translocation, either one of the 1BL/1RS or 2RL/2BS translocation or both of the translocations. Of importance was to check the stability of the translocation combinations. Several methods were used to identify plants of different types;
    - Within the used 2RL from KR99-139, a gene for red coleoptiles was present that could be used as a phenotypic marker for presence of this translocation. By using either KR99-139 or Topper as the back-crossing parent, it should be possible to use this marker as a determiner for presence or absence of the 2RL. By analyzing both the BC_1F_2 and BC_1F_3 from the backcrosses using both red and green coleoptile for identification it should be possible to determine homozygous and heterozygous presence or absence of 2RL.
    - Giemsa C-banding was used to verify the identification of lines that were homozygous for the 1RS and 2RL rye chromosome
    - Simple sequence repeat (SSR) markers were used to verify presence of 1RS and 2RL
- To identify resistance of wheat-rye translocation lines, some lines were analyzed for yellow rust, *Puccinia striiformis*, by seedling resistance test
- To investigate the presence of adult plants with resistance to stem rust of the race Ug99, some wheat-rye translocation lines were tested in Kenya

CHAPTER II

APPLICATION OF DIFFERENT TECHNIQUES: SELECTION OF RED AND GREEN COLEOPTILES, GIEMSA C-BANDING AND SIMPLE SEQUENCE REPEATS (SSR) MARKERS, FOR IDENTIFICATION AND ISOLATION OF WHEAT LINES WITH DIFFERENT COMBINATIONS OF RYE TRANSLOCATIONS (1RS AND 2RL)

INTRODUCTION

The Rye (*Secale cereale L.*) genome has played an important role as a source of genes for genetic improvement of bread wheat. Of the seven chromosomes of rye, the 1R and 2R chromosomes have been extensively used in wheat breeding and improvement. The wheat–rye translocations involving 1RS of rye is the most successfully used alien resource for wheat improvement and wheat breeding (Rabinovich, 1998). The 2RL rye chromosome in some genotypes have been observed to have a gene for red coleoptile colour that could be a simple marker for screening in some wheat backgrounds, in contrast to the green coleoptiles of the non-translocation lines (Hysing et al., 2007). The presence of the phenotypic marker, of the coleoptile colour gene(s), on chromosome 2R was first detected by Melz and Thiele (1990). The red coleoptile phenotype could function in thus of suppression of genes on the chromosomes 2A, 2B, 2D, 4B and 6A. However, the function of the red coleoptile phenotype could also be through absence of suppressors of anthocyanin pigments on the long arm of wheat chromosome 2B (Sutka, 1977; Melz et al., 1988). The wheat material selected for this study was one line with the red coleoptile that could be used as a phenotypic marker for homozygous presence of 2RL. Selections based on the coleoptile colour can be done directly in BC_1F_1 or in further generations to select those plants being homozygous for 2RL. Moreover, selections based on if the coleoptile is red or green can be carried out in two preceding generations, making it possible to screen for both heterozygousity and homozygosity of 2BL versus 2RL. In this study, BC_1F_2 and BC_1F_3 wheat-rye translocation lines obtained from selfing of BC_1F_1 lines from the backcross to Topper with 1RS– – 2RL+– were selected and investigated for red coleoptile in order to produce a line homozygous for 1B (1RS– –) and 2RL/2BS (2RL++). From the backcross to KR99-139, BC_1F_3 from the selfing of BC_1F_1 and BC_1F_2 lines with 1RS++ 2RL+– was selected for coleoptile colour. Since, in this BC_1F_3 lines the 2RL chromosome was present in heterozygous condition, therefore were used for green coleoptile selection. Lines showing green coleoptile colour in BC_1F_3 must then be homozygous for 1BL/1RS (1RS++) and 2B (2RL– –). In addition, materials

that have different rye (1R and 2R) translocations can be detected by the use of cytogenetical methods. Giemsa C-banding is a useful method for an efficient screening of chromosome aberration such as wheat-rye alien translocations as well as deletions (Friebe et al., 1991; Hohmann et al., 1994; Yamamori, 1994). The Giemsa C-banding technique is used to construct wheat karyotypes, and the karyotype is then utilized to identify individual chromosomes (Gill and Kimber, 1974). There is a characteristic C-banding pattern for the individual chromosomes in the somatic metaphases that is possible to use for distinguishing different species. The C-bands in the cereal chromosomes are usually present in the centromeric area and, additionally, may- or may not be present in interstitial or terminal regions or both. When, using any of the commonly used C-banding techniques, the rye C-bands stain more intensely than those of wheat (Weimarck 1974; Merker 1975). These features enable identification of all of the somatic metaphase chromosomes of rye and also allow recognition of rye chromosomes in wheat-rye addition lines (Gill and Kimber, 1974). Wheat and rye chromosomes involved in translocations have thus, been identified by C-banding (Gill and Kimber 1977; 1974).

Quick and reliable methods for identification of 1BL/1RS and 2RL/2BS translocated chromosomes in wheat breeding are of importance in practical breeding work. With increased availability of molecular markers for many agronomically important genes, and the establishment of wheat genotyping centers, molecular marker-assisted selection and the benefit of this method has received additional attention from wheat breeders (Koebner 1995; Shimizu et al. 1997). A fast and reliable marker system that is able to identify wheat–rye translocations involving 1RS and 2RL is helpful for efficient selection of lines of interest for wheat breeding programs. Until now, a number of rye-specific molecular markers have been identified (Saal and Wricke, 1999). In the present study, the BC_1F_3 wheat-rye translocation lines with combination of 1RS (++) and 2RL (++) were verified with SSR markers.

The objectives of this part of the study were to select lines containing different combinations of wheat-rye translocations; i.e. those lines having both translocation (1BL/1RS and 2RL/2BS), one of either of the translocations or none of the translocations. Differentiation of the lines was carried out by checking homozygosity for red or green coleoptile colour, Giemsa C-banding technique and presence of rye-specific SSR markers.

MATERIALS AND METHODS

Plant Materials

The line KR99-139 is homozygous for the two different wheat-rye translocations 1BL/1RS and 2RL/2BS. In this study, the line KR99-139 was crossed with the variety Topper to obtain a F_1 population (Figure 10). This F_1 population was then backcrossed to both KR99-139 and Topper. The chromosomal composition was determined by molecular markers at BAZ, Germany and the number of lines with each chromosomal composition has been given previously (page 26). For analyses of coleoptiles colour the 41 BC_1F_1 lines with 1RS--/2RL+- from the back-cross to Topper and the 20 BC_1F_1 lines with1RS++/2RL+- from the back-cross to KR99-139 were selected. Coleoptile colour was analyzed in BC_1F_2 (selfed BC_1F_1) in 12 seeds of each of the lines from the back-cross to Topper (a total of 12 x 41 seeds = 492 seeds). For the same back-cross, 108 plants of the BC_1F_2 with red coleoptiles were selected, grown in the green-house, selfed and again 12 seeds of each of these plants were tested for coleoptiles colour in BC_1F_3 (a total of 1296 seeds). As for the selected lines from the back-cross to KR99-139, 5 seeds of each line (a total of 100 plants of selfed BC_1F_1) were grown in the green-house, selfed again and thereafter 12 seeds of each of the 100 plants were analyzed for coleoptiles colour in BC_1F_3 (a total of 1200 seeds). A total of the 15 BC_1F_3 (backcross to KR99-139) wheat-rye translocations lines obtained, together with both the parents were also analyzed with Giemsa C-banding technique to evaluate homozygous/heterozygous presence/absence of 2RL and 1RS. In addition, molecular marker (SSR) analyses were used to determine presence or absence of the different rye chromosome arms in the BC_1F_3 wheat-rye translocation lines. A total of 24 lines supposed to be homozygous for both 1RS++ and 2RL++ together with both the parents, and 4 Tajik bread wheat varieties known as non-translocated wheat, were used for the molecular marker analysis, carried out as described below. The BC_1F_3 lines used were obtained through selfing of lines determined to be homozygous for both the translocation in BC_1F_1 by molecular analyses at BAZ, Germany.

Determination of red and green coleoptile

To perform the determination of coleoptile colour, 12 seeds from each individually harvested plant were taken and treated with Vitavax 750C fungicide for about 8-10 minutes. Thereafter the seeds were sown on one layer of Wettex and two layers of filter paper on Petri dishes in a germination chamber with 20°C and 16 hours days of full light with extra UV lamp and with 16°C and 8 hours

night for 5-6 days until germination. Water was given intermittently as required. The coleoptile colour was determined when the lengths of the plants were 1.5-2 cm, and the coleoptile colour of each plant was recorded. Plants with different coleoptile colours, red and green were counted to estimate the segregation ratios. Thereafter a number of plants were selected to be selfed in order to produce a new generation of the material. Selected plants were planted in the greenhouse in trays for 8-10 days. Thereafter the plants were placed in the vernalization room for 8 weeks, and then the plants were transferred to the greenhouse until maturity and harvesting. The Chi-square tests were used to evaluate fit between observed and expected segregation ratios (Table 3; Figure 13).

Cytological analysis with Giemsa C-banding technique

Pretreatment

The Giemsa C-banding technique was carried out similarly as has been described by several authors (Merker, 1973; Gill and Kimber, 1974; Gill et al., 1991). Five seeds selected randomly from each of 15 plants of the BC_1F_3 wheat-rye translocation lines together with the two parents, Topper and KR99-139, were analyzed. The seeds were germinated on wet paper on Wettex in Petri dishes in the germination chamber with following parameters: day of 16 hours light at 20°C and night of 8 hours darkness at 15°C for 3–4 days. Roots were excised when 1–2 cm long, and pretreated with 0.05% colchicine for 3-4 hours at room temperature. The roots were also taken to the ice water (in titer plates with 24 wells) after brief dipping in distilled water, and the ice water treatment gives better chromosome morphology and band contrast. A pre-treatment in ice water was done for 24 hours and therefore the samples were kept in a refrigerator. The colchicine pretreatment results in a lower mitotic index compared with ice water pretreatment but gives better chromosome morphology and band contrast. The next day, the excised roots were fixed in Carnoy's I for 1 hour at room temperature and then moved into the freezer at -20°C. After fixation, the roots were softened by cellulose + pectinase solution. The treatment was carried out in titer plates with 98 wells (10 ml enzyme solution for 24 wells). The roots were checked regularly for softness by taking them out and holding horizontally. When tips were bending down, they were softened. When treatments were ready, roots were taken back into the fixative in order to stop enzymes.

Preparation of the slides

The roots were pretreated with 45% acetic acid for 4-5 minutes. Every tip of the roots was cut with scalpel, and the meristematic tissue was squeezed out with the scalpel on the slide and squashed in 45% acetic acid and thereafter a cover slip was added. Gentle heating over a spirit flame helped to spread the chromosomes. The cover slip was pressed on to the slide by the use of a double folded filter paper and thereafter the slide was placed on dry ice (CO_2 ice block). The cover slips were removed with scalpel by freezing the slides, and the slides were immediately placed in 99% ethanol. The slides were dipped into 99% ethanol, changed twice; the first time they were kept only for a couple of minutes while the second time they were kept for 20-24 hours. Finally, the slides were air-dried about 10-20 minutes.

Acid and Basic treatment

The air-dried slides were incubated for 1.5 minute in 0.2 M HCL in a water bath at 50°C. Thereafter, the slides were washed briefly in distilled water and incubated for 7 minutes in a saturated Ba $(OH)_2$ at room temperature. In order to avoid crystallization of Ba $(OH)_2$, the slides were washed by distilled water. Incubation was performed in 2xSSC (Saline Sodium Citrate) in a water bath at 60°C for 55-60 minutes.

Staining

The slides were moved directly from the 2xSSC into a 2% staining solution of Giemsa for about 30-45 minutes. The slides were observed regularly under microscopy every few minutes for best contrast. In order to avoid crystallized dyes after staining the slides were rinsed with distilled water and air-dried.

Mounting

For making slides permanent, Canada Balsam, soaked in xyline was mounted for the slides. The mounted slides can be stored for several years without loss of contrast. All slides prepared were examined under a Nikon microscope to detect 1RS and 2RL.

DNA Extraction

Seeds were sown and grown in pots in the Alnarp greenhouse. DNA for SSR analysis was extracted from fresh leaves of 10 day-old seedlings using the cetyltrimethyl ammonium bromide (CTAB)

method as described by Doyle and Doyle, (1990) with some modifications. For each sample, about 40-45 mg of fresh leaves was taken from the first leaves and the leaves were placed with two metal bolls in an ependorf tube, where 350μl of CTAB buffer were added. Thereafter the leaves were crashed by using the crashing machine (RETSCH MM 400) for 2 minutes, and additionally 350μl CTAB buffer was added to the samples. Thereafter, samples were incubated in a water bath at 60°C for 60 minutes, and mixed once after 30 minutes. After incubation, the samples were cooled in room temperature, and 700μl of 24:1 Chloroform: Isomyl Alcohol were added to each ependorf tube and mixed very well by shaking tubes several times. Tubes were then centrifuged for 20 minutes at maximum speed of 14 000 rpm. After centrifugation three layers were obtained in the tubes: top - aqueous phase, middle - debris and proteins, and bottom - chloroform. The aqueous top layer (350μl) was taken and transferred into new ependorf tubes, and then 5μl RNase was added. The samples were incubated at 37°C for 30 minutes. After incubation, 0.54 volumes (204.12μl) of cold Isopropanol were added to each tube. The samples were centrifuged at maximum speed for 15 minutes. The supernatant was poured off carefully to avoid losing the DNA pellet and the tubes were inverted for air drying during 2-3 minutes. The pellets were washed with 600μl of washing solution (95% ethanol and 1M sodium acetate) at room temperature for 20 minutes. The samples were then centrifuged for 5 minutes at maximum speed, and the supernatant was poured off as well. Then 600μl of rinsing solution (95% ethanol and 7,5M ammonium acetate) was added and the tubes were turned upside down several times. The samples were centrifuged for 5 minutes at maximum speed, and again the supernatant was poured off. The samples were left to air dry overnight. The next day the extracted DNA was dissolved in 60μl TE buffer. The DNA concentration was measured with Nano Drop. From each sample, 2μl genomic DNA were loaded on 1% agarose gel in 1xTAE buffer, in order to check the quality and purity of extracted DNA. The gel was visualized using UV light and thereafter photographed. Finally, the DNA was diluted to concentration of 10 ng/μl, and the diluted DNA was used as stock solution for PCR reactions (Figure 11).

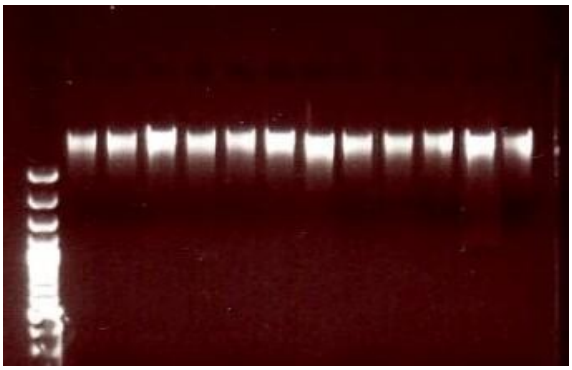

Figure 11. Evaluation of quality of DNA by gel-electrophoresis SSR analysis

Five rye-specific SSR markers to be selective for presence of 1RS and 2RL were used for verification of translocation events. Thus, the primer pairs SCM9, SCM39, SCM43, SCM69 and SCM75 (Saal and Wricke, 1999), were used to screen for rye fragments in the BC_1F_3 wheat-rye translocation lines. The PCR amplifications were carried out in 25μl volume containing 0.625 μl of forward and reverse primers, 0.2 μl dNTP, 0.2 μl Taq polymerase, 2.5μl 10xPCR buffer, 2.5μl 10ng/μl of DNA and 18.35μl sterile milipour water. The information about the five microsatellite rye specific SSR markers and PCR amplification conditions is summarized in Table 2. Amplified PCR products were separated by electrophoresis in 3% agarose gel. For the electrophoresis 1xTAE buffer was used and the agarose gel was stained with Ethidium bromide and photographed under ultraviolet light.

Table 2. SSR markers specific for 1RS and 2RL used for verification of wheat-rye translocation lines

SSR Marker	Primer pair sequence (5'-3')	Annealing temperature	PCR condition	Product size (bp)	Chromosome	Reference
SCM9	TGACAACCCCCTTTCCCTCGT TCATCGACGCTAAGGAGGACCC	60	94°C for 2 min; 95°C for 60 s; 60°C for 60 s, and 72°C for 60 s for 40 cycles	220	1RS	(Saal and Wricke, 1999)
SCM39	GACCTCAGTGGAGCCTCTAGGT GGACATCTGCCGTGACAATACC	60	94°C for 2 min; 95°C for 60 s; 60°C for 60 s, and 72°C for 60 s for 40 cycles	230	1RS	(Saal and Wricke, 1999)
SCM43	CTAGGGGATTACAGGGAGGGCA GTTCCCTTGTCCTACTCGTTACCG	60	94°C for 2 min; 95°C for 60 s; 60°C for 60 s, and 72°C for 60 s for 40 cycles	100	2RL	(Saal and Wricke, 1999)
SCM69	CTACCTGCTGTTCCCATTTGG GTGTGTAGAAGATGTTGTCCTGG	60	94°C for 2 min; 95°C for 60 s; 60°C for 60 s, and 72°C for 60 s for 40 cycles	144	2RL	(Saal and Wricke, 1999)
SCM75	TTTTCTATCTCAGCGATTCATGC TCCTGAGATCAAGTGCGTGTG	60	94°C for 2 min; 95°C for 60 s; 60°C for 60 s, and 72°C for 60 s for 40 cycles	191	2RL	(Saal and Wricke, 1999)

RESULTS

Screening of homozygous presence of 2RL through the use of red/green coleoptile selection in BC_1F_2 and BC_1F_3 from Topper x F_1 and BC_1F_3 from KR99-139 x F_1 wheat-rye translocation segregating population

The BC_1F_1 seeds were analysed before the start of this project at BAZ, Germany and the number of seeds with different combinations of homozygous/heterozygous presence of 1RS and 2RL was determined (as mentioned on page 26). A chi-square analysis of the segregation of this material shows significantly a 1:1:1:1 segregation in the material backcrossed to Topper but a slight deviation in the segregation in the material backcrossed to KR99-139 (Table 3). From each of the BC_1F_1, seeds were planted in the green-house and thereby lines from each of these seeds were created. Two of the lines were selected for the studies of coleoptiles colour; BC_1F_1 1RS--/2RL+- from the backcross to Topper and BC_1F_1 1RS++/2RL+- from the back-cross to KR99-139. Coleoptile colour was analysed in BC_1F_2 in the Topper back-crossed material. Of the analysed 492 seeds, 265 had a red coleoptile, 160 had a green coleoptile (Table 3) and the rest did not germinate. Depending on if the 2RL+- generate a red or green coleoptile, the expected segregation was 3:1 or 1:3 red:green coleoptiles. The obtained result corresponded better towards a 5/8:3/8 segregation, as shown below:

Observed:	265 – red coleoptile	160 – green coleoptile	= 425
Expected:	265.6 – red coleoptile	159.4 – green coleoptile	= 425

$\chi^2 = (265 - 265.6)^2/265.6 + (160 - 159.4)^2/159.4 = 0.004$, 1 d.f, P 0.94

A total of 108 plants with red coleoptile was selected, selfed and analyzed again for red coleoptiles in BC_1F_3. If only plants with 2RL++ had been selected with the red coleoptiles selection in BC_1F_2, then all BC_1F_3 selected should generate red coleoptiles. However, if all 2RL++ and 2RL+- was selected by the use of the red coleoptiles in BC_1F_2, then segregation ratio of 5/6:1/6 red:green coleoptiles was expected in BC_1F_3. As can be seen from Table 3 and Fig 12a, the segregation ratio was more similar to 3:1 than any of the expected ones.

For the KR99-139 back-crossed material, it was all selfed in the green-house and coleoptiles colour was analyzed in BC_1F_3. Here, the expected segregation pattern was either 5/6:1/6 or 3/8:5/8

red:green coleoptiles depending on if the 2RL+- resulted in red or green coleoptiles. The obtained segregation pattern was instead corresponding to 3:1 (Table 3, Fig 12b).

Due to the diverse results from the coleoptiles analyses a repetition of the analyses was made on the BC_1F_2 Topper back-crossed material, i.e. new plants were germinated and coleoptiles colour determined. This time 128:349 red:green coleoptiles were recorded which corresponded to a 1:3 ratio of red:green coleoptiles meaning that the 2RL+- were scored green.

Table 3. Segregation for chromosome combination in BC_1F_1 with SSR analysis, and in BC_1F_2 and BC_1F_3 populations with selection of red and green coleoptile, derived from cross of wheat-rye translocation and non-translocation parents

Topper x F_1

Generation	Chromosome combination	Number of plants		Expected ratio	Observed ratio	χ^2	d.f	*P*-value
		Red coleoptile	Green coleoptile					
BC_1F_1	1RS+–/2RL+– 1RS+–/2RL– – 1RS– –/2RL+– 1RS– –/2RL– –			1:1:1:1	37:40:41:40	0.076	3	0.99
BC_1F_2[A]	1RS– –/2RL++ 1RS– –/2RL+– 1RS– –/2RL– –	265	160	3:1	265:160	36.25	1	<.0001
BC_1F_3[B]	1RS– –/2RL++ or 1RS– –/2RL++ 1RS– –/2RL+– 1RS– –/2RL– –	987	309	all red	987:309	0.93 48	1	0.34 <.0001
BC_1F_2[C]	1RS– –/2RL++ 1RS– –/2RL+– 1RS– –/2RL– –	128	349	3:1	128:349	0.86	1	0.35

[A] - BC_1F_2 obtained from BC_1F_1 1RS– –/2RL+–
[B] - BC_1F_3 obtained from BC_1F_2 with red coleoptile
[C] - Repeated analysis; obtained from BC_1F_1 1RS– –/2RL+–

KR99-139 x F_1

Generation	Chromosome combination	Number of plants		Expected ratio	Observed ratio	χ^2	d.f	*P*-value
		Red coleoptile	Green coleoptile					
BC_1F_1	1RS++/2RL++ 1RS++/2RL+– 1RS+–/2RL++ 1RS– –/2RL– –			1:1:1:1	24:20:15:30	5.43	3	0.14
BC_1F_2	1RS++/2RL++ 1RS++/2RL+– 1RS++/2RL– –			**Selfed**				
BC_1F_3	1RS++/2RL++ 1RS++/2RL+– 1RS++/2RL– –	265	868	5/6:1/6 or 3/8:5/8	265:868	38 96	1	<.0001 <.0001

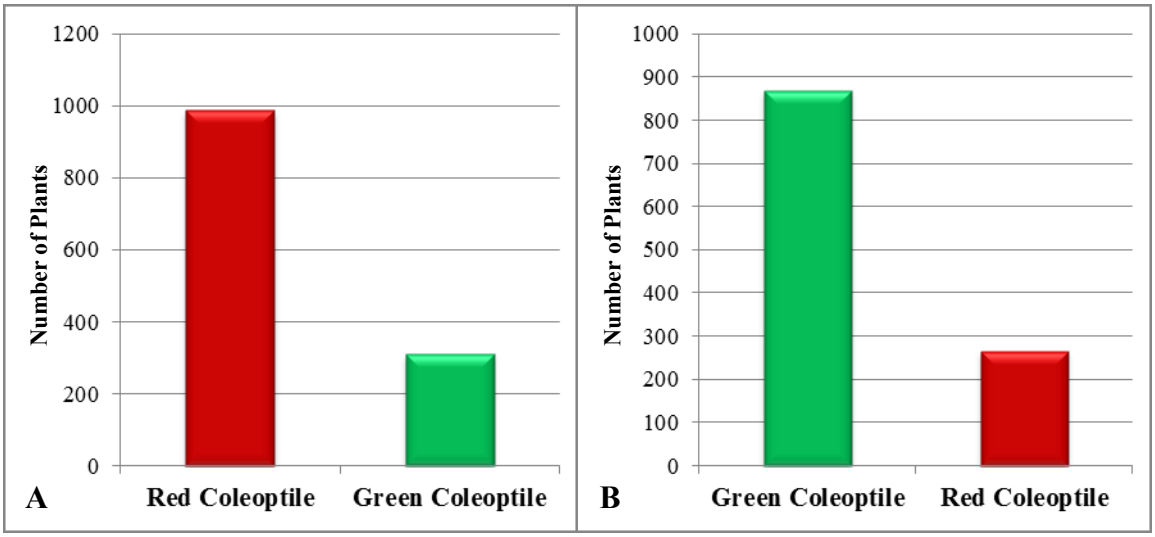

Figure 12. The results of analyses of green and red coleoptile colours in the BC_1F_3 wheat-rye translocation lines; A) Topper x F_1 and B) KR99-139 x F_1

Each selected BC_1F_3 plant with red and green coleoptiles was transplanted separately in different trays in the greenhouse and progressed to BC_1F_4 population.

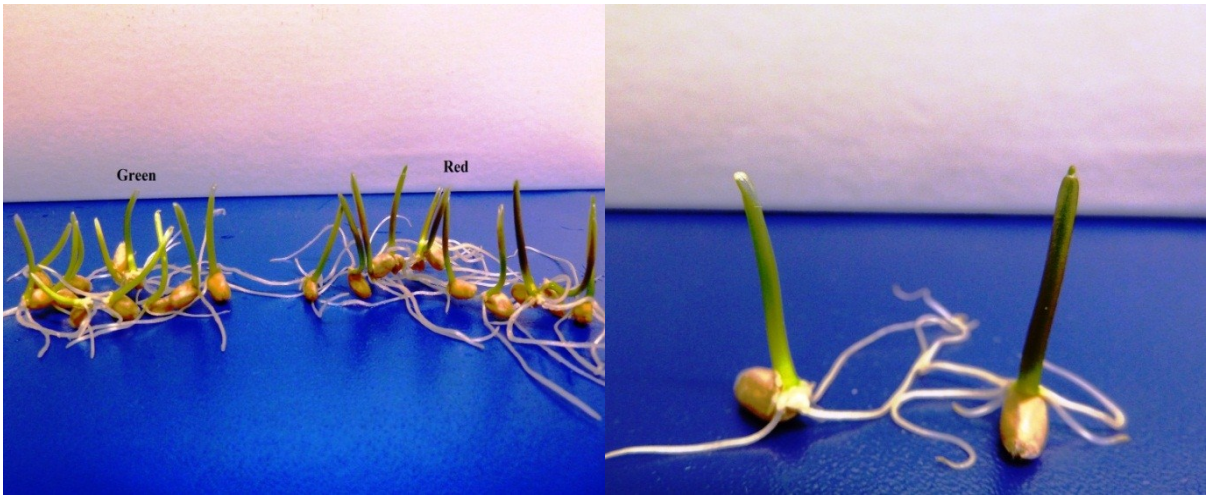

Figure 13. Green and Red Coleoptile differentiation in BC_1F_3 wheat-rye translocation seedlings

Identification of 1RS and 2RL with Giemsa C-banding techniques

The wheat-rye chromosomal compositions of individual plants from the BC_1F_3 segregating population differed with respect to the presence or absence of translocated 1BL/1RS and 2RL/2BS chromosomes. Giemsa C-banding techniques on the BC_1F_3 segregating population generally resulted in well-defined sharp, distinct bands in the wheat-rye translocation lines. Both the rye chromosome 1RS and 2RL were identified in wheat-rye translocation lines of the BC_1F_3 segregating populations. Example of Giemsa C-banding karyotypes for 1RS and 2RL chromosome are presented in Figure 14.

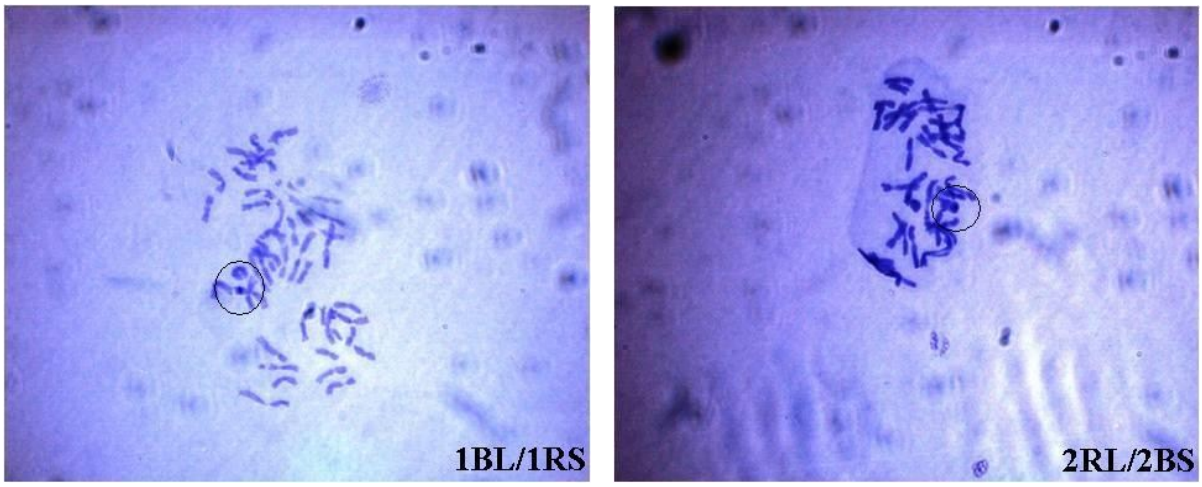

Figure 14. Distribution of 1RS and 2RL rye chromosomes by Giemsa C-banding technique in wheat background

From each of 15 single plants the BC_1F_3 wheat-rye translocations thought to be homozygous for 2RL and heterozygous for 1RS, 5 seeds were taken and analyzed. In some of the plants the chromosomes were problematic to identify. This was due to the preparation when cell division was not enough distributed in the plants.

The results of the Giemsa C-banding on the BC_1F_3 wheat-rye translocation lines showed 4 plants/lines homozygous for 1BL/1RS and 2RL/2BS, 8 plants/lines heterozygous for 1BL/1RS homozygous for 2RL/2BS and 3 plants/lines without 1BL/1RS and 2RL/2BS wheat-rye translocation (Table 4).

Table 4. Detection of presence of homozygous 2RL/2BS and heterozygous 1BL/1RS in the segregating BC_1F_3 wheat-rye translocation population and their parents revealed with Giemsa C-banding technique

№	Pedigree number and parent	1BL/1RS	2RL/2BS
1	Topper	– –	– –
2	KR99-139	++	++
3	1	+–	++
4	14	++	++
5	19	– –	– –
6	26	+–	++
7	32	+–	++
8	38	+–	++
9	39	++	++
10	43	+–	++
11	54	++	++
12	58	+–	++
13	62	– –	– –
14	77	++	++
15	79	– –	– –
16	84	+–	++
17	94	+–	++

+ Presence and – absence of translocated chromosomes

Verification of 1RS and 2RL with SSR markers

A total of five rye specific primer pairs were used to verify presence of 1RS and 2RL in the wheat-rye translocation lines. The PCR products amplified a reliable band with two of the primer pairs, SCM9 and SCM75, in the BC_1F_3 wheat-rye translocation lines as well as in KR99-139 line. The results of the analysis are summarized in Table 3.

Table 5. Specific primer pairs used to detect wheat-rye translocations 1RS and 2RL chromosome arms in the BC_1F_3

Primers	Base pairs	1BL/1RS			2RL/2BS		
		KR99-139	Topper	BC_1F_3	KR99-139	Topper	BC_1F_3
SCM9	220	+	-	+	-	-	-
SCM39	230	-	-	-	-	-	-
SCM43	100	-	-	-	-	-	-
SCM69	144	-	-	-	-	-	-
SCM75	191	-	-	-	+	-	+

- Absence of band, + Presence of band

The SCM9 primer, specific for 1RS was found to amplify a band of 220 bp. SSR band of 191 bp, amplified with primer pair SCM75 associated with 2RL was observed in both KR99-139 and the tested BC_1F_3 population (Figure 15). The bread wheat variety Topper without any rye chromosome showed no amplification with both SSR primer pairs (Figure 15 A, B). For the other tested primer pairs no clearly observed amplification was found either for Topper, Ormon, Tacikar, Ikbol, Oriyon varieties, and KR99-139 or of the BC_1F_3 plants.

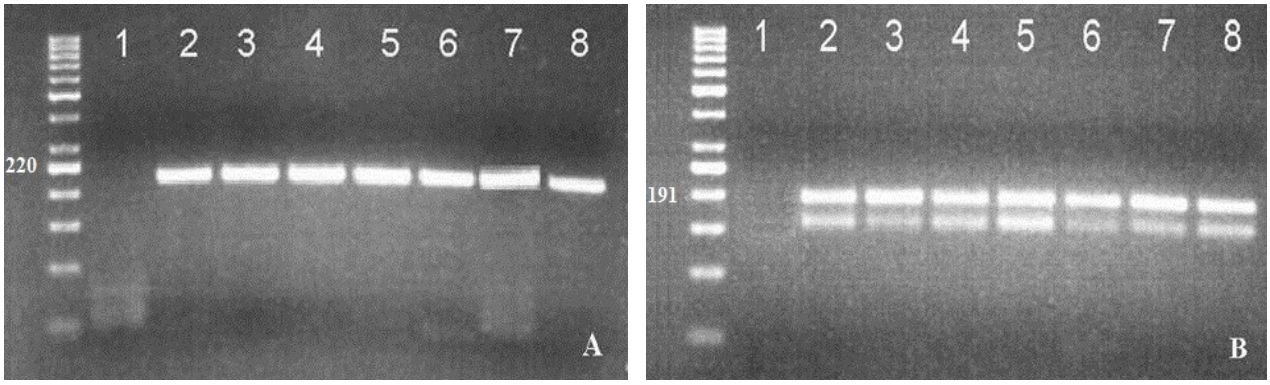

Figure 15. Detection of 1BL/1RS (A) and (B) 2RL/2BS wheat-rye double translocations in (1) Topper, (2) KR99-139, (3-8 BC_1F_3 segregating population)

Thus, there was no clear amplification by the means SCM39, SCM43 and SCM69 in neither non-translocation wheat lines nor rye translocation lines in the present study (Figure 15). PCR products amplified by SCM39, SCM43 and SCM69 could not separate the 1BL/1RS and 2RL/2BS from non-wheat-rye translocation lines.

DISSCUSSION

The rye genome contains many desirable genes that can be used for wheat improvement. Several rye genes have already been successfully introgressed into wheat (Friebe et al., 1996; Rabinovich, 1998). In order to detect the inheritance and distribution of 1RS and 2RL within a wheat breeding program several methods such as molecular markers, C-banding, ELISA, *in situ* hybridization, protein electrophoresis and disease resistance tests have been used to detect rye fragments in the bread wheat background (Gill and Kimber 1977; Heslop-Harrison et al. 1990; Weng et al. 2007; Zuniga et al. 2008).

In this study identification of the presence of 1RS and 2RL rye chromosomes was performed by using different techniques such as evaluation of segregation pattern, coleoptile selection, Giemsa C-banding and SSR molecular marker analysis. By the use of those methods it was possible to obtain complete information about presence of substitutions and genomic composition. The above indicated techniques were applied in BC_1F_2 and BC_1F_3 lines obtained through selfing of the BC_1F_1 and BC_1F_2 population respectively, and lines characterized to be homozygous for both 1BL/1RS and 2RL/2BS were detected. Coleoptiles were checked visually, and colours of the coleoptiles were recorded. The lines being homozygous for 2RL were clearly found to have the red coleoptile. Also lines being homozygous for not having 2RL were found to have green coleoptiles. The present results also clearly showed that coleoptiles colour was much more difficult to determine on lines being heterozygous for 2RL. By analyzing both BC_1F_2 and BC_1F_3 it was possible to show that 2RL++ were selected as having red coleoptiles but also some lines (but not all of them) with 2RL+- were selected as having red coleoptiles. Repetition of the analyzes showed that a more skilled and trained person doing the judgment of coleoptiles colour tended to lead to a more clear determination of only the 2RL++ as red coleoptiles. The conclusion from the coleoptiles colour analyses must however be that determination on two proceeding generations is a necessity for clear selection of homozygous lines. Presence of 1RS+- and 2RL++ rye chromosomes was also investigated in the

BC_1F_3 wheat-rye translocation lines by the use of Giemsa C-banding technique (Gill et al., 1991; Merker, 1973; Gill and Kimber, 1974). The analysis from the Giemsa C-banding technique showed that the heterozygous presence of the 1RS+- and homozygous presence of the 2RL++ rye chromosomes in the BC_1F_3 (KR99-139 x F1, 1RS+- and 2RL++) wheat-rye translocations populations. Homozygous and heterozygous presence of 2RL++ and 1RS+- respectively were found in several BC_1F_3 of the investigated plants. The rye chromosome 2RL indicated typical telomeric C-bandings, however in the same figure the chromosome of 1RS C-banding near the telomeric ends of chromosome was found (Figure 14). Moreover, the result of the Giemsa C-banding technique showed that the line KR99-139 was homozygous for both of the 1RS and 2RL translocated rye chromosomes, and in the variety Topper neither 1RS nor 2RL was identified (Table 4). Also not all of the wheat chromosomes were identified in this study, due to a general lack of chromosome-specific banding patterns and relatively uniform chromosome morphology. In fact the use of Giemsa C-banding technique in this study, mainly contributed to the detection of homozygous presence of 2RL in the BC_1F_3 wheat-rye translocation lines (KR99-139 x F1, 1RS+- and 2RL++). Promising and reliable information was verified in the BC_1F_3 wheat-rye translocation lines upon comparing the results of detecting 1RS and 2RL by using SSR marker analysis.

In conclusion, the results of the coleoptiles analyses showed the necessity of analyses in two proceeding generations to be able to select lines with homozygous presence/absence of 2RL. The Giemsa C-banding is a powerful technique that enabled to identify and classify presence of rye chromosome in BC_1F_3 wheat-rye translocation lines. Also, the two microsatellite (SSR) markers SCM9 and SCM75 could identify 1BL/1RS and 2RL/2BS and discriminate translocated from non-translocated lines in the present study. All the methods have proven to be powerful in detection of translocations. Moreover, the combination of several methods will help to avoid false-negative results. The expected output of the selection process was thereby confirmed. The results obtained here very much correspondent to what could be expected from analysis that had been performed in the BC_1F_1 before this project was started. However for creation of lines with homozygous presence of both the translocations, 1RS and 2RL (1BL/1RS++, 2RL/2BS++) one of each of the translocations (1BL/1RS++; 2RL/2BS-- and 1BL/1RS--, 2RL/2BS++) as well as no translocations (1BL/1RS--, 2RL/2BS--) selection based on red/green coleoptiles were found most useful and reliable.

CHAPTER III

YELLOW RUST SEEDLING RESISTANT TEST AND ADULT PLANT RESISTANT TO STEM RUST IN THE BC_1F_3 WHEAT-RYE TRANSLOCATION LINES AND THEIR PARENTS

INTRODUCTION

Wheat rusts are important foliar diseases of wheat that are causing millions of dollars in losses annually worldwide out in all wheat market classes (McIntosh, 2009; USDA). Rusts fungi are obligate biotrophic parasites that grow and reproduce on living plant tissue (Eckardt, 2006). The rust pathogens are adapted to many different cereal growing environments, they develop rapidly and their airborne spores are spread quickly over long distances. All rusts fungi are known as specialized pathogens and each rust species is divided into specialized forms having a specific host genotype to attack under particular environmental conditions (McIntosh, 2009; Hovmøller, 2001).

Stem rust caused by *Puccinia graminis* f. sp. *Tritici* is the most dangerous of the wheat diseases followed by yellow and leaf rusts. The yield losses that stem rust causes are varying between 30-100% in case of susceptible varieties (Leonard, 2001; Leonard and Szabo, 2005; Roelfs, 1985). The appearance of a new aggressive race, Ug99 (TTKSK), has threatened the global wheat production lately (Pretorius *et al.,* 2000; Singh *et al.,* 2006). It reminded the world of the continuous nature of breeding for rusts resistance and the need to increase diversity for rust resistance among commercially grown varieties. To achieve sustainable disease resistance in crops, it is often necessary to stay ahead of changing pathogens by searching for, understanding, and manipulating new sources of resistance. Breeding for disease resistance is an ongoing process, the success of which relies on the availability and utilization of genetically diverse sources of resistance. More than forty-five stem rust resistance loci have until now been genetically characterized and named (McIntosh et al. 2003; McIntosh et al., 1995; Singh and McIntosh, 1990).

Yellow rust caused by the obligate pathogen *Puccinia striiformis. f. spp. tritici* is the most economically important disease. Periodical causes of epidemic yellow rust disease on bread wheat, *Triticum aestivum,* are severely damaging the wheat production in cool and moist production regions (Singh et al. 2000). The yellow rust disease is highly destructive and can cause severe yield

losses of up to 100%. Resistant commercial varieties tend to become susceptible over time due to changes in the pathogen-host interaction (Chen, 2005). The rust diseases can be controlled by fungicide application although this is no way for small-scale farmers producing the bulk of wheat. The difficulties for small scale farmers to use pesticide are due to prohibitive costs in the local market in developing countries. The most environmentally and farmer friendly strategy for reducing yield losses is breeding for resistance to yellow rust and other diseases (Chen, 2005; Chen and Wood, 2004; Hardison, 1975). However, this method primarily depends upon the availability of resistant donor parent genotypes. Increased knowledge about the genetic basis of resistance to yellow rust in wheat varieties is useful as well as the understanding of the pathogen race composition and distribution (Boukhatem et al., 2002; Imtiaz et al., 2003; Singh et al., 2001).

Race specific resistance genes can be identified by comparing the reaction of the variety of interest to a set of pathogen isolates and comparing the reactions within a display of testers carrying known yellow rust genes (Dubin et al. 1989, Hovmøller 2007). About 40 wheat yellow rust resistance genes have been catalogued (Chen 2005; McIntosh et al. 1998 and 2007). Most of these genes are unique as pointed out by different chromosomal locations and differential response to races (Chen 2005). These resistance genes have often been identified from wild and related wheat species, e.g. from the rye genome (Chen et al. 2003; Hovmøller, 2007; McIntosh et al. 2004; Sharma et al., 1995; Xia et al., 2007). There are many examples in wheat where rye transfers have resulted in transfer of disease and pest resistance genes (Friebe et al. 1996). Wheat-rye translocated varieties and breeding lines carry several genes from a number of rye chromosomes e.g. from 1RS - *Yr9, Sr31* and *Lr26,* from 2RL - *Yr-2* and from 6RL - *Yr-3* (McIntosh et al., 2001). Rye is the most successfully used alien resource for wheat improvement.

The objective of this study was to identify resistance in wheat-rye translocation lines to yellow and stem rusts by using seedling test to yellow rust and adult plant resistance to stem rust.

MATERIAL AND METHODS

Plant materials

BC_1F_3 plants derived from the cross between Topper and KR99-139 were used for tests of seedling resistance against yellow rust and adult plant resistance against stem rust, i.e. the Ug99 race. The

materials used were the two parental lines and selected BC_1F_3 lines containing 1RS and 2RL combined, 1RS or 2RL single and without translocations. For the yellow rust seedling test, different host genotypes with known yellow rust resistance genes were included (Hovmøller, 2007).

Seedling resistance test to yellow rust

In the greenhouse, each line or plant were grown in plastic pots under controlled condition, until the appearance of the second leaf after about two weeks. Each entry was grown in standard peat soil in 9 cm square pots containing 10–12 plants in spore-proof greenhouse cabinets under daylight conditions, 18 hours day at 17°C and 6 hours night at 12°C. The plants were inoculated 12–14 days after sowing by fresh spores at the time of emergence of the second leaf. The plants were incubated in a dew chamber at 100% r.h. at 10–12°C for 24 h and transferred to a greenhouse for 14–16 days with light and temperature conditions as above (Hovmøller, 2007). The seedling resistance tests were conducted at Global Rust Reference Center, Denmark and 17 races/isolates were used. The races/isolates were selected based on their virulence spectrum and the list of races/isolates; origin, and sampling years are summarized in Table 6. Further, seedling responses of differential genotypes on the different races/isolates are presented in Table 7 (Hovmøller, 2007), and can be used as a characterization of the different races/isolates

The infection of *Puccinia striiformis* on the wheat seedlings were scored using a 0 to 9 scale 14-16 days after inoculation as described by McNeal et al., (1971).

0 – No symptoms
1 – Hypersensitive flecks without uredia
2 – Necrotic/Chlorotic flecks without uredia
3 – Necrotic/Chlorotic stripes without uredia
4 – Necrotic/Chlorotic stripes with trace uredia
5 – Necrotic/Chlorotic stripes with light uredia
6 – Necrotic/Chlorotic stripes with intermediate uredia
7 – Necrotic/Chlorotic stripes with moderate uredia
8 – Chlorotic stripes with abundant uredia
9 – Abundant uredia without necrosis/chlorosis

Table 6. List of origin and virulence profile of races/isolates that were used for seedling resistance test in the present study

Races/Isolates	Origin	Sampling year	Virulence profile	References
Ken 08/09	Kenya	2009	Yr 1, 2, 6, 7, 8, 9, 25, 27, Sd	(Hovmøller)*
CN4/08 (R109)	China	2008	Yr 1, 2, 3, 6, 7, 8, 9, 17, 25, 32, Sd	(Hovmøller)*
DK 122/09 R120	Denmark	2009	Yr 1, 2, 3, 4, 6, 9, 17, 25, 32, Sd, Su	(Hovmøller)*
R13 72/94	Denmark	1994	Yr 1, 2, 3, 9, 25, Sd	(Hovmøller, 2007)*
12/07 R99	Denmark	2007	Yr 1, 2, 3, 4, 9, 17, 25, 32, Sd, Su	(Hovmøller, 2007)*
Mix 28/06 R96	Denmark	2006	Yr 1, 3, 4, 6, 25, 32, Sd, Su, Sp	(Hovmøller, 2007)*
111/02 R52	Denmark	2002	Yr 1, 2, 3, 9, 15, 17, 25, Sd	(Hovmøller, 2007)*
R15 68/94 ha	Denmark	1994	Yr 3, 4, 6, 9, 25, Sd, Su	(Hovmøller, 2007)*
DK133/08 R113	Denmark	2008	Yr 3, 4, 6, 25, 27, 32, Sd, Su	(Hovmøller, 2007)*
R47 75.30c	UK	1975	Yr 25, 32, Sd, Sp	(Hovmøller, 2007)*
R72 E19b/04	Eritrea	2004	Yr 2, 6, 7, 8, 10, 24, 27, Su	(Hovmøller, 2007)*
R31 24/95	Denmark	1995	Yr 3, 4, 6, 25, 32, Sd, Su	(Hovmøller, 2007)*
71/93 R18	Denmark	1993	Yr 1, 2, 3, 4, 18, 25, 32, Sd	(Hovmøller, 2007)*
R118 Afg 4b/	Afghanistan	2009	Yr 1, 2, 3, 6, 7, 8, 25, Sd	(Hovmøller)*
R41 80/01??	Denmark	2007	Yr 1, 2, 3, 4, 6, 9, 17, 18, 25, Sd, Su	(Hovmøller, 2007)*
Mix 27 a/06	Denmark	2006	Yr 1, 2, 18, 25, 32, Sd, Sp	(Hovmøller, 2007)*
SE 100/09	Sweden	2009	Yr 6, 7, 8, 10	(Hovmøller)*

* Personal communication

Adult plant resistance to stem rust

The adult plant resistance was tested against stem rust race of Ug99 (TTKSK, isolate synonym of Ug99) in Njoro, Kenya established by the Kenyan Agricultural Research Institute in conjunction with CIMMYT and the Borlaug Global Rust Initiative. The nursery site was located at 0º20'S, 35º56'E, and 2.185 meter above sea level, with an average daily minimum temperature of 9.7ºC (night) and an average daily maximum temperature of 23.5ºC (noon) (Jin et al., 2007). A total of 28 BC_1F_3 wheat-rye translocation lines including their parents were tested against Ug99, *Puccinia graminis f. sp. tritici,* the most virulent race of the stem rust fungus yet to emerge. The material was placed in a vernalization room for about 7-8 weeks, and were then transformed and planted in the field (Figure 16). To generate a sufficient disease infection pressure, a mixture of susceptible wheat varieties were used as spreader rows between the plots along the main wind direction. The spreader rows were inoculated artificially, using a dusting with a mixture of urediniospores and talc powder at tillering/booting growing stage.

Figure 16. Adult plant resistance test to stem rust Ug99 in Njoro, Kenya

Scoring of the diseases severities in the adult plants was based on the modified Cobb scale (Peterson et al. 1948), and the reaction types were classified into the following categories (Roelfs et al., 1992):

R – Resistant, no visible infection or some chlorosis or necrosis, no uredia, 10%

MR – Moderately resistant, small uredia present and surrounded by either chlorotic or necrotic areas, 20-30%

MR-MS – Moderately Resistance to Moderately Susceptible, 30-40%

MS - Moderately susceptible, medium-sized uredia present and possibly surrounded by chlorotic areas, 40%

MSS - Moderately Susceptible to Susceptible, 40-50%

S – Susceptible, large uredia present, generally with little or no chlorosis and no necrosis, more than 50 %

Table 7. Infection types of differential genotypes to 17 races/isolates of *Puccinia striiformis*.

Varieties	Races/ Isolates	Ken 08/09	CN4/08 (R109)	DK 122/09 R120	R13 72/94	12/07 R99	Mix 28/06 R96	111/02 R52	R15 68/94 ha	DK133/08 R113	R47 75.30c	R72 E19b/04	R31 24/95	71/93 R18	R118 Afg 4b/	R41 80/01??	Mix 27 a/06	SE 100/09
Cartago	none	8	7	8	7	9	9	8	9	7	8	7	8	8	7	8	8	3
Chinese 166	Yr1	7	7	8	7	9	8	7	1	1	1	0	1	8	1	7	7	1
Kalyansona	Yr2, +	7	8	8	7	9	1	8	0	0	1	5	0	9	6	8	0	4
Heines VII	Yr2, +	7	7	8	6	8	2	7	1	2	1	4	1	7	5	7	1	0
Vilmorin 23	Yr3 +	2	7	7	7	7	8	6	8	8	1	2	7	7	3	7	2	0
Hybrid 46	Yr4 +	0	0	7	0	7	8	0	7	8	0	0	6	0	0	0	0	3
Suwon 92/Omar	So / Yr4	0	7	8	1	9	8	0	8	8	0	0	6	0	1	8	0	0
Heines Kolben	Yr6, (Yr2)	7	7	7	1	2	5	1	5	3	1	7	4	1	8	7	1	7
Heines Peko	Yr6, Yr2	7	7	7	1	2	1	1	1	3	0	5	0	1	4	7	0	1
Avocet Yr 6	Yr6	7	7	8	1	4	9	1	9	9	6	7	7	2	1	8	3	1
Lee	Yr7	7	7	2	2	2	2	1	2	2	2	7	3	2	7	2	2	7
Avocet Yr 7	Yr7	8	7	3	2	4	3	2	3	2	3	7	4	2	0	3	2	1
Compair	Yr8	7	4	0	0	0	1	0	1	0	2	5	1	1	7	3	0	5
Avocet Yr 8	Yr8	8	8	1	1	1	2	1	1	1	2	8	2	2	1	2	2	5
Sleipner	Yr9	0	8	8	7	9	2	8	8	1	0	0	0	1	1	8	0	0
Avocet Yr 9	Yr9	8	7	8	5	9	1	7	8	0	0	0	0	2	0	8	1	0
Moro	Yr10	2	0	0	0	0	1	0	0	0	1	2	0	2	1	0	0	4
Avocet Yr 10	Yr10	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
VPM1	Yr17, +	4	8	8	5	9	7	6	5	6	5	4	4	3	2	7	4	1
Carstens V	CV (Yr32, +)	3	8	8	1	7	7	0	6	9	9	2	6	7	1	2	7	0
Avocet Yr 32	Yr32	4	8	8	1	8	8	6	5	8	8	3	7	7	0	4	8	0
Strubes Dickkopf	Sd	5	7	7	5	8	7	7	8	7	5	5	6	8	6	7	7	4
Spaldings Prolific	Sp	1	0	0	1	4	3	0	3	3	7	0	3	2	1	2	7	0
Cortez	Yr15	0	0	0	0	1	0	7	0	0	0	0	0	0	0	0	0	0
Opata	Yr18, Yr27	8	5	5	4	5	5	4	4	5	5	5	3	4	3	5	4	4
Anja	Yr25 ?	6	7	8	7	8	7	8	9	7	4	1	8	8	5	8	8	1
Brigadier	Yr9, 17	4	6	8	0	7	8	5	1	0	0	0	0	1	0	6	0	0
Avocet Yr24	Yr24	2	2	2	1	3	2	2	2	2	3	2	3	2	0	3	3	0
Avocet Yr 5	Yr5	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Avocet S	none	7	8	8	8	8	8	8	9	9	8	8	7	8	0	9	9	3
Ambition	?	1	7	2	0	2	2	0	4	2	2	0	2	3	4	4	6	2
Bob White	Yr32+ ??	7	7	1	0	1	1	0	9	1	1	1	1	0	0	2	0	0
Avocet Yr18	Yr18	7	7	7	7	8	7	7	1	9	8	7	8	7	1	8	7	1

RESULTS

Seedling resistance test for yellow rust in BC_1F_3 and their parents

Lines of BC_1F_3 wheat-rye translocation together with their parents were inoculated with 17 races/isolates and screened in a seedling resistance test. In the seedling tests many of the BC_1F3 wheat-rye translocation lines were found susceptible against many of the tested races/isolates. The susceptible bread wheat variety Topper, BC_1F_3 without any wheat-rye translocations together with BC_1F_3 wheat-rye translocation lines that carry only 2RL/2BS were fully susceptible to all races of yellow rust, and their leaves were covered with uredinia (Figure 17). However the resistant double translocation line KR99-139 and BC1F3 wheat-rye translocations that either carries 1BL/1RS and 2RL/2BS or only with 1BL/1RS developed no visible symptoms for the following inoculation races/isolates; R15 68/94 ha, DK133/08 R113, R47 75.30c, R72 E19b/04, R31 24/95, R118 Afg 4b/ and SE 100/09. Scoring numbers of the BC_1F_3 wheat-rye translocations lines and their parents KR99-139 line and the variety Topper to yellow rust for the 17 tested races/isolates are presented in Table 8.

Table 8. Seedling infection scores of BC_1F_3 wheat-rye translocation lines and their parents to yellow rust

Races/Isolates	Topper	KR99-139	BC_1F_3 1R++ 2R++	BC_1F_3 1R++ 2R++	BC_1F_3 1R+- 2R++	BC_1F_3 1R+- 2R++	BC_1F_3 1R++ 2R+-	BC_1F_3 1R++ 2R+-	BC_1F_3 1R-- 2R--	BC_1F_3 1R-- 2R--
Ken 08/09	7	7	7	7	7	7	7	7	7	7
CN4/08 (R109)	8	8	8	8	8	8	8	8	8	8
DK 122/09 R120	8	8	8	8	8	8	8	8	8	8
R13 72/94	7	7	8	7	7	7	7	7	7	7
12/07 R99	8	8	7	8	8	8	8	8	8	8
Mix 28/06 R96	9	9	8	8	9	9	9	9	9	9
111/02 R52	7	7	7	7	7	7	7	8	8	8
R15 68/94 ha	8	0	0	0	8	8	0	0	8	8
DK133/08 R113	8	0	0	0	8	8	0	0	8	8
R47 75.30c	8	0	0	0	8	9	0	0	8	8
R72 E19b/04	9	0	0	0	7	8	0	0	9	9
R31 24/95	8	0	0	0	4	4	0	0	7	7
71/93 R18	8	7	7	7	7	7	7	7	8	9
R118 Afg 4b/	7	0	0	1	6	7	0	1	7	7
R41 80/01??	8	7	8	7	7	7	7	7	8	9
Mix 27 a/06	7	8	7	5	7	8	8	7	8	8
SE 100/09	7	1	0	0	4	5	0	0	6	6

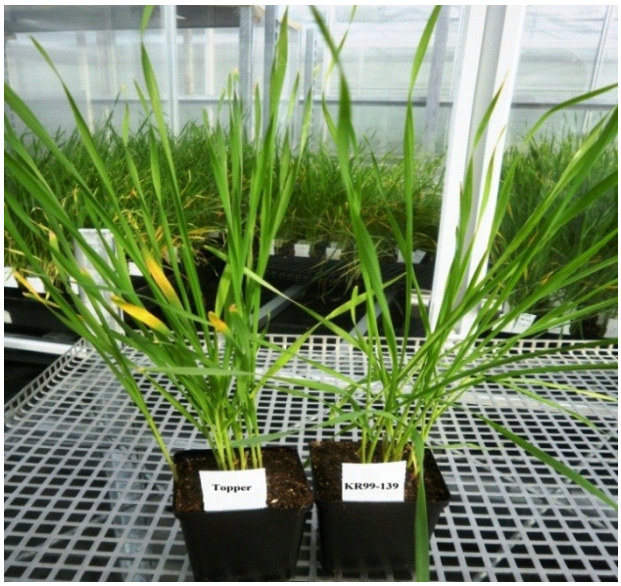

Figure 17. Reaction of seedling resistance test of two parents Topper (Susceptible) and KR99-136 (Resistance) to yellow rust *Puccinia striiformis f. spp. tritici*

Adult plant resistance to stem rust Ug99 race

In order to identify resistance sources for Ug99, BC_1F_3 wheat-rye translocation lines were grown under high stem rust Ug99 pressure at the Njoro research station of the Kenyan Agricultural Research Institute in Kenya. The assessments of the germplasm were carried out 2 times. The results of the adult plant resistance showed that 17 of the BC_1F_3 as well as both the parents were susceptible (S) for Ug99 and further 8 of the BC_1F_3 were moderately susceptible to susceptible (MSS). However among 30 tested BC1F3 lines two lines were found resistant against the stem rust, race Ug99 during both scorings for APR (Table 9).

During the assessment of APR, the tested materials ranged from 0-60% in disease severity. The two BC_1F_3 wheat-rye translocation lines that were found resistant against Ug99 were both being homozygous for 1RS++ and heterozygous for 2RL+–. The lines being homozygous for both the rye translocations (similar as the parent KR99-139) showed 10-50% disease severity, lines being 1RS++ and 2RL+– showed 0-30% disease severity, lines being 1RS+– and 2RL++ showed 15-50% disease severity, lines being 1RS+– and 2RL+– showed 10-50% disease severity and finally lines being 1RS– – and 2RL+– showed 5-60% disease severity (Table 9). Thus, the combination of 1RS++ and 2RL+– seemed to be the key issue for showing resistance towards Ug99.

Table 9. Adult plant resistance response and diseases severity of the BC_1F_3 segregating population against stem rust Ug99 in Kenya

#	Cultivar/lines	Rye Chromosome	Scoring date	
			21.10.2010	**05.11.2010**
1	Topper	**None**	20S	30S
2	KR99-139	**1RS++; 2RL++**	10S	10S
3	30	**1RS++; 2RL++**	20S	30S
4	34	**1RS++; 2RL++**	30S	40S
5	65	**1RS++; 2RL++**	15S	20S
6	81	**1RS++; 2RL++**	20MSS	30MSS
7	93	**1RS++; 2RL++**	30MSS	50MSS
8	7	**1RS++; 2RL+-**	10S	20S
9	37	**1RS++; 2RL+-**	20S	30S
10	45	**1RS++; 2RL+-**	R	R
11	51	**1RS++; 2RL+-**	20S	30S
12	63	**1RS++; 2RL+-**	R	10R
13	1	**1RS+-; 2RL++**	15S	30S
14	19	**1RS+-; 2RL++**	30MSS	50MSS
15	58	**1RS+-; 2RL++**	20S	50MSS
16	79	**1RS+-; 2RL++**	20S	40S
17	94	**1RS+-; 2RL++**	30S	50S
18	6	**1RS+-; 2RL+-**	30S	50MSS
19	27	**1RS+-; 2RL+-**	10S	30S
20	56	**1RS+-; 2RL+-**	20S	30S
21	73	**1RS+-; 2RL+-**	15S	40S
22	88	**1RS+-; 2RL+-**	5MSS	20MSS
23	10	**1RS- -; 2RL+-**	30S	40S
24	28	**1RS- -; 2RL+-**	20S	40S
25	29	**1RS- -; 2RL+-**	10S	20S
26	30	**1RS- -; 2RL+-**	5S	5S
27	45	**1RS- -; 2RL+-**	30MSS	60MSS
28	93	**1RS- -; 2RL+-**	20S	40S
29	95	**1RS- -; 2RL+-**	15S	20S
30	98	**1RS- -; 2RL+-**	30S	50MSS

++ designates of homozygous presence and +–heterozygous presence

DISCUSSION

There are two mechanisms of resistance to yellow rust; seedling resistance, which can be expressed in all stages of plant development, and adult plant resistance, which is expressed in adult plant stages (Chen, 2005). In this study, BC_1F_3 wheat-rye translocation lines including their parents were evaluated for seedling resistance towards yellow rust and adult plant resistance for stem rust, race Ug99.

Seedling resistance test of BC_1F_3 including parents was carried out using 17 races/isolates of yellow rust at the seedling stage. The races/isolates were collected from different origins/countries. Seedling resistances are functional from the onset of plant growth and effective throughout the life of the plant. These resistances are generally controlled by single genes and are very effective in the absence of matching virulence in the pathogen (Wallwork, 2009). Seedling resistance generally operates in a gene-for-gene manner with the avirulence of the pathogen, as described by Flor (1971). Due to limited time we were not able to conduct adult plant resistance evaluations in the field/or greenhouse against yellow rust. The yellow rust response in the BC_1F_3 wheat-rye translocation lines were shown to vary and the variety Topper was shown susceptible to all races/isolates and infection was scored to 7-8. The line KR99-139 was highly resistant towards seven races/isolates and for those lines the infection was scored 0 (Table 8). According to this study, the BC_1F_3 wheat-rye translocations lines containing (1RS++) and being either homozygous (2RL++) or heterozygous for (2RL+–) showed resistance against the same isolates/races as did KR99-139. The result thus indicated that KR99-139 carries resistance genes that were inherited to the BC_1F_3 wheat-rye translocation lines carrying 1RS. Probably, the most likely gene giving rise to the resistance reaction in KR99-139 and its offspring having the 1RS is the presence of the *Yr9* gene. The *Yr9* originates from rye, and it is present in many wheat varieties. The gene *Yr9* was originally derived from rye, *Secale cereale*, through chromosomal translocation of 1BL/1RS which are widely present in many wheat cultivars worldwide (Zeller, 1973; McIntosh et al., 1995; Schlegel and Korzun, 1997). The gene confers resistance to many races of yellow rust and has been incorporated into wheat cultivars in many countries. While comparing the virulence profile of *Yr9* with the races/isolates used in the present study it does, however, not matches totally. This might be explained by the fact that virulence for *Yr9* has occurred in most wheat producing areas worldwide, e.g. in Africa, Middle

East, Indian sub-continent, Tajikistan, China, USA, CWANA and Europe (Louwers et al., 1992; McIntosh et al., 1995; Wan et al., 2004; Bocra et al., 2009; Chen et al., 2002; 2009; Rahmatov et al., 2011a; Nazari et al., 2011). Since, the set of isolates used in the present study, are representatives from different origins worldwide, various combinations/reactions of avirulence/resistant and virulence/susceptible can occur in the tested lines. The *Yr9* is known to not provide enough resistance against a wide range of races/isolates. However *Yr9* was present in several of the isolates/races towards which the BC_1F_3 plants with 1RS++ showed resistance (Table 8). Another gene that matches very well with the resistance reactions obtained in the present material is *Yr1*. Thus a combination of the two mentioned genes might also be an explanation for the resistance as can the present of *Yr1* solely. To conclude, more studies are needed to understand exactly which genes that are responsible for the resistance reactions against yellow rust that we see in the material can be determined.

The adult plant resistance tests were conducted in the environmental conditions of Njoro, Kenya, a favorable environment for stem rust infection and a high severity of disease was observed in 2010. Thereby, high disease severities of about 80-100% were assessed on the susceptible check and spreader rows in the field of Njoro, Kenya. The infection levels were high enough to score adult plant resistance in the field. In two of the lines, carrying 1RS++ and 2RL+–, resistance against Ug99 was found, while the rest of the lines were found to be moderately susceptible to susceptible and susceptible. Further, lowest disease severity was also generally seen in the lines with the chromosomal combinations 1RS++ and 2RL+–. Lines with only 1RS++ or homozygous for 2RL was not showed similar low disease severity. This indicated that both homozygosity for 1RS and heterozygousity for 2RL were needed for resistance. Thus, these results indicated presence of several genes/QTLs to obtain resistance indicating possible epistatic effects of the genes involved. In order to clarify the background for the resistance, further studies are needed. Options are to create a mapping population in order to be able to understand the mechanisms of this resistance towards Ug99. Also, further validation to identify potential value of the resistance genes found against yellow rust and stem rust are necessary.

CONCLUSIONS

The 1BL/1RS and 2RL/2BS wheat - rye translocations have been of particular interest and are widely used in many bread wheat breeding programs. The following conclusions were made based on the study conducted:

- Assessment of coleoptiles, red and green used for selection of plants containing different combinations of presence or absence of 2RL was a suitable method to be used if two proceeding generations of the material was analyzed. The 2RL from KR99-139 carries gene for red coleoptile that was used as a phenotypic marker for presence of the 2RL translocation and might be used in wheat breeding. In this study, coleoptile colours helped in selecting lines with different sets/combination of 1RS and 2RL.
- By the use of Giemsa C-banding technique it was possible to detect 1BL/1RS and 2RL/2BS in BC_1F_3 and KR99-139 wheat-rye translocation lines. A Giemsa C-banding technique was a powerful technique that enabled identification and classification of the homozygous presence of 2RL++ and heterozygous presence of 1RS+– rye chromosome in BC_1F_3 wheat-rye translocation lines.
- Molecular markers proved to be an effective method for verification of 1BL/1RS and 2RL/2BS in BC_1F_3 wheat-rye translocation lines. Of the five rye specific SSR markers used, two were amplified reliable and distinguished easily translocation and non-translocation lines; SCM9 for 1RS and SCM75 for 2RL.The three SSR markers SCM39, SCM43 and SCM69 were not amplified when rye fragments in BC_1F_3 wheat-rye translocation lines were present.
- A total of 17 races/isolates of *Puccinia striiformis* were used for seedling resistance tests. The variety Topper and BC_1F_3 that carry 2RL/2BS or without any wheat-rye translocation were all fully susceptible to all 17 races/isolates for *Puccinia striiformis*. The KR99-139 and BC_1F_3 that carries wheat-rye translocation lines with combination of 1BL/1RS and 2RL/2BS or only 1BL/1RS were as identified highly resistant towards seven tested yellow rust races/isolates.
- The adult plant resistances to stem rust against Ug99 (TTKSK) race were conducted in Kenya, and we identified two resistance sources towards Ug99. The BC_1F_3 with homozygous presence of 1RS++ and heterozygous presence of 2RL-+ were identified as the most possible resistance sources towards Ug99 within the tested lines.

FUTURE RECOMENDATIONS

A translocated wheat material contains suitable genes from rye, and has a high potential for resistance against biotic and abiotic stresses. The following steps are proposed to continue the identification of novel resistance genes against yellow rust, stem rust as well as other diseases:

- Gene postulation and adult plant resistance is a key to provide complete genetic characterization of yellow rust and stem rust resistance. To recapitulate it is a prerequisite to conduct continuous gene postulation and adult plant resistance to identify genes which could contain resistance among the wheat-rye translocation lines
- Identification and characterization of genes/QTLs associated with resistance to yellow rust and stem rust. To further dissect resistance genes, major genes, interaction among genes as well as QTL alleles within the wheat-rye translocation materials should be evaluated, by performing genetic analysis of mapping populations
- Field trial for agronomic performances and other traits, should be carried out on the material when resistance genes for certain traits has been transformed to susceptible wheat genotypes

REFERENCES

Agriculture of Republic of Tajikistan. The statistical collection. 2009. Dushanbe,

Ali, S., and L.J. Francl. 2003. Population Race Structure of Pyrenophora tritici-repentis Prevalent on Wheat and Non cereal Grasses in the Great Plains. Plant Dis. 87: pp 418-422.

Biffen, R. H. 1905. Mendel's laws of inheritance and wheat breeding. J. Agric. Sci. pp 1: 4-8.

Bochra, Bahri,Oliver Kaltz, Marc Leconte, Claude de Vallavieille-Pope and Jérôme Enjalbert. 2009. Tracking costs of virulence in natural populations of the wheat pathogen, Puccinia striiformis f.sp.tritici. BMC Evolutionary Biology, pp 9:26

Borojevic, S. 1990. Principles and methods of plant breeding. Developments in Crop Science, Vol. 17, Elsevier Science Pub. Co., Amsterdam, The Netherlands.

Boukhatem, N., P.V. Baret and D. Mingeot, 2002. Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. Theoretical and Applied Genetics 104: pp 111-118.

Boyko, E.V., Gill K.S., Mickelson-Young L., Nasuda S., Raupp W.J., Yiegle J.N., Singh S., Hassawi D.S., Frity A.K., Namuth D. et al. 1999. A high-density genetic linkage map of Aegilops tauschii, the D-genome progenitor of bread wheat. Theor Appl Genet., 99: pp 16–26

Briggs, F.N., and R. W. Allard. 1953. The current status of the backcross method of plant breeding. Agron. J. 45:131–138

Chen, W.Q., Wu, L.R., Liu, T.G., Xu, S.C. 2009. Race dynamics, diversity and virulence evolution in *Puccinia striiformis f.sp. tritici*, the causal agent of wheat stripe rust in China from 2003 to 2007. Plant Dis 93: pp, 1093-1101

Chen, X. M. 2005. Epidemiology and control of yellow rust (Puccinia striiformis f. sp. tritici) on wheat. Can. J. Plant Pathol. 27: pp 314-337.

Chen, X. M., M. A. Soria, G. P. Yan, J. Sun and J. Dubcovsky. 2003. Development of sequenced tagged site and cleaved amplified polymorphic sequence markers for wheat yellow rust resistance gene Yr5. Crop Sci. 43: pp 2058-2064.

Chen, X.M., and Wood, D.A. 2004. Control of yellow rust of spring wheat with foliar fungicides, 2003. Fungicide and Nematicide Tests [serial online], Report 59:CF022. The American Phytopathological Society, St. Paul, Minn. doi: 10.1094/FN59.

Chen, X. M., Moore, M., Milus, E. A., Long, D. L., Line, R. F., Marshall, D., and Jackson, L. 2002. Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant Dis. 86: pp, 39-46

Clifford, B.C. 1974. Stable resistance to cereal disease: problems and progress. Rep. Welsh Plant Breeding Station.

Curtis, B.C., Rajaram S. and Macpherson H.G. (eds) 2002. Bread wheat improvement and production No. 30. Food and Agriculture Organization of the United Nations, Rome Italy pp 554.

Devos, K.M., J. Dubcovsky, J. Dvorak, C.N. Chinoy, and M.D. Gale. 1995. Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. Theor. Appl. Genet. 91: pp 282-288.

Doyle, J.J., Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: pp11–15

Dubin, H. J., R. W. Stubbs and R. Johnson. 1989. Postulated genes for resistance to yellow rust in selected CIMMYT and related wheat. Plant Dis. 73: pp 472-475.

Duveiller, E., Y.R. Kandel, R.C. Sharma, and S.M. Shrestha. 2005. Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95: pp 248-256

Dvorak, J., M.C. Luo, Z.L. Yang, and H.B. Zhang. 1998. The structure of the Aegilops tauschii gene pool and the evolution of hexaploid wheat. Theor. Appl. Genet. 97: pp 657- 670.

Eckardt, N.A. 2006. Identification of rust fungi avirulence elicitors. The Plant Cell 18: pp 1-3.

Ensermu, R., W. Mwangi, H. Verkujjl, M. Hassena, and Z. Alemayehu. 1998. Farmers Wheat Seed Sources and seed Management in Chilalo Awraja, Ethiopia, Mexico, D.F., IAR and CIIMYT.

Eshonova Z., Pett, B., Kosimov F., Muminjanov H.A., Rahmatov M. 2006. Evaluation of winter and facultative wheat varieties and breeding lines resistant to yellow rust in Tajikistan. 2nd Central Asian Cereals Conference, June 2006, Cholpon-Ata, Kyrgyz Republic, pp. 305 – 308

Eshonova Zebuniso, Mahbubjon Rahmatov, Ahadhon Ibrogimov, Munira Otambekova, Bahromiddin Khuseinov, Hafiz Muminjanov, Alexey Morgounov, Arnulf Merker Arne Hede. 2010. Monitoring and evaluation of Yellow Rust for breeding resistant varieties of

wheat in Tajikistan. Proceeding of 8th International Wheat Conference June 1-4, 2010 St. Petersburg, Russia. pp 249

Evans, T.T. and Peacock, W.J. 1981. Wheat science-today and tomorrow, Cambridge University Press, Cambridge, UK, 5: pp 75-91

Feldman, M., Lupton F.G.H., Miller T.E. 1995. Wheats. In: Smartt J, Simmonds N.W. (eds) Evolution of crops (2nd ed.). Longman Scientific, London, pp 184-192

Feldmann, M. 2001. Origin of cultivated wheat. In A.P. Bonjean, and W.J. Angus (eds) The world wheat book. A history of wheat breeding, Lavoiser Publishing, France. pp 3-56.

Feldman, M. and A.A. Levy. 2005. Allopolyploidy - a shaping force in the evolution of wheat genomes. Cytogenetic and Genome Research 109: pp 250-258.

Friebe, B., Jiang, J., Raupp, W.J., McIntosh, R.A. & Gill, B.S. 1996. Characterization of wheat – alien translocations conferring resistance to diseases and pests: current status. Euphytica 91: pp 59-87.

Friebe, B., Mukai Y., Dhaliwal H.S., Martin T.J. and Gill B.S. 1991. Identification of alien chromatin specifying resistance to wheat streak mosaic and Greenbug in wheat germplasm by C-banding and in situ hybridization. Theoretical and Applied Genetics 81: pp 381-389

Friebe, B., Heun M., Tuleen N., Zeller F.J., Gill B.S. 1994. Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci, 34: pp 621–625.

Gill, B.S., Kimber G. 1977. Recognition of translocations and alien chromosome transfers in wheat by the Giemsa C-banding technique. Crop Sci 17: pp 264–266

Gill, B.S., and B. Friebe. 2001. Cytogenetics, phylogeny and evolution of cultivated wheats. In A.P. Bonjean, and W.J. Angus (eds) The world wheat book. A history of wheat breeding, Lavoiser Publishing, France. pp 71-88.

Gill, B.S.; Kimber, G. 1974. Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. USA 71: pp 4086 4090

Gill, B. S. & Kimber, G. 1974. "The Giemsa C-banded karyotypes of rye," Proc. Nat. Acad. Sci. USA 71: pp 1247-1249

Gill, B. S. & Kimber, G. 1974. "A Giemsa C-banding technique for cereal chromosomes," Cereal Research Communications 2: pp 87-94.

Gill, B.S., B. Friebe and T.R. Endo. 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (Triticum aestivum). Genome 34: pp 830-839.

Green, G.J. and A.B. Campbell. 1979. Wheat varieties resistant to Puccinia graminis tritici in Western Canada: Their development, performance and economic value. Can. J. Plant Sci. 1: pp 3-11

Johnson, B.L. 1975. Identification of the apparent B genome donor of wheat. Can. J. Genet. Cytol. 17: pp 21-39.

Hardison, J.R. 1975. Control of Puccinia striiformis by two new systemic fungicides, Bay MEB 6447 and BAS 31702 F. Plant Dis. Rep. 59: pp 652–655

Harris, M. K. and Frederiksen R. A. 1984. Concepts and methods regarding host plant resistance to arthropods and pathogens. Annu. Rev. Phytopathol., 22: pp 247-72

Harlan, J.R. 1967. A wild wheat harvest in Turkey, Archaeology 19: pp 197–201.

Heslop-Harrison, J.S., Leith A.R., Schwarzacher T., Anamthawat-Jonsson K. 1990. Detection and characterisation of 1B/1R translocation in hexaploid wheat. Heredity 65: pp 385–392

Hillman, G.C. and Davies M.S. 1990. Domestication rates in wild-type wheats and barley under primitive cultivation, Biological Journal of the Linnean Society 39, pp 39–78.

Hohmann, U., Endo T.R., Gill K.S. and Gill B.S. 1994. Comparison of genetic and physical maps of group 7 chromosomes from Triticum aestivum. Molecular and General Genetics 2445: pp 644-653.

Hooker, A.L. 1967. The genetics and expression of resistance in plants to rusts of genus Puccinia. Ann. Rev. Phytopathology, 5: pp 163-182.

Hovmøller, M.S. 2001. Disease severity and pathotype dynamics of Puccinia striiformis f.sp.tritici in Denmark. Plant Pathology. 50: pp 181-189.

Hovmøller, M. S. 2007. Sources of seedling and adult plant resistance to Puccinia striifomis f. sp. tritici in European wheats. Plant Breeding 126: 225-233.

Hovmøller, M. S. and A. F. Justesen. 2007. Appearance of a typical Puccinia striiformis f. sp. tritici phenotypes in north-western Europe. Australian Journal of Agricultural Research 58: pp 518.

Hysing, S,C., Sai L. K. Hsam, Ravi P. Singh, Julio Huerta-Espino, Lesley A. Boyd, Robert M. D. Koebner Sue Cambron, Jerry W. Johnson, Daniel E. Bland, Erland Liljeroth and Arnulf Merker. 2007. Agronomic Performance and Multiple Disease Resistance in T2BS.2RL Wheat – Rye Translocation Lines. Crop Science 47: pp 254-260.

Imtiaz, M., M.G. Cromey, J.G. Hampton and M. Ahmad. 2003. Inheritance of durable adult plant resistance to yellow rust (Puccinia striiformis f. sp. tritici) in 'Otane' wheat (Triticum aestivum). New Zealand Journal of Crop and Horticultural Science 31: pp 23-31.

Jiang, J. and B.S. Gill. 1994. Different species-specific chromosome translocations in Triticum timopheevii and T. turgidum diphyletic origin of polyploid wheats. Chrom. Res. 2: pp 59-64.

Jin Y., Singh R. P., Ward R. W., Wanyera R., et al. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic Sr gene lines to race TTKS of *Puccinia graminis f. sp. tritici*. Plant Dis. 91: pp 1096-1099.

Johnson B.L., and H.S. Dhaliwal. 1976. Reproductive isolation of Triticum boeoticum and Triticum urartu and the origin of the tetraploid wheat. Am. J. Bot. 63: pp 1088-1094.

Kattermann, G. 1937. Zur Cytologie halmbehaarter Stämme aus Weizenroggenbastardierung. Züchter 9: pp 196-199.

Kimber, G. K., Sears, E.R. 1987. Evolution of the genus Triticeae and origin of cultivated wheat. Wheat and wheat improvement, Edition second. American Society of Agronomy, Inc., Crop Science of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin, USA. pp 154-164.

Kerby, K. and J. Kuspira. 1988. Cytological evidence bearing on the origin of the B genome in polyploid wheats. Genome 30: pp 36-43.

Knox, R.E., Menzies J.G., Howes N.K., Clarke J.M., Aung T. and Penner G.A. 2002. Genetic analysis of resistance to loose smut and an associated DNA marker in durum wheat doubled haploids. Canadian Journal of Plant Pathology 24: pp 316-322.

Koebner, R. M. D., 1995: Generation of PCR-based markers for the detection of rye chromatin in wheat background. Theor. Appl. Genet. 90: pp 740—745.

Lee, J.H., R.A. Graybosch, S.M. Kaeppler, and R.G. Sears. 1996. A PCR assay for detection of a 2RL.2BS wheat-rye chromosome translocation. Genome 39: pp 605–608.

Leonard, K.J. 2001. Stem rust – future enemy? in P.D. Peterson, ed., Stem Rust of Wheat: from Ancient Enemy to Modern Foe. APS Press, St. Paul, MN. pp 119-146

Leonard, K.J. and Szabo, L.J. 2005. Stem rust of small grains and grasses caused by Puccinia graminis. Molecular Plant Pathology 6: 99- 111.

Li, W.L., Zhang P, Fellers J.P., Friebe B., Gill B.S. 2004. Sequence composition, organization, and evolution of the core Triticeae genome. Plant J 40: pp 500-511

Louwers, J.M., Van Silfhout, C.H., Stubbs, R.W. 1992. Race analysis in wheat in developing countries. Report 1990-1992. IPO-DLO Report 92-11, pp 23

Lukaszewski, A.J. 1990. Frequency of 1RS.1AL and 1RS.1BL translocations in United States wheats. Crop Sci 30: pp 1151–1153

Lupton, F.G. and Johnson R. 1970. Breeding for mature-plant resistance to yellow rust in wheat. Ann. Applied Biology, 66: pp 137-143

Marsalis, M.A and Goldberg N.P. 2006. Leaf, stem, and yellow rust diseases of Wheat. New Mexico State University Guide A-415.

McFadden, E.S., Sears E.R. 1946. The origin of Triticum spelta and its free-threshing hexaploid relatives. *Journal of Heredity,* **37**:107-116.

McIntosh, R.A. 1983. A catalogue of gene symbols for wheat. Proc. Sixth Intern. Wheat Genet. Symp. 1983. Plant Germplasm Inst., Kyoto. pp 1197–1255.

McIntosh, R. A., G. E. Hart, K. M. Devos, M. D. Gale and W. J. Rogers. 1998. Catalogue of gene symbols for wheat. In: Proceedings of the 9th International Wheat Genetics Symposium. 2-7 August 1998, University of Saskatchewan, Saskatoon, Sask. Edited by A. E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask. Vol. 5. pp 1-235.

McIntosh, R.A., Hart G.E., Gale M.D. 2001. Catalogue of wheat symbols for wheat—2001 supplement (on line). In: Graingenes: a database for Triticeae and Avena. Available from http:// wheat.pw.usda.gov/ggpages/pubs.html

McIntosh, R.A., Yamazaki Y, Devos K.M., Dubcovsky J, Rogers W.J., Appels R. 2003. Catalogue of gene symbols for wheat. In: Pogna NE, Romano M, Pogna A, Galterio G (eds) Proceedings of the 10th international wheat genetics symposium. Paestum, Italy

McIntosh, R. A., K. M. Devos, J. Dubcovsky and W. J. Rogers. 2004. Catalogue of gene Symbols for wheat. 2004 supplement online. http://grain.jouy.inra.fr/ggpages/wgc/2004upd.htm1

McIntosh, R. A, K. M. Devos, J. Dubcovsky, W. J. Rogers, C. F. Morris, R. Appels and O. D. Anderson. 2006. Catalogue of gene symbols for wheat: 2006 supplement online. http://grain.jouy.inra.fr/ggpages/wgc/2006upd.htm1.

McIntosh, R.A., Wellings C.R., Park R.F. 1995 Wheat rusts: an atlas of resistance genes. CSIRO Publishing, Melbourne

McIntosh, R. A., K. M. Devos, J. Dubcovsky, W. J. Rogers, C. F. Morris, R. Appels and O. A. Anderson 2007. Catalogue of gene Symbols for wheat:

McIntosh, R.A. 2009. History and status of the wheat rusts. Borlaug Global Rust Initiative, Technical Workshop Cd. Obregon, Sonora, Mexico, March 17-20, 2009. pp 1-16.

McNeal, F.H., Konzac C.F., Smith E.P., Tate W.S., Russell T.S. 1971. A uniform system for recording and processing cereal research data. ARS-USDA, Washington D.C. pp 34–121Melz, G ., R . Schlegel & J . Sybenga. 1988 . Identification of the chromosomes in the 'Esto' set of rye trisomics . Plant Breeding 100: pp,169-172 .

Melz, G., and V. Thiele. 1990. Chromosome locations of genes controlling ‘purple leaf base’ in rye and wheat. Euphytica 49:155–159.

Mergoum, M., P.K. Singh, J.A. Anderson, R. J. Pen˜a, R.P. Singh, S.S. Xu, and J.K. Ransom. 2009. Spring Wheat Breeding, Handbook of plant breeding. pp 127-156

Merker, A. 1984. The rye genome in wheat breeding. Hereditas 100: pp 183-191.

Merker, A., 1982. ‘Veery’ - CIMMYT spring wheat with 1B/1R chromosome translocation. Cereal Res Comm 10: pp 105–106.

Merker, A. 1975. Chromosome composition of hexaploid triticale. Hereditas 80: pp 41-52

Merker, A., and P.O. Forsstro¨ m. 2000. Isolation of mildew resistant wheat-rye translocations from a double substitution line. Euphytica 115:167–172.

Merker, A. 1973. A Giemsa technique for rapid identification of chromosomes in Triticale. Hereditas 75: pp 280-282

Mitchell, M.J., R.H. Busch and H.W. Rines. 1992. Comparison of lines derived by anther culture and single seed descent in spring wheat cross. Crop Science. 32: pp 1446-1451

Mujeeb-Kazi, A., and R.L. Villareal. 2002. Wheat. In V.L. Chopra, and S. Prakash (eds) Evolution and adaptation of cereal crops. Science Publishers, Inc. Enfield USA. pp 43-96.

Muminjanov, H.A. Report of Wheat Breeding in Tajikistan. Dushanbe 2003.

Muminjanov, H. A. Berlin, R. Persson, M. Otambekova, Z. Bishaw. 2008. Focus on Seed Programs: The Tajikistan Seed Industry. Seed Unit, ICARDA, Aleppo, Syria, January pp 12.

Murray, T.D., Parry D.W. and Cattlin N.D. 1998. A colour handbook of diseases of small grain cereal crops. Manson Publishing Ltd. pp 142.

Nazari ,K., Hodson, D., and Hovmoller M. 2011. Yellow rust in CWANA in 2010 & 2011: The situation and measures taken to control it. Proceeding of 2011 Technical Workshop Borlaug Global Rust Initiative, St. Paul, Minnesota, USA, June 13-16, 2011, pp 24

Nelson, R. R. 1978. Genetics of horizontal resistance to plant diseases. Annu. Rev. Phytopathol., 16: pp 359-78.

O'MARA, J.G., 1947: The substitution of a specific *Secale cereale* chromosome for a specific *Triticum aestivum* chromosome. Genetics 32: pp 99-100

OECD-FAO Agricultural Outlook 2009-2018

Pagesse, P., 2001. Wheat: it's genetic diversity, history and prospects. In: The world wheatbook: A history of wheat breeding. Bonjean, A.P. and W.J. Angus (eds). Lavoisier Publishing, Paris. pp 1131-1184.

Peng, J.H., Sun, D., Nevo, E. 2011. Domestication evolution, genetics and genomics in wheat. Mol. Breeding 28: pp 281–301

Pett, B.; Muminjanov, H. 2004. Report on the project of GTZ-CIMMYT in Tajikistan (Russian). – Information Buletin. In: Seed production and breeding of wheat in Asia, no. 1 (06), Almaty. pp 10-22

Pett B., Muminjanov H., Morgunov A., Rahmatov M., Sarkisova T. 2005. Wheat Diseases & Pests Observation for Selection of Resistant Varieties in Tajikistan. Agromeridian, Theoretical and Applied Agricultural Research Journal, pp. 83-87.

Peterson, R.F. 1965. Origin and history of the wheat species. In: Polunin N (ed) Wheat:

Peterson, R.F., Campbell A.B., Hannah A.E. 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. Ca. J. Res. Sect. C. 26: pp 496-500.

Pretorius, Z.A., R.P. Singh, W.W. Wagoire and T.S. Payne. 2000. Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis. f.sp. tritici* in Uganda. Plant Disease. 84: pp 203.

Poehlman, J.M. and D.A. Sleeper. 1995. Breeding self-pollinated crops. "Breeding Field Crops". Fourt edition. Iowa state University prees/Ames

Rabinovich, S. V. 1998. Importance of wheat-rye translocations for breeding modern varieties of Triticum aestivum L. Euphytica 100: pp 323-340.

Rajaram, S., R. Villareal & A. Mujeeb-Kazi. 1990. The global impact of 1B/1R spring wheat. Agronomy abstracts. ASA, Madison, Wisconsin. pp 105.

Rajaram, S. 2001. Prospects and promise of wheat breeding in the 21st century. Euphytica 119: pp 3–15.

Rahmatov M.M., Huseinov B., Muminjanov H., Eshonova Z., Otambekova M., Hede A., Ibragimov A. 2008. Breeding of wheat varieties in Tajikistan. Proceeding of 18 General Congress of EUCARPIA, Valencia, Spain, pp 142

Rahmatov M., Muminjanov H., Otambekova M., Khusenov B., Eshonova Z., Ibragimov A., Yorov A., Hede A., Morgounov A. 2009. Breeding Rust Resistant Wheat Varieties in Tajikistan. Proceeding of 2009 Technical Workshop Borlaug Global Rust Initiative, Cd. Obregon, Sonora, Mexico, March 17-20, 2009, pp 258

Rahmatov M.M., Huseynov B., Otambekova M.G., Eshonova Z.Sh., Ibragimov A., Hede A., Morgunov A.I., Muminjanov H. 2010. Wheat breeding in Tajikistan. Journal Academy of Sciences of Tajikistan pp 67-78, In Russian

Rahmatov M.M., Muminjanov H., Eshonova Z., Ibrohimov A., Karimov M., Hovmøller M., Nazari K., Morgounov A., Hede A., Johansson E. 2011 a. Breeding, Survey and Epidemiology of Yellow Rust in Tajikistan in 2010. Proceeding of International Wheat Stripe Rust Symposium, Aleppo, Syria, April, 2011, pp 77

Rahmatov M., Muminjanov H., Eshonova Z., Morgounov A., Hede A., Johansson E. 2011 b. The national wheat breeding program for development of high yielding and rusts resistant of bread wheat's for Tajikistan. Proceeding of 2011 Technical Workshop Borlaug Global Rust Initiative, St. Paul, Minnesota, USA, June 13-16, 2011, pp 174

Reynolds, M.P., Borlaug N.E. 2006. Impacts of breeding on international collaborative wheat improvement. Journal of Agricultural Science 144: pp 3-17

Roelfs, A.P. 1985. The cereal rusts, Vol. II: diseases, distribution, epidemiology and control. Orlando (FL): Academic Press. Chapter 1, Wheat and rye stem rust. pp 3-37.

Roelfs, A.P., Singh R.P., Saari E.E. 1992. Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico, D.F.

Saal, B.G., Wricke G. 1999. Development of simple sequence repeats markers in rye (Secale cereale L.). Genome 42: pp 964–972

Saari, E.E. and J.M. Prescot. 1985. World distribution in relation to economic losses. In the Cereal Rusts. Vol. II pp 259-298 (Academic Press Orlando, Florida, USA)

Schlegel, R., Korzun, V. 1997. About the origin of 1RS.1BL wheat-rye chromosome translocations from Germany. Plant Breeding 116: pp 537–540

Sharma, S., J. M. Louwers, C. B. Karki and C. H. A. Snijders. 1995. Postulation of resistance genes to yellow rust in wild emmer wheat derivatives and advanced wheat lines from Nepal. Euphytica 8: pp 271-277.

Shimizu, Y., S. Nasuda, and T. R. Endo, 1997: Detection of the Sec-1 locus of rye by a PCR-based method. Genes Genet. Syst. 72: pp 97—203.

Singh, U.S., Mukhopadhyay A.N., Kumar J. and Chaube H.S. 1992. Plant diseases of international importance- diseases of cereals and pulses. Volume 1 Prentice-Hall, Inc.

Singh, R.P., and R.A. McIntosh. 1990. Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in four South African wheats. Theor. Appl. Genet. 79: pp 401-410.

Singh, R.P., Hodson D.P., Jin Y., Huerta-Espino J., Kinyua M.G., Wanyera R., Njau P. and Ward, R.W. 2006. Current status, likely migration and strategies to migrate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1: pp, 1-13.

Singh, R. P., J. C. Nelson, and M. E. Sorrells. 2000. Mapping Yr28 and other gene for resistance to yellow rust in wheat. Crop Sci. 40: pp 1148—1155.

Singh, R.P., J. Huerta-Espino and M. William. 2001. Slow rusting genes based resistance to leaf and yellow rusts in wheat. Eastwood, R., G. Hollamby, T. Rathjen and N. Gororo (eds).

Wheat Breeding Society of Australia: Proceedings of the Assembly 10, 16-21 September 2001, Mildura, Australia. pp 103-108.

Stoskopf, N.C., D.T. Tomes and B.R. Christie. 1993. Single seed descent and recurrent selection breeding methods. Plant breeding theory and practice. Westview press. Inc 5500 central Ave boulder, Colorado.

Sutka, J. 1977. The association of genes for purple coleoptile with chromosomes of the wheat variety Mironovskaya 808. Euphytica 26: pp, 475–479.

USDA, ARS. 2009. Cereal rusts. Cereal Disease Laboratory. St. Paul, MN 55108. Retrieved on April 23 2009, from http://www.ars.usda.gov/Main/docs.htm?docid=9854.

Van der Plank, J.E. 1968. Disease resistance of plants. Academic Press, New York, pp 206.

Van Zeist, W. and Casparie W.A. 1968. Wild einkorn wheat in northern Syria, Acta Botanica Neerlandica 17: pp 44–53.

Vavilov N.I. Five continents / N.I.Vavilov. Under tropics of Asia / A.N.Krasnov. The second edition, M.: A thought, 1987. pp 30-35.

Villareal, R.L, Banuelos O, Mujeeb-Kazi A. 1997. Agronomic performance of related durum wheat (Triticum turgidum L.) stocks possessing the chromosome substitution T1BL.1RS. Crop Sci 37: pp 1735–1740

Villareal, R.L., Banuelos, O., Mujeeb-Kazi, A., and Rajaram, S. 1998. Agronomic Performance of Chromosome 1B and T1BL.1RS Near-Isolines in the Spring Bread Wheat Seri M82, Euphytica, vol. 103: pp 195– 202.

Villareal, R.L,. Rajaram S., S., Mujeb-Kazi., and DelToro E. 1991. The effect of chromosome 1B/1R translocation on the yield potential of certain spring wheats (Triticum aestivum L). Plant Breed. 106: pp 77-81

Waines, J.G., and Barnhart D. 1992. Biosystematic research in *Aegilops* and *Triticum.* Hereditas, 116: pp 207–212.

Wan, A., Zhao, Z., Chen, X., He, Z., Jin, S., Jia, Q., Yao, G., Yang, J., Wang, B., Li, G., Bi, Y., Yuan , Z. 2004. Wheat stripe rust epidemic and virulence of Puccinia striiformis f.sp. tritici in China in 2002. Plant Dis 88: pp, 896-904Wallwork, H. 2009. The use of host plant resistance in disease control. In the book, Walters D. Disease Control in Crops. Blackwell Publishing Ltd, pp 122-141

Wanyera, R., Kinyua, M. G., Jin, Y., and Singh, R. P. 2006. The spread of stem rust caused by Puccinia graminis f. sp. tritici, with virulence on Sr31 in wheat in Eastern Africa. Plant Dis. 90: pp 113.

Watson, I.A. 1970. The utilization of wild species in the breeding of cultivated crops resistance to plant pathogens. In Frankel, O.H. and Bennett, E. (Eds.). Genetic resources in plants - their exploration nd conservation. I.B.P. Handbook № 11. Blackwell Sci. Publ., Oxford and Edinburgh. Chap. 38: pp 411-457.

Weng, Y, Azhaguvel P, Devkota R.N,, Rudd J.C. 2007. PCR based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat-rye translocations in wheat background. Plant Breed 126: pp 482–486

Weimarck, A. 1974. Elimination of wheat and rye chromosomes in a strain of octoploid Triticale as revealed by Giemsa banding technique. Hereditas 77. pp 281-286.

William H.M., R. Trethowan and E.M. Crosby-Galvan. 2007. Wheat breeding assisted by markers: CIMMYT's experience. Euphytica, 157: pp 307–319

Zeller, F.L. 1972. Cytologischer Nachweis einer Chromosomen subslitution in dem Weizenslamm Salztmunde 14 44 (*Triticum aestivum* L.). Z. Ptlanzenzuchtg 67: pp 90—94.

Zeller, F.J. 1973. 1B/1R wheat–rye chromosome substitutions and translocations. *In* Proceedings of the 4th International Wheat Genetics Symposium, held 6–11 August 1973, Columbia, Missouri. *Edited by* E.R. Sears and L.M.S. Sears. Missouri Agricultural Experiment Station, University of Missouri, Columbia. pp. 209–221

Zohary, D. 1999. Monophyletic vs. polyphyletic originof the crops on which agriculture was founded in the Near East, Genetic Resources and Crop Evolution 46: pp 133–142.

Yamamori, M. 1994. An N-band marker for gene Lr18 for resistance to leaf rust in wheat. Theoretical and Applied Genetics 89: pp 643-646.

Zohary, D. 1986. The origin and spread of agriculture in the old world. In: Baigozzi C, editor, The Origin and Domestication of Cultivated Plants, pp. 3–20, Elsevier,Amsterdam, Netherlands.

Zuniga, J., Soto B., Campos H. 2008. Using a gel-free PCR–ELISA for the molecular identification of wheat genotypes carrying wheat-rye translocations

Xia, X. C., Z. F. LI., G. Q. Li. and R. P. Singh. 2007. Yellow rust resistance in Chinese bread wheat varieties and lines. H.T. Buck et al. eds. Wheat Production in Stressed Environments. pp 77-82.