WINTER CEREAL TRUST

PROGRESS REPORT JULY 2012-JULY 2013

July 2013

1. TITLE OF PROJECT:

Marker-assisted introgression of different rust resistance genes as well as Fusarium Head Blight resistance into South African wheat cultivars

2. WINTER CEREAL TRUST PROJECT NUMBER:

WCT/W/2006/02

3. APPLICANTS:

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4. DURATION OF PROJECT:

1 January 2011 - 31 December 2015

5. ORIGINAL OBJECTIVES OF THE PROJECT:

The main objective of the research is:

1. Development of South African cultivars with durable Fusarium Head Blight (FHB) and rust resistance, adapted to South African conditions and with acceptable quality.

The main objective will be achieved through the following sub-objectives:

a. Adding two stem rust resistance genes (*Sr39* and adult plant resistance), effective against all currently known pathotypes of stem rust, including race TTKSK (Ug99) and related derivatives, to existing lines containing two leaf rust (*Lr34/Yr18/Sr57* and *Lr19*), one stem rust (*Sr26*) and two stripe rust (*QYr.sgi-2B.1* and *Yr18*) resistance genes to create a single line with the highest possible number of rust resistance genes.

b. Adding at least four rust resistance genes (*Lr34/Yr18/Sr57; Lr19, QYr.sgi-2B.1* and *Sr26*) from previously developed homozygous rust resistant lines to a Krokodil derived BC_2F_3 line containing four FHB resistance QTL (chromosomes 3B, 5A, 6B and 7A) using a rapid line conversion using a

two-generation backcross programme employing foreground selection for rust genes and FHB QTL in both background generations, background selection at non-target loci in the BC_2 generation and phenotypic selection for FHB and rust resistance and other desirable agronomic traits in subsequent selfed generations.

c. Crossing of the best rust resistant lines (developed in sub-objective a) with the best FHB resistant lines as well as lines containing both rust and FHB resistance in a South African background (developed in sub-objective b).

d. Rapid development of resistant cultivars through a combination of marker-assisted and phenotypic selection techniques.

6. SHORT INTRODUCTION

The success of any wheat breeding programme depends in part on the ability to combine the maximum number of favourable alleles present in the parents of a cross, especially effective and durable disease resistance and to identify those progeny. Wheat is influenced by a number of diseases, mainly caused by fungal pathogens, especially leaf, stem and stripe rust and Fusarium head blight. Selection for adult plant resistance genes can provide protection from rapid development of varietal susceptibility and reduce the potential impact of foliar diseases, particularly leaf, stem and stripe rust. The most cost-effective strategy for preventing disease epidemics depends on combining resistance genes that are effective against all predominant races of the pathogen. The focus of wheat breeders are shifting towards the identification and incorporation of race non-specific resistance genes that may provide only partial resistance but when used in combination with other genes can condition highly effective resistance. There exists a need to develop South African cultivars with durable rust and FHB resistance, adapted to South African conditions and with acceptable quality. Marker-assisted foreground and background selection can shorten this procedure considerably.

7. MODIFIED OBJECTIVES OF PROJECT

This project has two main objectives, namely i) Introduction of Ug99 resistance into existing rust resistant lines and ii) Combining FHB and rust resistance genes/QTL in a South African background. Since this project is already in its third year it can be expected that some adjustments had to be made throughout the project based on obtained results. Up-to-date no changes had to be made for meeting the first objective of the project regarding Ug99 resistance. However, several adjustments had to be made regarding the combination of FHB and rust resistance. The original

business plan was written based on the availability of data obtained from the PhD project of Mr Scott Sydenham from a previously WCT funded project "Marker-assisted backcross introgression of Fusarium Head Blight resistance into South African wheat cultivars". As described in the last year's progress report, final analysis of the FHB lines produced from Mr Sydenham's PhD project was not yet done by the time these lines were needed to start the second phase of the current project. Certain adjustments had to be made and new objectives were set for the current project. The main adjustments were as follows:

1. Mr Sydenham identified 40 BC_2F_2 wheat lines that contained either three or four FHB QTL. Final analysis to determine the percentage Krokodil genetic background of these lines has not been completed and without the percentage Krokodil background information the best possible lines could not be selected. Although the best lines could thus not be selected to make crosses with rust resistant lines, these 40 lines were in the meantime used to create a recombinant inbred line (RIL) population. The new objective was to self-pollinate these lines until homozygosity is reached and to use the best homozygous lines in crosses with the best rust resistant lines. Marker-assisted selection for both FHB resistant QTL and bread-making quality will be done after each self-pollination step in order to select the best possible lines for future use.
2. In order to still be able to combine rust and FHB resistance into a single line (in the absence of needed data from Mr Sydenham's PhD project), the two best UFS rust resistant lines S16(7.3) and S726(3.2) were used in a series of crosses with two FHB donor lines (Frontana and a CIMMYT line CM-82036). Rust resistance will thus be combined with both Type I and Type II FHB resistance. The best lines identified from these crosses will then be crossed with the rust resistant lines obtained from the first main objective of the project (lines containing Ug99 resistance).

This progress report will thus report on progress made with regard to the adjusted business plan and objectives.

8. OBJECTIVES FOR 2012/2013:

8.1 Introduction of Ug99 resistance into existing rust resistant lines

a. Production of double cross progeny (February - August 2012)

b. Marker-assisted selection (September 2012 - September 2013)

c. Phenotypic verification and seed production of progeny containing all possible marker alleles (September 2013 - December 2014)

8.2 Combining FHB and rust resistance genes/QTL in a South African background

a. Screen and identify possible parents for polymorphisms using FHB and rust specific markers (January - August 2012)
b. Production of F_1 progeny (September 2011 - January 2012)
c. Production of double cross progeny (February - August 2012)
d. Marker-assisted selection and data analysis from crossing the two best rust resistance lines with CM-82036 (Type II resistance) and Frontana (Type I resistance) (September 2012 - December 2013)
e. Development of RIL populations: Two rounds of self-pollination of BC_2F_3 individuals to produce BC_2F_5 individuals and marker screening of self-pollinated BC_2F_n populations for FHB resistance QTL and bread-making quality traits (January 2012 - December 2013)

8.3 Evaluation of rust and FHB resistant lines for bread-making qualities using marker-assisted selection and biochemical tests

a. Evaluate wheat experimental lines for the absence or presence of the selected rust and FHB resistance genes/QTL
b. Application of SDS-PAGE and SE-HPLC biochemical tests to determine certain bread-making quality characteristics
c. Screen molecular markers linked to protein quality traits to determine the presence or absence of certain high molecular weight-glutenin subunits (HMW-GS) and the 1BL.1RS translocation in the experimental wheat lines.

9. OBJECTIVES ACHIEVED DURING 2012:

9.1 INTRODUCTION OF UG99 RESISTANCE INTO EXISTING RUST RESISTANT LINES

Lines used in this project were obtained from different sources. Firstly wheat lines from a previous project at the UFS, containing different combinations of rust resistant genes were selected as possible parents. These lines were selected to contain between them all possible gene combinations of the original parental lines (*Sr2* from Blade, *Sr26* from Avocet*YrSp* and Blade, *QYr.sgi-2B.1 from* Kariega, *YrSp* from

Avocet*YrSp, Lr19* from CS-*Lr19*-149-299 and *Lr34/Yr18/Sr57* from Kariega and C*sLr19*-149-299). Secondly Kingbird containing adult plant resistance, was obtained from CIMMYT's 4th stem rust screening nursery (2010). Kingbird is also reported to contain *Sr2* resistance. Thirdly the line 2S#2/163 was obtained from the University of Adelaide in Australia. Line 2S#2/163 contains the seedling stem rust resistance gene *Sr39.* These lines were planted in the greenhouse for seed multiplication, marker screening and for use in the crossing scheme. Based on the marker data, two UFS rust resistant lines with the highest number of rust resistant genes were selected as parental lines for the crossing scheme, namely S16(7.3)/P1.5.1 and S726(3.2)/P1.3.1. The first line is homozygous for *QYr.sgi-2B.1, Lr19* and *Lr34/Yr18/Sr57* while the second line is homozygous for *Lr19, Lr34/Yr18/Sr57* and *Sr26*. *Sr2* was absent in all tested lines. Furthermore, Kingbird with APR from CIMMYT's collection and line 2S#2/163 from the University of Adelaide were selected as the other two parental lines. Selection was based on the marker profile of each line as well as the number of seed available after self-pollination. Results indicated that Kingbird showed higher levels of resistance towards stem rust than the other tested lines. Since *Sr2* is absent in the UFS rust resistant lines it was important to select at least one of the CIMMYT or University of Adelaide's lines that contain *Sr2* as parental line in the crossing scheme. Kingbird, containing both *Sr2* and *Lr34/Yr18/Sr57*, was selected as one of the parental lines. Line 2S#2/163 from the University of Adelaide and containing *Sr39* resistance was selected as one of the other parental lines. Kingbird was used as the female and S726(3.2)/P1.3.1 as the male in the first cross. Line 2S#2/163 was used as the female and S16(7.3)/P1.5.1 as the male in the second cross.

a. Production of double cross progeny (February - August 2012)

During March 2012, 150 seeds harvested from the Kingbird / S726(3.2) cross were planted in the greenhouse over a five week span, starting on 22 March 2012. These lines were used as female plants during the production of the double cross family. A total of 125 seedlings emerged from the 150 seeds planted. During April 2012, 120 seeds harvested from the Line 2S#2/163 / S16(7.3) cross were planted in the greenhouse over a three week span (to synchronise with the middle three weeks of the Kingbrid / S726(3.2) plants), starting on 6 April 2012. These lines were used as male plants during the production of the double cross family. A total of 112 seedlings emerged from the 120 seeds planted.

Leaf material was harvested from each seedling, freeze-dried and stored at -80°C for future use. DNA was extracted from more than 237 individual plants. In order to confirm that progeny produced form each F_1 cross, and to be used in the production of the double cross family, resulted from a cross and not self-pollination, each seedling was screened with a SSR marker only present in the male parental line used to produce the F_1 progeny. The resistance marker linked to *Sr26* was used to screen seedlings from the Kingbird / S726(3.2) cross while the resistance marker linked to *Lr34* was used to screen seedlings from the Line 2S#2/163 / S16(7.3) cross. Of the 125 tested seedlings of the Kingbird / S726(3.2) cross, 48 resulted from self-pollination, while eight of the 112 seedlings of the Line 2S#2/163 / S16(7.3) cross resulted from self-pollination. Seedlings that were identified as selfs were discarded and not used in the production of the double cross family. A total of 135 crosses were made between the two F_1 seedling families. Seed from the double cross family was harvested during the middle of August 2012.

b. Marker-assisted selection (September 2012 - September 2013)

Seed of the double cross family was harvested, thrashed and planted in August 2012. Leaf material was harvested from seedlings for DNA extractions and marker analyses. Plants were left to self-pollinate and seed harvested for future use. A total of 808 seedlings were screened for the presence of seven different rust resistance markers and the best lines identified for the next phase of the breeding scheme.

The following markers were screened: marker $STSLr19_{130}$ linked to *Lr19*; markers Sr26#43 (+) and BE 518379 (-) linked to *Sr26*; marker cssfr linked to *Lr34*; marker csSr2 linked to *Sr2*; markers Gwm148 and Gwm501 linked to *QYr.sgi-2B.1* and markers SR39F2/R3 (+) and BE 500705 (-) linked to *Sr39*.

The number of individual plants of the double cross population containing markers linked to the desired resistance gene(s)/QTL ranged from three individuals containing only one of the markers to one individual containing all seven markers. Of the 808 double cross progeny screened, three of the plants contained only one marker each, 139 plants contained two markers, 293 plants contained three markers, 274 plants contained four markers, 82 plants contained five markers, 16 plants contained six markers and one plant contained all seven screened markers. The best lines contained markers linked to all six genes of which *Sr26, Sr39* and one of the markers linked to the *QYr.sgi-2B.1* QTL (Gwm501) were in a heterozygous state and the rest in a homozygous state. Due to no markers being available that are linked to the *YrSp*

and APR resistance genes, the presence of these genes could not be confirmed using MAS.

Marker distribution in the sixteen individuals containing six of the seven markers varied. *Sr39* was heterozygous in 10 individuals and absent in six individuals. *Sr26* was heterozygous in 14 individuals and absent in two individuals. *Lr34* was heterozygous in nine individuals and homozygous present in the rest of the individuals. *Lr19* was absent in three of the individuals and *Sr2* in one of the individuals. Gwm501 was heterozygous in six individuals and absent in three of the individuals while Gwm148 was only absent in one individual. Based on molecular marker data, three stem rust (including resistance to Ug99 and its derivatives), two leaf rust and two stripe rust resistance genes/QTL were successfully pyramided into a single genotype.

9.2 COMBINING FHB AND RUST RESISTANCE GENES/QTL IN A SOUTH AFRICAN BACKGROUND

a. Screen and identify possible parents for polymorphisms using FHB and rust specific markers (January - August 2012)

The parental lines used in the directional crosses were all screened with the rust and FHB markers and results were confirmed using phenotypic screening in the greenhouse. The parental lines that were screened were: Kariega, Blade, Avocet*YrSp*, CS*Lr19*-149-299*,* CM-82036 and Frontana. These lines were screened using rust markers linked to the *QYr.sgi-2B.1* QTL of Kariega, *Lr19*, *Lr34/Yr18/Sr57, Sr26* and *Sr2* as well as FHB markers linked to FHB resistance QTL on chromosomes 3B, 5A, 6B and 7A present in CM-82036 and 3A present in Frontana. Results confirmed that all expected markers were present in the parental lines. Marker data also confirmed that the markers linked to the FHB QTL on chromosome 5A were present in the Chinese Spring derived line CS*Lr19*-149-299 and that Blade contained markers linked to the QTL on chromosome 6B. This data will be confirmed in future using phenotypic screening in the greenhouse. Marker data further indicated that CM-82036 contained markers linked to leaf and stripe rust resistance (*Lr34/*Yr18/Sr57). This was confirmed using phenotypic screening in the greenhouse. CM-82036 showed complete resistance towards stripe and leaf rust. Phenotypic screening furthermore indicated that the parental line S726(3.2) contained *YrSp* resistance, transferred from Avocet*YrSp*. There are no molecular markers available for this trait and it could only be confirmed using phenotypic screening. Unfortunately

this line was susceptible to stem rust, indicating that the *Sr26* gene was not present anymore, although marker data indicated otherwise.

b. Production of F_1 progeny (September 2011 - January 2012)

The two UFS rust resistant lines S16(7.3) and S726(3.2) were used in a series of crosses with two FHB donor lines (Frontana and a CIMMYT line CM-82036). CM-82036 shows type II resistance (resistance to spread of disease within the spike) and contain four FHB resistant QTL, namely *Fhb1* or *Qfhs.ndsu-3BS*, *Qfhs.ifa-5A*, *Fhb2* or the *6B-QTL* and the *7A-QTL*. Frontana shows Type I resistance (resistance to initial infection) and contains two QTL, namely the *3A-QTL* and the *5A-QTL*. Based on marker data of the self-pollinated lines, two of the best lines, S16(7.3) and S726(3.2), were selected for crosses with FHB resistant lines. Lines were selected based on the number of homozygous markers as well as the presence of all possible markers between the two lines. After another round of self-pollination MAS indicated that all markers segregated as expected, except for the marker linked to *Sr2* that could not be detected.

Rust resistant line S16(7.3) was crossed with the FHB resistant donor line CM-82036 (cross 1) while rust resistant line S726(3.2) was crossed with the FHB resistant donor line Frontana (cross 2). More than 600 seed were harvested from the first cross and 240 seed from the second cross. F_1 progeny from these two crosses were screened with one marker each to identify successful crosses.

c. Production of double cross progeny (February - August 2012)

A total of 153 F_1 progeny from cross 1 [S16(7.3) / CM-82036] was used as male parent and crossed with 240 progeny from cross 2 [S726(3.2) / Frontana]. A total of 215 directional crosses were made and seed from the double cross family harvested. Seed from the double cross family was planted, leaf material harvested for DNA analyses and plants self-pollinated and seed harvested for future use.

d. Marker-assisted selection and data analysis from crossing the two best rust resistance lines with CM-82036 (Type II resistance) and Frontana (Type I resistance) (September 2012 - December 2013)

Leaf material from the double cross progeny from the crosses between rust and FHB resistant lines was harvested and used for molecular marker analysis. Leaf material was sampled from more than a 1 000 double cross progeny. A total of 954 individuals of the double cross population were screened with five markers associated with rust

resistance genes (*Lr34/Yr18/Sr57, QYr.sgi-2B.1, Lr19* and *Sr26*) and with five markers associated with FHB resistance QTL. The following markers were used to screen for accumulated rust resistance genes/QTL: cssfr5 (*Lr34*), Gwm148 (*QYr.sgi-2B.1*), Gwm501 (*QYr.sgi-2B.1*), $STSLr19_{130}$ (*Lr19*) and Sr26#43(*Sr26*). The markers: DuPw227 (3A QTL), Barc133 (3B QTL), Gwm156, Gwm293, Barc197.2 (5A QTL), Gwm133, Gwm644 (6B QTL) and Gwm233 (7A QTL) were used to identify individuals containing FHB resistance QTL. Marker data was analysed to identify individuals with the highest number of resistance markers in a homozygous state.

Seed of the double cross family was harvested, thrashed and planted. Leaf material was harvested from seedlings for DNA extractions and marker analyses. Plants were left to self-pollinate and seed harvested for future use..

The number of individual plants of the double cross population containing markers linked to the desired resistance gene(s)/QTL ranged from eight individuals containing none of the markers to two individuals containing nine of the ten markers tested for. Of the 954 double cross progeny screened, 54 of the plants contained only one marker each, 163 plants contained two markers, 250 plants contained three markers, 214 plants contained four markers, 136 plants contained five markers, 88 plants contained six markers, 32 plants contained seven markers, seven plants contained eight markers and two plant contained nine screened markers. No lines were detected that contained all 10 markers.

The best two lines contained markers linked to all four rust resistant genes/QTL screened for, namely *QYr.sgi-2B.1, Lr19, Lr45/Yr18/Sr57* and *Sr39.* It furthermore also contained markers linked to the three major FHB genes/QTL, namely *Fhb1, Qfhs.ifa-5A* and *Fhb2*. The only marker missing was the marker linked to the minor QTL on chromosome 7A.

e. Development of RIL populations: Two rounds of self-pollination of BC_2F_3 individuals to produce BC_2F_5 individuals and marker screening of self-pollinated BC_2F_n populations for FHB resistance QTL and bread-making quality traits (January 2012 - December 2013)

BC_2F_2 seed were planted in the greenhouse in March 2012 and plants were self-pollinated to produce BC_2F_3 seed. The BC_2F_3 seed was harvested at the end of July 2012. Leaf material was harvested from the seedlings, freeze-dried and DNA extracted for molecular marker analysis. SSR markers linked to the four FHB QTL

were tested on these 40 lines and data analysed. The BC_2F_3 population was planted in October 2012 and BC_2F_4 seed harvested in December 2012 and seed planted in February 2013. Leaf material was harvested and DNA analysis on the BC_2F_4 generation is underway. Lines with the highest number of FHB QTL in a homozygous state will be identified and used in future breeding programmes. The BC_2F_4 seed from all 40 lines will also be made available to different breeding companies (Pannar, Sensako and ARC-SGI) for use in their breeding programmes. These 40 lines will also be tested for baking quality using molecular markers linked to genes involved in good bread-making characteristics as well as size-exclusion high performance liquid chromatography (SE-HPLC).

9.3 Evaluation of rust and FHB resistant lines for bread-making qualities using marker-assisted selection and biochemical tests

Rust resistant wheat lines developed at the University of the Free State by crossing the parental lines Kariega (*QYr.sgi-2B.1, Lr34/Yr18/Sr57*), Avocet*YrSp* (*YrSp, Sr26*), Blade (*Sr26, Sr2*) and CS-*Lr19*-149-299 (*Lr19, Lr34/Yr18/Sr57*), a derivative of Chinese Spring were tested. Furthermore, another group of wheat lines developed for FHB resistance using the parents Krokodil and CM-82036 (FHB resistance QTL on chromosomes 3B, 5A, 6B and 7A) were also tested. Fifty wheat lines compiled from the two groups (rust and FHB) containing the best combination of resistance genes, were selected for biochemical analyses and screened for certain quality-related markers. Size-exclusion high performance liquid chromatography (SE-HPLC) was performed to determine the percentage of large polymeric proteins (LPP). In general, a LPP value between 40-50% can be considered as an acceptable percentage for baking quality. These lines were also analysed by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) to determine the high molecular weight glutenin subunit (HMW-GS) profiles of each line. Furthermore, five SSR quality markers were screened to determine the quality of the lines and to see if there existed a correlation between SSR markers (DNA level) and SDS-PAGE (protein level) profiles. Correlations were detected between the different bread quality tests used. Furthermore, lines containing a high number of resistant markers and with good bread baking qualities were identified.

The aim of this part of the study was to identify rust and FHB resistant lines with good protein content. Rust and FHB resistant wheat lines developed during this project were tested. The best rust and FHB resistant lines were planted and self-pollinated. Lines were evaluated for the presence of five rust resistant genes/QTL (*Lr19,*

Lr34/Yr18/Sr57, QYr.sgi-2B.1, Sr2 and *Sr26*) and four FHB resistant genes/QTL (*Fhb1, Qfhs.ifa-5A-1, Qfhs.ifa-5A-2* and *Fhb2*) for two consecutive years. These selected rust and FHB resistant lines were also subjected to three biochemical tests namely SDS-PAGE, SE-HPLC and RP-HPLC. PCR-based markers linked to HMW-GS alleles and the IBL.1RS translocation, associated with weak dough strength, were also tested. Since the breeding programme was still in an early stage, only few seeds per line were available and therefore biochemical tests that can be performed using single seeds were selected. Results from SDS-PAGE and molecular markers linked to HMW-GS were similar and therefore lines were further evaluated using only molecular markers. No correlations were detected for the RP-HPLC data of lines therefore it was excluded during further analysis.

The rust and FHB resistance genes/QTL co-segregated based on their related parental lines. An additional round of self-pollination either led to higher levels of homozygosity for the selected traits or to the loss of traits due to recombination. MAS enabled the selection and enhancement of homozygous lines.

Only a few offspring of one of the rust resistant lines contained the *Sr26* gene, while the *Lr34/Yr18/Sr57* gene was present in all tested lines. None of the rust resistant lines contained the *Sr2* gene which is a major resistant gene against stem rust and especially effective against the threatening Ug99 race. The top ten rust resistant lines all had the same rust resistant genes/QTL present (*Lr19, Lr34/Yr18/Sr57* and *QYr.sgi-2B.1*) as well as the same protein quality alleles (*Ax2**, *Bx7+By8* and *Dx5+Dy10*). The LUPP% of these lines showed high levels of variation and was optimal (40% to 50%) for only one line.

High levels of variation were detected for the FHB resistant markers in the FHB resistant populations. The top ten lines contained all six markers although the level of homozygosity varied. Four of these lines expressed both the *Bx7+By8* and *Bx17+By18* alleles and only one line did not express the $Bx7^{OE}$ allele. None of these lines expressed the 1BL.1RS translocation. Two of these lines showed a desirable LUPP%.

Results indicated the preference towards rust or FHB resistance selection followed by selection for protein quality alleles and lastly the LUPP%. The top ten rust and FHB lines can serve as resistance sources in further breeding programmes.

10. OBJECTIVES NOT ACHIEVED DURING 2012:

Only one of the objectives set for 2013 will not be met at the end of December 2013, namely to start the production of the BC_1 individuals for combining FHB and rust resistance into a single South African wheat line. The reasons for not meeting this objective were given above. However, to compensate for the fact that the best FHB lines to be used in the crossing scheme could not be selected because the data was not available yet, the 40 best FHB lines were planted and self-pollinated twice to produce BC_2F_4 progeny. These 40 lines were furthermore screened with all FHB markers linked to FHB resistance and were also screened for backing quality. The BC_2F_4 seed were planted in June 2013 to produce BC_2F_5 seed. This will have the advantage that when data become available on the best FHB lines to be used in the breeding scheme, these lines would have undergone a few extra rounds of selfing, ensuring a higher possibility that all four FHB QTL will be in a homozygous state before the lines are being used in the crossing scheme. Furthermore, this will also open up the possibility to cross these homozygous FHB lines with previously developed the rust lines (additionally containing *Sr39* and APR resistance to Ug99). FHB resistance was also incorporated into the existing rust resistant lines using directional crosses and two FHB resistant parental lines, namely CM-82036 and Frontana.

11. GENERAL PROGRESS

Except for one objective, all objectives set for 2013 were achieved. Furthermore, additional crosses, marker screening and biochemical tests were incorporated that were not originally part of the project. The project is showing good overall progress. The double cross family [Kingbird / S726(3.2) // Line 2S#2/163 / S16(7.3)] were successfully produced, seed harvested, planted in the greenhouse and leaf material was harvested for DNA extraction and SSR analysis. A total of 808 individuals of the population were screened with markers linked to the different rust resistance genes and one line containing all seven markers were identified as well as an additional 16 lines that contained six of the seven markers. Furthermore, the double cross family [CM-82036 / S16(7.3) // Frontana / S726(3.2)] were successfully produced, seed harvested, planted in the greenhouse and leaf material was harvested for DNA extraction and SSR analysis. Two lines were identified that contained nine of the ten rust and FHB markers screened on the 954 individuals of the double cross population. The BC_2F_2 population developed from the Krokodil (recurrent parent) / CM-82036 (donor parent) crosses was advanced to a BC_2F_4 population. By the end of 2013 the best possible lines will be selected and used for the final crossing scheme of the project.