GERMPLASM DEVELOPMENT

GK 04/20: DEVELOPMENT OF RUST RESISTANT GERMPLASM PROGRESS REPORT APRIL 2012 – MARCH 2013

1. Project details

Number: GK 04/20
Title: Development of rust resistant germplasm
Duration: Ongoing
Status : Continuation of existing project
Project Leader: Mr Krishna Naicker

2. Objectives

Wheat rusts pose a significant threat to wheat production worldwide and are a serious concern in South Africa. Stripe, leaf and stem rusts are responsible for considerable yield and quality losses annually. The use of fungicides is effective in controlling the rusts. However, there are high costs involved with the use of fungicides and continuous usage could result in the development of fungicide resistant rust strains. Breeding wheat for rust resistance is, therefore, a more sustainable and cost effective method for farmers. New rust strains are constantly emerging, due to various factors and there is a need to use new genes and different gene combinations to prolong the resistance of race-specific genes. The strategy adopted in this pre-breeding programme is to use adult plant resistance (APR) or non-race specific resistance in combinations with race-specific genes to ensure durable protection against wheat rusts. The focus of this programme is to identify sources of rust resistance and incorporate gene combinations into well adapted wheat lines to develop rust resistant lines with pest resistance, high yields and good quality. Molecular markers have been used in the screenings to detect desired gene combinations in a shorter time period compared to field screenings. The objectives of this project are summarised below.

2.1. Long-term objectives

- The identification of new sources of resistance with single and durable resistance to the South African leaf, stem and stripe rust virulence spectrum.
- Manipulation of these genes so as to be of assistance to the breeders at the ARC-Small Grain Institute (ARC-SGI) in the breeding and the ultimate release of well adapted rust resistant wheat cultivars.
- To pyramid validated desirable genes into suitable combinations for the development of rust resistant germplasm for breeding programmes.
- Investigation of the combining ability of rust resistance genes with other important characteristics such as yield, quality, agronomy and insect resistance.

2.2. Short term objectives: April 2012 – March 2013

- To compile the 2012-spring and winter wheat crossing block and to ensure the proper planning and execution of crosses.
- To collaborate with the Biotechnology Laboratory to detect and select genes through marker-assisted selection (MAS).
- To evaluate breeding lines and advanced segregating populations in order to identify and select promising lines.
- To set disease nurseries for the crossing blocks in the field where early generation germplasm can be screened and selected for reasonable levels of resistance to rust diseases.
- To visit the different screening nurseries during the season to evaluate and select rust resistant lines for use in the breeding programme.

3. Report on the objectives: April 2012 – March 2013

3.1. Report on objective 2.2.1. To compile the 2012-spring and winter wheat crossing block and to ensure the proper planning and execution of crosses.

Five hundred and thirteen different crossing combinations were compiled and executed for the spring wheat pre-breeding programme. These crossing combinations were designed with the focus on stem rust resistance. Several of the crossing parents were lines developed by CIMMYT that contains APR to many stem rust pathotypes, including *Ug99*. The winter wheat crossing block produced 58 different crossing combinations. These crosses were developed to incorporate stripe rust resistance in combination with Russian wheat aphid resistance. Female parents for both crossing blocks consisted of cultivars and lines that were high yielding with good quality in order to incorporate disease resistance in these backgrounds. F_1 seed from the spring crossing combinations were multiplied in the glasshouse and the winter crossing combinations are currently being multiplied. Combinations from the winter and spring material will be screened at Bethlehem and Tygerhoek, respectively, during the 2013 season, and MAS will be used on specific material to select rust resistant material. Crossing parents contained a variety of different rust resistance genes and gene complexes. A selected number of these genes have been characterized and are indicated in Table 1.

Table 1. List of characterised rust resistance genes used in the spring and winter wheat pre-breeding programme to develop new gene combinations

Genes used in pre-breeding programme		
Stripe rust	**Leaf rust**	**Stem rust**
Yr3a, Yr3b, Yr4b, Yr5, Yr10, Yr15, Yr18, Yr29, Yr35, Yr38, YrSp,	*Lr9, Lr19, Lr22, Lr34, Lr35, Lr39, Lr41, Lr46, Lr52, Lr53, Lr56*	*Sr2, Sr20, Sr22, Sr26, Sr29, Sr31 Sr33, Sr35, Sr40, Sr45*

3.2. Report on objective 2.2.2. To collaborate with the Biotechnology Laboratory to detect and select genes through marker-assisted selection (MAS).

The use of molecular markers is a significant tool to combine effective rust resistance genes in single lines with durable resistance. An advantage of this technology is the ability to develop material with different desired genes in a shorter period of time compared to traditional breeding methods. Six hundred and eighty plants from 25 different populations were submitted to the biotechnology laboratory to identify the presence of 13 rust resistance genes in various combinations. These genes include *Sr2, Sr22, Sr26, Sr29, Sr31, Sr33, Sr35, Lr19, Lr22, Lr35, Lr39, Lr46/Yr29* and *Yr18/ Lr34.* Preliminary screenings for 5 of these genes identified plants with 3 different gene combinations and are shown in Figure 1. The remaining screenings are currently being conducted and more details are reported in project GK 09/17.

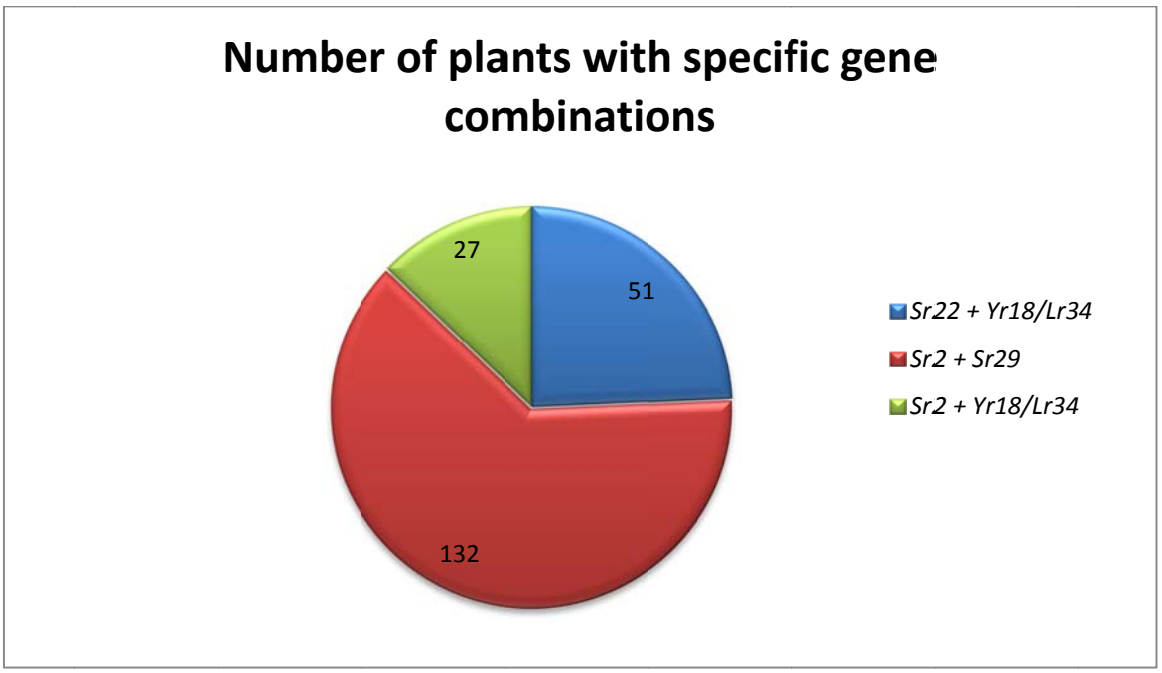

Figure 1. Number of plants containing 3 different gene combinations from preliminary data.

3.3. Report on objective 2.2.3. To evaluate breeding lines and advanced segregating populations in order to identify and select promising lines.

Five hundred and fifty five advanced breeding lines from 11 breeding populations were screened for seedling resistance to leaf, stem and stripe rust pathotypes. Three prevalent pathotypes, 3SA133, 3SA145 and 3SA146 were used for leaf rust evaluations. Stem rust evaluations were carried out using two pathotypes, 2SA88 and 2SA107, while a single pathotype, 6E22A^{+} was used to screen for stripe rust resistance. Plants were evaluated at the peak of disease infection and rated using a scale of 0 – 4. The results of these screenings are shown in Table 2. Lines with resistance, will be advanced in the breeding programmes. Levels of resistance to all three leaf rust pathotypes used in the evaulations were relatively low and varied between 0 and 22%. Similarly, the combined resistance with the two pathotypes used for stem rust ranged between 3 and 53%. The percentage range for stripe rust resistance varied between 3 and 44%. From these results, it can be concluded that new gene sources and gene combinations needs to be incorporated in these breeding programmes to develop lines with greater levels of rust resistance.

Table 2. Percentage of rust resistance lines from the breeding programmes using 6 rust pathotypes.

Breeding Populations Evaluated	**Percentage of resistant lines**							
	Leaf rust				**Stem rust**			**Stripe rust**
	3SA133	**3SA145**	**3SA146**	**Combined**	**2SA88**	**2SA107**	**Combined**	**6E22A^{+}**
Spring and irrigation Senior	41	10	22	**10**	34	15	**15**	3
Spring and Irrigation Elites	29	24	15	**0**	6	3	**3**	3
Winter Junior	42	28	7	**7**	50	56	**36**	5
Winter Elites	*	43	8	**4**	41	24	**24**	25
Winter Senior	33	7	7	**7**	24	19	**16**	13
Senior – Bultfontein	25	20	2	**2**	38	50	**21**	44
Junior - Bultfontein	30	45	11	**10**	37	39	**25**	3

Breeding Populations Evaluated	Percentage of resistant lines							
	Leaf rust				Stem rust			Stripe rust
	3SA133	3SA145	3SA146	Combined	2SA88	2SA107	Combined	6E22A+
Elites - Bultfontein	40	36	19	**19**	55	55	**48**	15
Intermediate Elites	39	33	29	**22**	68	75	**53**	11
Intermediate Junior	22	39	9	**6**	47	43	**18**	8
Intermediate Senior	26	22	14	**11**	50	51	**46**	10

* Evaluations are currently being conducted

Segregating lines showing different reaction types were omitted from this table

3.4. Report on objectives 2.2.4. & 2.2.5. To set disease nurseries for the crossing blocks in the field where early generation germplasm can be screened and selected for reasonable levels of resistance to rust diseases, and to visit the different screening nurseries during the season to evaluate and select rust resistant lines for use in the breeding programme

The incidence of the three rusts varies according to their climatic requirements. Stripe and leaf rusts are part of the problem in the summer rainfall area, whilst all three rusts are a concern in the winter rainfall area. Winter wheat crosses are therefore specifically designed to incorporate stripe and leaf rust resistance into good backgrounds and spring wheat crosses are designed to incorporate resistance for the three rusts.

Three hundred and six F_2 and 540 F_3 winter lines were planted in the hail-net enclosure in Bethlehem, to select for stripe and leaf rust resistant material. Plants were artificially inoculated by mixing a cocktail of spores of the predominant stripe and leaf rust pathotypes in mineral oil (Soltrol 170) and sprayed on plants. There were high levels of rust infections in the field and 158 single plants showing rust resistance were selected and harvested. Seven hundred and sixty two F_4 and 4602 F_6 lines were planted and evaluated under the floppy system in Bethlehem, to select suitable rust resistant genotypes. There were high levels of stripe rust infections and 349 resistant plants were selected and harvested.

One thousand seven hundred and eighty lines consisting of 682 F_2, 272 F_3 and 826 F_4 generations were planted at Tygerhoek in the Western Cape. There were low disease infections in the field and selections were made based on good agronomic characteristics. A total of 613 single plants were selected and harvested and selections will be repeated in the forthcoming season.

The Genebank at ARC–Small Grain Institute stores several thousand lines consisting of wheat, oat and barley in its collection. A certain number of these entries are evaluated annually to characterise their levels of resistance to all three rusts. In 2012, 1 286 lines were planted at three localities, Kransfontein (Free State), Tygerhoek (Western Cape) and Cedara (KwaZulu-Natal), to evaluate their resistance to stripe, leaf and stem rust. Rust infections resulted from natural infections. There were high levels of stripe rust infections at Kransfontein and high levels of stripe and leaf rust at Cedara and these entries were evaluated accordingly. However, there were very low levels of rust infections at Tygerhoek and evaluations could not be conducted at this site. This data has been submitted to the Genebank for entry in the database. Material with suitable resistance combinations will be used in the breeding programmes for the development of new cultivars.

4. Conclusion and future of the project

New races of wheat rusts continuously develop and overcome previously resistant genes. Chemical control plays an important role in controlling the rusts but breeding for genetic resistance remains the most effective, environment friendly and economic method. This pre-breeding programme addresses this challenge by exploring new genes as well as various gene combinations to provide durable protection to the rusts. Five

hundred and seventy one crossing combinations were made in the season for both spring and winter material to explore various gene combinations. These combinations will be evaluated in the forthcoming season and desirable material will be selected. The use of MAS will greatly assist in this regard to identify plants with the desirable gene combinations in a shorter period of time, thereby reducing the number of plants for field trials. Evaluating breeding lines and accessions from the Genebank has identified promising rust resistant material and these screenings will continue. Various lines with different gene combinations have been developed in this programme and will continue in the next season.

5. Objectives: April 2013 – March 2014

- To compile the 2013-spring and winter crossing block and to ensure the proper planning and execution of crosses.
- To collaborate with the Biotechnology Laboratory to detect and identify genes through marker-assisted selection (MAS).
- To evaluate breeding lines, advanced segregating populations and germplasm accessions in search of sources of resistance to rust diseases.
- To set disease nurseries for the crossing blocks in the field where early generation germplasm can be screened and selected for reasonable levels of resistance to rust diseases.

GK 04/20: DEVELOPMENT OF RUST RESISTANT GERMPLASM
PROGRESS REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 04/20
Title: Development of rust resistant germplasm
Duration: Ongoing
Status : Continuation of existing project
Project Leader: Mr Krishna Naicker

Stripe, leaf and stem rusts are responsible for considerable yield and quality losses annually in South Africa. The use of fungicides is effective in controlling the rusts. However, there are high costs involved with the use of fungicides and continuous usage could result in the development of fungicide resistant rust strains. Breeding wheat for rust resistance is therefore more sustainable and a cost effective method for farmers. Two crossing blocks were designed and executed to incorporate various rust resistance genes in spring and winter wheat germplasm. Specific crossing combinations were made resulting in 513 spring and 58 winter wheat genotypes. The use of molecular markers has been incorporated in the screenings, to track desired gene combinations in a shorter time period compared to field screenings. Six hundred and eighty plants from 25 populations were submitted to the Biotechnology Laboratory to detect the presence of 13 rust resistance genes in various combinations. Fifty one plants were identified to have gene combinations *Sr22* + *Yr18/Lr34*, 132 plants have gene combinations *Sr2* + *Sr29* and 27 plants have gene combinations *Sr2* + *Yr18/Lr34*. Further screening is still being conducted to identify other gene combinations. Five hundred and fifty advanced breeding lines from 11 populations were screened for seedling resistance to leaf, stem and stripe rust using prevalent pathotypes. The levels of rust resistance were relatively low and new gene sources and gene combinations are needed in the breeding programmes to increase the levels of rust resistance. Six thousand two hundred and ten winter wheat entries from different generations were evaluated at Bethlehem and 507 plants with stripe and leaf rust resistance were identified and selected. One thousand seven hundred and eighty entries were evaluated at Tygerhoek and 613 plants were selected for good agronomic characteristics, since there were poor rust infections in the field. Three localities, Kransfontein, Cedara and Tygerhoek were used to screen 1 286 entries from the Genebank. Trials at Kransfontein and Cedara had good disease infections and the entries were evaluated, but data could not be recorded at Tygerhoek due to low disease infections. Evaluating breeding lines and accessions from the Genebank has identified promising rust resistant material and these screenings will continue. Various lines with different gene combinations have been developed in this programme and will continue in the next season.

GK 04/24: RESISTANCE AS AN ALTERNATIVE IN COST-EFFECTIVE MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SMALL GRAINS
PROGRESS REPORT APRIL 2012 – MARCH 2013

1. Project details

Number:	GK 04/24
Title:	Resistance as an alternative in cost-effective management of Fusarium head blight in small grains
Duration:	2002 - 2013
Status:	Final report
Project leader:	Ms Cathy de Villiers

2. Objectives

2.1 Long-term objectives

The long-term objectives of the project are as follows:

- To determine the level of genetic resistance of imported germplasm to Fusarium Head Blight (FHB).
- To release a line/cultivar with Fusarium Head Blight resistance, either to the plant breeders or the industry.

2.2 Short term objectives: April 2012 - March 2013

The short term objectives for the 2012/2013 season were as follows:

- Identify effective FHB resistant donors for a backcross programme.
- Obtain the resistant donors and test the resistant sources to FHB for Type 1 and Type 2 resistance.
- Use resistant sources for a crossing block, together with the current cultivar spectrum.
- Technology transfer.

3. Report on the objectives: April 2012 – March 2013

Identify effective FHB resistant donors for use in the backcross programme.

All Scab nurseries imported from CIMMYT, Mexico were evaluated in the field under irrigation at ARC-Small Grain Institute (ARC-SGI) during 2012. The plants were artificially inoculated during anthesis and subsequently evaluated during the peak of disease infection (Table 1).

Table 1. Combined data for the different Scab nurseries imported from CIMMYT, Mexico that were evaluated under irrigation in Bethlehem

Classification	SRSN 1 - 7	SRSN 8	SRSN 9	SRSN 10	SRSN 11	SRSN 12
Very Susceptible	13	9	5	2	1	2
Susceptible	36	2	4	2	2	3
Moderately Susceptible	61	18	22	11	12	7
Moderately Resistant	49	5	20	5	8	8
Resistant	79	11	12	11	21	16
No ScoreNo Germination	51	5	11	6	3	1
Total	**289**	**50**	**74**	**37**	**47**	**37**

From the 534 entries that were screened, a total of 150 entries were found to be resistant to scab. All imported entries will be screened using local *Fusarium* isolates to confirm resistance.

Evaluation of Rust, Aphid and Scab (RAS) nursery lines for scab resistance

One thousand two hundred and eighty six entries from the Rust-, Aphid- and Scab nursery (RAS) were also evaluated in the field for scab resistance (Table 2).

Table 2. Data for the RAS nurseries tested in the field under irrigation in Bethlehem

Classification	Amount
Very Susceptible	265
Susceptible	207
Moderately Susceptible	293
Moderately Resistant	130
Resistant	201
No Score/No Germination	190
Total	**1286**

Only 1 096 lines were evaluated, in that 190 lines did not germinate and some did not have scores. Of these lines, 201 and 130 lines showed resistance and moderate resistance, respectively.

Evaluation of irrigation lines in Bethlehem

The irrigation lines were also evaluated under irrigation in Bethlehem (Table 3).

Table 3. Classification on the irrigation lines tested in Bethlehem

Classification	Entries
Very Susceptible	36
Susceptible	16
Moderately Susceptible	14
Moderately Resistant	3
Resistant	0
No Score/No Germination	3
Total	**72**

Similar to the RAS nursery, most of the irrigation entries screened, were susceptible and/or very susceptible to scab. These results imply that there is a need to address challenges with scab under irrigation, to avoid severe epidemics, should disease conditions become favourable.

Evaluation of the 13th scab nursery from CIMMYT (FHBSN)

The 13th Scab nursery from CIMMYT (FHBSN) was planted under quarantine conditions in the wire enclosure at Small Grain Institute in 2011. These lines were evaluated for heading date, maturity, plant height, hectolitre mass (HLM) and yield in tons per hectare (t/ha) in the same year. During 2012, these entries were tested in the glasshouse, using 5 virulent isolates *of F. graminearum*. A randomized complete block design was used with twenty entries and four replications per entry. Results are presented in Table 4. Entry number 3 was not tested, because there was not enough heads to be inoculated.

As indicated in the previous report, the number of days to heading for the entries ranged from 74 to 85, while the number of days to heading, for the local check (Kariega), was between 79 and 82 days. Maturity of the different entries differed between 118 and 124 days. Plant height is an important agronomic trait, since tall plants are at a higher risk of lodging when planted under irrigation. The height of the entries evaluated, varied between 85 and 108 cm, and the hectoliter mass (HLM) varied between 71.2 and 77.8. The HLM of Kariega was 75.0 and all entries below 73.2 are not acceptable, which includes entries 3, 4, 11, 13 and 14. The 0 in the HLM column indicate that there was not enough seed to test the HLM. The yield varied between 2.5 t/ha to 4.1 t/ha respectively, whilst the local check, Kariega had a yield of 3.1 t/ha.

With regards to plants that were inoculated with Fusarium isolates, entries 1, 2, 15 and 17 showed susceptible reaction, while entries 11 and 13 showed resistance. The LSD at 5% level for the glasshouse trial was at 64.17 and the CV% at 21.1. These entries were also tested for Russian wheat aphid (RWA) resistance, since RWA is becoming a serious problem under irrigation. All of the entries evaluated were susceptible to RWA. These entries were also tested at Njoro, Kenya for resistance to stem rust, in particular *UG99,* and all the entries were susceptible to stem rust.

Table 4. Combined data for the evaluation of the 13th Scab nursery (CIMMYT) for agronomic traits in the field in 2011 and for resistance in the glasshouse in 2012

Entry	Cross Name	Heading date (days)	Maturity (days)	Plant height (cm)	HLM	Yield (t/ha)	Resistance level mean	Resistance group
1	LOCAL CHECK – KARIEGA***	82	124	95	75.0	3.1	388.2	S
2	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	82	124	104	77.5	4.0	432.4	S
3	WUH1/VEE#5//CBRD/3/URES/PRL//BAV92*	82	124	95	71.2	3.5	not evaluated	not evaluated
4	EMB16/CBRD//CBRD	82	119	102	71.8	3.5	95.6	MS
5	80456/YANGMAI 5//SHA5/WEAVER/3/PRINIA	74	120	102	73.5	3.5	79	MR
6	NG8675/CBRD	82	124	101	0	2.5	39.3	MR
7	NG8675/CBRD//SHA5/WEAVER	80	124	85	0	2.5	98.8	MS
8	CATBIRD	83	123	91	0	2.7	62.7	MR
9	TRAP#1/BOW//TAIGU DERIVATIVE	83	123	90	0	2.1	37	MR
10	TNMU/6/CEP80111/CEP81165/5/IAC5/4/YKT406/3/AG/ASN//ATR	84	124	95	77.8	3.6	173.8	MS
11	WUH1/VEE#5//CBRD	84	122	86	72.8	4.1	32.1	R
12	BAU/MILAN//CBRD	85	124	91	73.8	3.3	224.9	MS
13	SHA3/CBRD	82	123	85	71.9	2.8	33.4	R
14	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)	82	122	92	71.9	3.8	158.6	MS
15	HUW234+LR34/PRINIA//KRONSTAD F2004	83	123	95	75.4	3.4	402.6	S
16	KINDE	82	122	96	75.0	3.1	275.7	MS
17	PFAU/WEAVER*2//TRANSFER#12,P88.272.2	82	122	91	75.1	3.8	443.2	S
18	SUMAI #3**	78	118	108	0	2.5	not included	Not included
19	GAMENYA***	79	118	104	0	2.6	827.8	HS
20	GONDO/CBRD	79	119	90	0	2.7	56.3	MR

HS = highly susceptible, S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant

* Not enough heads were formed to inoculate entry number 3

** Sumai 3 was not included since resistance is known.

***Kariega and Gamenya were both used as susceptible checks as well as to check the virulence of the inoculum.

Obtain resistant donors and test the resistant sources to FHB for Type 1 and Type 2 resistance.

Resistant sources including Sumai 3, Frontana and other CIMMYT lines were imported from CIMMYT, Mexico and were submitted to the Biotechnology Laboratory for testing with DNA markers. Since all these lines were resistant to FHB, genotyping data showed that these lines had a different source of resistance from that of Sumai 3 and Frontana.

A total of 203 isolates (Dundee - 102, Frankfort - 27, Groblersdal - 57 and Vaalharts - 17) were submitted to the Biotechnology Laboratory for testing with species specific PCR primers.

The *F. graminearum* isolate, PPRI 7721 obtained from the ARC-Plant Protection Institute in Pretoria, was used as a positive control for Fg16 and Fg16N primers. The isolates were tested for *Fusarium graminearum, F. pseudograminearum, F. boothii, F. culmorum* and *F. meridionale.* The primers were used to test the most prevalent species occurring in South Africa, according to Boutigny *et al*. (2011). The results show that from the 203 isolates tested, 139 isolates were confirmed to be in the *Fusarium* group that consisted of 134 *F. graminearum* isolates, one *F. pseudograminearum* isolate and four *F. boothii* isolates. No isolates that belonged to any of the *F. culmorum* and *F. meridionale* were identified. The remaining 64 isolates did not fall in the *Fusarium* group and will not be tested, since only the *Fusarium* group isolates are being used in the programme.

Fusarium single spore isolates may produce mycotoxins including trichothecenes, moniliformin and fumonisins. According to literature, *Fusarium graminearum* produce type B trichothecenes including DON, acetylated derivatives include 3-acetyl (3-ADON) and 15-acetyl (15-ADON) and NIV chemotypes. All the isolates were chemotyped, but only 133 isolates were positively identified. The results show that DON was the predominant chemotype in all the localities with a total of 122 isolates testing positive for DNO from the 203 samples tested. Six isolates from Dundee tested positive for NIV and 5 of these tested positive for both DON and NIV.

Use resistant sources for a crossing block, together with the current cultivar spectrum.

Since Sumai 3 and Frontana are well-known resistance sources to scab, they were included in a backcross (BC) programme, together with five different sources previously tested for scab resistance.

The backcrosses (BC) were completed (Table 5).

Table 5. The crossing combinations together with the backcross stage of each of the entries

FEMALE \ MALE	Sumai #3	Frontana	9th-10	9th-36	9th-37	9th-42	10th-36	11th-47
Baviaans	BC3F1	BC3F1	BC3F1	BC3F1	BC3F1	BC3F1	BC3F1	BC1F1
Buffels		BC2F1	BC1F1	BC2F1	BC1F1	B2F1	BC2F1	
Duzi	BC3F1	BC2F1	BC3F1	BC3F 1	BC3F1	BC3F1	BC3F1	BC1F1
Kariega	BC1F1	BC3F1	BC3F1	BC3F1	BC3F1	BC1F1		BC1F1
Marico	BC1F1	BC1F1	BC3F1	BC1F1		BC1F1		BC1F1

No crosses were made since no pollen was available

Total seed set per entry per cross combination is indicated in Table 6. The seeds are to be multiplied and supplied to Wimpie du Toit at Vaalharts, where selections will be made.

Table 6. Total seed count per cross combination

FEMALE \ MALE	Sumai #3	Frontana	9th-10	9th-36	9th-37	9th-42	10th-36	11th-47
Baviaans	157	122	50	136	143	64	216	115
Buffels		166	22	85	193	95	156	
Duzi	167	227	165	81	216	163	328	66
Kariega	55	179	53	37	176	236		149
Marico	54	138	58	21		45		80

No crosses made

Seed totals of 327.3 grams and 141.04 grams of F_2 and F_3 crossing combinations, respectively, were given to Wimpie du Toit during 2012 for selections. From the F_2 crossing combinations, 136 plants were selected and will be continued in the breeding programme in 2013 as F_3 entries. From the F_3 crossing combinations, 14 single plants were selected and will be included in the F_4 population in 2013. A further 35 single F_3 plants were selected from 117 entries based on good agronomic traits. These single plants will be included in the F_4 population in 2013.

The current BC_2F_2 seed will be given to Wimpie du Toit for selections during the 2013 season. The BC_3F_1, BC_2F_1 and the F_1 seed will be multiplied in the glasshouse during 2013 and will be submitted to Wimpie du Toit for single plant selections in 2014 at Vaalharts.

Cross combinations between rust and *fusarium* resistance were planted under the irrigation system at Bethlehem. A number of 226 entries were planted and 186 of these entries were selected for agronomic traits. These entries will be supplied to Wimpie du Toit to make selections for the breeding programme.

Transfer technology, to disseminate research findings to farmers and publish information.

The following popular articles were published in 2012:

- Article in “Kommuniek”, “Fusarium-aarskroei een van belangrikste koringsiektes", June 2012, pp. 24-26.
- Article in “SA Grain”, "Voorkoms van aarskroei in Suid-Afrika”, September 2012, pp 125.

Farmers’ Days

Presentations were given at Allgro farmers’ day on Fusarium head blight on irrigation wheat on the following dates:

- Clarens – 13 June 2012
- Lydenburg - 1 October 2012
- Burgersfort - 2 October 2012
- Marble Hall and Groblersdal - 3 October 2012
- Atlanta - 4 October 2012
- Brits - 9 October 2012
- Potgietersrus and Naboomspruit - 10 October 2012
- Koedoeskop - 11 October 2012
- Brits – 5 March 2013
- Koedoeskop – 6 March 2013
- Allgro Conference – 14 March 2013
- Atlanta – 19 March 2013
- Thabazimbi – 20 March 2013
- Vaalharts – 11 April 2013
- Ohrigstad – 17 April 2013

Conference presentations:

- Presented at the Allgro Conference on Fusarium head blight on wheat in March 2013.
- Co-authored the following presentation: "SSR marker haplotype comparison of five CIMMYT resistance lines with seven well-known Fusarium head blight resistant wheat sources indicates resistance novelty" presented at the National Head Blight Forum in Orlando Florida, USA, December 2012.
- Co-authored a presentation at the SASPP conference in January 2013 titled "Screening of germplasm to identify material resistant to Stripe rust, Leaf rust, Stem rust, Russian Wheat Aphid and Fusarium head blight.

Reference:

Boutigny, A., Ward, T.J., Van Coller G.J., Flett, B., Lamprecht, S.C., O'Donnel, K., Viljoen A. 2011. Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific difference in host preference. Fungal Genetics and Biology 48, p 914 -920.

GK 04/24: RESISTANCE AS AN ALTERNATIVE IN COST-EFFECTIVE MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SMALL GRAINS
PROGRESS REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 04/24
Title: Resistance as an alternative in cost-effective management of Fusarium head blight in small grains
Duration: 2002 - 2013
Status: Final report
Project leader: Mrs Cathy de Villiers

In 2012, five objectives were set for the project and are summarised below. Technology transfer and the short term objectives are also included in the summary.

Field evaluations:
Five hundred and thirty four entries from different scab nurseries were evaluated for scab in the field under irrigation and a total of 150 lines showed resistance to scab. The RAS nursery consisted of 1 286 entries and 201 of these entries showed resistance. Irrigation lines were also screened for scab in the field and none of the lines showed resistance. Therefore, there is a growing need to include scab resistance in the irrigation breeding programme, since scab is a significant problem under irrigation. Entries that showed resistance are to be tested again in the field to confirm resistance.

Glasshouse:
The 13th scab nursery (FHBSN) was planted in the quarantine facility and was evaluated for agronomic and quality parameters in 2011. The nursery was evaluated for scab resistance in the glasshouse in 2012 and the data showed that 5 lines were moderately resistant and 2 lines were resistant (entries 11 and 13).

Seed total of 327.3 grams and 141.04 grams of F_2 and F_3 crossing combination seeds, respectively, were given to Wimpie du Toit in 2012 for selections. From the F_2 crossing combinations, 136 plants were selected and will continue in the breeding programme in 2013 as F_3 entries. From the F_3 crossing combinations, 14 single plants were selected and will be included in the F_4 population in 2013. A further 35 single F_3 plants were selected from 117 entries based on good agronomic traits. These single plants will be included in the F_4 population in 2013.

The current BC_2F_2 seed will be given to Wimpie du Toit for selections during the 2013 season. The BC_3F_1, BC_2F_1 and the F_1 seed will be multiplied in the glasshouse during 2013 and will be submitted to Wimpie du Toit for single plant selections in 2014 at Vaalharts.

Crossing combinations between rust and *fusarium* resistance were planted under the irrigation system at Bethlehem. A number of 226 entries were planted and 186 of these entries were selected for agronomic traits. These entries will be supplied to Wimpie du Toit to make selections for the breeding programme.

Laboratory:
Two hundred and three single spore isolates from Frankfort, Dundee, Groblersdal and Vaalharts were tested in the Biotechnology Laboratory, for species specific PCR primers and mycotoxin production. The results showed that 139 isolates were in the *Fusarium* group that consisted of 134 *F. graminearum* isolates, on *F. pseudograminearum* isolate and four *F. boothii* isolates. Mycotoxin analysis was done and 133 isolates tested positive for mycotoxin production. DON was the predominant chemotype in all the localities where NIV and DON + NIV were produced from 6 and 5 isolates respectively.

A total of 139 isolates were tested against the *Fusarium* species and the mycotoxins they produce. The 3-ADON chemotype was absent in all localities whereas the 15-ADON chemotype were found in *F. graminearum*, *F. pseudograminearum* and *F. boothii* isolates. The data show that of the 139 *F. graminearum* samples tested, 108 samples produced 15-ADON, 6 produced NIV and 5 produced DON and NIV. In the *F. graminearum* samples all the NIV producing isolates were from Dundee. The *F. pseudograminearum* isolated from Groblersdal produced the 15-ADON mycotoxin. Four *F. boothii* isolates produced the 15-ADON mycotoxin isolated from infected kernels from Groblersdal. This data correlates with the findings of Boutigny *et al.* (2011) where 85% of the samples collected were *F. graminearum* and the predominant trichothecene types also showed the 15-ADON as predominant.

Technology transfer:

To disseminate the research findings on FHB, 2 popular articles were published during the past year, and fifteen presentations were made at the Allgro farmers' days on the following topic: "Fusarium head blight on irrigation wheat." Three conference presentations were also made: one at the Allgro Conference, one during the Fusarium Head Blight Forum in Florida, USA and one during the Southern African Society of Plant Pathology.

GK 05/04: USE OF RUSSIAN WHEAT APHID RESISTANT GERMPLASM FOR THE DEVELOPMENT OF RUSSIAN WHEAT APHID RESISTANT BREEDER'S LINES
INTERIM PROGRESS REPORT APRIL 2013 – JULY 2013

1. Project details

Number:	GK 05/04
Title:	Use of Russian Wheat Aphid resistant germplasm for the development of Russian Wheat Aphid resistant breeder's lines
Duration:	2013 - 2017
Status:	Interim Progress Report
Project leader:	Dr Vicki Tolmay

2. Objectives

2.1 Long-term objectives

- Identify the most suitable sources of Russian wheat aphid (RWA) resistance genes for deployment in cultivars in the field and transfer resistance from suitable sources to well-adapted wheat lines for use by breeders in mainstream breeding programmes.
- Maintain international collaboration set up through project GK05/12 and ensure flow of RWA resistant germplasm between collaborators.
- Over time, and as reliable markers become available, replace the shuttle of RWA germplasm abroadfor phenotypic screening with other RWA biotypes, with marker assisted selection (MAS).
- Provide assistance and follow-up to breeders with regard to the use and suitability of RWA resistant lines that have been incorporated into breeding programmes.

2.2 Short term objectives: April 2013 - March 2014

- Continue to collaborate with CIMMYT-Turkey, USDA-ARS, ICARDA, Murdoch University, CSIRO, KARI Kenya, University of Minnesota and other possible collaborators (Argentina) to screen South African germplasm with other biotypes of Russian wheat aphid.
  a. Murdoch University: Obtain DH population [PI94365/EGA-Gregory] from Mehmet to phenotypically screen with RWASA1, RWASA2 and RWASA3
  b. Murdoch University: Scott Sydenham to visit Australia to screen RWA populations with newly developed markers. 4 x PI94365 populations and 1 x 50 line set
  c. Murdoch University: Identify 4 populations [PI94365 as RWA donor]. Plant, extract DNA, export to Murdoch University to test newly developed marker.
- Use both phenotypic screening and MAS to combine different RWA resistance genes, as well as RWA resistance and *Yr* resistance, in adapted germplasm for use as parent lines by breeding programmes.
  d. Progress with crossing combinations
  e. Test 50 plants each of Nossob single-plant-derived lines 2, 8, 14 and 28 to select most resistant plants for seed increase. Can markers confirm *Yr* resistance?
- Plant all accessions that may need to be exported in future in the greenhouse for inspection by the National Department so as to comply with the quarantine regulations.
- Continue developing RWA resistance mapping populations and screen phenotypically when generated.
- Screen a nursery from the ARC-Small Grain Institute germplasm collection (2013 RAS) for seedling resistance to RWASA2 in the greenhouse.

3. Report on the objectives: April 2013 – July 2013

Continue to collaborate with CIMMYT-Turkey, USDA-ARS, ICARDA, Murdoch University, CSIRO, KARI Kenya, University of Minnesota and other possible collaborators (Argentina) to screen South African germplasm with other biotypes of Russian wheat aphid.

As part of the ongoing collaboration with Murdoch University in Western Australia, a doubled haploid population (200 lines) has been imported. The experimental design for phenotypic evaluation in Bethlehem has been completed and is being verified by scientists at Murdoch University. Germplasm will be evaluated during July/August 2013 using all three biotypes in a split plot design experiment. One hundred and fifty plants of of each of four populations namely, CIMMYT14/Duzi F_2, CIMMYT/Matlabas F_2, CIMMYT14/Elands and CIMMYT14/PAN3144 have been planted. DNA will be extracted and shipped to Australia during July 2013.

Use both phenotypic screening and MAS to combine different RWA resistance genes, as well as RWA resistance and Yr resistance, in adapted germplasm for use as parent lines by breeding programmes.

Crosses using Matlabas, Duzi, Baviaans and Buffels as female parent and a selection of previously unused RWA resistant donor accessions are continuing. Selected F_4 plants of the combination Elands/PAN3144 have been indentified and are being grown out in the greenhouse. The plants will be evaluated for resistance to pre-harvest sprouting. Those with the best resistance will be maintained and used as the female parent in selected crosses in future.

Plant all accessions that may need to be exported in future in the greenhouse for inspection by the National Department so as to comply with the quarantine regulations.

This objective is ongoing

Continue developing RWA resistance mapping populations and screen phenotypically when generated.

Evaluation of the genotypic data generated from these populations is ongoing. Correlations between the phenotypic evaluation and the marker data will follow.

Screen a nursery from the ARC-Small Grain Institute germplasm collection (2013 RAS) for seedling resistance to RWASA2 in the greenhouse.

The nursery has been received from the SGI Germplasm Collection and evaluation is scheduled to take place from August 2013.

4. Future of the project

This project is on track and progressing satisfactorily. A presentation entitled “Host plant resistance for RWA control: It’s complicated” will be presented at the Entomological Society of Southern Africa’s bi-annual Congress which will be held in Potchefstroom from 30 June to 4 July 2013.

GK 05/04:	USE OF RUSSIAN WHEAT APHID RESISTANT GERMPLASM FOR THE DEVELOPMENT OF RUSSIAN WHEAT APHID RESISTANT BREEDER'S LINES INTERIM PROGRESS REPORT APRIL 2013 – JULY 2013

Summary

Number: GK 05/04
Title: Use of Russian Wheat Aphid resistant germplasm for the development of Russian Wheat Aphid resistant breeder's lines
Duration: 2013 - 2017
Status: Interim Progress Report
Project leader: Dr Vicki Tolmay

This project is on track and progressing satisfactorily. As part of the ongoing collaboration with Murdoch University in Western Australia, a doubled haploid population (200 lines) has been imported. The experimental design for phenotypic evaluation in Bethlehem has been completed and is being verified by scientists at Murdoch University. Germplasm will be evaluated during July/August 2013 using all three biotypes in a split plot design experiment. One hundred and fifty plants of of each of four populations namely, CIMMYT14/Duzi F_2, CIMMYT/Matlabas F_2, CIMMYT14/Elands and CIMMYT14/PAN3144 have been planted. DNA will be extracted and shipped to Australia during July 2013. Crosses using Matlabas, Duzi, Baviaans and Buffels as female parent and a selection of previously unused RWA resistant donor accessions are continuing. Selected F_4 plants of the combination Elands/PAN3144 have been indentified and are being grown out in the greenhouse. The plants will be evaluated for resistance to pre-harvest sprouting. Those with the best resistance will be maintained and used as the female parent in selected crosses in future. Evaluation of the genotypic data generated from these populations is ongoing. Correlations between the phenotypic evaluation and the marker data will follow. The nursery has been received from the SGI Germplasm Collection and evaluation is scheduled to take place from August 2013. A presentation entitled "Host plant resistance for RWA control: It's complicated" will be presented at the Entomological Society of Southern Africa's bi-annual Congress which will be held in Potchefstroom from 30 June to 4 July 2013.

GK 05/12: PRE-BREEDING FOR RUSSIAN WHEAT APHID RESISTANCE TO BIOTYPE B
FINAL REPORT APRIL 2012 – MARCH 2013

1. Project details

Number: GK 05/12
Title: Pre-breeding for Russian wheat aphid resistance to Biotype B
Duration: 2005 - 2013
Status: Final Report
Project leader: Dr Vicki Tolmay

2. Objectives

2.1 Long-term objective

The long-term objective of the project is as follows:

- To shuttle Russian wheat aphid resistant germplasm abroad to be screened with other, known Russian wheat aphid biotypes to pre-emptively ensure that resistance that is effective against other biotypes besides the known South African one is included in South African wheat germplasm.

2.2 Short term objectives: April 2012 - March 2013

The short term objectives for the 2012/2013 report period were as follows:

- To use both phenotypic screening and marker-assisted selection (MAS) to combine different RWA resistance genes, as well as RWA resistance and *Yr* resistance, in adapted germplasm for use as parent lines by breeding programmes.
  - Progress with crossing combinations
  - Follow-up evaluation of Nossob single-plant-derived lines 20, 21, 22, 24, 31, 55, 62, 63, 64 and 65 under field conditions.
  - Test 50 plants each of Nossob single-plant-derived lines 2, 8, 14 and 28 to select most resistant plants for seed increase. Can markers confirm *Yr* resistance?
  - Plant and evaluate remaining seed of SA463/*5 Karee (22) // Kariega /// 95 BHM 178.5 Sr35/*5 Kariega potentially containing *Dn5, Dn8, Dn9, Sr24*, and *Sr35* in addition to *Lr34/Yr18*
  - Plant and evaluate seed of PI 225227/*4 Kariega //17 BHM 40.11 Sr22/*5 Kariega F_6 potentially containing unknown RWA resistance gene and *Sr22* in addition to *Lr34/Yr18*
  - Re-evaluate 154 lines rescued from 2010 RAS during 2010 and 2011 to confirm resistance.
- Plant all accessions that may need to be exported in future in the glasshouse, for inspection by the National Department so as to comply with the quarantine regulations
  - PI 243642, PI 225217, PI 366549, PI 245462, Plant and test for RWASA2 resistance
- Continue to collaborate with CIMMYT-Turkey, USDA-ARS, ICARDA, Murdoch University, CSIRO, KARI Kenya, University of Minnesota and other possible collaborators (Argentina) to screen South African germplasm with other biotypes of Russian wheat aphid.
- Visit ICARDA, Syria to evaluate South African lines for resistance to RWA biotypes occurring there
- Continue developing RWA resistance mapping populations and screen phenotypically.
- To screen a nursery from the ARC-Small Grain Institute germplasm collection for seedling resistance to RWASA2 in the glasshouse.
- Compile a final detailed project report

3. Report on the objectives: April 2012 - March 2013

General remark

During the report period additional new RWA biotype(s) (RWASA3 and others) were confirmed. A number of months research time was lost with no phenotypic screening taking place from May 2012 until November 2012 as a result of the unclear status regarding the distribution and prevalence of the different RWA biotypes. In December 2012 screening was resumed, using RWASA3 for the majority of evaluations. In this report the specific biotype used for particular goals is listed with the results; however, some goals were postponed to wait for more information on the distribution of the different biotypes. In March 2013 Dr Astrid Jankielsohn recommended the use of RWASA2 for screening most of the material, based on results from Project GK 05/16 which show RWASA2 to be the most widely distributed biotype in the wheat production areas of South Africa.

3.1 To use both phenotypic screening and marker assisted selection (MAS) to combine different RWA resistance genes, as well as RWA resistance and Yr resistance, in adapted germplasm for use as parent lines by breeding programmes

Progress with crossing combinations: New crossing combinations generated

One hundred and nineteen crosses were made during the year, using Matlabas as female parent and accessions with resistance to RWASA2 as resistance donor, pollen parents. Lines used as pollen parents are listed in Table 1 with their pedigree (where available), characteristics of interest, possible genes and the number of seeds generated.

Table 1. Pedigree, possible genes and/or characteristic(s) of interest of pollen parents used in crosses with Matlabas during 2012/2013 as well as the number of seed generated

Pollen parent	Pedigree and/or characteristics of interest	Possible genes	# of seeds
Ankor	Akron/Halt//4*Akron [RWASA2=4,6,7; R, MR]	*Dn4*	51
Bond CL	Yumar//TXGH-12588-120*4/FS-2 [RWASA2=5; R]	*Dn4*	13
CORWA	Sumner/CO-820026//PI372129/3/TAM-107 [RWASA2=7; MR]	*Dn4*	17
Hatcher	Yuma/PI372129//TAM200/3/4*Yuma/4/KS91H184/Vista [RWASA2=4; R]	*Dn4*	57
Kingbird	Not tested for RWA resistance	*Sr(Ug99)*	26
MTRWA92-93	PI 372129/*2Pondera [RWASA2=4; R]	*Dn4*	12
MTRWA92-114	PI 372129/*2Pondera [RWASA2=3; HR]	*Dn4*	16
MTRWA92-115	PI 372129/*2Pondera [RWASA2=3,4, 7; HR, R, MR]	*Dn4*	31
MTRWA92-120	PI 372129/*2Pondera [RWASA2=4,7; R, MR]	*Dn4*	22
MTRWA92-123	PI 372129/*2Pondera [RWASA2=3; HR]	*Dn4*	22
MTRWA92-145	PI 372129/*2Newana [RWASA2=5; R]	*Dn4*	33
MTRWA92-149	PI 372129/*2Newana [RWASA2=3,4; HR, R]	*Dn4*	46
MTRWA92-160	PI 372129/*2Newana [RWASA2=3; HR]	*Dn4*	12
Prowers99	CO-850060/PI372129//5*Lamar [RWASA2 = 7; MR]	*Dn4*	41
Ripper	PI220127/P5//TAM200/KS87H66(CO940606)/CO850034/PI372129//5*TAM107(TAM107R-2) [RWASA2=3,4,5; HR, R]	*Dn4,Dnx*	78
T99/5	SST 333/3/SXL/VEE"S"//SA1684/*4 MOLOPO(77) [RWASA1 & RWASA2=4, R]	*Dn1,Dn2,APR*	54

Pollen parent	Pedigree and/or characteristics of interest	Possible genes	# of seeds
T99/07	Aus1408/Oranje [RWASA2=4; R]	*Dn?*	19
T00/03	661L 1-33/TUGELA-DN	*Dn1*	22
T01/51	Aus1408//SA1684/*4Molopo(2)	*Dn?*	25
T01/52	Aus1408//SA1684/*4Molopo(2)	*Dn?*	22
T05/02	SA463/*4 Molen	*Dn5,Dn8,Dn9*	4
V809	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWASA2=6; R. AA Sr35; Sr24]	*Dn5,Dn8,Dn9, Sr24, Sr35*	6
V815	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWASA2=6; R. AA Sr35; Sr24; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr24, Sr35*	17
V821	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=6; AA Sr35; Sr24; Aa Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr24, Sr35*	15
V822	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=MR; AA Sr35; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr35*	14
V833	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=4; +Sr24]	*Dn5,Dn8,Dn9, Sr24*	24
V837	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=R; Aa Sr35; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr35*	4
V838	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=5; Sr24]	*Dn5,Dn8,Dn9, Sr24*	23
V842	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=6; AA Sr35; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr35*	2
V866	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=MR; Sr24]	*Dn5,Dn8,Dn9, Sr24*	24
V868	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=5; AA Sr35; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr35*	8
V902	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=4; Aa Sr35]	*Dn5,Dn8,Dn9, Sr35*	6
V938	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=4; Aa Lr34; Sr24]	*Dn5,Dn8,Dn9, Lr34, Sr24*	4
V948	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=5; Aa Sr35; Sr24; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr24, Sr35*	9
V990	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA = 5 = R; Aa Sr35; AA Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr35*	1
V995	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega [RWA=4; AA Sr35; Sr24; Aa Lr34]	*Dn5,Dn8,Dn9, Lr34, Sr24, Sr35*	8
Yuma	NS14/NS25//2*Vona [RWASA2=9; S]	-	43
Yumar	Yuma/PI372129//CO-850034/3/4*Yuma [RWASA2=4,5; R]	*Dn4*	40

Progress with crossing combinations: Seed multiplication and agronomic characterisation of genotypes containing rust and RWA resistance

Ten spring lines containing good Russian wheat aphid resistance (phenotypicaly screened with RWASA2), as well as homozygous resistance for *Lr34/Yr18* (MAS) and *Sr35* (MAS) which were identified during the previous year, were planted out under the wire-enclosure. The lines are listed in Table 2. Single plants were harvested within each line. It appears that there is likely a yield penalty associated with the multi-resistant lines. The germplasm will be evaluated under irrigated conditions in the 2013/2014 season, after which a germplasm release will be done. This material is particularily valuable as it contains not only the Lr34/Yr18/Sr57/Pm38 multipathogen resistance, but has additional important major genes as well.

Table 2. Plant height, total mass seed harvested, number of plants and mean mass per plant of ten spring lines containing resistance to RWASA2 as well as *Lr34*/*Yr18* and *Sr35*

Line	Plant height (cm)	Total mass (g) seed harvested	Number of plants	Mean mass (g) per plant	Pedigree
8-02-8	83.75	233.5	44	5.31	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-08-1	84.25	288.1	42	6.86	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-08-2	80.75	215.7	29	7.44	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-10-3	81.75	276.3	35	7.89	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-10-7	83.75	275.1	41	6.71	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-11-1	82.25	250.9	41	6.12	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-12-1	79.25	260.4	34	7.66	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-17-4	85.75	312.2	45	6.94	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
8-28-6	79	153	32	4.78	Kariega/*4 PI225227//334BHM171Sr35/*5Kariega
SAPBA Set 125	84.25	231.6	22	10.53	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega
Kariega	90.4	116.1	9	12.90	

Fifteen lines are listed in Table 3. These lines, which originate from either National Germplasm Collection or the RWA resistance pre-breeding programme, are being made available as a segregating nursery for public use, based on specific RWA resistance characteristics.

Table 3. Fifteen lines with Russian wheat aphid resistance being made available as a segregating nursery for public use

SA #	Name	Pedigree	Additional information
SA8236	T99/21	SAULESKU 28/TUGELA-DN	RWASA1=R Segregating, RWASA2=R Segregating
SA8256	T00/6	AUS22498/*5 KAREE	RWASA1=MR Segregating, RWASA2=R Segregating
-	V839	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega	RWASA2 = R, AA Sr35, +Sr24
-	V851	SA463/*5Karee-22//Kariega///334BHM171Sr35/*5Kariega	RWASA2=MR, AA Sr35, AA Lr34
-		Matlabas///Elands//CItr2401/*4Kariega	F2 Bulk
-		Matlabas/Iran15	F2 Bulk
-		Matlabas//2007CIMMYTRWASet17	F2 Bulk, RWAArgentina=R, (USA)RWA2=MR, RWAKenya=MR
SA8797	Aus21905	TAKARI=PI483058	RWASA2=R Segregating, (USA)RWA1=S
SA10436	Katya-A-1	KHEBROS/BEZOSTAYA-1	RWASA3=R
SA10382	Chokka		RWASA3=MR Segregating, APR Field
SA10751	IAS 63 (80098 *T. aestivum*)		RWASA2=MR Segregating, APR Field
SA10906	CI 14971		RWASA2=R Segregating, APR Field
SA11442	35IBWSN-479	WEAVER/9/KT/BAGE//F	RWASA2=R Segregating, APR Field
SA11606	14HRWSN-119	PASTOR//MUNIA/ALTAR 84	RWASA2=R Segregating, APR Field
SA12172	12 SAWYT-49	MTRWA92.1	RWASA2=R, APR Field

Follow-up evaluation of Nossob single-plant-derived lines 20, 21, 22, 24, 31, 55, 62, 63, 64 and 65 under field conditions.

The ten single-plant-derived lines of the cultivar Nossob, which was released but not commercialised, were planted in Bethlehem in the field for evaluation during the 2012/2013 season. No selections were made from this material and this objective will be discontinued.

Test 50 plants each of Nossob single-plant-derived lines 2, 8, 14 and 28 to select most resistant plants for seed increase. Can markers confirm Yr resistance?

Nossob lines 2, 8, 14, and 28 were not evaluated, due to the change in biotype. Consequently the evaluation for Yr resistance with molecular markers was not done as the seed was not planted. These lines will be planted in 2013 and will be evaluated with RWASA2.

*Plant and evaluate remaining seed of SA463/*5 Karee (22) // Kariega /// 95 BHM 178.5 Sr35/*5 Kariega potentially containing Dn5, Dn8, Dn9, Sr24, and Sr35 in addition to Lr34/Yr18*

This germplasm originates from combinations generated between 2001 and 2006 [WCT funded Project GK 05/04]. Twenty four lines containing the target resistance genes have been selected from the population. The F_4 generation will be planted in Bethelehem under the wire-enclosure in 2013 for data collection and the F_5 generation will be planted in the field in 2014. It is expected that a germplasm release will be made from this material in 2015.

*Plant and evaluate seed of PI 225227/*4 Kariega //17 BHM 40.11 Sr22/*5 Kariega F6 potentially containing unknown RWA resistance gene and Sr22 in addition to Lr34/Yr18*

RWA resistance evaluation has been completed for this set of germplasm, however MAS for the presence of *Sr22* has not yet been completed due to ongoing marker optimisation.

3.2 To plant all accessions that may need to be exported in future in the glasshouse, for inspection by the National Department so as to comply with the quarantine regulations

All plants in Glasshouse 4 were inspected by the Quarantine Service on 8 March 2012 and 27 November 2012. The MTA's signed during the previous report period made it possible to provide germplasm obtained from USDA-ARS, Colorado State University and Kansas State University to Sensako for the development of DH's with the purpose of creating a stable, standardised differential set on which to characterise RWA biotypes. Development of these DH's is currently underway.

3.3 To continue to collaborate with CIMMYT-Turkey, USDA-ARS, ICARDA, Murdoch University, CSIRO, KARI Kenya and other possible collaborators (Argentina) to screen South African germplasm with other biotypes of Russian wheat aphid

Due to close collaboration built up with the International Winter Wheat Improvement Programme (IWWIP) 63 segregating populations (F_2 stage) were obtained from IWWIP in 2011. Single plant selections were made from the F_2 germplasm, based on glasshouse evaluation for RWA resistance. F_3 plants will be evaluated again in 2013/2014.

Selections were made from four of the international nurseries obtained from CIMMYT Turkey in order to include germplasm with effective disease resistance in the RWA resistance pre-breeding action (Table 4). Nine lines were selected from the 19th FAWWON (Facultative and Annual Winter Wheat Observation Nursery) for irrigation, two from the 19th FAWWON for semi-arid production. Two lines were selected from the 3rd WWSRRN (Winter Wheat Stem Rust Resistance Nursery) and 12 lines from the 1st WWYRRN (Winter Wheat Yellow Rust Resistance Nursery).

Table 4. Lines selected from International nurseries to utilise in the RWA-resistance pre-breeding programme

Identity	Pedigree
19FAWWON-IR24	Owl*2/Shiroodi
19FAWWON-IR25	CH111.14422
19FAWWON-IR35	JI5418/MARAS//SHARK/F4105W2.1
19FAWWON-IR127	Chamran/5/Bez/4/On/6*Ph//Kf/3/Tob"s"/Napo//No66/6/Spn/Mcd//Cama/3/Nzt/4/Urles*2/Prl"s";
19FAWWON-IR137	Owl/Shiroodi/5/Owl/4/Bloudan/3/Bb/2*7C//Y50E/Kal*3
19FAWWON-IR159	FIORINA
19FAWWON-IR161	SIMANO
19FAWWON-IR207	T.AST/SPRW//BEZ/CGN/4/INIA//HBGN/DRC/3/BEZ/5/CHIL/2*STAR
19FAWWON-IR213	4WON-IR-257/5/YMH/HYS//HYS/TUR3055/3/DGA/4/VPM/MOS
19FAWWON-SA326	FRTL/NEMURA
19FAWWON-SA376	F5H80/5/KVZ/3/BB/CHA//TOR/4/TEMU47,E2E1 F2MBP-6H-5H-4H-2H
3WWSRRN3	SULTAN
3WWSRRN54	Owl//Ombul/Alamo
1WWYRRN5	DORADE-5
1WWYRRN6	338-K1-1//ANB/BUC/3/KIRGIZ
1WWYRRN7	RSK/CA8055//CHAM6
1WWYRRN11	VORONA/OPATA//PYN/BAU
1WWYRRN12	F4141-W-1-1/PASTOR//PYN/BAU
1WWYRRN16	338-K1-1//ANB/BUC/3/GS50A
1WWYRRN19	BILINMIYEN96.55
1WWYRRN23	AU/3/MINN//HK/38MA/4/YMH/ERA/5/PMF//CNO/GLL/6/KAUZ//ALTAR 84/AOS
1WWYRRN26	JUP/4/CLLF/3/II14.53/ODIN//CI13431/5/IL-75-2534
1WWYRRN33	AGRI/NAC//ATTILA
1WWYRRN38	AGRI/NAC//KAUZ
1WWYRRN44	OK81306//ANB/BUC/3/[SAULESKU 43]

A discussion was held between Dr Reneé Prins, Dr Mehmet Cakir, Scott Sydenham and Dr Vicki Tolmay at ORT International Airport on 2 November 2012 regarding RWA molecular marker development. As an outflow of this meeting, a visit is planned for August/September 2013, when Scott Sydenham will visit Dr Cakir's laboratory in Western Australia, to screen South African germplasm with RWA resistance markers recently developed by Dr Cakir. Dr Tolmay will screen DH populations developed for this marker validation using RWASA1, RWASA2 and RWASA3 in Bethlehem.

Dr Vicki Tolmay, Dr Mehmet Cakir and Mr Scott Sydenham on 2 November 2012

3.4 To visit ICARDA, Syria to evaluate South African lines for resistance to RWA biotypes occurring there.

The visit to Syria was not possible due to the continuing unstable political situation in the country. However, discussions were held with Dr Mustapha el Bouhsinni, the RWA scientist for ICARDA during a workshop held in October 2012 in Addis Ababa in Ethiopia.

3.5 To continue developing RWA resistance mapping populations and screen phenotypically.

The mapping population A1–A89 and B1–B139 progressed one generation during the report period. The following generation will be evaluated phenotypically using RWASA2. In addition to the mapping population being developed and evaluated with Dr Toi Tsilo, a second set of germplasm was planted and screened during the current year.

The resistance gene *Dny* reportedly contained in PI 586956 and Stanton was included in the experiment undertaken to confirm virulence of RWASA3 on *Dn4,* as *Dny* was overcome in the USA at the same time as *Dn4*. In confirming SSR markers useful for identifying *Dn4*-containing lines, a marker was found that appeared to differentiate between *Dn4* and *Dny*. Additional SSR markers were investigated in the adjacent areas of chromosome 1D. An F_2 and a BC_3F_2 populations of a cross between recurring parent #68 [Babax/KS94U276//Babax] and PI 586956 were evaluated, using RWASA1 and were used as mapping populations (2013). A cross between Matlabas and Stanton, a *Dny*-containing cultivar developed at Kansas State University, was also evaluated for RWASA1 and will be used in confirming the markers.

3.6 To screen a nursery from the ARC-Small Grain Institute germplasm collection for seedling resistance to RWASA2 in the glasshouse.

The 2012 RAS Nursery was screened using RWASA3. The 1 286 lines were evaluated during the report period. Eighty-five lines tested resistant to RWASA3 and 214 showed segregation (or contained 2 or more plants that escaped infestation). Results will be confirmed in a follow-up screening of resistant lines. These lines will be useful resource to diversify RWA resistance in SA.

3.7 Additional achievements and contributions from this project.

Scientific articles

Tolmay VL, Jankielsohn A, Sydenham SL, 2012. Resistance evaluation of wheat germplasm containing *Dn4* or *Dny* against Russian wheat aphid biotype RWASA3. Journal of Applied Entomology Article first published online: 5 OCT 2012, DOI: 10.1111/jen.12008. JAE Impact Factor: 1.311.

Oral Congress Contributions

Tolmay VL, Jankielsohn A, Sydenham SL, 2013. Evaluation of *Dn4* and *Dny* containing germplasm with Russian wheat aphid (RWA) biotype RWASA3. Combined Congress, 21 - 24 January 2013, University of KwaZulu-Natal (Westville campus), South Africa.

Tolmay VL, 2012. Wheat research in South Africa: Helping farmers grow their crop. Wheat for food security in Africa: Science and policy dialogue about the future of wheat in Africa. 8 - 12 October 2012, United Nations Conference Centre, Addis Ababa, Ethiopia.

4. Future of the project

This project is now concluded. Specific activities arising from research conducted in this project will be undertaken in the new project GK 05/04, which commenced in April 2013. This new project is entitled “Use of Russian Wheat Aphid resistant germplasm for the development of Russian Wheat Aphid resistant breeders’ lines”.

GK 05/12: PRE-BREEDING FOR RUSSIAN WHEAT APHID RESISTANCE TO BIOTYPE B
FINAL REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 05/12
Title: Pre-breeding for Russian wheat aphid resistance to Biotype B
Duration: 2005 - 2013
Status: Final report
Project leader: Dr Vicki Tolmay

During the report period, more new RWA biotype(s) (RWASA3 and others) were confirmed (see Project GK 05/16), impacting on GK 05/12 by causing changes in the biotype used for general phenotypic screening activities. The biotype used for each activity is therefore listed with each activity. One hundred and nineteen crosses were made during the year, using Matlabas as female parent and accessions with resistance to RWASA2 as resistance donor, pollen parents. Ten spring lines containing good Russian wheat aphid resistance (phenotypic screening with RWASA2) as well as homozygous resistance for *Lr34/Yr18* (MAS) and *Sr35* (MAS), which were identified during the previous year, were planted out under the wire-enclosure. The lines are listed in Table 2. Single plants were harvested within each line. It appears that there is likely a yield penalty associated with the multi-resistant lines. The germplasm will be evaluated under irrigated conditions in the 2013/2014 season, after which a germplasm release will be done. This material is particularily valuable as it contains not only the *Lr34/Yr18/Sr57/Pm38* multipathogen resistance, but has additional important major genes as well. These lines will be planted in 2013 and will be evaluated with RWASA2. Twenty four lines containing the target resistance genes have been selected from the population of SA463/*5 Karee (22) // Kariega /// 95 BHM 178.5 Sr35/*5 Kariega for further evaluation and development as germplasm release. The MTA's signed during the previous report period made it possible to provide germplasm obtained from USDA-ARS, Colorado State University and Kansas State University to Sensako for the development of DH's with the purpose of creating a stable, standardised differential set on which to characterise RWA biotypes. Development of these DH's is currently still underway. Single plant selections were made from the F_2 germplasm obtained from IWWIP in 2011, based on glasshouse evaluation for RWASA2 resistance. Twenty-five lines were selected from four of the international nurseries obtained from CIMMYT Turkey, in order to include germplasm with effective disease resistance in the RWA resistance pre-breeding action. A discussion was held between Dr Reneé Prins, Dr Mehmet Cakir, Scott Sydenham and Dr Vicki Tolmay at ORT International Airport on 2 November 2012, regarding RWA molecular marker development. It is unlikely that the visit to build a collaborative relationship with scientists at the International Centre for Agricultural Research in the Dry Areas (ICARDA), will ever take place due to the security situation in Syria. However, discussions were held with Dr Mustapha el Bouhsinni, the RWA scientist for ICARDA during a workshop held in October 2012 in Addis Ababa in Ethiopia. It was confirmed that, in addition to *Dn4,* the resistance gene *Dny* reportedly contained in PI 586956 and Stanton was also overcome by RWASA3. Potential markers for *Dny* were identified and germplasm previously developed in GK 05/12 is being utilised as mapping populations. One thousand, two hundred and eighty-six lines from the National Germplasm Collection were evaluated with RWASA3 during the report period. Eighty-five lines tested resistant and 214 showed segregation to RWASA3. This project is now concluded. Specific activities arising from research conducted in this project, will be undertaken in the new project GK 05/04 which commenced in April 2013. This new project is entitled "Use of Russian Wheat Aphid resistant germplasm for the development of Russian Wheat Aphid resistant breeders' lines".

GK 09/07: THE COLLECTION AND MAINTENANCE OF UNIQUE SMALL GRAIN GERMPLASM PROGRESS REPORT APRIL 2012 – MARCH 2013

1. Project details

Number: GK 09/07
Title: The collection and maintenance of unique small grain germplasm
Duration: Ongoing
Status: Continuation of existing project
Project leaders: Dr Eben von Well and mr Moses P Ncala

The mandate of this project is to maintain small grain germplasm accessions in the Genebank and to acquire new sources of germplasm with important agronomic traits and disease and pest resistance in order to enhance the fast tracking of the development of wheat cultivars with high yield and durable resistance. To achieve this, ARC-Small Grain Institute (ARC-SGI) collaborates with various international institutions like CIMMYT Mexico, CIMMYT Turkey and Syria, as well as breeding companies and universities in Uruguay, Australia, Germany and the USA for germplasm exchange. ARC-SGI receives germplasm collections containing important genes annually from CIMMYT Mexico, CIMMYT Turkey and the USA. This collection plays a vital role in food security for South Africa and Sub-Sahara African countries.

2. Objectives

2.1 Long-term objective

The aim of the new Germplasm Collection unit is to maintain high-viability accessions for long period storage. This ensures that seeds placed in storage are of high quality and ensure maximum longevity. The small grain germplasm collection in total holds 17 221 small grain accessions that consist of wheat (*Triticum sp.*) accessions (15 045), Oats (*Avena sativa*) (676), Barley (*Hordeum vulgare*) (1006), Rye (*Secale cereale*) (57) and Triticale (*Triticosecale*) (437).

2.2 Short term objectives: April 2012 - March 2013

- To regenerate germplasm for the conservation and maintenance of important genetic resources for food and agriculture. Regeneration is the renewal of germplasm accessions by sowing and harvesting fresh seeds that possess as high genetic purity as the original sample. Germplasm regeneration is the most critical operation in Genebank management.
- To request small grain germplasm through internal, national and international sources. The main reason for acquiring new germplasm is to ensure that sufficient diversity is available to meet current and future needs for research and food production in South Africa.
- To co-ordinate import and export of seed samples for the different programmes at ARC-SGI. The seed samples designed for export has to comply with the phytosanitary requirements specified by Directorate: Agricultural Product Inspection Services (APIS), which falls under the Department of Agriculture, Forestry and Fisheries. For the export of germplasm, the fields from which germplasm intended for export are produced, are registered. These fields are inspected by the officials of the Directorate: APIS during the active growth stages. After harvesting, seed samples are submitted to the APIS officials by the ARC-Plant Protection Research Institute (PPRI) for tests against Karnal Bunt (*Tilletia indica*) before export.
- To maintain and keep all information readily available on a computer database. Genetic resource work involves the management of documentation and storage of information and the update of the database.

- To maintain the seed samples in a viable condition with low temperature storage facilities. This is done by drying the seeds to low moisture content (between 3 to 6%) over a period of 6 to 8 weeks. After drying, the seeds are sealed in aluminum foil bags and stored in deep-freezers at a temperature of -18°C.

3. Report on the objectives: April 2012 - March 2013

3.1 Report on the objective 2.2.1

Regeneration

During the 2012/2013 season, two nurseries were maintained for germination rejuvenation. The first was the Germplasm Nursery 2012, consisting of 1 710 accessions. The nursery consisted of wheat (1 687 entries) and barley (23). Data was collected from them to update the Genebank database. The other was the Germplasm Introduction Nursery 2012, consisting of 157 accessions obtained from abroad and was used for seed generation. The composition of small grain types planted during the 2012/13 growing season is shown in Table 1 below.

Table 1. Number of different small grain types planted during the 2012/2013 season

Small grain type	Accessions
Wheat	1 687
Barley	23
Failed to germinate	72 (wheat)
Total	**1 710**

Six RAS nurseries (Rust, Aphids and Scab) consisting of 1 286 entries, were planted for characterisation and data collection on pest and disease (Russian Wheat Aphid, different rusts (Yr, Sr and Lr), Septoria and Scab) reactions at different localities. The localities used for pest and disease characterisation were Bethlehem for RWA evaluation, Cedara for Lr, Elliot for Yr, Tygerhoek for Sr and Septoria, and Bethlehem for Scab. At Tygerhoek, the nursery was planted in two adjacent blocks and rust infestation was adequate for discernible rating among the accessions in the first block, but very low in the second block. In total, only accessions that received high infestation were rated and their ratings are included in this report. The results of these screenings are reported on in the results by other projects (GK 04/20, GK 04/24 and GK 05/12).

From these evaluations 15 accessions (Table 2) exhibited moderate to high resistance to RWASA2. These accessions are available for public request and use.

Table 2. List of resistant germplasm lines available for public request

S/n	SA number	Name	Pedigree	Resistance
1	SA 8236	T99/21	SAULESKU 28/Tugela DN	RWASA1=R Segregating, RWASA2=R Segregating
2	SA 8256	T00/6	AUS 22498/*5 Karee	RWASA1=MR Segregating, RWASA2=R Segregating
3	-	V839	SA 463/*5Karee-22//Kariega/// 334BHM171Sr35/*5Kariega	RWASA2=R, AA Sr35, +Sr24
4	-	V851	SA 463/*5Karee-22//Kariega/// 334BHM171Sr35/*5Kariega	RWASA2=MR, AA Sr35, AA Lr34
5	-	-	Matlabas///Elands//CItr	F2 Bulk

S/n	SA number	Name	Pedigree	Resistance
			2401/*4Kariega F2 Bulk	
6	-	-	Matlabas/Iran 15 F2 Bulk	F2 Bulk
7	-	-	Matlabas//2007 CIMMYT RWA Set 17	F2 Bulk, RWAArgentina=R, (USA)RWA2=MR, RWAKenya=MR
8	SA 8797	AUS 21905	TAKARI = PI 483058	RWASA2=R Segregating, (USA)RWA1=S
9	SA 10436	Katya-A-1	KHEBROS/BEZOSTAYA-1	RWASA3=R
10	SA 10382	Chokka	-	RWASA3=MR Segregating, APR
11	SA 10751	IAS 63 (80098 T.aest)	-	RWASA2=MR Segregating, APR Field
12	SA 10906	CI 14971	-	RWASA2=R Segregating, APR Field
13	SA 11442	35IBWSN-479	WEAVER/9/KT/BAGE//F	RWASA2=R Segregating APR Field
14	SA 11606	14HRWSN-119	PASTOR//MUNIA/ALTAR 84	RWASA2=R Segregating APR Field
15	SA 12172	12SAWYT-49	MTRWA92.1	RWASA2=R, APR Field

Wheat (Triticum sp.)
A total of 1 521 accessions were harvested from rejuvenation conducted during the 2012/2013 season. Data were taken and observations made on material segregating, as well as entries with possible genetic mixtures.

Barley (Hordeum vulgare)
Twenty three accessions were harvested for germination rejuvenation during the season and processed.

Table 3. Number of germplasm accessions rejuvenated per crop

Seed regeneration:	Nurseries consists of:	Total entries:
2012 Germplasm Nursery	- wheat (1 687 entries) - barley (23 entries)	1 710 entries planted in total and 1 615 entries harvested.
Germplasm Introductions 2012	157 Accessions introduced from various countries.	157 accessions planted in total: - 157 accessions harvested.

3.2 Report on the objectives 2.2.2 and 2.2.3

Internal (ARC-SGI) germplasm request:
One hundred and ninety eight accessions were requested internally by scientists for research purposes at ARC-SGI and 198 accessions were supplied (Table 4).

Table 4. Internal germplasm request from genebank

Requested by	Accession requested	Supplied
Dr Toi Tsilo – Old Cultivars	- Adam Tas, 1990	Yes to all
	- Adeste, 1965	
	- Baard 46, 1946	

Requested by	Accession requested	Supplied
	- Baard India, 1925	
	- Barta, 1970	
	- Basoeto, 1953	
	- Belinda, 1970	
	- Bella, 1967	
	- Benita, 1974	
	- Betana,1953	
	- Betana,1959	
	- Betmark, 1953	
	- Betta, 1970	
	- Betta-DN, 1994	
	- Bobriet, 1919	
	- Bobs, 1896, AUS	
	- Bokveld, 1938	
	- Bona,1964	
	- Bontaar, 1923	
	- Caledon Baard, 1888	
	- Chokka, 1994	
	- Daeraad, 1950	
	- Delta, 1953	
	- Dias, 1994	
	- Dipka, 1978	
	- Dromedaris, 1952	
	- Du Toits, 1876	
	- Duiker, 1949	
	- Ecksteen, 1888	
	- Eksteen, ?	
	- Elan, 1971	
	- Eleksie, 1959	
	- Elize, 1975	
	- Elrina, 1976	
	- Farrertrou, 1933	
	- Flameks, 1962	
	- Flamink, 1979	
	- Florence Aurore, 1920	
	- Gamka,1982	
	- Gamtoos, 1983	
	- Gewone Scheepers, 1925	
	- Goudveld, 1951	
	- Gouritz,1978	

Requested by	Accession requested	Supplied
	- Harts, 1994	
	- Heemraad,1965	
	- Helene,1975	
	- Hoopvol, 1948	
	- Hugenoot, 1994	
	- Impala, 1948	
	- Indies, ?	
	- Inia 66, 1986	
	- Janitor, 1967	
	- K20, 1967	
	- Kalyansona, 1972	
	- Karee, 1983	
	- Kasarwali, 1933	
	- Kasteel, 1967	
	- Kenia Governor, 1929	
	- Kenia Sokkies, 1950	
	- Kenya, ?	
	- Klein Wit, ?	
	- Kleintrou, 1916	
	- Knoppies Caledon, 1930	
	- Knoppies Duimpies, ?	
	- Knoppies, 1930	
	- Koalisie,1933	
	- Kruger, 1935	
	- Kwarta, 1965	
	- Lalkasarwali, 1929	
	- Lee Mida, 1965	
	- Letaba, 1994	
	- Liesbeeck, 1976	
	- Loerie, ?	
	- Magaliesberg, 1950	
	- Malgas, 1938	
	- Malson 1992	
	- Maluti, 1950	
	- Marico"S", ?	
	- Mentana,1920	
	- Molen, 1985	
	- Molopo, 1994	
	- Moni, ?	
	- Myburghs, 1878	

Requested by	Accession requested	Supplied
	- Nantes, 1994	
	- Niekerks, 1905	
	- Oom Charl, 1994	
	- Orania, 1988	
	- Oranja, 1994	
	- Ou Baard, 1924	
	- Palala, 1980	
	- Palmiet, 1983	
	- Pelgrim, 1933	
	- Penkop, 1951	
	- Poenskop, ?	
	- Primrose, 1914	
	- Punjab, 1940	
	- Quality,1923	
	- Queen Fan, 1924	
	- Raats, ?	
	- Rama, ?	
	- Red Egyptian, 1892	
	- Red Victory, 1948	
	- Regent, 1940	
	- Rheebok,1965	
	- Ritters, 1924	
	- Roodts, 1920	
	- Rooi Egipties, 1894	
	- Rooi Gysie, ?	
	- Rooi Indies,1940	
	- Rooi Kleinkoring, 1912	
	- Rooi Kleintrou, ?	
	- Rooi Llama, 1931	
	- Rooi Spitskop, 1951	
	- Rooi Stormberg, 1948	
	- Rooikleinkoring, 1912	
	- Scheepers 69, 1969	
	- Scheepers, 1925	
	- Scheepers, 1972	
	- Skemer,1965	
	- Sokkies, 1950	
	- Sonderend, 1974	
	- South African 43, ?	
	- Spilhaus, 1930	

Requested by	Accession requested	Supplied
	- Spoetnik, 1958	
	- SST 101, 1978	
	- SST 102, 1978	
	- SST 107, 1994	
	- SST 11, ?	
	- SST 121 ZA Pretorius	
	- SST 121, ?	
	- SST 124, 1994	
	- SST 136, ?	
	- SST 16, 1976	
	- SST 22, ?	
	- SST 23, 1981	
	- SST 25, 1994	
	- SST 3 (R), 1979	
	- SST 3,1973	
	- SST 33, 1979	
	- SST 333, 1994	
	- SST 38, 1973	
	- SST 4, ?	
	- SST 44, 1994	
	- SST 55, 1994	
	- SST 6, 1973	
	- SST 66, 1979	
	- SST 8, ?	
	- SST 822, 1993	
	- SST 825, 1994	
	- SST 86, 1994	
	- SST 102, 1978	
	- SST 2, 1976	
	- T4, 1965	
	- T-4, 1979	
	- T7, 1965	
	- T8, 1965	
	- Terblanche, 1938	
	- Tobari 66, 1969	
	- Tokwe, 1968	
	- Tolletjies, 1929	
	- Transvaal, 1905	
	- Tugela, 1985	
	- Tugela-DN (Dn1),1992	

Requested by	Accession requested	Supplied
	- Uitstaande Gluretty, 1920	
	- Unie 17, 1914	
	- Unie 23, 1914	
	- Unie 31, 1914	
	- Unie 52 STB, 1914	
	- Unie 52, 1914	
	- Unie 54, 1980	
	- Unie 57, 1931	
	- Unie 7, 1914	
	- USGEN 14 C.P.P., 1989	
	- USGEN 18, 1993	
	- USGEN 19 ?	
	- Van Dyk, 1924	
	- Verbeterde Kenia, 1940	
	- Vondeling, 1931	
	- Vorentoe, 1945	
	- Wesselsbron, ?	
	- Wilge, 1980	
	- Wit Wol, 1905	
	- Wit Wolkoring,1905	
	- Witspitskop, 1951	
	- Witwol ex Leliefontein, ?	
	- Wolkoring, 1917	
	- Zambezi,1966	
	- Zaragoza 75, 1978	
Mr Barend Wentzel	- Chinese Spring	
	- Opata 85	
	- Gabo	
	- Glenlea	
	- Pavon 76	
	- Halberd	
	- Renan	
	- Seri 82	
	- Marquis	
Mrs Cathy de Villiers	- Shu Chou Wheat no 3	

National germplasm requests

Germplasm requested for research purposes at National level from the ARC-SGI Genebankwere 193 accessions (Table 5).

Table 5. National germplasm request from ARC-SGI

Requested by	Accession	Supplied
Dr Francois Koekemoer, Sensako (Pty) Ltd, Bethlehem	- The same list as requested by Dr T Tsilo	Yes
Dr Eduard Venter, Senior Lecturer: Plant Biotechnology, Department of Pant and Biotechnology, University of Johannesburg	- Tugela - Tugela DN - Gamtoos - Gamtoos DN	Yes to all
Mr Francois Burger	- Tugela	Yes

Germplasm requested at national level for research purposes from PANNAR (29 accessions) and SENSAKO (63 accessions). Requests are still pending, only 5 accessions from PANNAR were received (Table 6).

Table 6. Germplasm requests from PANNAR and SENSAKO

Requested from	Accession	Supplied
Dr Willem Boshof, PANNAR Seed (Pty) Ltd, Bainsvlei, to identify possible rust resistance genes contained in these cultivars, by ARC-SGI (Krishna Naicker, Scott Sydenham, Drs Tarekegn Terefe and Toi Tsilo).	- PAN 3118 - PAN 3120 - PAN 3122 - PAN 3144 - PAN 3161 - PAN 3172 - PAN 3179 - PAN 3191 - PAN 3195 - PAN 3198 - PAN 3211 - PAN 3232 - PAN 3235 - PAN 3349 - PAN 3355 - PAN 3364 - PAN 3368 - PAN 3377 - PAN 3379 - PAN 3404 - PAN 3408 - PAN 3434 - PAN 3471 - PAN 3478 - PAN 3489 - PAN 3490 - PAN 3492 - PAN 3497	Yes Yes Yes Yes Yes
Dr Francois Koekemoer, Sensako (Pty) Ltd, Bethlehem, to identify possible rust resistance genes contained in these cultivars by ARC-SGI (Krishna Naicker, Scott Sydenham, Drs Tarekegn Terefe and Toi Tsilo).	- SST 096 - SST 107 - SST 124 - SST 15 - SST 26 - SST 27 - SST 308 - SST 319	Not received yet.

Requested from	Accession	Supplied
	- SST 322 - SST 333 - SST 334 - SST 347 - SST 35 - SST 356 - SST 363 - SST 366 - SST 367 - SST 374 - SST 38 - SST 387 - SST 398 - SST 399 - SST 44 - SST 47 - SST 56 - SST 57 - SST 64 - SST 65 - SST 66 - SST 67 - SST 75 - SST 802 - SST 805 - SST 806 - SST 822 - SST 825 - SST 835 - SST 843 - SST 866 - SST 867 - SST 87 - SST 874 - SST 875 - SST 876 - SST 877 - SST 878 - SST 88 - SST 884 - SST 885 - SST 886 - SST 895 - SST 896 - SST 935 - SST 936 - SST 94 - SST 946 - SST 954 - SST 963 - SST 964 - SST 966 - SST 972	

Requested from	Accession	Supplied
	- SST 983	
	- Afgri 75-3	

Germplasm exported

A total of 269 germplasm accessions were supplied from ARC-SGI Genebank to foreign collaborators (Table 7). The seeds were subjected to phytosanitary tests and treatments (before shipment) as specified in the import permit of the requesting organization. All the material was covered by signed Standard Material Transfer Agreements.

Table 7. Germplasm requests from ARC-SGI Genebank by foreign collaborators

Request by:	Germplasm:	Comment:
Dr Ruth Wanyera, KARI, Njoro, Kenya	- 265 ARC-SGI breeding lines	On behalf of Prof Klaus Pakendorf, Dr Eben von Well, Robbie Lindeque and Cathy de Villiers for Ug99 resistance screening in Kenya.
Prof Miriam Kinyua, Chepkoilel University College, Eldoret, Kenya	- 3 radiated materials - 1 control	On behalf of Dr Eben von Well for Ug99 screening of the Mutation Breeding Project.

Germplasm imported

Germplasm accessions were imported from international research centres to enhance the genetic base of ARC-SGI's Genebank. Eleven nurseries along with 174 germplasm accessions were imported to meet the needs of scientists (Table 8). The nurseries will be planted in June-July 2013. One nursery (Quaker 2013) is still pending.

Table 8. Germplasm requested from abroad

Imported from:	Germplasm requested:	Received	Correspondences:
Dr Harold Bockelman, USDA-ARS, National Small Grains Collection, Aberdeen, Idaho 83210, USA	- PI 660065 - PI 660066 - PI 660067 - PI 660068 - PI 660069 - PI 660070 - PI 660071 - PI 660072 - PI 660073 - PI 660074 - PI 660075 - PI 660076 - PI 660077 - PI 660078 - PI 660079 - PI 660080 - PI 660081 - PI 660082 - PI 660083 - PI 660084 - PI 660085	Yes	On behalf of Dr Toi Tsilo

Imported from:	Germplasm requested:	Received	Correspondences:
	- PI 660086 - PI 660087 - PI 660088 - PI 660089 - PI 660090 - PI 660091 - PI 660092 - PI 660093 - PI 660094 - PI 660095 - PI 660096 - PI 660097 - PI 660098 - PI 660099 - PI 660100 - PI 660101 - PI 660102 - PI 660103 - PI 660104 - PI 660105 - PI 660106 - PI 660107 - PI 660108 - PI 660109 - PI 660110 - PI 660111 - PI 660112 - PI 660113 - PI 660114 - PI 660115 - PI 660116 - PI 660117 - PI 660118 - PI 660119 - PI 660120 - PI 660121 - PI 660122 - PI 660123 - PI 660124 - PI 660125 - PI 660126 - PI 660127 - PI 660128 - PI 660129 - PI 660130 - PI 660131 - PI 660132 - PI 660133 - PI 660134 - PI 183527		
Dr Harold Bockelman, USDA-ARS, National Small Grains Collection, Aberdeen, Idaho 83210, USA	- Outlook	Yes	On behalf of Dr Vicki Tolmay and Mr Scott Sydenham

Imported from:	Germplasm requested:	Received	Correspondences:
National Wheat Improvement Program of Turkey (CIMMYT, ICARDA	- 12 TY-SA 8004 - 12 TY-SA 8007 - 12 TY-SA 8009 - 12 TY-SA 8011 - 12 TY-SA 8013 - 12 TY-SA 8021 - 12 TY-SA 8022 - 12 TY-SA 8027 - 12 TY-SA 8035 - 12 TY-SA 8071 - 12 TY-SA 8078 - 12 TY-SA 8096 - 12 TY-SA 8101 - 12 TY-SA 8103 - 12 TY-SA 8112 - 12 TY-SA 8113 - 12 TY-SA 8131 - 12 TY-SA 8135 - 12 TY-SA 8143 - 12 TY-SA 8145 - 12 TY-SA 8149 - 12 TY-SA 8160 - 12 TY-SA 8168 - 12 TY-SA 8178 - 12 TY-SA 8179 - 12 TY-SA 8221 - 14th IWWYT-SA 9902 - 14th IWWYT-SA 9908 - 14th IWWYT-SA 9917	Yes	On behalf of Mr Robbie Lindeque
CIMMYT International Wheat Nurseries, Mexico	- ACA 303 - Amadina CRG682 - Bluesky-Ocan - Darius - Fengmal - Insignia - Norin 61, PI235236	Yes	On behalf of Mr Barend Wentzel
Dr Harold Bockelman, USDA-ARS, National Small Grains Collection, Aberdeen, Idaho 83210, USA	- PI TR 12ID SD - ND 2710	Yes	On behalf of Mrs Cathy de Villiers
CIMMYT International Wheat Nurseries, Mexico	- TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile - TID: 925312 L.coleoptile	Yes	On behalf of Dr JAN Asiwe

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CIMMYT International Wheat Nurseries, Mexico	Various CIMMYT Nurseries for 2013 season at ARC-SGI: - 46 IBWSN - 24 HRWSN - 31 SAWSN - 7 STEMRRSN - 8 HTWSN - 15 FHBSN - 23 ISEPTON	Yes	On behalf of the ARC-SGI International Nurseries, Prof Sakkie Pretorius, University of Free State and Willem Botes, University of Stellenbosch
National Wheat Improvement Program of Turkey (CIMMYT, ICARDA & Oregon State University)	Various International Nurseries- Winter/Facultative Bread Wheat for 2013 season at ARC-SGI: - 20 FAWWON-IR - 20 FAWWON-SA - 4 WWSRRN	Yes	On behalf of the ARC-SGI International Nurseries
Dr Stephen Harrison, Wheat and Oat Breeding, Louisiana State University, Baton Rouge, LA 70803-2110, USA	- 2013 QION (Quaker International Oat Nursery) for 2012	Pending	On behalf of the ARC-SGI International

Imported from:	Germplasm requested:	Received	Correspondences:
	season.		Nurseries

3.3 Report on the objective 2.2.4

Total accessions of the Germplasm Collection

The total number of germplasm accessions (17 221) in ARC-SGI Genebank is indicated in Figure 1 below. Wheat germplasm consists of (15 045) 87.4% of the Genebank collections, while the remaining 12.6% consist of barley (1 006), oats (676), rye (57) and triticale (437) (Figure 1).

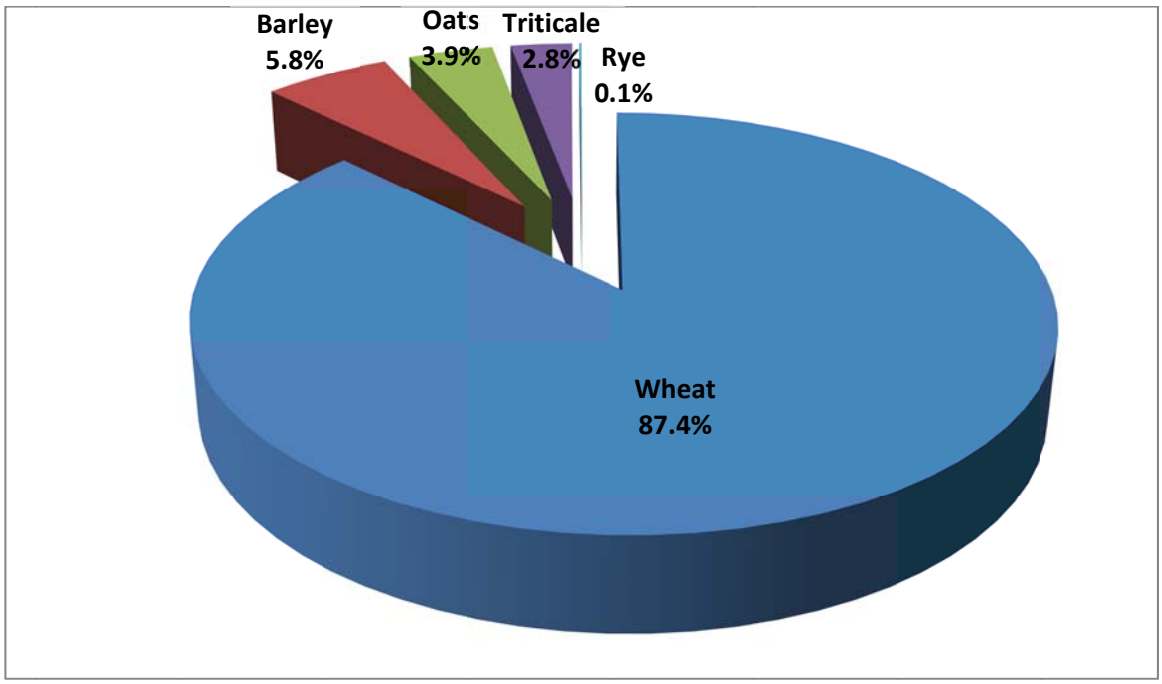


Figure 1. Germplasm collections on crop basis in ARC-SGI Genebank

3.4 Report on the objective 2.2.5

Germplasm Development facilities

Two hundred and twenty accessions that were planted in the 2011 rejuvenation trial were dried to a moisture content of 3-6% and placed in the freezers.

4. Future of the project

This is an ongoing long-term project that is of high interest and value to national and international agricultural organizations. The ideal is to continue the maintenance and collection of germplasm, making it possible to develop new breeds of small grain cultivars.

5. Objectives April 2013 – March 2014

During the 2013/2014 season, the Germplasm Collection will continue with the following:

- Regeneration of the germplasm collection will take place this season. Approximately 1 500 entries of the germplasm collection will be planted this year.
- To supply and request germplasm for SGI researchers;
- To co-ordinate the import and export of seed samples with specific characteristics and comply with the protocol of the Directorate: Agricultural Product Inspection Services;
- To continue with the characterisation and update of the database of the germplasm collection;
- To maintain the Germplasm Collection in a viable condition.

GK 09/07 THE COLLECTION AND MAINTENANCE OF UNIQUE SMALL GRAIN GERMPLASM PROGRESS REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 09/07
Title: The collection and maintenance of unique small grain germplasm
Duration: Ongoing
Status: Continuation of existing project
Project leader: Dr Eben von Well and Mr Moses P Ncala

The purpose of the new Germplasm Collection unit is to maintain high-viability accessions for long period storage, as well as to introduce new germplasm with good pest and agronomic traits. All the targets indicated for 2012/2013 were accomplished under the prevailing environmental conditions and budgetary constraints.

Two nurseries, the first consisting of 1 710 accessions in total were planted for data collection and 1 638 accessions were successfully harvested. The second nursery consisting of 157 accessions from Germplasm Introduction Nursery from abroad was planted for seed generation. Six RAS nurseries (Rust, Aphids and Scab) consisting of 1 286 entries were planted for characterisation and data collection on pest (Russian wheat aphid), different rusts (Yr, Sr and Lr), Septoria and Scab reactions at different localities.

One hundred and ninety eight accessions were internally supplied by ARC-SGI Genebank to its researchers, while 193 accessions were nationally requested from ARC-SGI Genebank. Seeds requested from PANNAR (29) and SENSAKO (63) were not supplied. A total of 11 nurseries along with 174 germplasm accessions were imported for next season's planting in June-July 2013. The Genebank at Small Grain Institute has a total collection of 17 221, comprising of wheat (15 045 accessions), oats (676), barley (1 006), rye (57) and triticale (437).

GK 09/08: THE INTRODUCTION AND EVALUATION OF NEW INTERNATIONAL GERMPLASM PROGRESS REPORT APRIL 2012 – MARCH 2013

1. Project details

Number: GK 09/08
Title: The introduction and evaluation of new international germplasm
Duration: Continuous
Status: Continuation of existing project
Project leaders: Dr Eben von Well and Mr Moses P Ncala

2. Objectives

2.1 Long-term objectives

To enhance the genetic diversity of a variety of winter and spring wheat and oats through the introduction of exotic germplasm accessions with desirable traits.

2.2. Short term objectives: April 2012 - March 2013

- Evaluate introduced international germplasm for agronomic and disease resistance characteristics.
- Close collaboration with APIS (Agricultural Product Inspections Services) to ensure no foreign disease enters South Africa.
- Make all entries available to researchers for selection.
- Retain all selected lines as new accessions to the Germplasm Bank.

3. Report on the objectives: April 2012 - March 2013

3.1 Report on objective 2.2.1

To enhance genetic variation of the small grain collection in ARC's Genebank for the fast tracking of the development of high yielding and pest resistant cultivars, germplasm nurseries were introduced from three international research centres (Table 1).

Table 1. Wheat nurseries introduced from International Research Centres during 2012-2013

Source	Number of nurseries
CIMMYT – Mexico	8
CIMMYT/ICARDA – Turkey	4
USA	1
Total	**13**

The 13 nurseries were planted at ARC-Small Grain Institute (ARC-SGI) Bethlehem. These consisted of 1 677 entries arising from spring wheat (8), winter wheat (4), oats (1), as indicated in Table 2. The nurseries were planted in July 2012 under irrigation in Bethlehem. The trials were planted in 4 m rows with 45 cm between rows. The nurseries were subjected to the strict quarantine regulations recommended by the Department of Agriculture, Forestry and Fisheries (DAFF), Pretoria. Pest infestation was natural and disease ratings, as well as agronomic evaluations were conducted. All disease and agronomic traits such as yellow rust and powdery mildew, plant height, flowering date and yield were collected from emergence until

maturity. Near maturity, breeders, pathologist and agronomists made selections before the harvesting of nurseries commenced. Harvesting was executed from December 2012 to early January, 2013.

Table 2. Composition of the nurseries

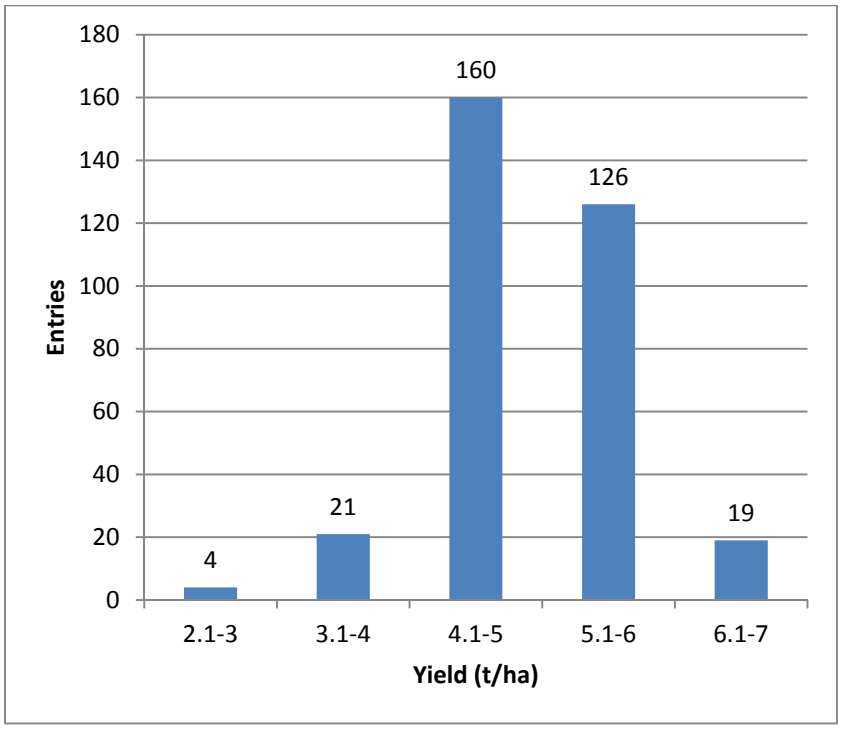
Nursery Type	Number of nurseries	Entries
Spring wheat	8	987
Winter wheat	4	471
Oats	1	219
Total	**13**	**1 677**

Parts of the agronomic data collected from the evaluation of the 13 nurseries are presented in Figures 1-5. In general, there were remarkable differences among the nurseries in their agronomic performances, thus indicating high genetic variability within and among the various nurseries evaluated. The mean grain yield (ton/ha) of most of the nurseries varied between 0.5 - 6 ton/ha with some high performing accessions giving up to 6 ton/ha. The data also indicate that plant height showed differences (80-120cm) among the nurseries (Figures 1-13), thus indicating that there is genetic variation available for improvement of the trait within and among various nurseries.

45th International Bread Wheat Screening Nursery 2012 (45th IBWSN 2012)

This spring wheat screening nursery consisted of 350 accessions.

A



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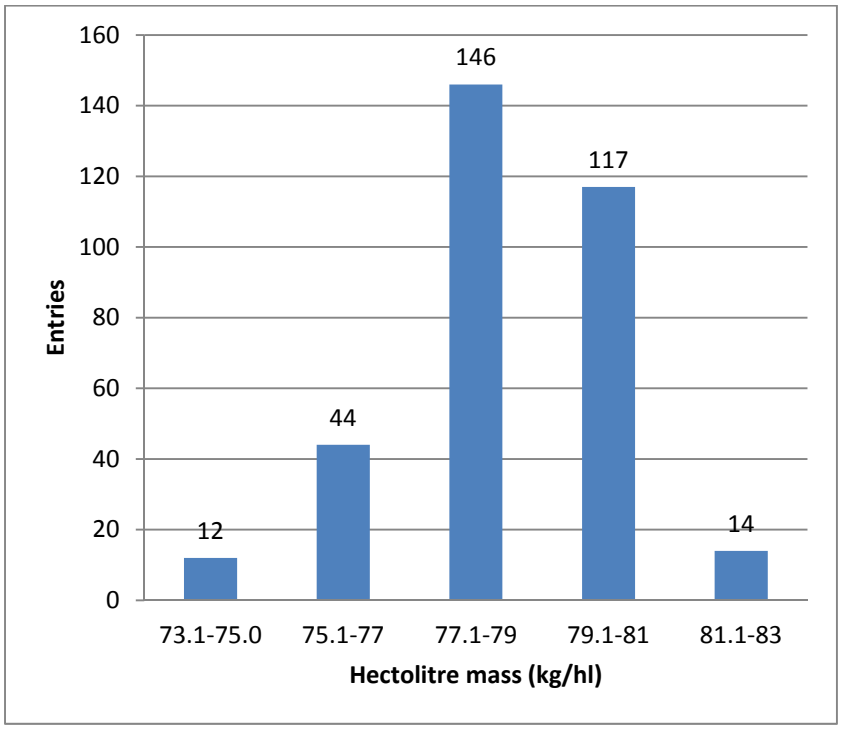

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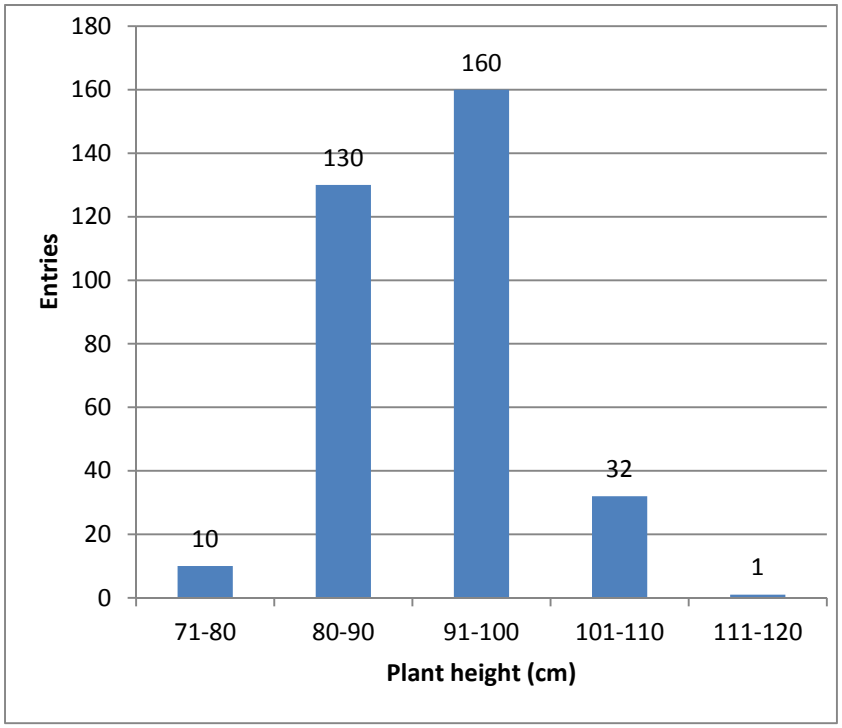
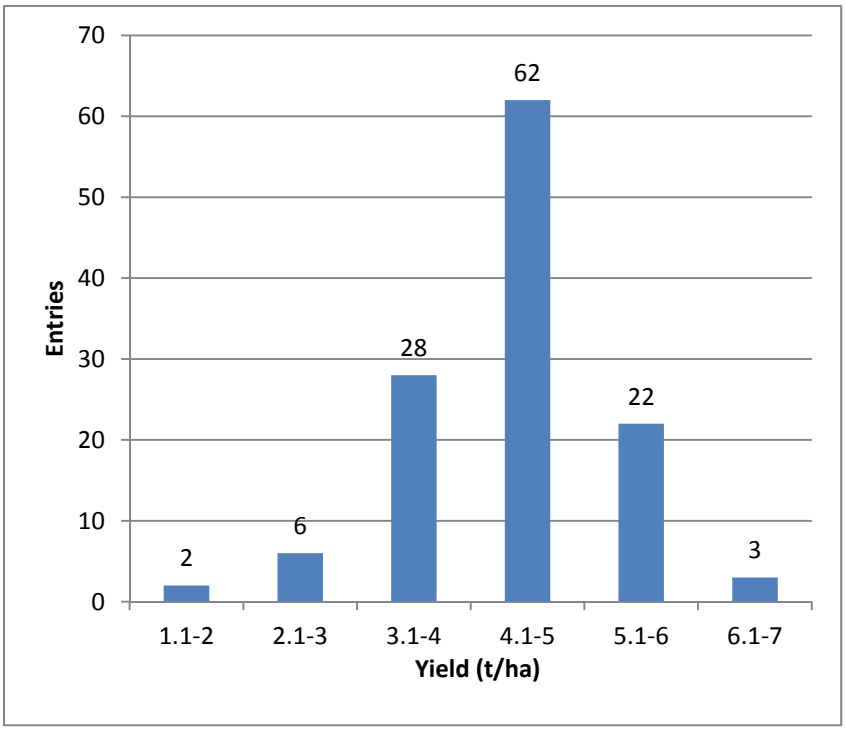


Figure 1. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in the 45th IBWSN 2012 at Bethlehem

6th Stem Rust Resistance Screening Nursery 2012 (6th STEMRRSN 2012)

This spring wheat screening nursery consisted of 129 accessions.

A



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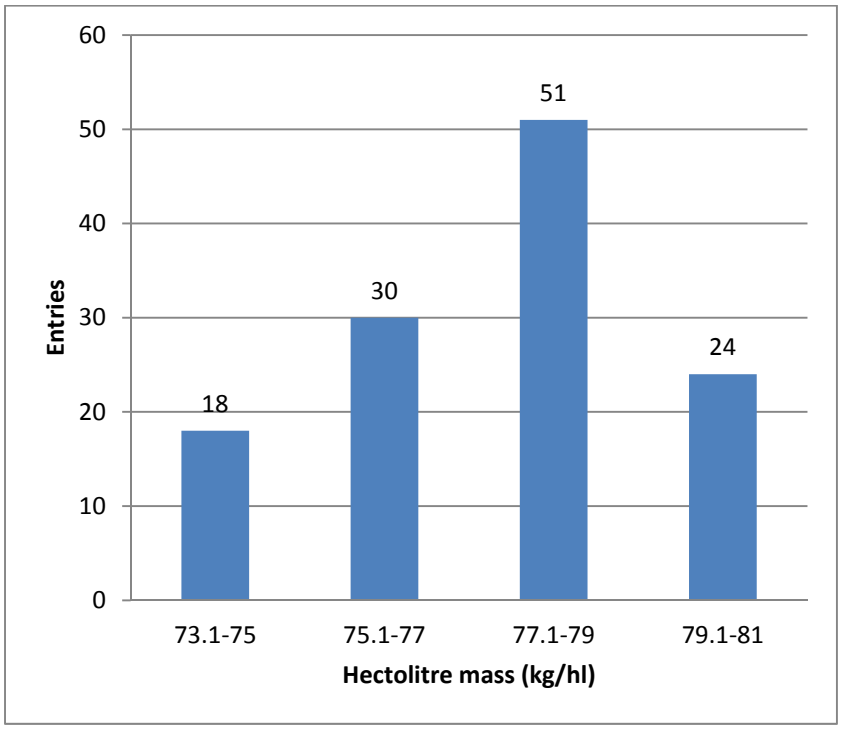

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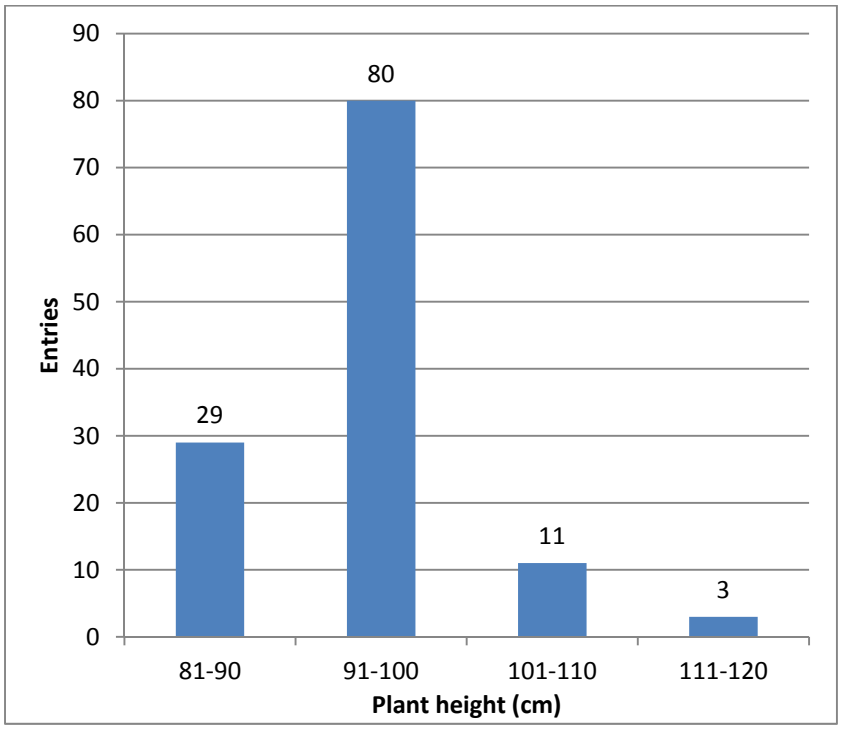
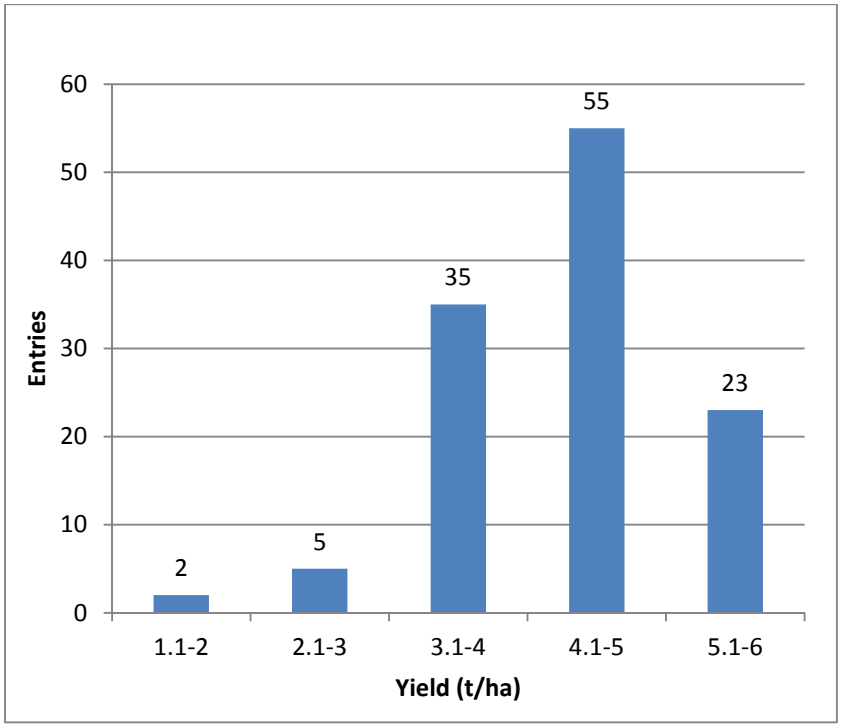


Figure 2. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in the 6th STEMRRSN 2012 at Bethlehem

7th Heat Tolerant Wheat Screening Nursery 2012 (7th HTWSN 2012)

This spring wheat screening nursery consisted of 126 accessions.

A



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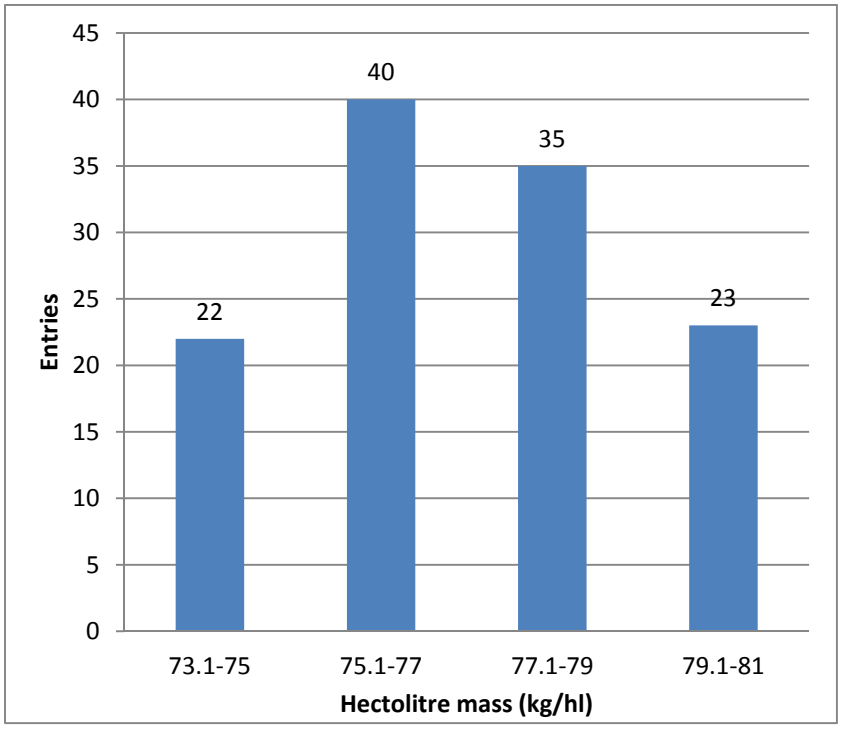

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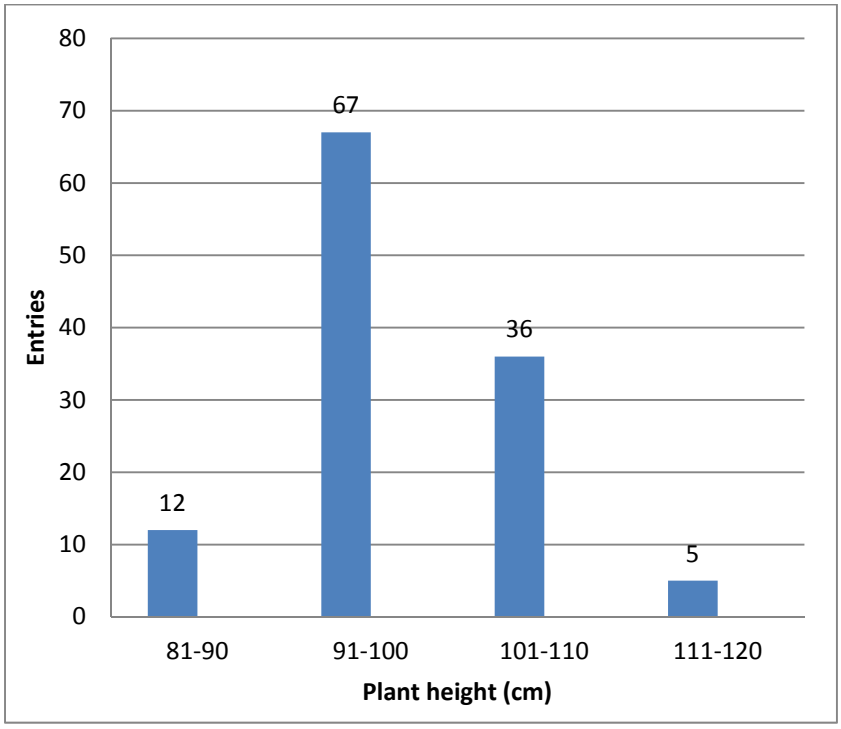
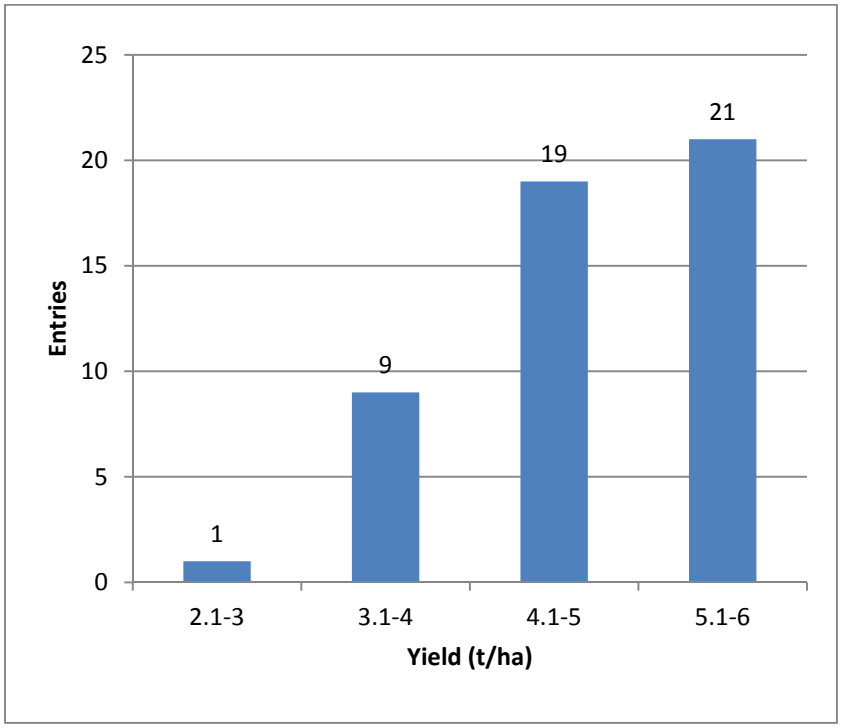


Figure 3. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in the 7th HTWSN 2012 at Bethlehem

22ed International Septoria Observation Nursery 2012 (22ed ISEPTON 2012)

This spring wheat observation nursery consisted of 52 accessions.

A



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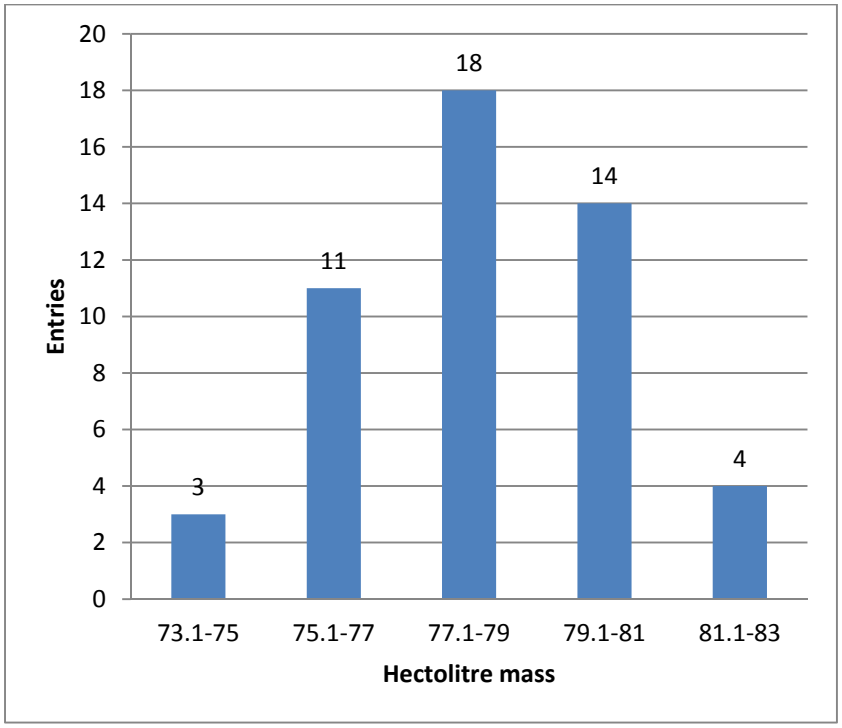

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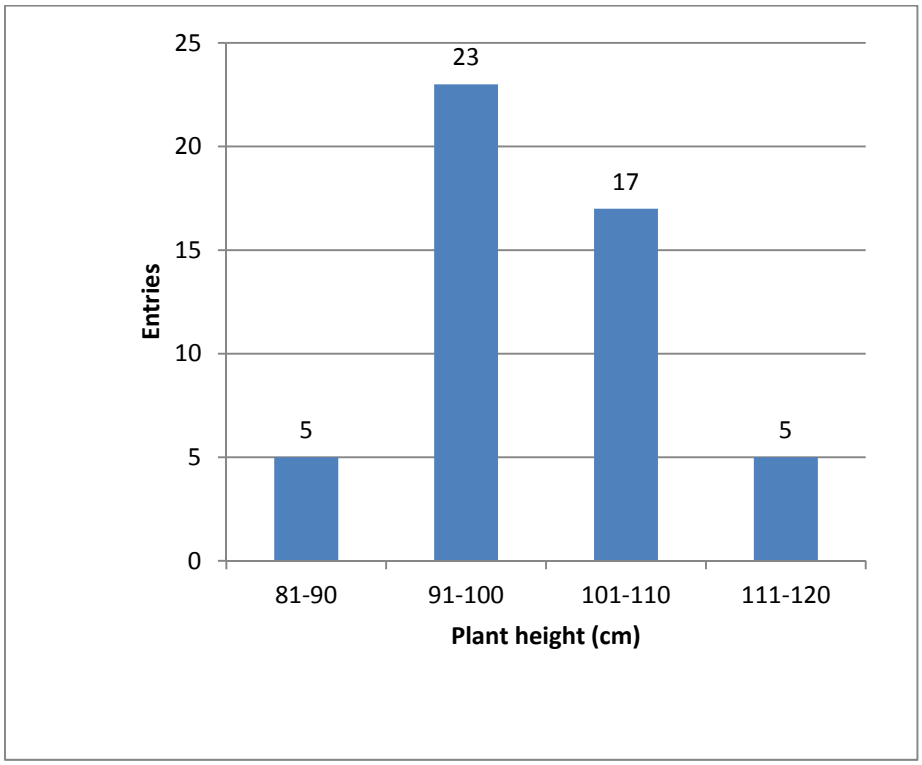
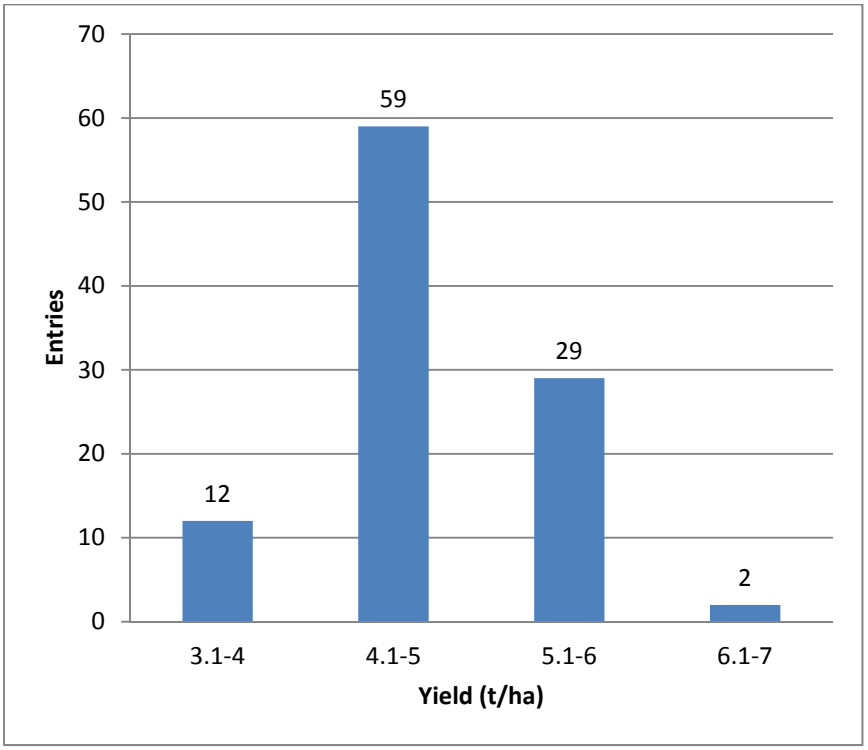


Figure 4. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 22^{ed} ISEPTON 2012 at Bethlehem

14th Fusarium Head Blight Screening Nursery 2012 (14th FHBSN 2012)

This spring wheat screening nursery consisted of 24 accessions.

A



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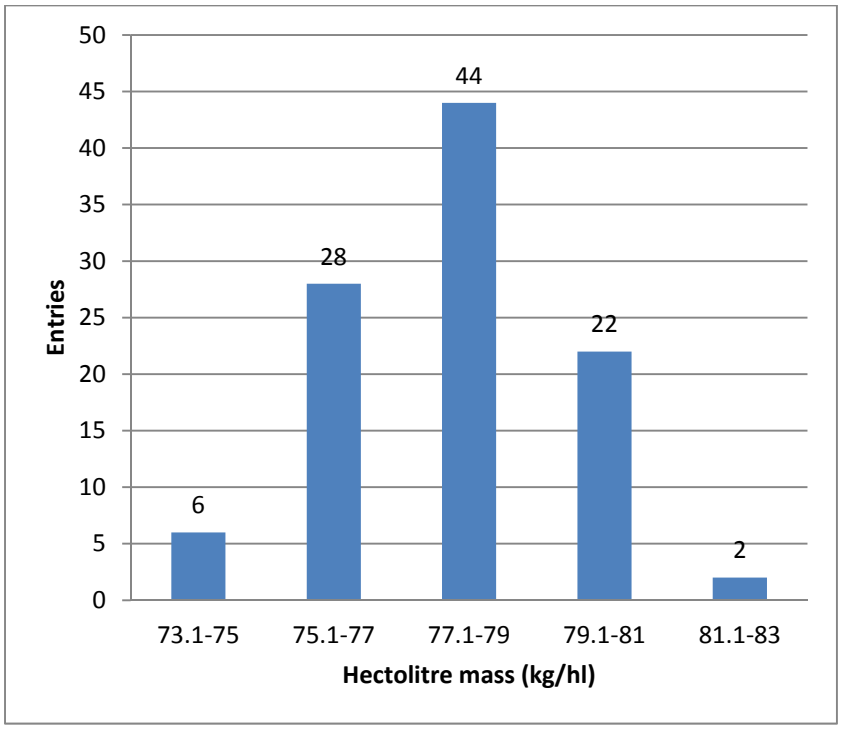

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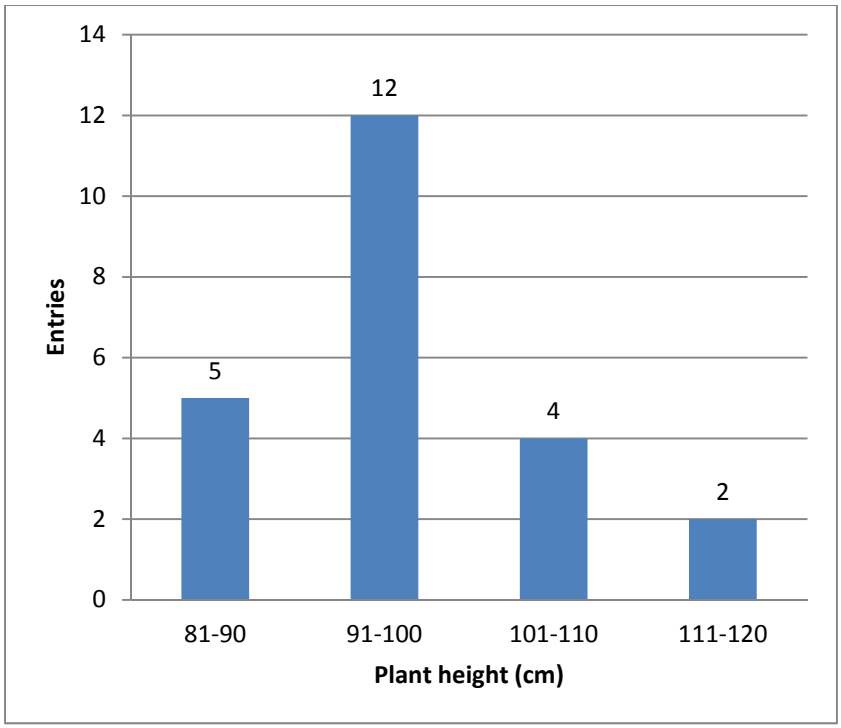
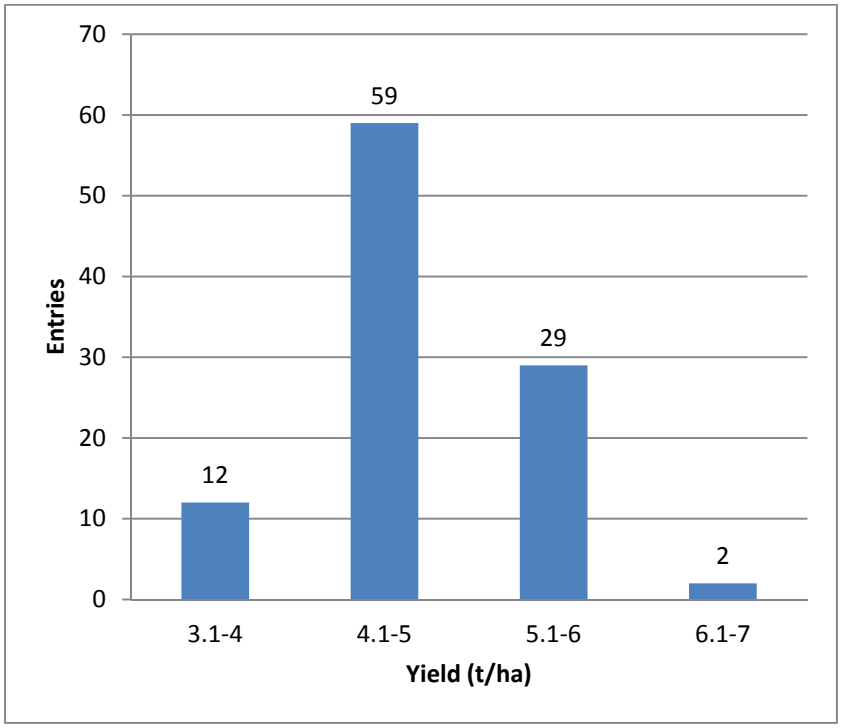


Figure 5. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 14th FHBSN 2012 at Bethlehem

30th Semi-Arid Wheat Screening Nursery 2012 (30th SAWSN 2012)

This spring wheat screening nursery consisted of 108 accessions.

A



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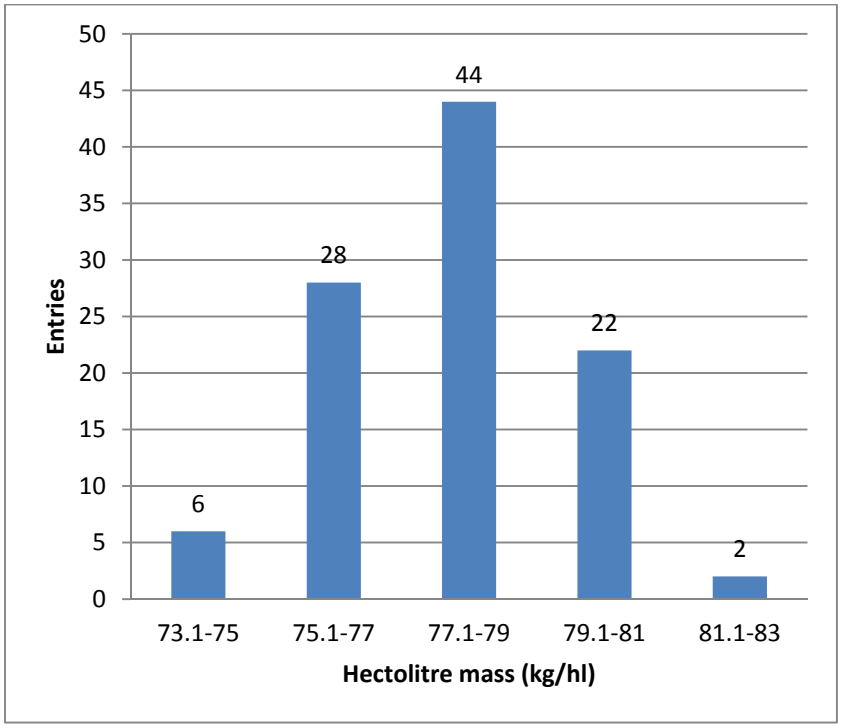

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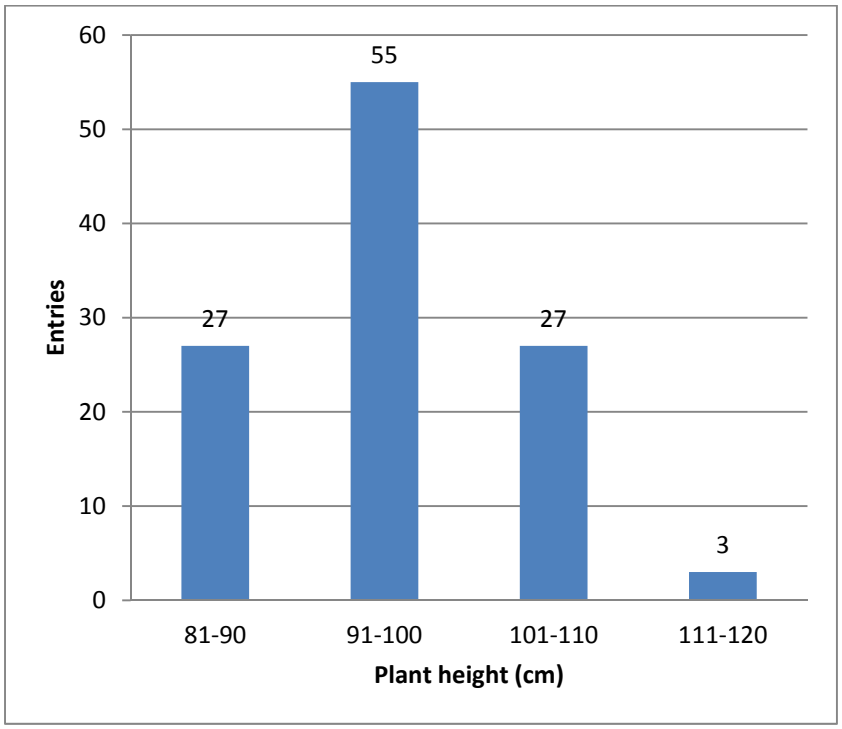
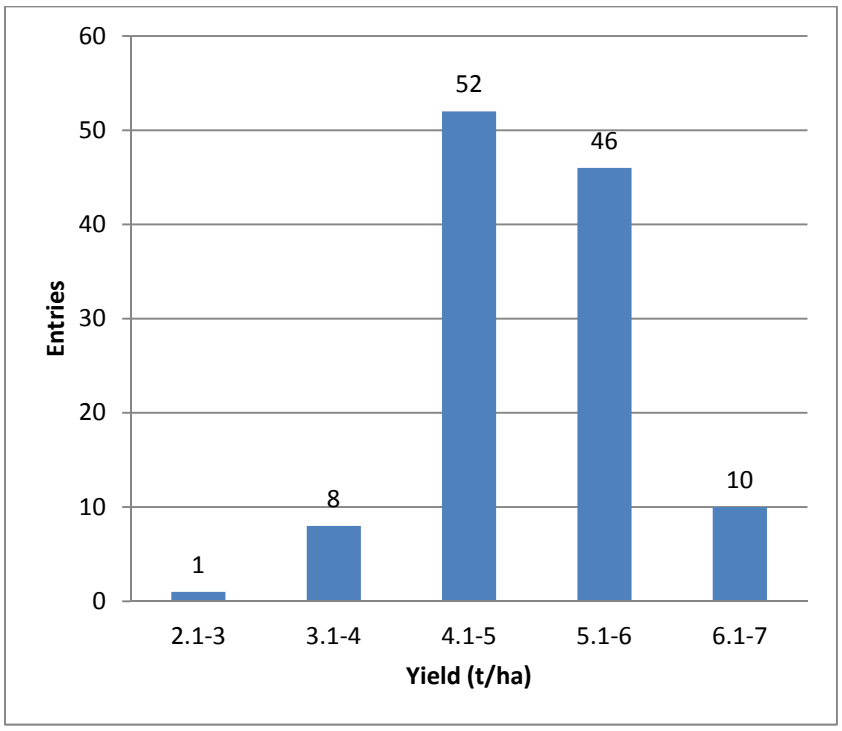


Figure 6. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 30th SAWSN 2012 at Bethlehem

CIMMYT Mexico Core Germplasm (CIMCOG)

This spring wheat nursery consisted of 120 accessions.

A



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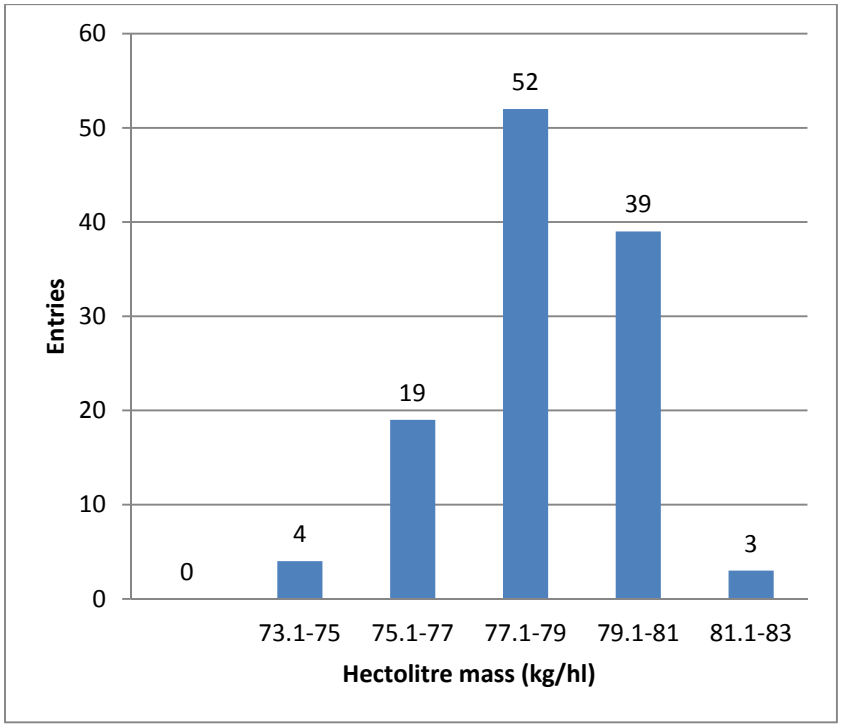

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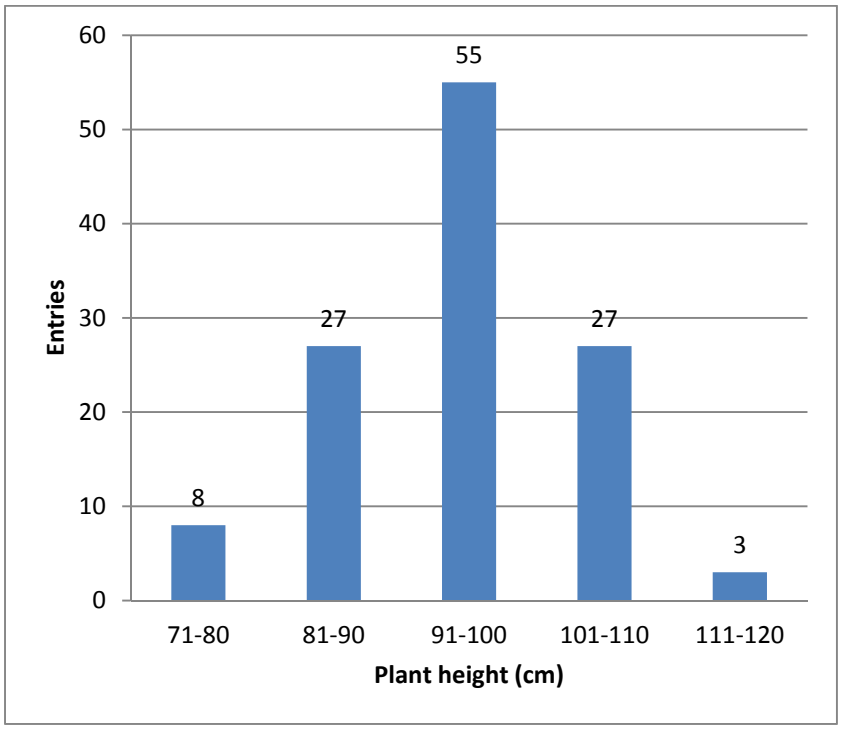
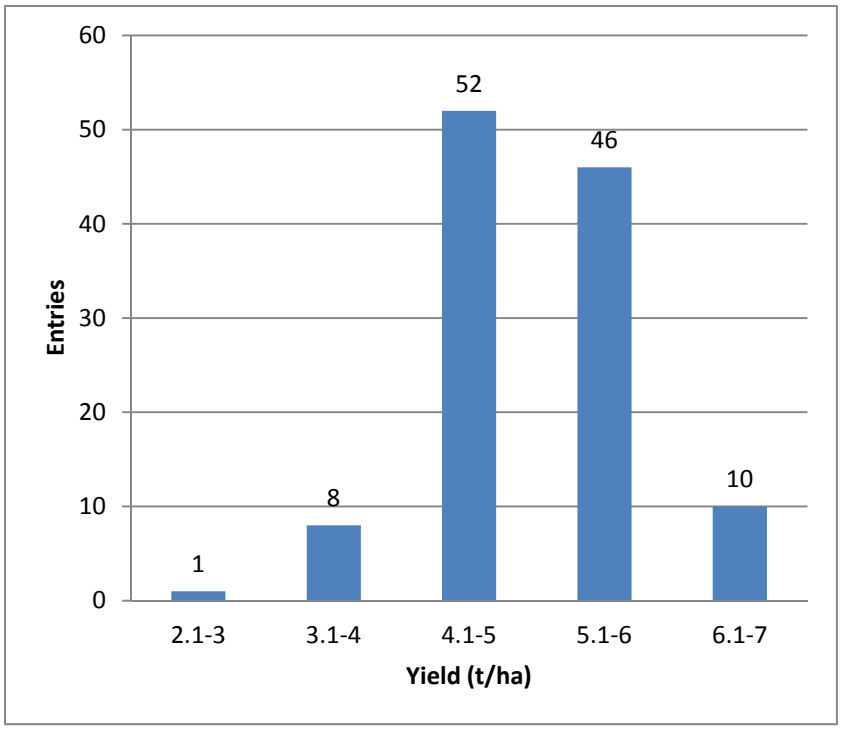


Figure 7. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in CIMCOG at Bethlehem

23rd High Rainfall Wheat Screening Nursery 2012 (23RD HRWSN 2012)

This spring wheat screening nursery consisted of 123 accessions.

A



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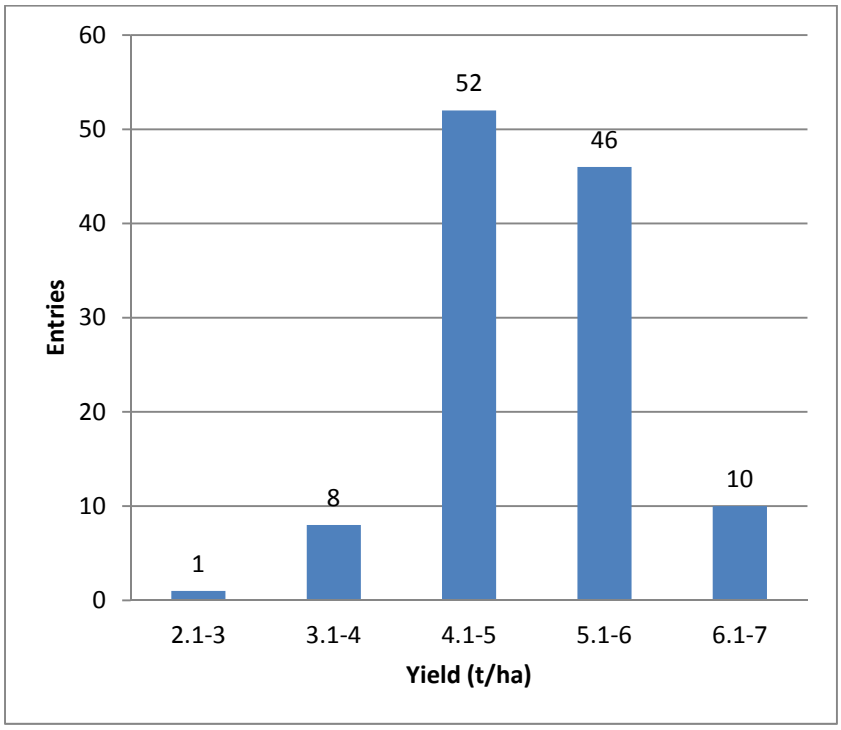

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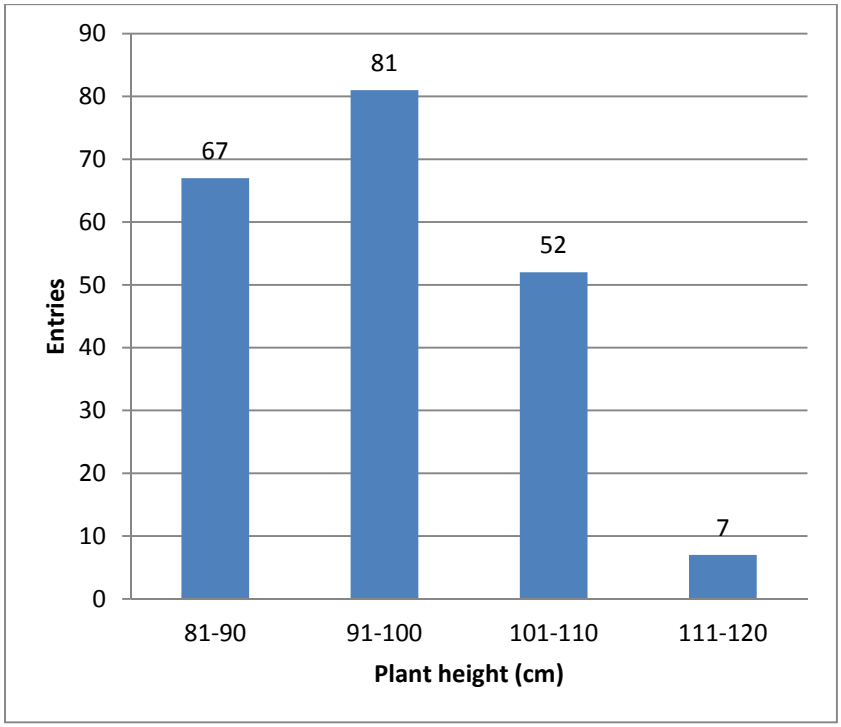
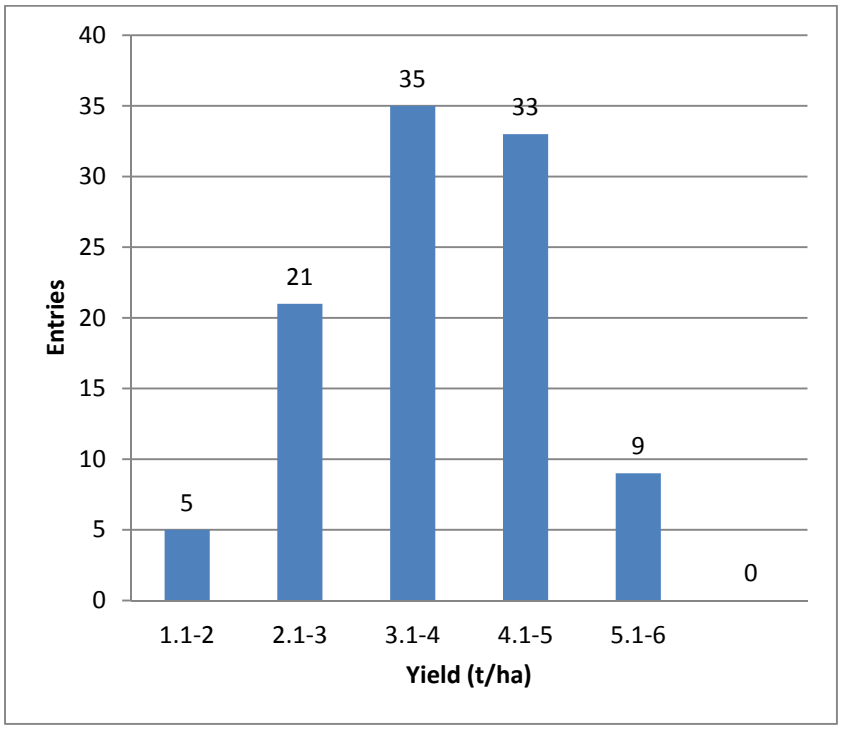


Figure 8. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 23RD HRWSN 2012 at Bethlehem

19th Facultative and Winter Wheat Observation Nursery-Irrigated 2012 (19TH FAWWON-IR 2012)

This facultative and winter wheat observation nursery consisted of 234 accessions.

A



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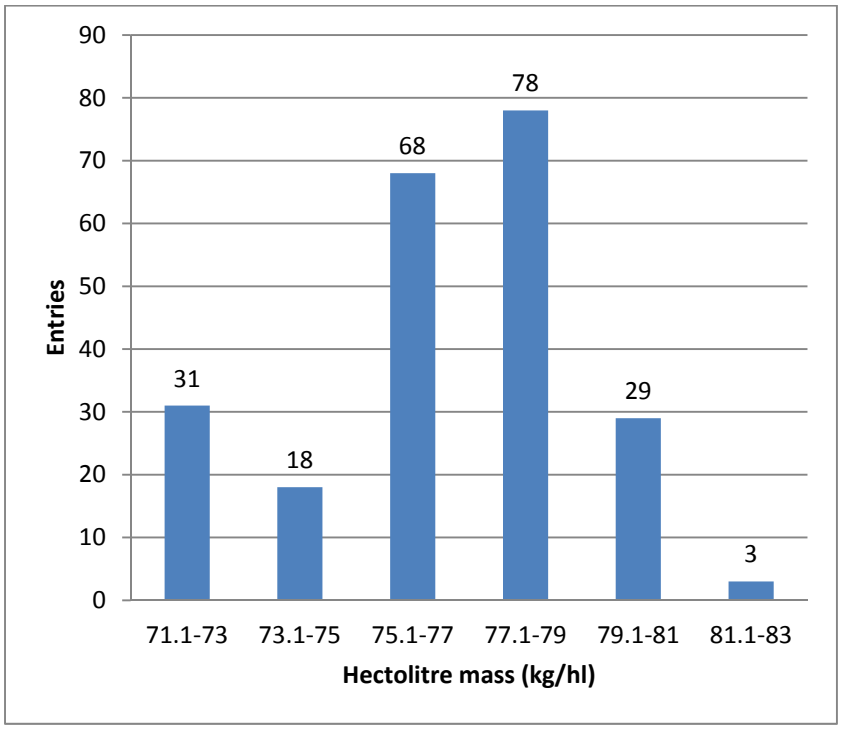

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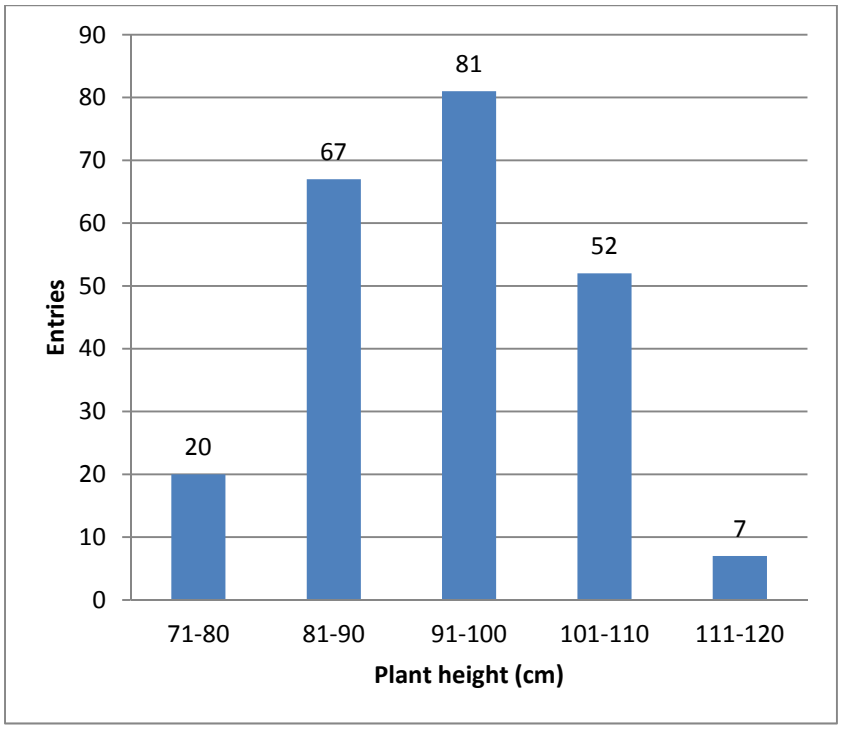
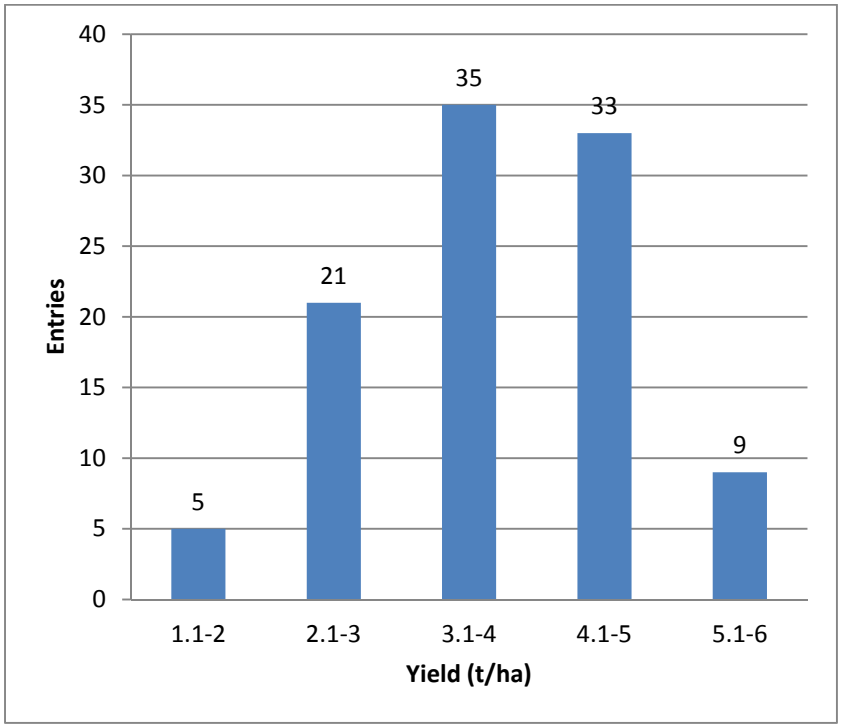


Figure 9. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 19th FAWWON-IR 2012 at Bethlehem

19th Facultative and Winter Wheat Observation Nursery-Semi Arid 2012 (19TH FAWWON-SA 2012)

This facultative and winter wheat observation nursery consisted of 108 accessions.

A



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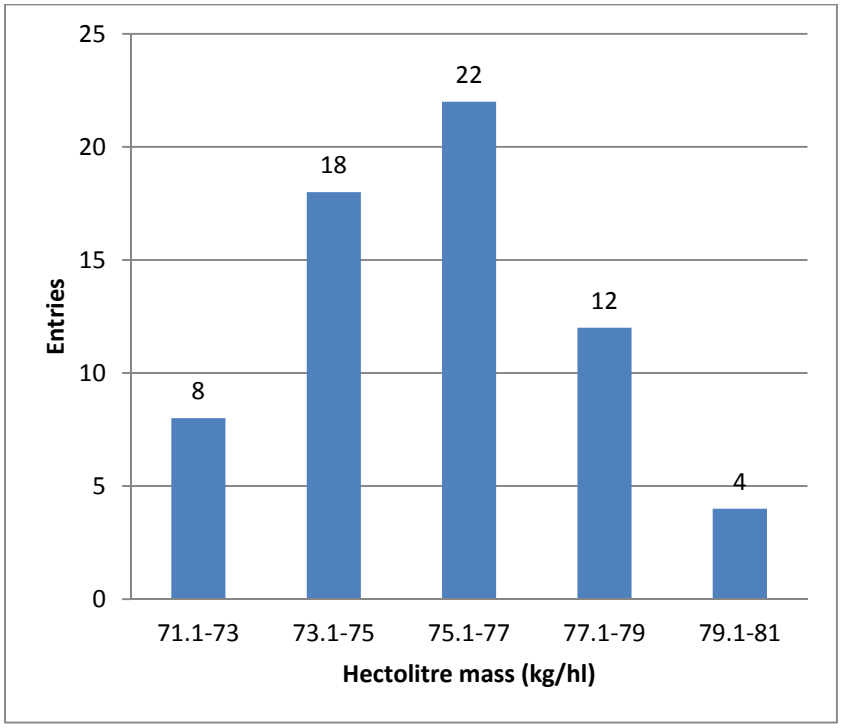

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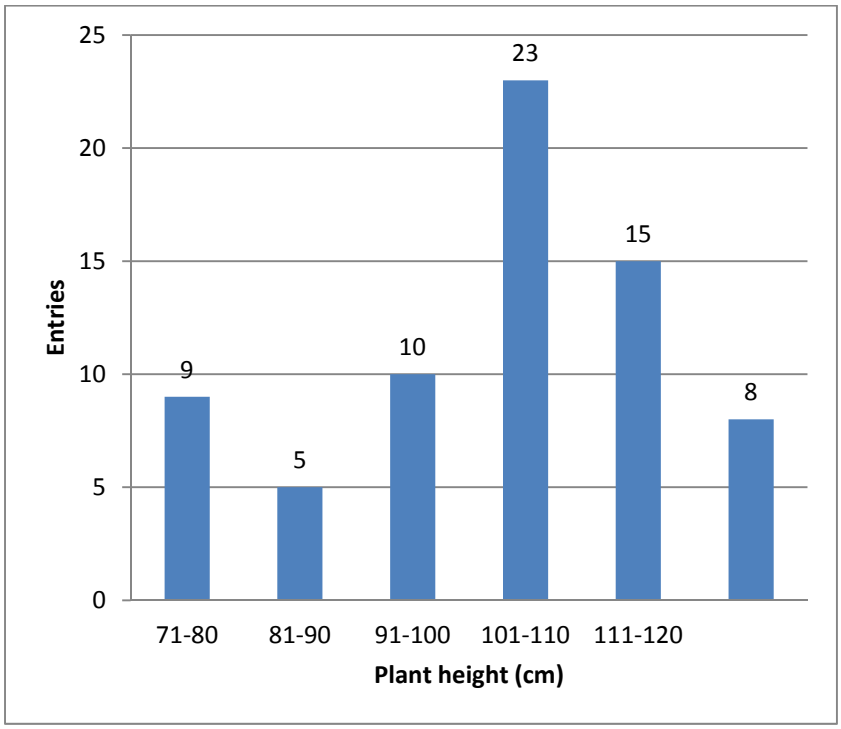
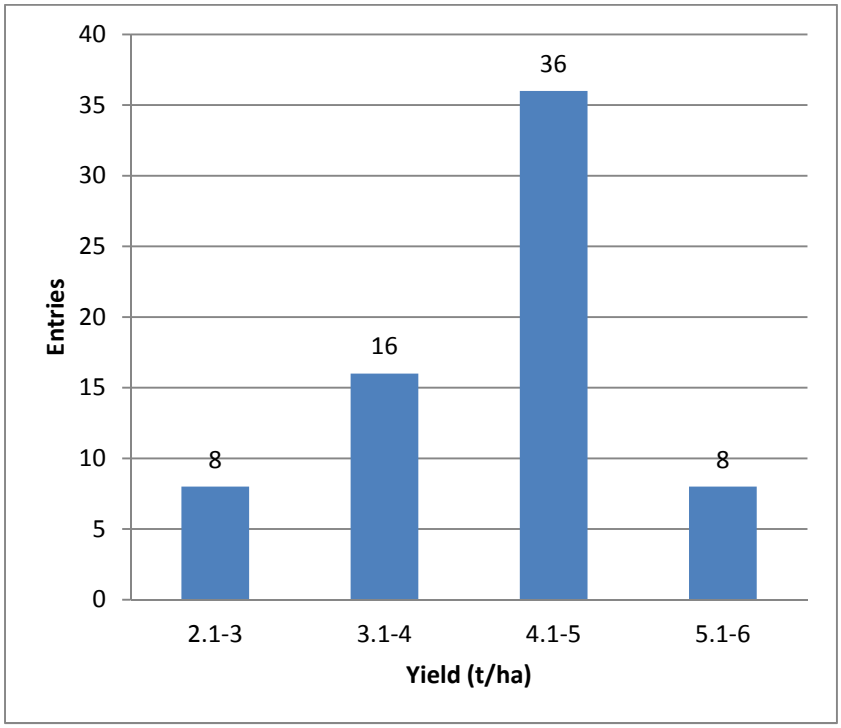


Figure 10. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 19th FAWWON-SA 2012 at Bethlehem

3rd Winter Wheat Stem Rust Resistance Nursery 2012 (3RD WWSRRN 2012)

This winter wheat nursery consisted of 75 accessions.

A



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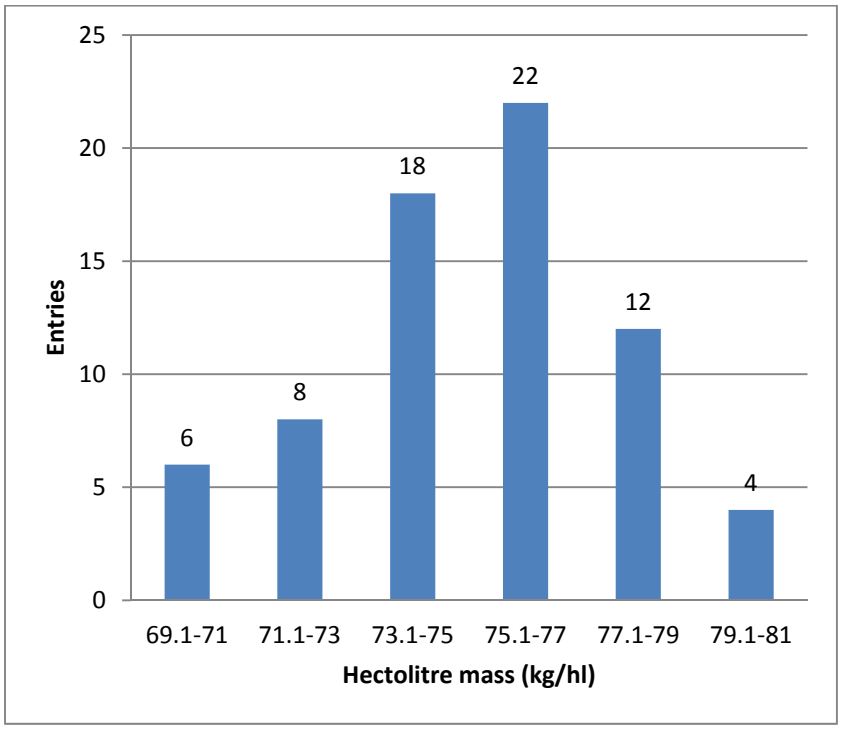

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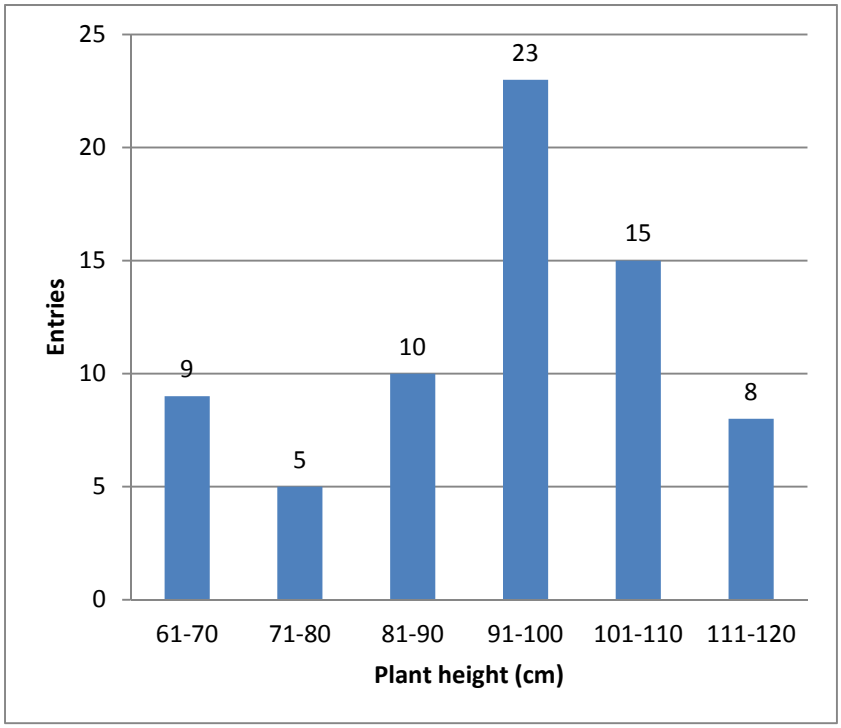
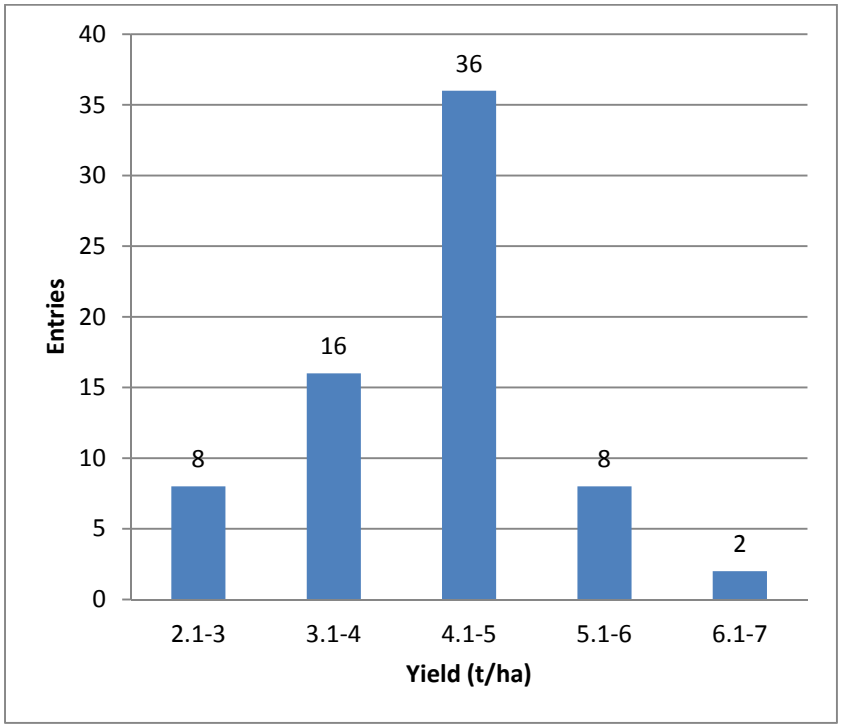


Figure 11. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 3rd WWSRRN 2012 at Bethlehem

1st Winter Wheat Yellow Rust Resistance Nursery 2012 (1ST WWYRRN 2012)

This winter wheat nursery consisted of 75 accessions.

A



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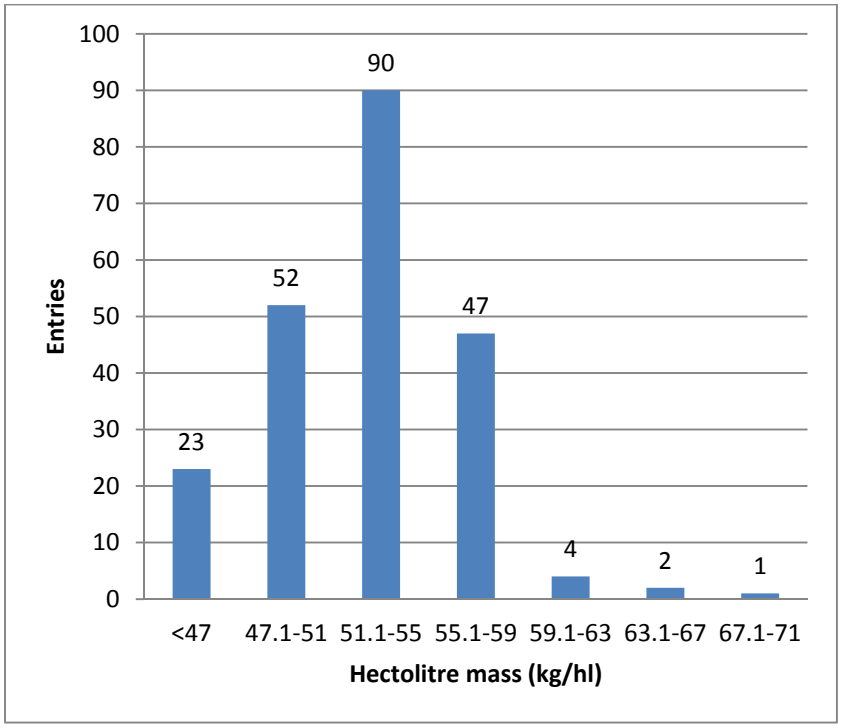

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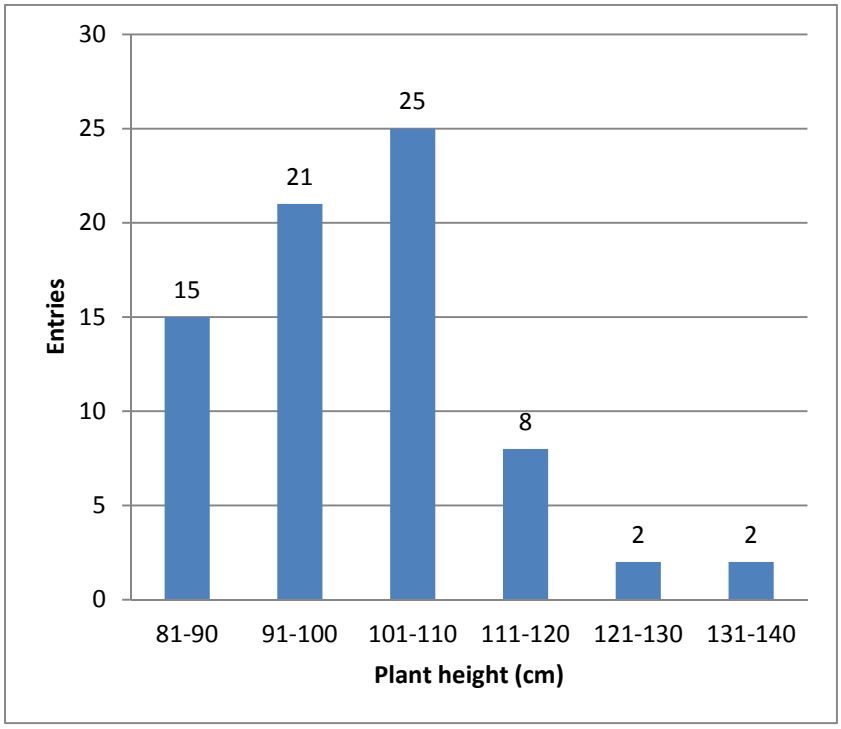
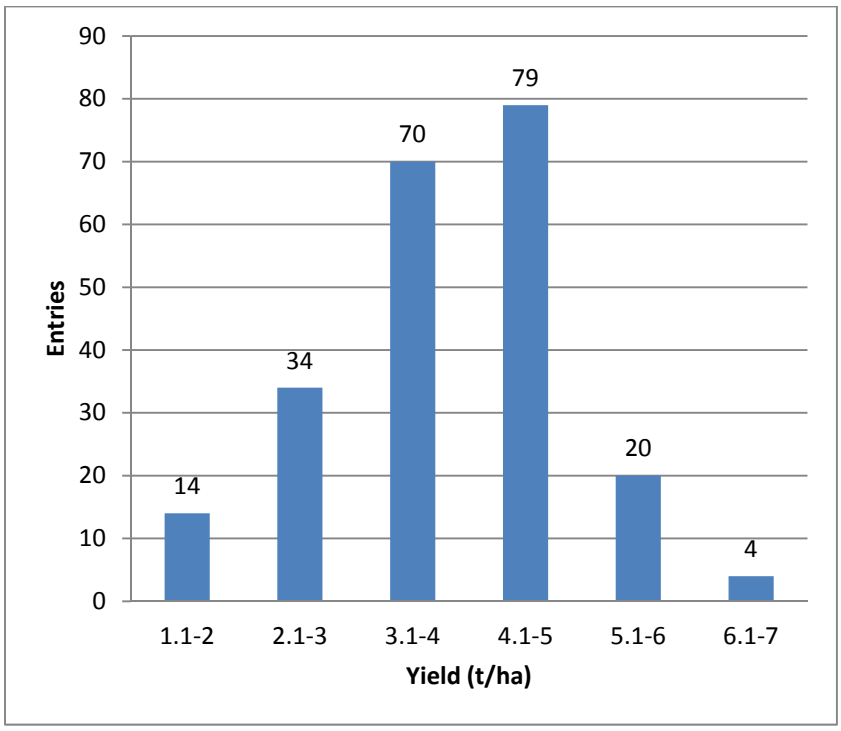


Figure 12. Frequency distribution of (A) grain yield, (B) hectolitre mass and (C) plant height of germplasm accessions evaluated in 1st WWYRRN 2012 at Bethlehem

2012 Quaker International Oat Nursery (2012 QION)

This oat nursery consisted of 231 accessions.

A



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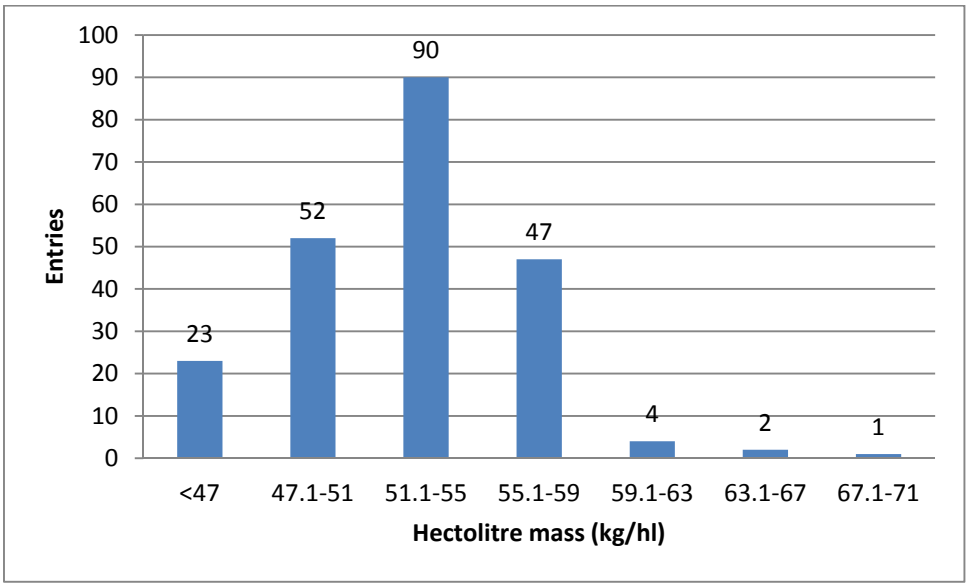

Figure 13. Frequency distribution of (A) grain yield and (B) hectolitre mass of germplasm accessions evaluated in 2012 QION at Bethlehem

3.2 Report on objective 2.2.2

One thousand four hundred and fifty eight entries from wheat nurseries and 219 entries from oat nurseries were planted and evaluated under quarantine conditions as specified and required by the Department of Agriculture, Forestry and Fisheries (DAFF). Observations were made against present exotic diseases. The nurseries were well maintained and no foreign disease or insect pest was recorded.

3.3 Report on objective 2.2.3

The introduction of international germplasm is to fast track the development of adapted cultivars in South Africa. Near maturity when the evaluations are complete, breeders, pathologists, entomologists and agronomists made selections before harvesting all the accessions in December 2012 and January 2013. The total number of selections made in various nurseries is shown in Table 3. No selections were made from the oat nursery. The compete oat nursery will be planted in field trials in the 2013/2014 season.

Table 3. Number of small grain entries planted and selected

Nursery Type	Entries planted	Entries Selected
Spring wheat	987	377
Winter wheat	471	58
Oats	219	-
Total	**1 677**	**435**

3.4 Report on objective 2.2.4

The seeds of all the selections made, as well as those not selected, were harvested, processed and stored in the Germplasm Bank as new accessions. The data collected were used to update the Genebank database.

4. Future of the project

The project will continue to obtain new international germplasm with important traits to broaden genetic diversity of the small grain Genebank at ARC-SGI and for fast tracking of the development of high yielding and pest resistant cultivars.

5. Objectives April 2013 – March 2014

International germplasm exchange is an important activity in enhancing genetic variability of local germplasm at ARC-SGI. This project with all its activities will continue in the following season and the following objectives will continue in 2013/2014.

- Comply with APIS requirements.
- Make new germplasm available to all researchers.
- Evaluate new germplasm for agronomic and disease resistance characteristics.
- Retain all selected lines as new accessions to the Germplasm Bank.

GK 09/08: THE INTRODUCTION AND EVALUATION OF NEW INTERNATIONAL GERMPLASM PROGRESS REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 09/08
Title: The introduction and evaluation of new international germplasm
Duration: Ongoing
Status: Continuation of existing project
Project leader: Dr Eben von Well and Mr Moses P Ncala

The objective of the project is to enhance genetic diversity by introducing new germplasm lines with good genetic and agronomic backgrounds. During the 2012/13 cropping season, 1 458 entries from eight spring and four winter wheat nurseries and 231 from an oat nursery were evaluated under quarantine conditions and no foreign insect pest or diseases were observed within and among the nurseries. A total of 377 and 58 selections were made by various scientists from spring and winter wheat nurseries, respectively. These selections will be tested in hot spots for biotic stress resistance. Part of the seeds harvested from the selected accessions were cleaned and stored in the Germplasm Bank.

GK 09/13: THE APPLICATION OF TISSUE CULTURE TECHNIQUES IN THE WHEAT BREEDING PROGRAMME TO ADDRESS COMPLEX GENETIC CHARACTERISTICS
FINAL REPORT APRIL 2012 – MARCH 2013

1. Project details

Number:	GK 09/13
Title:	The application of tissue culture techniques in the wheat breeding programme to address complex genetic characteristics
Duration:	Until March 2013
Status:	Final Report
Project leader:	Ms Tsepiso Hlongoane

2. Objectives

2.1 Long-term objective

The long-term objective of the project is as follows:

- To hasten the attainment of complex genetic characteristics to homozygous state in order to produce non-segregating lines, through doubled haploid technology.

2.2 Short term objectives: April 2012 - March 2013

The short term objectives for the 2012/2013 report period were as follows:

- To produce doubled-haploids from pyramided cross combinations identified with molecular markers by the Biotechnology Unit.
- To multiply doubled-haploids produced and store it in the germplasm collection for easy access by the breeders.

3. Report on objectives: April 2012 – March 2013

General remark
The double-haploid technique is a micro-technique that is currently gaining global acceptance to fast track and shorten the time needed for the attainment of homozygosity of cross combinations. The double haploid technique is a tool to fix complex genetic characteristics. The techniques employ two commonly used methods, embryo culture obtained by maize-wheat hybridization *a*nd another culture (androgenesis). The embryo culture technique is currently being used at ARC-SGI.

3.1 To produce doubled-haploids from pyramided cross combinations identified with molecular markers by the Biotechnology unit

For the first five months of the reporting period, several growing media were tested and optimised using the germplasm developed through project GK 04/20 for rust resistance. After optimisation, selected crosses of Tug-DN/Elands F_1 and Elands/Flamink F_1 were used for wheat x maize crosses to induce haploid embryos. At ear emergence, wheat spikes on intact plants were conventionally emasculated and pollinated with fresh maize, the fourth or fifth day after emasculation. The pollinated wheat spikes were sprayed with 213 mg/l 2,4-D solution in order to maintain embryo growth to the stage suitable for embryo rescue (Fig. 1). At 21 days after pollination, immature embryos were aseptically excised from all seed set on the wheat spikes and transferred onto Murashige & Skoog (MS) culture medium. Following regeneration, haploid plantlets (Fig. 2)

were transferred from the MS medium into jiffy pots for hardening (Fig. 3). After one week, plants were transplanted into pots and transferred to the cold room at (4°C) for vernalization for a period of 6 weeks. These haploids produced, contain only one set of chromosomes and they are smaller and exhibit a lower plant vigor compared to donor plants, and are sterile due to the inability of their chromosomes to pair during meiosis. In order to propagate them through seed and to include them in the breeding programme, their fertility has to be restored with induced chromosome doubling, using colchicine. After colchicine treatment, fertility was restored and seed was produced (Fig. 4). The results obtained are shown in Table 1.

Figure 1. Emasculated spikes covered with polythene bags and envelopes

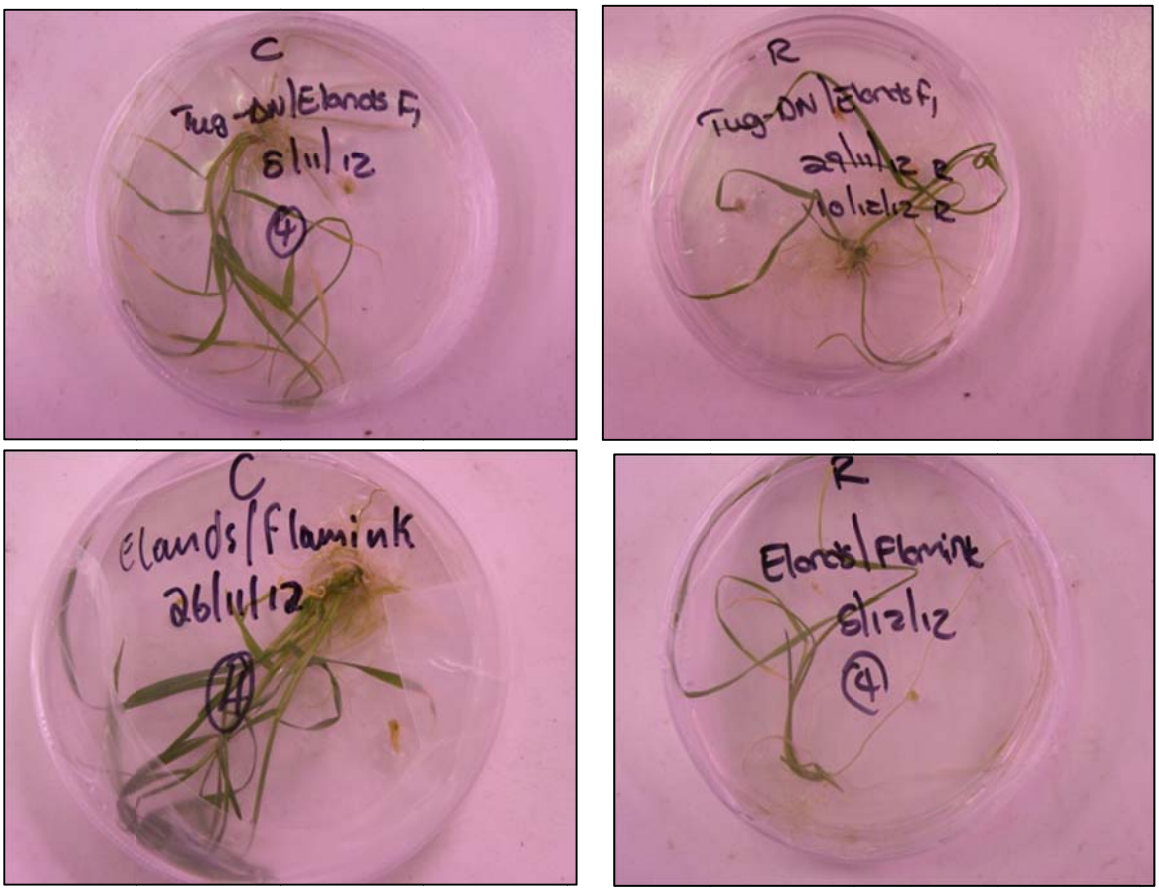

Figure 2. Completely regenerated haploid seedling with green leaves and root presence.

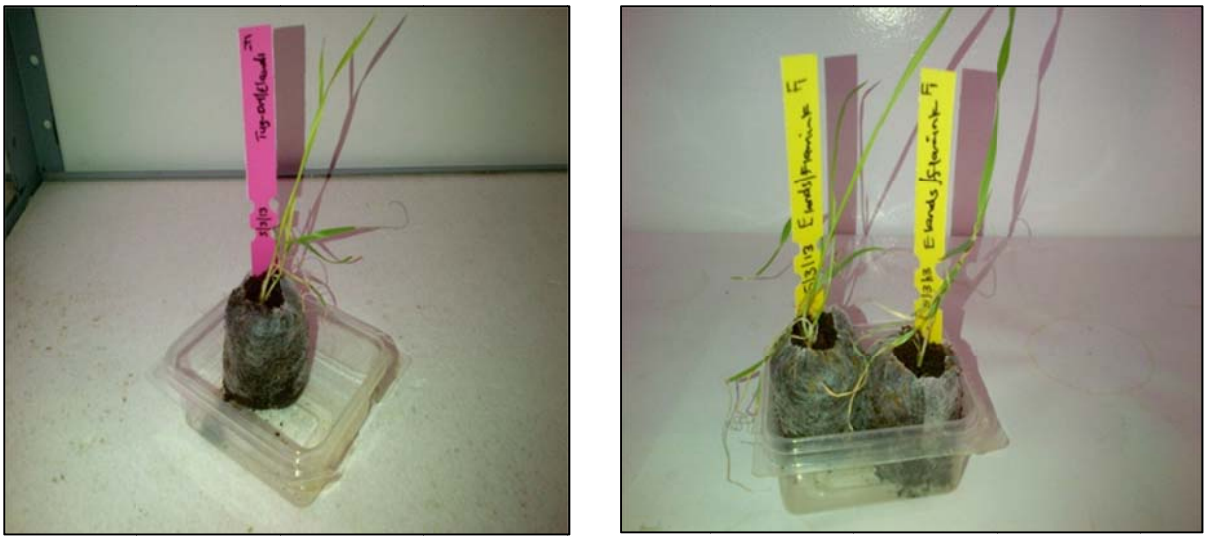

Figure 3. Plantlets transferred from the MS medium into jiffy pot for hardening.

Figure 4. The end product showing seed set on colchicine treated plants.

Table 1. List of plant undergoing double haploid production

Crosses	# of plants in growth room	# of plants in cold room	# of plants in cubicle awaiting colchicine	# plants treated	Total
Tug-DN/Elands	6	0	19	127	152
Elands/Flamink	16	10	50	38	114

Achievements on objective 1:

- The figures given in this report are those of the surviving plants
- 127 Tug-DN/Elands plants have been treated with colchicine
- 60 plants have been harvested, while others are still producing seed
- 38 Elands/Flamink have been treated with colchicine, while 50 are awaiting colchicine treatment

Doubled haploid plants from these two crosses will be used as mapping populations for the genetic analysis of important traits present in Elands. The technology utilised to achieve the objective of this project, was optimised and was highly efficient and it will be used in future.

3.2 *To multiply doubled-haploids produced and store it in the germplasm collection for easy access by the breeders.*

Following chromosome doubling, the doubled haploid plants were selfed for further multiplication.

Achievements on objective 2:

- 17 doubled haploid plants from the previous report were grown in the glasshouse for multiplication purposes
- Table 2 illustrates the number of seeds obtained from the 14 of the 17 lines that were utilised for multiplication purposes
- The resulting lines were utilised for optimisation of the technology and the technique was successfully applied

The obtained doubled haploids are homozygous at all loci and can be candidate breeding lines to undergo final evaluation and yield trials. Doubled haploid seeds were multiplied and a large number of seeds were obtained (Table 2) and stored for easy access. The Tug-DN/Elands plants are yet to be multiplied.

Table 2. List of lines with multiplied double haploid seeds

Sample number	Lines	Number of seeds obtained
1	K370	>1000
2	K516	>100
3	K327	>500
4	K303	>1000
5	K187	>1000
6	K85	>100
7	K80	>1000
8	K82	7
9	K89	>500
10	K11/11	1
11	K1/11	>300
12	V1	>500
13	V5	24
14	K481	2

Additional achievements

Training

The newly employed Laboratory assistant in the DH Laboratory, Mr Tebogo Oliphant, four students from Potchefstroom College of Agriculture and the three interns from NRF/SAASTA were trained on the doubled haploid techniques and optimisation of the protocols, as well as management of plants in the glasshouse.

4. Future of the project

This project is now concluded. The double haploid will now be implemented as a service on a demand basis. The production of doubled haploids using embryos developed from wheat crossed with maize pollen will continue at ARC-SGI.

GK 09/13: THE APPLICATION OF TISSUE CULTURE TECHNIQUES IN THE WHEAT BREEDING PROGRAMME TO ADDRESS COMPLEX GENETIC CHARACTERISTICS
FINAL REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 09/13
Title: The application of tissue culture techniques in the wheat breeding programme to address complex genetic characteristics
Duration: Until March 2013
Status: Final report
Project leader: Ms Tsepiso Hlongoane

This project uses the wheat by maize cross system as one of the methods for production of haploid wheat embryo. Out of 17 doubled haploid (DH) lines obtained, 14 were successfully multiplied and stored for easy access. The obtained doubled haploids are homozygous at all loci and can be breeding lines to undergo trait evaluation in multiple trials.

The doubled haploid protocol was successfully optimised during this reporting period. Of the 152 Tug-DN/Elands plantlets, 60 seeds and were harvested, while other plants are producing seeds. All the doubled haploid seeds will be multiplied and stored for easy access. Of 114 Elands/Flamink, 38 plants were treated with colchicine, while 50 are still waiting for colchicine treatment. Doubled haploid plants from these two crosses will be used as mapping populations for the genetic analysis of important traits present in Elands. The technology utilised to achieve the objective of this project was optimized. The newly appointed Laboratory assistant, Mr Tebogo Oliphant has been trained on the technique.

Double haploid is and will continue to be a very efficient tool for the production of completely homozygous lines from heterozygous donor plants and it will be used at SGI, mainly for the winter wheat. Its application will be combined with several other biotechnological techniques such as genetic transformation and improved backcrossing to enable novel breeding achievements.

GK 09/17: APPLICATION OF MOLECULAR AND TISSUE CULTURE TECHNIQUES TO SOLVE PROBLEMS IN DISEASE RESISTANCE OF WHEAT
PROGRESS REPORT APRIL 2012 – MARCH 2013

1. Project details

Number: GK 09/17
Title: Application of molecular and tissue culture techniques to solve problems in disease resistance of wheat and barley
Duration: Ongoing
Status: Continuation of existing project
Project leaders: Dr Toi Tsilo and mr Scott Sydenham

2. Objectives

2.1 Long- term objectives

The long-term objectives of the application of molecular and tissue culture techniques project are as follows:

- To validate useful, diagnostic markers for application in marker-assisted selection (MAS) at the Agricultural Research Council (ARC)-Small Grain Institute (SGI).
  a) Pest resistance in Wheat
     i) Russian wheat aphid
     ii) Leaf rust
     iii) Stem- and Stripe rust
     iv) Fusarium Head Blight
  b) Bread making quality

- To create a number of specific designed mapping populations to map and characterise new sources of disease resistance
  a) Map pest resistance in Wheat
     i) Russian wheat aphid
     ii) Leaf rust
     iii) Stem rust
     iv) Stripe rust

2.2 Short term objectives: 2012/2013

The short term objectives for the 2012/2013 report period were as follows:

- Identification of single spore *Fusarium* isolates.
- Molecular identification of viruses.
- Collaborate with national and international researchers to improve molecular techniques for the application of molecular marker-assisted selection in plant breeding.
- Generate and develop mapping populations segregating for important traits.
- Perform genetic mapping analyses on existing mapping populations and recommend germplasm to the ARC-SGI plant breeders, geneticists, pathologists, and entomologists.
- Assist the ARC-SGI Plant Breeders with MAS to stack genes or to screen segregating populations arising from crosses made for resistance to wheat diseases, insect pests and agronomic traits:
  - *Fusarium* head blight (FHB) resistance
  - Rust

- Russian wheat aphid (RWA)
- Agronomic and quality traits

3. Report on objectives: April 2012 – March 2013

3.1 Objective 2.1: Identification of single spore Fusarium isolates

A total of 203 isolates from 4 locations (Dundee - 102, Frankfort - 27, Groblersdal - 57 and Vaalharts - 17) were evaluated for their species specificity using species specific PCR primers (Table 1).

Table 1. PCR primers used for the identification of *Fusarium* species

Primer name	Sequence (5'-3')	Tested for species
Fg16F	CTACGGATATGTTGCGTCAA	*F. graminearum*
Fg16R	GGTAGGTATCCGACATGGCAA	*F. graminearum*
Fg16NF	ACAGATGACAAGATTCAGGCACA	*F. graminearum*
Fg16NR	TTCTTTGACATCTGTTCAACC CA	*F. graminearum*
F. boothii F	ATTGGTGTTGCCTTCGCC	*F. boothii*
F.boothii R	AAGGTCTTAAGCGCTTCG	*F. boothii*
FP1-1	CGGGGTAGTTTCACATTTCCG	*F. pseudograminearum*
FP1-2	GAGAATGTGATGACGACAATA	*F. pseudograminearum*
EF1	ATGGGTAAGGA(A/G)GACAAGAC	Within *Fusarium* group
EF2	GGA(G/A)GTACCAGT(G/C)ATCAT	Within *Fusarium* group

The *F. graminearum* isolate PPRI 7721 was obtained from the ARC-Plant Protection Institute in Pretoria and it was used as a positive control for Fg16 and Fg16N primers. The isolates were tested against *Fusarium graminearum, F. pseudograminearum, F. boothii, F. culmorum* and *F. meridionale*. The results showed that of the 203 isolates tested, 139 isolates were confirmed to be in the *Fusarium* group that consisted of 134 *F. graminearum* isolates, one *F. pseudograminearum* isolate and four *F. boothii* isolates. Species-specific primers for *F. culmorum* and *F. meridionale* were also used in the PCR reaction, but there were no isolates that belonged to any of these species (Table 2). There was no amplification in 64 isolates and the resulting isolates were not tested further. Only positively identified *Fusarium* group isolates were targeted for use in the FHB resistance screening and breeding programmes (Table 2).

Table 2. The PCR results for the identification of *Fusarium* species

Locality	Number of isolates tested	*F. graminearum*	*F. pseudograminearum*	*F. boothii*	*F. culmorum*	*F. meridionale*
Dundee	102	96	0	0	0	0
Frankfort	27	15	0	0	0	0
Groblersdal	57	13	1	4	0	0
Vaalharts	17	10	0	0	0	0
Total	**203**	**134**	**1**	**4**	**0**	**0**

Fusarium single spore isolates may produce mycotoxins including trichothecenes, moniliformin and fumonisins. *Fusarium graminearum* produces type B trichothecenes including DON, acetylated derivatives including 3-acetyl (3-ADON) and 15-acetyl (15-ADON), and NIV chemotypes. The 139 isolates tested

positive for the *Fusarium* group were tested for mycotoxin production. The presence of mycotoxins was tested using mycotoxin specific primers (Table 3). The primer sequences in Table 3 were adapted from Scoz et al. (2009).

Table 3. Primers used for mycotoxin analysis

Primer set	Primer name	Sequence (5'-3')	Amplicon size (basis pairs)
*tri*12	12CON	CATGAGCATGGTGATGTC	410
tri12	12NF	TCTCCTCGTTGTATCTGG	670
tri12	12-15F	TACAGCGGTCGCAACTTC	840
tri12	12-3F	CTTTGGCAAGCCCGTGCA	
*tri*13	13F	CATCATGAGACTTGTGCRGTTTGGG	282
*tri*13	13DONR	GCTAGATCGATTGTTGCATTGAG	
*tri*13	13R	TTGAAAGCTCCAATGTCGTG	
*tri*13	13NIVF	CCAAATCCGAAAACCGCAG	312

Primer sets *tri*12 and *tri*13 were used to determine the presence of 3-ADON, 15-ADON and NIV chemotypes. The data for the chemotyping for the different localities was identified using the *tri*13 primer set (Table 4). Chemotyping was conducted on all isolates and only 133 isolates were positively chemotyped for the localities. The results showed that DON was the predominant chemotype in all the localities, with a total of 122 isolates out of 203 samples tested showing the presence of DON (Table 4). The results further showed that 6 and 5 isolates from Dundee tested positive for DON and NIV, respectively (Table 4).

Table 4. Chemotyping of isolates in four localities

Locality	Number of isolates	DON	NIV	DON + NIV
Dundee	102	84	6	5
Frankfort	27	15	0	0
Groblersdal	57	13	0	0
Vaalharts	17	10	0	0
Total	**203**	**122**	**6**	**5**

The tri12 primer set was used for the identification of chemotypes produced by the targeted *Fusarium* species (Table 5).

Table 5. *Fusarium* species and the chemotypes they produced

***Fusarium* species**	**Number of the group of the *Fusarium* isolates**	**3-ADON**	**15-ADON**	**NIV**	**DON + NIV**
F. graminearum	134	0	108	6	5
F. pseudograminearum	1	0	1	0	0
F. boothii	4	0	4	0	0
Total	**139**	**0**	**113**	**6**	**5**

A total of 139 isolates were evaluated for their species specificity and the mycotoxins they produce. The 3-ADON chemotype was absent in all localities, whereas the 15-ADON chemotype were found in *F. graminearum*, *F. pseudograminearum* and *F. boothii* isolates. The data showed that of the 139 *F. graminearum* samples tested, 108 samples produced 15-ADON, 6 produced NIV and 5 produced DON and NIV. In the *F. graminearum* samples all the NIV producing isolates were from Dundee. The *F. pseudograminearum* species isolated from Groblersdal produced the 15-ADON mycotoxin. Four *F. boothii* isolates produced the 15-ADON mycotoxin isolated from infected kernels from Groblersdal. This data correlates with the findings by Boutigny et al. (2011) whereby 85% of the samples collected were *F. graminearum* and of these, the predominant trichothecene types showed that 15-ADON was predominant.
.

3.2 Objective 2.2: Molecular identification of viruses

A number of virus infected wheat samples were obtained and supplied by Dr Goddy Prinsloo. The PCR markers for the simulations detection of eight different viruses were purchased. The total viral RNA extraction and the RT-PCR procedure are still being optimised. Results on the molecular identification of viruses causing infection on wheat samples will be provided in the next Winter Cereal Trust report.

3.3 Objective 2.3: Collaborate with national and international researchers to improve molecular techniques for the application of molecular marker-assisted selection in plant breeding

Further collaboration was established with Dr G Bai and other colleques from the USA (USDA-ARS), during the National Fusarium Head blight Forum in Orlando in December 2012. Dr G Bai has over the past few years given the ARC-SGI Biotechnology Unit a number of new promising Fusarium head blight resistant sources, that are being used to develop and release FHB resistant germplasm.

At the same FHB Forum, further networking was established with Dr Stine Petersen from the NC State University who runs a FHB screening nursery. Dr Stine presented a poster in which digital software was used to predict the amount of fusarium damaged wheat kernels in a representative sample photograph. The discussion was around the use of digital software and how accurate it can predict the amount of fusarium damaged kernels (FDK) in a wheat grain sample and how it can be purchased. In future, SGI will aim at exploring this collaboration and also to purchase this software and make use of this time saving application for FDK analysis.

A collaboration has been established with Dr Mehmet Cakirt, an associate Professor of the Genomics for Discovery and Breeding in Crops Division at the School of Biological Sciences and Biotechnology within the Faculty of Sciences and Engineering, Murdoch University, Perth, Western Australia. Dr Mehmet has invited Mr Scott Sydenham to Australia for a few weeks during late 2013. The purpose of the international travel will be to exchange important RWA resistant germplasm, fine map a number of markers linked to important RWA resistance genes, further develop mapping populations and to attend a SNP and DArT marker training course.

Dr Toi Tsilo attended the Borlaug Global Rust Initiatives in 2012, and also made several research visits, to the Chinese Academy of Agricultural Sciences (CAAS) and several other research organisations.

Between September 5-7, 2012, Dr Tsilo visited the Institute of Crop Sciences, CAAS. The reason of the visit was to establish collaboration with Prof. Xianchun Xia on germplasm exchange, discovery and utilisation. An agreement was reached between Drs Xia and Tsilo, both reseachers (Photo 1) will take advantage of the funding options that are available for a cooperative research project between China and South Africa. The draft proposal will focus on Fusarium head blight, stripe rust, wheat end-use quality, agronomic traits and yield. Phase one of the project will include germplasm exchange and evaluation for phenotype and genotype. Phase two will include trait discovery using structural, comparative and functional genomics with emphasis on sequencing, annotation, gene silencing tools, and transformation tools.

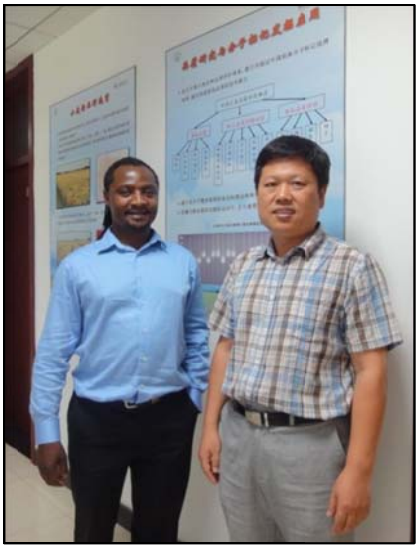

Photo 1. Dr Tsilo and Dr Xianchun Xia after a laboratory tour

On the 7th of September, 2012, Drs Tsilo and Martin Nyachoti (professor of Animal Science at the University of Manitoba, Canada) met with the Managing Editors of the Journal of Integrative Agriculture (JIA), formally known as Agricultural Sciences in China, which is sponsored by the Chinese Academy of Agricultural Sciences (CAAS) (Photo 2). A meeting was held with the editors to discuss the role of the journal and how to increase the impact factor and the quality of articles published by the journal. The discussion will help the journal to publish influential papers that will significantly advance scientific understanding of agriculture. Both Tsilo and Nyachoti are Editorial Board Members and Editors of JIA.

Photo 2. left-right: Dr Ning Wang (Managing Editor), Dr Yi-min Zhang (Director of Editorial Dept.), Dr Tsilo, Dr Nyachoti, and Dr Juan Zhang (Managing Editor).

Prof. Yinsuo Jia, Director of the Centre of Resistance Physiology and Molecular Breeding at Hebei Academy of Agriculture and Forestry Sciences, invited Dr Tsilo and introduced him to the government of Hebei Province. Several meetings and a gala dinner were held. During meetings, the government officials of Hebei Province showed their dedication and support for collaborative research between Hebei Province and South Africa. Furthermore, Prof Jia has vested interest in South African agriculture and he holds an adjunct professorship with the University of Fort Hare, Alice. Wheat and maize seeds were obtained from Hebei Province and will be used for research purposes. Two of the wheat lines from China are already being crossed to some of the SA cultivars. Prof Jia plans on visiting SGI in July 2013.

A strain of the rust pathogen, Ug99, remains a serious threat to wheat production worldwide. SGI researchers established a collaborative research network with several researchers from the United State Department of Agriculture Cereal Disease Laboratory, University of Minnesota, and Agriculture and Agri-Food Canada to characterise the stem rust resistance gene *Sr42* that gives a high level of resistance to Ug99. In this collaborative research, useful and diagnostic DNA markers associated with the *Sr42* gene on chromosome 6D of wheat were identified and made publicly available to other researchers world-wide. This work is documented in the journal of Theoretical and Applied Genetics (DOI: 10.1007/s00122-012-1874-y) with a title "Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42*". Also, a research collaborative work is underway with the same collaborators to study the genetics of stem rust resistance in durum wheat line, I-39. The I-39 line has high levels of resistance to Ug99 and has the potential to diversify Ug99 resistance in durum wheat. A mapping population was phenotyped with two stem rust races and it was found to segregate for resistance. Genotyping of the parents and selected progenies resulted in the identification of single nucleotide polymorphic (SNP) markers, using the KBiosciences Competitive Allele-Specific PCR (KASP) SNP genotyping system.

Dr Toi Tsilo and his colleagues at SGI obtained 70 wheat accessions from Dr Xianming Chen's group at Washington State University. These accessions have yellow rust resistant sources derived from 23 countries. Twenty-eight of these accessions have resistance genes that are effective at all stages of plant growth. Forty-two accessions have either adult plant resistance or seedling resistance to yellow rust. These accessions will be valuable resources to diversify yellow rust resistance in South African wheat germplasm. Accessions are being used at SGI to pyramid resistance into current commercial wheat cultivars that are grown in areas where the yellow rust pathogen is most predominant.

Seven wheat accessions from Dr Steven Xu's group at USDA-ARS in Fargo were also recieved. Four of these accessions carry the stem rust resistance gene *Sr39*. In the past, the *Sr39* gene was not used in wheat breeding, because the gene is linked with other genes that have adverse effects on grain yield. The wheat accessions obtained from Dr Xu only contain *Sr39* with no adverse effect on grain yield. These accessions are being used to diversify and ensure durable stem rust resistance in South African wheat germplasm.

3.4 Objective 2.4: Generate and develop mapping populations segregating for important traits

The following mapping populations are under development:

FHB populations: Eight populations are being developed to map new sources of FHB resistance. For the reporting period, generations were advanced to F_5 in the glasshouse. All five of the new sources of resistance used to create the mapping populations were found to be different from Sumai 3 and Frontana, as discussed earlier in this report. Each population has about 150-200 lines.

Stem rust populations: Ten populations are being developed to map new sources of adult-plant resistance to stem rust. For this reporting period, generations were advanced to F_4 in the glasshouse. Crosses were also created between Baviaans and Baviaans mutant. The mutant was generated by Dr Eben von Well and has resistance to Ug99. The F_1 and F_2 lines were generated during this reporting period. The objective of studying this mutant is to generate a population that will be used to identify on which chromosome mutation occurred and on chromosome region. Details of this work will be included in the next reporting period, when more phenotypic and genotypic data are collected and analysed.

Pre-harvest sprouting: Two crosses involving Tugela-DN and Elands and Elands and Flamink were created and the F_1 seeds were send for double haploid production.

RWA populations: A set of 228 BC_5F_3 lines were created to map RWA resistance present in the germplasm CI 2401. This population is being studied for RWA resistance with close collaboration with Dr Vicki Tolmay.

From all these mapping populations, DNA markers linked to resistance genes will be identified and also made available for public use in marker assisted selection and pyramiding schemes.

3.5 Objective 2.5: Perform genetic mapping analyses on existing mapping populations and recommend germplasm to the ARC-SGI plant breeders, geneticists, pathologists, and entomologists.

Two mapping populations, a BC_3F_3 (186 plants) and F_3 (103 plants) segregating for the RWA resistance gene *Dny* (Stanton) have been planted out in the glasshouse RWA readings for each plant reaction against RWASA1 have been taken and DNA extractions was done on this material. The two populations are currently being screened with a number of SSR markers on chromosome 1D and other chromosomes. This is the first attempt to map the *Dny* gene position.

A collaborative research work involving several researchers with Agri-Food Canada in Winnipeg, Manitoba, researchers with USAD-ARS (Cereal Disease Laboratory), and researchers from the University of Minnesota, has resulted in the mapping of the *Sr42* gene and the DNA markers linked to the gene were

identified and the work was published. The next step is to incorporate the *Sr42* gene into several cultivars and breeding lines that are adapted to South Africa, to broaden stem rust resistance in South African germplasm.

3.6 Objective 2.6: Assist the ARC-SGI plant breeders with MAS to stack genes or to screening segregating populations arising from crosses made for resistance to wheat diseases and insect pests

3.6.1 *Fusarium* head blight (FHB) resistance

Five lines phenotypically selected from the 9th and 10th CIMMYT FHB screening nurseries have now been extensively haplo-typed. Twenty SSR markers that are linked to a number of FHB resistance QTL/genes that are present in multiple different FHB Donor sources were used to make haplo-type comparisons (Table 6). The eight FHB donor sources used for comparisons were, Sumai 3, Frontana, Asozaria III, Baisanyuehuang, Huangcandou, Huangfangzhu, Haiyanzhong and Wangshuibai. The different marker haplo-types observed across the different polymorphic markers, are presented in Table 7.

Table 6. SSR markers used for haplotype comparison of eight FHB resistance sources

Targeted Resistant source	SSR Marker	Target QTL/Gene
Sumai 3	*Xgwm389*	*3B QTL/Fhb1*
	Xgwm533	
	UMN-10	
	Xbarc133	
	Xgwm493	
Wangshuibai	*Xgwm156*	*5A QTL/Fhb5*
	Xbarc197	
	Xgwm304	
	Xgwm415	
	Xgwm293	
Sumai 3	*Xgwm133*	*6B QTL/Fhb2*
	Xgwm644	
Frontana	*Dupw227*	3A QTL
Sumai 3	*Xwmc17*	*7A-Fhb7AC*
Huangfangzhu	*Xbarc121*	*7A-QTL*
	Xgwm276	
Huangcandou	*Xgwm261*	*2D-QTL/Rht8*
Wangshuibai	*Xhbg226*	*4B-Fhb4*
	Xgwm149	
Haiyanzhong	*Xwmc702*	*7D QTL*
	Xwmc121	
	Xcfd46	

Table 7. Molecular haplo-type data of each of the polymorphic FHB specific DNA marker screened on 16 wheat varieties

		FHB resistant varieties								*CIMMYT selections/ Test lines*				
QTL/gene	**Varieties/Marker**	***Sumai 3***	***Frontana***	***ASO***	***BAI***	***HCD***	***HFZ***	***HYZ***	***WSB***	**9SRSN-10**	**9SRSN-36**	**9SRSN-37**	**9SRSN-42**	**10SRSN-36**
3B-Fhb1	***Xgwm389***	140	120	140	140	140	140	140	140	150	120	120	120	null
	Xgwm533	120	120	150	150	150	140	150	150	120	130	120	120	120
	UMN-10	240	260	260	240	240	260	260	240	260	260	240	260	250
	Xbarc133	140	110	140	100	140	100	100	100	null	120	120	120	130
	Xgwm493	150	null	180	220	220	220	220	220	150	150	150	null	150
5A-QTL/5A-Fhb5	***Xgwm156***	290	310	320	320	320	320	320	340	290	290	290	320	320
	Xbarc197	185	170	185	185	185	185	185	185	Null	185	185	185	185
	Xgwm415	130	130	140	140	140	140	140	140	130	130	120	130	130
	Xgwm304	200	200	220	220	220	220	220	230	190	190	190	null	190
	Xgwm293	190	200	null	170	200	200	200	200	190	190	180	null	180
6B-Fhb2	***Xgwm133***	100	120	130	130	110	110	130	130	110	110	130	110	110
	Xgwm644	150	160	180	180	180	180	180	180	180	160	150	160	160
3A-QTL	***Dupw227***	190	175	190	190	190	190	190	190	190	190	190	190	190
7A-Fhb7AC	***Xwmc17***	170	170	180	180	180	180	null	180	170	170	170	170	Null
2D-QTL/Rht8	***Xgwm261***	175	150	192	192	192	175	192	192	null	175	150	150	150
4B-Fhb4	***Xgwm149***	165	165	175	175	175	175	175	155	165	175	null	175	175
7D QTL	***Xwmc702***	200	200	200	200	200	180	180	180	180	180	180	180	180
	Xcfd46	180	170	180	180	180	190	180	180	180	180	170	170	180

ASO- Asozaira **BAI-** Baisanyuehuang **HCD-** Huangcandou **HFZ –** Huangfangzhu **HYZ-** Haiyanzhong **WSB-** Wangshuibai

3.6.2 Rust

Marker-assisted selection:
About 710 lines received from project GK 04/20 were genotyped for several genes and results are presented in Table 8. DNA was extracted from all the samples using CTAB DNA extraction protocol. Screening was conducted using diagnostic markers for *Sr2*, *Sr22 Sr26*, *Sr31, Lr34* and *Yr29.* The PCR products were resolved on agarose gel electrophoresis (Fig. 1). Figure 1 illustrates an example of samples that were screened using the *Lr34* marker, which were resolved on agarose gel electrophoresis.

Table 8. Results of 710 samples screened by diagnostic markers for *Sr2, Sr22, Sr26, Sr31, Lr34* and *Yr29*

Genes present	Number of samples screened	Number of lines with genes present
Sr2	214	164
Sr22	74	72
Sr26	158	10
Sr31	33	0
Lr34	219	197
Yr29	159	155

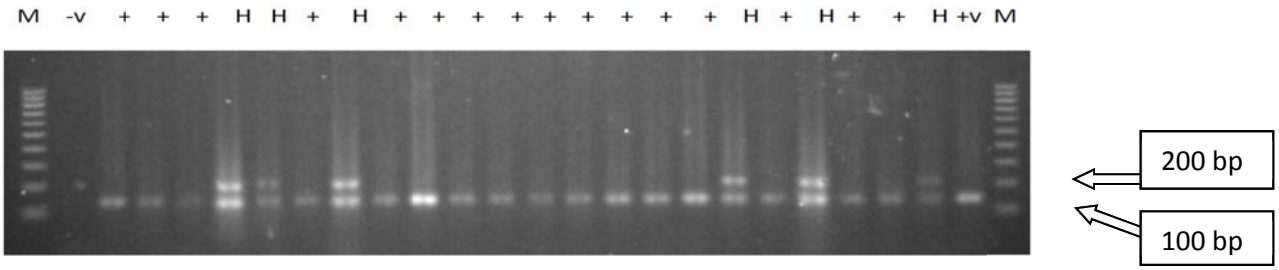

Figure 1. Illustration of samples screened with *Lr34* marker resolved on a 1% agarose gel. M: 100 bp DNA ladder; (+): presence of Lr34; (H): heterozygous; (+v): positive control and (-v): negative control.

Four-hundred and five lines were obtained from the breeding programme to screen for resistance to rust. DNA was extracted from 405 samples and screening was conducted using diagnostic markers for *Sr2, Sr26* and *Lr34*. The PCR products were visualised on agarose gel electrophoresis (Fig. 2). Results are presented on Table 9.

Table 9. Results of 405 samples screened with diagnostic markers for *Sr2, Sr26* and *Lr34*

Genes present	Number of samples screened	Number of lines with genes present
Sr2	405	266
Sr26	405	265
Lr34	405	197

Figure 2 illustrates an example of samples that were screened using the *Sr2* diagnostic marker which were resolved on agarose gel electrophoresis.

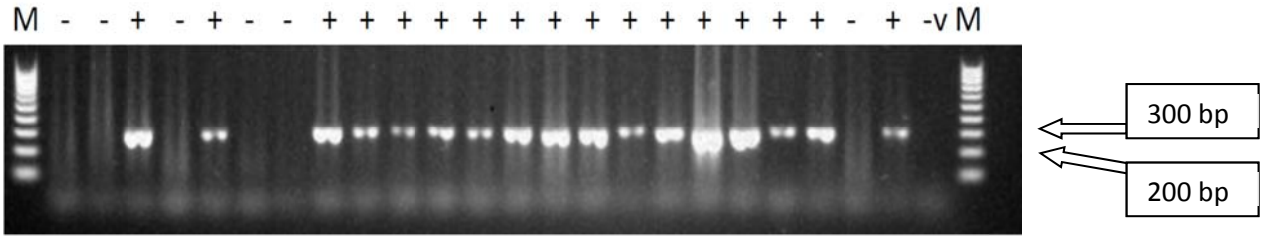

Figure 2. Illustration of samples 314-336 screened with the *Sr2* marker (csSr2) resolved on a 1% agarose gel. M: 100 bp DNA ladder; (-): *Sr2* absent; (+): *Sr2* present; (-v): negative control.

Gene Pyramiding Scheme:
Several backcrosses were made to pyramid three stem rust resistance genes (*Sr2*, *Sr25*, *Sr26*) into Baviaans, Buffels, Kariega, Kwartel, Tankwa, and Ratel. Some of the backgrounds have reached BC_2F_1 generation. DNA was extracted from about 600 BC_1F_1 lines and screening was done with diagnostic markers for all three the genes. Only 91 lines had a combination of all three genes. The BC_2F_1 crosses were made with selected lines. After obtaining homozygous lines for all three genes, seeds will be increased and made avaible to breeders. The target genes *Sr25* and *Sr26* came from the new sources that showed that these two genes don't have an adverse effect on grain yield and quality. Other genes targeted are *Sr39* and *Sr42* and material at F_1 generation is available.

Sources of *Yr45* and *Yr52* were crossed with Matlabas.

3.6.3 Russian wheat aphid (RWA)

***Dn4* marker validation:**
Twenty five different *Dn4* containing genotypes/lines, three *Dny* containing genotypes/lines were screened with the two published *Dn4* markers (GWM106 and GWM337) and 12 other SSRs on chromosome 1D. The other 12 SSR markers were selected from the wheat consensus map and are upstream and downstream of the *Dn4* locus. There is a need to identify, map and develop diagnostic markers for RWA resistance gene components or QTL present on chromosome 1D. Some markers gave important differences between *Dn4* and *Dny,* containing genotypes that will be studied further. This study has led to a number of other potential studies and future publications.

RWA, leaf, stripe and stem rust pyramiding:
Twelve lines contained phenotypically confirmed RWA resistance donor source of MR or R, and marker confirmed combinations of stem rust genes *Sr35* and leaf rust *Lr34*/*Yr18*. These 12 lines were selected after screening 224 different lines. These lines are currently going through SDS page and HPLC quality analysis. Other relevant markers on this material are still to be run.

Twenty two lines contained phenotypically confirmed RWA resistance donor source of MR or R scores, and marker confirmed combinations of rust resistance genes *Sr24, Sr35* and *Lr34*/*Yr18*. These 22 lines were selected after screening 642 different lines. These selected lines are to be planted out in 2013 for seed multiplication and quality testing.

3.6.4 Agronomic traits

Sixty different genotypes were supplied by breeder, mr Robbie Lindique. These included 30 South African cultivars, three international checks and 27 imported CIMMYT lines, which were genotyped with markers for photoperiod sensitive genes, height genes (dwarfing genes), vernalisation genes and the different form/types of ALMT1 gene (aluminum tolerance gene). Importantly a number of different types of the promoter region of the Aluminum Tolerance ALMT1 gene were observed. Most of the South African cultivars had the Type I and Type II variants of ALMT1 which confers sensitivity to Aluminum. However, there were one or two important exceptions that contained Types IV and V, which confer moderate to high tolerance to Aluminum. Genotyping for these genes will be expanded further in the coming year.

4. Conference out-puts

In order to bring our research results to the public, congresses and conferences were attended. A list of conferences that were attended is shown below:

4.1 National Fusarium head blight Forum 2012

A poster titled "SSR Marker Haplotypes Confirm Five novel FHB Resistance Sources from the CIMMYT Scab Resistance Screening Nursery" was presented at the National Fusarium Head Blight forum, held in Orlando, Florida in USA, December 4-7, 2012. Results of the extensive marker haplotype comparison of five new novel FHB resistance sources with eight well known resistance sources of FHB resistance were presented. A large amount of interest was generated during the poster sessions.

4.2 *Combined Congress 2013*

One oral presentation titled "Numerous Haplotypes for RWA Resistance Gene *Dn4* Identified" was presented by Mr. Scott Sydenham and Dr Vicki Tolmay at the 2013 Combined Congress at the KZN University, Westville, Durban. This paper presented the unique results of a number of different marker haplotypes identified for the Russian wheat aphid resistance gene *Dn4* locus.

4.3 International Crop Science Congress 2012

A presentation titled "Polymeric Proteins and their Association with Grain Yield in Hard Red Spring Wheat" was made at the 6th International Crop Science Congress. Bento Goncalves, RS, Brazil. (August 06-10, 2012). Co-authored by Tsilo, T.J., J-B. Ohm, G.A. Hareland, and J.A. Anderson.

4.4 Borlaug Global Rust Initiative 2012

The following two presentations were made at the conference: one was titled "QTL Influencing Adult Plant Resistance to Leaf Rust in MN98550-5/MN99394-1 Wheat Mapping Population" co-authored by Tsilo, T.J., and J.A. Anderson. Another one was titled "Pyramiding Stem Rust Resistance Genes in CIMMYT Wheat Germplasm" co-authored by Tsilo T.J., M. Dilawari, S. Liu, R.P. Singh, and J.A. Anderson.

5. Scientific Journal Article

One manuscript article was published and has the following reference: Ghazvini, H., C.W. Hiebert, T. Zegeye, S. Liu, M. Dilawari, **T. Tsilo**, J.A. Anderson, M.N. Rouse, Y. Jin, T. Fetch. 2012. Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42.* Theoretical and Applied Genetics 125(4):817-824. TAG Impact Factor: 3.814

6. Popular Media Articles

An article was published in the November/December issue of Koringfokuis/Wheat Focus, authored by mr Scott Sydenham titled "WHEAT-CSI: Cereal Science Investigation". The article explained the processes from leaf sampling, DNA extraction, PCR reactions to Marker-assisted selections done at the ARC-SGI under the Biotechnology project GK 09/17. The aim of this article was to inform wheat producers, that ARC-SGI is using a number of molecular techniques to further improve the wheat cultivars of ARC-SGI.

7. Objectives for April 2013- March 2014

- Identification of single spore Fusarium isolates.
- Molecular identification of Viruses

- Collaborate with other researchers national and international to improve molecular techniques for the application of molecular marker-assisted selection in plant breeding.
- Generate and develop mapping populations segregating for important traits.
- Recommend germplasm to the ARC-SGI plant breeders, geneticists, pathologists, and entomologists.
- Pyramid genes for rust resistance using diagnostic DNA markers.
- Assist the ARC-SGI plant breeders with MAS to stack genes or to screening segregating population arising from crosses made for resistance to wheat diseases and insect pests:
  - Fusarium head blight (FHB) resistance
  - Rust
  - Russian wheat aphid (RWA)
  - Agronomic and quality traits

GK09/17: APPLICATION OF MOLECULAR AND TISSUE CULTURE TECHNIQUES TO SOLVE PROBLEMS IN DISEASE RESISTANCE OF WHEAT AND BARLEY
PROGRESS REPORT APRIL 2012 – MARCH 2013

Summary

Number: GK 09/17
Title: Application of molecular and tissue culture techniques to solve problems in disease resistance of wheat
Duration: Ongoing
Status: Continuation of existing project
Project Leaders: Dr Toi Tsilo and Mr Scott Sydenham

During the past year, project GK 09/17 has met all its short term objectives set out for the year. The project has further helped pre-breeders working on rusts, Fusarium head blight and Russian wheat aphid resistance and recently for agronomic traits; make high value line selections, aided by the molecular marker data. This fast and efficient selection of high value pre-breeding lines was a further improvement on the outputs and highlights for ARC-SGI in 2012. A number of promising high quality line selections containing RWA resistance and a number of stem, leaf and stripe rust resistance genes have been identified as a result of 09/17. This assistance by the Biotechnology Unit led to speedy selection of pre-breeding lines, thereby increasing genetic gains, saving time and more importantly, leading to early reduction of breeding populations. A number of important mapping populations for stem rust, Fusarium head blight, pre-harvest sprouting and Russian wheat aphid resistance are being developed further to gain an understanding of the genetic resistance present in unique lines. Of the 203 Fusarium isolates tested, 139 isolates were confirmed to be in the *Fusarium* group that consisted of *134 F. graminearum* isolates, one *F. pseudograminearum* isolate and four *F. boothii* isolates. Important collaboration was secured with several scientists from USDA-ARS, Murdoch University, Chinese Academy of Agricultural Sciences, Hebei Academy of Agriculture and Forestry Sciences, University of Minnesota, Agri-Food Canada, Washington State University. The haplotyping study showed that the five new sources of FHB were different from Sumai 3 and Frontana and will diversify the sources of resistance to FHB in SA. The following genes: *Sr2*, *Sr26* and *Lr34* were present in most of the breeding lines submitted by breeders. The *Sr2*, *Sr22*, *Lr34* and *Yr29* genes were present in most of the germplasm lines submitted by pre-breeders. The project successfully obtained BC_2F_1 generation of backcrossing material pyramiding a combination of three genes (*Sr2*, *Sr25*, *Sr26*) into SGI cultivars. The 34 lines containing resistance to RWA, stem rust and leaf rust were identified and this material will undergo germplasm release after quality analysis and field testing. Genotypes supplied by breeders differ in their level of tolerance to Aluminum, based on the differences present at the Aluminum tolerance gene. During the course of 2012, as a result of the marker work and research activities done in GK 09/17, one national and three international conferences were attended and one article was published in the journal of Theoretical and Applied genetics.

GK 09/19: USING PHENOTYPIC SCREENING AND MARKER-ASSISTED BACKCROSSING TO DEVELOP DIVERSE FHB AND DON RESISTANT ADAPTED GERMPLASM
PROGRESS REPORT APRIL 2013 – JUNE 2013

1. Project details

Number:	GK 09/19
Title:	Using phenotypic screening and marker-assisted backcrossing to develop diverse FHB and DON resistant adapted germplasm
Duration:	2013 - 2019
Status:	New project
Project leaders:	Scott Sydenham and Cathy de Villiers

2. Objectives

2.1 Long-term objectives

The long-term objectives of the diverse Fusarium head blight (FHB) resistant germplasm pre-breeding programme are as follows:

- Making use of two backcross pre-breeding programmes, one phenotypic screening based and the other Marker-assisted based.
- To successfully pyramid/stack a number of FHB resistance QTL/genes from diverse donor sources into top performing South African wheat cultivars that are well adapted to irrigation production areas
- While retaining the highest recurrent parent genome percentage resulting in an increased resistance to FHB disease, reduction in Deoxynivalenol (DON) mycotoxin contamination, reduced yield loss and kernel damage.

2.2 Short term objectives: 2012/2013

The short term objectives from April 2013 to June 2013 are as follows:

2.2.1 Objectives of Phenotypic pre-breeding programme:

- Determine Fusarium head blight (FHB) and Deoxynivalenol (DON) resistance in imported wheat lines/cultivars
- Combine FHB resistance in our current wheat irrigation cultivar spectrum
- Supply resistant germplasm for further development to interesting parties

2.2.2 Objectives of Marker-assisted backcross pre-breeding programme:

- Develop diverse FHB germplasm by using marker-assisted backcross breeding of well characterized FHB resistance sources
- Develop near-isogenic lines in South African brackgrounds for easier FHB resistance introduction into elite breeding lines
- Supply resistant germplasm for further development

3. Report on these objectives from April 2013 – May 2013:

3.1 Phenotypic pre-breeding programme:

Determine FHB and DON resistance in imported wheat lines/cultivars

In 2012, seven new novel FHB resistance donors were imported to South Africa namely: Asozaira III, Baisanyehuang, Huangcando, Hunagfangshu, Haiyanzhong, Wangshuibai and Heyne. These lines are being planted for crossings in the glasshouse (Objective 2), and will be used in a field trial for FHB screenings and evaluations this season. In June 2013, five additional resistance sources namely, Fu mai 3, Ning 7840, Shinchunaga, Wan Nian 2, Yang Mai 1, were imported and theyare to be planted and multiplied this season and they are to be used in 2014's crossing block.

Combine FHB resistance in our current wheat irrigation cultivar spectrum

Figure 1 below illustrates the 2013/2014 crossing block. In May 2013 most SGI lines were crossed with the resistance source Heyne. However the crossings with entries 1, 2, 3, 4, 5 and 6 are on-going but slow due to the light sensitivity of these exotic entries (Figure 1).

		MALE						
FEMALE	Cultivar/Line	No 1	No 2	No 3	No 4	No 5	No 6	No 7
	Baviaans							
	Semi Kortgat							
	Duzi							
	Kariega							
	Marico							

Figure 1. Crossing block of five SGI lines used as female (recurrent parents) and seven FHB resistance Donors used as male parents.

List for entries:

No 1 Hunagfangshu
No 2 Haiyanzhong
No 3 Asozaira III
No 4 Baisanyehuang
No 5 Huangcandou
No 6 Wangshuibai
No 7 Heyne

Supply resistant germplasm for further development

Six of the resistant sources mentioned above have already been made available in specific germplasm requests by SENSKO (Francois Koekemoer) and PANNAR (Willem Boshoff). Ten seeds per entry/line were supplied already in November/December 2012 covered by transfer agreements between these two companies and ARC-SGI.

No releases of specific developed material as yet.

3.2 Marker-assisted backcross pre-breeding programme:

Develop diverse FHB germplasm using marker-assisted backcross breeding of well characterized FHB resistance sources

Between April 2013 and May 2013 over 930 lines containing potential FHB resistance QTL from different combinations have been supplied to the Biotech facilities. These lines were mostly previously developed BC_2F_2 material that needed confirmation of the presence of FHB resistance QTL. DNA was extracted from these 930 entries and FHB resistance (MAS) screening with specific linked SSR markers has started in June 2013.

Develop near-isogenic lines in South African backgrounds for easier FHB resistance introduction into elite breeding lines

Since the project started in April 2013, nothing to report yet as it is too early in the project.

Supply resistant germplasm for further development

As objective 3 of Phenotypic pre-breeding programme, additional information requests were received early in 2013 by Dr. R Prins, assistance was required in obtaining articles which describe the FHB resistance QTL and markers present in a few of the newly selected FHB resistance imports and was supplied by Mr. Scott Sydenham.