

## ANNEXURE B

### **Establishment of a molecular marker service laboratory for routine application of marker-assisted selection in South African wheat breeding programs.**

#### **1 BUSINESS PLAN:**

##### Participants:

Pannar and Sensako participated since 2011 and ARC-SGI joined the 2012 cycle. Each breeding program has its own unique identity, genotypes they work with, breeding approaches and greenhouse capacity and therefore a unique strategy was designed for each.

##### IP:

Confidentiality agreements have been signed between CenGen and Sensako and Pannar, respectively. A confidentiality agreement still needs to be reached with ARC-SGI. Only leaf material will be received by CenGen for destructive analysis. All parties also agreed NOT TO distribute the gene sources for which Prof ZA Pretorius had to sign MTAs

##### Management of finances:

The employment of a full-time researcher in 2012 significantly improved CenGen's capacity to establish a sustainable service. Part-time technical assistance is required to process a large number of samples in a short period of time. In many instances only 3 weeks are available to process the samples before the plants start to produce ears for crossing. It is also anticipated that more than one breeding program will send material at the same time. The running costs for Task 1 and 2 are for developmental costs and expenses such as the purchase of test primers which may not necessarily pass the quality tests for further use. CenGen strives to treat each program equally with an accountant checking the allocation every second month, but it is also essential that we are not too rigid in this allocation. The running costs allocated to each breeding program for Task 3 (actual breeding schemes) are similarly managed within CenGen's books. After the initial year, an agreement was reached in 2011 within the group that we should request the same amount for each program and that additional needs will be addressed by the programs themselves. During 2012, we realised that this is not realistic as there is a difference in capacity and that this may become inhibitive to the successful application of MAS in RSA breeding programs. The financial request for 2014 is calculated yet again based on the breeding schemes developed for each breeding program and the experience of 2013 and will only allow for a small increase to make provision for inflation. As we are working with plants it could also happen that a specific screening need to be postponed to the following year and it is proposed that the unused funds are then offered to the programs that have more samples available for testing but we need to formalize this. No further progress has been made in this regard and it is clear that the different programs have different success levels in incorporating a MAS strategy. Dealing with the financial management is one of the most challenging aspects of the project as the main aim is to be fair to each program but also not to lose momentum due to differences in capacity.

##### Sustainability of DNA service lab:

In order to be able to do a reasonable assessment of the implementation of a DNA service to the breeding programs, initial support for a 6-year period is requested to allow a conventional breeding cycle to reach at least the F<sub>6</sub> stage. As all the targets could not be met in 2011 due to the restricted budget, we therefore request for the project to run until 2017. Annually new cycles of MAS will be implemented, which will not be completed at the time of the final report, but it will lay a foundation for a follow-up project. Critical annual evaluation of the project combined with new information becoming available on markers, marker based technologies and their application in breeding programs should make it possible to make a sound decision on whether to extend the program at least a year before the final report.

#### Target traits:

In general, there is an overall trend to utilise the same type of desirable genes for genotype building. However, as expected, each breeding programs' strategy and available resources vary and therefore a breeding scheme was designed based on very specific requirements. As we are only starting this work a lot of preliminary testing will be required, which will then eventually influence the final outcome of the project and follow-up strategies. The current project will be influenced by the breeders' requirements, marker availability for the genes they are interested in, marker informativeness in specific genetic backgrounds and the ability to get cooperation from the owners of the gene sources. At the outset the focus will be, as proven world-wide, to mostly work on single gene traits or well-characterised QTL for which marker-trait associations have been proven. A sufficient number of markers, that meet these criteria, have already been identified. In cases where perfect markers are not available, two flanking markers will be used to increase the efficiency of the markers as a selection tool. Most of the markers are already applied in CenGen and UFS research programs and optimised for use in the laboratory.

#### High throughput technologies:

Automated genotyping services are essential to the routine application of MAS (Yunbi 2010). DNA platforms such as the ABI3130xl (16 capillary) and ABI3730xl (96 capillary) are used to provide an automated genotyping analysis to ensure the generation of many SSR/STS data points in a short period of time. CenGen makes use of the ABI platforms at the Central Analytical Sequencing Facility of the University of Stellenbosch (<http://www.sun.ac.za/SAF>). They process a large number of samples *per annum* from across the country, which is much more cost-effective as the running cost per sample is significantly lower than when one needs to maintain an ABI for a specific project only. CenGen will continue with the approach of utilising central service facilities as far as possible. However, in some cases it may be necessary in the future to develop the automated equipment infrastructure at CenGen such as smaller types of robotic systems for DNA extractions and real-time PCR technology. Training was received to utilise the real-time platform purchased in 2013 and as reported in the research project progress has been made to convert some of the marker systems e.g. *Sr26* to this platform. *Capacity building should be seen as one of the components of this project required to ensure sustainability in the longer run.*

At the moment Diversity Arrays does not yet provide a low-density diversity array (DArT) service designed according to the SA breeders needs, but new developments will be closely monitored (<http://www.diversityarrays.com>). It is widely accepted amongst wheat breeders that have utilised MAS for a while that SNP technology will also in future make a significant contribution to improving the efficiency of MAS with the added advantage of a reduction in costs (Kuchel 2010 personal communication). An International Wheat SNP working group with the primary goal to facilitate open-access marker technologies based on SNPs by providing an organizational structure, communication and for making the results, both materials and information, available to the broader wheat community, has been established (Akhunov 2010). A lot of new SNP markers can now be accessed through the Cereal Genomics website ([www.cerealsdb.uk.net](http://www.cerealsdb.uk.net)). Access to unpublished SNP markers for some of the genes we are currently selecting for using the ABI system has been obtained through Dr. Susanne Dreisigacker (CIMMYT). At the same time our UFS-research group with Dr Lesley Boyd (NIAB, Cambridge) was successful in securing a BBSRC-SCPRID project in which we obtained the basic equipment needed to run the KASPAR SNPs system. The equipment was installed and we received training at the end of May 2013 to use these new platforms. Optimisation of protocols will first take place in our BBSRC-SCPRID research project but it is estimated that implementation of this technology will start to take place towards the end of 2013.

#### High costs:

All scientists are in agreement that the costs of MAS are one of the major limitations of the technology. As the allele frequency of some of the target genes/traits is expected to be low (such as the improved version of the *Sr26* translocation), the initial costs are also higher in the first generations. As the allele frequencies of target alleles are increased (we already see an increase in 2013 vs 2012), more markers can be tested at the same time in a specific genotype, which will result in a reduction of costs per data point. Increased allele frequencies will

also be obtained by feeding back lines developed in this project to the crossing blocks of the respective programs.

#### Problematic areas:

All gene interactions in specific backgrounds are not known and also not which genes produce additive effects (Hospital 2009; Laugdahl 2010.) Once we have obtained specific gene combinations and they can be evaluated in the field it is possible that the line MAY NOT react as expected. Although care is taken to limit the combination of e.g. more than one translocation line, it cannot be predicted which genes may affect yield or quality negatively and we expect that some combinations will need further backcrosses before it will result in a commercial cultivar. It is also possible that some combinations may not be desirable at all. Once we have this type of feedback those gene sources will be reconsidered in future.

In terms of wheat quality it is not clear which specific marker alleles need to be targeted by SA breeders as a test such as an alveograph in effect 'test' many different genes contributing to quality, simultaneously. Several DNA markers are available for instance to select for specific high molecular weight glutenin subunits in wheat (Liu et al 2008). Literature states that subunits Dx5 and Dy10 of the *Glu-D1* locus are required for strong dough and good bread making quality and that the opposite is true for Dx2+Dy12. However, several of the South African cultivars of acceptable quality, carry the latter combination (Jan Cilliers, personal communication). We therefore rather propose to include these markers in an assessment of which alleles are present (Task 2) before applying it on a routine basis for selection. Another example is the mutation of either the Puroindoline-a (*Pina*) gene or Puroindoline-b (*Pinb*) gene that results in hard grain texture, which is one of the most important phenotypes related to milling, baking and noodle making. A deletion mutation of *Pina* (*Pina-D1b*) is widely distributed among common wheat cultivars (Ikeda et al 2010). The presence of known hardness alleles was studied in a representative sample of 373 bread wheat lines from the breeding program at CIMMYT. The PINA-null mutation (*Pina-D1b*) was the most frequent hardness allele and present in 283 of the 328 lines with hard endosperm. All other hard wheat had the glycine to serine mutation in PINB (*Pinb-D1b*). A study of historically important CIMMYT bread wheat lines showed that *Pina-D1b* has been the dominating hardness allele since the inception of the wheat breeding program in Mexico (Lillemo et al 2006). In the USA they have selected for the *pinB-D1b* allele for grain texture but no information exists in SA whether this is an allele we should target for the South African milling and baking requirements. In short, there are thus markers for traits available which have not been properly assessed before in SA and we first would like to establish the frequency of these mutations in our material. A potential PhD candidate is currently busy with a literature study to investigate the possibility to map some of the important milling and baking quality traits in the Kariega X Avocet S population and already increased the seed for the first rounds of field trials (privately funded). The outcomes of this study can then be implemented in future in this project.

Timely delivery of marker data by CenGen is also dependant on the timely reception of material. The breeders are working with a living organism with growing cycles that is influenced by genotype and environmental conditions. We are therefore prepared that they may need to adapt some of the schedules.

The securing of money abroad through the BBSRC-funded project to establish SNP technology as part of the MSI project, which will allow a higher throughput and a significant reduction in cost for MAS in wheat.

#### MAS strategy:

Breeders are faced by many complex choices in the design of efficient crossing and selection strategies aimed at combining desired alleles into a single target genotype (Wang et al 2007). Substantial planning went into the participating breeding programs' crossing and breeding schemes to ensure that the relevant population sizes were chosen to recover a sufficient number of the target genotypes, but at the same time an effort was made to limit advanced population sizes by implementing allele enrichment steps in early generations or during the haploid phase of doubled haploid (DH) production (Bonnett et al 2005; Kuchel et al 2005, 2007; Wang et al 2007; William et al 2007; Yunbi et al 2010). In order to achieve a sufficient number of DH plants that can go through to the field screenings, the numbers have to be increased significantly to obtain enough plants that can serve as haploid donors using the wheat X maize system. Should the microspore technique work well, it will reduce the number of plants that needs to be screened with markers considerably.

We will not implement background selection on a routine basis. It will only be considered, if it becomes evident that in certain combinations it is required to recover the receiver background. We will follow the work that is being done on genome-wide selection strategies (Piyasatian et al 2007; Sorrels 2010), but is of the opinion that it is not an option until thousands of SNPs have been identified and a platform for routine application has been established. This type of approach may be necessary to target complex traits such as yield.

**PROGRESS FOR REPORT PERIOD 1 JULY 2012 - 30 JUNE 2013 IN ITALICS (Laboratory activities were summarised up to 12<sup>th</sup> of July 2013):**

**TASK 1: Gene donor material and cultivars that will be used to transfer genes to.**

It is foreseen that this task will be performed on an annual basis as new gene sources or marker information for gene sources become available.

**1.1. Requisition of gene donors:**

Although CenGen and UFS assisted in the requisition of useful donor sources from abroad and will attempt to do so in future, the decision was made that each breeding program will take responsibility for bringing in donor material from international researchers and SA breeders and multiplying it. This is an ongoing task as useful gene sources are identified and characterised worldwide and made available on a continuous basis. For the material brought in by Prof ZA Pretorius a genetic material transfer agreement has been signed with the University of Adelaide (Australia). *Rust resistance sources were given to all three breeding programs. These resistance sources will only be used in the context of this project as we need to provide quarantees to the international donors of the resistance sources that we are good stewards of these gene sources. The breeders also actively sourced other useful gene donors as required by their specific program, internationally. In the UFS research program doubled haploids combining the Yr-QTL derived from Karioga and Cappelle Desprez were developed. DNA of these DH lines was extracted and characterization is currently underway. The best lines will be given to the breeders, which will save time in terms of making only a single cross instead of two to combine these QTL in their respective backgrounds. The increased frequencies of target alleles will also fast-track marker screens.*

**1.2. Provision of cultivars/lines in crossing blocks for task 2.2:**

On an annual basis breeders will provide background cultivars (estimate 10-20 cultivars per breeding program), tag individual plants and send leaf material (destructive analysis) to CenGen for a DNA fingerprint and marker screen with gene-associated markers (see TASK 2.2).

**1.3. Pure seed stocks:**

Breeders harvest seed of these individual plants and keep as pure seed-stock for crosses with donor material in crossing block.

**TASK 2: DNA characterization of germplasm**

**2.1. Information:**

Literature and internet search for marker sequence availability for markers not used thus far and suggested by breeders will be done on a continuous basis. Breeders will be made aware of new developments and a conscious effort thus far was also made to help the breeders 'assimilate' this information. Breeders will also be visited annually.

*R Prins was in regular email and telephone contact with the breeders. She also met with Pannar and Sensako, whilst teaching at UFS in Bloemfontein. The breeder of ARC-SGI already had other commitments at the time of her visit. ARC-SGI and Sensako breeders visited CenGen as well.*

**2.2 Annual marker screens of cultivars in crossing blocks:**

Donor and receiver material will be screened annually with markers for use in gene tagging in order to determine marker informativeness and to generate a preliminary DNA fingerprint. The design of specific crosses for genotype building will depend whether the relevant markers are informative in these specific combinations.

*Gene-associated markers:*

Here the focus will be a bit broader than the actual schemes in Task 3, as markers for traits such a HMW-glutenin alleles (quality), reduced height, Russian wheat aphid, etc. will be included to establish a knowledge base on the alleles present in the breeding programs for possible implementation as needed along with the targets set in Task 3. It is not yet known for many of the traits for which markers have been published which ones are required in the South African conditions, especially some of the quality characteristics.

Preliminary fingerprints will point out any variation within the five receiver plants per cultivar. If variation does occur, a specific plant will be chosen as representative of the cultivar. It will also point out diversity and in cases where a cross is made with an unadapted background, the fingerprint can then be used to select for the adapted background in cases where field trials reveal that specific individuals need an additional backcross to recover the adapted background. This will be done on a very limited scale and mostly in cases where the gene source has a wild species' origin. It is also during this stage that new markers for traits such a quality traits will be tested.

*This step is also used to fine-tune laboratory operations and therefore more work may be generated in a specific program due to repetition of data sets. As we become more familiar with the marker sets used and the allele ranges of these marker sets, the allocation to each program will become more equal.*

## **Task 2: General**

*Samples processed for Task 2 for the period 16<sup>th</sup> July 2012- 12<sup>th</sup> July 2013*

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
<i>DNA extractions</i>	0
<i>Fluorescent PCR</i>	366.5
<i>Multiplex Fluorescent PCR</i>	112.5
<i>ABI runs</i>	192
<i>PCR</i>	284
<i>Restriction digests</i>	18
<i>Agarose gels</i>	20

**Task 2: Pannar**

Samples processed for Task 2 for the period 16<sup>th</sup> July 2012- 12<sup>th</sup> July 2013

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
DNA extractions	280
Fluorescent PCR	212
Multiplex Fluorescent PCR	180
ABI runs	908
PCR	198
Restriction digests	35
Agarose gels	9

**Task 2: Sensako**

Samples processed for Task 2 for the period 16<sup>th</sup> July 2012- 12<sup>th</sup> July 2013

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
DNA extractions	542
Fluorescent PCR	2933
Multiplex Fluorescent PCR	624
ABI runs	1033
PCR	0
Restriction digests	154
Agarose gels	4

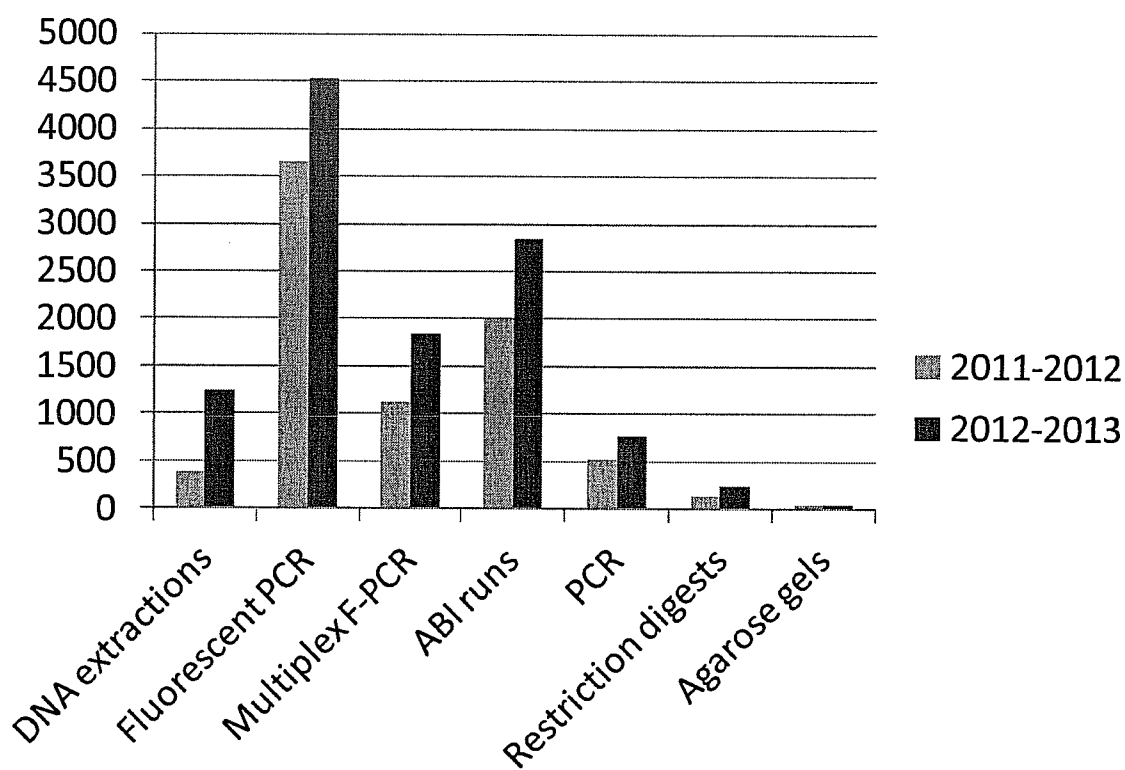
**Task 2 ARC-SGI:**

Samples processed for Task 2 for the period 16<sup>th</sup> July 2012- 12<sup>th</sup> July 2013

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
DNA extractions	424
Fluorescent PCR	1024
Multiplex Fluorescent PCR	928
ABI runs	704
PCR	290
Restriction digests	37
Agarose gels	11

Comparison of total activities related to Task 2 for 2012 and 2013

<b>Activity</b>	<b>Year 1 2012</b>	<b>Year 2 2013</b>
	<b>24 July 2011- 16 July 2012</b>	<b>16 July 2012 - 12 July 2013</b>
<i>DNA extractions</i>	390	1246
<i>Fluorescent PCR</i>	3657	4536
<i>Multiplex F-PCR</i>	1130	1845
<i>ABI runs</i>	2008	2837
<i>PCR</i>	528	772
<i>Restriction digests</i>	132	244
<i>Agarose gels</i>	45	44



### **2.3 High throughput screening capacity development:**

Emphasis is placed to develop capacity and methodologies to handle and screen thousands of plants from different sources in an efficient way within the required time-period. Experimental design will aim at reducing man-handling of the samples thereby limiting the chances for the introduction of mistakes e.g. sample switches, incorrect labelling and wrong identity assignments. Practical laboratory aspects such as 1) the collaboration with the breeders to develop an efficient sample and data tracking system that can feed back into their databases, 2) sample provision in 96-well format, 3) the routine usage of a simplified DNA extraction protocols in a plate format, 4) optimisation of experimental conditions for specific sets of markers to assist in automated allele assignment, will receive attention.

#### **DNA extractions:**

*The 96-well plate extraction protocol is now used routinely. The same format will be maintained for the SNP platforms, but 4x 96-well plates will be combined in a 384-well format for further analysis. To achieve the cost reduction it is thus essential that the breeders generate enough plants in a specific cross to fill at least 4x 96-well plates.*

*We are still struggling to grind the leaves to fine powder before the DNA extraction protocol can commence. The current scientific equipment such as the in-house Tissue-lyser has not been designed for the number of samples we process and breaks frequently. We are investigating alternative options such as modifying a 'Paint-shaker' that is more robust. Alternate options such as a Genogrinder is quite costly (>R250 000) and we first would like to see whether a local engineer cannot assist us with a cheaper solution. It is also anticipated that if we can get the grinding process more even that the PCR amplifications will also be more consistent.*

#### **PCRs:**

*PCRs are done in the same format as the extraction plates and single tube handling are kept at a minimum.*

#### **Automated capillary electrophoresis on ABI platform:**

*The laboratory is in daily contact with the Central Analytical Facility (<http://www.sun.ac.za/SAF>). They are made aware of the fact when they receive samples that the breeders need to do crosses on and where it is thus necessary to have a high turn-over. In general we receive data within 48 hours of submission and it seems difficult to get to a 24 hour turn-over. As the ABI platform is very sensitive, we worked with them to improve quality control in order to detect any problems associated with the run itself.*

*By converting those markers that qualify for Real-time PCR and KASPar SNP technology we will also gain in turn-over time, as the 'electrophoresis' step is then eliminated. The technology will also help us to optimise PCR conditions for PCR markers.*

#### **Data analysis:**

*Marker sets have been designed around specific gene/QTL or gene/QTL sets. This is probably the most time-consuming step in the whole procedure as the raw data files received from the ABI needs careful inspection. Often we have to work with markers that do not have optimum profiles for automatic scoring by the software and many hours are needed to ensure that we call the alleles correctly. The breeders that are becoming familiar with dealing with allele scores and have been very helpful in populating the breeding scheme management files with the molecular data they received. However, CenGen still works very closely with the breeders to manage their data files.*



***New laboratory facilities:***

*CenGen has made a significant investment in 2012 in developing better infrastructure and the new facility became operational in August 2012. The bigger facility allowed expansion of the CenGen team to seven people. A part-time BSc student studying at UNISA (Cheryl le Roux) specifically assists Dr Agenbag with technical assistance in the MSL project. The improved facility also allows the separation of processes to separate rooms improving experimental quality control and the incorporation of new technologies. Capacity has also been increased for full-time student training with the joining of full-time PhD student (Hester van Schalkwyk. Post-graduate students of SU(maize) and UFS (wheat) have already been hosted for short periods to come and work at CenGen.*

***New equipment:***

*The new Real-Time PCR and SNPLine Light equipment will result in a higher throughput and reduction in costs.*

**TASK 3: MAS schemes**

Based on available information detail planning has been already done in cooperation with breeders. We first would like to test the systems and capacity (greenhouse; laboratory; peak periods) with the projects as presented here, before we plan further crosses. *However, to ensure the successful implementation of MAS, it is envisaged that follow-up strategies will start in each consecutive year depending on the availability of funding.*

**Marker-traits:**

As we are implementing technology we will initially focus on markers and traits that:

- 1) were developed and/or tested in-house,
- 2) that has a proven track record of reliable trait-marker associations e.g. published on the MASwheat database (<http://maswheat.ucdavis.edu>), or
- 3) have been used by international breeders successfully (Gupta et al 2010).

Most of the proven marker–trait associations are for disease resistance [stem, stripe and leaf rust, fusarium head blight (FHB)]. The focus on rust resistance will be on utilising adult plant rust resistance genes of which several have been identified in a WCT-funded project ('Development and application of marker-assisted selection systems for adult-plant resistance to rust diseases of wheat'). As soon as information is obtained via the public domain or through validation in Task 2, as to which alleles should be selected for in terms of traits such as quality, these markers can be implemented at any stage as most of the receiver backgrounds will be cultivars of acceptable milling and baking quality.

As stated before, the focus at the start will be on target selection and only in rare cases where the material demonstrate inferiority adopt an additional backcross cycle supported by background-selection as the cost is quite inhibitive to apply it on a routine basis.

Primers will be labelled according the requirements for each breeding program in order to load as many markers as possible in one lane. Therefore a situation may occur where the same marker may be labelled with a different colour for a specific purpose.

Breeders are also requested to add a identity tag i.e. MAS to the lines that were developed via the MAS route to enable us to measure the impact of this investment, especially as some of the material will also enter the conventional breeding program via the crossing block or advanced yield trials without any further marker testing.

The following abbreviations are used in the tables for target traits:

FHB = fusarium head blight resistance

LrAPR = adult plant resistance to leaf rust

Lr = leaf rust resistance

YrAPR = adult plant resistance to stripe rust

Sr = UG 99 effective stem rust genes

Dn=Russian wheat aphid resistance

PHS = preharvest sprouting resistance

### TASK 3A: PANNAR

At present 26 breeding schemes are in progress. The schemes involve a combination of conventional and MAS strategy including stacking of specific target alleles through selective generation enrichment aiming at increasing the frequency of favourable alleles in breeding lines and future releases. Follow-up strategies will start in each consecutive year depending on greenhouse capacity and funding.

*Samples processed for Task 3 for the period 16<sup>th</sup> July 2012-12<sup>th</sup> July 2013:*

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
<i>DNA extractions</i>	5612
<i>Fluorescent PCR</i>	21265.7
<i>Multiplex Fluorescent PCR</i>	3829
<i>ABI runs</i>	8176
<i>PCR</i>	936
<i>Restriction digests</i>	2392
<i>Agarose gels</i>	30.5

**TASK 3B: Sensako**

Nineteen schemes have been designed using a DH approach with allele enrichment at the haploid stage. Follow-up strategies will start in each consecutive year depending on greenhouse, DH capacity and funding.

*Samples processed for Task 3 for the period 16<sup>th</sup> July 2012-12<sup>th</sup> July 2013:*

<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
<i>DNA extractions</i>	1490
<i>Fluorescent PCR</i>	6352
<i>Multiplex Fluorescent PCR</i>	2512
<i>ABI runs</i>	3287
<i>PCR</i>	379
<i>Restriction digests</i>	1190
<i>Agarose gels</i>	12

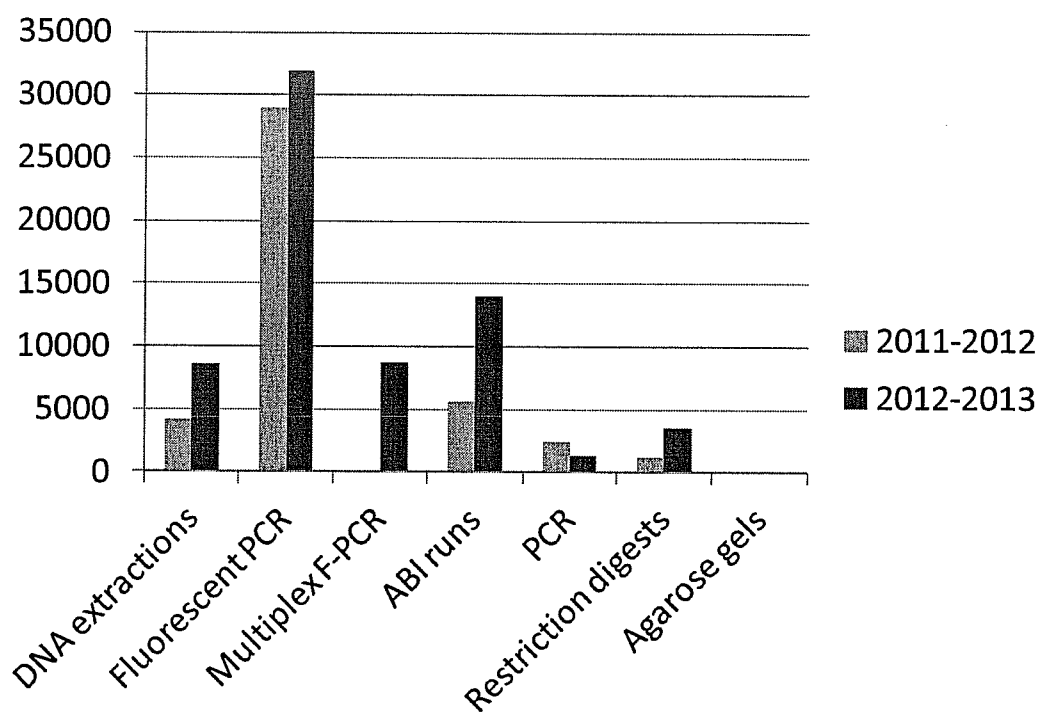
**TASK 3C: ARC-SGI**

The ARC-SGI started off in 2012 with five breeding schemes incorporating MAS.

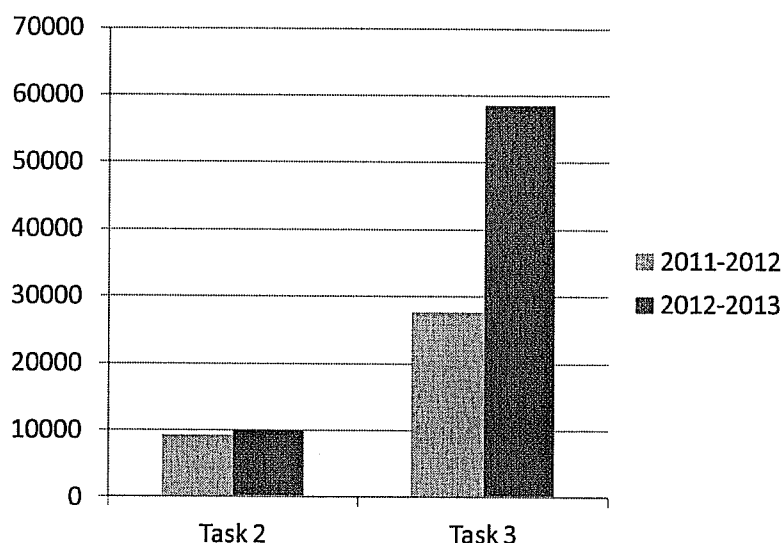
<b>Activity</b>	<b>16 July 2012 - 12 July 2013</b>
<i>DNA extractions</i>	1520
<i>Fluorescent PCR</i>	4348
<i>Multiplex Fluorescent PCR</i>	2405
<i>ABI runs</i>	2496
<i>PCR</i>	0
<i>Restriction digests</i>	0
<i>Agarose gels</i>	1

**TOTAL NUMBER OF ACTIVITIES (Task 3) in MSL for the 16<sup>th</sup> July 2012-12<sup>th</sup> July 2013:**

<b>Activity</b>	<b>Year 1 2012</b>	<b>Year 2 2013</b>
	<b>24 July 2011- 16 July 2012</b>	<b>16 July 2012 -12 July 2013</b>
<i>DNA extractions</i>	4179	8622
<i>Fluorescent PCR</i>	28961	31966
<i>Multiplex F-PCR</i>	0	8746
<i>ABI runs</i>	5696	13959
<i>PCR</i>	2464	1315
<i>Restriction digests</i>	1216	3582
<i>Agarose gels</i>	107	44



Although there was an increase in laboratory activity for Task 3 with > 8000 plants being processed in the lab, the most notable improvement was in the number of data points being generated as indicated in the graph below.



The data points generated for Task 2 stayed fairly stable as expected and planned. However we needed to accelerated the outputs for Task 3 to meet the challenges faced by the breeders that they need to make crosses and selections within a certain period, which left us with limited time to process the samples. Almost 60 000 data points were generated through:

- 1) Streamlining processes further in the lab such as the development of multiplex PCRs where more than one marker was amplified at the same time.
- 2) The fact that breeding material are coming through where more than one gene has been stacked i.e. the frequencies of desirable alleles have been increased and therefore one sample is screened with many markers which also allow an increase of data points.

**The output achieved is comparable with the reported data points generated by CIMMYT's marker lab, when this project was submitted in 2011.**

***Overall comment:***

*These tables do not reflect the considerable amount of time spent to manage data files and breeding schemes but give an indication of the practical outputs.*

*The breeders can now compare their genotypic data sets obtained through MAS with their phenotypic data gathered in field trial. This information allows informed decision on line selection, thereby avoiding carrying on with lines that do not carry target genes.*

**Anticipated outcomes:**

- Identification of specific crosses that need to be made to introduce the required genes. This will be program-dependent and will vary for each (*Achieved in 2013*)
- Marker optimisation and validation (*Achieved in 2013*)
- Marker implementation on a significant scale (*Implementation has already been achieved in 2012. Some programs have embraced the technology fully, whilst others are still struggling to incorporate it routinely. It has been recognised that the capacity at the breeding programs (especially greenhouse capacity are in itself a limitation to the success of this project.)*)
- Routine screenings for MAS (*More than 8000 plants were processed this year with almost 60 000 data points generated.*)
- Capacity building in high-throughput routine service provision (*The allocating of a full-time researcher to this project is a major step forward. We also brought in a student on a part-time basis to assist with the technical work. Through our collaboration with Dr Lesley Boyd, we were successful in securing the first SNP platform that has been proven to work well for wheat which will also impact positively on the work we do for SA wheat breeders. We have received training and access to SNP assays of importance and this will be implemented in the latter part of 2013.*)