CHAPTER 6

PRELIMINARY STUDY ON THE DEVELOPMENT OF CAPS MARKERS FOR FUTURE APPLICATION IN MAS STRATEGIES IN THE SOUTHERN HIGHLAND TANZANIAN MAIZE HYBRID BREEDING PROGRAM

Introduction

Most plant traits are quantitative i.e. controlled by many genes (QTLs) together with environmental factors (Babu *et al.,* 2003), however, some traits are controlled by one or few genes (Young *et al.,* 1999). In classical genetic improvement programs selection is carried out based on phenotypes but without knowing which genes are actually selected. The development of molecular markers was therefore greeted with great enthusiasm as it was seen as a major breakthrough promising to overcome this key limitation (Liu and Cordes, 2004). Well-designed studies using genetic markers will undoubtedly accelerate identification of genes linked to quantitative trait loci (QTLs) for marker-assisted selection (Morgante and Salamini, 2003).

It is documented that for most traits, the location and characteristics of genes are unknown. Therefore identification of tightly linked molecular marker is an important step for molecular characterization of plant genes (Yu *et al.,* 1994). The identification of molecular markers linked to specific genes is usually a three-step process: (1) Assessing the mode of inheritance of the plant trait and the molecular marker; (2) Verification of the linkage between the marker and the trait through segregation analysis (Hayes and Goddard, 2003); and (3) Calculation of the recombination fraction and linkage distance (Yu *et al.,* 1994).

Maize crop is severely attacked by gray leaf spot (GLS) disease caused by the fungus *Cercospora zea maydis,* (Tehon and Daniels, 1925). To date, the GLS control methods in use are: field sanitation, crop rotation, chemicals and host resistance. Host resistance, however, is considered the best option for managing GLS as it is environmental friendly, inexpensive and very effective (Bubeck *et al.,* 1993; Saghai-Maroof *et al.,* 1996). Sources of GLS resistance in maize are available in the cultivated crop (Bubeck *et al.,* 1993; Dunkle and Levy, 2000; Gordon *et al.,* 2004) and in the wild maize relatives (Gevers and Lake, 1994). All these genes are important for initiation of marker assisted selection (MAS) in concert with backcrossing in maize breeding programs. It is documented that when wild germplasm is used as a donor parent in backcross breeding, there are problems of linkage drag, whereby an undesirable trait becomes tightly linked with desirable genes

(Peleman and Van der Voort, 2003; Claudio De Giovanni *et al.,* 2003). Thus, the availability of molecular markers linked to the GLS resistant genes and low genetic distance within the cultivated maize germplasm as compared to wild relatives (Rick *et al.*, 1976; Gevers and Lake, 1994) could overcome linkage drag problems, sterility problems associated with cytoplasm and increase the efficacy of MAS. Also since GLS resistance is quantitatively inherited with narrow sense heritability (Mahn, 1977), MAS could be efficiently used to select maize genotypes resistant to GLS. Several published papers such as that of Pratt *et al*. (2003) suggested that it is important to breed for GLS host resistance by using both conventional and molecular marker assisted selection. They viewed it as important components of integrated pest management (IPM) of disease control strategies. Similar studies of using MAS to select GLS resistant hybrids were proposed by Lehmensiek *et al*. (20001), Bubeck *et al*. (1993) Gordon *et al*. (2004) etc. They further added that MAS is able to pyramiding quantitative resistance factors.

In Tanzania, GLS is an important maize disease hindering maize production. In order to control this disease, the Tanzanian Maize Breeding Program produces many hybrids yearly and screen them phenotypically for GLS resistance in multi-environments. However, pitfalls of phenotypic selection are firstly, this type of selection is not very effective for lowly inherited traits like GLS resistance. Secondly, susceptible genotypes can be selected for which have escaped the disease by chance, and thirdly, selections of GLS resistant genotypes using developed molecular markers from different backgrounds become less effective and reliable when used across other backgrounds. Thus, the main aim of the preliminary study was therefore to develop cleaved amplified polymorphic sequence markers (CAPS) putatively linked to GLS resistance that in future, after proper testing in Tanzania, can be used in MAS strategies in the Tanzanian Maize Breeding Program.

Materials and methods

Plant material

The population for molecular markers development linked to GLS resistant genes using Tanzanian germplasm was developed at Uyole Agricultural Research Farm, in the Mbeya Region, Tanzania, for two consecutive seasons, during the 2002 and 2003

rain seasons. In the 2002 rain season, the inbred line, P62145, a highly GLS resistant commercial line was crossed with line P103, a highly GLS susceptible inbred (Lyimo per. comm.*[1]). The resulting $F_{1:2}$ population was selfed to produce an $F_{2:3}$ progeny which was segregating for the GLS resistant genes in the 2003 rain season. When the $F_{2:3}$ plants were about 88 days old, they were morphologically scored for GLS disease on individual plant basis according to Donahue *et al.* (1991) (scale where 1 = no GLS symptoms, with an increment of 0.25, to 9 = highly GLS susceptible). During 2004, the $F_{2:3}$ plants were grown to produce $F_{3:4}$ in the green house of the Pretoria University, South Africa, and DNA were extracted from each plant.

DNA extraction

Genomic DNA was extracted from leaves of each GLS scored $F_{3:4}$ plant as described by Doyle and Doyle (1987). Total genomic DNA was extracted from approximately 100 mg of leaf tissue using 5 % (w/v) Cetyltrimethyl ammonium bromide (CTAB) [0.1 M Tris-HCL (pH 8.0), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 1% (w/v) soluble Polyvinylpyrrolidone (PVP) and 0.2 % (v/v) 2-Mercaptoethanol]. Genomic DNA of each plant was precipitated by using either ice-cold isopropanol or 95 % (v/v) ethyl alcohol. The genomic DNA pellets were dissolved in either 100 µl double distilled water or in low TE buffer [10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA] and then stored at -20 °C. Quantification of the genomic DNA was done by using a spectrophotometer.

GLS resistant and susceptible bulks

Two bulks were prepared for GLS marker development and analysis. The resistant bulk (GLSRB), was prepared from the most resistant individuals by pooling together equal DNA concentrations of six $F_{2:3}$ plants (plant numbers 1, 8, 11, 28, 32 and 35) that phenotypically showed no symptoms of GLS disease and were rated as GLS score of one. The susceptible bulk (GLSSB), was prepared in a similar way but in this

[1] *N.G. Lyimo. Uyole Agricultural Research Institute, Box 400 Mbeya, Tanzania.

case the six most susceptible $F_{2:3}$ individuals (plant numbers 67, 69, 105, 106, 108 and 112) were selected on the basis that they highly succumbed to GLS and had a GLS score of nine.

AFLP Analysis for production of bands of interest

Amplified fragment length polymorphism (AFLP) was carried out as described in the IRDye™ Fluorescent AFLP® Kit for large Plant genome analysis with minor modifications. Briefly, a total reaction volume of 12.5µl was used for digestions, and each sample contained 100 ng genomic DNA, 1.0 µl *EcoR*1/*Mse*1 enzyme mix [1.25 U/µl each in 10 mM Tris-HCL (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA, 50 % (v/v) glycerol, 0.15 % Triton X-100] and 2.5 µl of 5x reaction buffer [50 mM Tris- HCL (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate]. These were mixed gently, centrifuged briefly, incubated in the water bath at 37 °C for 2 hours and then placed at 70 °C (15 min) to inactivate the restriction enzymes. The second step involved ligation of the adaptor sequences to the restricted DNA fragments.

Ligation was done by mixing 12.0 µl of the adapter mix and T4 DNA ligase [5 U/µl in 10 mM Tris- HCL (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 50 mM KCL, 200 µg/ml BSA, 50 % (v/v) glycerol] to the previous tube. The mixture was centrifuged and incubated at 20 °C for 2 hours. This was followed by performing a 1:10 dilution of the ligation mixture by mixing 10 µl of the mixture and 90 µl of TE buffer [10 mM Tris-HCL (pH 8.0), 1.0 mM EDTA]. Preselective amplification involved mixing 2.5 µl of the diluted (1:10) ligation mixture from the step above, 20 µl AFLP preamplification primer mixture, 2.5 µl of 10x PCR reaction buffer and Taq DNA polymerase (5 U/µl) in a total volume of 25.0 µl. These were mixed gently and then amplified using a thermocycler Gene Amp PCR® System 9700 Model. The preamplification PCR profile was: 20 cycles at 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1 min. The preselective products were diluted 1:260 by taking 10 µl of the preamplification product and adding 250 µl of ddH_2O or low TE [10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA]. The selective step had a total reaction volume of 20.0 µl,

with the following consumables: 7.0 µl diluted preamplification DNA template, 2.0 µl 10x PCR buffer, 2.0 µl dNTP (2.5 mM), 1.2 µl $MgCl_2$ (25 mM), 0.8 µl *EcoR*1-ACA (1 mM), 0.5 µl *Mse*1 (6 mM), 0.12 µl Taq polymerase (5 U/µl) and finally 6.38 µl. The PCR reaction for selective was also done in a thermocyler Gene Amp PCR® System 9700 model.

AFLP primer screening and selective amplification

The primers used for pre-amplification and amplification were similar to those described by Vos *et al.* (1995) with *EcoR*1/*Mse*1 extensions ACA/CTG, ACA/CAG, ACA/CCG, ACA/CCC and ACA/CGC. Only two primer combinations (ACA/CGC and ACA/CCG) from the five screened produced polymorphisms between the GLS resistant and GLS susceptible samples, and were thus used in this preliminary study. The *EcoR*1 primers were 5' labelled with infrared dye (1 µM IRDye700 or IRDye 800, LI-COR, Lincoln, NE, USA). The PCR profile for selective amplification was: one cycle of 94 °C for 10 s, followed by 13 cycles of 65 °C for 30 s, with 0.7 °C decrease/cycle. Then there was 23 cycles of 94 °C for 10 s, 56 °C for 30 s and 72 °C for 1 min, with 1 s decrease /cycle. Finally, one cycle of primer elongation at 72 °C for 1 min.

Electrophoresis and excision of fragments

AFLP fragments were resolved in 8 % LongRanger™ polyacrylamide gels the LI-COR IR2 automated DNA analyser (LI-COR, Lincoln, NE, USA) using a 0.4 mm thickness gel which is suitable for fragment cutting. The AFLP gel was scanned using an Odyssey Infrared Imager instrument (LI-COR Biosciences, Lincoln, Ne, USA) in order to facilitate the cutting of polymorphic bands from the gel. The polymorphic fragments present in the resistant parent and resistant bulk but absent in the susceptible parent and susceptible bulk were excised for cloning. All the exercised fragments were "squashed" in 50 µl low TE [10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA] or ddH_2O and left at 4 °C for a week to facilitate elution of DNA from the gel. Thereafter the samples were centrifuged at 1200 rpm for 5 min and recovered on agarose gel before re-amplification of fragments using the AFLP primer specific to

the fragment. After amplification, each AFLP fragment was verified for purity on a 3 % agarose gel containing ethidium bromide (0.5 μg/ml) and visualised under UV light. Each band on the agarose was excised and recovered with the QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany). The excised and purified fragments were cloned into pGEM®- T Easy Vector (Promega) for transformation to competent JM109 *E. coli* cells according to the manufacturer's instructions. The competent cells were prepared adopting the Hanahan (1985) method. The presence of insertions was assessed in a restriction digestion of plasmids following an alkaline lysis preparation (QIAprep 8 mini prep Kit Qiagen) and the sizes of the positively cloned fragments were verified using colony PCR (Gussouw and Clarkson, 1989). The amplicons were subjected to cycle sequencing of both strands using the BigDye™ Dye Terminator Kit (Perkin-Elmer, USA) and the SP6 (5'-ATTCTATAGTGTCACCTAAAT-3') and T7 (5'-TAATACGACTCACTATAGGG-3') primer sets (Promega, USA). Sequencing of clones was done by Macrogen Biotechnology Company, USA.

Primer Design and synthesis

The obtained sequences were subjected to homology searching using BLASTNn and BLASTx (http://www.ncbi.nlm.nih.gov or for further information please see Altschul *et al.*, 1997), and multiple sequence alignment was conducted using CLUSTALW software (http://www.ebi.ac.uk/clustalw). Primer design was done using the obtained sequence information and the Primer 3 software package (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Results

Five AFLP primer combinations were used in Chapter 3 for the prediction of best line combiners and heterosis in Tanzania maize breeding lines. From the initial screening of the breeding lines, primer *EcoR*1-ACA+*Mse*1-CGC had a marker index (MI) of 16.5 and *EcoR*1-ACA+*Mse*1-CCG had a MI of 20.4 (Table 3.3). Two of these, *EcoR*1-ACA+*Mse*1-CGC and *EcoR*1-ACA+*Mse*1-CCG were utilized in the development of putative markers, and screened against the DNA from GLS resistant

parents and GLS resistant bulk and the GLS susceptible parents and GLS susceptible bulk (Figure 6.1).

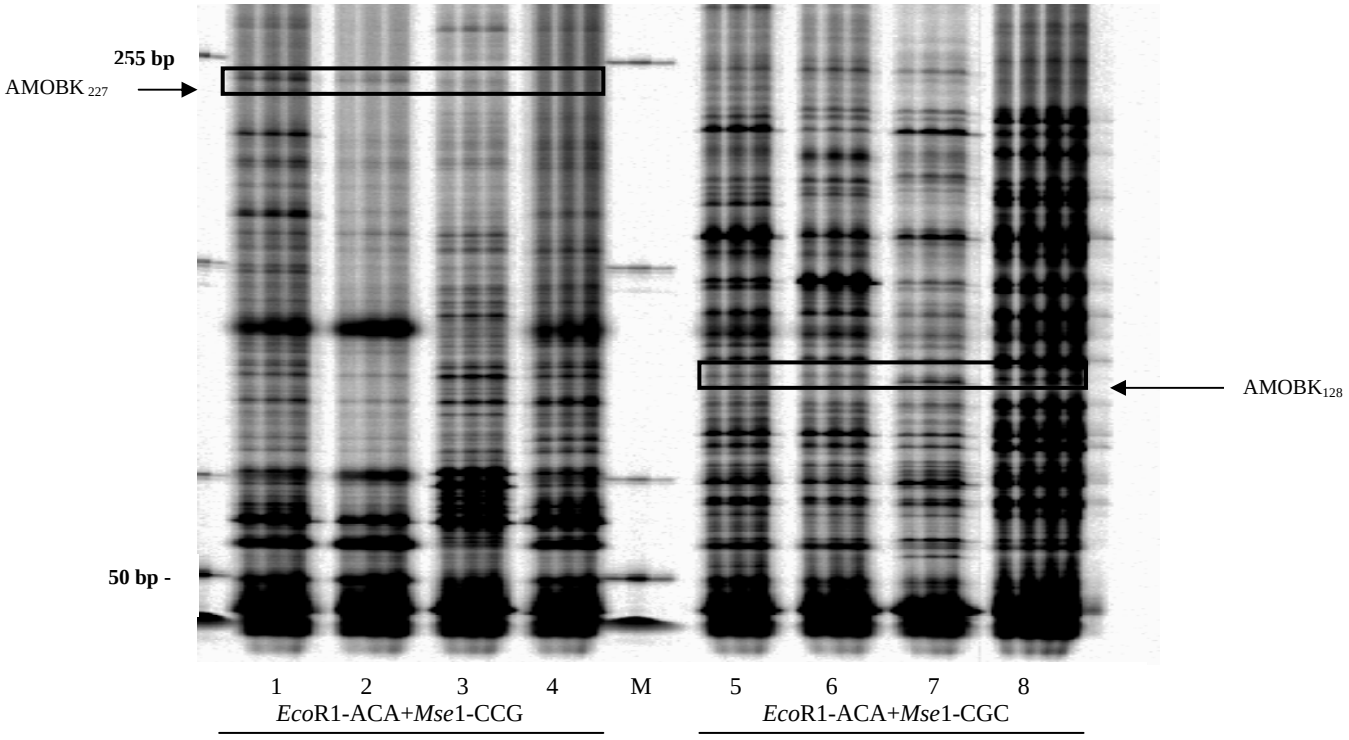

Figure 6. 1. A part of the AFLP gel obtained after AFLP analysis of the susceptible and resistant maize DNA with primer combinations *Eco*R1-ACA+*Mse*1-CGC and *Eco*R1-ACA+*Mse*1-CCG. Where 1 and 5 = GLS resistant parent; 2 and 6 = GLS resistant bulk; 3 and 7 = GLS susceptible parent; 4 and 8 = GLS susceptible bulk; and M = 100 bp marker. Also indicated are two of the polymorphic fragments that were excised, cloned and sequenced characterized, i.e. $AMOBK_{227}$ (227 bp in size) and $AMOBK_{128}$ (128 bp in size).

After analysis of the AFLP profiles, 15 polymorphic bands were obtained which discerned between the resistant and susceptible GLS bulks (Figure 6.1). Of these, four bands were putatively linked in repulsion phase to GLS resistance, while 11 fragments were putatively linked in coupling phase. AFLP primer combination *EcoR*1-ACA+*Mse*1-CGC resulted in polymorphic fragments of approximate sizes of 452 bp and 260 bp in coupling phase, while 190 bp and 260 bp were in repulsion phase. Furthermore, two polymorphic bands in repulsion phase (i.e., sizes 128 bp and 174 bp) were obtained with primer combination *EcoR*1-ACA+*Mse*1-CCG and nine

polymorphic fragments (i.e., sizes 373 bp, 316 bp, 276 bp, 227 bp, 219 bp, 214 bp, 208 bp, 82 bp and 78 bp) were linked in coupling phase (Figure 6.2).

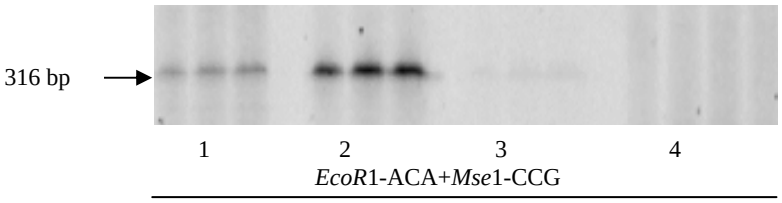

Figure 6. 2. An enlarged section illustrating the polymorphic fragment $AMOBK_{316}$ that discerns between the GLS resistant parent and GLS resistant bulk *versus* GLS susceptible parent and GLS susceptible bulk after analysis using *EcoR*1-ACA+*Mse*1-CCG primer combination. Where 1 = GLS resistant parent; 2 = GLS resistant bulk; 3 = GLS susceptible parent and 4 = GLS susceptible bulk.

Of these putatively GLS linked markers, 15 fragments were excised from the polyacrylamide gels after scanning with the Odyssey Infrared Imager. These were purified, cloned and sequenced. After removal of the vector sequences, the putative sequence annotation and alignment followed. Ten of these sequences were omitted due to poor sequence quality or sequence length making them uninformative (not shown). Surprisingly, the remaining five sequences all showed significant homology to a partial 18S rRNA gene (E-value of $2e^{-69}$) and a partial ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene region (E-value of $2e^{-67}$) of an uncultured soil fungus when using BLASTn (not shown), but to the hypothetical protein 3 from *Microplitis demolitor* bracovirus (E-value of $7e^{-14}$) when using the BLASTx search (Figure 6.3).

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Query  270  QETAMTMITPSYLGDTIEYSSYASNALGALPYGRPAGGREFTSDYLG----DTIEY  115
            QE AM MI PSYLG  IEYSSYAS ALGALPYGRPAGGREF SD+L     DT EY
Sbjct  37   QEPAMXMIPPSYLGAXIEYSSYASXALGALPYGRPAGGREFXSDFLQMPFLDTEEY  92
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Figure 6. 3. Clone AMOBK276 exhibiting significant homology to the hypothetical protein 3 from *Microplitis demolitor* bracovirus (E-value of $7e^{-14}$) when using BLASTx search.

The sequences were then aligned using multiple alignment software (Figure 6.4).

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CLUSTAL W (1.83) multiple sequence alignment

AMOBK227          ------------------------------------------------------------
AMOBK219          ------------------------------------------------------------
AMOBK452          GGGATAAACNTGGATGCCATTGGCGATTGAGCCGACGTCGCATGCTCCCGGCCGCCATGG 60
AMOBK316          ------------------------------------------------------------
AMOBK276          ------------------------------------------------------------

AMOBK227          ------------------------------------------------------------
AMOBK219          ------------------------------------------------------------
AMOBK452          CGGCCGCGGGAATTCGATTGATGAGTCCTGAGTAACCGAGAAGAAAATCATCAGGAACCA 120
AMOBK316          ------------------------------------------------------------
AMOBK276          ------------------------------------------------------------

AMOBK227          ------------------------------------------------------------
AMOBK219          ------------------------------------------------------------
AMOBK452          CACAGCCAATGCCAAAGCAAAGGTCGTGTTGATATCTGAAGTTGGGACGATACGCCATTT 180
AMOBK316          --------GGGGGAGTATGAGNAANNCGATATGAAGGGGTATTGGGTAACCAA------C 46
AMOBK276          -------------------ATAAACGCG-TAGATAATGCGATTGGGCCGACG-------T 33

AMOBK227          ----------------------------------AAAAGGGCATACATGTGTGATGAGCC 26
AMOBK219          ----------------------------------AAAAAGCGATACTTGGGCGATGAGCC 26
AMOBK452          CGTATGTTCGCCGCCGATTACGGAAGCCACATAGGCCACATGATCTACCGGCAGGAAGTC 240
AMOBK316          CGCATGCGTTCAGCCG-CCGTGGCGGCCGACCGCAGGCTCGACCATATAGGGAGAGCTCC 105
AMOBK276          CGCATGCTCCCGGCCG-CCATGGCGGCCGCCTGCAGG-TCGACCATAT-GGGAGAGCTCC 90
                                                                 *       *

AMOBK227          GA-CGTGCATGCTCCCGGCCGCCATG-----GCGGCCGC--GGGAATTCGATATCA-TA- 76
AMOBK219          GA-CGTGCATGCTCCCGGCCGCCATG-----GCGGCCGC--GGGAATTCGATATCACTA- 77
AMOBK452          CATCGCGTTCATCACCAGTACCCACACGAATACGGTTACTCAGGACTCATCAATCACTA- 299
AMOBK316          CAACGCAGTTGGATGCA-TAGCTTTGAGTATTCTATAGTG-TCACCTAAATAATCACTAT 163
AMOBK276          CAACGC-GTTGGATGCA-TAGCTT-GAGTATTCTATAGTG-TCACCTAAATAATCACTA- 145
                    * **          *     *          *             *     **** **

AMOBK227          GTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA 136
AMOBK219          GTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA 137
AMOBK452          GTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA 359
AMOBK316          GTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA 223
AMOBK276          GTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA 205
                  ************************************************************

AMOBK227          TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGC-TGT 195
AMOBK219          TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGC-TGT 196
AMOBK452          TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGTGTGC 419
AMOBK316          TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGC-TGT 282
AMOBK276          TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGC-TGT 264
                  ***************************************************  **

AMOBK227          -TTCCTGA-------------- 202
AMOBK219          -TTCCTGAT------------- 204
AMOBK452          GTTCCTGAA------------- 428
AMOBK316          -TTCCTGATTTAAAGACGATTT 303
AMOBK276          -TTCCTGACAGA---------- 275
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Figure 6. 4. Nucleic acid alignment of five cloned fragments putatively linked to GLS resistance. Asterisks (*) represent regions of sequence consensus, while gap (-) represents openings. Forward and reverse primers are indicated in red bold text.

The multiple sequence alignment showed significant sequence consensus (i.e., 115 bp) between the different cloned fragments ($AMOBK_{219}$, $AMOBK_{227}$, $AMOBK_{276}$, $AMOBK_{316}$ and $AMOBK_{452}$) in the region with significant homology to the partial 18S rRNA gene (E-value of $2e^{-69}$) and ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene regions (E-value of $2e^{-67}$) of an uncultured soil fungus. The reason for this is still puzzling, since the clones were all different in size when verified using colony PCR technology (not shown).

Primers were then designed using Primer 3 software (Figure 6.4). Since all the targeted fragments are putatively linked to GLS resistance in coupling phase, two strategies were followed during primer design, namely specific primers (i.e., outside the consensus region) and generic primers (i.e., targeting the consensus region). The primers will in future be tested on Tanzanian populations presently in preparation.

To conclude, although the predictions for these primers to be "useful" as GLS linked markers are low, since the excised fragments shared a surprisingly high sequence identity, they will still be tested once the populations for testing are available. However, the search for more putative markers is ongoing, but due to time constraints the results will not be included in the thesis document.

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CHAPTER 7

CONCLUSION

Maize is the staple food for more that 60 % of Tanzanians, but its production and productivity are highly reduced by gray leaf spot disease especially in the Southern highlands of Tanzania. This disease significantly reduces grain yield, kernel quality and silage quality. In order to manage this disease, however, different GLS control strategies such as the use of fungicides, field sanitation, crop rotation, removal of field debris and combination of methods are widely applied. But these control measures are either expensive or biologically unfriendly to the environment or less effective. Furthermore, adoption of exotic maize varieties usually fail due to poor adaptation. Thus, the Tanzanian maize research has put more efforts to breed maize hybrid varieties that are resistant to GLS by using conventional breeding methods which may not be always very effective for traits like GLS resistance that is poorly to moderately inherited. Despite all these efforts, there are still few commercial hybrids which are insensitive to GLS pathogen in Tanzania.

This study, therefore, aimed to produce more GLS resistant hybrids for commercial use by integrating molecular marker technology and conventional resistance breeding which is much safer to the environment, more effective than other control methods and an inexpensive strategy of GLS control. Also, the study aimed to increase the farmers choice of growing different types of GLS insensitive hybrids and ensure a constant supply of GLS resistant hybrids in case of GLS hybrid breakdown of resistance. Furthermore, no molecular data on maize is available for Tanzanian maize cultivars which could assist plant breeders to choose inbreds with regard to carrying combining ability in the production of commercial maize hybrid varieties. Thus, the identification of best inbred combiners is still a major challenge to maize breeders in Tanzania. Many breeding strategies such as crossing parents from different heterotic groups, pedigrees, use of tester lines, etc have been used extensively. Lack of progress in breeding for resistance to GLS has been attributed to the limited effectiveness of phenotype-based selection due to the impact of environmental factors. Hence there is a need to combine GLS resistant genes and high yielding traits in hybrids. Complementation of molecular marker and phenotypic selections could therefore increase the efficiency of breeding maize cultivars resistant to GLS. This study assessed the genetic diversity of highly/moderately GLS resistant

inbreds of Tanzanian germplasm and then predicts the potential of these parents to produce high yielding GLS resistant hybrids. The assessment of genetic variation aimed at testing the genetic variation of the Tanzanian inbreds as a way to ensure against genetic erosion of the present gene pools.

In this study the efficacy of AFLP marker system for grouping inbred lines into genetically similar clusters was assessed. AFLP fingerprinting of genotypes was complemented/supplemented with an investigation which aimed to study the associations between AFLP based genetic distances and F_1 morphological data that included many agronomically important traits like 50 % silking, ear length, rows/ear, kernels/row yield and GLS ratings. Furthermore, this study aimed to develop cleaved amplified polymorphic CAPS marker bands putatively linked to GLS resistant genes which in future can be tested and applied in marker-assisted selection (MAS) to identify high yielding GLS resistant hybrids in an efficient way and/or in marker based backcrossing programs to develop parents in a shorter period of time.

DNA fingerprinting of the 21 inbred lines using 5 AFLP primer combinations detected a total of 259 AFLP marker bands of which 83.2 % were polymorphic and 16.8 % were monomorphic. The average genetic distance (GD) of all the 21 lines was 25.5. The GD of pair wise line crosses varied from a minimum of 0.14 to a maximum of approximately 0.5. Theoretically, the GDs of 0.5 crosses are expected to produce high yielding commercial hybrids, but such crosses will require field observations to confirm their validity in GLS “hot spot” studied zone. The study results exhibited that the AFLP marker as a fingerprinting tool showed higher r(GD, f) than the correlations recorded with SSR and RFLP in previous studies which also proves its superiority and power on assaying a lot of genetic loci.

The UPGMA dendrogram grouped together the assayed lines into three main clusters and four outliers. The AFLP results effectively grouped the lines according to the established heterotic groups but with very minor discrepancies since the established heterotic groupings are all based on morphological data. Morphological data are not capable of

identifying those traits that are masked/unadapted/recessive. Results also revealed that the inheritance of resistance to GLS is low.

In addition to the AFLP study, a pair wise genetic analysis of the inbred lines with their F_1 morphological data revealed the genetic distance of intergroup crosses was much higher than the mean genetic distance of intragroup crosses. This implied that higher yielding hybrids are predicted from intergroup (i.e. between populations) crosses than from intragroup crosses. Results also identified crosses between heterotic groups such as those which exhibited high pair wise GD of more than 0.40. These crosses are line 21 (P62145) crossed with the following lines: line 13 (K53015213), line 14 (K37581011), line 16 (CML37) and line 18 (P621111), as well as crosses of line 13 (K53015213**) x** line 14 (K37581011). These crosses with high pair wise GDs are predicted to produce higher yields due to their genetic dissimilarities, and line P62145 showed the best general combining ability (GCA) in this study.

A genotype x environment analysis (G x E) of the 225 highly/moderately GLS resistant hybrids evaluated in multi-environments revealed that hybrids 90, 45, and 48 were the top yielding and consistently exhibited lowest GLS susceptibility across locations and years. Also hybrids 72, 189 and 107 recorded higher yields and good GLS tolerance. Finally, in a preliminary study 5 cleaved amplified polymorphic sequence (CAPS) markers putatively linked to GLS resistant genes in the studied maize population was developed.

In conclusion, the AFLP DNA fingerprinting of the Tanzanian lines have proved to be powerful tools, highly reproducible, could assay a lot of genetic loci and can be effectively used for characterization of lines and clustering of germplasm according to their genetic similarities. Similar AFLPs studies could be conducted in other Tanzanian maize breeding programs for the protection of breeders' rights of the released varieties and/ or elite commercial inbred lines and for the prediction of best inbred combinations for commercial hybrids use. Intergroup crosses especially with high GD-MPH associations should be the main target for the production of commercial hybrid varieties

as they always show high level of heterosis which is a function of crossing genetically dissimilar lines. Intragroup crosses generally exhibit low pair wise GD-MPH associations. Hence intragroup hybrids are always not suitable for commercial hybrid production. They may also produce hybrids that are more prone to both biotic and abiotic stresses and also may suffer more from the deleterious effects of inbreeding depression and degeneration in comparison to intergroup crosses. However, the intragroup crosses could be useful in the production of modified single crosses, three way crosses and single hybrid seed production if the inbreds used in the crosses exhibit a certain level of heterosis or complement/supplement each other for the traits that are agronomically important for the resulting hybrids. Furthermore, some intragroup hybrids might also be used to make silage as they can exhibit more vegetative growth as the main sink instead of ears.

Finally, the G x E study results showed that GLS disease is highly influenced by both weather factors and locations and thus it is important that new varieties should be tested in different weather conditions as well as locations. Highly GLS resistant with high level of general and specific combing ability should be used. Also testing locations should be truly representative of all the areas that are agro-ecologically different in terms altitude, annual precipitation, soil type, temperature, with regard to disease occurrence and be done over seasons until significant year effects in terms of GLS severity and incidence are revealed. Lastly, characterization of the GLS pathogen is imperative since information on virulence of isolates is needed for long term management strategies against the pathogen.

ANNEXURE