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# Increasing line combining ability and gray leaf spot resistance in maize by integrating conventional with DNA marker technology

by

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**CO-PROMOTOR**: Dr. N.G. Lyimo

## DECLARATION

I the undersigned hereby declare that this thesis submitted herewith for the degree Doctor of Philosophy to the University of Pretoria, contains my own independent work as hitherto not been submitted for any degree at any other university or faculty.

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Date

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# INCREASING LINE COMBINING ABILITY AND GRAY LEAF SPOT RESISTANCE IN MAIZE BY COMBINING CONVENTIONAL BREEDING WITH DNA MARKER TECHNOLOGY

**BY**

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SUPERVISOR: Prof. A-M Oberholster
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## ABSTRACT

Maize is the staple food for the majority of Tanzanians. However, maize production in the Southern highlands of Tanzania (SHT) is highly reduced by gray leaf spot disease (GLS) caused by the fungus *Cercospora zea maydis*. GLS reduces grain yield, kernel and silage quality. The most common GLS control methods in Tanzania include amongst others; fungicides, crop rotation, field sanitation, host resistance. These methods except host resistance are, however, either expensive or less effective or unsafe to the environment. Furthermore, conventional breeding strategies are not very effective for traits, which are lowly inherited such as GLS resistance. Lastly, to date there are few GLS resistant commercial hybrids in SHT. Thus, this study aimed to produce more commercial GLS resistant hybrids, increase farmers' hybrid choices of growing genetically different GLS insensitive hybrids, which will also provide a constant supply of GLS resistant maize cultivars in case of GLS resistance breakdown due to new GLS pathotypes. This research combined conventional breeding with molecular technologies to increase the efficacy of selecting GLS resistant hybrids and assist breeders in

predicting best inbred combinations for commercial hybrid production. Studies conducted to meet the main aims were on; the prediction of best line combiners and heterosis in Tanzanian maize breeding lines through the use of amplified fragment length polymorphism, (AFLP), an association of AFLPs and the performance of phenotypic traits in maize, evaluation of maize hybrids for gray leaf spot resistance in multienvironments and finally a preliminary study on gray leaf spot PCR-based marker development with the long term objective of implementing cleaved amplified polymorphic markers (CAPS) in a marker assisted selection (MAS) strategy in the SHT maize breeding programme.

Results from the study revealed that pairwise GD (genetic distance) of the lines varied from a GD of 0.13 to 0.5. High coancentry coefficients were exhibited by these lines. Joint data analyses showed that there were tighter associations between line GD and $F_1$ traits or MPH in the intergroup than in the intragroup crosses. Combined analyses revealed that hybrids 48, 90 and 45 recorded higher stable yields and consistently low GLS scores in multienvironments. Fifteen CAPS marker bands were identified that are putatively linked to the GLS resistant genes.

In summary, it was noted that strong selection during inbreeding programs should be avoided as it reduces germplasm variability. Local landraces/varieties can be improved by introgressing desirable genes into them. AFLP marker system could be effectively used for inbred genetic diversity studies in Tanzania. Intergroup crosses with high GD-MPH should be the main target for commercial hybrid production but field testing of

them is inevitable to confirm their yielding potentials. Intergroups and intragroup crosses with low GD-MPH should be discarded to avoid field costs. Better $F_1$ hybrid performance predictions can be achieved by integrating inbred GD and $F_1$ phenotypic data. Hybrids with low GLS/high GLS resistance could be used to produce other breeding populations. Hybrids 45, 48 and 90 can be commercially preleased. Lastly a study to characterize the GLS fungus in the SHT is imperative since information on virulence of isolates is needed for long term breeding strategies against the fungus. Finally, the SHT maize germplasm has potential GLS resistant inbred lines which could be used in the deployment of genes to susceptible lines and in the development of commercial GLS resistant hybrids/open pollinated varieties/doubled haploid hybrids.

***Keywords:*** AFLP, dendrogram, GLS, GD, genetic diversity, germplasm.

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# ABBREVIATIONS

| | |
|---|---|
| AFLP | Amplified fragment length polymorphism |
| AMOVA | Analysis of molecule variance |
| APS | Ammonium persulphate |
| ASAP | Allele specific associated primers |
| CAPS | Cleaved amplified polymorphic markers |
| CIMMYT | Centro international de Mejoramiento de Mazy Trigo |
| CTAB | Cetyltrimethyl ammonium bromide |
| DAPS | Days after planting to silking |
| EDTA | Ethylene diamine tetra acetate |
| G x E | Genotype x environment |
| GCA | General combining ability |
| GAS | Gene assisted selection |
| GD | Genetic distance |
| GLS | Gray leaf spot disease |
| LD | Linkage disequilibrium |
| LE | Linkage equilibrium |
| MABC | Marker assisted backcrossing |
| MAS | Marker assisted selection |
| MI | Marker index |
| PCR | Polymerase chain reaction |
| PIC | Polymorphism information content |
| QTL | Quantitative trait loci |
| RAPD | Random Amplified polymorphic DNA |
| RCBD | Randomized complete block design |
| RFLP | Restriction fragment length polymorphism |
| SCA | Specific combining ability |
| SCAR | Sequence characterized amplified region |
| SHT | Southern highlands of Tanzania |
| SSR | Simple sequence repeats |
| STS | Sequence tagged sites |
| UMC | a molecular marker which is linked to GLS resistant genes in maize |

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# DEDICATION

I hereby dedicate my thesis to my LORD JESUS CHRIST, the SAVIOUR of the whole human race and THE POWER OF OUR RESSURECTION. In him we live, we walk and without him nothing can be done. So it is only God and God alone who makes all things possible including the accomplishment of this thesis.