

CHAPTER 1

GENERAL INTRODUCTION

Maize (*zea mays* L.) is a staple food for millions of people (dietary energy consumption i.e. kcal/person/day) in several African countries, Asia and South America (FAO; 2004). While in the USA and Europe animal feeding is by far the largest user of maize (USDA, 1986). Maize is among the most important coarse grain consumed by both human beings and livestock. In 1996, the world's average maize yield was 4.1 MT/ha, while in Africa in the same year; the average production was 1.7 MT/ha (Moshi *et al.*, 1997). These figures show that only the USA and Europe reported a commercially viable yield, which came about through the use of hybrids together with improved management practices (USDA, 1996). So the question remains what are the future prospects in Africa? Bantayehu (1985) reported that the available technologies are inadequate, while Olson and Sander (1988) said the adoptions of improved management are necessary.

Maize has been improved from a wild grass to one of the most productive crops. Its breeding work, especially for hybrid development started in the early 1900s with the work of Shull (1909) and others. Maize breeding has been effective in developing improved varieties and hybrids to meet the rapidly changing cultural and environmental

conditions of this century. The development of the commercial seed industry is in testimony of successful breeding methods that have been followed for the economic production of acceptable high quality hybrid seed (Hallauer and Miranda, 1988).

Agricultural crops get damaged if they are not protected against diseases (Hagenboom, 1993). Crop protection, therefore, is necessary not only for the maintenance of production capacity, but also to prevent negative effects on the quality of the products (Hagenboom, 1993; Ayliffe and Lagudah, 2004; Hall, 2003). For example, fungal diseases are economically important to man because they reduce both crop yield and quality. Consequently, many governments use a lot of their resources to control crop pathogens. Losses in maize yield bring famine, reduce industrial productivities, cause unbalanced diets, malnutrition and even death etc. It is clear that crop losses are very high and every effort should be taken to reduce them. Crop protection may be realized by different means: chemical, biological, plant resistance, molecular techniques, etc. Of these, plant resistance is highly preferable. It is inexpensive as compared to others, best for the farmer, and biologically safe to the environment (Parlevliet, 1993; Gevers and Lake, 1994).

Gray leaf spot (GLS) is one of maize foliar diseases that is currently recognized as the most yield limiting disease of maize world wide (Ward and Nowell, 1998; Dunkle and Levy, 2000; Gordon *et al.*, 2004), especially in the USA and Africa. GLS is caused by the fungus *Cercospora zea maydis*. This disease was first reported in the USA in 1925 (Tehon and Daniels, 1925). The disease has continued to expand its geographic distribution and increased its intensity over the past 25 years (Ward *et al.*, 1999). In addition to the USA and Africa, GLS has been reported in Asia (Coates and White, 1994) and South America (Latterell and Rossi, 1983).

GLS is of major importance in Africa with its resultant severe reductions in grain yield and quality. This disease poses serious repercussions concerning the food security and nutrition of the African countries further widening the food deficits in these nations (Ward *et al.*, 1999). The impact of this disease can be ascribed to the fact that maize is

the major staple food for most of the indigenous rural populations of Africa (CIMMYT, 1990). Estimates of yield losses attributed to GLS for example in South Africa, are frequently as high as 30-60 percent for moderately resistant and susceptible hybrids (Ward *et al.*, 1997). Other deleterious effects of GLS are increased lodging; reduced grain quality, poor silage quality and no mechanical harvesting can be done on the highly infected hybrids (Gevers and Lake; 1994, Ward *et al.*, 1999).

An outbreak of this disease in Kwazulu Natal, South Africa resulted in the first official report from the African continent (Gevers *et al.*, 1994). In Africa, GLS has now become pandemic (Nowell, 1997). Large areas of farmland currently under maize production in other geographical areas are potentially at risk, should the pathogen be introduced and environmental conditions become favourable to this disease. The current rise in the incidence and spread of the GLS has been ascribed to conservation tillage, increase in plant debris from season to season and use of genetically vulnerable maize genotypes (Roane 1950; Ward *et al.*, 1994). Furthermore, the disease rise in many countries has been aggravated by the fact that many national maize breeding programs have released numerous inbreds, hybrids, and germplasm with no direct or conscious selection for resistance to GLS. (Elwinger *et al.*, 1990; Huff *et al.*, 1988).

In Tanzania, maize is the most important staple food grown and consumed. Approximately 1.7 million hectares are annually cultivated with maize which accounts for about 60 percent of the total area planted with cereal crops (Moshi *et al.*, 1997). Although grain yields are highest in the highlands of Tanzania, where there is sufficient and reliable rainfall, maize production per unit area is still low. This low production is a result of a variety of constraints including diseases, poor soils, poor weed control, pests (Moshi *et al.*, 1997), etc. Among these production constraints, gray leaf spot disease is the most yield limiting factor in the Southern Highlands of Tanzania (SHT).

Gray leaf spot disease was reported for the first time in Tanzania (SHT) during the 1994/95-rain season (Lyimo and Mohammed, pers. Comm.). But a recent study at Uyole research institute revealed that only one (or at most two) commercial hybrid (UH615) are

resistant to GLS. However, some inbreds such as P62145 and others developed from population 62 (an open pollinated variety) are good sources of GLS resistance with potential to be deployed effectively in the SHT maize breeding program for the production of GLS insensitive maize cultivars as they express different type of resistances to GLS. For example highly resistant inbreds show fleck type of lesions, while moderately resistant display chlorosis, and highly susceptible materials exhibit necrotic spots. This disease has now been reported from other regions in the country and is still moving northward following the major corn belt of Tanzania. Thus, it is possible that if GLS disease is not managed properly it may spread to the rest of the country. Hence the hypothesis of this study was that hybrids in the SHT highly succumbed to GLS disease. GLS management measures are therefore necessary for increased and sustained maize production.

Current control measures of GLS like field sanitation, use of fungicides, crop rotation, combination of methods, etc, are not very effective (Dunkle and Levy, 2000; Ward *et al.*, 1997) and are either uneconomical or environmentally unsound (Ward and Nowell, 1998; Elwinger *et al.*, 1990) or both. Furthermore, the pathogen may develop resistance to the fungicides (Ward *et al.*, 1999). Breeding for disease resistance using conventional methods seem to be somehow limited by the quantitative nature of the inheritance of GLS resistance (Saghai Marroof *et al.*, 1996). Thus, the need to integrate conventional breeding strategies (i.e. phenotypic-based selection) with molecular techniques such as marker assisted selection (MAS). These strategies seem to be the most feasible approaches for breeding resistance with quantitatively inherited traits like GLS. Conventional technique is very effective for highly inherited traits while molecular methods are effective for lowly inherited traits. So integrating phenotypic data with molecular information seem to increase the efficacy of selection as they complement/supplementation each other. The advantage of molecular selection is to pyramid GLS resistant quantitative loci in the selection. Also, molecular strategies are able to identify those hybrids which have escaped the disease by chance during the selection process.

In view of the seriousness, destructiveness, the rapid spread, crop losses due to GLS and the importance of maize to the Tanzanians (as the most staple food and or /cash crop), there is thus an urgent need to manage this disease. The crop losses due to gray leaf spot disease stipulated this study. Thus the main objective of the study was to effectively control GLS disease in the studied area by using an efficient, cost effective, more durable, friendly and safe to the environment method of integrating conventional with molecular techniques of maize breeding. Furthermore, this research investigated the effects of genotype x environment on GLS, yield and other agronomically important traits of the studied materials across multi-locations. Also, this study aimed to identify the most suitable locations for the hybrid production and their stability of performance in many environments. Finally, this work aimed to produce commercial GLS resistant hybrids for the farmers in the SHT, to increase farmer's choice of growing GLS resistant hybrids and or in case of GLS resistance break down. In order to achieve the study main goal, it was important to assess the genetic diversity or composition of the SHT breeding materials and then apply this knowledge to meet the research objectives. This study combined conventional with molecular techniques of plant breeding to produce high yielding GLS insensitive hybrids in an efficient manner. The potential benefits of this study is to apply molecular techniques with phenotypic-based selection that are able to increase the efficacy of identifying best line combinations with maximum heterosis, and also improves screening procedures for identifying hybrids with stable GLS genetical resistance across environments. Thus, the main objectives of this study were:

1. To predict best line combinations for the production of commercial GLS resistant hybrids in the SHT. Hence it was necessary to study the genetic diversity of the selected moderately and highly GLS resistant maize inbreds by applying AFLP-DNA fingerprinting protocol.
2. To compare and correlate genetic distance (GD) of the maize lines with important morphological traits of their F₁ progeny.
3. To evaluate hybrids for GLS resistance in multi-environments by using phenotypic-based traits in order to assess their yields, identify their adaptation areas for optimum production and evaluate their stability of performance.

4. To do a preliminary study of developing putative PCR based molecular marker bands linked to GLS resistant genes which will be used in marker-assisted selection with phenotypic selection as a way of increasing the efficacy of selecting GLS resistant hybrids.

Chapter two describes a detailed review of the available literature concerning GLS disease in maize. This chapter has several sections which deal with the discovery and the outbreak of GLS in the world, the biology of the GLS causative fungus *Cercospora zea maydis*, the development GLS epidemiology, symptoms and importance of GLS, the current control methods of GLS and lastly the use of knowledge of DNA molecular markers in maize resistance breeding. The rationale of this review is to generate knowledge, guidance, direction and methodologies, which could assist breeders to develop maize hybrids with durable GLS resistance in different environments by improving the current conventional methodologies and integrating with modern technologies of biotechnology.

In Chapter three, I assayed the genetic diversity of the selected SHT maize germplasm by using the AFLP-DNA fingerprinting protocol (by applying the knowledge of pairwise genetic distance of lines) so as to predict the potential of these inbreds to produce high yielding GLS resistant hybrids. Pairwise genetic distance of lines is based on the theory that genetically dissimilar parents produce high yielding hybrids when crossed. DNA fingerprinting using the AFLP protocol was also important as a way of assessing the existing genetic variation in the selected germplasm and to ensure against depletion of the existing gene pools. This chapter forms the basis of my thesis for marker development as some AFLP bands could be linked to certain genes of interest which might be used as putative markers for GLS-genotype selection purposes. The other objective of AFLP-DNA fingerprinting was to investigate the usefulness and the potential of AFLP data to determining the best line combinations for increased hybrid heterosis. Information from inbred DNA fingerprinting also helps to give information on heterotic groupings which the later assists to give information on general and specific combining ability and possibly prediction of best line combiners.

Chapter four looked at the inbred AFLP-GDs and their F₁ phenotypic trait associations. It also investigated the inheritance of GLS resistance in maize. The information obtained from chapter four could complement data of chapter three required to predict best line combinations for commercial GLS resistant hybrid production. Furthermore, this chapter generated information of correlations between agronomically important traits that can be simultaneously selected for and fixed in inbred lines especially when they are tightly linked during inbreeding programs.

Chapter five focused on production, evaluation and identification of GLS resistant hybrids in multi-environments. The rationale of this chapter was that resistance breeding appears to be superior to other control methods such as use of fungicides, cultural control, and crop rotation. The limitations of the latter control strategies are that they are either expensive or less effective or both. Such demerits prompted the use of resistance breeding as the best GLS control method. It is important to note that information of pairwise GDs of inbred lines that were predicted using AFLP-fingerprinting (chapter three) were then combined with studies of correlations of GDs of inbred lines with morphological data and inheritance of GLS resistance (chapter four) enabled to identify high yielding crosses of maize which had high level of GLS resistance. Furthermore, evaluation of hybrids for GLS resistance was necessary in order to quantify the effects of environment and G x E on the phenotypic expression of different hybrid traits. These hybrids were evaluated in “hot spot” GLS disease pressure areas across locations and seasons in the SHT as a prerequisite for evaluating genotypes for GLS resistance. Other objectives of this chapter were to identify areas of adaptation of these hybrids for their optimum commercial production, to increase the number and choice of GLS resistant hybrids for the farmers in the SHT and finally, aimed to ensure a constant supply of GLS insensitive hybrids in case of GLS resistance breakdown of the commercial hybrids.

In Chapter six, Putative cleaved amplified polymorphic molecular marker bands were developed, which are tightly linked to GLS resistant OTLs in maize. The rationale for developing these putative markers was due to the fact that currently, in Tanzania, to the

best of my knowledge, there are no molecular markers that have been developed in the past /on going work which are linked to the GLS insensitive genes. Thus, this chapter attempted a preliminary study on the development of CAPS markers for future application in MAS strategies in the SHT maize hybrid breeding program. Some of the merits of molecular selection are that it is able to identify those genotypes which have escaped the disease by chance. Also, molecular marker selections help to pyramid GLS resistant factors during the selection process. The importance of PCR-based molecular markers over other DNA markers is that a lot of genotypes can be screened for the gene of interest in a shorter period of time and at a lower cost. The development of markers using the Tanzanian population was significant to advancement in the SHT breeding program, as markers from different backgrounds usually become less effective and reliable when used across other backgrounds.

Finally, it is my believe that combining MAS and phenotypic selection to identify GLS resistant hybrids, as a long term strategy in the SHT breeding work will optimize the efficacy of selection gains for the breeding program when compared with phenotypic or marker selection alone. I anticipate that combining molecular technology with phenotypic selections could complement each other and effectively improve breeding for resistant to GLS disease.

Lastly, this thesis presents the first molecular-genetic study in maize in the SHT. It explored the knowledge and application of the associations between parental GD and F₁ crosses for the identification of best line combinations which could be used to produce commercial GLS resistant hybrids. The impact of this research will directly result in the germplasm genetic improvement as well as release of GLS resistant hybrids.

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