CHAPTER 2

LITERATURE REVIEW

Gray leaf spot (GLS) of maize is a foliar disease caused by the fungus *Cercospora zea maydis* (Tehon and Daniels, 1925). The disease was first described from samples taken in Illinois, USA, in 1925. There were a few reports of the disease in the 1940s (Arndt, 1943; Lehman, 1944; Roane, 1950). In 1950s (Roane, 1950) and 1960s (Kingsland, 1963) the disease was relatively rare. However, during the early 1970s the number of outbreaks reported increased (Latterell and Rossi, 1983). It is now a disease of agricultural importance in the warmer regions of USA (Latterell and Rossi, 1983; Bubeck *et al.,* 1993, Saghai Maroof *et al.,* 1996; Wang *et al.,* 1998) and in South Africa (Gevers *et al.*, 1994; Ward *et al.,* 1997).

Significant outbreaks of GLS have also been reported in South America, for example in Peru, Colombia, Brazil, Trinidad (Chupp, 1953). In South Africa, the disease was first observed at Greytown areas during the 1988/89 season and then at Cedara in 1992 (Ward and Nowell, 1997; Gevers *et al.,* 1994). It has since spread throughout the province of Kwazulu-Natal and in neighbouring provinces and countries (Ward and Nowell, 1997). In Tanzania, it was first reported during the 1994/95-rain season (Lyimo and Mohammed pers. Comm.).

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2.1 The genus *Cercospora*

Cercospora Fresenius, is one of the genera of the fungi imperfecti (fungi which contain conidial or imperfecti stage). This genus has relatively broad and large conidia. Mature conidia are 70-180 µm long and 5-6 µm wide at the base. They are tapering to 2-3 µm at the apex (Latterell and Rosi, 1983). As with most fungi, however, these dimensions as well as those of conidiophores vary considerably with the conditions under which these structures are formed especially with respect to natural or artificial substrate, moisture, light and age of stroma. Stromatic tissue of the fungus is normally found in the substomatal chambers of the infected host tissues. These stromatic tissues later produce conidiophores that merge in clusters of up to 20 in number. Conidiophores can be found emerging from leaf blades, sheaths and husks (Latterell and Rosi, 1983). The principle characteristics of this genus include the following parts:

(1) **Conidia:** These are the principle source of the characters, which separate *Cercospora* from other genera, and that differentiates species within the genus itself (Chupp, 1953). Most true species of *Cercospora* have their conidia hyaline or pale coloured (Chupp, 1953). About 6-10 septa and germinate readily (within 3 hours) from any cell, three or four germ tubes called "appresoria" typically emerging during infection process on corn tissues. It should be noted that spermagonia (bodies containing permatia), the presumed male gametes of the sexual state of the pathogen (Latterell and Rossi, 1977) develop in mature lesions from the stromatic cells in each substomatal cavity. They are filiform, obclavate, straight or curved not cylindrical, tips acute to obtuse, multiseptate, and borne singly and terminally or by the further growth of conidiophores.

The **base of the conidia**: The form of the basal end of the conidium of the genus *Cercospora* is probably the most dependable character on which differences in species of *Cercospora* are based (Chupp, 1953). For example if one species is found in any given country/environment with truncate base the same base will differentiate it wherever collections are made.

(2) The **conidiophores**. These are the pathogen structures which arise singly from procumbet heads or in fascicles, from interlacing threads or from a distinct troma. Conidiophores are septate or unicellular less dependable than the conidia in furnishing stable characters of the genus (Chupp, 1953). They, however have a number of specific variations, which should be used to determine the identity of any given specimen. For example colour. The depth and uniformity can be used in any quick identification. Some conidiophores have the same colour throughout the entire length; others are pale near the tip. But most of them are usually black, simple, arising in clusters and bursting out of leaf tissue, bearing conidia successively on new growing tips (Chupp, 1953).

(3) Their **mycelia** are internal or external, filamentous, branched, septate, hyaline or coloured.

(4) **Stroma** are lacking to prominent. When present pale coloured to black, globular, irregular or elongated, mostly borne superficially on the affected tissue or finally become erumpent.

2.2 The African population structure of *Cercospora zea maydis*

In order to determine the African population structure of C*ercospora zea maydis* and its relationship to the species that occur in the USA (Dunkle and Levy, 2000) collected several disease samples and fungal cultures at [CIMMYT] Mexico DF, in Zimbabwe, Uganda, South Africa and Zambia. These samples were analysed using Amplified Fragment Length Polymorphism (AFLP) and restriction of internal transcribed spacer (ITS) regions and 5.8s ribosomal DNA (rDNA). They were also morphological and culturally characterised. Results of these analyses revealed that; (a) The ITS and rDNA regions were monomorphic, further indicating genetic homology and very similar haplotypes among the African isolates; (b) genetic diversity within the African population was limited. The average within population similarity was 97.6 percent; (c) the primary taxonomic characters of the African isolates were similar to those of the

isolates from the United States of America; (d) isolates of Africa and the group II isolates of the USA from the eastern USA did not produce detectable quantities of Cercosporin crystal (a photo-activated phytotoxin produced by many species of *Cercospora* (Daub, 1982) over a period of 3 weeks and both grew at a substantially slower rate than isolates of *Cercospora zea maydis* group I; (e) based on morphological characteristics and molecular-genetics criteria, the African population of *Cercospora zea maydis* is equivalent to the population of *Cercospora zea maydis* designated group II. These results show that the African population of *Cercospora zea maydis* has probably a limited or no genetic diversity.

Studies using AFLPs and the ITS region on genetic variability of USA isolates (Dunkle and Levy, 2000; Wang *et al.,* 1998) showed that the pathogen population consists of two taxonomically indistinct but genetically distinct sibling species that are partially sympatric, and thus, are considered sibling species. A genetic distance of approximately 80 percent separated these groups from each other, and within group similarity were approximately 93 (group I) and 94 percent (group II) respectively. They also reported that the mechanisms by which genetic diversity might arise in *Cercospora zea maydis* are unknown. Para sexuality has never been demonstrated in this species and the involvement of the sexual stage in the life is debatable. Latterell and Rossi (1977) observed spermagonia bearing spermatia which are considered as sexual reproductive structures in some cultures. But their role, if any, is unknown (Dunkle and Levy, 2000). However, other investigators (Beckerman and Payne, 1982) did not observe these putative sexual reproductive structures.

2.3 Variability of virulence of *Cercospora zea maydis*

There is little evidence that the virulence of *Cercospora zea maydis* is changing (Thompson, 1987; Wang *et al.,* 1998; Dunkle and Levy, 2000) or that races of the pathogen exist. However, Latterell and Rossi (1974) described variation in certain structural, cultural and metabolic characteristic among the isolates of *Cercospora zea maydis* and concluded that such variation also may be expressed in pathogenecity or

aggressiveness. However, Bair and Ayers (1986) reported variation in components of parasitic fitness (disease deficiency and virulence measured as lesion length). Dunkle and Levy (2000) reported that isolates within both groups I and II of the USA exhibit a range of aggressiveness when inoculated on maize hybrids but disease symptoms were identical and no significant differences were noted in the severity of the disease incited by both groups. They concluded that the substantial genetic difference between the two groups in the USA was not expressed as differential virulence. While Wang *et al.* (1998) showed that the relative disease severity ratings of hybrids with a range of phenotypes did not change significantly from location to location. They also observed neither of the siblings of *Cercospora* species had selected maize genotypes which are more susceptible to one than.

Also note that in field trials, significant G x E (hybrid x location) interaction is frequently observed (Bair and Ayers, 1986; Bubeck *et al.,* 1993; Carson, 1997; Huff *et al.,* 1988 and Thompson, 1987). Such variation may be due to differential sensitivities of maize genotypes to environmental factors and thus are predisposed to more severe infection by GLS development. Such sensitivities usually have an impact on disease severity, since the QTL effects associated with resistance to GLS are inconsistent over environment (Bubeck *et al.,* 1993; Wang *et al.,* 1998) resulting from genotype x environmental interaction, random variation within environment or in some cases false positives (Bubeck *et al.,* 1993). Although numerous instances of G x E interactions have been detected by classical quantitative genetics analysis, recent results from RFLP mapping tentatively suggest very little environment by QTLS interaction (Tanksley, 1993).

2.4 Variability of *Cercospora zea maydis* in East Africa

Phyllogenetic analysis using AFLP on the East African population structure of *Cercospora zea maydis* (Okori *et al*., 2003) revealed two major clusters. One large cluster comprised 75 % African and USA group II isolates and the second cluster had four USA group I. Similar grouping was observed with RFLP data. Analysis of

molecular variation (AMOVA) based on AFLP data revealed a significant population structure between American and African populations (ϕF_{st} = 0.07). No population structure was detected among African isolates (ϕF_{st} = 0.01). But a strong and significant structure was obtained between the two pathotypes (ϕF_{st} = 0.19). The AMOVA using RFLP data showed absence of population structure among African populations (ϕF_{st} = 0.01). The Okori *et al*. (2003) study reported that gene flow among African populations was high 0.45. These findings suggest that group II pathotype is predominant in East Africa and gene flow appears to be fundamental evolutionary force accounting for the current genetic structure.

2.5 Disease epidemiology and development

GLS disease development is favoured by extended periods of overcast days, warm temperatures and high relative humidity (Beckman and Payne, 1982; Rupe *et al.,* 1982). High relative humidity, suitable air temperatures, host susceptibility and the presence of a source of inoculum are the conditions necessary to cause a GLS epiphytotic. The absolute rate of change of GLS with respect to time is often best described by the logistic model in which the absolute rate of disease increase dy/dt is a function of ry (1-y) (Jenco 1995; Nutter and Jenco, 1992; Nutter *et al.,* 1994; Ward *et al.,* 1999). Logits = $\log_e$ (p/1-p) i.e. the natural logarithm of the odds corresponding to the probability p of a specified outcome given the existence of a stated attribute (international dictionary of medicine and biology volume 3 in John Willey and sons 1986) and is conveniently expressed as the difference of the two logits. Thus, the rate of disease development of GLS epidemics is driven by three factors that interact in time and space: (1) the initial amount of inoculum (y); (2) the rate of within season pathogen reproduction (r); and (3) the proportion of healthy tissue remaining to be infected (1-y). It follows that the higher the initial level of inoculum the faster the GLS development will occur with respect to time.

Several studies have shown that environmental factors have tremendous impact on the rate of within season gray leaf spot disease development. In Ohio, deNazareno *et al.*

(1992) reported that the rate of GLS progress (r) ranged from 0.13 to 0.17 logits per day (favourable) for disease development and 0.02 to 0.06 logits per day (less favourable) for disease development. In Iowa, Nutter and Stromberg (1999) obtained some what higher estimates of disease increase with rates of disease development ranging from 0.07 in 1991 (moderate favourable) to 0.28 logits per day in 1992 (extremely favourable). In South Africa, Ward *et al.* (1999) reported apparent infection rates of up to 0.16 logits per day during 1991/92-rain season (highly favourable) and up to 0.10 logits per day (moderately favourable). It was suggested that higher apparent infection rates in moderately favourable seasons coupled with longer growing seasons help to explain as to why disease severities are generally higher by the end of the growing season in Africa as compared with the United States (Ward *et al*., 1999; Nutter and Stromberg, 1999).

Studies of the spread of *Cercospora zea maydis* from infected maize debris in the East African soil surface and its progress over time showed that the amount of infected residue on the soil surface in the maize planting was highly significant (Asea *et al*., 2002). The distance from the infected residue significantly affected the foliar disease but the direction from the residue inoculum source did not nor was the distance by direction interaction significant. However, interactions between amount of residue and distance were significant. The percent leaf area affected by GLS, the area under disease progress curve and disease intercepts decreased with distance.

2.6 Symptoms of GLS on maize plants

Symptoms of GLS on susceptible maize are grey to tan linear rectangular lesions delimited by major veins on the maize leaves. Lesions are completely opaque when viewed through transmitted light on leaf, sheath and husk tissue (Smith and White, 1987; Latterell and Rossi, 1983). This is a characteristic of *Cercospora* infection on graminaceous hosts (Latterell and Rossi, 1983). Within 2 weeks such lesions gradually elongate appearing as streaks before developing their dark grayish brown or tan rectangular shape. The lesions remain tan until dense sporulating under humid conditions and then produce a greyish cast, hence the common name. Lesions develop

slowly as compared to other foliar pathogens and are pin- point in size surrounded by a "yellow halo" (Latterell and Rossi, 1983). They require about 2-3 weeks for full expansion. Mature lesions are 1-10 cm long and 2-5 mm wide (Ayers *et al.,* 1984; Latterell and Rossi, 1983; Stromberg, 1986). If adjacent interveinal areas are infected, two single lesions may coalesce to form one broad one. Lesion development usually begins on the lower leaves producing conidia that serve as inoculum for the upper leaves. As many lesions form they coalesce and become more difficult to distinguish and blighting results until all leaves are killed (Stromberg, 1986). Gray leaf spot resistant genotypes display fleck type lesions (Ayers *et al.,* 1984; Latterell and Rossi, 1983), while moderate resistance materials exhibit chlorotic lesions (Roane *et al.,* 1974) and susceptible plants show necrotic spots (Ayers *et al.,* 1984; Huff *et al.,* 1988; Latterell and Rossi, 1983; Saghai Maroof *et al.,* 1993).

2.7 Effects of GLS on maize crop

Losses associated with gray leaf spot occur when photosynthetic leaves are rendered non-functional due to lesions and/or the blighting of the leaves. The blighting and premature death of leaves severely limit radiation interception as well as the production and translocation of photosynthates to developing kernels (Ward *et al.,* 1999). Leaves of susceptible genotypes may become severely blighted or killed as early as 30 days prior to physiological maturity (Jenco, 1995; Ward, 1996).

Gray leaf spot has resulted significant losses in income to grain producers and adversely affects yield and quality of silage maize (Ward and Nowell, 1994; Donahue *et al.,* 1991). This is especially true for the upper eight to nine leaves that contribute 75 to 90% of the photosynthates for grainfill (Allison and Watson, 1996). The components of yield which are most affected by GLS are (a) number of kernels per ear and (b) kernel size. Thus the little food manufactured is diverted from roots and stalks in greater amounts than normal at the expense of the ear, which is the major sink at that time. Ears are incompletely filled with the photosynthates from the leaves and mature prematurely Donahue *et al.,* 1991). Consequently stalk deterioration and lodging and breakages occur (Latterell and

Rossi, 1983; Shurteff and Perdesen, 1991; Ayers *et al.,* 1984; Stromberg and Donahue, 1986). This can also lead to plant death (Donahue *et al.,* 1991).

Percent yield loss is defined as the difference between the attainable yield and actual yield/attainable yield x 100 (Nutter *et al.,* 1993). Using this definition to quantify the yield losses caused by GLS, different loss estimates have been reported. Donahue *et al.* (1991), Ayers *et al.* (1984) and Beckman *et al.* (1981) reported loss due to GLS to be 10-25 percent of the yield potential in endemic areas but can be much higher. Gevers *et al.* (1994) observed up to 40 percent whereas Ward and Nowell (1994) and Ward *et al.* (1997) indicated yield losses up to 50-65 percent with reduction in both yield and quality of silage. Latterell and Rossi (1983) reported the loss due to GLS approaching 80 to 100 percent in epiphytotics. No mechanical harvesting of maize can be done on GLS infected fields. From literature it is clear that GLS causes enormous maize crop losses in both endemic and epidemic situations. This problem has posed many scientists around the world to embark on different research programs looking for solutions to the problem (Ward and Nowell, 1994, 1998; Bubeck *et al.,* 1993). Some of GLS control methods documented include the following:

2.8 Control of GLS disease

2.8.1 Elimination of the corn debris and avoid no till and other conservation practices

Debris serves as good over wintering source of inoculum for the GLS (deNazareno *et al.,* 1992; Ureta, 1985; Payne and Waldron, 1983). It is the fungus within the infected debris from the previous crop that produced conidia following long periods of warm humid weather. Disease levels increase with the amount of residues on the soil surface (deNazareno *et al.,* 1992). Ward *et al.* (1997) used four treatments e.g. no till, chisel, chisel and disc and conventional ploughing leaving stubble residues of 3, 26, 41 and 82 percent on the soil surface. Their results showed that the effective tillage practices of GLS development depended on the amount of stubble remaining on the surface and the

prevailing weather conditions following tillage treatments. These results also revealed that the safest GLS control option is to avoid no till and other conservation practices especially when maize follows maize (deNazareno *et al*., 1992) and that the weather pattern has the greatest effect on the disease development (Payne and Waldron, 1983).

2.8.2 Crop rotation

This cultural practice has been emphasized by several studies (Smith and White, 1987; Ward and Nowell, 1994; Thorson, 1989; Latterell and Rossi, 1983). They said avoiding the inoculum by rotation to another crop would reduce the level of inoculum in the soil. Thus crop rotation can be a promising an alternative control method for two reasons; (a) the pathogen does not survive in diseased maize debris beyond one year (Latterell and Rossi, 1983), and (b) *Cercospora zea maydis* is pathogenic only to maize among crop species (Latterell and Rossi, 1983). However, (Gevers *et al.,* 1994; Gevers and Lake, 1994) reported that discontinuation of conservation tillage is not a viable control option and crop rotation may not be an effective control method.

2.8.3 Use of chemicals

Thorson (1989) reported that fungicides have been observed to reduce GLS severity in the USA, but the application of fungicides is probably uneconomical except for seed producers (Ward *et al.,* 1999). The objective of fungicides is to delay the onset and the rate of development of the disease until the crop reaches physiological maturity. Fungicide application was most effective when spraying commenced as disease severity levels reached 2 to 3 percent of the leaf area blighted, and when lesions were restricted to the basal five leaves of the maize plant. The number of spray application will therefore be a function of the stage of growth of the maize crop when it is initially infected by the pathogen (Ward *et al.,* 1997). With early infections, more fungicide treatments were necessary to provide protection to physiological maturity. Infections, which may occur from shortly before or soon after tasselling, two fungicide applications may be necessary to delay disease development (Ward and Nowell, 1994).

Approximately 3-4 weeks after tasselling, a single fungicide application should be sufficient (Ward *et al.,* 1994). These results indicate that fungicides are highly effective in controlling the development of GLS. Examples of fungicides are carbendazim, flutriafol, difenoconazole, benlate, etc. Although foliar applications of fungicides are effective control but the pathogen may develop resistance to the fungicides and may not be rewarding to grain producers (Ward *et al.,* 1999).

2.8.4 Combination of methods

This refers to the use of fungicides and less susceptible cultivars. Lless susceptible hybrids to GLS disease have been identified (Ward *et al.,* 1994; Ward and Nowell, 1998). However, resistance of less susceptible hybrids may not be adequate to minimize significant yield loss under high disease pressure. A fewer of these hybrids with low susceptibility are high yielding and should be selected for areas subject to heavy disease pressure (Ward and Nowell, 1994). In many cases these hybrids may need timous application of fungicides (Ward *et al.,* 1994). In fact, this is one type of integrated pest management.

2.8.5 Use of GLS resistant cultivars

The adverse economical and ecological effects of mouldboard tillage, use of fungicides and other methods made genetic resistance the best control strategy for GLS disease (Ward *et al.,* 1999; Lipps and Pratt, 1989; Hohls *et al.,* 1995; Saghai Maroof *et al.,* 1996; Coates and White, 1994). Despite the fact that there are several GLS resistant germplasm that have been identified (Ayers *et al.,* 1984; Thompson *et al.,* 1987; Dunkle and Levy, 2000) a high level of resistance has not been incorporated into a commercial hybrid (Ward and Nowell, 1994; Ward *et al.,* 1997; Dunkle and Levy, 2000). But there are increasing number of hybrids, which are less susceptible to GLS (Stromberg and Donahue, 1986; Gevers *et al.,* 1994; Ward *et al.,* 1997). Genetic resistance for the control of GLS is highly effective, easy to use and cost effective method and will play a major role in providing a long term solution to the problem (Lipps and Pratt, 1989;

Elwinger *et al.,* 1990; Gevers and Lake, 1994; Ward and Nowell, 1998; Bubeck *et al.,* 1993). The quantitative nature of the inheritance of GLS resistance was determined by Manh (1977) based on GLS disease rating in F_2 and F_3 generations from a cross between a resistant line (Va14) and a susceptible line (H98). He concluded that additive genetic effects account for 82-96 percent of total variability among generations, with a narrow sense heritability of 31 percent. Other investigators (Ayers *et al.,* 1984; Donahue *et al.,* 1991; Huff *et al.,* 1988; Ulrich *et al.,* 1990; Freppon *et al.,* 1994) reported similar results. While Gevers and Lake, 1994, Elwinger *et al.,* 1990 and Bubeck *et al.,* 1993) added that some genes exhibited major gene with dominant effect. They also identified some outstanding GLS resistant hybrids of 0.46 for the cross of K054W x R055W. Both the GCA (i.e. the general performance of a line in hybrid combinations) and SCA (i.e. best line x line performance) were also highly significant. They noted that R0465W, S081W and R0558W had high GCA indicating greater additivity in some lines. The importance of the additive component of variance suggests that active selection for resistance to GLS in these materials as well as use of the most resistant lines in crosses or in back cross programs should be very successful.

But it should be known that breeding maize for GLS resistance has been limited by (1) quantitative nature of the inheritance of GLS (Ayers *et al.,* 1984; Saghai *et al.,* 1996) and (2) limitation of selection under less than optimum disease pressure (Payne and Waldron, 1983). So the presence of heavy disease pressure is an essential prerequisite to evaluate the levels of GLS resistance (Saghai Maroof *et al.,* 1996).

2.8.6 Use of molecular techniques

Identification of genes controlling traits is critical in order to determine the effect of these genes or quantitative trait loci (QTLs), to study the molecular mechanism of individual genes and to facilitate the transfer of desirable traits in marker assisted breeding programs. Quantitative identification across populations should allow opportunities for marker assisted selection (Bubeck *et al.,* 1993; Lehmensiek *et al.*, 2001: Gordon *et al.,* 2004) and pyramiding of genes from different backgrounds into one

line (Bubeck *et al.,* 1993).

2.9 Early genetic studies for GLS resistance

Early studies of temperate adapted germplasm addressed the genetic basis of resistance to GLS by diallel and generation mean analyses and concluded that the resistance is under additive genetic control with some dominance effects (Thompson *et al*., 1987; Ulrich *et al*., 1990; Gevers *et al*., 1994; Coates and White, 1998). Genotypes were selected in these studies resulting in a fixed model (Griffing, 1956). The conclusions reached were therefore applied only for the selected inbred lines. Gevers and Lake (1994) suggested that a single gene in South African germplasm conferred resistance to GLS, but their results have not been confirmed (Gordon *et al*., 2004).

2.10 QTLs identification for GLS resistance studies

QTL mapping studies have made limited progress in identifying consensus QTL for resistance to *Cercospora zea maydis* (Bubeck *et al*., 1993; Saghai Maroof *et al*., 1996; Clement *et al*., 2000; Lehmensiek *et al*., 2001; Gordon *et al*., 2004). Bubeck *et al*. (1993) used two different inbreds, NC250A and DENT, as source of resistance to GLS in the $F_{2:3}$ mapping populations. They identified QTL on five different chromosomes but only one of these, on the short arm of the chromosome 2 was consistent over three environments. Saghai Maroof *et al*. (1996) employed selective genotyping to identify three QTL on chromosome 1, 4 and 8 that collectively explained 44-46 percent of the variation across two generations F_2, and $F_{2:3}$, across two seasons in one location. In test for epistatic interactions, they demonstrated that the QTLs on chromosome 4 had little or no effect when the QTL on chromosome 1 was homozygous**.** In addition, the QTL on chromosome 8 displayed recessive gene action. Using the inbred 061 as a resistance source, Clement *et al*. (2000) evaluated a BC_1S_1 population for 2 year at one site and 1 year at a second site. They found five QTLs all from the resistant parent, which were significantly associated with GLS resistance in both years and locations. Lehmensiek *et al*. (2001) used bulked segregant analysis to identify QTLs on chromosome 2, 3 and 5

associated with resistance to GLS in an F_2 population derived from proprietary parental lines. These four studies collectively utilized five resistant inbreds and chromosome 1, bin 1.05/1.06 is nearest to a consensus QTLs identified, with three of the five inbreds contributing GLS resistance from this region. Gordon *et al*. (2004) reported several markers on chromosome 2L (long arm) and 4L were deemed to be significantly associated with GLS resistance in maize. They documented that such inbred line like V061Y is a good source of resistance to be deployed effectively in USA and South Africa and that intervals on chromosome 2L and 4L were detected in all tests and can be considered them to be suitable QTLs for MAS.

2.11 Linkages between GLS resistance QTL and other disease resistance genes

Quantitative resistance to several maize diseases has been reported (Saghai Maroof *et al.,* 1996). The separation of quantitative resistance into a few QTL enabled to examine their relationship to previously identified maize genes conferring qualitative resistance to other diseases. The chromosomal location of a *Cochliobulus carbonum* race-1 gene that has been previously cloned by transposon tagging (Johal and Briggs, 1992) is also located on chromosome 1 (QTL1) which had largest effect on GLS resistance. *QTL*8 was also found to be closely linked to two *Helminthosporium turcicum* resistant genes, *Ht*2 (Zaitlin *et al.,* 1992) and *hm*1 (Simcor and Bennetzen, 1993). These three genes appear to belong to a resistance gene cluster since neither *Ht*2 nor *Hm*1 *per se* contribute to GLS resistance. Significant effects of RFLP markers on resistance to *Gibberella zea* were found on chromosome 1, 2, 4, 5, and 10 (Pe *et al.,* 1993). Freymark *et al.,* (1993) reported that chromosome 2, 4 and 8 each had at least one marker with some resistance to *Helminthosporium turcicum* at $\propto$= 0.05 level of significance.

2.12 Molecular markers

Molecular markers should not be considered as normal genes as they usually do not have any biological effect and instead can be thought as constant ‘land marks’ in the genome.

They are identifiable DNA sequences (Russell and Thompson, 1989) that were found at specific locations of the genome and transmitted by standard laws of inheritance from one generation to the next. They relied on DNA assays in contrast to morphological markers and biochemical markers. Many molecular markers have been identified in the intergenic DNA within the introns of the genes; a few have been recorded within the recording sequences (exons) of the genes (Tom and Read, 1999).

2.13 Types of DNA markers

Different types of molecular markers exist. The principles, potential power, advantages/ disadvantages and their application in different area of science can be reviewed (Dogson *et al.,* 1997; Rafalki, 2002; Buitkamp and Epplen, 1996; Botha and Venter, 2000). There are two classes of DNA markers. Those based on DNA-DNA hybridisation and those on the polymerase chain reaction (PCR) amplification of genomic sequences (Cobb and Clarkson, 1994; Botha and Venter, 2000; Liu and Cordes, 2004).

2.13.1 Amplified fragment length polymorphism (AFLP)

The amplified fragment length polymorphism technique was developed by Zabeau and Vos (1993). An AFLP is a highly reproducible multiplex assay and has the ability to produce large number of polymorphic loci (Myburg *et al.,* 2001; Botha and Venter, 2000). They are used extensively for high resolution genetic linkage mapping (Blears *et al.,* 1998), fingerprinting in plants, analyse genetic diversity in many species (Mackill *et al*., 1996; Maughan *et al*., 1996). Furthermore, the AFLP is a robust and rapid technique for analysing many genetic loci and can be used to facilitate breeding programs based on combining and selecting new combinations of genotypes to maximize the rate of line improvement (Liu and Cordes, 2004).

This technique involves three steps, namely (1) restriction digestion of genomic DNA and ligation of oligonucleotide adaptors; (2) preselection and selection amplification of

restriction fragments; and (3) gel analysis of amplified fragments (Vos *et al.,* 1995). The fragment sizes range from 60 to 1500 base pair (50 to 100 fragments are co-amplified per AFLP cycle (AFLP Perkin-Elmer, 1996). The amplified fragments are radioactively or fluorescently labelled and separated on sequencing gels. Results can be silver stained to save on costs. PCR amplification of restriction fragments is achieved by using the ligated adapters on to restriction ends as target sites for primer annealing. The preselection and selection is achieved by using primers that extend into the restriction fragments amplifying only those fragments in which the primer extensions match the nucleotide flanking the restriction sites (Vos *et al.,* 1995). Like RAPDs, AFLP markers are inherited as dominant markers although software packages are now available (AFLP QuantaPro, Keygene) for codominant scoring of AFLP bands (Liu and Cordes, 2004). Codominant scoring is also possible if PCR markers are designed by cutting individual bands of interest from the AFLP gel sequence and design specific primers for each band. This results in one or more codominant markers that can be scored using traditional analyses (Liu *et al.,* 1999).

2.13.2 Sequence characterised amplified region (SCAR)

Problems that are associated with RAPD analysis have necessitated scientists (Demeke and Adams, 1994; Xu and Bakalinsky, 1996) to develop a new marker system that can be used in routine screening. The SCARs also known as sequence tagged sites (STS) or allele specific associated primers (ASAP) (Kelly, 1995; Schachermayr, *et al.,* 1994; Gu *et al.,* 1995 and Olson *et al.,* 1989). A SCAR is a genomic DNA fragment at a single genetically defined locus that is identifiable by PCR amplification using a pair of specific primers (Paran and Michelmore, 1993).A SCAR differs from RAPDs in that it makes use of a set of specific oligonucleotides that amplify a specific region of the genome, usually of low copy number (Weedern *et al.,* 1994; Ohmori *et al.,* 1996; Talbert *et al.*, 1994). When designing a SCAR primer, the RAPD fragment linked to the gene of interest is cloned and then sequenced. The first 10 to 15 bases, together with the RAPD primer sequence, are used to design a more robust primer that results in a less complex banding pattern than the original RAPD primer (Procunier *et al.,* 1997;

Schachermayr *et al*., 1994; Ohmori *et al*., 1996; Dedryver *et al*., 1996). It is also possible to design primers from internal sequences where there are point mutations, deletions or insertions (Feuillet *et al*., 1995).

The advantages of SCARs over RAPDs are that they are more reproducible and show less variability between labs (Schachermayr *et al.,* 1994; Paran and Michelmore, 1994; Melotto *et al.,* 1996), can be used as markers and mapped onto a genetic map as physical landmarks (Paran and Michelmore, 1993). Can be used in position cloning of genes (Salentjin *et al*., 1995) etc. One of the limitations of SCARs is the need to sequence before the primer can be designed whereby more time is needed in cloning the fragment (Talbert *et al.,* 1994). Conversion of a RAPD and a RFLP marker to a SCAR marker can be tedious and difficult (Botha and Venter, 2000; Blake *et al.,* 1996). Tsumura *et al.,* 1997 and McDernott *et al.,* 1994 reported that SCARs can amplify more than one copy of an allele and not all RAPD markers can be converted to SCAR markers (Paran and Michelmore, 1993; Botha and Venter, 2000).

2.13.3 Microsatellite or simple sequence repeats (SSR)

Although some RFLPs as well as many RAPD markers recognize more than one locus in a given genome, the number of loci recognized is limited as is their polymophic content (Winter and Kahl, 1995). Markers derived from small, tandemly arranged repetitive elements overcome this limitation (Winter and Kahl, 1995). Such markers are called microsatellite or minisatellite or simple tandem repeats (STR) or simple sequence repeats (SSR) because their sequence organisation resembles the tandemly arrangement of classical satellite DNA. These sequences are tandemly dispersed throughout the eukaryotic genomes and are often highly polymorphic due to variation in the number of repeats (Litt and Luty, 1989; Tautz, 1989). SSR have been found inside gene coding regions, introns and in the non-coding gene sequences (Liu *et al.,* 2001). Tandemly repeated basic motifs of 2 to 6 base pair (Saiki *et al*., 1988) such as (GA)n, (GT)n, (TG)n or (AAT)n are very common for SSRs. The SSR loci can be amplified by the PCR using primers, which are complementary to the region flanking the repeats.

Microsatellites are highly polymorphic and provide codominant markers with Mendelian inheritance (Beckman and Soller, 1990). These markers are able to detect a much higher level of genetic variation than any other class of markers and are crucial for genetic analysis of organisms with narrow genetic base (Kam-morgan *et al*., 1989).

In plants it has been demonstrated that SSRs are highly informative locus specific markers in many species (Taramingo and Tingey, 1996; Wu and Tanksley, 1993). Other uses of SSRs include linkage analysis (Lothrop *et al.,* 1989; Wells *et al*., 1989), identification of species and cultivars (Weising *et al*., 1998; 1991a), marker assisted breeding (Beckman and Soller, 1990) etc. Due to their varying repeat numbers at a given locus the elements frequently change their length by slippage mispairing during replication and other less understandable processes (Jeffreys *et al*., 1988; Zischeler *et al*., 1992). The surrounding single copy sequences are normally not affected and therefore provide a variable source of polymorphisms for many purposes. One advantage of microsatellite is that primers developed for a particular species can be applicable across a wide range of taxa (Moore *et al*., 1995; Schloetter *et al*., 1991). They seem to provide readily detectable markers for agronomically important genes and quantitatively inherited traits and can facilitate their handling in segregating population (Rodel *et al*., 1998).

2.14 Application of molecular marker technology

Molecular markers provide a remarkable improvement in the efficiency and sophistication of plant breeding and are currently the most important application of molecular biology to plant breeding (Langridge and Chalmers, 1998). Molecular markers allow the construction of high-density linkage maps (Jones *et al.,* 2003; Mitchel *et al.,* 1997, Diwan *et al.,* 2000). Fine mapping of QTLs has sometimes revealed the presence of tightly linked loci affecting the same trait (Fridman *et al.,* 2002; Steinmetz *et al.,* 2002). Molecular markers can be used to test and select traits without performing length field trials. Most importantly molecular markers play greater role in identifying different genes responsible for desirable traits. One method of using molecular markers

for selecting desirable genes is in marker-assisted selection breeding programs. Usually breeders use coupling phase markers (markers only present in resistant plants) to select for plant traits in backcrossing procedures (Kelly, 1995). Haley *et al.* (1994a) demonstrated that the efficacy of MAS could be improved dramatically by selecting against a repulsion phase markers (markers linked to coupling phase to the susceptibility allele). Molecular markers therefore provide an important genetic tool where traditional studies have been difficult (Keim *et al.,* 1990). Markers facilitate establishing genetic linkage among markers and linkages between markers and QTLs.

2.15 The success of MAS

The success of MAS is influenced by relationship between the markers and the genes of interest (Dekkers*,* 2003; Blears *et al*., 1998) as follows:
(a) Gene assisted selection (GAS). In this case molecular markers are located within the gene of interest (Fridman *et al.,* 2002; Steinmetz *et al.,* 2002). On the other hand it is most difficult to find these kinds of markers.
(b) The marker is in Linkage disequilibrium (LD). LD is the tendency of certain combinations of alleles (e.g. M_1 and Q_1) to be inherited together (Morgante and Salamini, 2003; Weir, 1996; Thornberry *et al.,* 2001). Population wide LD can be found when markers and genes of interest are physically very close to each other and or when lines or breeds have been crossed in recent generation. Selection using these markers can be called LD-MAS. So additional work is needed to determine the extent of LD in crop species and to provide guidelines on the marker density needed to reveal significant associations. (c) The marker is in a linkage Equilibrium (LE). Selection using these markers can be called LE-MAS. This is the most difficult situation of applying MAS and that's why there is an argument that if Fisher's infinitesimal model (many genes with small effects) applies, the efficiency of MAS will always be inferior to that of phenotypic selection even if all genes are known and mapped (Bernardo, 2001).

2.16 Limitations in utilising marker loci-QTLs associations

A current problem in the use of molecular markers is that they are often only effective in the genetic backgrounds in which they were developed. When screening other backgrounds they are less effective and reliable. Routine testing is thus needed to determine the efficacy of markers in other genetic backgrounds (A-M Botha pers. comm.). Others include sample size. The larger the sample size the higher the proportion of additive genetic variance likely to be detected by markers. Markers are effectively used when the linkage between the marker and QTL are tightly linked especially in GAS (Fridman *et al.,* 2002; Steinmetz *et al.,* 2002) and in LD (Weir, 1996; Thornberry *et al.,* 2001; Morgante and Salamini, 2003).

2.17 Marker loci-QTLs associations for GLS resistance in maize

One method of controlling GLS is to develop hybrids with genetic resistance (Dunkle and Levy, 2000; Coates and White, 1998; Gevers and Lake, 1994; Bubeck *et al*., 1993). A number of recent investigations utilized isozyme, allozyme, and or RFLP/RAPD markers to identify and characterize gene action of QTL (Beavis *et al.,* 1991; Keim *et al.,* 1990; Beamount *et al.,* 1996; Saghai Maroof *et al.,* 1996). The QTL were identified on the basis of marker associations with GLS means over all ratings taken over environments (Bubeck *et al.,* 1993). Results showed that individual markers accounted for 4 to 26 percent of the phenotypic variation. Quantitative trait loci associated with GLS were inconsistent over environment (Bubeck *et al.,* 1993; Wang *et al.,* 1998). Bubeck *et al.* (1993) observed that one region on chromosome 2 was associated with GLS resistance in three populations. Additive genetic variance was displayed for nearly all markers associated with GLS resistance. Heterozygosity calculated as percentage of heterozygous loci over all RFLP markers or percentages of marker loci heterozygous in individual plants and mean trait value was not significantly correlated with GLS ratings**.**

2.18 Correlations between mean GLS scores over environments

Pairwise comparisons (Bubeck *et al.,* 1993) of any two subsequent GLS ratings resulted in positive correlations ($p \leq 0.05$) ranging from 0.17 to 0.82 for 3 populations. As the number of days between ratings increased the correlations tended to decrease suggesting that different genes could be involved in early season resistance versus late season resistance. Gray leaf spot often does not begin to develop until several days after flowering and in general late maturing lines tend to be more resistant than early lines (Bubeck *et al.,* 1993). Saghai Maroof *et al.,* (1996) and Bubeck *et al.,* (1993) did preliminary investigations of QTL for number of days after planting to silking (DAPS) to determine whether any regions were similar to those for GLS resistance. Results showed that the region on chromosome 4 (markers P10200597, P10200595, BNL7.65 and UMC15) were associated with both DAPS and GLS entry means over environments. In addition, markers on chromosome 2, 3, and 8 were significantly associated with DAPS in one location and were associated with GLS means in the same location in previous year. Therefore some QTLs for DAPS and GLS means do appear to correspond, resulting from pleiotropic effect or linkage of genes controlling the two traits.

In summary, from literature review, it is revealed that GLS of corn is not yet completely solved in many parts of the world including Tanzania (Lyimo per. comm.). GLS disease is still causing enormous maize yield losses in both endemic and epidemic conditions (Ward *et al.,* 1997; Dunkle and Levy, 2000). This situation is worse in those countries that use maize as a main staple food or as an export crop. Such losses bring famine, food shortages, etc which are always aggravated by bad weather**.** The current control measures of GLS are not very effective and are either uneconomical or environmentally unsound or both. Breeding for disease resistance by using the normal conventional methods seem to be somehow limited by the quantitative nature of inheritance of the GLS resistance (Bubeck *et al.,* 1993; Gevers *et al*., 1994; Pratt *et al*., 2003). It would therefore be wise to consider both phenotypic and molecular (MAS) as complementary breeding tools for efficient resistance breeding to GLS.

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