Theor Appl Genet (2001) 103:797–803

A. Lehmensiek · A.M. Esterhuizen · D. van Staden
S.W. Nelson · A.E. Retief

Genetic mapping of gray leaf spot (GLS) resistance genes in maize

Received: 10.10.00 / Accepted: 26.01.01

Abstract Bulked segregant analysis was used to identify amplified fragment length polymorphism markers (AFLPs) linked to quantitative trait loci (QTLs) involved in the resistance to gray leaf spot (GLS) in maize. By using ten AFLP primer combinations 11 polymorphic markers were identified and converted to sequence-specific PCR markers. Five of the 11 converted AFLPs were linked to three GLS resistance QTLs. The markers were mapped to maize chromosomes 1, 3 and 5 using existing linkage maps of two commercially available recombinant inbred-line populations. Converted restriction fragment length polymorphism markers and microsatellite markers were used to obtain a more-precise localization for the detected QTLs. The QTL on chromosome 1 was localized in bin 1.05/06 and had a LOD score of 21. A variance of 37% was explained by the QTL. Two peaks were visible on chromosome 5, one was localized in bin 5.03/04 and the other in bin 5.05/06. Both peaks had a LOD score of 5, and 11% of the variance was explained by the QTLs. A variance of 8–10% was explained by the QTL on chromosome 3 (bin 3.04). The consistency of the QTLs was tested across two F_2 populations planted in consecutive years.

Keywords Gray leaf spot · Maize · Bulked segregant analysis · AFLPs · QTL analysis

Introduction

Gray leaf spot (GLS) of maize, caused by the fungus *Cercospora zeae-maydis* (Tehon and Daniels 1925), has become a major threat throughout the maize-growing regions of the United States during the past decade and appears to be increasing each year (Wang et al. 1998). In South Africa the disease was first observed in KwaZulu-Natal in 1988 and has since spread rapidly to neighbouring provinces and countries, reducing grain yields by 30 to 60%, depending on hybrid susceptibility and favourable weather conditions (Ward et al. 1997). GLS is an extremely environmentally sensitive disease requiring high humidities and extended leaf wetness. Symptoms of GLS are normally first observed on the lower leaves. Typical mature GLS lesions are gray to tan in color, sharply rectangular, long and narrow, and run parallel to the leaf veins (Latterell and Rossi 1983; Ward et al. 1999).

Methods to control GLS include the discontinuation of conservation tillage, the use of crop rotation, the application of foliar fungicides, and growing hybrids with resistance (Latterell and Rossi 1983; Ward et al. 1997; Coates and White 1998). As it is important to preserve the economic and environmental advantages of conservation tillage systems, tillage is not a viable control option and crop rotation may not be an effective control. Foliar-applied fungicides are an effective control, but may not be economical for grain production. Furthermore, the pathogen may develop resistance to the fungicides (Ward et al. 1999). Host resistance is therefore expected to be the most cost-effective, efficient and acceptable control (Gevers and Lake 1994; Saghai Maroof et al. 1996; Coates and White 1998; Ward et al. 1999).

The development of GLS is highly dependent on environmental effects, and field assessment of the disease is problematic. Recovery through conventional breeding is therefore difficult and, to-date, only a few high-yielding maize hybrids resistant to GLS are available in South Africa.

Both additive and non-additive genetic effects play a major role in the resistance mechanism in South African maize-breeding material (Gevers et al. 1994). Hohls et al. (1995) found that GLS in maize can be expressed in terms of an additive-dominance model, with dominance almost complete. Breeding material, presumably

Communicated by G. Wenzel

A. Lehmensiek · D. van Staden · A.E. Retief (✉)
Department of Genetics, University of Stellenbosch,
Private Bag X1, Matieland 7602, R.S.A.
e-mail: aretief@mrc.ac.za
Fax: 00-27-21-8085833

A. Esterhuizen · S.W. Nelson
Sensako, P.O. Box 3295, Brits 0250, R.S.A.

originating from teosinte germplas, which exhibited levels of resistance due to a major gene, *GLS1*, was also identified (Gevers and Lake 1994).

Two studies in the USA have examined quantitative trait loci (QTLs) associated with GLS resistance (Bubeck et al. 1993; Saghai Maroof et al. 1996). Both used restriction fragment length polymorphism (RFLP) analysis to identify markers linked to GLS resistance. Bubeck et al. (1993) identified QTLs associated with resistance on all maize chromosomes. The QTLs associated with GLS resistance were inconsistent over environments. Saghai Maroof et al. (1996) identified QTLs located on chromosomes 1, 4 and 8 which had large effects on GLS resistance and were remarkably consistent across three disease evaluations over 2 years and two generations. Smaller QTLs effects were found on chromosomes 2 and 5.

DNA markers can rapidly be linked to important plant genes by bulked segregant analysis (BSA) (Michelmore et al. 1991) and this is a valid and reliable method for targeting QTLs (Chagué et al. 1997). Our objective was to map GLS resistance QTLs using a Zimbabwean (Seed Co. Ltd.) resistant inbred line by making use of BSA together with the amplified fragment length polymorphism (AFLP) technique. This technique was used as it has the capacity to inspect a large number of loci for polymorphism simultaneously in any background or complexity, including pooled DNA samples (Vos et al. 1995). Putative AFLP markers were converted to sequence-specific PCR markers and QTL analysis was performed using a F_2 population. The converted AFLP markers were mapped to the maize chromosomes using existing linkage maps of two commercially available recombinant inbred line (RIL) populations (Burr et al. 1988). Once the map positions of the QTLs had been identified, converted RFLP and microsatellite markers from the chromosomal regions were used to obtain a more precise localization of the QTLs. To test the consistency of the detected QTLs across seasons, the markers flanking each QTL were amplified on selected plants of two F_2 populations planted in consecutive years and a standard linear regression analysis was performed.

Materials and methods

Plant material and GLS disease evaluations

The plant material used in this study was obtained from the seed company Sensako, South Africa. F_2 plants were visually assessed and scored for resistance on a rating scale of 1–9, where 1 is most resistant and 9 is highly susceptible. Although the plants were grown in field plots with naturally infested corn debris, each plant was artificial inoculated to ensure high disease pressure. Inoculum was obtained from dry infected leaves which had been harvested in the previous season. The leaves had been ground with a hammer and stored at 4°C. Approximately 3 g of the inoculum was placed in the whorl of each plant at the 8-leaf stage of growth. This procedure was repeated after 10 days. At least three rows of the susceptible parent were planted in each generation to determine the progress the disease had made. The first disease ratings were recorded when the susceptible parent had a GLS score of 7 and, on average, two to three ratings were taken.

The F_1 single cross between a GLS-resistant male parent (Seed Co. Ltd., Zimbabwe) and a susceptible female parent (Sensako, South Africa) was backcrossed to the susceptible parent during the summer of 1995/6. During the winter of 1996 the backcrossed generation was selfed to produce a segregating F_2 generation, which was planted at Hillcrest, KwaZulu-Natal, in the 1996/7 season. From the F_2 population ten resistant (score 1) and ten susceptible (score 9) plants were chosen for bulk segregant analysis. F_2 populations were also planted at Hillcrest in 1998, 1999 and 2000, and scored for GLS resistance. Of the 1998 F_2 population, 230 scored plants were used in linkage analysis and QTL mapping. Plants of the 1999 and 2000 F_2 population were selected and used in the regression analysis to test the consistency of the QTLs.

Two publicly available recombinant inbred families T323× CM37 and CO159×Tx303, consisting of 48 and 41 lines respectively (Burr et al. 1988), were used to map cloned AFLP fragments.

Leave tissues samples from individual plants were used for DNA extractions.

AFLP analysis

The AFLP methodology was used essentially as described by Vos et al. (1995) with minor modifications. Genomic DNA (150 ng) was digested with *Mse*I and *Mlu*I in a total volume of 50 µl at 37°C for 1 h. The 50-µl digested DNA mixture was supplemented with 10 µl of adapter/ligation solution, containing 50 pmol of the *Mse* adapter and 5 pmol of the 5′-biotinylated *Mlu* adapter, 1.2 µl of 10 mM ATP, 1× One-Phor-All Buffer PLUS (Pharmacia Biotech) and 1U of T4 DNA ligase, and incubated overnight at 37°C. The complexity of the DNA mixture was reduced by selecting the biotinylated *Mlu* fragments using streptavidine beads (Dynal). The remaining fragments were suspended in 100 µl of TE buffer and stored at –20°C. One microliter of the biotinylated DNA fragments was added to 100 µM of each dNTP, 2 mM $MgCl_2$, 1× NH_4 buffer (Bioline), 0.5 U of *Taq* DNA polymerase (BIOTA polymerase, Bioline), 30 ng of radioactively labelled *Mse* primer and 30 ng of *Mlu* primer in a total volume of 20 µl. The *Mse* primers and the *Mlu* primers had 3 and 2 selective nucleotides, respectively (Table 1). The PCR-cycle profile was performed in a Hybaid PCR Express thermocycler. The cycle profile used for amplification was as follows: one cycle of 72°C for 1 min, one cycle of 94°C for 2 min, followed by 12 cycles of 94°C for 20 s, 65°C for 30 s, and 72°C for 2 min, followed by 25 cycles of 94°C for 20 s, 56°C for 30 s and 72°C for 2 min, and one cycle at 72°C for 30 min. After amplification 10 µl of formamide-loading buffer was added to each sample. Four microliters of each sample were loaded on 4% polyacrylamide denaturing sequencing gels. Gels were run at 60 W for approximately 2 h. The gels were transferred to Whatman paper, dried, and exposed to X-ray film overnight.

Conversion of AFLPs to sequence-specific PCR markers

Autoradiography glo-stickers (Bel-Art products) were used to mark the dried gel for orientation purposes. A specific AFLP frag-

Table 1 Primer combinations used in AFLP analysis. The *Mlu*-5 primer was employed in combination with the ten *Mse* primers

Name of primer	Sequence
Mlu-5	5′-GAC TGC GTA ACC GCG TGC-3′
Mse-1	5′-GAT GAG TCC TGA GTA AGA A-3′
Mse-2	5′-GAT GAG TCC TGA GTA AAC A-3′
Mse-3	5′-GAT GAG TCC TGA GTA AAC C-3 ′
Mse-4	5′-GAT GAG TCC TGA GTA ACC G-3′
Mse-5	5′-GAT GAG TCC TGA GTA AGG C-3′
Mse-6	5′-GAT GAG TCC TGA GTA ATT G-3′
Mse-7	5′-GAT GAG TCC TGA GTA ATA C-3′
Mse-8	5′-GAT GAG TCC TGA GTA AGA G-3′
Mse-9	5′-GAT GAG TCC TGA GTA ACA T-3′
Mse-10	5′-GAT GAG TCC TGA GTA ACA C-3′

ment was excised with a scalpel and incubated at 37°C overnight in 50 µl of TE buffer. One microliter of the TE buffer containing the excised DNA fragment was amplified with the same set of AFLP primers. Amplification products were electrophoresed at 80 V in a 1.5% low-melting-point agarose gel. The desired fragments were excised from the gel. The DNA was extracted by phenol/chloroform extraction and ethanol-precipitated. A pGem-T Easy Vector System II (Promega) was used to clone the fragments. Plasmids were extracted using the Perkin Elmer Miniprep kit and sequenced with an ABI Prism 377 automatic sequencer. Primers were commercially synthesized. The primers were used with 20 ng of genomic plant DNA in the PCR reaction as described under AFLP analysis. The cycle profile used for amplification was as follows: one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 45–68°C (depending on the primer pair) for 30 s, and 72°C for 2 min, and one cycle at 72°C for 10 min. The amplification products were electrophoresed either in 1.5–2% agarose gels or polyacrylamide gels. Restriction enzyme digestions were carried out directly on 20 µl of the amplification products and digested fragments were electrophoresed in 2% agarose gels.

Conversion of RFLPs and analysis

RFLP probes received from the University of Missouri, Columbia, Mo., USA were sequenced and two 20-bp primers were commercially synthesized for each probe. The primers were used with 20 ng of genomic plant DNA in the PCR reaction as described under AFLP analysis. The following cycle-profile was performed in a Hybaid PCR Express thermocycler: one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 54–60°C (depending on the primer pair) for 30 s and 72°C for 2 min, and one cycle at 72°C for 10 min. The amplification products were electrophoresed in 2% agarose gels. Restriction enzyme digestions were carried out as described under 'Conversion of AFLP markers.'

Microsatellite analysis

The microsatellite primer sequences were obtained from the Maize Database website (http://www.agron.missouri.edu). The primers were used with 10 ng of genomic plant DNA in the PCR reaction, as described under AFLP analysis. The PCR-cycle profile (one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 45–70°C (depending on the primer pair) for 30 s and 72°C for 30 s, and one cycle at 72°C for 30 min) was performed in a Hybaid PCR Express thermocycler. The amplification products were electrophoresed in 2% agarose gels or polyacrylamide gels.

Linkage analysis and QTL mapping

Linkage analysis was performed with the software package MAPMAKER/EXP version 3.0b (Lander et al. 1987; Lincoln et al. 1992a). The data files used in linkage analysis with the RIL populations were obtained from the Maize Database website (http://www.agron. missouri.edu). To include a locus in a linkage group, a minimum LOD threshold of 3.0 was used.

The chromosomal location of the QTLs was determined by interval mapping (Lander and Botstein 1989) using MAPMAKER/QTL version 1.1b (Paterson et al. 1988; Lincoln et al. 1992b) at a LOD threshold of 2.0, and by composite interval mapping (Zeng 1994) using the program QTL Cartographer version 1.13 (Baston et al. 1994, 1997).

Linear regression analysis

Linkage of molecular markers to genetic factors responsible for GLS resistance was investigated by standard ANOVA (analysis of variance) for linear regression of GLS scores on genotypes for each marker, scored as 1, 2 and 3 for the homozygous resistant, heterozygous and homozygous susceptible allele, respectively. The regression of GLS score on marker genotype was employed to calculate the proportion of the total phenotypic variance explained by each marker, and used the standard *F*-statistic. All calculations were performed on a spreadsheet using the program Microsoft Excel 97.

Results

AFLP analysis

Bulked segregant analysis was used to identify AFLP markers linked to GLS resistance QTLs. Equal volumes of standardized DNA of ten GLS-resistant (score 1) and ten GLS-susceptible (score 9) F_2 plants, of the population planted in 1996, were pooled. Ten AFLP primer combinations (Table 1) were used to screen the *Mse*I/*Mlu*I-digested parent and bulk DNA, and an average of 45 bands per primer combination were observed. Approximately 50% of the fragments were found to be polymorphic between the parental lines. An example of a DNA fingerprinting gel produced using the primer combination *Mlu*-5/*Mse*-5 is given in Fig. 1A.

Eleven distinct fragments were polymorphic in the bulks. These fragments, ranging in size from 175 to 770 base pairs, were also polymorphic in the parental lines. AFLP analysis indicated that these fragments were present on average in seven of the ten plants of one bulk and absent in the other bulk.

The 11 polymorphic AFLP fragments were converted to sequence-specific PCR markers and five of these markers were polymorphic between the parental lines. The markers were added to the University of Stellenbosch maize database, and designated as numbers us40, us41, us42, us44 and us45 (Table 2). Markers

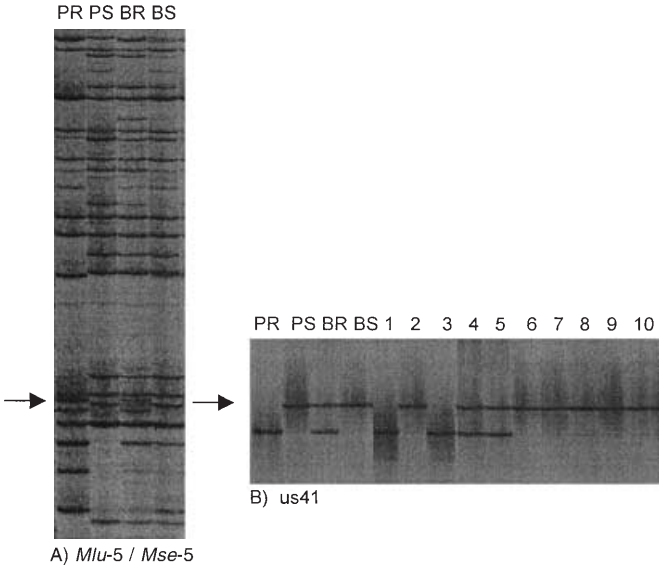

Fig. 1A DNA fingerprinting gel produced using the primer combination *Mlu*-5/*Mse*-5. The polymorphic fragment is indicated. **B** The derived sequence-specific PCR marker us41 is shown (*PR*=resistant parent, *PS*=susceptible parent, *BR*=resistant bulk, *BS*=suscepible bulk, 1–5 =five of the ten individual plants making up the resistant bulk, and 6–10 =five of the ten individual plants making up the susceptible bulk)

Table 2 Primer sequences of converted AFLP markers

AFLP primer combinations	Name of converted AFLP marker	Primer sequences of the converted AFLP markers
Mlu-5/*Mse*-5	us40	5′-GTACAGCGGCCTGACGTGAT-3′ 5′-CAGTTGTAGCGGCCTGTGAA-3′
Mlu-5/*Mse*-5	us41	5′-GGTTGCTTGTCCAAGATC-3′ 5′-GCGATAATCTACTGGAGC-3′
Mlu-5/*Mse*-6	us42	5′-CCTACGAGAACTCATC-3′ 5′-CTTGACGATGGCTTGATG-3′
Mlu-5/*Mse*-8	us44	5′-TATAGCCTCGGAAGGTCGGT-3′ 5′-ACCACAGTCCGCTGACGTGT-3′
Mlu-5/*Mse*-10	us45	5′-GGCGACGACAGGCTCTTCTT-3′ 5′-TGTTGTGGCGTATGATCCTC-3′

Table 3 Primer sequences of the converted RFLP markers on chromosome 1 and the restriction enzymes with which polymorphisms were obtained

Name of RFLP probe	Primer sequences of the converted RFLP markers	Restriction enzyme
asg30	5′-CTCATTCTCTACTCTCGCAG-3′ 5′-GGCATGAACACCTCCAGTAT-3′	*Cfo*I
bnl5.59	5′-GTCGCTGGATGGATTGGTTC-3′ 5′-CTCATAGCCTAGATACGGTG-3′	*Tru*91
umc58	5′-TGGACGAGGTCAGCAGCTAG-3′ 5′-CCTGAATATGCGTCGTGTGA-3′	
php20855	5′-TGCGTCGTTGATGTCGGTTC-3′ 5′-CTGTACTCGGTGTTCTGCCT-3′	*Hpa*II

us40, us41 and us42 could be visualized on a polyacrylamide gel as co-dominant markers (example Fig. 1B). Marker us44 was also co-dominant in the parental lines after digestion with the restriction enzyme *Cfo*I. Marker us45 was dominant in the parental lines.

The five markers were amplified on the 230 plants of the 1998 F_2 population and linkage analysis was performed with MAPMAKER/EXP. Two linkage groups were identified; one group included the two markers us44 and us45, and the other included markers us40 and us42. The linkage distance between the markers us44 and us45 was 10.4 cM (LOD 22.83), and between markers us40 and us42 was 8.2 cM (LOD 55.41).

Linkage was confirmed when using the genotype data and disease scores of the 230 F_2 plants with the program MAPMAKER/QTL. A LOD value of 18.12 and a variance of 43% were calculated with the markers us44 and us45, and a LOD value of 4.85 and a variance of 10% were calculated with the markers us40 and us42.

As us41 was not linked to the other markers, QTL analysis could not be performed. A standard ANOVA for the linear regression of GLS score on marker genotypes was used to determine whether an association between the marker and GLS resistance could be detected (results are presented under the heading ‘Consistency of the QTLs’).

Mapping of the us markers using RIL populations

Two publicly available recombinant inbred families (Burr et al. 1988), already mapped for more than 1000 markers, were used to map the us markers on the maize chromosomes. Four markers (us40, us41, us44 and us45) were polymorphic between the parents of at least one of the RIL populations. The four polymorphic markers were amplified on the DNA of the individual plants of the RIL populations. The genotype data were added to the existing datafiles of the RIL populations and linkage analysis was performed. Markers us44 and us45 were mapped on chromosome 1 (bin 1.04/05). Marker us40 mapped on chromosome 5 (bin 5.04) and marker us41 on chromosome 3 (bin 3.04). LOD scores >7 were obtained with flanking markers on the RIL maize maps. As marker us42 showed linkage with marker us40 in the F_2 population, it could be inferred that this marker is also localized on chromosome 5.

Linkage analysis and QTL mapping

To obtain a more-precise localization of the QTLs on chromosomes 1 and 5, converted RFLP and microsatellite markers from the chromosomal regions were used. Primer sequences for four converted RFLP markers were identified from sequenced RFLP probes (Table 3). A size difference was detected with one of the converted RFLP markers (umc58) whereas site differences were observed with the other markers (asg30, bnl5.59 and php20855). Polymorphisms were also observed between the parental lines with five and seven microsatellite markers on chromosomes 1 and 5, respectively. Linkage maps were constructed with MAPMAKER/EXP based on 11 markers, including us44 and us45 on chromosome 1 (Fig. 2A), and nine markers, including us40 and us42, on chromosome 5 (Fig. 2B).

Linkage was confirmed when using the genotype data and disease scores of the 230 F_2 plants with the pro-

grams MAPMAKER/QTL and QTL Cartographer. As the phenotypic GLS data showed deviations from the normal distribution, the data was $\log_{10}$-transformed prior to analysis. This transformation did not alter the QTL that was identified, and the original data was therefore used in QTL analysis. Using interval mapping, the highest peak with a LOD value of 20.7 was identified on chromosome 1 between markers us44 and bnl5.59, 3.1-cM proximal to marker us44 (dotted line, Fig. 3A). The phenotypic variance explained by the QTL on chromosome 1 (QTL1) was 36.7%. Two peaks were visible on chromosome 5 (dotted line, Fig. 3B). A LOD value of 4.3 was calculated for the highest peak between markers mmc0282 and bnlg1847, 3.6-cM proximal to marker mmc0282. The second peak was located between markers bnlg557 and bnlg150, 4.6-cM proximal to marker bnlg557, and the LOD value for the second peak was 4.08. A phenotypic variance of 10.6% was given for the highest peak and 10.5% was given for the lower peak. By examining QTL1 and the QTL on chromosome 5 (QTL5) simultaneously, the cumulative variance explained was 46.6%.

Further analysis using composite interval mapping resulted in peaks localized between the same markers as those identified by interval mapping (solid line, Fig. 3). The LOD scores calculated using composite interval mapping were, however, lower (15.49 for the peak on chromosome 1, and 1.78 and 0.9 for the highest and lowest peak on chromosome 5, respectively).

The gene action of the QTLs was tested using MAPMAKER/QTL and the free model accounted for most of the variance, with 37% (LOD 20.87) for QTL1 and 10.6% (LOD 5.19) and 10.6% (LOD 4.82) for the two peaks of QTL5, respectively.

Consistency of the QTLs

To determine the consistency of the QTLs on chromosomes 1 and 5 the markers flanking the QTLs (Fig. 3) were amplified on the F_2 populations planted in 1999 and 2000. Furthermore, marker us41 on chromosome 3 was tested on the 1998, 1999 and 2000 populations to determine whether an association between the marker and GLS resistance could be detected. To limit the number of progeny to be genotyped, selective genotyping, introduced by Lander and Botstein (1989), was used. DNA was extracted from 111 plants of the 1999 F_2 population and 48 plants of the 2000 F_2 population.

A standard ANOVA for linear regression of the GLS score on marker genotypes was used to calculate the proportion of the total phenotypic variance explained by each marker. The results obtained with the 1999 and 2000 F_2 populations are given in Table 4. In comparison, the regression-analysis results obtained with the 1998 F_2 population are also given. Both markers on chromosome 1 showed a highly significant (P<0.001) association with GLS resistance and accounted for 26–40% of the variance, whereas on chromosome 5 only marker bnlg150 explained a significant amount of the variance in the

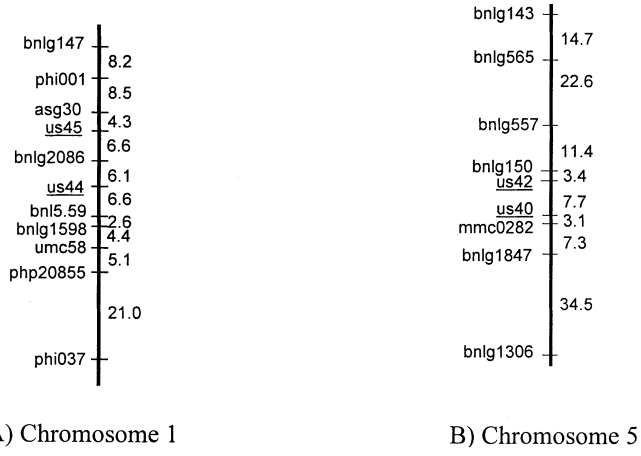

Fig. 2 Linkage group on chromosome 1 **A** and chromosome 5 **B** obtained by linkage analysis of 230 F_2 plants using MAPMAKER/EXP. Distances are given in centiMorgans. The converted AFLP markers are *underlined*

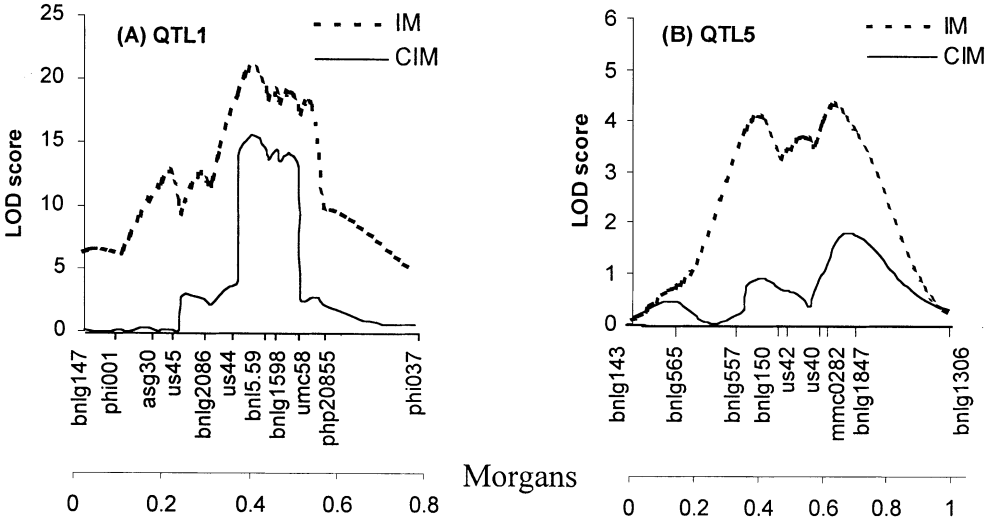

Fig. 3 Interval mapping (IM – *dotted line*) and composite interval mapping (CIM – *black line*) results on chromosome 1 **A** and chromosome 5 **B**

Table 4 Linear regression analysis results for the association between markers and GLS resistance in the 1998, 1999 and 2000 F_2 populations. R^2=proportion of phenotypic variation explained by the markers, *F*=Fisher F-ratio, *P*=significance

Bin marker		1998 F_2 population			1999 F_2 population			2000 F_2 population		
		R^2	*F*	*P*	R^2	*F*	*P*	R^2	*F*	*P*
Chromosome 1										
1.05	us44	0.306	93.263	<0.001*	0.401	50.249	<0.001*	0.262	15.971	<0.001*
1.06	bnlg1598	0.274	85.301	<0.001*	0.327	51.926	<0.001*	0.315	21.105	<0.001*
Chromosome 5										
5.03	bnlg557	0.093	23.304	<0.001*	0.008	0.763	0.385	0.121	6.302	0.016*
5.04	bnlg150	0.082	20.283	<0.001*	0.075	8.810	0.004*	0.205	11.849	0.001*
5.05	mmc0282	0.099	24.968	<0.001*	0.014	1.594	0.209	0.078	3.897	0.054
5.06	bnlg1847	0.101	25.255	<0.001*	0.010	0.843	0.361	0.042	2.021	0.162
Chromosome 3										
3.04	us41	0.007	1.626	0.204	0.098	11.606	0.001*	0.082	4.104	0.049*

* Significant at the *P*=0.05 probability level

1999 and 2000 populations (8%, *P*=0.004, and 21%, P=0.001, respectively). Marker us41 on chromosome 3 accounted for 10% (*P*=0.001) and 8% (*P*=0.049) of the variance in the 1999 and 2000 populations, respectively, and could therefore be linked to GLS resistance in these populations but not to the 1998 population (*P*=0.204).

Discussion

Resistance to GLS is an essential trait in most maize improvement programs (Schechert et al. 1999). To-date only a few high-yielding maize hybrids resistant to GLS are available in South Africa. Recovery through conventional breeding is difficult, because the development of GLS is highly dependent on environmental effects, field assessment of the disease is problematic, and the heritability of resistance is relatively low. Molecular markers linked to the resistance genes may thus be useful to plant breeders to support the introgression of the resistance alleles into elite high yielding inbred lines. Furthermore, this can be done without inoculation and at an early stage of plant development.

BSA has been used in a number of studies to identify markers linked to QTLs (Chagué et al. 1997; William et al. 1997; Somers et al. 1998; Quarrie et al. 1999). In this study, BSA was used together with the AFLP technique. Eleven polymorphic AFLP fragments were detected with ten AFLP primer combinations. Five of the 11 AFLP fragments were converted to reliable sequence-specific PCR markers and designated as numbers us40, us41, us42, us44 and us45. In further studies, polymorphisms between the parental lines may be obtained with the six remaining markers.

Conversion of the AFLP fragments to sequence-specific PCR markers made the screening of the plants of the F_2 population easier, faster and less expensive. Furthermore, most of the dominant markers were converted into co-dominant markers. It has been stated that the use of dominant markers in linkage analysis and QTL mapping using an F_2 population can lead to errors, as the amount of information produced by each datapoint is decreased in situations where heterozygous genotypes are found (Beaumont et al. 1996; Schondelmaier et al. 1996; Jiang and Zeng 1997).

Converted RFLP and microsatellite markers were used to obtain a more-precise localization of the QTLs on chromosomes 1 and 5. The number of publicly available microsatellite markers is increasing very rapidly. Over 1000 microsatellite primer pairs have already been published for maize and can be accessed via the Internet. The increasing number of available microsatellite primer pairs, and the high number of polymorphisms detected with these markers, make them very useful to study genome regions of particular interest.

QTL mapping indicated that one QTL is present on chromosome 1 (QTL1) and at least one QTL, but probably two, are present on chromosome 5 (QTL5). QTL1 had the largest effect on GLS resistance and explained 37% of the variance. A smaller QTL effect was explained by QTL5 (11%). It is worth mentioning that both QTL1 and the highest peak of QTL5 localized to the regions where the QTLs for GLS resistance, introgressed from the inbred line Va14, were reported by Saghai Maroof et al. (1996). Interestingly, they assumed that the QTL on chromosome 5 was a false-positive QTL, as it was not reproducible in their F_3 populations. QTLs in common across different mapping populations have been reported by Bubeck et al. (1993), who detected one region on chromosome 2 associated with GLS resistance in three different populations.

It has been found that the consistency of the identification of QTLs in one population across seasons is low (Bubeck et al. 1993; Tuberosa et al. 1998). In our study, the consistency of the QTLs was tested on selected plants of two F_2 populations planted in consecutive years. The results of the regression analysis indicated that the highest proportion of the variance was accounted for by the markers on chromosome 1 in all populations. Only the flanking

markers for one of the peaks of QTL5 (Fig. 3B) explained a significant amount of the variance in the 1999 and 2000 populations. This could confirm that two QTLs are present on chromosome 5 and that only one of the two QTLs was detectable in these populations. Furthermore, regression analysis confirmed the presence of a QTL on chromosome 3 (QTL3). The detection of QTL3 in the 1999 and the 2000 populations, but not the 1998 population, could indicate that the environment has an effect on QTL3. Since only one marker for the QTL on chromosome 3 was used in regression analysis, the precise localization of the QTL could not be determined. The distance between the marker and the GLS resistance QTL may still be considerable, and therefore the QTL effects calculated could be under-estimated. Further studies would alleviate this problem. Although the genetic effects calculated for each marker by using regression analysis were fairly consistent, the difference in the number of plants used per population in the regression analysis was large. The calculated genetic effects could, therefore, be biased and should only be used as an indication of the presence or absence of a QTL.

In this study, a major GLS resistance QTL was mapped on chromosome 1, and two minor GLS resistance QTLs were mapped on chromosomes 3 and 5 using a resistant Zimbabwean (Seed Co. Ltd.) inbred line. The next step in this study will be fine-mapping of the QTLs to obtain markers closer to the resistance genes. These markers could be useful to breeders in marker-assisted selection programs. The ultimate achievement of this project would be the molecular cloning of the genes involved in GLS resistance.

Acknowledgements This research was supported in part by funds provided by the seed company Sensako and the South African National Research Foundation.

References

Baston CJ, Weir BS, Zeng Z-B (1994) Zmap-a QTL cartographer. In: Smith C, Gavora JS, Benkel B, Chesnais J, Fairfull W, Gibson JP, Kennedy BW, Burnside EB (eds) Proceed 5th World Congress on Genetic Applied to Livestock Production: Computing Strategies and Software. Published by the organizing committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada, Vol 22, pp 65–66

Baston CJ, Weir BS, Zeng Z-B (1997) QTL Cartographer: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, North Carolina

Beaumont VH, Mantet J, Rocheford TR, Widholm JM (1996) Comparison of RAPD and RFLP markers for mapping F_2 generations in maize (*Zea mays* L.). Theor Appl Genet 93:606–612

Bubeck DM, Goodman MM, Beavis WD, Grant D (1993) Quantitative trait loci controlling resistance to gray leaf spot in maize. Crop Sci 33:838–847

Burr B, Burr FA, Thompson KH, Albertson MA, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Chaguè V, Mercier JC, Guènard M, de Courcel A, Vedel F (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor Appl Genet 95:671–677

Coates ST, White DG (1998) Inheritance of resistance to gray leaf spot in crosses involving selected resistant inbred lines of corn. Phytopathology 88:972–982

Gevers HO, Lake JK (1994) *GLS1* – a major gene for resistance to gray leaf spot in maize. S Afr J Science 90:377–379

Gevers HO, Lake JK, Hohls T (1994) Diallel cross analysis of resistance to gray leaf spot in maize. Plant Dis 78:379–383

Hohls T, Shanahan PE, Clarke GPY, Gevers HO (1995) Genetic analyses of grey leaf spot infection of maize in a single-location 12×12 diallel. S Afr J Plant Soil 12:133–139

Jiang C, Zeng ZB (1997) Mapping quantitative trait loci with dominant and missing markers in various crosses from two inbred lines. Genetica 101:47–58

Lander E, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Latterell FM, Rossi AE (1983) Gray leaf spot of corn: a disease on the move. Plant Dis 67:842–847

Lincoln S, Daley MJ, Lander E (1992a) Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report, 3rd edn

Lincoln S, Daley MJ, Lander E (1992b) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report, 2nd edn

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete RFLP linkage map. Nature 335:721–726

Quarrie SA, Lazic-Lancic V, Kovacevic D, Steed A, Pekic S (1999) Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. J Exp Bot 50:1299–1306

Saghai Maroof MA, Yue YG, Xiang ZX, Stromberg EL, Rufener GK (1996) Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. Theor Appl Genet 93:539–546

Schechert AW, Welz HG, Geiger HH (1999) QTL for resistance to *Setosphaeria turcica* in tropical maize. Crop Sci 39:514–523

Schondelmaier J, Steinbrucken G, Jung C (1996) Integration of AFLP markers into a linkage map of sugar beet (*Beta-vulgaris* L). Plant Breed 115:231–237

Somers, DJ, Friesen KRD, Rakow G (1998) Identification of molecular markers associated with linoleic acid desaturation in *Brassica napus*. Theor Appl Genet 96:897–903

Tehon LR, Daniels E (1925) Notes on parasitic fungi of Illinois. Mycologia 17:240–249

Tuberosa R, Sanguineti MC, Landi P, Salvi S, Casarini E, Conti S (1998) RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (*Zea mays* L.) Theor Appl Genet 97:744–755

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang J, Levy M, Dunkle LD (1998) Sibling Species of *Cercospora* associated with gray leaf spot of maize. Phytopathology 88:1269–1275

Ward JMJ, Laing MD, Cairns ALP (1997) Management practices to reduce gray leaf spot of maize. Crop Sci 37:1257–1262

Ward JMJ, Stromberg EL, Nowell DC, Nutter FW Jr (1999) Gray leaf spot: a disease of global importance in maize production. Plant Dis 83: 884–895

William HM, Hoisington D, Singh RP, González-de-León D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253–260

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468