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Full Length Research Paper

Quantitative trait loci (QTLs) for resistance to gray leaf spot and common rust diseases of maize

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Gray leaf spot and common rust diseases can greatly reduce grain yield of maize in susceptible genotypes by between 10 and 70% on average. Control of these diseases through conventional measures has been quite ineffective and difficult to sustain. The most feasible way to control them is by breeding and deploying resistant maize genotypes. This study was carried out to evaluate germplasm for QTLs associated with resistance to GLS and common rust diseases by use of microsatellite markers and artificial inoculation with the two pathogens. A total of 41 genotypes comprising 23 recombinant inbred lines, 14 parental inbred lines and 4 hybrid checks were screened using 28 SSR markers and disease pressure by artificial inoculation (Tables 1 and 2). Out of the 14 parental inbred lines, only 4 were found to carry the QTL associating positively for the two diseases, and 10 out of the 23 recombinant inbred lines with possible lineage from any of the 14 parents, were positively associated with the traits and seven of the markers used (Table 3). GLS QTLs were significant for two markers; bnlg1258 with a LOD score of 16.0 and umc2019 with a LOD score of 17.9 from regions 2.06 and 2.08 of chromosome 2, respectively. Significant QTLs for common rust resistance were identified in three regions of chromosome 10, corresponding to markers phi054 with a LOD score of 14.0 at bin 10.00, umc1319 with a LOD score of 4.0 at bin 10.02 and bnlg1451 with a LOD score of 14.3 at bin 10.03. The effects of these QTLs were different from genotype to genotype. The disease severity scores (scale of 1-5) of artificial inoculation ranged from 1.5 to 2.5 for gray leaf spot with a mean of 1.88 and a range of 1.5 to 3.0 with a mean of 1.74 for common rust. All the inbred lines scored better for the two diseases than the four hybrid checks which scored 3.5. The most resistant genotypes showed a score of 1.5 for gray leaf spot and common rust. All parents showed a score of less than 2.5 for GLS and common rust. Parental genotypes MAL40, MAL9, MAL13, MAL41, MAL11, MAL19, MAL23-2, MAL24, and MAL19-1, carried QTLs associated with resistance to grey leaf spot and common rust and thus were identified as sources of resistance conferred to the inbred lines. The selected lines are being used to make single hybrids, double crosses, three way hybrids and synthetics resistant to diseases. The marker data was also used to analyze the diversity of the genotypes studied, with relevance to immune/resistant, tolerant or susceptible to the two diseases. Using the GLS genotypic data, 13 genotypes clustered into 11 groups, and using the common rust data, the 13 genotypes clustered into 12 clusters. This indicates that almost each of these genotypes was grouped in a cluster that contained lines that did not have positive association of marker and trait data. These results indicated that the putative QTLs for GLS are associated with the 13 genotypes and two markers in chromosome 2 (bnlg1258 and umc2019), whereas those of common rust are associated with the 13 genotypes and three markers on chromosome 10 (phi054, umc1319, and bnlg1451).

Key word: GLS, CR, QTLs, resistance, SSR, maize.

INTRODUCTION

Grey leaf spot disease

Gray leaf spot (GLS) caused by the fungal pathogen *Cercospora zeae-maydis* (Tehon and Daniels, 1925), is one of the major disease constraints to maize production in many parts of the World including Kenya. It was first

reported in Kenya in 1993, and by 1995 it had significantly contributed to yield losses of about 15% in the western part of the country. Since then, the disease has spread to other maize agro-ecological zones of Rift valley, Central, and Eastern regions. When susceptible genotypes are affected, it may lead to epidemics and yield losses of over 50%. For instance, yield losses of between 30 and 60% due to GLS infection have been reported in South Africa (Ward et al., 1997). However, documented yield losses of maize attributed to GLS vary from 11 to 69% (Ward et al., 1999), with estimated losses as high as 100% when severe epidemics contribute to loss of total photosynthetic leaf area, increased stalk lodging, and premature plant death (Latterell and Rossi, 1983). Under severe disease pressure a toxin called cercosporin is produced which causes extensive blighting of the upper leaves resulting in significant yield losses (Lipps, 1987). This disease is most severe and damaging during high relative humidity and prolonged late-season rains (Beckman and Payne, 1983). The extent of the damage has been found to be dependent on the hybrid affected and prevailing environmental conditions (Ward et al., 1999). Increased incidence of GLS in Africa has been associated with continuous cultivation of maize, and use of susceptible maize cultivars (Gevers et al., 1994; De Nazareno et al., 1993).

GLS disease epidemics have been managed conventionally through deep tillage to burry previous maize residue, fungicide application, and field hygiene (Ward et al., 1997). However, these measures have not been efficient in the management of GLS (Bigirwa et al., 2001). A reduction in conservation tillage would have to be universally adopted to have an economic impact on GLS epidemics (Lipps et al., 1996). Fungicide application is costly and not practical in most operations for the resource-poor farmers. Most hybrids currently in production in Kenya are susceptible to GLS (Ininda et al., 2004). Availability and adoption of resistant hybrids would provide a cost-effective means of controlling GLS. Development of improved maize lines with resistance to multiple foliar pathogens (including GLS, Turcicum blight and common rust) has been commenced in Kenya, through the use of resistance sources from Kenya, CIMMYT, IITA and South Africa. This has been accomplished by introgressing resistance regions from donors into elite maize germplasm. But GLS disease resistance in these sources is quantitatively controlled, necessitating the search for molecular markers linked to quantitative trait loci for resistance to GLS that have aided breeding efforts by augmenting conventional phenotypic selection. Many studies have reported on how resistance to the disease is controlled. In some temperate adapted lines, the genetic basis of resistance to *C. zeae-maydis* has been reported to be under additive genetic control, with some dominance effects (Coates and White, 1998; Gevers and Lake, 1994). Most of the sources of resistance to *C. zeae-maydis* identified and used in maize have genes for resistance inherited in a quantitative manner (Gevers et al., 1994; Lehmensiek et al., 2001; Clements et al., 2000). The genetic study of Mann (1977) using a generation mean analysis concluded that additive genetic effects account-ted for 82 to 96% of the total variation in conditioning GLS resistance among generations, although dominance and epistasis provided some contribution. Major resis-tance factors have been mapped to at least three different chromosomes, with some of the quantitative trait loci consistently expressed across environments and having large effects on GLS resistance (Clements et al., 2000).

A major constraint in breeding for GLS resistance is the high degree of genotype-environment interactions observed during artificial inoculation experiments. Molecular markers linked to QTLs that control resistance may be useful for plant breeders to support the introgression of the resistance alleles into elite-yielding inbred lines. The objectives of this study were to identify QTLs linked to GLS resistance in 37 inbred lines and to associate the QTLs with the phenotypic trait.

Common rust

Common rust caused by *Puccinia sorghi* is a common fungal disease of maize in Kenyan medium to high altitude zones. Common rust may cause extensive yellowing and premature desiccation of maize foliage, resulting in leaf necrosis, and complete destruction of photosynthetic areas. In extreme cases, heavy rust infestations may result in stunting, incomplete ear tip fill, and pustules on ear husks, reducing marketability and yield. On average, common rust reduces yield by up to 40%. Conventionally, control of common rust disease has been through cultural and chemical measures. The major cultural method used is timing of the rainy seasons, while chemical sprays use strobilurin and sterol-inhibiting fungicides which have some systemic properties. However, these measures are limited by the unpredictable weather conditions, and the environmental side effects. In addition, chemicals require to be used in a program that minimizes the development of resistant strains of the rust fungus and to maximize efficacy. This increases the cost of production, hence putting an additional burden of resource poor farmers.

Breeding for resistance has been identified as a better method of managing the common rust disease. There are two types of resistance against *P. sorghi*: partial and race-specific. In some studies more than 25 dominant resistance (*Rp*) genes were found to be involved in race-specific resistance and organized in complex loci at chromosomes 3, 4, and 10 (Hooker, 1985; Delaney et al., 1998). Richter et al. (1995) found that within these complex loci, novel resistance specificities are generated by genetic re-assortment events, such as unequal crossing-

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Table 1. Disease scores for forty-one genotypes (23 recombinant inbred lines, 14 parental lines and 4 hybrid checks) evaluated.

No.	Genotype	Code	Description	GLS*	E.T*	RUST*
1	MAL8	Pop1	Parent	2.0	2.0	1.5
2	MAL10	Pop2	Parent	2.5	3.0	1.5
3	MAL14	Pop3	Parent	1.5	2.0	1.5
4	MAL13	Pop4	Parent	2.5	2.0	1.5
5	MAL40	Pop5	Parent	1.5	2.5	1.5
6	MAL9	Pop6	Parent	1.5	2.0	1.5
7	MAL41	Pop7	Parent	2.0	2.5	2.0
8	MAL19	Pop8	Parent	1.5	1.5	1.5
9	MAL24	Pop9	Inbred	1.5	2.0	1.5
10	MAL15	Pop10	Parent	2.5	2.0	2.0
11	MAL26	Pop11	Inbred	1.5	2.0	1.5
12	MAL49	Pop12	Inbred	1.5	2.0	1.5
13	MAL1	Pop13	Parent	1.5	2.0	1.5
14	MAL5	Pop14	Parent	1.5	2.0	1.5
15	MAL6	Pop15	Parent	2.0	4.5	1.5
16	MAL11	Pop16	Parent	1.5	2.0	1.5
17	MAL16	Pop17	Parent	2.0	3.5	2.5
18	$S_5$39-10-2-2-1	Pop18	Inbred	1.5	2.0	1.5
19	MAL44	Pop19	Inbred	2.0	2.5	2.0
20	MAL45	Pop20	Inbred	2.0	2.0	2.0
21	MAL46	Pop21	Inbred	2.0	2.0	1.5
22	MAL47	Pop22	Inbred	2.0	2.5	2.0
23	MAL48	Pop23	Inbred	2.0	2.5	2.5
24	MAL43	Pop24	Inbred	1.5	2.5	2.5
25	MAL25	Pop25	Inbred	1.5	2.0	1.5
26	MAL15-1	Pop26	Inbred	2.5	2.0	2.0
27	MAL23-2	Pop27	Inbred	1.5	1.5	1.5
28	MAL19-1	Pop28	Inbred	1.5	2.0	1.5
29	S496–21-1-1	Pop29	Inbred	1.5	2.0	2.0
30	S496–6-1-1	Pop30	Inbred	1.5	2.0	2.0
31	S496–15-1-1	Pop31	Inbred	1.5	2.0	2.0
32	MAL24-1	Pop32	Inbred	2.0	2.5	1.5
33	MAL50	Pop33	Inbred	2.5	2.5	1.5
34	Z426-43, Z387-4-1	Pop34	Inbred	1.5	2.5	1.5
35	MAL52	Pop35	Inbred	2.5	3.0	1.5
36	MAL53	Pop36	Inbred	2.5	2.5	1.5
37	MAL54	Pop37	Inbred	1.5	2.0	1.5
38	H614	Pop38	Check	1.5	2.0	1.5
39	H627	Pop39	Check	1.5	2.0	2.0
40	H623	Pop40	Check	2.5	2.5	1.5
41	PH3253	Pop41	Check	3.5	3.5	2.0

*GLS = Gray leaf spot disease, E.T. = Turcicum leaf blight disease, Rust = Common rust disease.

over or gene conversion. Pyramiding of multiple closely linked genes into “compound” genes has been proposed as a possible means of constructing more durable race specific resistance inherited by complex loci against common rust in maize (Hu and Hulbert, 1996). Depending on the materials employed in generation mean analyses, the prevalent mode of gene action varies between additive and dominant for resistance against *P. sorghi* (Kim and Brewbaker, 1977; Randle et al., 1994). Other studies, utilizing different maize genotypes have identified resistance QTLs on chromosomes 1, 3, and 8 (Thomas et al., 1998).

Table 2. List of the SSR primers used in the study.

Bin	Marker	Repeat motif	Primer sequences F 5'-3' R 5'-3'
Chromosome 2			
2.07-2.08	Bnlg1329	AG(14)	ATAGAATGGGATGTGGGCAA//AAGCAGACTATGCTATGCTACGCC
2.07	umc2019	(GTG)4	GACATGGACTGCCTTCAAATGAT//ATAGCTTTTCTCAGTAAGCGCCAG
2.07	umc2402	(CAT)4	CACCGAGGAGAACAGAGCCTTA//CCAAGAGCAAACCGAAGAAGAAG
2.08	bnlg1258	AG(24)	GGTGAGATCGTCAGGGAAAA//GAGAAGGAACCTGATGCTGC
2.08	bnlg1316	AG(13)	CGAAACAGAGCCCAAAAGAC//GATCCGCGTCTAGCCCCT
2.08	bnlg1767	AG(16)	AATTTCACGGTAGGGACACG//AATCCGCGTGTTTTCATAGG
2.09	bnlg1520	AG(22)	TCCTCTTGCTCTCCATGTCC//ACAGCTGCGTAGCTTCTTCC
2.08-2.09	umc1230	(TAA)8	GCGATTTCAACTATTTGTGGTAAAGG//GTACGACCGTTGAAACTGTTGTTTT
2.09	umc1252	(CCA)4	GCGTCGGAGAAGTACATCAAGTTT//CTTCTGCATCATCATCATCGTCTT
2.09	umc1525	(CGA)4	TTTGTGCCGAATATAAATGTGACG//AATAATATCAAATGGCGCCAAGC
2.09	umc1551	(AGCC)4	CACCGGAACACCTTCTTACAGTTT//CGAAACCTTCTCGTGATGAGC
2.09	umc1256	(CAT)5	CATCTCGACCTTTGACATTCTCCT//AGAAGACGATGATGATGATGCAGA
2.06	umc2023	(AGC)5	TCAGTCCCATTATATTCACCGACC//TCCTCTTCTTTTCCTCTCAGAGCC
	Phi127	AGAC	ATATGCATTGCCTGGAACTGGAAGGA//AATTCAAACACGCCTCCCGAGTGT
Bin	**Marker**	**Repeat motif**	**Primer sequences F 5'-3' R 5'-3'**
Chromosome 10			
10.0	phi041	AGCC	TTGGCTCCCAGCGCCGCAAA//GATCCAGAGCGATTTGACGGCA
10.0	phi117	ACC	ATCGGATCGGCTGCCGTCAAA//AGACACGACGGTGTGTCCATC
10.0	phi118	AGG	GAAAGCGGAGAGAGGGCTTCAA//TTGGGATGTGATGTGAGAGCTTGCT
10.01	bnlg1451	AG(34)	TGATCGATGGCTCAATCAGT//ATCTGGAACACCGTCGTCTC
10.01	umc1291	(CGT)4	CAAGTCGTGATCATGCGTAGGTAG//ACTGCTCCAGGGTGAACTGAAC
10.01	umc1319	(ACC)5	TGAGAGCCACCTTCTTGAGCTACT//TTCCTTGAAGGCGAAGGTAGGTAT
10.01	umc2018	(CCT)7	TAGCCAAGCTTCTCCCTAGCTTTT//GCAGTTGGAGGAGGAGCAGAC
10.01	umc2053	(CGA)4	ATCTCTCCCTCGCTCTCCTTCTC//AGCAGCAGGTTGGTCGAATG
10.02	phi059	ACC	AAGCTAATTAAGGCCGGTCATCCC//TCCGTGTACTCGGCGGACTC
10.02	phi063	TATC	GGCGGCGGTGCTGGTAG//CAGCTAGCCGCTAGATATACGCT
10.03	bnlg1712	AG(20)	CTCAGGCTTCACGTGGGTTT//GTTACACTCCCCTGCCAAAA
10.03	bnlg1716	AG(28)	AAATAACCAGAACATGCCGC//CGCAACTTTCATCGAGTTGA
10.03	phi054	AG	AGAAAAGAGAGTGTGCAATTGTGATAGAG//AATGGGTGCCTCGCACCAAG

Table 3. Parental and recombinant inbred lines positively associating with both GLS and rust resistance.

Genotype	Description	GLS* score	E.T* score	RUST* score
MAL14	Parent	1.5	2.0	1.5
MAL40	Parent	1.5	2.5	1.5
MAL1	Parent	1.5	2.0	1.5
MAL5	Parent	1.5	2.0	1.5
MAL19	Parent	1.5	1.5	1.5
MAL24	Recombinant inbred line	1.5	2.0	1.5
MAL26	Recombinant inbred line	1.5	2.0	1.5
MAL26-1	Recombinant inbred line	1.5	2.0	2.0
$S_5$39-10-2-2-1	Recombinant inbred line	1.5	2.0	1.5
MAL19-1	Recombinant inbred line	1.5	2.0	1.5
S496–21-1-1	Recombinant inbred line	1.5	2.0	2.0
S496–6-1-1	Recombinant inbred line	1.5	2.0	2.0
Z426-43, Z387-4-1	Recombinant inbred line	1.5	2.5	1.5

*GLS = Gray leaf spot disease, E.T. = Turcicum leaf blight disease, Rust = Common rust disease.

MATERIALS AND METHODS

Genetic material

A total of 37 inbred lines collected from Kenya, CIMMYT, IITA and South Africa were used in the study. Out of these, 14 genotypes were parental lines and 23 were recombinant inbred lines. In addition, 4 hybrids were included as checks. The genotypes were previously selected using disease severity scores for GLS and common rust. Planting was done in the field at Kakamega in Western Kenya for GLS experiments, and at Muguga for common rust experiments. The experiments were laid down in a randomized complete block design with three replicates. Each plant was artificially inoculated with the GLS and common rust pathogens at 6 - 7 leaf stage, using about 10 g of shreaded infected maize leaves, and this was repeated three times at a 7-day interval. GLS and common rust severity was assessed by rating leaves from the ear leaf using the percent leaf area affected scale developed by Smith (1989). This scale assigns a percent leaf area affected score on the basis of visual estimates of the percentage of leaf surface area covered by lesions. Data were also collected for common rust (*Puccinia sorghi*) using the same scale. Severity scores based on percent leaf area affected were converted to a scale of 1 - 5 where 1 = very small necrotic lesions on leaves; 2 = light necrosis covering <40 percent of plant; 3 = moderate necrosis on leaves 60% of leaf area; 4 = severe necrosis on about 80% of leaf area; and 5 = very severe necrosis on more than 90 percent of leaf area or dead plants.

SSR analysis

DNA extraction, quality and concentration checks were carried out as described in Danson et al. (2006). A total of 28 polymorphic markers contained in chromosome 2 and 10. GLS QTLs were screened for in chromosome 2 while those of common rust in chromosome 10. PCR reactions and conditions, gel electrophoresis, and data collection were done as previously described (Danson et al., 2006). Fragments were separated using metaphor agarose electrophoresis, and also by capillary electrophoresis using ABI genotyper and data extracted using genemapper 3.7 software. Genetic data were analyzed using Popgene 3.1 program, while QTL analysis was carried out by interval mapping using MapQTL 5.0. Linkage groups were determined by a log-likelihood (LOD) threshold of 3.0 and linkage maps constructed using pairwise recombination estimates between 0.0 and 0.5. A LOD score larger than 3.0 with Kosambi's mapping function was taken as QTL. Linkage groups were scanned for QTLs at 4-cM intervals with LOD thresholds corresponding to a genome-wide error rate of 5% calculated by 2000 permutations of the data. Marker cofactors were selected by the automatic cofactor selection option in MapQTL 5.0 and then modified according to the program instructions (Van et al., 2004) to finish with a set of cofactor loci closest to the significant maxima in the QTL likelihood map.

RESULTS

Disease scores

Disease reactions are shown for GLS and common rust. The disease scores for the two diseases ranged from 1.5 (resistant), 2.0 to 2.5 (tolerant) and above 3.0 (susceptible). Ten lines were resistant to both diseases, while none was susceptible to both. Generally 27% of the genotypes were resistant to both GLS and common rust. None of the genotypes was immune to the diseases (i.e. score of 1.0). The means for GLS and rust scores were 1.878 and 1.743 respectively.

SSR analysis

The peaks were extracted and analyzed using genemapper 3.7, and allele sizes ranged from 102 to 192. The combined probabilities for all alleles showed significant deviation from the expected frequencies ($p < 0.001$) in 67% of the loci. Twenty five per cent of the loci were monomorphic and 8% were not significant ($p < 0.05$). The analysis indicated homozygote excess at all the loci with significant deviation from the expected homozygosity. Null alleles were present in 20% of the loci as was suggested by the general excess of homozygotes for most allele size classes. However, there was no evidence for scoring errors due to stuttering, nor evidence for large allele dropout in 95% of the loci. The presence of null alleles suggested the possibility of a population in Hardy-Weinberg equilibrium with the affected loci. The marker data was also used to analyze the diversity of the genotypes studied, with relevance to immune/resistant, tolerant or susceptible to the two diseases. Using the GLS genotypic data, 13 genotypes clustered into 11 groups, and using the common rust data, the 13 genotypes clustered into 12 clusters (Figures 1 and 2).

QTL analysis

Two microsatellite markers out of fourteen used to detect QTLS located on chromosome 2, bnlg1258 at position 2.06 and umc2019 at position 2.08 had large effects on GLS resistance. Marker bnlg1258 had a LOD score of 16.0 and explained 73% of the variance while marker umc2019 had a LOD score of 17.9 and explained the later explained 91% of the variance. Similarly, three markers out of fourteen used to detect QTLs located on chromosome 10, phi054 at position 10.0, umc1319 at position 10.02 and bnlg1451 at position 10.03 had large effects for common rust disease resistance. Marker phi054 had a LOD score of 14.0 and explained 47% of the variance, marker umc1319 had a LOD score of 4.0 and explained 48% of the variance, while marker bnlg1451 had a LOD score of 14.3 and explained 56% of the variance. These QTLs were used to select genotypes resistant to both GLS and common rust. Thirteen lines had positive marker trait associations for the two traits. Four of them were parental lines (MAL14, MAL40, MAL1, and MAL5), while nine were inbred lines (MAL19, MAL24, MAL26, MAL26-1, $S_5$39-10-2-2-1, MAL19-1, S_4 96–21-1-1, S^4 96–6-1-1, and Z426-43 Z387-4-1).

DISCUSSION

The data showed an association between 5 markers and phenotypic traits for GLS and common rust in 4 parental

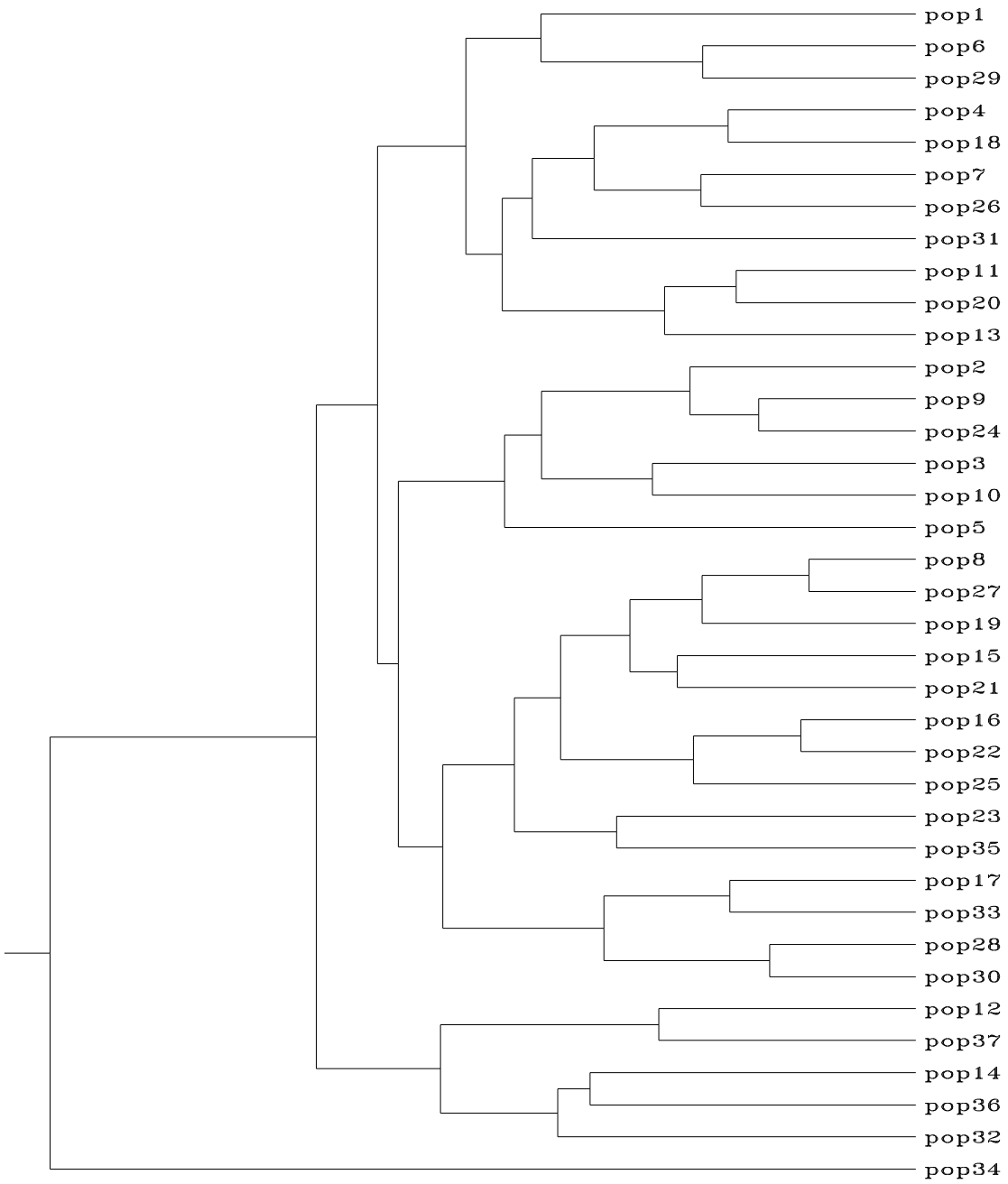

Figure 1. Dendrogram constructed from genetic distances based on Nei's (1978) and UPGMA method using gray leaf spot marker-trait data.

lines and 9 nine inbred lines. The perfect association of genotypic data and the phenotypic traits for the two diseases suggest a possibility of the inbred lines having originated from the studied parental lines. The main objective of this study was to identify molecular markers linked to quantitative trait loci responsible for conferring resistance of maize genotypes to *C. zeae-maydis* and *Puccinia sorghi,* the causative agents of gray leaf spot and common rust diseases. Out of the 14 parental lines studied, only 4 were found to carry the QTL associating positively for the two diseases, and out of the 23 inbred lines with possible lineage from any of the 14 parents, were positively associated with the traits and five of the markers used. GLS QTLs were significant for two markers bnlg1258 and umc2019 from regions 2.06 and 2.08 of chromosome 2 respectively. However, the QTLs were located in different positions in different individual genotypes, and also the sizes and effects of the QTLs

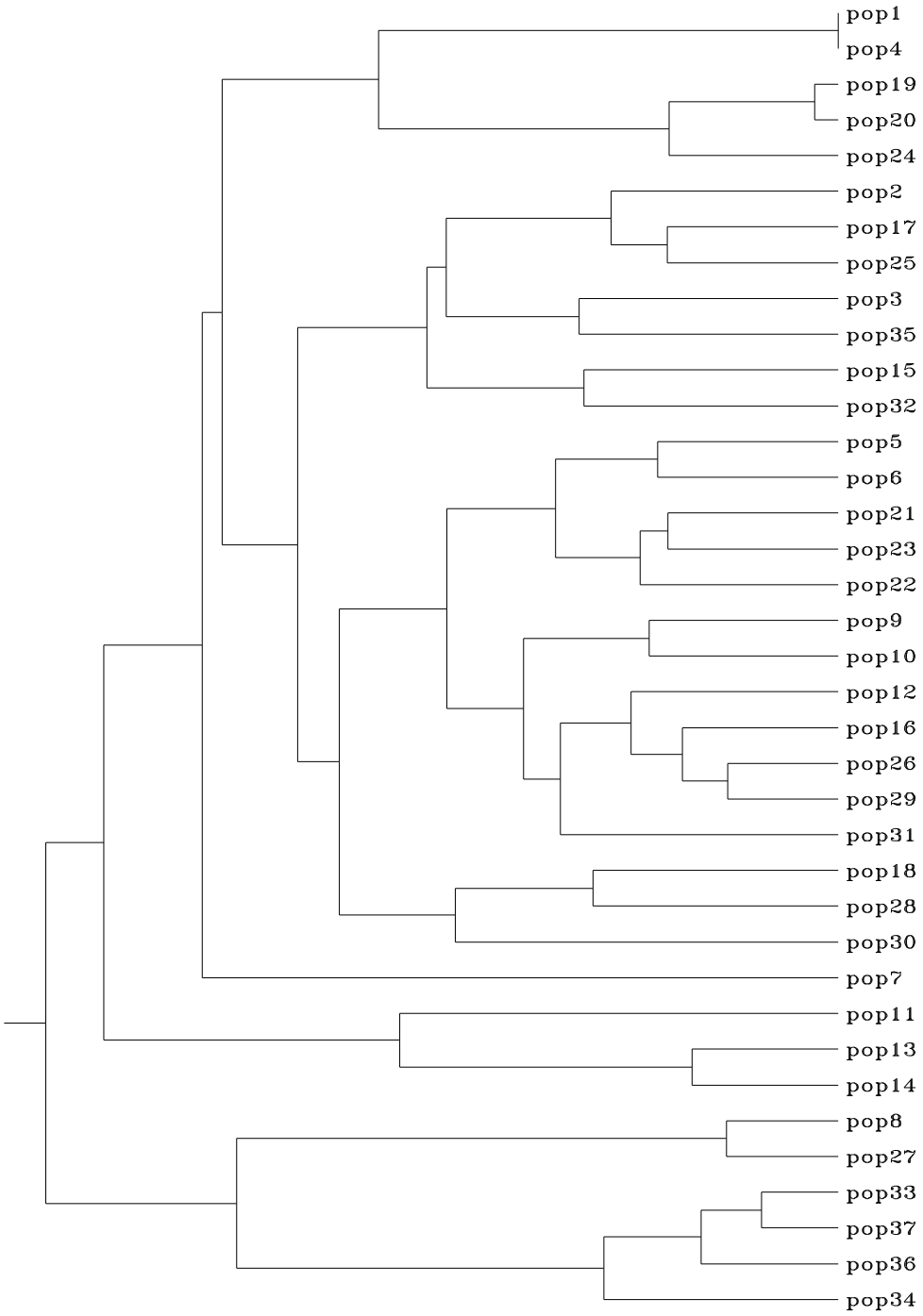

Figure 2. Dendrogram constructed from genetic distances based on Nei's (1978) and UPGMA method using common rust marker-trait data. Checks were not included in the clustering.

differed from genotype to genotype. Similarly, significant QTLs for common rust resistance were identified in three regions of chromosome 10, corresponding to markers phi054 at 10.00, umc1319 at 10.02, and bnlg1451 at 10.03. The positions, sizes and effects of these QTLs were also different from genotype to genotype. The difference in the number of possible recombinations and heterozygote fragments arising from allele frequencies was an indicator of the quantitative nature of the QTLS. Distantly placed markers were found to associate with QTLs that have close effect on the traits. This suggests possible occurrence of multiple number of crossovers within the chromosome. Furthermore there was a marked deviation from Hardy-Weinberg equilibrium, suggesting

linkage of QTLs and lack of segregating QTLs of minimum effect on the trait. The results show that the possible parental sources of resistance to GLS and common rust that carry QTLs of significant sizes and effects are MAL40, MAL1, MAL5, MAL14, MAL15, MAL19, and MAL23.

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