QTL Mapping for Gray Leaf Spot Resistance in a Tropical Maize Population

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Abstract

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A tropical gray leaf spot (GLS)-resistant line, YML 32, was crossed to a temperate GLS-susceptible line, Ye 478, to produce an F_{2:3} population for the identification of quantitative trait loci (QTL) associated with resistance to GLS. The population was evaluated for GLS disease resistance and flowering time at two locations in Yunnan province. Seven QTL using GLS disease scores and six QTL using flowering time were identified on chromosomes 2, 3, 4, 5, and 8 in the YML 32 × Ye 478 maize population. All QTL, except one identified on chromosome 2 using flowering time, were overlapped with the QTL for GLS disease scores. The

results indicated that QTL for flowering time in this population strongly corresponded to QTL for GLS resistance. Among the QTL, *qRglS.yaas-8-1/qFt.yaas-8* with the largest genetic effect accounted for 17.9 to 18.1 and 11.0 to 21.42% of variations for GLS disease scores and flowering time, respectively, and these should be very useful for improving resistance to GLS, especially in subtropical maize breeding programs. The QTL effects for resistance to GLS were predominantly additive in nature, with a dominance effect having been found for two QTL on the basis of joint segregation genetic analysis and QTL analysis.

Gray leaf spot (GLS), caused by *Cercospora zae-maydis* (Tehon and Daniels 1925) and *C. zeina-maydis* (Crous et al. 2006; Liu and Xu 2013), is a global threat to maize production (Katwal et al. 2013; Ward et al. 1999). In the 1960s and 1970s, the disease became of concern in the eastern United States. In the 1980s and 1990s, as reduced tillage gained popularity, GLS became a noticeable problem in most of the maize-growing areas of the midwestern and eastern United States. Now, the disease has become an international problem, from Africa to Asia and in North and South America, and has caused huge maize yield losses (Crous and Braun 2003). Documented yield losses of maize attributed to GLS varied from 11 to 69% (Ward et al. 1999), with estimated losses as high as 100% when severe epidemics occurred. The GLS damage has been linked to loss of photosynthetic capability and premature plant death (Latterell and Rossi 1983; McGee 1988). Because of broad distribution of the disease and severity of losses to maize production, GLS control has become a major breeding objective in many maize improvement programs worldwide (Menkir and Ayodele 2005; Nzuve et al. 2013).

The most common GLS control methods include the application of fungicides, cultural practices (i.e., crop rotation and field sanitation), and host resistance (Ward et al. 1999). Because all other methods are not highly effective or are detrimental to the environment, host resistance, which is considered to be environmentally friendly, inexpensive, and highly effective, seems to be the best means of reducing yield losses caused by GLS (Shi et al. 2014).

Introgression of resistance genes from donors into elite maize germplasm has proved to be effective in improving disease resistance (Gordon et al. 2004). Previous studies have shown additive genetic effects to be more important than dominance effects (Clements

et al. 2000; Coates and White 1994; Donahue et al. 1991; Elwinger et al. 1990; Gevers et al. 1994; Gordon et al. 2006). Other studies have indicated that resistance to GLS in maize germplasm was controlled by multiple genes (Balestre et al. 2012; Danson et al. 2008; Donahue et al. 1991; Gevers et al. 1994; Huff et al. 1988). Thus, it is quite logical to search for molecular markers linked to quantitative trait loci (QTL) offering resistance to GLS to speed up the development of resistant inbreds or hybrids through marker-assisted selection (MAS). Knowledge of genetic effects and nature of resistance QTL is important for introducing favorable alleles into susceptible materials and for optimal utilization of genetic variability (Pozar et al. 2009).

Several important QTL for GLS resistance have successfully been identified in maize under various environments in different resistant germplasms (Balint-Kurti et al. 2008; Danson et al. 2008; Saghai Maroof et al. 1996; Zhang et al. 2012). For example, Saghai Maroof et al. (1996) identified QTL for resistance to GLS on chromosomes 1, 4, and 8 in the Va14 × B73 cross and these QTL explained 35.0 to 56.0, 14.3, and 7.7 to 11.0%, respectively, of the variation for resistance to GLS. Gordon et al. (2004) identified two QTL associated with GLS resistance on chromosomes 2 and 4, which together explained 40 to 47% of the total GLS resistance variation in an F_{2:4} population derived from the VO613Y × Pa405 cross. Similar reports have been published by other researchers, with resistance QTL also identified on chromosomes 1, 3, 5, 7, 9, and 10 (Balint-Kurti et al. 2008; Benson et al. 2015; Berger et al. 2014; Clements et al. 2000; Lehmensiek et al. 2001; Messmer et al. 2009; Pozar et al. 2009; Xu et al. 2014; Zhang et al. 2012; Zwonitzer et al. 2010). Because many GLS resistance QTL have been detected in different populations and environments, Shi et al. (2007) constructed an integrated GLS resistance QTL map and found that 26 real and 7 consensus QTL were located in 1.06, 2.06, 3.04, 4.06, 4.08, 5.03, and 8.06 bins.

Previous studies reported that GLS resistance correlated to flowering time and later-maturing plants showed high resistance (Balint-Kurti et al. 2008; Benson et al. 2015; Bubeck et al. 1993; Clements et al. 2000; Zwonitzer et al. 2010). The flowering times were highly associated with GLS resistance in the KB population developed from the tropical inbred Ki14 compared with IBM population developed from the temperate inbred B73 (Zwonitzer et al. 2010). Messmer et al. (2009) found that QTL corresponding to flowering time in

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the population of CML444 × SC Malawi did not overlap with GLS resistance QTL identified in that study, although it was close to the main-effect GLS resistance QTL observed in multiple environments. Further studies demonstrated that QTL for flowering time in the population of CML444 × SC Malawi did not correspond to GLS resistance QTL (Berger et al. 2014).

Although many putative GLS resistance QTL have been identified, subtropical and tropical maize possess high levels of unexplored genetic diversity, and it is valuable to identify additional sources of resistance in elite breeding germplasm utilized in these environments. Because of their ability to combine differences among different maize lines, and their capacity to develop elite hybrids, different GLS-resistant germplasms and QTL for GLS resistance existing in those resources need to be explored for their proper utilization in hybrid maize breeding programs (Chevin and Hospital 2008). With the identification of good combiners between Suwan 1 (tropical germplasms) and Reid or non-Reid (temperate germplasm) heterotic groups (Fan et al. 2014), tropical GLS-resistant germplasm can be very valuable in hybrid development, especially when this can be utilized in subtropical environments to increase yields in the presence of many biotic and abiotic stresses. It's important to screen additional germplasm, especially tropical maize germplasm, for GLS resistance and identify their resistance QTL.

A tropical inbred line, YML 32, which is highly resistant to GLS, was developed at the Institute of Food Crops, Yunnan Academy of Agricultural Sciences, from Suwan 1. Suwan 1 not only propelled Thailand to become the fourth largest maize exporting country in 1986 but it has also been used as a source material or as direct release in Africa, Asia, Oceania, and South America (Sriwatanapongse et al. 1993). In southwest China, including Yunnan, Guangxi, and Guizhou, YML 32 has been successfully used as a source material for the development of GLS-resistant hybrids such as Yunrui 1. Temperate inbred line Ye 478, which is highly susceptible to GLS, is one of the most widely used parental lines in hybrid maize breeding programs in China. To date, more than 40 maize hybrids released for production in China have been developed from Ye 478. Among the released hybrids, Zhengdan 958, formed from a derived parental line, is one of the most influential hybrids and covered 38.76 million ha in China in 2006 (Dai and E 2010). We have found that YML 32 always produces GLS-resistant hybrids, and the genetic analysis of GLS resistance and GLS-resistant QTL identification would provide useful information for development of maize hybrids and the development of a MAS breeding program. In this study, the tropical inbred line YML 32 (resistant to GLS) was crossed to the susceptible temperate inbred line Ye 478 to produce an $F_{2:3}$ population for the identification of QTL associated with resistance to GLS. The objectives were to (i) study the genetic effects of QTL imparting resistance to GLS, (ii) identify QTL associated with resistance to GLS in tropical maize germplasm; and (iii) verify whether the flowering time QTL in this tropical population were associated with the GLS resistance.

Materials and Methods

Plant materials. In 2009, the resistant inbred YML 32, used as female parent, was crossed with the highly susceptible inbred Ye 478 at Jinghong, Yunnan, China in the winter season. In 2010, The F_1 was selfed in Kunming, Yunnan, during the summer season. The F_2 population was grown at Jinghong in winter. Then, 204 F_2 individuals were self-pollinated to generate 204 $F_{2:3}$ lines in conformity with QTL methods that used F_2 -derived F_3 lines (Zhang and Xu 2004).

Field trials. Both Baoshan (99°0' N, 24°55' E, 1,870 m above sea level [masl]) and Dehong (24°26' N, 98°35' E, 914 masl) are hotspots for GLS in China and experience severe GLS epidemics almost every year. These two locations are optimal sites for natural inoculation of maize with *C. zea-maydis* (Xu et al. 2014) and for germplasm evaluation for GLS resistance (Liu and Xu 2013; Wu et al. 2009; Xu et al. 2014; Zhang et al. 2012). In 2011 and 2012, parents, F_1 , and the 204 $F_{2:3}$ lines were planted in a randomized complete-block design, with three replicates at each location. Plots were planted as single rows with a row spacing of 0.7 m and row length of 4.0 m. Distance between two adjacent plants was 0.44 m and the population density

was approximately 64,500 plants/ha. Trials were managed according to standard local practices.

GLS tests. The data of both plant response to the infection collected at day 28 after flowering and the flowering time were used for genetic analysis and QTL mapping. The evaluation of leaf infection with GLS followed the standard 1-to-9 scale reported by Saghai Maroof et al. (1993). The GLS resistance was evaluated using disease index and disease severity on a scale of 1 to 9, where 1 = highly resistant (none or few gray spots on leaves or lesion area being less than 5% of total leaf area), 3 = resistant (a small number of spots on leaves or lesion area = 6 to 10% of total leaf area), 5 = moderately resistant (medium number of spots on leaves or lesion area = 11 to 30% of total leaf area), 7 = susceptible (a large number of spots on leaves or lesion area = 31 to 70% of total leaf area), and 9 = highly susceptible (large lesion area on leaves or leaf death). Infected leaves corresponding to different categories are displayed in Figure 1. Date of anthesis (namely, date with pollen release in 50% of plants in a row) was recorded as the flowering date and days from planting to anthesis were used for flowering time.

Analysis of GLS resistance and QTL genetic effect estimation. Analysis of variance (ANOVA) for resistance was conducted via PROC GLM of SAS 9.1 software (SAS Institute Inc. 2004). The joint segregation analysis of P_1 , P_2 , F_1 , and $F_{2:3}$ with a mixed major gene plus polygene model was used to analyze the GLS resistance of the YML 32 × Ye 478 maize population (Zhang and Xu 2004). This model had five subgenetic models: one pair of major genes (A model), two pairs of major genes (B model), polygenes (C model), one pair of major genes plus polygenes (D model), and two pairs of major genes plus polygenes (E model). The assumptions for the A, B, C, D, and E models were as follows: diploid nuclear inheritance with no maternal or cytoplasmic effects; no interaction or linkage between major genes and polygenes, and no selection; the genetic effect of polygenes and the effect of the environment in any segregating population followed a normal distribution; and variances within the P_1 , P_2 , and F_1 populations were equal. Based on these assumptions, 24 subgenetic models, in total, from the five genetic models involving additive, dominance, equal, epistasis, and negative effect were established (Wang and Gai 2001).

The generation parameters and component distributions were estimated with the maximum-likelihood method and iterated expectation and conditional maximization (IECM) algorithm (Dempster et al. 1977; McLachlan and Basford 1988; Wang and Gai 2001; Zhang and Xu 2004; Zhang et al. 2003). In the joint segregation analysis of the mixed genetic model, Akaike information criterion (AIC) was employed to determine which submodel best fit the data. A goodness-of-fit test was used to determine whether or not the selected model sufficiently explained the data. The best-fitting genetic model was selected according to both AIC and the results from goodness-of-fit tests (homogeneity test U_1^2 , U_2^2 , and U_3^2 ; Smirnov [nW^2] test; and Kolmogorov [D^n] test). From AIC, the models with the lowest AIC

Fig. 1. Leaves infected by *Cercospora zea-maydis* corresponding to different scales and grades. HR = highly resistant to gray leaf spot (GLS), R = resistant to GLS, M = moderately resistant to GLS, S = susceptible to GLS, and HS = highly susceptible to GLS.

value or the relatively lower AIC value were chosen as the best-fit models. According to the test, the model with the lowest number of statistics among the best-fit models was selected as the best-fit model.

The genetic variances were estimated via the least-squares method according to the optimal genetic model (Gai and Wang 1998). In the mixed inheritance model, the phenotypic value (p) can be expressed as the summation of population mean (m), major gene effect (g), polygene effect (c), and environmental effect (e); that is, $p = m + g + c + e$, where g varies with different major genotypes and c and e are normally distributed variables. Based on this model, the phenotypic variation (σ_p^2) can be expressed as major gene variation (σ_{mg}^2), polygenic variation (σ_{pg}^2), and environmental variation (σ_e^2). $\sigma_p^2 = \sigma_{mg}^2 + \sigma_{pg}^2 + \sigma_e^2$; heritability of major gene (h_{mg}^2) and heritability of polygene (h_{pg}^2) were calculated, respectively, as

$$h_{mg}^2 (\%) = \left(\sigma_{mg}^2 / \sigma_p^2 \right) \times 100$$

$$h_{pg}^2 (\%) = \left(\sigma_{pg}^2 / \sigma_p^2 \right) \times 100$$

Genotyping. Based on GLS resistance data at various sites in both 2011 and 2012, equal amounts of DNA from the five most susceptible lines were mixed to form the susceptible bulk and equal amounts of DNA from the five most resistant lines were mixed to form the resistant bulk for screening the polymorphic marker. In total, 760 simple-sequence repeats (SSR) were retrieved from the Maize Genetics and Genomics Database (<http://www.maizegdb.org/>) and synthesized by Sangon Biotech (Shanghai, China). In total, 167 polymorphic SSR markers (21.9%) between two parents were used for genotyping the 204 individuals of the F_2 population, which were selfed to generate $F_{2.3}$ lines for phenotyping. A modified polymerase chain reaction (PCR) protocol according to McCouch et al. (2002) was used. The protocol consisted of a total volume of 10 μ l containing 20 ng of genomic DNA, 0.3 μ M each primer, 1.0 μ l of 10 $\times$ Taq DNA polymerase buffer (20 mM $MgCl_2$), 0.1 mM each dNTP (TransGen Biotech), and 1 U of Taq DNA polymerase (TransGen Biotech). The reaction profile with Mastercycler gradient (Eppendorf) was 5 min at 94°C; with 34 cycles of 45 s at 95°C, 45 s at 57°C annealing, and 1 min at 72°C; and 10 min at 72°C for final extension. The PCR products were separated via electrophoresis on 6% polyacrylamide gel.

Table 1. Analysis of variances for gray leaf spot (GLS) resistance^a

Source	DF	SS	MS	F value	Pr > F
Lines	203	6674.37	32.88	33.34	<0.0001**
Location	1	1165.34	1165.34	1193.42	0.0007**
Year	1	506.92	506.92	510.44	0.0623
Repeat	2	14.19	7.09	7.27	0.0086**
Lines $\times$ location	203	475.69	2.34	2.38	0.0232*
Lines $\times$ year	203	150.71	0.74	0.77	0.0854
Location $\times$ year	1	177.34	177.34	181.61	0.4587
Error	1837	1793.77	0.98	...	...

^a DF = degree of freedom, SS = sum of squares, MS = mean squares, and Pr = probability; * and ** indicate significance at 5 and 1% probability levels, respectively.

Map construction and QTL detection. A linkage map was constructed by use of IciMapping v3.1 software with polymorphic SSR markers between two parents (Wang et al. 2011). Linkage groups were identified using the Group command to identify linkage groups with a logarithm of odds (LOD) score of 3.0, and recombination frequency was converted into centimorgans using the Kosambi mapping function (Kosambi 1943). The QTL detection was performed with Breeding View in IB Workflow System (<https://www.integratedbreeding.net/breeding-management-system>) by inclusive composite-interval mapping (CIM) and the results were verified by the IciMapping v3.1 software with ICIM-ADD mapping method (Wang et al. 2011). A LOD of 3.0 was used to declare a QTL as significant. The percentages of phenotypic variance explained (R^2) by individual QTL and additive effects at LOD peaks were obtained through stepwise regression. Adjacent QTL on the same chromosome were considered different when the distances between the curve peaks were >20 centimorgans (cM). The QTL overlapping within intervals of 20 cM on the same chromosome at both locations were considered to be the same. The CIM method was used to detect QTL for the quantitative traits because of its greater efficiency of QTL detection. This method uses stepwise regression for identifying the significant flanking markers, one-way scanning to identify additive effect, and two-way scanning to identify digenic-epistatic effect (Murray et al. 2013).

Results

Phenotypic analysis of GLS response and flowering time. Results from both locations (Baoshan and Dehong) in the 2 years (2011 and 2012) showed that resistant parental line YML 32 expressed either resistance or high resistance, with disease scores of 1 to 3, whereas susceptible parental line Ye 478 was highly susceptible, with disease scores of 7 to 9. Because ANOVA (Table 1) showed that there were no significant differences in GLS disease scores between 2011 and 2012, average GLS disease scores from both years were used for the rest of the analyses.

The GLS disease scores showed wide segregation among $F_{2.3}$ lines and the average GLS disease score (3.76) and flowering time (79 days) at both locations were close to the midparent value (Table 2). The correlation coefficient of GLS disease scores and flowering time were -0.82 ($P < 0.01$) and -0.85 ($P < 0.01$) at Baoshan and Dehong, respectively. The results indicated that flowering time is strongly correlated with GLS resistance in this population. The frequency distribution of GLS disease scores and flowering time of the $F_{2.3}$ lines at the two locations showed continuous variation, confirming quantitative inheritance for GLS resistance (Fig. 2). The GLS disease scores and flowering time presented positive and negative skewness at both locations, indicating that complementary gene interactions might condition GLS resistance, with dominance genes playing an important role (Jayaramachandran et al. 2010; Samak et al. 2011). This means that intensive selection for GLS resistance in segregating generations would ensure rapid gains but, on the other hand, gains would be slow if mild selection is practiced.

Estimation of heritability for resistance to GLS. Statistical analysis of the average GLS resistance ratings for 204 $F_{2.3}$ lines across years and locations indicated the presence of significant differences among genotypes and between locations (Table 1). The

Table 2. Phenotypes of gray leaf spot (GLS) disease score and flowering time in the YML 32 $\times$ Ye 478 lines and the parents in Baoshan and Dehong locations

Location	Trait ^a	Parent		$F_{2.3}$ line			
		YML 32	Ye 478	Average $\pm$ SD ^b	Range	Skewness	Kurtosis
Baoshan	GLS	1.00	8.00	4.65 $\pm$ 1.89	1.00–8.89	0.16	–0.84
	FT	79	71	75.00 $\pm$ 2.07	70.00–80.00	–0.29	–0.56
Dehong	GLS	1.00	9.00	2.87 $\pm$ 1.59	1.00–7.20	0.95	0.06
	FT	86	79	83.09 $\pm$ 1.68	79.00–86.00	–0.75	–0.12
Average	GLS	1.00	8.50	3.76 $\pm$ 1.65	1.00–7.95	0.53	–0.51
	FT	83	75	79.16 $\pm$ 1.74	74.50–82.50	–0.51	–0.51

^a GLS = the disease scores of gray leaf spot and FT = the flowering time.

^b SD = standard deviation.

maximum-likelihood estimators and AIC values of five types of genetic models and 24 submodels (Table 3), including one major gene (A), two major genes (B), polygenes (C), one major gene plus polygene (D), and two major genes plus polygenes (E), were acquired by IECM arithmetic to jointly analyze the GLS resistance of the four generations (i.e., P_1 , P_2 , F_1 , and $F_{2.3}$) from the YML 32 $\times$ Ye 478 population using the mixed major gene plus polygene inheritance model (Gai and Wang 1998). A set of goodness-of-fit tests (U_1^2 , U_2^2 , U_3^2 , ${}_nW^2$, and D'') were further conducted for selecting an optimal model; the genetic models with relatively low AIC values are given in Table 4. According to the AIC criterion, model MX2-AD-AD was the optimal genetic model. This model has a smaller AIC value than those of the other 23 subgenetic models. The F_1 reaches 0.01 and 0.05

significance levels according to U_3^2 and ${}_nW^2$ tests, and $F_{2.3}$ reaches 0.05 significance levels based on the U_3^2 test. This indicated that the GLS resistance of maize fitted the MX2-AD-AD genetic model; namely, with two additive-dominance effect major genes plus an additive-dominance effect polygene. Broad-sense heritability for GLS resistance was estimated to be as high as 0.926 using the least-squares method based on the optimal genetic model (Table 3).

Construction of the linkage map. The $F_{2.3}$ lines derived from YML 32 $\times$ Ye 478 were used to construct a linkage map. The linkage map was constructed using ICIM (Wang et al. 2011), including 10 linkage groups, and spanned a total of 3,614.3 cM in genome, with an average of 10.0 cM between every two adjacent markers.

Fig. 2. Distribution of gray leaf spot (GLS) disease scores and flowering time in the $F_{2.3}$ population. GLS = GLS disease scores and FT = flowering time. **A**, Distribution of GLS disease scores and flowering time in the $F_{2.3}$ population in Baoshan and **B**, distribution of GLS disease scores and flowering time in the $F_{2.3}$ population in Dehong.

Table 3. Maximum log-likelihood (MLV) and Akaike's information criterion (AIC) values in various genetic models of four generations for gray leaf spot (GLS) resistance^a

Model ^b	MLV	AIC	Variation		Heritability	
			Major gene	Polygene	Major gene	Polygene
1MG-AD	-400.2	812.5	1.589	...	0.583	...
1MG-A	-411.5	832.9	1.226	...	0.449	...
1MG-EAD	-400.9	811.8	1.512	...	0.554	...
1MG-AEND	-416.0	841.9	0.000	...	0.000	...
2MG-ADI	-392.5	806.9	2.319	...	0.850	...
2MG-AD	-392.9	799.9	2.214	...	0.811	...
2MG-A	-405.6	821.2	1.629	...	0.597	...
2MG-EA	-405.5	819.1	1.626	...	0.596	...
2MG-AED	-397.9	805.8	1.869	...	0.685	...
2MG-EEAD	-397.7	803.4	1.818	...	0.667	...
PG-ADI	-399.4	810.8	...	2.487	...	0.912
PG-AD	-399.4	808.9	...	2.486	...	0.911
MX1-AD-ADI	-386.8	789.7	1.667	0.820	0.611	0.301
MX1-AD-AD	-410.0	834.1	1.705	0.783	0.625	0.287
MX1-A-AD	-394.7	801.4	2.012	0.445	0.738	0.163
MX1-EAD-AD	-399.4	810.9	0.019	2.467	0.007	0.904
MX1-AEND-AD	-399.4	810.9	0.019	2.467	0.007	0.904
MX2-ADI-ADI	-378.4	780.8	2.554	0.000	0.936	0.000
MX2-ADI-AD	-380.1	778.2	2.547	0.000	0.934	0.000
MX2-AD-AD	-379.6	769.2	2.527	0.000	0.926	0.000
MX2-A-AD	-394.5	795.0	2.024	0.433	0.742	0.159
MX2-EAED-AD	-387.6	779.3	2.330	0.140	0.854	0.051
MX2-AED-AD	-399.4	804.9	0.017	2.470	0.006	0.905
MX2-EEAD-AD	-388.4	780.8	1.908	0.578	0.699	0.212

^a Symbol: ... indicates data not available.

^b In these models, 1MG and 2MG represent one major gene and two major gene models, respectively; MX1 represents a mixed model with one major gene and polygene; MX2 indicates a mixed model with two major gene and polygene; PG represents polygene; and A, D, E, I, and N represent the genes with additive, dominance, equal, epistatic, and negative effects, respectively. For example, model MX2-AD-AD means a mixed model with two major genes of additive-dominance effects and additive-dominance polygene.

Among the 167 polymorphic SSR markers (Supplementary Table S1) between the two parents, 17, 20, 22, 28, 19, 12, 13, 13, 15, and 8 markers were located on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively. The linkage map is well established because the bins of markers used and listed as Supplementary Table S1 were evenly distributed on chromosomes compared with other IBM maps. Therefore, the linkage map was suitable for QTL detection.

QTL and their genetic effects for GLS resistance. Seven QTL related to GLS disease scores and six QTL corresponding to flowering time were detected from the population of YML 32 × Ye 478, with different numbers of QTL being recorded at the two locations. At Baoshan, five QTL (*qRgls.yaas-2*, *qRgls.yaas-3-1*, *qRgls.yaas-4*, *qRgls.yaas-5*, and *qRgls.yaas-8-1*) for GLS disease scores were detected in bins 2.06, 3.04, 4.06, 5.04, and 8.04, respectively (Table 5; Fig. 3). These five QTL showed additive effects from 0.36 to 1.13 and explained 1.8 to 17.9% of the variation of GLS disease scores. In addition to additive effects, a dominance effect (0.60) was also found for QTL *qRgls.yaas-5*, which explained 1.8% of the variation of GLS disease scores. As for the flowering time, three QTL (*qFt.yaas-3*, *qFt.yaas-4*, and *qFt.yaas-8*) were detected in bins 3.05, 4.06, and 8.04, respectively (Table 6). These three QTL showed additive effects from 0.71 to 0.99 and explained 5.92 to 11.48% of the variation of flowering time. In addition to additive effects, a dominance effect (0.56) was also found for QTL *qFt.yaas-8*. Two of the three QTL for flowering time were overlapped with the QTL for

GLS disease scores, which indicated that these two QTL (*qRgls.yaas-4/qFt.yaas-4*, and *qRgls.yaas-8-1/qFt.yaas-8*) were likely at the same chromosome location and corresponded to both GLS resistance and flowering time.

At Dehong, four QTL for resistance to GLS (*qRgls.yaas-3-2*, *qRgls.yaas-4*, *qRgls.yaas-8-1*, and *qRgls.yaas-8-2*) were identified in bins of 3.05, 4.06, 8.04, and 8.01, respectively (Table 5; Fig. 3). These four QTL accounted for 1.8 to 18.1% of the variation of GLS disease scores. The additive effects of these QTL were 0.30 to 0.96. Similar to Baoshan, the QTL *qRgls.yaas-3-2* also showed a dominance effect of 0.43, explaining 1.8% of the variation of GLS disease scores. As for the flowering time, four QTL (*qFt.yaas-2-1*, *qFt.yaas-4*, *qFt.yaas-5*, and *qFt.yaas-8*) were identified in bins of 2.06, 4.06, 5.04, and 8.04, respectively (Table 6). The QTL (*qFt.yaas-2-1* and *qFt.yaas-5*) for flowering time were not overlapped with the QTL for the GLS disease scores. These four QTL accounted for 0.15 to 21.42% of the variation of flowering time. The additive effects of these QTL were 0.05 to 1.10. The QTL *qFt.yaas-5* showed a dominance effect of 0.59. The result from Dehong location further showed that the two QTL (*qRgls.yaas-4/qFt.yaas-4* and *qRgls.yaas-8-1/qFt.yaas-8*) were likely at the same chromosome and corresponded to both GLS resistance and flowering time. However, the two QTL for GLS disease scores (*qRgls.yaas-3-2* and *qRgls.yaas-8-1*) did not associate with flowering time, and the QTL *qFt.yaas-2-1* for flowering time did not corresponded to GLS resistance.

When the average value of GLS disease scores was used from the two locations across 2 years, five QTL (namely, *qRgls.yaas-2*, *qRgls.yaas-3-2*, *qRgls.yaas-4*, *qRgls.yaas-5*, and *qRgls.yaas-8-1*) were detected in bins 2.06, 3.05, 4.06, 5.04, and 8.04, respectively (Table 5; Fig. 3). As for the flowering time, four QTL (namely, *qFt.yaas-2-2*, *qFt.yaas-3*, *qFt.yaas-4*, and *qFt.yaas-8*) were detected in bins 2.06, 3.05, 4.06, and 8.04, respectively (Table 6). These four QTL for flowering time overlapped the QTL for the GLS disease scores, which indicated that the four (*qRgls.yaas-2/qFt.yaas-2-2*, *qRgls.yaas-3-2/qFt.yaas-3*, *qRgls.yaas-4/qFt.yaas-4*, and *qRgls.yaas-8-1/qFt.yaas-8*) were likely at the same chromosome locations and corresponded to both GLS resistance and flowering time. The results from averages seemed to detect most of the QTL in different locations, suggesting that multiple locations and multiple tests would give more reliable results.

By examining sources of the GLS resistance, we found that the QTL *qRgls.yaas-8-1* and *qFt.yaas-8*, with the largest additive effect, were derived from the resistant parent YML 32, suggesting that the tropical line YML 32 was a good source for resistance to GLS, while there were five resistance QTL alleles identified to be originated from the susceptible parent Ye 478 (Tables 5 and 6). Comparing results from both locations, we found that *qRgls.yaas-8-1* and *qFt.yaas-8* had the largest effect, which was consistent at both locations. These results further confirmed that there was an important QTL for resistance to GLS from the resistant parent YML 32, as found in our previous study (Zhang et al. 2012). Thus, YML 32 derived from the well-known maize germplasm Suwan 1 could serve as a good source of resistance to GLS in breeding programs. Among the QTL for the GLS disease scores and flowering time, only two QTL (*qRgls.yaas-4/qFt.yaas-4* and *qRgls.yaas-8-1/qFt.yaas-8*) were identified at both locations. The fact that different QTL were detected at the two locations implied that environmental factors or experimental errors might have affected QTL detection, especially the QTL with smaller effects.

The two QTL *qRgls.yaas-5* (bin 5.04) and *qRgls.yaas-8-1* (bin 8.04) for GLS disease scores identified from YML 32 were not identified in the consensus GLS resistance QTL in bins 5.03 and 8.06 in the previous studies (Shi et al. 2007). Among the five QTL (*qRgls.yaas-2*, *qRgls.yaas-3-1*, *qRgls.yaas-3-2*, *qRgls.yaas-4*, and *qRgls.yaas-8-2*) for GLS disease scores identified from Ye 478, *qRgls.yaas-3-2* (bin 3.05) and *qRgls.yaas-8-2* (bin 8.01) were also not located in the consensus GLS resistance QTL in bins 3.04, and 8.06 in previous studies (Shi et al. 2007). These results indicated that *qRgls.yaas-3-2*, *qRgls.yaas-5*, *qRgls.yaas-8-1*, and *qRgls.yaas-8-2*

Table 4. Tests of gray leaf spot (GLS) resistance for goodness-of-fit of genetic models with lower Akaike's information criterion (AIC) values in the cross of YML 32 × Ye 478^a

Model, population ^b	U_1^2	U_2^2	U_3^2	nW^2	D^a
MX1-AD-ADI					
P ₁	0.0000	0.1562	2.5000	0.1667	0.5000
F ₁	0.0000	0.3616	5.7851*	0.1285	0.4584
P ₂	0.0000	0.1562	2.5000	0.1667	0.5000
F _{2:3}	0.0152	0.0055	0.6008	0.0703	0.0065
MX2-ADI-ADI					
P ₁	0.0000	0.1562	2.5000	0.1667	0.5000
F ₁	0.0000	0.4819	7.7097**	0.1468	0.4792
P ₂	0.0000	0.1562	2.5000	0.1667	0.5000
F _{2:3}	0.0002	0.0010	0.0046	0.0167	0.0039
MX2-ADI-AD					
P ₁	0.6602	1.1057	1.1220	0.2217	0.6659
F ₁	0.0941	0.0505	4.3558*	0.1181	0.3726
P ₂	0.6602	1.1057	1.1220	0.2217	0.6659
F _{2:3}	0.0047	0.0070	0.0048	0.0178	0.0032
MX2-AD-AD					
P ₁	0.0308	0.0481	2.4236	0.1692	0.4642
F ₁	0.0035	0.4978	6.7214**	0.1378*	0.4810
P ₂	0.0308	0.0481	2.4236	0.1692	0.4642
F _{2:3}	0.0033	0.0007	0.0129*	0.0240	0.0031
MX2-EAED-AD					
P ₁	0.1169	0.4946	2.2162	0.1764	0.5698
F ₁	0.0311	0.1357	4.6526*	0.1183	0.4064
P ₂	0.1169	0.4946	2.2162	0.1764	0.5698
F _{2:3}	0.0962	0.0484	0.1034	0.0713	0.0042
MX2-EEAD-AD					
P ₁	0.0051	0.2148	2.4872	0.1671	0.5146
F ₁	0.0010	0.3215	5.7254*	0.1280	0.4510
P ₂	0.0051	0.2148	2.4872	0.1671	0.5146
F _{2:3}	0.0204	0.0000	0.2834	0.0540	0.0114

^a U_1^2 , U_2^2 , and U_3^2 = homogeneity tests, nW^2 = Smirnov test, and D^a = Kolmogorov test; * and ** indicate significance at 5 and 1% probability levels, respectively.

^b In these models, MX1 represents a mixed model with one major gene and polygene; MX2 indicates a mixed model with two major gene and polygene; and A, D, E, and I represent the genes with additive, dominance, equal, epistatic, and negative effects, respectively. For example, model MX2-AD-AD means a mixed model with two major genes of additive-dominance effects and additive-dominance polygene.

Fig. 3. Positions of quantitative trait loci (QTL) for gray leaf spot resistance in Baoshan and Dehong locations.

might be new GLS resistance source for use in maize breeding program.

Discussion

Elwinger et al. (1990) had suggested that dominance should be included in models to fully explain the inheritance of GLS resistance. The results from our study offered strong evidence that dominance-effect QTL played a role in controlling resistance to GLS (Table 5). In addition, the results from the joint segregation analysis in this study suggested that GLS resistance fitted the MX2-AD-AD genetic model. This result further suggested that additive-dominance effects were a major component of GLS resistance and that there might be two major genes and polygenes controlling GLS resistance in the YML 32 × Ye 478 population. Both joint segregation genetic analysis and QTL analysis suggested that both additive and dominance effects existed in YML 32 × Ye 478, which agreed with the findings of Elwinger et al. (1990) that GLS resistance was controlled by major genes with additive effects and polygenes with additive-dominance effects.

It is widely known that disease resistance can be inherited from a resistant parent. However, previous research also demonstrated that a highly susceptible parent also has the possibility of improving disease resistance in the offspring (Wang et al. 2012). Our previous study (Zhang et al. 2012) showed that a detected GLS resistance

QTL, *qRgls3*, was derived from the susceptible parent Q 11 and it could account for 10.73% of the variation for GLS resistance at Dehong. To confirm this finding, this study used a different susceptible parent (Ye 478) to cross with the same GLS-resistant parent (YML 32). The results showed that some QTL for resistance to GLS were derived from the susceptible parent Ye 478 in the YML 32 × Ye 478 population. This further demonstrated that susceptible plants may also contain valuable resistance genes or QTL for improving disease resistance as reported in cotton (Wang et al. 2012), cacao (Crouzillat et al. 2000), pepper (Lefebvre and Palloix 1996), maize (Bubeck et al. 1993; Freymark et al. 1994), mung bean (Young et al. 1993), and tomato (Danesh et al. 1994). Therefore, in a maize breeding program, it is recommended that the background of the susceptible parent be carefully considered because of the potential of finding different QTL for resistance to GLS.

In a previous study (Zhang et al. 2012), a major QTL on chromosome 8 was found to confer GLS resistance in the YML 32 × Q 11 population. In this study, the major QTL *qRgls.yaas-8-1* and *qFt.yaas-8* was detected on the same chromosome from the YML 32 × Ye 478 population in both locations. These results further demonstrated that the inbred line YML 32 derived from Suwan 1 could be a valuable source of GLS resistance in maize breeding programs aimed at improving GLS resistance worldwide. It has all the advantages of a tropical maize germplasm such as high stress tolerance, a

Table 5. Quantitative trait loci (QTL) detected for gray leaf spot (GLS) disease scores in the F_{2:3} population derived from the cross of YML 32 × Ye 478^a

Location, QTL	Bin	Flanking markers	Position (cM)	LOD	Additive effect	Dominant effect	Source of resistance	R ² (%)
Baoshan								
<i>qRgls.yaas-2</i>	2.06	umc2192-umc1755	141.1	2.60	0.46	—	Ye 478	2.9
<i>qRgls.yaas-3-1</i>	3.04	umc1174-umc1773	152.0	6.96	0.80	—	Ye 478	9.0
<i>qRgls.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	7.32	0.80	—	Ye 478	9.1
<i>qRgls.yaas-5</i>	5.04	umc2164-umc1332	17.5	3.00	0.36	0.60	YML 32	1.8
<i>qRgls.yaas-8-1</i>	8.04	umc1997-umc1505	248.2	11.49	1.13	—	YML 32	17.9
Dehong								
<i>qRgls.yaas-3-2</i>	3.05	umc1167-phi053	148.6	2.26	0.30	0.43	Ye 478	1.8
<i>qRgls.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	5.67	0.63	—	Ye 478	7.8
<i>qRgls.yaas-8-1</i>	8.04	umc1997-umc1505	248.2	10.60	0.96	—	YML 32	18.1
<i>qRgls.yaas-8-2</i>	8.01	umc1154-phi015	0.0	3.67	0.51	—	Ye 478	5.1
Average								
<i>qRgls.yaas-2</i>	2.06	umc2192-umc1755	141.1	4.18	0.53	—	Ye 478	5.1
<i>qRgls.yaas-3-2</i>	3.05	umc1167-phi053	148.6	5.89	0.62	—	Ye 478	7.1
<i>qRgls.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	6.52	0.65	—	Ye 478	7.8
<i>qRgls.yaas-5</i>	5.04	umc2164-umc1332	17.5	2.31	0.18	0.53	YML 32	0.6
<i>qRgls.yaas-8-1</i>	8.04	umc1997-umc1505	248.2	12.69	1.04	—	YML 32	19.9

^a Bin = chromosome bin number, cM = centimorgans, LOD = logarithm of odds, Additive effect represents half the difference between the means of the homozygous classes at a given QTL, Dominance effect represents the difference between the mean of the heterozygous class and the mean of the homozygous classes at a given QTL, and — indicates no dominance effect on resistance.

Table 6. Quantitative trait loci (QTL) detected for flowering time in the F_{2:3} population derived from the cross of YML 32 × Ye 478^a

Location, QTL	Bin	Flanking markers	Position (cM)	LOD	Additive effect	Dominant effect	Source of resistance	R ² (%)
Baoshan								
<i>qFt.yaas-3</i>	3.05	umc1167-phi053	148.6	4.41	0.71	—	Ye 478	5.92
<i>qFt.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	7.77	0.99	—	Ye 478	11.48
<i>qFt.yaas-8</i>	8.04	umc1997-umc1505	248.2	7.24	0.97	0.56	YML 32	11.00
Dehong								
<i>qFt.yaas-2-1</i>	2.06	umc2194- umc2254	139.6	3.48	0.50	—	Ye 478	4.46
<i>qFt.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	5.12	0.61	—	Ye 478	6.58
<i>qFt.yaas-5</i>	5.04	umc2164-umc1332	17.5	2.02	0.05	0.59	Ye 478	0.15
<i>qFt.yaas-8</i>	8.04	umc1997-umc1505	248.2	12.39	1.10	—	YML32	21.42
Average								
<i>qFt.yaas-2-2</i>	2.06	umc2192-umc1755	141.1	4.38	0.58	—	Ye 478	5.58
<i>qFt.yaas-3</i>	3.05	umc1167-phi053	148.6	5.70	0.67	—	Ye 478	7.39
<i>qFt.yaas-4</i>	4.06	umc1142-bnlg1621	211.6	7.16	0.76	—	Ye 478	9.34
<i>qFt.yaas-8</i>	8.04	umc1997-umc1505	248.2	12.01	1.10	—	YML 32	19.68

^a Bin = chromosome bin number, cM = centimorgans, LOD = logarithm of odds, Additive effect represents half the difference between the means of the homozygous classes at a given QTL, Dominance effect represents the difference between the mean of the heterozygous class and the mean of the homozygous classes at a given QTL, and — indicates no dominance effect on resistance.

strong root system, and long stay green period, as compared with temperate maize germplasm, and can be widely utilized in FAO maize maturity zones of 700 and above. More importantly, all the released hybrids such as Yunrui 1, Yunrui 2, Yunde 3, and so on developed from the inbred line YML 32 possessed a high level of resistance to GLS. Maize breeders worldwide (Fan et al. 2014; Goodman 2004) have been trying to incorporate tropical maize germplasm into elite temperate maize germplasm to increase genetic variability for disease and pest resistance or yield. In our breeding program, we used YML 32 as the recurrent parent being crossed with temperate inbreds. We found that the progenies of YML 32 were not only highly resistant to GLS but also recovered most favorable traits such as yield, plant height, and so on from the temperate elite germplasm. This result not only confirmed that YML 32 was a very good source germplasm for GLS resistance but also further indicated that YML 32 had good combining ability with temperate germplasm for maize breeding aiming at improving disease resistance as well as keeping the most favorable traits of elite local germplasm.

Previous studies showed that GLS resistance correlated with flowering time (Balint-Kurti et al. 2008; Benson et al. 2015; Bubeck et al. 1993; Clements et al. 2000; Zwonitzer et al. 2010). In this study, the correlation coefficient of GLS disease scores and flowering were -0.82 and -0.85 at Baoshan and Dehong, respectively. Among all identified QTL, five QTL (*qRgls.yaas-2/qFt.yaas-2-2*, *qRgls.yaas-3-2/qFt.yaas-3*, *qRgls.yaas-4/qFt.yaas-4*, *qRgls.yaas-5/qFt.yaas-5*, and *qRgls.yaas-8-1/qFt.yaas-8*) corresponded to GLS resistance and flowering time. These results further demonstrated that flowering time is strongly correlated with GLS resistance. However, the major QTL *qRgls.yaas-8-1/qFt.yaas-8* was not the same as the QTL *vtg1* (bin 8.05) for flowering time due to the different position on the chromosome (Salvi et al. 2007). Except for QTL *qFt.yaas-2-1*, identified on chromosome 2 at the Dehong location, all other QTL for flowering time were overlapped with the QTL for GLS disease scores. Therefore, the QTL for resistance to GLS are also QTL for flowering time and selection for flowering time is also selection for QTL to GLS resistance. However, Berger et al. (2014) thought that QTL for flowering time in the population of CML444 × SC Malawi did not correspond to QTL for GLS resistance. These contrary results might indicate either that the correlation between the flowering time and GLS resistance might vary from germplasm to germplasm or more markers may be needed to distinguish QTL based on GLS disease score and flowering time. Careful selection for maturity and disease resistance can be utilized in tandem for identifying new elite breeding germplasm adapted for the different maize maturity zones.

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