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RESEARCH

Combining Ability Analysis of RILs Developed from a YML32 × Q11 Cross for Grain Yield and Resistance to Gray Leaf Spot

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ABSTRACT

The development of resistant lines and hybrids is an economical way to control disease and improve yield stability. The objectives of this study were (i) to investigate if differences in resistance to gray leaf spot (GLS, caused by *Cercospora zeina*) exist among recombinant inbred lines (RILs) with and without the quantitative trait locus encompassing the resistance-carrying GZ204/IDP5 DNA segment (RDNAS) and to determine its effect on grain yield, and (ii) to determine general combining ability and specific combining ability effects for grain yield and GLS scores (GLSS). Four RILs (three with RDNAS [RL1_1, RL1_2, and RL2_1] and one without RDNAS [RL2_2]) were developed via marker-assisted selection from a cross between YML32 and Q11—an elite line susceptible to GLS. The four RILs and the susceptible parent (Q11) were crossed as testers with 13 maize (*Zea mays* L.) lines of known heterotic groups (Suwan1, Reid, and non-Reid). The three RDNAS-carrying RILs showed reduced GLSS and improved grain yield stability, but grain yield itself was not significantly increased. These three RILs also showed negative general combining ability effects for GLSS. RL2_1 was the best line for improving GLS resistance. The RILs possessing the RDNAS in crosses with lines from the Suwan1 heterotic group had lower GLSS than those from Reid and non-Reid heterotic groups, suggesting that resistance genes or quantitative trait loci, in addition to RDNAS, might be present in Suwan1.

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Abbreviations: GCA, general combining ability; GLS, gray leaf spot; GLSS, gray leaf spot scores; GY, grain yield; MAS, marker-assisted selection; NCII, North Carolina Design II; PCR, polymerase chain reaction; QTL, quantitative trait locus/loci; RDNAS, resistance-carrying GZ204/IDP5 DNA segment; RIL, recombinant inbred line; SCA, specific combining ability; SS, sum of squares.

Gray leaf spot (GLS), caused by two species (*Cercospora zeae-maydis* and *C. zeina*) is a major disease that affects maize (*Zea mays* L.) production globally (Ward et al., 1999; Katwal et al., 2013; Liu et al., 2016). Originally, the causal agent of GLS was reported as *Cercospora zeae-maydis* with two variants (Wang et al., 1998), but later studies recognized the variants as two distinct species (Crous et al., 2006). *Cercospora zeae-maydis* occurs in the US Corn Belt, Mexico, Brazil, and North China, whereas *C. zeina* occurs in the Eastern United States, Africa (Dunkle and Levy, 2000; Okori et al., 2003; Meisel et al., 2009), Brazil, and Southwest China (Liu and Xu, 2013).

The use of reduced tillage practices and the planting of susceptible genotypes are associated with increased yield losses that can reach up to 100% (Ward et al., 1999; Crous and Braun, 2003). Studies have shown that GLS adversely affects photosynthesis and grain filling in maize (Latterell and Rossi, 1983; Menkir and Ayodele, 2005).

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Host-plant resistance is a cost-effective measure for controlling disease. Many quantitative trait loci (QTL) associated with resistance to GLS in maize have been identified under various environments and in different genetic backgrounds (Saghai Maroof et al., 1996; Balint-Kurti et al., 2008; Danson et al., 2008; Zhang et al., 2012). Saghai Maroof et al. (1996) identified QTL for resistance to GLS on chromosomes 1, 4, and 8 in a Va14 × B73 cross; these QTL explained, respectively, 35.0 to 56.0, 14.3, and 7.7 to 11.0% of the variation for resistance to GLS. Shi et al. (2007) conducted a meta-analysis of all the mapped QTL for GLS and constructed an integrated QTL map. Twenty-six QTL and seven consensus QTL mapped to bins 1.06, 2.06, 3.04, 4.06, 4.08, 5.03, and 8.06 on six maize chromosomes. Balint-Kurti et al. (2008) detected five QTL for resistance to GLS in chromosome bins 1.05, 2.04, 4.05, 9.03, and 9.05; the QTL in the 9.03 bin had the largest effect and explained 12.0% of the total phenotypic variation. Pozar et al. (2009) found three QTL in bins 1.05, 1.07, and 3.07, which were effective in reducing GLS severity. Zhang et al. (2012) found four QTL for resistance to GLS in the cross Y32 × Q11 that were located on chromosomes 1, 2, 5, and 8 and mainly contributed additive genetic effects. A major QTL, designated as *qRgls1*, on chromosome 8 was fine-mapped to a 1.4-Mb segment designated as GZ204/IDP5, or the "resistance DNA segment" (RDNAS), from the resistant parent YML32, which reduced GLS scores (GLSS) by 19.70 to 61.28% (Zhang et al., 2012). Berger et al. (2014) identified seven QTL for resistance to GLS caused by *C. zeina* in the recombinant inbred population formed from CML444 × SC Malawi, of which four QTL (bins 1.10, 4.08, 9.04–9.05, 10.06–10.07) were contributed by the resistant parent CML444 and three (bins 6.06–6.07, 7.02–7.03, 9.06) by the susceptible parent SC Malawi. Berger et al. (2014) placed QTL for GLS from 11 previous studies on the IBM2005 map and recognized chromosomes 1, 2, 4, 5, and 7 as hotspots for resistance QTL to GLS.

The introgression of resistance genes from donors into elite maize germplasm is an effective method to improve germplasm (Godshalk and Kauffmann, 1995; Gordon et al., 2004; Liu et al., 2016), and the use of marker-assisted selection (MAS) has been shown to improve selection efficiency for several traits (e.g., disease resistance, grain protein quality, and pro-vitamin A content) (Pozar et al., 2009; Prasanna et al., 2010; Liu et al., 2015). The use of MAS for the development of quality-protein maize (QPM) led to the release of Vivek QPM Hybrid 9 containing the *o2* gene in India (Prasanna et al., 2010). Marker-assisted selection has also been used to improve drought tolerance of maize lines and populations developed at CIMMYT (Ribaut and Ragot, 2006).

Knowledge of combining ability for disease resistance is important for an effective breeding program aimed at improving yield stability (Hallauer and Miranda, 1988; Menkir and Ayodele, 2005). North Carolina Design II (NCII) has been widely used for estimating general combining ability (GCA), specific combining ability (SCA), and certain other genetic parameters related to heterosis (Menkir et al., 2004; Wu et al., 2007; Derera et al., 2008). Using the line × tester design, Menkir et al. (2004) classified 23 of 38 inbred lines into two heterotic groups, and Wu et al. (2007) used the NCII to classify 27 maize lines into four heterotic groups. Fan et al. (2008, 2015) used the line × tester design for estimating GCA and SCA of both tropical and temperate germplasm and identified a new maize heterotic group, Suwan1. Suwan1 is a tropical maize population from Thailand; its use led to Thailand becoming the fourth largest maize-exporting country in the world in 1986, and it also either served as a source material or was released directly in Africa, Asia, Oceania, and South America (Sriwatanapongse et al., 1993).

The inbred line YML32, also known as Y32 and developed from Suwan1, is highly resistant to GLS. In Southwest China (Yunnan, Guangxi, and Guizhou), YML32 has been used to develop GLS-resistant hybrids, such as Yunrui 1. Q11, derived from a US hybrid, is another key inbred line used for developing commercial maize hybrids adapted to Southwest China, but this inbred line is susceptible to GLS.

Resistance to GLS in maize is primarily attributable to additive effects, with moderate to high narrow-sense heritability estimates (Gordon et al., 2006; Derera et al., 2008; Zhang et al., 2012), which would make MAS a good option for the transfer of resistance genes or QTL in a germplasm improvement program. It is desirable (i) to know if the identified RDNAS can be effectively transferred via MAS to improve resistance to GLS in a locally adapted, elite maize line; (ii) to learn whether or not hybrids containing the RDNAS would possess improved resistance to GLS; and (iii) to ascertain if lines or hybrids with the introgressed RDNAS would affect grain yield (GY). We developed four recombinant inbred lines (RILs, three containing the RDNAS and one without the RDNAS) from the backcross population (YML32 × Q11) × Q11 for evaluation in this study. Objectives of this study were (i) to investigate if RILs with the RDNAS (RL1_1, RL1_2, and RL2_1) and without the RDNAS (RL2_2) differed in GLSS and GY; and (ii) to determine GCA of the RILs and the recurrent parent line (Q11) and the SCA of the crosses made between certain testers (male) and selected elite lines (female), using the NCII, to detect any differences attributable to RDNAS in GLSS and GY.

MATERIALS AND METHODS

Plant Materials

The four RILs (RL1_1, RL1_2, RL2_1, and RL2_2) were developed via pedigree selection from the backcross population

(YML32 × Q11) × Q11, as outlined in Fig. 1. Q11 was used as recurrent parent because of its importance in developing commercial maize hybrids in China, and the four RILs were selected on the basis of the presence (RL1_1, RL1_2, and RL2_1) or absence of the RDNAS from among the BC_1– and BC_2–derived lines (Fig. 1). Five testers (male; i.e., four RILs and a local elite maize line [Q11] that is susceptible to GLS) were crossed with 13 inbred lines (female) to generate 65 F_1 crosses according to the NCII design (see Table 1 for a list of testers and lines). The 13 male lines were selected on the basis of genetic diversity and desirable agronomic characteristics. During flowering time, pollen was collected from individual plants of each RIL and Q11 and was used to bulk-pollinate each of the 13 female inbred lines.

Marker Preparation and RIL Selection

Marker-assisted selection was used at several stages of the breeding program. The procedure for MAS is shown in Fig. 1. The details regarding marker preparation and utilization are as follows: leaves were collected from the field at the three-leaf stage. A modified cetyl trimethyl ammonium bromide (CTAB) method (Dellaporta et al., 1983) was used to extract DNA from the leaves. The GZ204 and IDP5 markers identified the RDNAS carrying resistance to GLS (Zhang et al., 2012). The forward primer for marker GZ204 was 5′-ACGAAGTGGGAAGGGAGA-3′, and the reverse primer was 5′-GTGCCTGTGACAGCAACC-3′; for marker IDP5, the forward primer was 5′-GAGACAATGAAGGCAGAT-3′ and the reverse primers was 5′-TTGTGGACCAACTATGAG-3′.

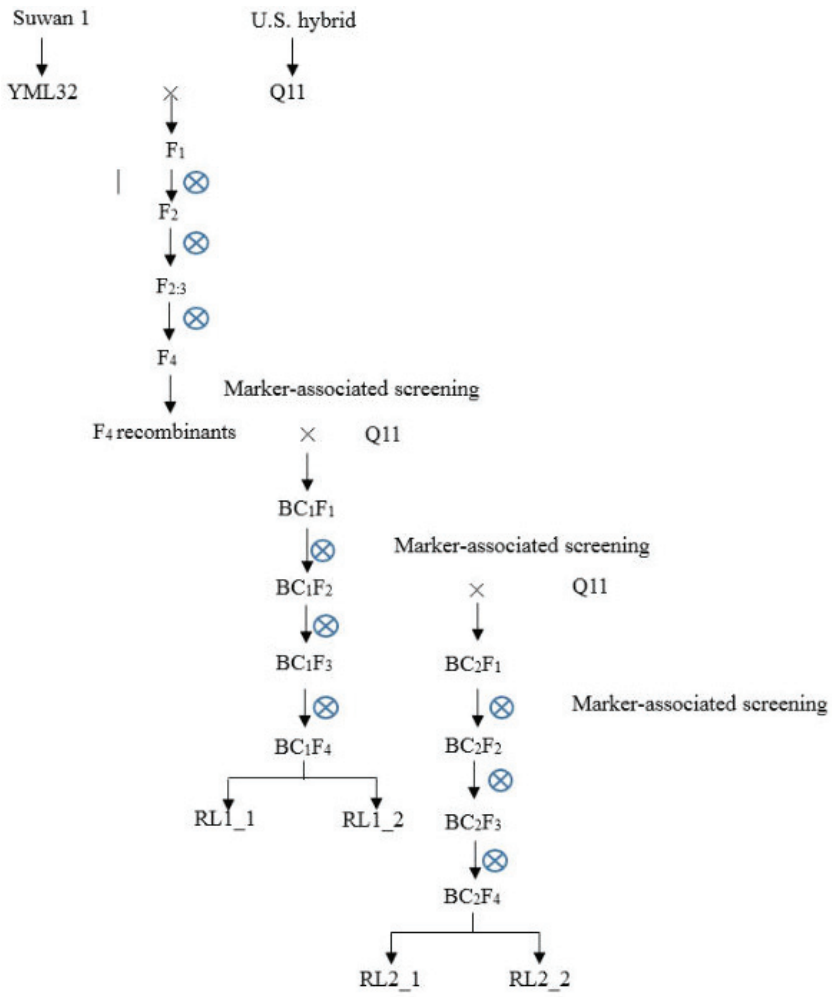

Fig. 1. Procedure for developing the four recombinant inbred lines from a cross with gray leaf spot-resistant line YML32 and susceptible line Q11.

The reaction mixture for genotyping with the GZ204 and IDP5 markers consisted of a total volume of 15 μL, containing 30 ng genomic DNA, 0.2 μM of each primer, 1.5 μL of 10× *Taq* DNA polymerase buffer (20 mM $MgCl_2$), 0.1 mM of each deoxynucleotide (TransGen Biotech), and 1 U of *Taq* DNA polymerase (TransGen Biotech). The reaction profile with a Mastercycler gradient (Eppendorf) was performed using the following protocol: the initial hold was at 95°C for 5 min. The second hold started with a denaturation step at 95°C for 1 min, annealing at 64°C for 1 min (19 cycles, reducing 0.5°C $cycle^{-1}$), and an extension step at 72°C for 1 min. The third hold began with a denaturation temperature of 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 19 cycles. The final extension step was done at 72°C for 10 min. Polymerase chain reaction (PCR) products were separated via electrophoresis on a 6% polyacrylamide gel.

From 42 backcross plants screened via the RDNAS-associated markers, four RILs with favorable agronomic traits were obtained. The four RILs included RL1_1 from YML32/Q11-BC1-1-1-1-1, RL1_2 from YML32/Q11-BC1-1-1-1-2, RL2_1 from YML32/Q11-BC2-1-1-1-1–1, all three carrying the GZ204/IDP5 (RDNAS); and RL2_2 from YML32/Q11-BC2-1-1-1-1–2 without the GZ204/IDP5 (RDNAS) (see Table 1). The PCR results for the four RLs were given in Fig. 1 (Supplemental Fig S1).

Field Trials and GLS Data Collection

Three locations (Dehong, 24°26′ N, 98°35′ E, 914 m asl; Kunming, 25°23′ N, 102°9′ E, 1970 m asl; and Wenshan, 23°60′ N, 104°4′ E, 1570 m asl) are considered hotspots for GLS in China. Thus, they are regarded as optimal sites for natural infection of maize by *C. zeina* (Liu et al., 2016) and for germplasm evaluation for GLS resistance (Wu et al., 2009; Zhang et al., 2012; Liu and Xu, 2013; Xu et al., 2014).

The 65 crosses were evaluated at Dehong, Kunming, and Wenshan in Yunnan Province in 2016. A randomized complete block design with three replications was employed at each location. Each experimental plot consisted of two 3-m-long rows with an inter-row spacing of 0.70 m and 14 plants per row. The overall plant density was ~62,142 plants ha^{-1}. Trials were managed according to standard local practices. Ten plants from the middle of each row were sampled and GY per plot was computed. After harvest, the kernels were air dried until constant moisture of 130 g kg^{-1} was achieved. Grain yield per plant was determined and mean GY of the 10 plants was calculated. The GY per plot was computed as the mean GY per plant multiplied by the total number of plants in the plot.

Table 1. The pedigree of 13 lines and five testers and their ecotype information.

Line	Pedigree	Heterotic group	Ecotype
CML312	S89500-F2-2-2-1-1-B	Reid	Subtropical
CML373	P43SR-4-1-1-2-1-B-8-1-B	Reid	Subtropical
CML384	P502-C1-771-2-2-1-3-B	Reid	Subtropical
CML395	90323B-1-B-1-B	Reid	Subtropical
YML46	Selected from Suwan1	Suwan1	Tropical
YML226	(CML226/(CATETO DC1276/7619))F2-25-1-B-1	non-Reid	Tropical
D39	Selected from Suwan1	Suwan1	Tropical
TRL2	Derived from a US hybrid	non-Reid	Tropical
3760	Derived from a South African hybrid	non-Reid	Tropical
Zheng58	Derived from Ye 478	Reid	Temperate
Y1218	HuangZaoSi × WeiChun	non-Reid	Temperate
Chang 7-2	V59 × HuangZaoSi	non-Reid	Temperate
Huang C	Yugoslavia O2/Huangxiao 162/Zi330/Mobai 1	non-Reid	Temperate
Tester		–	–
RL1_1	Y32/Q11-BC1-1-1-1-1 with GZ204/IDP5	–	–
RL1_2	Y32/Q11-BC1-1-1-1-2 with GZ204/IDP5	–	–
RL2_1	Y32/Q11-BC2-1-1-1-1-1 with GZ204/IDP5	–	–
RL2_2	Y32/Q11-BC2-1-1-1-1-2 without GZ204/IDP5	–	–
Q11	Recurrent parent, without GZ204/IDP5	Reid	Tropical

The GLSS were recorded 28 d after flowering on a whole-plot basis using a 1-to-9 scale, where 1= highly resistant (none or few gray spots on leaves and/or lesion area < 5% of total leaf area), 3 = resistant (a few gray spots on leaves and/or lesion area = 6–10% of total leaf area), 5 = moderately resistant (intermediate number of gray spots on leaves and/or lesion area = 11–30% of total leaf area), 7 = susceptible (a large number of gray spots on leaves and/or lesion area = 31–70% of total leaf area), and 9 = highly susceptible (large lesion area on leaves and/or lesion area = 71–100% of total leaf area) (Saghai Maroof et al., 1993). Based on performance in GLS evaluations, the test cross Chang 7-2 × Q11 was selected to serve as a susceptible check, and CML 312 × Q11 was selected as a resistant check.

Statistical Analysis

The general linear model below was used for ANOVA of GLSS and GY:

$$Y_{ijkl} = \mu + \alpha_l + b(a)_{kl} + \upsilon_{ij} + (\alpha\upsilon)_{ijl} + e_{ijkl}$$

where Y_{ijkl} us observed value from each experimental unit; μ is the population mean; α_l is the location effect; $b(a)_{kl}$ is the replication within location effect; υ_{ij} is the F_1 hybrid effect ($l_i + t_j + lt_{ij}$, where l_i is the ith line effect, t_j is the jth tester effect, and lt_{ij} is the ith line × jth tester interaction effect); $(\alpha\upsilon)_{ijl}$ is the ijth F_1 hybrid × lth location interaction effect; and e_{ijkl} is the residual effect.

The locations were considered a random sample of all possible locations within Southwest China, as each location represented a unique environment. Statistical significance of various sources of variation was tested as follows: line × location interaction was used for lines, tester × location interaction for testers, and line × tester × location interaction for line × tester interaction (Table 2). The significance of location was tested against replications within location mean square. The significance of replication within location and interactions of line × location, tester × location, and line × tester × location was tested against the overall experimental error term (Table 2).

Combining ability analysis was conducted according to the model and method used by Fan et al. (2009). Data analyses were conducted using the SAS 9.1.3 software package (SAS Institute, 2005).

RESULTS AND DISCUSSION

Analyses of Variance for GLSS and GY across Locations

Gray Leaf Spot Score

The ANOVA results for GLSS showed that, with the exception of replication within location and the three-way interaction (line × tester × location), mean squares for all sources of variation (location, line, tester, and line × tester, line × location, and tester × location interactions) were statistically significant (Table 2). The significant line × tester interaction suggested that lines differed in their reaction to *C. zeina* in crosses with different testers, which meant that specific crosses (hybrids), rather than

Table 2. Analysis of variance (mean squares) for gray leaf spot resistance scores (GLSS) and grain yield (GY) at three locations.

Source of variation	df	GLSS†	GY‡
Location (Loc)	2	351.94*	63.13
Replication(Loc)	6	1.17	0.43**
Line	12	50.92**	2.00**
Tester	4	16.38**	0.23
Line × Tester	48	2.07**	0.43**
Line × Loc	24	7.88**	1.35**
Tester × Loc	8	3.01*	0.70**
Line × Tester × Loc	96	1.17	0.31**
Error	384	1.24	0.17

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

† The GLS scores were recorded using a 1-to-9 scale, where 1 = highly resistant, 3 = resistant, 5 = moderately resistant, 7 = susceptible, and 9 = highly susceptible.

‡ Grain yield (kg) per plot.

lines or testers, resistant to GLS should be identified. The significant line × location and tester × location interactions implied that gene expression relative to GLSS in both lines (13 lines) and testers (four RILs and Q11) was differentially modified by location. As pointed out by Kang (1997), gene expression is environmentally induced and regulated. If the activity of an enzyme (gene product) is environment sensitive, norms of reaction (an array of phenotypes) are observed (Kang, 1997). Thus, differences in GLSS performance of testers and lines could partly be explained by their significant interaction with location. These interactions also meant that for a reliable evaluation of lines and testers for GLSS, multiple-location data would be necessary for decision making relative to GLSS.

The nonsignificant line × tester × location interaction in our study was consistent with the findings of Derera et al. (2008), who evaluated 72 hybrids for resistance to GLS across several environments via NCII design and found that the ranks of hybrids for resistance to GLS across environments were not significantly different. The implications of these results are that to identify hybrids resistant to GLSS, evaluation for resistance to GLS might not need to be done in multiple locations and that it would be possible to select hybrids with high levels of resistance to GLSS by testing at a location showing a reasonably high level of fungal infection.

Grain Yield

Mean squares for GY were significant for replication within location, line, line × tester interaction, line × location interaction, tester × location interaction, and line × tester × location interaction. As was the case for GLSS, the crosses (line × tester interaction) differed in performance with respect to GY. The significant line × location and tester × location interactions implied that for a reliable evaluation of GY of lines and testers per se, multiple-location data would be needed. Because the line × tester × location interaction was also significant for GY, performance of hybrids must also be measured across multiple locations. This interaction also implied that it should be possible to identify hybrids or crosses with consistent (stable) performance across locations and those specifically adapted to individual locations (Yan and Kang, 2003).

The mean squares for location and tester (i.e., male parents) were statistically nonsignificant, but those for line × location and tester × location interactions were significant. These results implied that instead of comparing mean GYs of individual locations and of individual testers, two-way interaction (line × location and tester × location) GY means should be compared. It was interesting to note also that, although there were significant differences among testers with respect to GLSS, no significant differences were detected among testers with respect to GY, making the GLSS and GY traits independent of each other. Further discussion of this relationship with respect to presence or absence of RDNAS is below.

RDNAS vs. No RDNAS Relative to GLSS and GY among Testers (RILs and Q11) across and at Individual Locations

The mean GLSS and mean GY of the five testers used for making crosses with 13 lines across three locations (Fig. 2) revealed the following:

1. The three RILs with RDNAS had significantly lower mean GLSS (3.77–4.38) than the recurrent parent Q11 (4.79); RL2_1 had the lowest mean GLSS (3.77) among the three RDNAS-carrying RILs (Fig. 2).
2. RL2_2 (without RDNAS) showed a similar level of GLS resistance as RL1_1 and RL1_2 (with RDNAS), and it also had significantly lower mean GLSS than Q11 (Fig. 2). However, RL2_1 had significantly lower GLSS than RL2_2 (Fig. 2). Overall, these results meant that RDNAS was not solely responsible for contributing toward resistance to GLS.

The mean GLSS of the five testers for individual locations (Table 3) showed the following:

1. The GLS scores at Dehong and Wenshan, which experienced relatively low disease pressure, were <5 (1–9 scale). Disease pressure at Kunming was relatively high, as the GLS scores were >5, which improved differentiation of lines with and without RDNAS.
2. Among the three RILs containing the RDNAS, RL2_1 had significantly lower mean GLSS than Q11, the most susceptible line, at all three locations (Table 3), indicating that RL2_1 was the best GLS resistance-contributing line, and that it could be used in breeding programs aimed at improving resistance to GLS.
3. Although RL2_2 did not have the RDNAS, it had similar GLSS levels as RL1_1 and RL1_2 and showed significantly lower GLSS than that of Q11 at Wenshan and Dehong (Table 3), the locations with light disease pressure, but RL2_2 was not significantly different in GLSS than Q11 at Kunming, the location with high disease pressure.

Furthermore, the three RILs containing RDNAS showed different GLSS levels at different locations. For example, at Kunming, RL2_1 had significant lower GLSS than RL1_2; at Wenshan, RL_2_1 showed significantly lower GLSS than RL1_1 and RL1_2, whereas at Dehong, no significant differences were found among the three RLs. This result is consistent with the fact that tester × location interaction was significant (Table 2). An explanation for this has been provided previously; gene (RDNAS) expression relative to GLSS in different RILs was differentially modified by location (Kang, 1997).

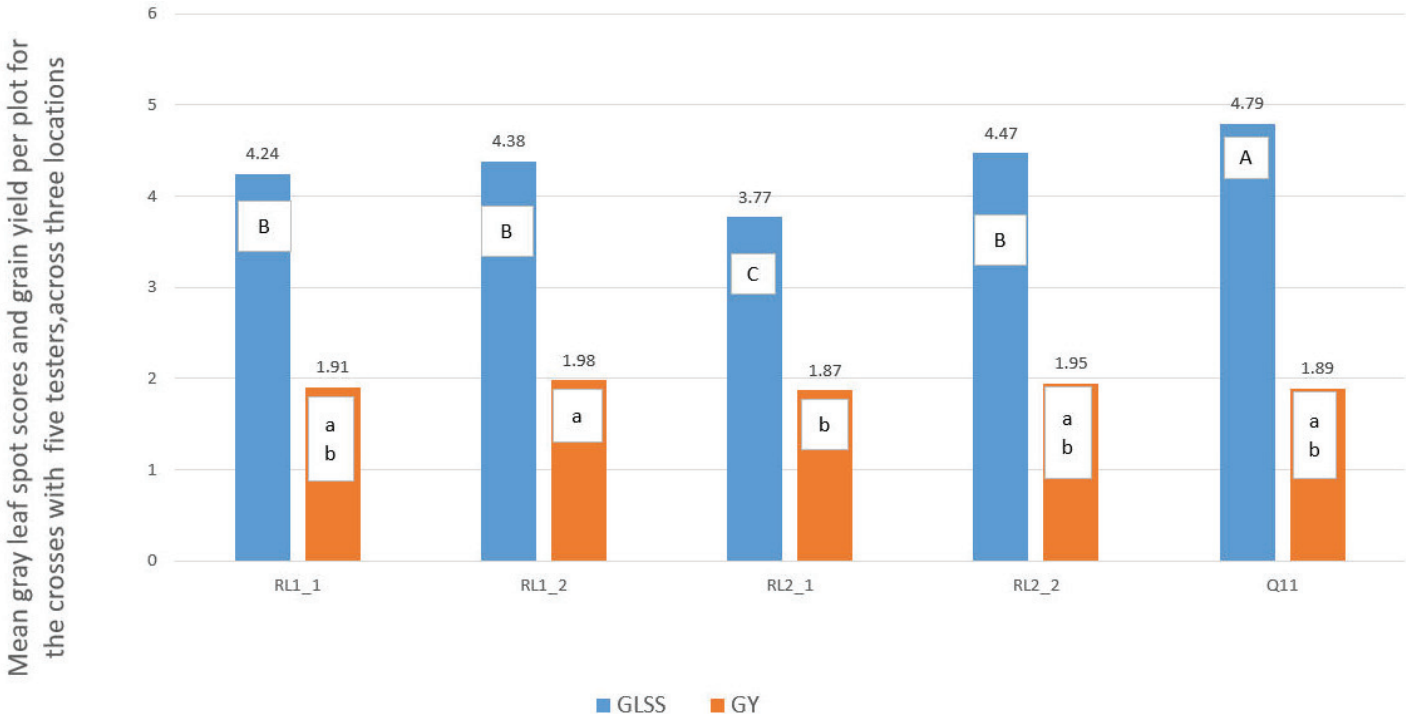

Fig. 2. Mean gray leaf spot scores (GLSS) and mean grain yield (GY) of five testers from all crosses between the testers and 13 lines across three locations. The bars with different letters are statistically significant at the $\alpha = 0.05$ level.

We feel that, in our study, the defense response of RL1_1 and RL1_2 probably was not sufficient enough to limit infection by their influence on the latency period or incubation period for the pathogen and might have led to more leaf area being affected under more severe disease pressure at Kunming. Zhang et al. (2017) fine-mapped ~130 Mb QTL and identified five putative genes on chromosome 8 in teosinte (*Z. mays* ssp. *parviglumis*). By comparing with the B73 reference genome, Zhang et al. (2017) found that the QTL had two alleles; one increased resistance to GLS, and the other increased susceptibility to GLS. Given the current data, field observation, and previous reports, we offer the following plausible explanation for the results.

First, in the three RILs with RDNAS (QTL), there may be two major genes, with one gene increasing resistance to GLS, and the other increasing susceptibility to GLS. In RL2_1 (the best GLS-resistant line), as described by Zhang et al. (2017), the gene for increased susceptibility might have been missed because of recombination (double crossing over). In RL1_1 and RL1_2, the gene increasing susceptibility might have been retained.

Second, the resistance genes could be just outside one of the two flanking markers relative to the RDNAS. In RL1_1 and RL1_2, the flanking DNA might be from Q11 and might have expressed the same resistance level as RL2_2.

The reason why RL2_2 had a lower level of GLSS than Q11 at Dehong and Wenshan could be that it contained additional resistance genes from YML32 or Q11. Zhang et al. (2012) reported that, in addition to RDNAS, three other DNA segments carrying resistance to GLS were involved, of which two were contributed by YML32 and one by Q11. Similar results have been reported in other studies (Berger et al., 2014; Liu et al., 2016).

Table 3. The mean of all crosses, by tester, for gray leaf spot scores (GLSS) and mean grain yield (GY) per plot (kg) at three locations.

Location	Tester†	GLSS‡	GY
			kg plot^{-1}
Dehong	RL1_1	3.31	1.79
	RL1_2	3.31	2.12
	RL2_1	3.21	1.92
	RL2_2	3.21	1.79
	Q11	3.67	1.74
	LSD0.05§	0.40	0.15
Kunming	RL1_1	5.67	1.48
	RL1_2	6.33	1.40
	RL2_1	5.15	1.24
	RL2_2	5.92	1.39
	Q11	6.23	1.37
	LSD0.05	0.65	0.23
Wenshan	RL1_1	3.77	2.47
	RL1_2	3.77	2.42
	RL2_1	2.95	2.45
	RL2_2	4.03	2.66
	Q11	4.49	2.57
	LSD0.05	0.40	0.17

† RL1_1 and RL1_2, recombinant lines from the BC_1 population; RL2_1 and RL2_2, recombinant lines from the BC_2 population (same as in Fig. 1 and Table 1).

‡ Gray leaf spot scores were recorded on a 1-to-9 scale, where 1 = highly resistant, 3 = resistant, 5 = moderately resistant, 7 = susceptible, and 9 = highly susceptible.

§ LSD0.05 is the least significant difference at $\alpha = 0.05$ level.

GCA Effects on GLSS and GY for RILs with and without RDNAS and Q11 at Different Locations

The GCA effects of the five testers on GLSS and GY are given in Fig. 3A and 3B, respectively. In addition to the GCA effects of testers (Fig. 3A), the GCA effects of the 13 lines are also provided in Supplemental Fig. S2. The results (Supplemental Fig. S2) showed that the hybrids formed from subtropical lines, which had been selected for improved resistance to GLS in Latin America and Africa (D. Jeffers, personal communication, 2016), and usually had lower GLSS than those from the temperate lines (Supplemental Fig. S1), suggesting that introducing tropical and subtropical germplasm was an effective way of improving resistance of lines or/and hybrids to GLS. In this case, the subtropical germplasm would need to be introduced into a yellow germplasm background, as the four lines are white grain color. Figure 3A revealed that RL2_1 had the lowest GCA effects for GLSS at Wenshan and Kunming and had GLSS as low as that of RL1_2 at Dehong. Further, the GCA effects of the recurrent parent (Q11) for GLSS were positive (i.e., in the susceptible direction) at the three locations (Fig. 3A). At Kunming (the location with moderately high disease pressure), the GCA effect of RL2_2 (no RDNAS) was positive, whereas the GCA effects of RL1_1 and RL1_2 were significantly lower than those of RL2_2 ($\alpha = 0.05$) (Fig. 3A). Furthermore, the GCA effects of RL1_1 and RL1_2 for GLSS were significantly lower than that of RL2_2 at Kunming, the location with relatively high disease pressure. These results suggested that the resistance to GLS of crosses with RL1_1, RL1_2, and RL2_2 (Table 3) might be controlled by different genetic mechanisms. The lower GLSS for RL1_1 and RL1_2 could be attributable to RDNAS, whereas the lower GCA of RL2_2 (no RDNAS) for GLSS than that of Q11 at Dehong and Wenshan was likely attributable to QTL other than RDNAS.

Derera et al. (2008) studied gene action controlling GLS resistance using southern African maize germplasm. They found that both GCA and SCA effects were

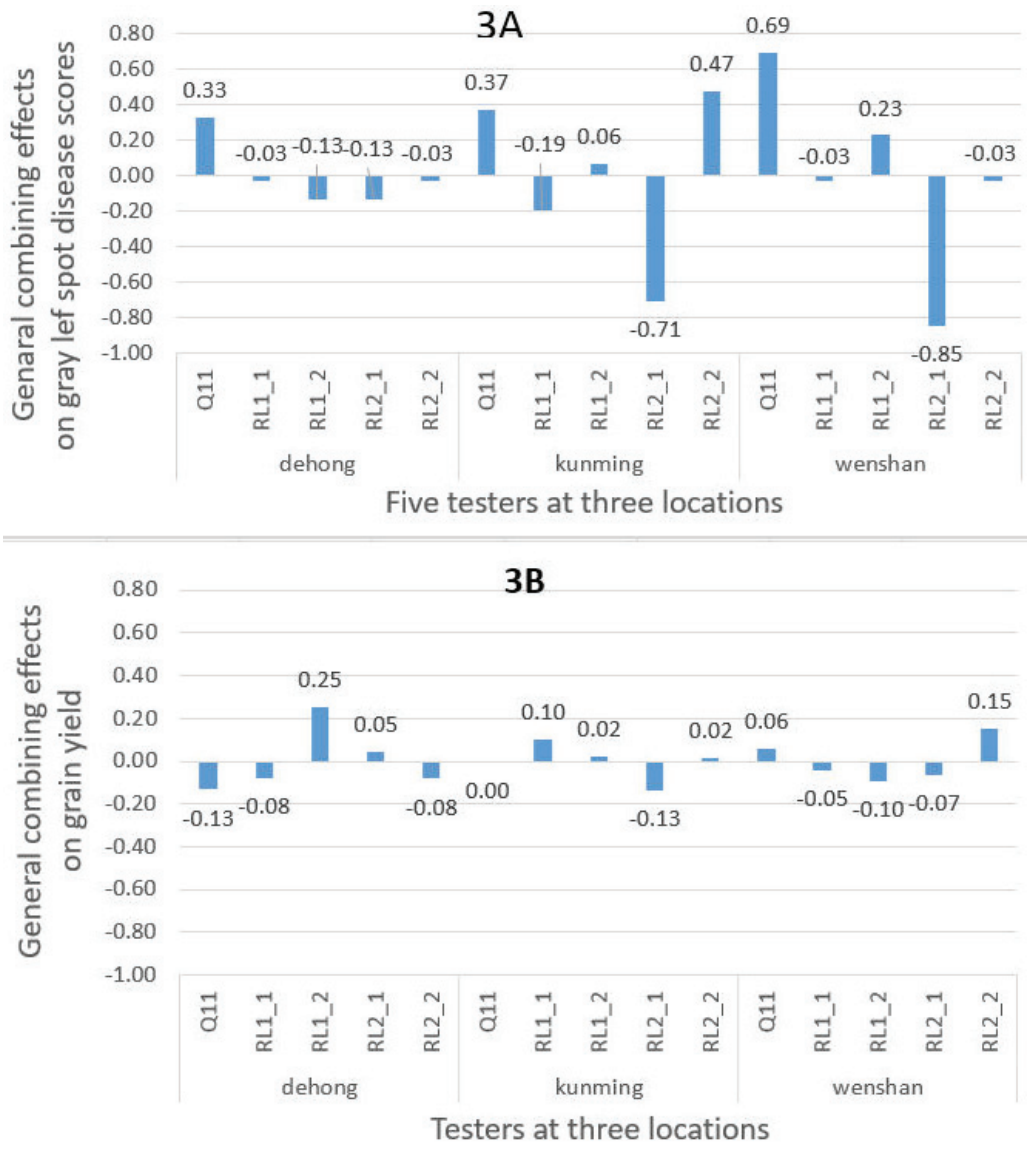

Fig. 3. General combing ability effects of five testers at three locations for (3A) gray leaf spot scores and (3B) grain yield in kilograms per plot.

significant for resistance to GLS, and with GCA being more important than SCA, they concluded that additive effects were more important than nonadditive effects. In comparing the sums of squares (SS) for lines and testers (which represent GCA) and for line × tester interaction (which represent SCA) (Table 2), we found the GCA SS (676.5 or 87.2% of total GCA and SCA SS) to be 6.8 larger than SCA SS (99.4 or 12.8% of total GCA and SCA SS). Because the study by Derera et al. (2008) and our study used different germplasm, it would seem that GCA for resistance to GLS is quite predominant in maize germplasm. The predominance of GCA effects for GLS resistance should allow breeders to focus on development of lines resistant to GLS.

Role of SCA in Hybrid Resistance to GLS

The distribution of resistance to GLS for all 65 crosses is shown in Fig. 4. The resistant and susceptible checks, CML 312 × Q11 and Chang 7–2 × Q11, had a spread of four points on the 1-to-9 scale. Had disease pressure at Dehong and Wenshan been higher, a greater spread between the checks would have been expected. The SCA effects for GLSS for the 65 crosses at the three individual locations are shown in Fig. 5. The results revealed the following:

1. The crosses with low GLSS usually corresponded with low negative (favorable) SCA effects, suggesting that SCA is an important component of resistance to GLS in maize hybrids.
2. Whether the testers had RDNAS (i.e., RL1_1, RL1_2, RL2_1) or not (i.e., RL2_2 and Q11), some crosses were produced with GLS scores <3.5 (Fig. 4) or with negative SCA effects (Fig. 5). This suggested that, in addition to the specific RDNAS transferred via MAS, other DNA segment(s) from the resistant RILs or other gene(s) also likely contributed to GLS resistance. As mentioned above, the four subtropical lines (CML 312, CML 373, CML 384, and CML 395) also carried resistance to GLS. These results are consistent with the findings of Zhang et al. (2012), who found that although 19 to 62% reduction in GLSS was attributed to the RDNAS, three other QTL that came from both resistant and susceptible parents also contributed to resistance to GLS. That susceptible parental lines contribute resistance QTL for GLS has also been reported by other researchers (Zhang et al., 2012; Berger et al., 2014; Liu et al., 2016).

GY and GLSS of the Crosses between the Five Testers and 13 Lines from Three Heterotic Groups

Mean GLSS values for the five testers in crosses with lines from three heterotic groups at the three locations are given in Fig. 6. The GLS scores were consistently lower, across locations, for the crosses between the three testers containing the RDNAS and lines from Suwan1 heterotic group than for those between the

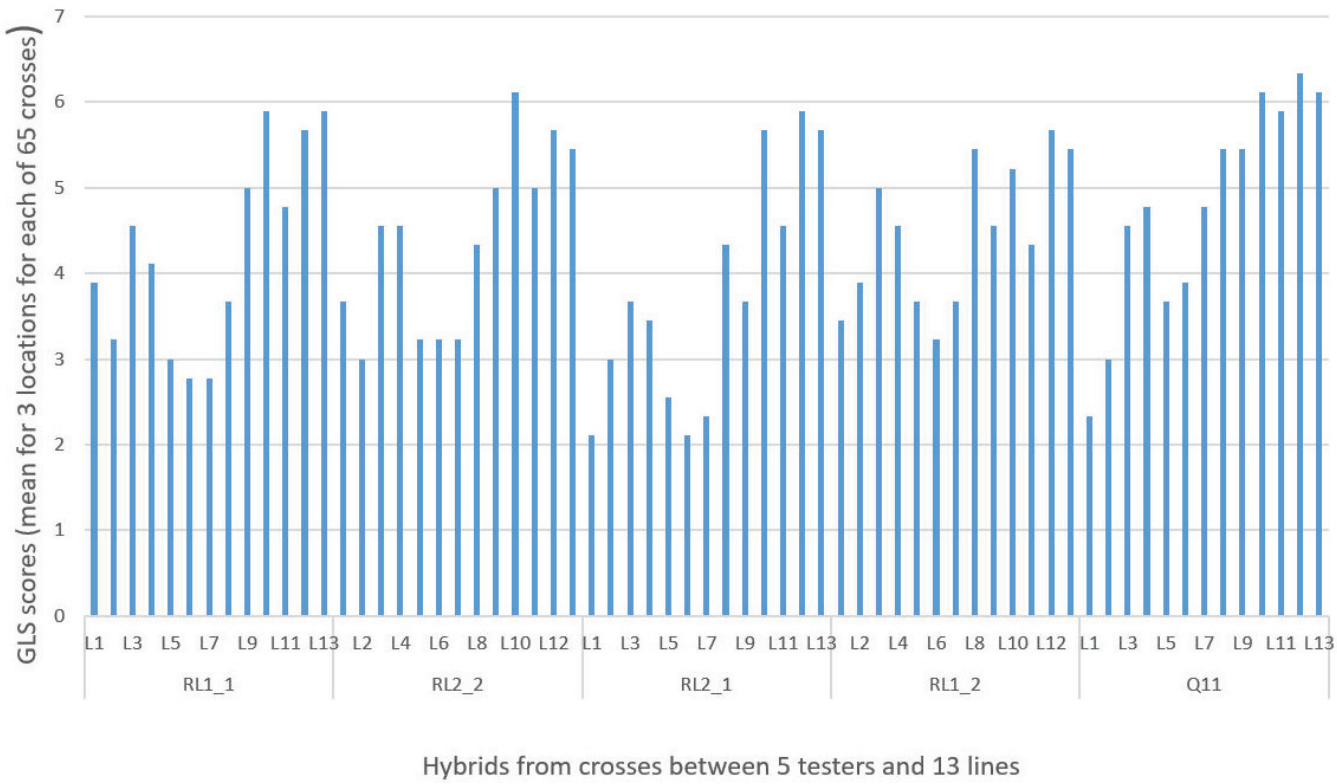

Fig. 4. Distributions of the gray leaf spot (GLS) scores from the 65 crosses across three locations.

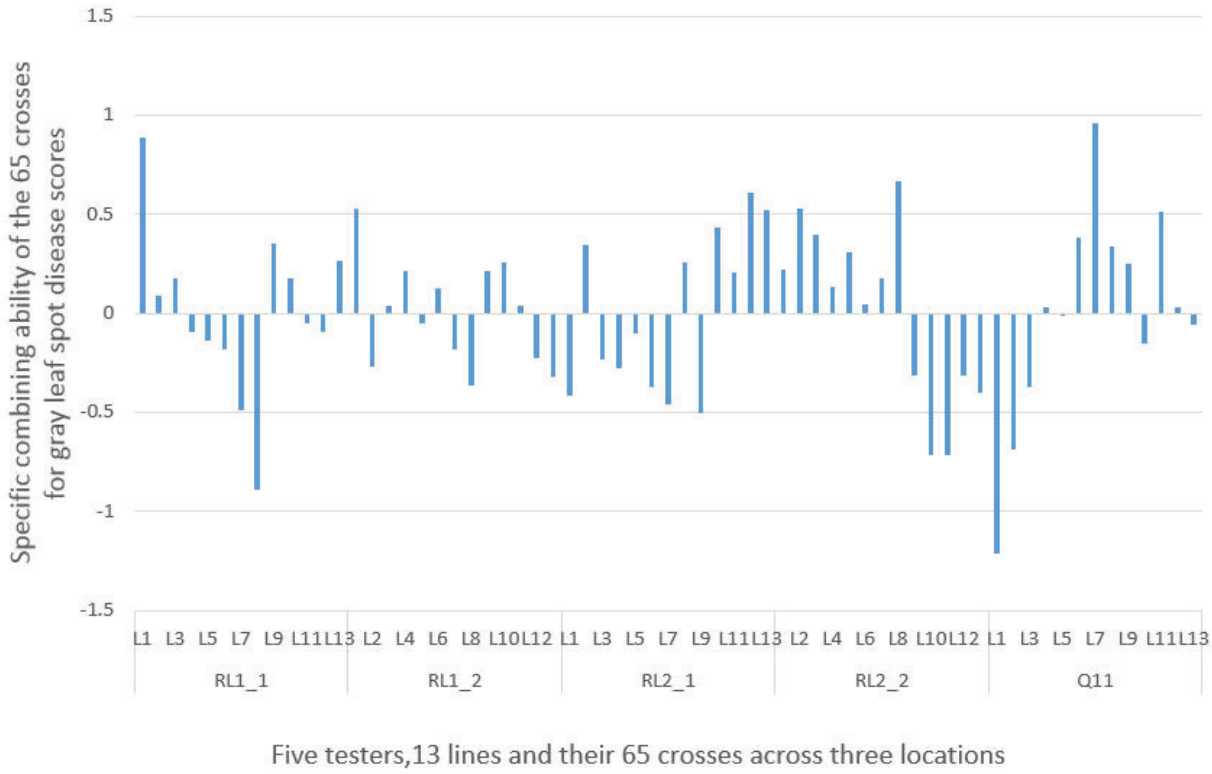

Fig. 5. Specific combining ability effects of 65 crosses between five testers and 13 lines for gray leaf spot scores across locations.

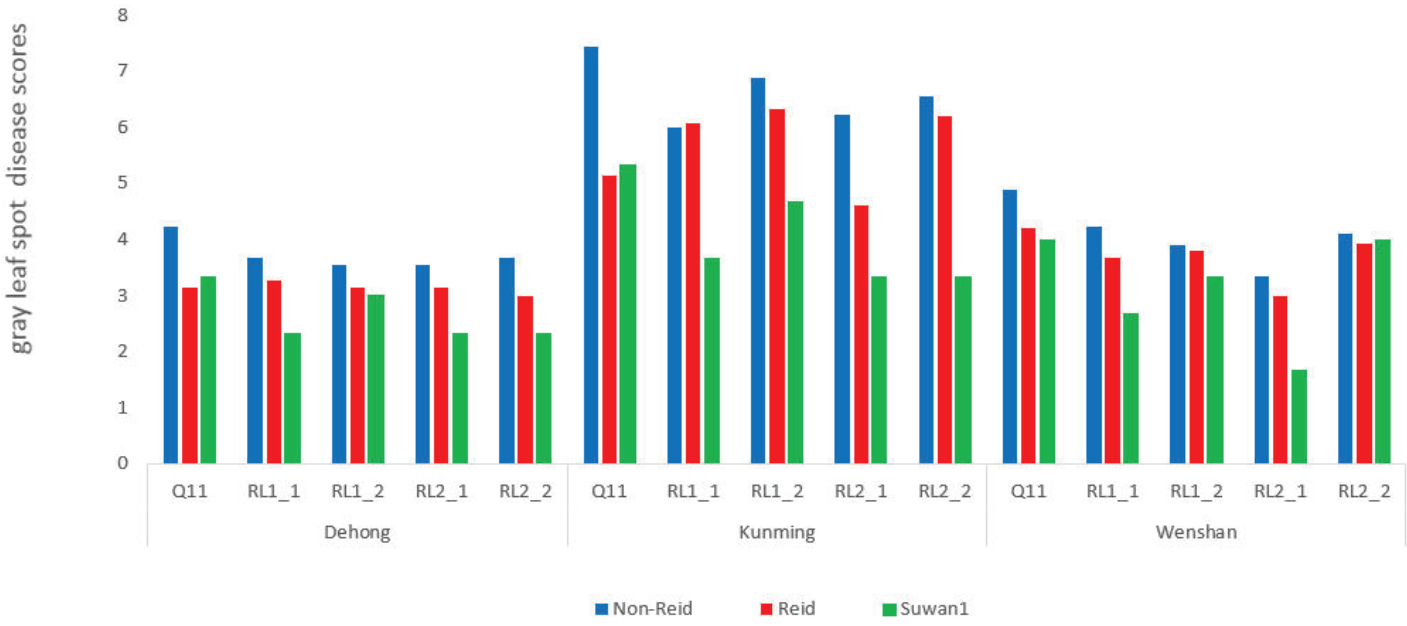

Fig. 6. The mean gray leaf spot resistance scores for testers crossed with lines from three different heterotic groups at three locations.

testers and lines from Reid and non-Reid heterotic groups. These results suggested that

1. The lines developed from Suwan1 or other germplasm may contain additional QTL offering resistance to GLS, as reported in other crosses from previous studies (Derera et al., 2008; Zhang et al., 2012; Berger et al., 2014; Liu et al., 2016). It is possible that the crosses between lines from the Suwan1 heterotic group either had stronger resistance QTL than the lines from the other two heterotic groups or had QTL in addition to the RDNAS, which accentuated the level of resistance to GLS in the crosses.
2. Regardless of environmental differences, the resistance genes in the RDNAS might be expressed or transcribed differentially, or they might have interacted differentially in the crosses representing the different heterotic groups.

On the basis of studies by Zwonitzer et al. (2010) and Zhang et al. (2012), Berger et al. (2014) argued that multiple resistance genes might exist in a population. From the results of our study, it is highly likely that RILs developed from YML32 could contain multiple resistance genes located on different chromosomes; the lower GLSS in the crosses with lines from Suwan1 might be attributed to the presence of additional genes or alleles that condition resistance to GLS.

Upon further analyzing mean GY and mean GLSS and evaluating the differences resulting from the five testers crossed with the lines from the three heterotic groups, we found that, in general, GYs of crosses between the lines from different heterotic groups and five different testers (i.e., four RILs and Q11) were different (Table 4). When crossed with non-Reid heterotic group lines, RL1_2 and RL2_1 showed higher GY than with Q11. When crossed with Reid and Suwan1 heterotic group lines, no significant differences were found between the two RILs (testers RL1_2 and RL2_1) and Q11, but RL2_1 had lower GY than Q11. Standard deviations of the GY of the crosses of testers (RL1_1, RL1_2, RL_2_1, RL2_2, and Q11) from those of lines from the three heterotic groups were, respectively, 0.15, 0.08, 0.12, 0.15, and 0.23. These results indicated that integration of RDNAS via MAS did not consistently improve the GY across environments but improved yield stability of the crosses developed from Q11. By integrating the RDNAS via MAS, no linkage drag was observed and yield potential was maintained.

In summary, the RILs containing the RDNAS had lower GLSS than that of recurrent parent Q11 at Wenshan with low disease pressure (GLSS < 5). At Kunming, the location with relatively higher disease pressure (GLS > 5), RL2_1 had significantly lower GLSS than Q11 and had significantly lower GCA for GLSS than RL2_2. This result suggested that the RDNAS was at least partly responsible for lowering GLSS in RL2_1. As for the other two RL lines (RL1_2 and RL_1_2) with RDNAS, although their GLSS was not significantly lower than that of Q11 at all locations, the GCA effects of the RL1_1 and RL1_2 for GLSS were significantly lower than that of RL2_2 at Kunming (the location with high disease pressure). These results also suggested that RDNAS was partly responsible for resistance to GLS in RL1_1 and RL1_2. In addition, since RL2_2 showed lower GLSS at Kunming than at Wenshan and Dehong, QTL different from RDNAS might be responsible for lowering the GLSS in RL2_2.

The GY was not greatly changed with the introgression of the RDNAS into local elite lines, but GY stability had been improved. The RL2_1 was identified as the best inbred line for use in maize breeding programs aimed at improving GLS resistance. The RILs containing the RDNAS, when crossed with Suwan1-derived inbreds, gave lower GLSS than those for the crosses between testers and most of the lines from the other two heterotic groups, except crosses between RL2_1 and Reid lines. Mean GLSS and combining ability results suggested that genetic components other than the RDNAS might be involved in determining resistance to GLS in the crosses. Further, use of lines derived from Suwan1 could help enhance resistance to GLS. Basically, the deployment of Suwan1-derived lines and testers containing RDNAS should be useful in greatly improving hybrid resistance to GLS.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Table 4. Comparison of mean grain yield (GY) and mean gray leaf spot resistance scores (GLSS) for crosses between five testers, and the mean of the lines from three heterotic groups.

Heterotic group of female lines	Tester (male)	GY	GLSS	Resistance rank
		Mean	Mean	
Non-Reid	RL1_1 (R)†	1.83ABC‡	4.63BC‡	2
Non-Reid	RL1_2 (R)	1.91AB	4.78B	4
Non-Reid	RL2_1 (R)	1.96A	4.37C	1
Non-Reid	RL2_2 (no R)†	1.81BC	4.78B	3
Non-Reid	Q11 (no R)	1.70C	5.52A	5
Reid	RL1_1 (R)	1.93AB	4.33A	3
Reid	RL1_2 (R)	2.07A	4.42A	5
Reid	RL2_1 (R)	1.80B	3.58B	1
Reid	RL2_2 (no R)	2.06A	4.38A	4
Reid	Q11 (no R)	2.04A	4.16A	2
Suwan1	RL1_1 (R)	2.13A	2.89BC	2
Suwan1	RL1_2 (R)	1.95AB	3.67AB	4
Suwan1	RL2_1 (R)	1.76B	2.44BC	1
Suwan1	RL2_2 (no R)	2.08A	3.22BC	3
Suwan1	Q11 (no R)	2.13A	4.22A	5

† R, RDNAS present; no R, RDNAS not present.

‡ Different letters represent significant difference between means according to Duncan's multiple range test at $\alpha = 0.05$.

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