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Genetic association of agronomic traits with partial resistance to gray leaf spot in elite maize germplasm

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Abstract

Studies on gray leaf spot (GLS) of maize have reported inconsistencies in the relationship between partial disease resistance and agronomic traits. Understanding this variation could facilitate the use of agronomic traits as a basis for selection to improve partial resistance to GLS. Coinheritance of nine agronomic traits with partial resistance to GLS was examined among 48 maize (*Zea mays* L.) inbred lines artificially infected with *Cercospora zeina* in field evaluations across nine environments in western Kenya in 2013 and 2014. Five measures of disease severity and two disease resistance components were evaluated for their association with agronomic traits. Standardized area under disease progress curve (SAUDPC) was the most efficient in delineating differences in GLS severity between genotypes, whereas latent period (LP) was the least effective. Among maize genotypes, values of SAUDPC ranged from 29.3 to 97.9 across environments. Genotypic and phenotypic correlations were strongest between SAUDPC and the absolute rate of disease increase (ρ; $r = .71$), final percent diseased leaf area ($r = .66$) and International Maize and Wheat Improvement Center (CIMMYT) disease severity grade ($r = .60$), but weakest between SAUDPC and LP ($r = -.19$). Correlations of SAUDPC were significant ($P = .05$) with eight of the 11 agronomic traits examined, with the strongest being between SAUDPC and the stay-green characteristic (SGR; $r = -.87$), days to maturity (DTM; $r = -.60$) and ear/plant height ratio ($r = -.52$). Genotypic and phenotypic coefficients of correlation

Abbreviations: AIC, Akaike's information criterion; BCFI, Bentley's comparative fit index; CDSG, International Maize and Wheat Improvement Center disease severity grade; CIMMYT, International Maize and Wheat Improvement Center; CMA, causal mediation analysis; DTA, days to anthesis; DTM, days to maturity; DTS, days to silking; EH, ear height; EH/PH, ear/plant height ratio; EPP, number of ears per plant; FPDLA, final percent disease leaf area; GLS, gray leaf spot; H^2, broad-sense heritability; LP, latent period; LPP, number of leaves per plant; PDLA, percent disease leaf area; $PDLA_{IP}$, percent disease leaf area at the inflection point; PH, plant height; QTL, quantitative trait loci; RMSEA, root mean square error approximation; SAUDPC, standardized area under the disease progress curve; SE, standard error; SGR, stay-green characteristic; SMI, silking–maturity interval; ρ, absolute rate of disease increase.

of SAUDPC with agronomic traits were all negative. Overall, absolute genotypic correlations were numerically larger than the corresponding coefficients of phenotypic correlation with the magnitude and direction of coheritability estimates mimicking trends in genotypic and phenotypic correlations. Causal mediation analysis indicated that covariation of GLS resistance with agronomic traits was mainly due to direct effects of days to anthesis and DTM and indirect effects of SGR and silking–maturity interval.

1 | INTRODUCTION

Genotypic correlation between traits is often an indicator of the direction and magnitude of correlated response to selection, relative efficiency of indirect selection based on correlated secondary traits and thus, facilitates computation of multiple trait selection indices (Falconer & Mackay, 1996). Correlation of traits indicates linkage and/or pleiotropy of pertinent genes, whose segregation accounts for phenotypic covariation. However, pleiotropy may be absent if traits are influenced to a similar extent by environmental conditions manifested as nonheritable correlation. This phenomenon, referred to as environmental correlation, directly contributes to deviation of phenotypic or genotypic correlation from coheritability due to the confounding effects of the environment (Holland, 2006; Vasquez-Kool, 2019). Since coheritability depicts the concurrent or recursive inheritance of traits, it reflects the effectiveness of tandem selection and facilitates the development of appropriate methods to incorporate multiple desirable traits into new germplasm.

Previous studies on the relationship between partial resistance to gray leaf spot (GLS) in maize (*Zea mays* L.) and agronomic traits have reported mixed results. For example, Rupe et al. (1982) indicated that plant maturity was critical in late-season development of GLS, with initial symptoms not appearing until plants approached anthesis. A 3-wk delay in planting caused a corresponding delay in the appearance of symptoms. However, Hilty et al. (1979) reported concurrence of initial disease symptoms with silk emergence regardless of genotype maturity, wherein the number of GLS lesions increased as plants approached senescence. Examination of stomatal penetration by the GLS pathogen in maize demonstrated that host resistance is ontogenic, regardless of the level of varietal resistance (Gwinn et al., 1987). Further, the discordance between early and late-disease ratings among genotypes suggested that early and late-season resistance involved different loci (Bubeck et al., 1993). Coates and White (1994) corroborated this finding when they reported a different gene action for resistance in early and late-disease ratings. Understanding the inconsistency between GLS resistance and agronomic traits can facilitate the identification of agronomic traits that could serve as surrogate for resistance, particularly in early generation trials where more emphasis is often placed on the phenology of genotypes.

A significant negative phenotypic correlation between GLS severity and days to anthesis (DTA) was reported by Derera et al. (2008). However, this observation did not sufficiently explain the protection of late-maturing hybrids against GLS. It was hypothesized that environmental factors regulated the strength of the relationship between earliness and resistance since the correlation was significant in only one of three trial environments (Balint-Kurti et al., 2008). Similarly, Gordon et al. (2006) observed a negative but weak and nonsignificant correlation between GLS severity and days to silking (DTS) and concluded that resistance was not significantly affected by earliness of flowering as previously reported by Menkir and Ayodele (2005). The observed correlation between GLS resistance and plant maturity in molecular markers studies has equally yielded mixed results. For example, a quantitative trait locus (QTL) on chromosome 4 associated with DTS (Bubeck et al., 1993) was linked to a QTL for resistance in a separate study (Saghai Maroof et al., 1993). However, in a subsequent study, QTLs associated with GLS resistance did not colocalize with those associated with DTA (Balint-Kurti et al., 2008). Clement et al. (2000) observed that only one of the four QTLs associated with ear/total plant height ratio (EH/PH) was associated with ear height (EH) and GLS resistance. While some of the above studies indicate that DTA, days to maturity (DTM), and EH/PH are correlated with each other and with GLS resistance, there are no reports that describe the direct and indirect effect of agronomic traits on GLS resistance.

Other aspects of maize phenology that have also been investigated for phenotypic association with GLS resistance include PH, EH, ear aspect (EA, i.e., visual appeal of a dehusked ear), grain yield, and plant aspect (PA, i.e., visual appeal of a plant). Menkir and Ayodele (2005) reported that GLS severity was significantly correlated with PA, EA, and grain yield, but not with plant height (PH) and EH. However, the relevance of PA is unclear given that highly susceptible genotypes are bound to have a lower visual appeal. Although Clement et al. (2000) reported that GLS severity was correlated with EH/PH, the correlations were weak and nonsignificant in four of the 12 environments included in their study. In addition, only one out of the four QTLs

associated with EH/PH colocalized with a QTL for GLS resistance (Clement et al., 2000). A better understanding of the inconsistency between GLS resistance and plant maturity or EH/PH can facilitate selection of breeding approaches that simultaneously improve resistance and agronomic traits to optimize yield.

Since its conceptualization by Wright (1934), path coefficient analysis has been used extensively in genetics and other sciences to decompose the total effects of component independent traits and/or variables into direct and indirect effects on correlated traits and/or variables. In its essence, path analysis is a form of structural equation modeling (SAM), a powerful tool for describing the relationships between three or more variables (Dudley et al., 2004). Causal mediation analysis (CMA) is another form of SAM that describes the strength of the sequence by which antecedent variables affect mediating variable(s) that ultimately influence a dependent variable (Shrout & Bolger, 2002). Comparative analysis of the effectiveness of classical path analysis and CMA in modeling mechanisms underlying coinheritance of traits is lacking in plant genetics. In addition, path analysis models in plant genetic studies usually exclude endogenous mediation variables, despite this being the standard approach in contemporary SAM in other fields (Shrout & Bolger, 2002). Prediction of dependent variables is greatly improved when mediation (intervening) variables are included in SAM as mediator(s) of indirect effects of causal (independent) variable(s). While studies indicate that DTA, DTS, DTM, and EH/PH are significantly correlated with each other and with GLS resistance (Clement et al., 2000; Derera et al., 2008; Menkir & Ayodele, 2005), there are no reports of direct and indirect effects of maize phenology on GLS resistance. Similarly, no studies have been conducted on the coheritability or genotypic correlation of partial resistance to GLS with these agronomic traits.

Given the above considerations, the goal of this study was to determine the prospect of using of agronomic traits as a basis for truncated, tandem, or index selection aimed at improving partial resistance to GLS. The specific objective of the study was to use genotypic correlations and coheritability estimates in CMA to examine and develop an empirical construct of the genetic association between agronomic traits and partial resistance to GLS in maize.

Core Ideas

- Resistance correlated with stay green characteristic, days to maturity, and ear to plant height ratio.
- Coheritability of earliness and ear to plant height ratio with disease resistance is moderate.
- Days to anthesis indirectly mediates genetic correlation of stay green characteristic with resistance.
- Effects of days to silking and days to maturity on partial resistance to gray leaf spot were more or less direct.

2 | MATERIALS AND METHODS

2.1 | Genotypes, planting, field layout and inoculation

Forty-eight inbred lines from the International Maize and Wheat Improvement Center (CIMMYT) germplasm collection were used in this study (Supplemental Table S1). These genotypes are among the most genetically diverse in the world and are considered useful for broadening the genetic base of other maize germplasms (Fan et al., 2010). These genotypes were evaluated in nine environments in western Kenya in 2013 and 2014, with each environment representing a single location–year–season combination (Table 1). In each environment, inbred lines were planted in plots consisting of three 3-m rows spaced 0.75-m apart. Within the rows, seeds were sown in hills spaced 0.25-m apart by placing two kernels per hill and covering with a 2- to 3-cm layer of topsoil. Each hill received 5 g of diammonium phosphate fertilizer (DAP-46) at planting.

To reduce inter-plot interference, plots were separated from each other by two rows of a non-host, sorghum (*Sorghum bicolor* L. Moench) cultivar Seredo (Kenya Seed Co. Ltd). Inbred lines were evaluated in a randomized complete block design with four replications and layouts of each plot were generated with PROC PLAN of SAS version 8.2. To limit the risk of early natural infection of plants with GLS before artificial inoculation, planting was carried out immediately at the start of the growing season in plots that were at least 75 m away from sites where maize had been grown in the previous season. Besides pesticide application, all standard agronomic and cultural practices recommended for maize were followed in all environments. To limit specific host × pathogen interaction that would confound host genotype × environment interaction, a wildtype mixed population of *C. zeina* was used to inoculate plants between V2 and V3 growth stage as described by Nyanapah et al. (2020).

2.2 | Data collection

2.2.1 | Disease assessment and resistance components

Five measures of disease severity, that is, percent diseased leaf area (PDLA), CIMMYT (1985) disease severity grade (CDSG), final PDLA (FPDLA), standardized area under

TABLE 1 Locations and weather attributes of environments used to characterize quantitative resistance of gray leaf spot of maize in western Kenya

Season[a]	Location	Longitude	Latitude	Rainfall	Mean day temp.	Mean night temp.	RH[b]	Environment[c]
				mm	˚C		%	
2013								
LR	Kadongo	34˚35′56″ E	0˚12′08″ S	812	32.4	16.8	58	E1
	Kapuonja	34˚35′54″ E	0˚00′24″ S	765	28.6	14.0	60	E2
	Kehancha	34˚36′54″ E	1˚11′38″ S	861	32.7	17.8	61	E3
SR	Kadongo	34˚46′04″ E	0˚41′02″ S	963	28.2	15.1	58	E4
	Kapuonja	34˚35′56″ E	0˚12′08″ S	659	30.2	16.8	59	E5
	Kehancha	34˚36′54″ E	1˚11′38″ S	773	32.6	19.6	59	E6
2014								
LR	Kadongo	34˚43′18″ E	0˚31′28″ S	862	29.4	14.8	58	E7
	Kapuonja	34˚35′56″ E	0˚12′08″ S	798	28.7	18.2	62	E8
	Kehancha	34˚36′54″ E	1˚11′36″ S	804	29.8	15.6	60	E9

[a]The long rains (LR) season is from March to July, while the short rains (SR) season is from August to November. Values of weather variables are either total (rainfall) or means (temperature and relative humidity) across the entire season.
[b]RH, Relative humidity.
[c]E refers to the serial number of an environment.

disease progress curve (SAUDPC), and percent diseased leaf area at the inflection point ($PDLA_{IP}$) and two disease resistance components, that is, late period (LP) and the weighted mean absolute rate of disease increase (ρ), were quantified and reported as described by Nyanapah et al. (2020).

2.2.2 | Agronomic traits

Eleven agronomic traits were recorded for test genotypes using six tagged plants randomly selected in each plot except for DTA, DTS, and DTM, which were measured on all plants in a plot.

Plant height was recorded as the height (cm) between the plant base and point of insertion of the lowest tassel branch, EH was recorded as the height (cm) between the plant base to the point of insertion of the topmost ear, ET/PH was calculated as the ear height divided by the plant height, and the number of leaves per plant (LPP) was recorded by dividing the total number of leaves in the middle row of each plot by the total number of plants in a row at the R4 stage (Ritchie et al., 1993). The number of ears per plant (EPP) was recorded by dividing the total number of ears in the middle row of each plot by the total number of plants in that particular row at the R6 growth stage, DTA was recorded as the number of days from sowing to the day when 50% of the plants in each plot started to shed pollen, DTS was recorded as the number of days from sowing to the day when ears in 50% of plants in each plot started to expose their silk (i.e., attained the R3 growth stage), DTM was recorded as the number of days from sowing to the day when ears in 50% of plants in each plot attained physiological maturity (R6 growth stage), anthesis–silking interval was recorded as the difference between DTS and DTA, and the silking–maturity interval (SMI) recorded as the difference (d) between DTM and DTS. The stay-green characteristic (SGR) was rated on a scale of 1 to 10 at R6 as described by Bekavac et al. (2007).

2.3 | Statistical analyses

All statistical analyses were conducted based on single-plot data composed of the mean of measurements taken over six sampled plants for all traits except for DTP, DTS, and DTM. Data for the latter three traits comprised of a single measurement taken from the entire plot.

2.3.1 | Exploratory data analysis and processing

Unless otherwise stated, all parameters were estimated under the following assumptions: (a) inbred lines are fully inbred, that is, inbreeding coefficient of a line = 1.0, (b) effect of inbred sets is fixed, and (c) effects of genotypes, environments, and replications are random. A series of exploratory analyses were conducted to test and correct for heterogeneity of error variances. Each location–season–year data were first analyzed separately as a distinct environment before combining for appropriate analyses over environments.

Single environment analysis of variation (ANOVA) for partitioning out structured plot error was based on the model: $Y_{ijk} = \mu + G_i + R_j + e_{ijk}$, where Y_{ijk} is the observation on plot k with the replication j of genotype i (i.e., a particular

inbred entry), μ is the mean of all plots in the set and environment (i.e., mean over all replications and all genotypes), G is the effect of genotype i, R is the effect of replication j in the environment, and eijk is random effect of plot error (i.e., residual). Residuals for individual environments were then inspected for normality using the Shapiro–Wilk test and data corrected for normality and subjected Levene test for homogeneity of error variance for combined ANOVA over environments (SAS Institute, 1999). Data violating the normality assumptions of ANOVA were subjected to the corrective transformations depending on the nature of the violation (Supplemental Table S2). Where entry means and their corresponding standard errors were computed based on transformed observations, the values were back-transformed to present them in the original units of measurement for the considered traits.

Evaluation of disease assessment and resistance component variables

The most useful disease assessment or resistance component variable to quantify disease resistance for inclusion in the genetic analyses was determined as described by Nyanapah et al. (2020). The ANOVA of appropriately corrected data of each trait nested within trial environments were all based on the following linear model: $Y_{ijkx} = \mu_x + G_{ix} + R_{jx} + e_{ijkx}$, where x is the trait of interest, and all other variables are as described above. The ANOVA of each trait pooled over trial environments was based on the following model: $Y_{ijkx} = \mu_x + E_{kx} + G_{ix} + \mathrm{RE}_{jkx} + \mathrm{GE}_{ikx} + e_{ijkx}$, where E_{kx} is the effect of environment k on trait x, RE_{jkx} is the effect of replication j within environment k on trait x, GE_{ikx} is the effect of the interaction between genotype i and environment k on trait x, and all other variables are as described above.

Estimation of variance and covariance components

Analysis of variance for appropriately processed data was implemented to facilitate additive decomposition of components of variance attributed to the different sources of variation of phenotypes (SAS Institute, 1994). Actual coefficients of expected mean squares and cross products were obtained to estimate variance and covariance components by equating actual mean squares corresponding to specific sources of variation and their expected values (Hallauer et al., 2010). Covariance components and their asymptotic variance–covariance matrices were estimated based on results of multivariate analysis of variance (MANOVA) invoked with METHOD = TYPE1 option of multivariate PROC GLM. Estimates of the phenotypic and genotypic components of covariance between variate x (i.e., resistance ratings) and covariate y (i.e., agronomic trait ratings) were extracted from MANOVA results using the method-of-moments by equating expected mean sum of cross products to their actual values (Mode & Robinson, 1959).

2.3.2 | Estimation of repeatability and broad-sense heritability

Repeatability and broad-sense heritability were estimated as described by Campbell and Lipps (1998). Standard errors (SE) of the genotypic component of variance were approximated and subsequently used to approximate SE of the broad sense heritability estimates. The heritability estimates were declared significant if values exceeded 1.96 SE (Campbell & Lipps, 1998). The approximate 95% confidence interval (CI) of repeatability estimates were computed as the estimates ± 1.96 SE. Repeatability estimates were declared significantly different from zero if the approximate 95% CI did not include zero.

2.3.3 | Estimation of association between partial resistance and agronomic traits

Coefficients of phenotypic correlation ($r_{p_{xy}}$) between trait pairs x and y within environments were calculated as described by Sodini et al. (2018). Here, SEs of the estimated phenotypic correlation coefficients were approximated using the jackknife method (Singh et al., 1997) and an approximate 95% CI for the correlations was calculated as described above. Phenotypic correlation coefficients were declared significant if their approximate 95% CI did not include a value of zero. Genotypic correlations between resistance and agronomic traits were estimated from genotypic covariances and phenotypic components of variance for the corresponding traits using the method of moments. Standard errors of genotypic correlations and approximate 95% CI were calculated as described above. Coheritability ($C_{h_{xy}}$) between trait pairs were computed from phenotypic and genotypic variances as described by Vasquez-Kool (2019). Similarly, SE of and coheritability estimates were calculated as described by Vasquez-Kool (2019) and estimates of coheritability were declared significant as described above.

2.3.4 | Structural equation modeling

Model assumptions

Six elements were emphasized in the validation of path analysis and causal mediation models: (a) no skewness or kurtosis in the joint distribution of the variables, (b) all variables were continuous, (c) there were negligible (i.e., < .05) missing data, (d) there was only first-order relationships between variables, (e) there was no multi-collinearity among the independent variables, and (f) residuals of all covariances were small and centered around zero. In addition, every mediation variable (and their residual terms) was assigned a scale as described by Yung (2010).

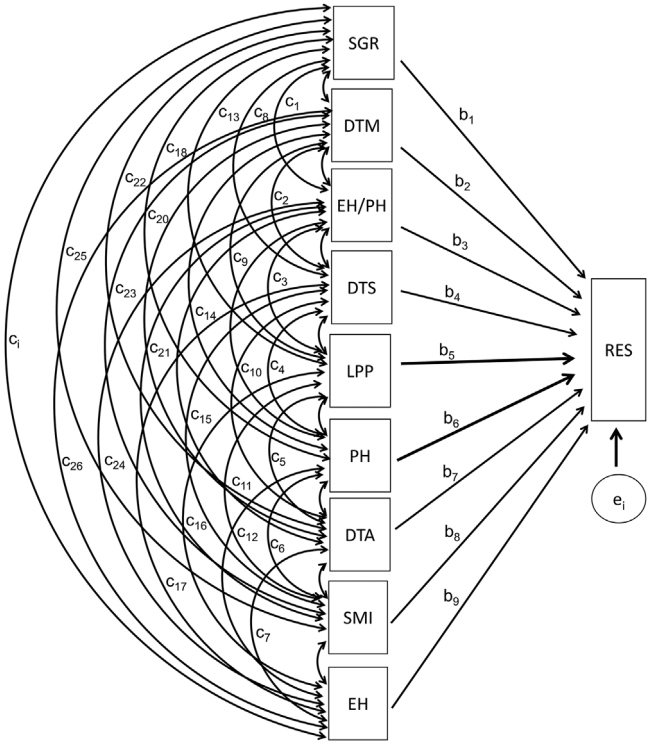

FIGURE 1 Simple path diagram depicting the relationship between gray leaf spot (GLS) resistance and agronomic traits for 48 elite maize inbred lines artificially inoculated with *Cercospora zeina* and evaluated across nine environments in western Kenya. Parameters b_1 to b_9 are path coefficients for the relationship between pertinent agronomic traits and resistance to GLS; c_1 to c_3 are covariance values between pertinent exogenous variables; and e_i represents the errors associated with measurement of pertinent variables

Variable specification

To avoid bias in selection of predictor variables included the causal mediation analysis, relationships between SAUDPC and agronomic traits were pretested using best-subsets regression implemented with PROC REG (SAS Institute, 1999). Out of the 2^{10} possible SAUDPC prediction models, only agronomic traits within the nine-predictor-variable model with the largest chi-square score were advanced to causal mediation analysis. Based on this criterion, EPP and anthesis–silking interval were excluded from the structural equation analysis.

Basic path analysis model specification

The basic path model was designed based on the hypothesis that all agronomic variables retained after the best-subsets regression significantly affected partial resistance to GLS. The hypothesized paths and in the basic multiple regression model (Figure 1) were set up by entering each selected agronomic trait with resistance (i.e., inverse of SAUPDC) using the RAM syntax of PROC CALIS.

Causal mediation model specification

Conceptual framework for the tentative causal mediation model (Figure 2) was based on a mixture of intuition, established theory, known aspects of maize phenology, and the temporal sequence of measurement of the retained agronomic variables. For instance, EH/PH is computed after measurement of EH and PH. Thus, EH/PH was presumed to mediate effects of EH and PH. Similarly, SMI is the difference between DTS and DTM. Thus, SMI was the presumed mediator of the effects of DTS and DTM. Previous studies have reported that flowering of maize terminates internode elongation, increase in PH, and production of new leaves (Fournier & Bruno, 2000). Thus, DTA preceded PH, EH, and LPP. Other studies have shown that the duration of vegetative growth in maize affects SGR (Bekavac et al., 2007). Thus, DTA was hypothesized to be a predictor of SGR since it marks truncation of the vegetative phase of growth. In addition, increased number of leaves has previously been linked to low placement of the primary ear in maize (Muirhead & Shaver, 1985). Thus, LPP was regarded as an additional predictor of EH/PH. Contribution of exogenous variables to prediction of resistance, computed as the inverse of SAUDPC, was tested by principal component analysis using PROC PRINCOMP. To optimize model parsimony, only exogenous variables that predicted resistance with eigenvalues > 1.0 and collectively accounted for under 90% of the total variation in resistance were included as mediators. Based on these criteria, only DTS and DTM were retained as direct predictors of resistance (Figure 2).

Structural equations corresponding to hypothesized causal mediation model were transcribed directly in the LINEQS statement of PROC CALIS in SAS by entering the exogenous (DTA, DTS, and DTM) and endogenous (EH, EH/PH, PH, LPP, SGR, and SMI) variables with resistance as the response variable. Precision of path coefficient estimates was optimized by entering observed values of exogenous variables into the mediation path model as the true score plus a measurement error term attributed to variation in accuracy of measurements (Yung, 2010). This approach was formalized by the function, $f(\phi) = \phi + e_\phi$, to create a latent predictor variable $f(\phi)$, which is the true, but unobserved score that equals the observed score, ϕ, plus a presumed measurement error, e_ϕ. To minimize risk of under-identification, some error covariances and variances were constrained using the PCOV and PVAR statements of PROC CALIS as described by Yung (2010).

Model estimation and evaluation

Standardized model parameters and accompanying statistics for statistical validation based on the default maximum likelihood method were subsequently estimated using PROC CALIS. To limit complication of the tentative causal mediation model, only covariance parameters justified by theoretical or substantive logic were added to the model. For

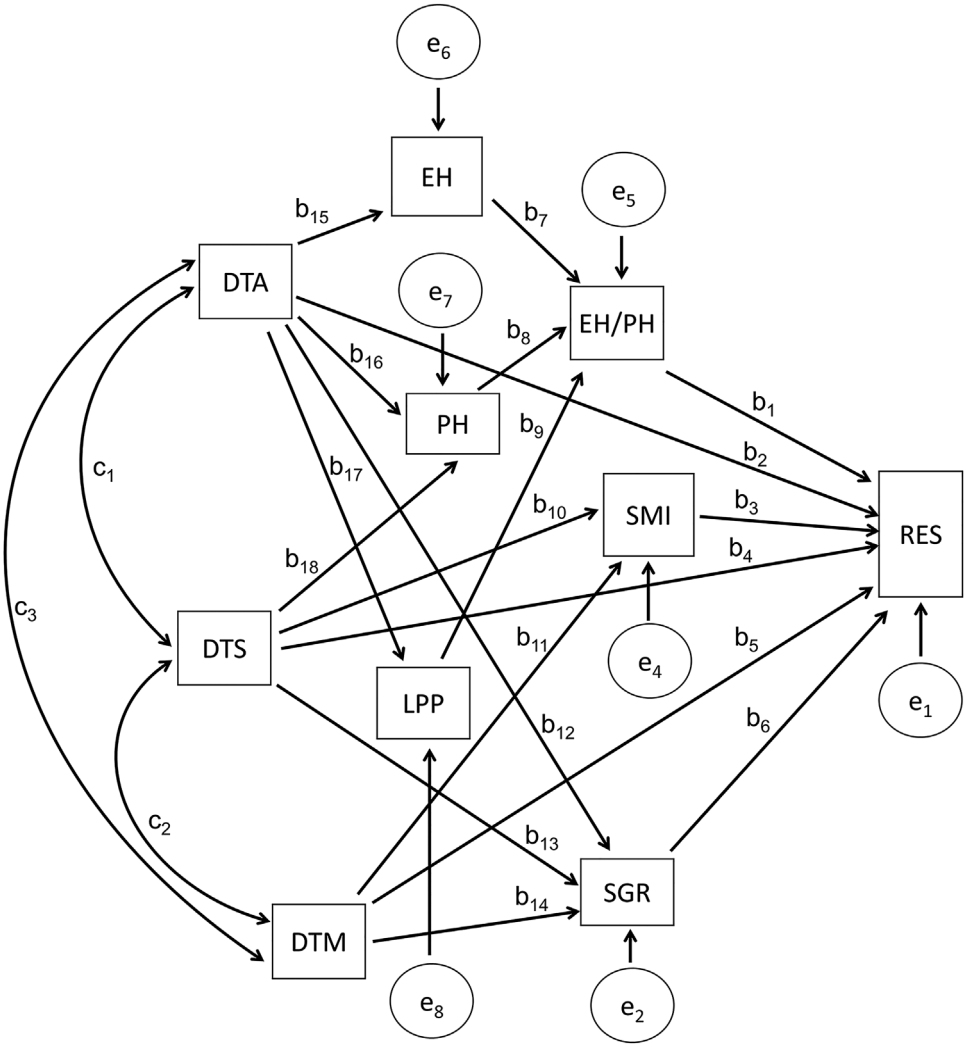

FIGURE 2 Tentative mediation path diagram of the relationship between gray leaf spot (GLS) resistance and agronomic traits for 48 elite maize inbred lines artificially infected with *Cercospora zeina* and evaluated across nine environments in western Kenya. RES, resistance computed as inverse of standardized area under disease progress curve (i.e., RES = 1/SAUDPC); PH, plant height; EH, ear height; EH/PH, ear height relative to plant height; DTA, days to anthesis; DT, days to silking; DTM, days to maturity; LPP, number of leaves per plant; SMI, silking-physiological maturity interval; SGR, stay-green characteristic. Parameters b_1 to b_9 are path coefficients for the relationship between pertinent agronomic traits and resistance to GLS; c_1 to c_3 are covariance values between pertinent exogenous variables; and e_1 to e_8 are errors associated with measurement of pertinent variables.

example, DTA and DTS are assumed to have a part of their correlation beyond their common latent origins. As such, this "extra" correlation was conceptualized in the model as a correlation (or covariance) between the errors of the two variables represented by a double-headed arrow connecting the two variables as shown in the path diagram. Similarly, QTL associated with flowering of maize and PH are pleiotropic and height-related traits are genetically correlated with flowering of maize (Cui et al., 2017). As such, PH and EH were connected to DTA by double arrows to represent covariance of their errors. The logic justified path specifications that included correlated and explicit error parameters (Figure 2).

To assess the overall performance of the path model, the ON(PNLY) option in the FITINDEX Statement of PROC CALIS was used to customize output of model fit summary with only the five most useful statistics; (a) adjusted goodness-of-fit index (AGFI), (b) Akaike's information criterion (AIC), (c) Bentley's comparative fit index (BCFI), (d) root mean squared error approximation (RMSEA), and (e) mean square residual (SRMSR). Small SRMSR and RMSEA (i.e., < 0.05) and large AGFI and BCFI (i.e., > 0.9) values were deemed indicators of good model fit. The AIC values were compared to determine which of the multiple models best balanced model for the considered dataset, with smaller values indicating better fit and a model with a ΔAIC (the

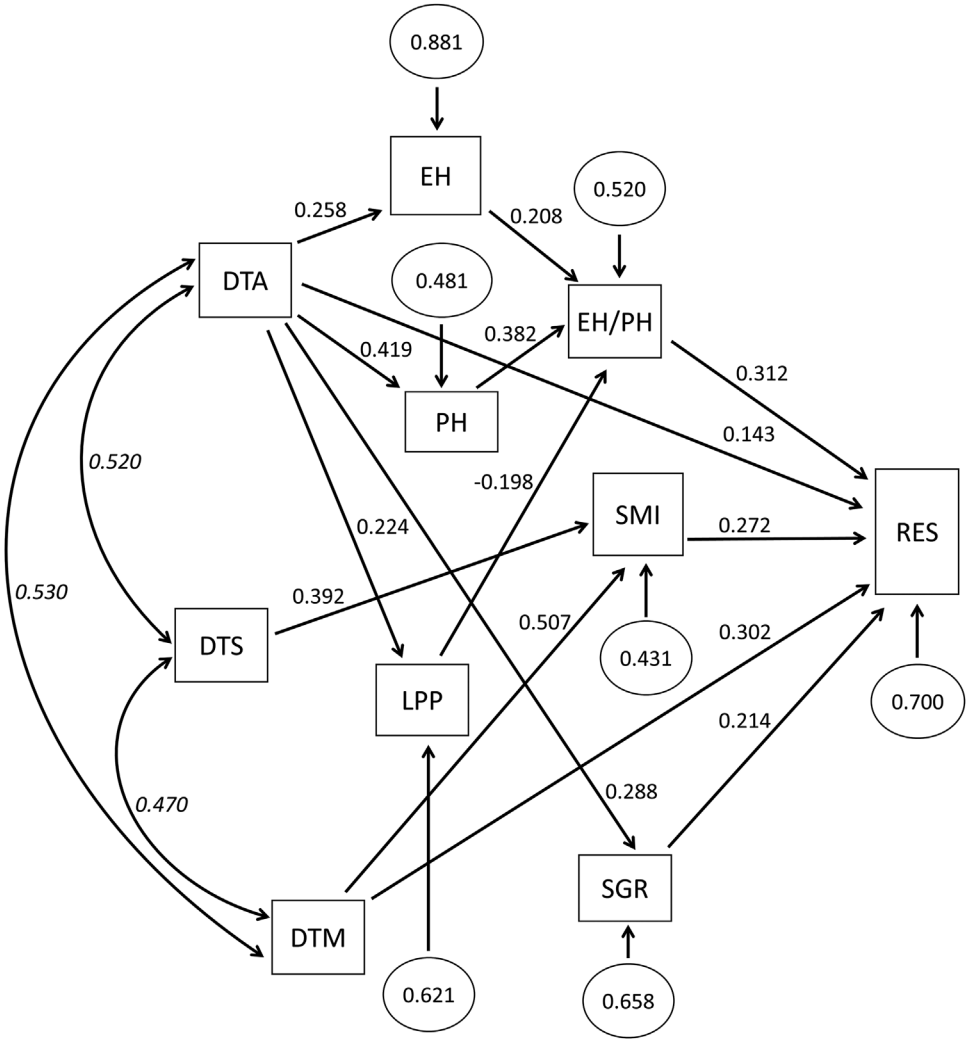

FIGURE 3 Revised mediation path diagram of the relationship between gray leaf spot (GLS) resistance and agronomic traits for 48 elite maize inbred lines artificially infected with *Cercospora zeina* and evaluated across nine environments in western Kenya. RES, resistance computed as inverse of standardized area under disease progress curve (i.e., RES = 1/SAUDPC); PH, plant height; EH, ear height; EH/PH, ear height relative to plant height; DTA, days to anthesis; DT, days to silking; DTM, days to maturity; LPP, number of leaves per plant; SMI, silking-physiological maturity interval; SGR, stay-green characteristic. Romanized values are path coefficients for the relationship between pertinent agronomic traits and resistance to GLS; italicized values are covariance values between pertinent exogenous variables; Romanized values within ovals are errors associated with measurement of pertinent variables

difference between the two AIC values being compared) >2 being deemed significantly better.

Model modification

Only the CMA model was modified with initial fit statistics. To test potential for improvement of model fit and guide model modification, Lagrange multiplier test indices and Wald statistics were extracted using the MODIFICATION option of PROC CALIS. Model modification was then performed by: (a) by freeing parameters fixed a priori (b) by fixing parameters freed in the tentative model as described elsewhere (SAS Institute, 1999), and/or (c) by omitting parameters whose estimates were not significant ($\alpha = .05$) from the revised model (Figure 3).

3 | RESULTS

3.1 | Disease assessment variable to quantify GLS resistance

Disease severity across all the environments ranged from 57% recorded in E3 to 70% that was recorded in E7 (Figure 4). Environments E1, E2, E7, E8, and E9 were highly favorable (i.e., severity >65%) for disease development, whereas E3, E4, E5, and E6 were moderately favorable (50% > severity ≤ 65%) to disease development.

Of the six disease variables used to quantify resistance, SAUDPC explained the greatest proportion of the variation (R^2 = 89.4%) in the ANOVA model. Among other factors

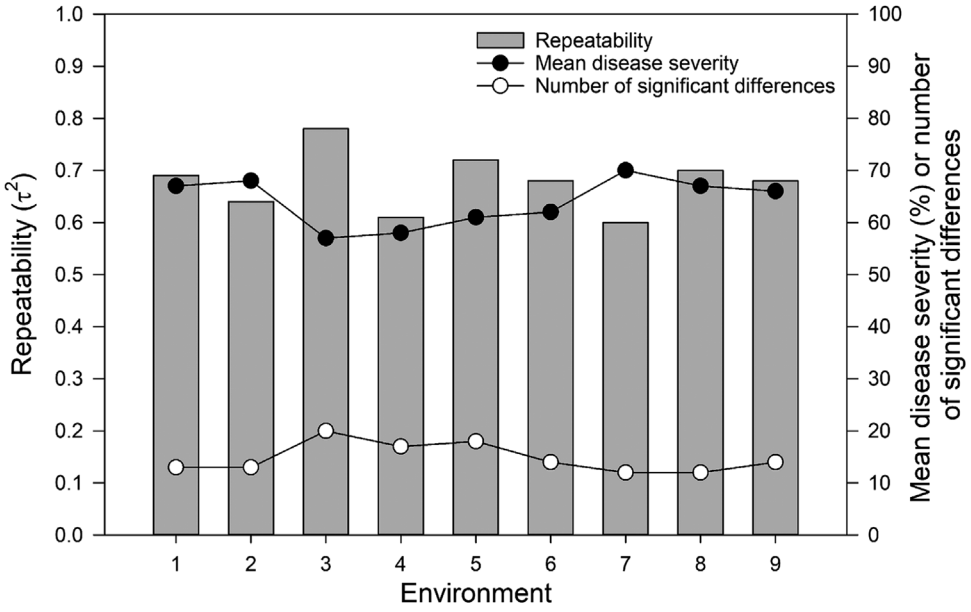

FIGURE 4 Estimates of repeatability, mean disease severity and number of significant differences among standardized area under the disease progress curve scores of 48 elite maize inbred lines artificially infected with *Cercospora zeina* and evaluated across nine environments in western Kenya. Mean disease severity is the average gray leaf spot severity across all inbred lines in each environment.

(Nyanapah et al., 2020), SAUDPC was thus selected as the primary disease variable for subsequent evaluation of the relationship between GLS resistance and agronomic traits.

3.2 | Repeatability estimates

Estimates of repeatability (τ^2) ranged from .09 to .82 depending on the environment (Table 2). Among disease variables, τ^2 were lowest for LP and highest for SAUDPC and CDSG (τ^2 = .68) across environments. Estimates of repeatability were significantly different from zero for all disease variables except for LP. Among agronomic traits, estimates were lowest for SGR (τ^2 = .35) and highest for PH (τ^2 = .77) across environments (Table 2).

Comparison of τ^2 across environments showed that lower estimates were more common in environments that exhibited lower rather that higher within-trial variability of SAUDPC (Figure 4). For example, τ^2 were lower in E2 and E7, which also had a corresponding lower number of significant differences among entries. In contrast, τ^2 was highest in E3 and E5, which also had the highest number of significant differences in SAUDPC values among entries. In general, high τ^2 for SAUDPC were observed in environments that were moderately favorable for disease development (i.e., intermediate mean severity), such as E5 and E6 (Figure 4). However, the highest τ^2 for SAUDPC was observed in E3, which had the broadest range of disease severity among inbred lines, even though this environment had the lowest mean disease severity. The lowest τ^2 for SAUDPC was observed in E7, which had the highest mean disease severity but less within-environment variability of inbred disease severity (Figure 4).

3.3 | Broad-sense heritability estimates

Estimates of broad-sense heritability (H^2) of GLS severity and agronomic traits pooled over all environments were all significantly ($P \leq .05$) different from zero except for LP and ranged from .22 (for $PDLA_{IP}$) to .78 (for LPP; Table 3). Among the disease variables, H^2 were high (i.e., >.65) for SAUDPC and FPDLA but low (i.e., ≤.45) for $PDLA_{IP}$ and LP. Similarly, among agronomic traits, H^2 were high (≥.70) for LPP and PH and low (≤.47) for EH and SGR.

Estimates of H^2 were also affected by the how favorable trial environments were to disease development. For example, H^2 increased when data were pooled over three environments with moderate levels of disease (i.e., E3, E4, and E5) for all traits except FPDLA, $PDLA_{IP}$, EH/PH, DTS, and SMI. In contrast, H^2 decreased for all traits except SAUDPC and ρ, when data were pooled over three environments most favorable for disease development (i.e., E1, E2, and E7; Table 3). Regardless of the favorability of environmental conditions for disease development, H^2 remained consistently higher for SAUDPC than FPDLA among disease variables and consistently higher for LPP than PH among agronomic traits evaluated (Table 3).

TABLE 2 Estimates of repeatability for gray leaf spot (GLS) resistance assessment and agronomic traits for elite maize inbred lines artificially infected with *Cercospora zeina* in nine environments in western Kenya

Variable	Repeatability in environment[a]									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	Mean
GLS assessment[b]										
SAUDPC	.69*	.64*	.78*	.61*	.72*	.68*	.60*	.70*	.68*	.68
ρ	.56*	.59*	.62*	.53*	.61*	.57*	.55*	.59*	.55*	.57
FPDLA	.63*	.70*	.69*	.61*	.59*	.73*	.68*	.68*	.61*	.66
CDSG	.68*	.70*	.66*	.67*	.75*	.64*	.71*	.68*	.59*	.68
$PDLA_{ip}$	.61*	.57*	.62*	.48*	.60*	.47*	.51*	.47*	.59*	.55
LP	.17	.24*	.15	.21	.18	.23	.12	.09	.12	.17
Agronomic trait[c]										
SGR	.38*	.35*	.32*	.34*	.41*	.37*	.33*	.29*	.32*	.35
DTM	.49*	.43*	.46*	.45*	.38*	.38*	.46*	.35*	.40*	.42
EH/PH	.51*	.52*	.57*	.56*	.59*	.57*	.55*	.53*	.43*	.54
DTS	.45*	.47*	.45*	.54*	.46*	.54*	.42*	.47*	.50*	.48
LPP	.64*	.65*	.56*	.62*	.66*	.68*	.64*	.59*	.63*	.63
PH	.82*	.74*	.82*	.74*	.75*	.78*	.76*	.69*	.81*	.77
DTA	.69*	.74*	.67*	.66*	.70*	.68*	.67*	.69*	.64*	.68
SMI	.60*	.65*	.59*	.66*	.57*	.66*	.55*	.54*	.59*	.60
EH	.57*	.58*	.48*	.51*	.53*	.49*	.56*	.47*	.48*	.52

Note. Means are average of repeatability estimates across all environments.

[a]E denotes the serial number of environment and specific details of the environments are presented in Table 1.

[b]SAUDPC is standardized area under disease progress curve; ρ is the weighted mean absolute rate of disease increase; FPDLA is final percent diseased leaf area; CDSG is CIMMYT (1985) disease severity grade; $PDLA_{ip}$ is percent diseased leaf area at inflection point; and LP is latent period.

[c]SGR is the Stay-green characteristic; DTM is days to maturity; EH/PH is ear height relative to plant height; DTS is days to silking; LPP is number of leaves per plant; PH is plant height; DTA is days to anthesis; SMI is silking-physiological maturity interval; and EH is ear height.

*Significant at the .05 probability level.

3.4 | Phenotypic correlation and components of covariance estimates

Absolute values of coefficients of phenotypic correlation of SAUDPC with agronomic traits ranged from .16 (with EH) to .84 (with SGR) with different levels of statistical significance (Table 4). Among the disease variables examined, phenotypic correlation of SAUDPC was strongest with CDSG ($r = .60$; $P < .01$) and weakest with LP ($|r| = .19$; $P > .05$). Out of the agronomic traits examined, the strongest correlation was between SGR and CDSG ($|r| = .96$; $P < .01$), whereas the weakest correlation was between EH and LP ($r = .02$; $P > .05$; Table 4).

Phenotypic correlations between SAUDPC and agronomic traits were all negative ranging from −.84 to −.28, and significant ($P < .01$) except with EH ($r = -.16$; $P > .05$). In contrast, phenotypic correlations between LP and agronomic traits were all positive, weak ($.02 \leq r \leq .21$), and nonsignificant. Phenotypic correlations of the remaining disease assessment variables with all agronomic traits followed the same trends as those between SAUDPC and agronomic traits but with slightly lower coefficients except for correlation of FPDLA and CDSG with SGR, where coefficients were much higher ($r = -.92$ and $r = -.96$, respectively; Table 4). Estimates of phenotypic components of covariance between SAUDPC and agronomic traits ranged from −127.6 to −0.78 (Supplemental Table S3).

3.5 | Genotypic correlation estimates

Absolute values of the coefficients of genotypic correlations were generally larger than their corresponding phenotypic values and ranged from .05 to .89 (Table 4). Further, the strength and direction of genotypic correlations were broadly similar to the trends observed for estimates of phenotypic correlations. For example, genotypic correlations between SAUDPC and all the other traits ranged from −.16 (with EH) to −.87 (with SGR) with all these values being significant ($P < .05$) except for SAUDPC with LP and EH (Table 4). Negative, moderate (−.31) to strong (−.87), and significant ($P < .05$) genotypic correlations were observed between SAUDPC and all agronomic traits except with EH ($r = -.16$; $P > .05$). In contrast, genotypic correlations between LP and agronomic traits were all positive, weak, and nonsignificant except those with SGR, where coefficient was the strongest and significant ($r = .42$; $P < .01$).

TABLE 3 Mean, standard error (Std error), and estimates of broad-sense heritability (H^2) for gray leaf spot (GLS) resistance assessment and agronomic traits for 48 elite maize inbred lines artificially infected with *Cercospora zeina* in western Kenya

Variable	Mean	Std error	H^2[a]		
			Pooled over all 9 environments	Pooled over 3 least GLS favorable environments	Pooled over 3 most GLS favorable environments
GLS assessment[a]					
SAUDPC	51.7	8.4	.67*	.75*	.72*
ρ	0.01	0.10	.56*	.62*	.59*
FPDLA	63.2	10.3	.69*	.67*	.59*
CDSG	2.2	0.4	.64*	.69*	.61*
$PDLA_{IP}$	37.4	4.4	.22*	.17*	.13*
LP	23.6	4.9	.33*	.41*	.31*
Agronomic trait[b]					
SGR	95.3	9.8	.45*	.51*	.43*
DTM	0.35	0.02	.52*	.53*	.45*
EH/PH	60.1	5.7	.47*	.42*	.38*
DTS	12.6	1.1	.57*	.55*	.46*
LPP	104.0	11.3	.78*	.80*	.67*
PH	51.5	5.6	.70*	.69*	.64*
DTA	32.1	2.9	.61*	.62*	.56*
SMI	36.4	3.7	.51*	.49*	.42*
EH	95.3	9.8	.45*	.51*	.43*

[a]E3, E4, and E5 were the three moderately favorable environments, whereas E1, E2, and E7 (See Table 1) were the three most favorable environments for disease development.

[b]SAUDPC is standardized area under disease progress curve; ρ is the weighted mean absolute rate of disease increase; FPDLA is final percent diseased leaf area; CDSG is CIMMYT (1985) disease severity grade; $PDLA_{IP}$ is percent diseased leaf area at inflection point; and LP is latent period.

[c]SGR is the Stay-green characteristics; DTM is days to maturity; EH/PH is ear height relative to plant height; DTS is days to silking; LPP is number of leaves per plant; PH is plant height; DTA is days to anthesis; SMI is silking-physiological maturity interval; and EH is ear height.

*Significant at the .05 probability level.

Genotypic correlations of the remaining disease variables with agronomic traits followed the same trends as those between SAUDPC and agronomic traits but were slightly lower except for correlation of FPDLA with DTM and LPP for which the coefficients were much higher ($r = -.65$ and $r = -.42$, respectively; Table 4). The strongest genetic correlation between disease variables and agronomic traits was observed between SAUDPC and SGR ($|r| = .87$; $P < .01$), whereas the weakest correlation was between LP and SMI ($r = .05$; $P > .05$; Table 4). Among disease variables, coefficients of genotypic correlation were strongest between SAUDPC and ρ ($r = .71$; $P < .01$) and weakest between $PDLA_{IP}$ and LP ($|r| = .18$; $P > .05$). Genotypic components of covariance involving SAUDPC were strongest with DTM (estimate = −77.90) and weakest with EH (estimate = −4.62) (Supplemental Table S3).

3.6 | Coheritability estimates

Trends in coheritability followed a pattern similar to those of phenotypic and genetic correlations, with coheritability of SAUDPC with agronomic traits being negative and significant except for coheritability of SAUDPC with EH and LPP (Table 5). Coheritability values involving LP and agronomic traits were all positive and nonsignificant. Similarly, coheritability of ρ, FPDLA and CDSG with SGR while negative, were nonsignificant. The highest estimate involving SAUDPC occurred with DTA ($|C_{h_{xy}}| = 0.46$; $P < .05$), whereas the lowest was between LP and LPP ($C_{h_{xy}} = 0.09$; $P > .05$). The disease variable that had the strongest $C_{h_{xy}}$ with a given agronomic trait varied within the study. For example, coheritability of SAUDPC were strongest with DTA ($|C_{h_{xy}}| = 0.46$; $P < .05$), whereas those of ρ were strongest with SMI ($|C_{h_{xy}}| = 0.44$; $P < .05$; Table 5). Similarly, absolute values of coheritability of FPDLA were strongest with DTM ($|C_{h_{xy}}| = 0.48$; $P < .05$), whereas those of ρ were strongest with SMI and DTM ($|C_{h_{xy}}| = 0.43$; $P < .05$) (Table 5).

3.7 | CMA model fit indices

The adjusted goodness-of-fit for the revised CMA model was higher ($R^2 = 89.2\%$) than that of either the basic path analysis model ($R^2 = 81.9\%$) or the tentative CMA model ($R^2 = 45.3\%$;

TABLE 4 Estimates of coefficients of phenotypic (above diagonal) and genotypic (below diagonal) correlations among six gray leaf spot resistance assessments and agronomic traits for 48 inbred lines artificially infected with *Cercospora zeina* across nine environments in western Kenya

	Disease variable[a]						Agronomic trait[b]								
	SAUDPC	**ρ**	**FPDLA**	**CDSG**	**$PDLA_{IP}$**	**LP**	**SGR**	**DTM**	**EH/PH**	**DTS**	**LPP**	**PH**	**DTA**	**SMI**	**EH**
SAUDPC	…	.45**	.49**	.60**	.38**	−.19	−.84**	−.58**	−.49**	−.45**	−.34**	−.31*	−.29*	−.28*	−.16
ρ	.71**	…	.55**	.48*	.58*	−.10	−.69**	−.48*	−.42**	−.38*	−.27*	−.27*	−.32*	−.28*	−.14
FPDLA	.66**	.60**	…	.65**	.48**	−.25*	−.92**	−.61**	−.36*	−.47**	−.39**	−.24*	−.24*	−.31**	−.14
CDSG	.59**	.52**	.70**	…	.42**	−.20	−.96**	−.52**	−.34*	−.49**	−.22*	−.18	−.29*	−.25*	−.08
$PDLA_{IP}$	.43**	.60**	.51*	.46*	…	−.16	−.55**	−.46*	−.37*	−.34*	−.19	−.21	−.19	−.24**	−.11
LP	−.21	−.21	−.30*	−.32*	−.18	…	.21	.18	.12	.12	.04	.08	.04	.03	.02
SGR	−.87**	−.76**	−.81**	−.75**	−.57*	.42*	…	.47	.54*	.24*	.46*	.26*	.51*	.31**	.42**
DTM	−.60**	−.52*	−.65**	−.52**	−.47**	.18	.59**	…	.18	.47**	.38*	.69**	.53**	.82**	.43**
EH/PH	−.52**	−.52*	−.37*	−.42*	−.41**	.13	.63**	.18	…	.31*	−.19	.28*	.56	.32*	.61**
DTS	−.47**	−.41*	−.49**	−.50**	−.41**	.16	.45**	.61**	.36*	…	.40*	.44**	.52**	.58**	.31*
LPP	−.35*	−.28*	−.42**	−.23*	−.37*	.08	.07	.68**	−.12	.52**	…	.35*	.40*	.91**	.23*
PH	−.33**	−.28*	−.25*	−.19	−.25*	.16	.28*	.51**	.29*	.76**	.58**	…	.68**	.58**	.38**
DTA	−.30*	−.34**	−.25*	−.30**	−.32*	.09	.57*	.69**	.53**	.61**	.40*	.80**	…	.52**	.18
SMI	−.31*	−.30*	−.32*	−.25*	−.28**	.05	.38**	.52**	.36*	.67**	.89**	.62**	.64**	…	.28*
EH	−.16	−.15	−.15	−.08	−.18	.07	.49*	.52*	.77**	.32*	.28*	.51**	.22*	.31*	…

[a]SAUDPC is standardized area under disease progress curve; LP is latent period; ρ is the weighted mean absolute rate of disease increase; CDSG is CIMMYT (1985) disease severity grade; $PDLA_{IP}$ is percent diseased leaf area at inflection point, and FPDLA is final percent diseased leaf area.

[b]PH is plant height; EH is ear height; EH/PH is ear height relative to plant height; DTA is days to anthesis; DT is days to silking; DTM is days to maturity; LPP is number of leaves per plant; SMI is silking–physiological maturity interval, and SGR is the stay-green characteristic.

*Significant at the .05 probability level. **Significant at the .01 probability level.

TABLE 5 Estimates of coheritability of gray leaf spot severity resistance assessment with agronomic traits among 48 elite maize inbred lines artificially infected with *Cercospora zeina* across nine environments in western Kenya

	Disease assessment variable[b]					
Agronomic trait[a]	SAUDPC	ρ	FPDLA	CDSG	$PDLA_{IP}$	LP
SGR	−.29*	−.14	−.17	−.14	−.27*	.16
DTM	−.40**	−.42**	−.48**	−.43**	−.30*	.20
EH/PH	−.43**	−.30*	−.43**	−.33*	−.29*	.19
DTS	−.42**	−.37*	−.44**	−.36**	−.28*	.12
LPP	−.21	−.31*	−.38*	−.25*	−.27*	.09
PH	−.39*	−.39*	−.40*	−.33*	−.22*	.19
DTA	−.46**	−.38*	−.43**	−.41*	−.29*	.20
SMI	−.40**	−.44**	−.41*	−.43*	−.35*	.23
EH	−.20	−.19	−.18	−.20	−.16	.18

[a]SGR is the Stay-green characteristics; DTM is days to maturity; EH/PH is ear height relative to plant height; DTS is days to silking; LPP is number of leaves per plant; PH is plant height; DTA is days to anthesis; SMI is silking–physiological maturity interval; and EH is ear height.

[b]SAUDPC is standardized area under disease progress curve; ρ is the weighted mean absolute rate of disease increase; FPDLA is final percent diseased leaf area; CDSG is CIMMYT (1985) disease severity grade; $PDLA_{IP}$ is percent diseased leaf area at inflection point; and LP is the latent period.

*Significant at the .05 probability level. **Significant at the .01 probability level.

TABLE 6 Estimates of goodness of fit indices from basic path analysis, tentative causal mediation, and revised causal mediation modeling of association of agronomic traits with partial resistance to gray leaf spot among 48 maize inbred lines evaluated across nine environments in western Kenya

	Model configuration		
Fit statistic[a]	Basic path model (Figure 1)	Tentative causal mediation model (Figure 2)	Revised causal mediation model (Figure 4)
AGFI	0.819	0.453	0.892
AIC	27.944	30.864	23.621
BCFI	0.689	0.784	0.906
RMSEA	0.066	0.011	0.030
SRMSR	0.035	0.042	0.042

[a]AGFI is adjusted goodness of fit; AIC is Akaike information criterion; BCFI is Bentler's comparative fit index; RMSEA is root mean square error of approximation; and SRMSR is standardized root mean square residual.

Table 6). In addition, model fit statistics indicated a better fit to the data for the revised causal model (AIC = 23.6) than either the basic path model (AIC = 27.9) or the tentative model (AIC = 30.8). Similarly, BCFI values were substantially higher for the revised model than either the basic path or tentative causal model (Table 6). Further, errors for the revised CMA model (RMSEA < 0.05) also indicated good fit to the data. Thus, the revised CMA model (Figure 3) was used to determine the effect of exogenous and endogenous agronomic traits on disease resistance.

Exogenous agronomic traits, DTA (P = .0322) and DTM (P = .0022) had a significant positive direct effect on disease resistance (Supplemental Table S4), with the effects of DTM (estimate = .302) being about two times higher than the effect of DTA (estimate = .143; Table 7). While the exogenous variable DTS had a significant positive genetic correlation with SAUDPC (Table 4), its direct effect on resistance was not significant (P = .6353) in the revised causal mediation model (Table 7). Within the revised model, the effects of endogenous variables mediated the effects of exogenous variables or other endogenous variables. For example, the endogenous variable SMI significantly mediated the effects of DTS (P = .0042) and DTM (P = .0494) on disease resistance (Table 7). Similarly, the endogenous variable SGR significantly (P = .0223) mediated the effects of DTA on disease resistance. The effects of the endogenous variables EH, PH, and LPP were all mediated by EH/PH. However, the sum of indirect effects of DTA through these endogenous variables (estimate = .081) was slightly less than its direct and/or unmediated effect (estimate = .143) on disease resistance. While an increase in DTA increased LPP, most of the additional leaves were produced above the ear, thus decreasing EH/PH. Overall, exogenous effects on resistance were mainly driven by their indirect effects, particularly those mediated by EH/PH and SMI. However, the direct effect of DTM on resistance was nearly the same as the cumulative indirect effects of all endogenous variables (Table 7).

4 | DISCUSSION

4.1 | Consistency of partial resistance measurements

Estimates of repeatability show the reliability of phenotype prediction based on previous measurements and set the upper bounds of heritability (Falconer & Mackay, 1996). In

TABLE 7 Standardized estimates of direct and indirect effects of agronomic traits on gray leaf spot resistance from revised causal mediation modeling of association of agronomic traits with partial resistance (RES) among 48 maize inbred lines evaluated across nine environments in western Kenya. Std error, standard error

Path[a]	Effect[a]	Estimate	Std error	*t*-value	*P*-value
DTA ⟶ RES	Total	.158	0.015	4.275	.0042
	Direct	.143	0.013	4.252	.0322
	Indirect (sum)	.115	0.014	2.863	.0524
	via EH and EH/PH	.017	0.020	7.436	.0051
	via PH and EH/PH	.050	0.017	5.788	.0064
	via LPP and EH/PH	−.014	0.002	4.078	.0141
	via SGR	.062	0.015	4.594	.0223
DTS ⟶ RES	Total	.146	0.016	8.042	.0080
	Direct	.000	0.000	0.000	.6353
	Indirect via SMI	.146	0.014	6.699	.0042
DTM ⟶ RES	Total	.409	0.015	1.886	.0931
	Direct	.302	0.017	6.956	.0022
	Indirect via SMI	.137	0.013	3.640	.0494

[a]DTA is days to anthesis; DTS is days to silking; DTM is days to maturity; EH is ear height; PH is plant height; EH/PH is ear height relative to plant height; LPP is number of leaves per plant; SGR is the stay-green characteristic; and SMI is silking-physiological maturity interval. Exogenous variables are DTA, DTS, and DTM, whereas endogenous variables are EH, EH/PH, PH, LPP, SGR and SMI. RES = resistance computed as inverse of standardized area under disease progress curve (i.e., RES = 1/SAUDPC).

this study, we observed moderate to high repeatability of SAUDPC, CDSG, and FPDLA (Table 2). Thus, these disease variables have higher heritability compared with other disease assessment variables. The lower repeatability of LP relative to other disease variables suggests that uncharacterized random perturbations of environmental variations had stronger influence on phenotypes of LP. Thus, genotype × environment interactions resulted in a weaker correlation between LP across environments. Often, the precise measurement of highly repeatable traits improves only marginally with repeated measurements. However, the accuracy of traits with low repeatability is usually greatly increased with multiple measurements. Thus, few duplicated measurements should be sufficient for assessment of SAUDPC, but delineation of inbred lines based on LP would require more trial environments or replications per environment. The highest repeatability of SAUDPC occurred in an environment where inbred reactions to GLS were most diverse (Figure 4) rather than in an environment with the largest mean disease severity (Table 2). Thus, phenotypic variation was more responsive to the range of GLS severity among inbred lines than to the average GLS severity pooled over all inbreds. Consequently, environments with broader entry reactions to GLS should be more appropriate for evaluation of genotypes those with the largest overall GLS severity. This is probably because the former allows for a more accurate rating of resistance and increased detection of significant differences between genotypes.

4.2 | Phenotypic correlates of resistance to GLS

The weak phenotypic correlation between resistance and PH observed in this study was somewhat unexpected since biotic or abiotic stress is expected to decrease plant height. This observation suggests that covariance of resistance with PH is driven by physiological mechanisms activated by genetic and/or environmental cues not associated with PH. The significant negative correlation of SAUDPC with DTA as well as EH/PH (Table 4) indicates that genotypes that shed pollen later or those whose ears were relatively farther from the ground than they were from the tassel were more resistant and vice-versa. Similar findings were reported by Menkir and Ayodele (2005). However, DTA and EH/PH are likely to be less useful predictors of resistance given the weak to moderate correlation of these traits with GLS severity. Nonetheless, this finding partly suggests that partitioning and/or remobilization of photo-assimilates for plant defense is likely to increase with increased EH and EH/PH, since leaves below the ear contribute relatively less to grain fill than those above the ear (Subedi & Ma, 2005).

In this study, the significant negative phenotypic correlations of SAUDPC with DTM, DTA, and DTS are inconsistent with findings by Balint-Kurti et al. (2008), but are in general agreement with other reports (Pozar et al, 2009; Zwonitzer et al., 2010). The increase in GLS severity with increased plant earliness could be due to either: (a) termination of

disease intensification by premature plant senescence, (b) concurrent response of both traits to similar environmental cues, or (c) regulation of the two traits by identical or related genetic systems. The likelihood of the first explanation is supported by increased transcription of factors associated with senescence following exposure of plants to environmental stress (Robatzek & Somssich, 2002). However, the strong negative correlation of SAUDPC with SGR suggests that undesirable SGR ratings may confound the expression of stress-accelerated plant senescence. The second explanation is based on previous association of covariance between genetically unrelated traits with environmental correlation, particularly if such traits have low estimates of heritability (Conner & Hartl, 2004). The third explanation corresponds to pleiotropy and/or linkage of the genes (Hallauer et al., 2010) and is the default argument whenever phenotypic correlations closely mirror their genetic counterparts in traits with high heritability. While we did not specifically estimate narrow sense heritability of the agronomic traits without GLS infection in this study, broad-sense heritability estimates of the examined traits were noticeably moderate to high (Table 3). Further, heritability of resistance to GLS and several aspects of maize phenology has been rated as moderate to high (Gordon et al., 2006; Hallauer et al, 2010).

4.3 | Genotypic correlates of resistance to GLS

Absolute values of nearly all coefficients of genotypic correlation were numerically larger than the corresponding phenotypic correlations. This suggests that the latter could be surrogate measures of genetic correlation of GLS with agronomic traits. Thus, there is no need for elaborate mating designs to generate genotypes with known family structures for estimation of genetic correlation, which makes sampling of large sets of genetic materials unnecessary (Holland, 2006). In addition, the weaker covariance of genotype × environment and error effects suggest that genetic mechanisms underlying resistance and the examined agronomic traits are largely similar and, thus, resistance should respond to selection targeting the agronomic traits. This observation could be due to (a) low heritability of one or both of the correlated traits, (b) regulation of the physiological pathways conditioning the correlated traits by similar genetic and environmental factors, or (c) insufficient sample size and, hence, large sampling errors typical of estimates of genetic correlation (Cheverud, 1988). The first hypothesis is less likely since due to the high estimates of H^2 for SAUDPC (Table 3). Although the second and third propositions cannot be directly tested based on design of the present study, large differences between genotypic and phenotypic correlations can be caused by both phenomena (Cheverud, 1988).

The strongest genetic correlations were recorded between SAUDPC and SGR, followed by those between SAUDPC and DTM and EH/PH (Table 4). Other studies have suggested that similar genetic mechanisms condition GLS resistance and some agronomic traits in maize (Clements et al., 2000; Menkir & Ayodele, 2005). This is probably due to linkage disequilibrium or pleiotropic effects. Previous mapping of QTL for both resistance to GLS and plant maturity to regions on chromosomes 2, 3, 4, and 8 of the maize genome (Bubeck et al., 1993) appear to validate this hypothesis. In addition, Clement et al. (2000) identified QTL on chromosome 1 that regulated both GLS resistance and EH/PH. Similarly, Pozar et al. (2009) mapped a QTL for both GLS resistance and plant maturity on chromosome 3, whereas QTLs for resistance and flowering time colocalized on chromosome 1 (Zwonitzer et al., 2010). Gray leaf spot progresses most rapidly after flowering (Rupe et al., 1982), which marks the transition from channeling of photo-assimilates from vegetative to reproductive development (Subedi & Ma, 2005). This reduces the proportion of metabolites devoted to plant defense. Thus, the strong correlation of resistance with late maturity is possibly due to pleiotropy of basic genes that delay grain fill and prolong photosynthesis while simultaneously slowing down plant senescence. This subsequently enhances and prolongs the use of photo-assimilates for plant defense (Pozar et al., 2009). The strong negative correlation between susceptibility to GLS resistance (i.e., SAUDPC) and SGR (Table 4) seems to support this hypothesis. However, colocalization of QTLs for GLS resistance and late maturity (Zwonitzer et al., 2010) suggests that tight linkage of loci conditioning the two traits cannot be ignored.

Selection for increased EH/PH, DTM, and SGR should result in a corresponding increase in GL resistance if their correlation is due to linkage and/or pleiotropy. However, genetic correlations between disease resistance and the three traits may not necessarily lead to a greater response of resistance to indirect selection based on indices developed using these secondary traits. Indirect selection is more efficient only if heritability of the primary trait is lower (Hallauer et al., 2010) and if the secondary traits are weakly correlated with each other. Inclusion of highly correlated secondary traits in a selection index reduces precision of the index because sampling variance associated with covariance estimates are often higher. While we did not find studies that compared heritability of resistance with that of EH/PH, DTM, or SGR, some previous studies (Balint-Kurti et al., 2008; Hallauer et al, 2010) show that estimates of heritability of EH/PH and DTM are similar to that of resistance. Additionally, the weak to moderate correlations have previously been reported among these three agronomic traits (Farias-Neto & Miranda, 2001). Thus, the potential for exploitation of the observed genetic corrections in indirect selection for partial resistance to GLS will likely be moderate.

4.4 | Coinheritance of resistance to GLS with agronomic traits

Coheritability estimates measure the extent of joint inheritance of two traits and thus, they are more useful than coefficients of genotypic correlation that reflect the genetic relationship between traits only in the current rather than future generations. The significant coheritability of SAUDPC with SGR (Table 5), suggests that these traits are jointly transmittable in pairs across generations and are thus, amenable to tandem selection for improvement of GLS resistance. In this case, selection for SGR would have greater success in development of genotypes with low SAUDPC rather than low ρ, FPDLA, and CDSG, since coheritability estimates involving the latter disease variables were nonsignificant and relatively small. However, premature senescence of highly susceptible genetic materials due to biotic stress could confound the benefits of the coheritability of SAUDPC with SGR in breeding for GLS resistance. Thus, additional studies are needed to better establish the implication of this observation in maize breeding programs. The extremely low coheritability of LP with agronomic traits suggests it is unlikely that index selection based on agronomic traits would improve LP. While some studies (e.g., Menkir & Ayodele, 2005) have documented genetic correlation of GLS severity with EH/PH, DTM, DTS, and DTA, to our knowledge, this is the first report of coheritability resistance to GLS with agronomic traits.

4.5 | Phenological basis of coinheritance of GLS resistance with agronomic traits

Causal mediation analysis revealed that only EH/PH, DTM, and SGR accounted for most of the hypothesized effects of agronomic traits on resistance. Furthermore, this analysis indicated that overall effect of DTA on GLS resistance was partly due to negative mediation of its indirect effects by LPP via EH/PH. Previous reports indicate that increased LPP and SGR produce healthier maize due to increased moisture retention and prolonged photosynthesis (Bekavac et al., 2007). Increased SGR probably also prolongs transcription of mRNAs and assimilation or remobilization and translocation of metabolites that enhance plant defense. Moreover, increased leafiness enhances lignification of maize stalks and leaves (Dijak et al., 1999), which possibly constrains tissue penetration and invasion by *C. zeina*.

Earlier reports indicated that leafy maize have relatively lower EH/PH (Dijak et al., 1999). While this increased yield and resistance to logging, it was shown to decrease partitioning of leaf metabolites towards plant defense. Our results suggest that increased LPP would reduce EH/PH (Table 7; Figure 3). However, the latter trait was also significantly increased by PH and EH that led to increased resistance to GLS. Thus, cultivars with increased LPP and desirable EH/PH for GLS resistance can only be developed by specialized breeding strategies such as the multiple population model (Burdon & Namkoong, 1983) or restricted index selection approach (Holbrook et al., 1989). The unifying theme in both breeding concepts is the breakage of linkage drag followed by sourcing desired alleles of the correlated traits by independent culling within separate populations. Inbred lines can then be developed from the diverse populations and crossed to produce hybrids with both desirable EH/PH and GLS resistance, which would otherwise be impossible from tandem selection within a single population. In maize, DTM has been shown to be positively correlated with yield (Iqbal et al., 2011). Thus, increased GLS resistance associated with the direct and indirect effects of increased DTM should result in a complementary increase in yield. However, late-maturing maize also increase production costs and vulnerability to drought. Thus, the positive effect of increased DTM on resistance presents a dilemma in breeding maize for drought escape since it would compromise earliness.

5 | CONCLUSION

Phenotypic variation in GLS resistance was more sensitive to genetic background of the inbred lines evaluated than to the favorability of the environment for disease development. Genetically, the strongest agronomic correlates of resistance were SGR, DTM, EH/PH, and DTS, of which the latter three manifested the highest coheritability with resistance. Phenologically, the genetic correlation of GLS resistance with agronomic traits was mediated primarily by the direct effects of DTA and DTM and indirect effects of SMI and SGR on disease resistance.

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AUTHOR CONTRIBUTIONS

James O. Nyanapah: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Writing – original draft. Patrick O. Ayiecho: Project administration; Supervision; Writing – review & editing. Julius O. Nyabundi: Project administration; Supervision; Writing – review & editing. Washington Otieno: Supervision;

Writing – review & editing. Peter S. Ojiambo: Methodology; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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