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Genetic gain and cost efficiency of marker-assisted selection of maize for improved resistance to multiple foliar pathogens

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Abstract Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum*, gray leaf spot (GLS) caused by *Cercospora zeae-maydis* and maize streak caused by maize streak *Mastrevirus* (MSV) are the most destructive foliar diseases limiting maize production in sub-Saharan Africa. Most foliar diseases of maize are managed using quantitative (partial) resistance, and previous studies have reported quantitative trait loci associated with host resistance (rQTL). Our objective was to compare the genetic gain and costs resulting from phenotypic, genotypic, and marker-assisted selection of partially inbred lines derived from many families for resistance to infection by three foliar pathogens. We developed a population of 410 F2:3 families by crossing inbred line CML202 with a breeding line designated VP31. These families were planted in nurseries inoculated separately with each pathogen. We conducted one cycle of early generation pedigree selection using three different procedures, phenotypic, genotypic, and marker/phenotypic index, for improvement of resistance to each pathogen. We used simple sequence repeat (SSR) markers flanking six target rQTL associated with partial resistance. Broad- and narrow-sense heritability estimates were also obtained for the F2:3 families, and selected and non-selected F2:4 families. Genetic gains resulting from the selection procedures were determined. Gene action of the candidate rQTL was determined using orthogonal contrasts. Estimates of costs based on lower boundary values indicated that the cost of marker-based selection was lower than that of phenotypic selection. Our results indicate that molecular markers linked to target rQTL can facilitate pyramiding resistance to multiple diseases during early generation pedigree selection.

Keywords Gray leaf spot · Selection methods · Maize streak virus · Selection efficiency · Northern corn leaf blight

Abbreviations

CIMMYT	International Maize and Wheat Improvement Center
GLS	Gray leaf spot

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MAS	Marker-assisted selection
MSV	Maize streak virus
NCLB	Northern corn leaf blight
rQTL	Resistance quantitative trait locus/loci
SSR	Simple sequence repeat

Introduction

Foliar diseases limit maize (*Zea mays* L.) productivity worldwide. Gray leaf spot (caused by *Cercospora zeae-maydis*) and northern corn leaf blight (caused by *Exserohilum turcicum*) are cosmopolitan fungal diseases, occurring world-wide (Pratt and Gordon 2006) and maize streak *Mastrevirus* (MSV) infects maize throughout sub-Saharan Africa (Bosque-Perez 2000). These diseases may occur simultaneously and recurring epidemics are common due to favorable weather conditions, planting of susceptible cultivars and continuous maize cropping (Bigirwa et al. 2001; Pratt and Gordon 2006).

Most foliar diseases of maize can be successfully managed using quantitative (partial) host resistance. Quantitative traits are reported to have the most potential for marker-based (genotypic) selection because conventional (phenotypic) selection of traits with relatively low heritability is costly and often ineffective, due in part to confounding environmental effects. Marker-based or marker-assisted selection indices that combine phenotypic and genotypic data (MAS indices) have been widely adopted by the private sector, but only a few reports in the scientific literature have documented the efficacy of MAS for improving quantitative traits in maize (Bernardo 2008). The cost of generating marker data has declined, but breeding programs in the public sector have lagged behind in the adoption of marker technology because of funding concerns or physical resource limitations. In addition, expression of quantitative trait loci (QTL) effects may be inconsistent across genetic backgrounds or environments. In spite of these limitations, several simulation studies have suggested a greater potential for application of markers to improve quantitative traits (Lande and Thompson 1990; Dudley 1993; Hospital and Charcosset 1997; Moreau et al. 1998; Nicholas 2006).

Quantitative trait loci conditioning resistance to plant pathogens (rQTL) have been discovered and reviewed by several authors (Pratt and Gordon 2006; Balint-Kurti and Johal 2008; Redinbaugh and Pratt 2009). Many of the reported rQTL have not been precisely mapped and display inconsistent effects (Wisser et al. 2006). To date only a few rQTL conferring resistance to these pathogens have been validated (Abalo et al. 2009; Asea et al. 2009). Breeders must first document the reproducibility of candidate rQTL in unique populations and target environments of interest before considering them targets for selection.

Pyramiding multiple alleles for quantitative resistance has been proposed for improvement of host resistance (Asea et al. 2009; Poland et al. 2009). Combining multiple resistance alleles needed to confer sufficient levels of resistance to multiple pathogens presents a sizeable challenge to breeders. Field screening of large populations requires considerable capacity and resources. The influence of variable environmental conditions on expression of resistance will be ever-present, as will potential interactions among resistance genes. Unfavorable linkages and multiple sources of useful QTL alleles will also decrease the frequency of usable segregants with all the desired QTL alleles (Bernardo 2008). Any combination of these factors can severely impact the accuracy of selection and thus genetic gain for improved resistance.

Stringent phenotypic selection is not generally practiced in resistance-breeding schemes during early generations, or may be limited to traits that are highly heritable and easily handled (Simmonds and Smartt 1999). MAS for partial resistance of low to moderate narrow-sense heritability during early generations would be feasible if selection was known to be effective and affordable.

Recent studies have examined the affordability and effectiveness of using MAS for improvement of maize. Abalo et al. (2009) reported that MAS was a cost-effective alternative to conventional selection for maize streak resistance in Uganda. Economic analyses by Dreher et al. (2003) and Morris et al. (2003) regarding selection of quality-protein maize using theoretical breeding schemes determined that MAS was faster, but more costly, than phenotypic selection. They concluded that in addition to differences in efficiencies, there may also be differences in relative costs depending on the trait to be selected. Yousef and

Juvik (2001) compared marker-assisted and phenotypic selection for multiple traits in composite populations of sweet maize. It was determined that MAS resulted in significantly higher gain than did phenotypic selection, and that MAS was most useful when traits were difficult and costly to measure.

Other studies of MAS in maize have shown promising results, although relative costs were not examined. Flint-Garcia et al. (2003) demonstrated that MAS was a potentially valuable selection tool compared with phenotypic selection for stalk strength (rind penetrometer resistance) and European corn borer (*Ostrinia nubilalis*) resistance. They showed that rQTL in several populations were effective in improving both traits related to stalk lodging. In all populations, MAS was effective in selecting for both resistance and susceptibility. Selection studies with rust rQTL in barley (Castro et al. 2003) and black mold (*Alternaria alternata*) rQTL in tomato (Robert et al. 2001) have demonstrated the value of MAS for pyramiding resistance alleles associated with individual diseases, but to date the usefulness of MAS for pyramiding resistance to multiple pathogens has not been examined.

Several rQTL are likely targets for a MAS program aimed at improving resistance to the causal agents of GLS, NCLB and maize streak. One rQTL (*msv1*) explained half (or more) of the phenotypic variation detected for resistance to MSV infection in four diverse tropical inbreds (Pratt and Gordon 2006). Similarly, three QTL regions conditioning resistance to NCLB during the adult plant stage, and two for GLS, are common in different sources of resistance. A summary of consistent rQTL regions from different studies is shown in Fig. 1. Asea et al. (2009) validated three of these rQTL using flanking SSR markers that were significantly associated with disease resistance and confirmed that one marker significantly associated with disease resistance was linked to the other two rQTL (bin 2.09 for GLS resistance and bin 3.06 for NCLB resistance). We propose the three validated rQTL (MSV—bin 1.04, *umc1169-bnlg2086*; NCLB—bin 5.04, *umc1221-phi330507*; GLS—4.08, *umc1086-umc1559*) will be highly suitable target rQTL for MAS. Two other rQTL with one of two flanking markers significantly associated with resistance were confirmed (GLS—2.09, *umc1551-umc2077*; NCLB—*3.06*, *umc1644-umc2169*), although less consistent, and may be potentially useful for selection purposes (Asea et al. 2009). One rQTL (NCLB—8.04, *umc1724-mmc0181*) was contributed by the susceptible parent, and it too has been included in the present study.

Our goal is to compare the effectiveness of selection procedures that are, or are not, dependent on molecular-marker technology for improvement of resistance to multiple foliar pathogens of maize during early generation selection. Our specific objectives were to: (1) estimate the broad- and narrow-sense heritabilities of partial resistance to *E. turcicum*, *C. zeae-maydis* and MSV, (2) compare genetic gains associated with one cycle of selection for resistance to these pathogens using phenotype-based (conventional), marker-based (genotypic) and combined phenotype-plus marker-based selection procedures, (3) compare the associated costs for the different selection procedures, and (4) characterize modes of gene action of the above rQTL for GLS, NCLB and MSV.

Materials and methods

Plant materials

Partial inbred lines were developed from a cross between CML202 and an F2:4 line (VP31) with known resistance to GLS (Gordon et al. 2004). CML202 has a relatively high level of partial resistance to *E. turcicum* and MSV (Welz et al. 1998; Schechert et al. 1999). The inbred line is also widely used in many tropical breeding programs for production of hybrids and new inbred lines due to its excellent combining ability for both disease resistance and yield (Schechert et al. 1999). CML202 has white, flint-dent kernels and was developed by the International Maize and Wheat Improvement Center (CIMMYT) with tropical background originating from West Africa (Welz et al. 1998). It is late maturing and generally well adapted to growing conditions in the humid mid-altitude zones of eastern and southern Africa. The GLS resistance source VP31 was derived from a resistant line selected from a cross between single plants of South African inbred VO613Y and Corn Belt inbred Pa405, which are resistant and susceptible to infection by *C. zeae-maydis*, respectively (Gordon et al. 2004). VP31 is yellow-grained with dent kernels and matures much earlier than CML202. Crosses were made between

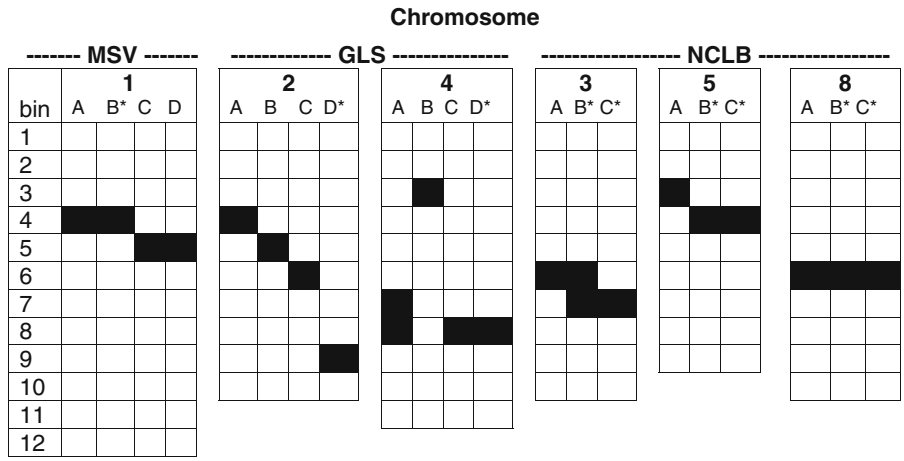

Fig. 1 Chromosomal bin positions of the major consensus QTL regions for resistance to maize streak virus (*MSV*), gray leaf spot (*GLS*) and northern corn leaf blight (*NCLB*) in different mapping populations (*A*, *B*, *C*, *D*). MSV: Kyetere et al. (1999) (*A*), Welz et al. (1998) (*B*), Pernet et al. (1999a) (*C*) and Pernet et al. (1999b) (*D*); GLS: Bubeck et al. (1993) (*A*), Clements et al. (2000) (*B*), Saghai-Maroof et al. (1996) (*C*) and Gordon et al. (2004) (*D*); NCLB: Freymark et al. (1993) (*A*), Schechert et al. (1999) (*B*) and Welz et al. (1999a, b) (*C*). *Study utilized resistant parent in this study. Where consensus QTL did not exist, resistant parent QTL was used

inbred lines CML202 and VP31 using controlled pollination. The F1 plants were self-pollinated and F2 plants were grown and self-pollinated in winter nurseries in Puerto Rico, Hawaii and at Wooster, Ohio (greenhouse) to generate F2-derived F3 (F2:3) families.

Early generation selection for resistance to infection by multiple pathogens

To help eliminate negative agronomic attributes, mild culling (10%) for late maturity, stalk lodging, and plant height was employed among F3 families prior to selection for host resistance. This was intended to emulate standard breeding procedures that would be undertaken during pedigree breeding. All F2:3 families were evaluated independently for resistance to infection by three foliar pathogens during the 2003 growing season in Wooster, Ohio (*C. zeae-maydis*); Namulonge, Uganda (*E. turcicum*); and CIMMYT, Zimbabwe (maize streak) using controlled inoculation protocols. Field plots were established and inoculations and disease severity assessments were performed using the methods described by Asea et al. (2009). Field management and inoculation procedures were as reported in Asea et al. (2009). The most resistant families were selected at 10% selection intensity. This resulted in 38 families being selected for GLS resistance, 37 families for MSV resistance and 38 families for NCLB resistance. This selection intensity corresponded to a truncation point of 1.0 standard deviations from the mean for GLS and NCLB and 2.0 standard deviations for maize streak. Using flanking markers linked to desired parental alleles at each locus, 38 families were selected from the genotyping conducted. Concomitantly, an equal number of families were randomly selected to produce an unselected control population using a random number generation procedure. Some of the randomly selected families overlapped with those previously selected for other traits, resulting in only 26 random selections that did not overlap. This process resulted in a total of 228 F2:4 families combined from phenotyping, genotyping and random selections that were grown and inoculated together in the same trial. The F2:4 families were evaluated in the same locations as F2:3 families except for the NCLB disease nursery that was conducted in Wooster instead of Namulonge. Comparisons were then made between the following treatments: (1) phenotype-based (phenotypic) selection, where selections were based only on field evaluation following artificial inoculation, (2) marker-based (genotypic) selection, where selections were based only on rQTL, (3) combined phenotypic and genotypic or marker/phenotypic index, and (4) random selection. The marker/

phenotypic index combined both phenotypic value and genotypic (QTL) information. We assumed that because some of the minor QTL for a given trait were not known, their contribution would be captured through phenotypic selection. The term "marker-based selection" is used interchangeably with genotypic selection.

Heritability and gene action

Broad-sense heritability for disease resistance was calculated from standard analysis of variance with total variation apportioned into genotypic and phenotypic components. Broad-sense heritability was estimated by variance component analysis of the F2:3 and F2:4 families as described by Holland (2003): $h^2 = \sigma_g^2 \Big/ \left(\sigma_e^2 / r \cdot e + \sigma_{ge}^2 \Big/ e + \sigma_g^2\right)$ where σ_g^2 = genotypic variance component, σ_{ge}^2 = genotype × environment variance component, σ_e^2 = experimental error variance component, e = number of environments and r = number of replications per environment. Narrow-sense heritability estimates were computed by calculating the correlation coefficients between F2:3 and F2:4 family means of mid- and late-epiphytotic ratings and standardized area under the disease progress curve (SAUDPC) as described by Asea et al. (2009). Correlation rather than regression coefficients were used as estimates of heritability because the parents and progenies were evaluated in different years and/or environments. Parent–offspring correlation has been recommended as a more reliable method than regression for estimating narrow-sense heritability for self-pollinating crops because it reduces the scale of phenotypic variation in progeny relative to the parent due to the environmental differences (Foolad et al. 2002). The standard error (SE) for each h^2 estimate was calculated as SE $[h^2(\text{F2:4})] = [(1 - r_{\text{F2:4}}^2)/(n - 2)]^{1/2}$, where n is the number of F2:4 families and r is the correlation coefficient (Foolad et al. 2002).

Orthogonal contrasts were used to test for significance and estimates of additive and dominance gene action associated with each locus using the PROC GLM procedure of SAS (SAS, 2003). For tests of epistasis, the statistical model was $X_{ij} = \mu + \text{M1}_i + \text{M2}_j + (\text{M1}_k{*}\text{M2}_j) + \varepsilon_{ijk}$, where M1 and M2 were markers on different loci. The F test for epistasis was equal to the mean square for marker interaction divided by the mean square for the residual error. Genotypic analysis was conducted using the procedure described earlier (Asea et al. 2009).

Marker analysis

Selection of desired resistant parental alleles at the rQTL was performed using flanking molecular markers that were polymorphic between the parents and associated with resistance (Asea et al. 2009). The net marker score was calculated by summing across marker loci the product of the coded marker genotype (0, 0.5 and 1) as described for marker-based selections (Lande and Thompson 1990; Eathington et al. 1997; Moreau et al. 2000). A value of 0 was given to a locus lacking a resistance allele, 0.5 for heterozygous and 1 for homozygous resistant loci. The selection index used for ranking the families incorporated the information from both the phenotypic and genotypic data through a combined score of the form $I_i = b_z Z_i + b_m M_i$, as described by Eathington et al. (1997), where I_i is the index value for the ith family, Z_i is the phenotypic mean, b_z is the weight given to the phenotypic mean, M_i is the net molecular score, and b_m is the weight given to the molecular score. In each index, b_z was equal to one, while b_m was calculated as $[(1/h^2) - 1]/(1 - p)$, where p is the proportion of additive genetic variance explained by the marker (Lande and Thompson 1990). Because additive genetic variance explained by each marker was less than 30%, broad-sense heritability estimates were moderately high, and each consensus rQTL was considered important, the value of b_m was approximately the same for all the loci. The sums of selection indices across each rQTL for families were ranked and used to select the highest ranking families equivalent to those selected previously using the phenotype-based selection method for disease resistance.

Statistical analysis

Statistical analyses were performed for each disease independently. Genetic and error variance were estimated using the SAS PROC GLM procedure. To obtain the performance of the genotypic value of each family, analyses of variance were conducted on a family-mean basis and total variation was apportioned into the effects of replication, genotypes and

errors for each disease using: $Y_{ij} = \mu + B_i + F_j + \varepsilon_{ij}$, where μ = overall mean, B_i = effect of block, F_j = family effect, and ε_{ij} = experimental error.

Associations between individual marker loci and disease severity were tested with single-factor analysis of variance using the SAS PROC GLM procedure, with a threshold significance level of $P = 0.05$. For genotypic analysis, ten F2:3 plants per family and five F2:4 plants per family were sampled and pooled as described by Asea et al. (2009). For tests of marker–trait association within the population, the statistical model used was the same as that described by Coaker et al. (2002).

The realized genetic gain (R) was calculated as the difference between the mean of selections (k) and the grand mean of all (n) genotypes: $R = (\Sigma_k Y)/k - (\Sigma Y)/n$, where Σ_k = summation over the k selections, Σ = summation over all genotypes and n = genotypes tested in both seasons before and after selections. The standard error of R was calculated as: $[(n - k)/nk]^{1/2}$(SEM), where SEM is the standard error of the mean for an individual genotype in the test of F2:4 families and $(n - k)$ was equal to the number of genotypes that were not selected. Predicted gains under selection were calculated as described by Allard (1964) as $G_s = (k)\ (\sigma_A)(\sigma_a^2)/(\sigma_A^2)$ where k = selection differential, σ_A = phenotypic standard deviation of the trait mean, σ_a^2 = genetic component arising from genetic differences among families and σ_A^2 = total phenotypic variance. The relative efficiency of marker-based selection over phenotypic selection was calculated as $RE_{MBS:PS} = \frac{\sqrt{V_M/V_A}}{h}$, where V_M is marker variance due to marker scores, V_A is additive genetic variance and was calculated using narrow-sense heritability based on parent-offspring correlation, and h is the square root of heritability. The relative efficiency of marker-assisted selection over phenotypic selection was calculated as $RE_{MAS:PS} = \sqrt{\frac{V_M/V_A}{h^2} + \frac{(1-V_M/V_A)}{1-h^2(V_M/V_A)}}$, where h^2 is the heritability of the trait (Bernardo 2002).

Economic analysis

The economic analysis was based on a spreadsheet budget using actual costs at the time similar to one used by Dreher et al. (2003) that provided a basis for comparing costs of different selection schemes for disease resistance. Additional costs added to estimate the cost of selection for disease resistance included the cost of inoculum preparation and performing inoculations, and the cost of insect rearing and infestations to elicit maize streak. Our costs did not include capital costs both in the laboratory and field because all the research was conducted using existing facilities.

Results

Natural conditions were favorable for development of GLS in Ohio and NCLB in Uganda and Ohio disease nurseries during both seasons of evaluation. Families in the trials displayed a wide range of disease severity, typical of partial resistance. Controlled artificial inoculation using viruliferous leafhoppers at the CIMMYT, Zimbabwe, site resulted in infection of all families. Differential reactions of the families ranging from highly resistant to mostly susceptible were observed. Evaluations of F2:3 families resulted in a population mean of 36% leaf area affected (PLAA) (11.8 standard deviations) for GLS, 32% PLAA (16.3 standard deviations) for NCLB and 4.0 (0–5 scale) (0.8 standard deviations) for maize streak. Disease severity values for GLS were continuously distributed with significant ($P < 0.01$) transgressive segregation occurring in both seasons' trials. The most resistant F2:3 family had 8% final PLAA and the most susceptible family had 80% PLAA. Overall, 156 of the 410 families had final PLAA less than 30% (less than either parent) and 87 families had final severity scores of greater than 45% PLAA. The susceptible checks Pa405 and B73 were severely affected (>70% PLAA at the final rating; 63 days after inoculation). Disease severity of parental lines was intermediate and similar, ranging from 29 to 32% final PLAA in both seasons.

A large portion of F2:3 families were extensively blighted by NCLB (>50% final PLAA). The resistant and susceptible parents differed significantly ($P < 0.01$) for severity of NCLB in both seasons. The distribution of severity ratings did not fit a normal curve and transgressive segregation was observed for resistance in the F2:3 but not the F3:4 families.

Evaluations of maize streak severity following artificial inoculation showed infection of all F2:3 and F2:4 families and checks. Severity values of families ranged from resistant to susceptible during both seasons. The majority of the F2:3 families had disease scores above 2.0 and 105 displayed a score of 5.0. The F3:4 mean was 2.2 and the final ratings of 35 families were not significantly different from that of the resistant parent. Across two seasons, the mean rating was 2.5 for CML202 and 3.1 for VP31.

Heritability estimates

Variance components were used to estimate broad-sense heritability for each disease. Broad-sense heritability estimates based on disease severity for GLS and maize streak were higher in F2:4 than F2:3 families but estimates for NCLB were similar across generations. Heritability estimates for all diseases ranged from 0.42 to 0.90 and were more variable for maize streak than for NCLB or GLS. The lowest and highest estimates of heritability across the two generations were obtained for maize streak (Table 1). In both seasons, estimates of heritability were similar for mid-epiphytotic and late epiphytotic disease ratings, indicating similarity in genotypic responses as the season progressed (Tables 1, 2).

There was variation in the magnitude of narrow-sense heritability estimates for the three diseases. Narrow-sense heritability estimates based on the correlation coefficients from parent–offspring regression at final disease ratings were 0.22, 0.25 and 0.39 for maize streak, NCLB and GLS, respectively. At all disease assessment times from early to late epiphytotic stages, heritability estimates were greater for GLS ($0.34 \leq r_p \leq 0.39$) compared to NCLB ($0.06 \leq r_p \leq 0.25$) and maize streak ($0.22 \leq r_p \leq 0.29$) (Table 3). Heritability increased at later ratings, corresponding perhaps to increased disease severity as the season progressed. The highest heritability estimates were attained from the SAUDPC that accounted for the season-long disease progress. Overall, there were modest, but significant, similarities between the F2:3 families and the corresponding F2:4 families in reaction to GLS, NCLB and maize streak, as evidenced by low to moderate correlation coefficients ($0.06 \leq r_p \leq 0.39$, $P < 0.0001$). There were particularly significant correlations between families for the disease resistance at late epiphytotic stages.

Table 1 Population mean values, predicted genetic gains and associated genetic parameters of F2:3 families evaluated independently for reaction to *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus in Ohio, Uganda and CIMMYT Zimbabwe, respectively

	GLS			NCLB			MSV
	Sev^{a}_{53}	Sev_{62}	SAUDPC[b]	Sev_{54}	Sev_{65}	SAUDPC	
Genetic materials							
F2:3	30.0	36.0	30.8	25.9	32.1	23.3	4.2
Mean	33.7	39.6	34.2	33.7	39.6	34.2	4.2
SD	10.1	11.8	9.8	15.3	16.3	13.3	
Genetic parameters (F2:3 families)							
σ^2_F	67.6	110.0	67.4	161.3	191.1	126.1	0.2
σ^2_e	54.0	33.0	29.6	29.3	172.5	113.5	0.6
H^2	0.70	0.77	0.69	0.9	0.7	0.7	0.4
ΔG (H^2)	13.6	16.2	12.0	20.7	23.2	18.8	0.7
ΔG (h^2)	6.6	8.2	6.8	1.5	8.4	6.5	0.4

σ^2_F, σ^2_e, H^2 = Estimates of variances between families, residuals and broad-sense heritability

ΔG (H^2), ΔG (h^2) = Predicted genetic gains calculated using broad-sense and narrow-sense heritability, respectively

[a] Sev_{53}, Sev_{62}, Sev_{55}, Sev_{65} = severity assessed 53 and 62 days after inoculation for GLS, 54 and 65 days after inoculation for NCLB

[b] SAUDPC = standardized area under the disease progress curve

Table 2 Genetic means of F2:4 families evaluated at mid and maximum epiphytotic stages and associated realized genetic gains for different selection procedures for resistance to *Cercospora zeae-maydis*, *Exserohilum turcicum* and maize streak virus

Selection treatment	Disease ratings			Actual realized genetic gain		
	Mid epiphytotic	Late epiphytotic	SAUDPC[a]	Mid epiphytotic	Late epiphytotic	SAUDPC
MSV (1–5 scale)				Top 10%		
Phenotype	1.9 ± 0.2	2.0 ± 0.2	–	−0.3 ± 0.03[b]	−0.4 ± 0.03	–
Genotype	1.7 ± 0.1	1.9 ± 0.1	–	−0.5 ± 0.02	−0.5 ± 0.02	–
Random	2.1 ± 0.1	2.3 ± 0.1	–	−0.1 ± 0.01	−0.1 ± 0.01	–
Phen and gen[c]	1.7 ± 0.1	1.8 ± 0.2	–	−0.5 ± 0.02	−0.6 ± 0.03	–
F2:4 mean	2.2 ± 0.1	2.4 ± 0.1	–			
GLS (0–100% PLAA)						
Phenotype	23.6 ± 1.1	30.5 ± 1.6	23.8 ± 1.2	−3.4 ± 0.2	−5.0 ± 0.2	−3.5 ± 0.2
Genotype	23.3 ± 1.4	30.5 ± 2.1	23.3 ± 1.4	−3.7 ± 0.2	−5.0 ± 0.3	−4.0 ± 0.2
Random	27.8 ± 0.8	35.7 ± 1.1	28.1 ± 0.9	0.8 ± 0.1	0.2 ± 0.1	0.8 ± 0.1
Phen & gen	19.2 ± 1.1	24.2 ± 1.0	19.2 ± 1.0	−7.8 ± 0.2	−11.3 ± 0.1	−8.1 ± 0.1
F2:4 mean	27.0 ± 0.5	35.5 ± 0.8	27.3 ± 0.6			
NCLB (0–100% PLAA)						
Phenotype	7.8 ± 1.1	10.3 ± 1.5	6.6 ± 1.0	−2.6 ± 0.2	−3.8 ± 0.2	−2.8 ± 0.2
Genotype	9.4 ± 1.5	11.8 ± 1.6	7.9 ± 1.1	−1.0 ± 0.2	−2.3 ± 0.2	−1.5 ± 0.2
Random	9.7 ± 0.9	13.8 ± 1.3	9.2 ± 0.9	−0.7 ± 0.1	−0.3 ± 0.1	−0.2 ± 0.1
Phen and gen[c]	4.1 ± 1.2	6.1 ± 1.5	3.9 ± 1.1	−6.3 ± 0.2	−4.6 ± 0.2	−5.5 ± 0.2
F2:4 mean	10.4 ± 0.6	14.1 ± 0.8	9.4 ± 0.6			

[a] *SAUDPC* standardized area under the disease progress curve

[b] Negative realized gains indicate gain in resistance and positive gains indicate loss in resistance compared to overall mean of the population

[c] Phen & gen = Marker/phenotypic selection for GLS and NCLB calculated using only markers significantly associated with resistance

Table 3 Relative efficiencies of marker-based selection and marker-assisted selection compared with phenotypic selection for maize diseases

Trait	V_M/V_A			Efficiency over phenotypic selection					
				Marker-based selection			Marker/phenotypic selection		
	Sev_1[a]	Sev_2	SAUDPC	Sev_1	Sev_2	SAUDPC[b]	Sev_1	Sev_2	SAUDPC
GLS	0.65	0.49	0.60	0.91	0.79	0.87	1.04	1.02	1.03
MSV	0.85	0.64	–	0.97	0.85	–	1.02	1.01	–
NCLB	0.05	0.03	0.28	0.29	0.21	0.66	1.02	1.00	1.05

[a] Sev_1 and Sev_2 = mid and late disease severity assessment for GLS, NCLB and MSV

[b] SAUDPC = standardized area under the disease progress curve

Genetic gains under selection

There were differences in the amount of genetic gain realized for disease resistance at mid, final and overall epiphytotic assessments during both seasons. Overall, the genetic gains were higher at late season disease severity, presumably due to maximum differences in the reactions of families associated with this assessment time (Table 1). Comparatively higher genetic gains were realized for GLS than for NCLB and maize streak for all rating times.

Realized genetic gain varied with the selection treatments employed, indicating differences in their effectiveness (Table 1). For GLS, genotypic selection

reduced disease severity by 3.7 and 5.0% PLAA from F2:3 to F2:4 families at mid and final disease assessment times, respectively. Phenotypic selection reduced disease severity by almost the same amount (no significant difference with genotypic), and random selection resulted in essentially no change. Similar results were obtained for maize streak where slightly higher genetic gains were realized for genotypic selection over phenotypic selection. For NCLB resistance, phenotypic selection resulted in a population with lower mean disease ratings compared to either genotypic or random selection. Marker/phenotypic index achieved the highest genetic gains for all three diseases. The mean disease severity was reduced by 11% for GLS, 6% for NCLB and 0.6 (1–5 scale) for maize streak using epiphytotic assessments from the overall mean of each disease evaluated in the F2:4 families. In most cases, random selection (simulating no selection) resulted in higher disease severity compared to the overall mean of the F2:4 families and was, as anticipated, not effective for improving disease resistance.

Predicted genetic gains and selection efficiency

Predicted genetic gains for disease resistance calculated using both narrow-sense and broad-sense heritability for the three diseases were higher than actual realized gains for all ratings. Predicted genetic gains were similar at late season epiphytotic and overall disease ratings for both GLS and NCLB ranging from 7 to 8% PLAA (Table 2). Variance component estimates used to calculate predicted genetic gains are shown in Table 1. NCLB, with the highest heritability, had a predicted gain from phenotypic selection that was correspondingly higher relative to other selection methods.

Calculation of relative efficiencies of selection for the three diseases indicated that marker-based selection was generally less efficient than phenotypic selection (Table 3). The efficiencies for marker-based selection were practically equal to those for phenotypic selection for maize streak and GLS at both assessment dates. The lower values reflect correspondingly lower variance explained by the markers and moderately high heritability for the disease resistance. These results indicate that marker-based selection will be most valuable in situations where there is poor disease development or selections must be made in off-season nurseries where disease may be absent, and consequently the heritability during selection is zero or very low. In all cases, the efficiencies of marker/phenotypic selection were superior to only phenotypic selection.

Gene action

Analysis of gene action using orthogonal contrasts showed that mostly dominant gene action was present for QTL associated with all three diseases, although the magnitude of gene action varied with the particular QTL. The QTL in bin 5.04 for resistance to *E. turcicum* showed consistently higher significant effects for dominance compared to the QTL in bin 3.06 (Table 4). Dominance was expressed starting from early disease ratings, about 2 weeks post-anthesis to maximum epiphytotic near-leaf senescence. In general, the values of dominant effects were greater for the overall disease rating expressed as SAUDPC than for individual ratings. Consistently stronger additive gene action was detected for the rQTL position on chromosome 8 (bin 8.06). Resistance due to the presence of alleles from rQTL positions 3.06 and 5.04 was maintained from mid- and late-season ratings and no evidence for epistatic interactions was detected among these loci. For GLS, dominant gene action was prevalent at bin 4.08 while the effects of VP31 alleles at bin 2.09 were mainly additive. Gene action for the MSV resistance target QTL on chromosome 1 (*msv1*; bin 1.04–1.05) also appeared to be partially dominant, consistent with earlier reports (Kyetere et al. 1999).

Cost comparison of selection schemes

A comparison was also made between phenotypic and genotypic selection schemes relative to their costs. During the 2003/04 seasons, field costs totaled USD 5,300 per acre at Ohio, USA. This value was only for one disease nursery and represented lower boundary costs because it was mainly based on overhead costs. Our estimates indicated that costs of genotyping were lower than phenotypic selection for disease resistance even when all the traits were selected simultaneously. These results are in agreement with those of Abalo et al. (2009) who examined cost efficiency of selection for MSV resistance. In addition, the cost of genotyping is more likely to

Table 4 Genetic effects associated with putative QTL affecting resistance to MSV, GLS and NCLB on severity in 410 F2:3 families and 202 F2:4 families evaluated for each disease separately at CIMMYT Zimbabwe, OARDC Ohio and Namulonge Uganda, respectively

Chromosome bin			F2:3			F3:4		
Position	Marker	Parameter	Sev_{53}[a]	Sev_{62}	SAUDPC[b]	Sev_{55}	Sev_{63}	SAUDPC
MSV (1–5 scale)								
1.04	umc1169	a	0.07	–	–	0.19**	0.15*	–
		d	0.30**	–	–	0.82***	0.55***	–
		Action	D[c]			D	D	
1.04	bnlg2086	a	0.07	–	–	0.04	0.00	–
		d	0.39**	–	–	1.03***	0.81***	–
		Action	D			D	D	
GLS (0–100%)								
2.09	umc2077	a	1.09	2.07	–	1.65***	1.42*	1.54***
		d	−0.36	−0.68	–	2.30***	1.93*	0.77
		Action						A
2.09	umc1551	a	−0.48	−0.20	–	1.41***	1.27*	1.24**
		d	−0.39	0.55	–	0.81	−0.79	0.77
		Action				A	A	A
4.08	umc1086	a	−1.58	−1.20	–	0.22	0.70	0.15
		d	−4.35	−5.89	–	−2.04**	−4.07***	−2.38***
		Action				D	D	D
4.08	umc1559	a	−0.37	0.33	–	0.24	0.57	0.15
		d	−4.04	−5.19	–	−3.00***	−4.67***	−3.25***
		Action				D	D	D
NCLB (0–100%)								
3.06	umc1644	a	0.03*	0.03**	0.43*	0.10**	−0.10**	0.10***
		d	0.08**	0.07**	1.23**	0.05	0.07	0.06
		Action	D	D	D	A	A	A
3.06	umc2169	a	−0.01	−0.01	−0.21	0.00	−0.03	−0.01
		d	−0.07*	−0.06**	−1.01**	−0.04	−0.06	−0.05
		Action	D	D	D			
5.04	umc1221	a	0.02	0.01	0.43*	0.03**	0.09**	0.09**
		d	0.10***	0.06**	1.23**	0.24***	0.22***	0.21***
		Action	D	D	D	D	D	D
5.04	phi330507	a	0.03*	0.02*	0.46*	0.11**	0.10**	0.10**
		d	0.10***	0.07**	1.36***	0.20***	0.17***	0.18***
		Action	D	D	D	D	D	D
8.06	umc1724	a	−0.04*	0.24*	−0.51**	−0.02	−0.01	−0.02
		d	−0.05	0.26*	−0.63	0.01	−0.03	−0.01
		Action	A	A	A			
8.06	mmc0181	a	−0.06***	−0.05***	−0.95***	−0.02	−0.03	−0.02
		d	−0.01	−0.02	−0.18	0.01	−0.01	0.00
		Action	A	A	A			

[a] Sev_{53}, Sev_{63}, Sev_{55}, Sev_{62} = severity assessed 53 and 62 days after inoculation for GLS in first season and 55 and 62 days during the second

[b] SAUDPC = standardized area under the disease progress curve

[c] *D* dominant gene action, *A* additive gene action

benefit from economies of scale when sample size increases. In disease nurseries, time commitment during inoculations and disease ratings, in addition to other agronomic field requirements, result in substantial labor costs. These factors together increased field costs for conventional selection relative to genotyping costs for the same traits. These costs are likely to vary in different programs (primarily due to differences in labor costs), but the results indicated that genotyping was relatively cheaper than selection under field conditions for the three disease traits.

We used markers associated with previously validated QTL regions and the cost of initial QTL mapping was not considered as a factor in cost comparison. It was evident that the efficiency of genotypic selection compared to that of phenotype-based was higher based on cost-effectiveness and the time required to obtain data. Not only was genotyping cost-effective, but it also resulted in higher genetic gains for the population during one generation of selection for resistance to GLS and NCLB.

Discussion

Our results demonstrated the effectiveness of major rQTL for improving host resistance to maize foliar pathogens, as postulated by Welz and Geiger (2000). The magnitude of the genetic gains obtained from selections in response to infections varied among the three diseases, but in all cases genetic gains using marker/phenotypic index were highest. These results support the speculation of Lande and Thompson (1990) that MAS is expected to be more effective when the breeding value of an individual or a line is predicted by an index determined by both the marker score and the phenotypic value of a trait.

In this study we demonstrated that selection for candidate QTL associated with resistance substantially increased the level of host resistance. Experience obtained in this study also indicates that markers linked to major resistance loci can facilitate pyramiding of resistant alleles for different diseases by selecting desirable recombination events in addition to bringing unlinked genes together.

The estimates of broad-sense heritability for all diseases were moderate to high (0.58 to 0.90) suggesting that reasonable progress in selection is possible for disease resistance traits. The estimates of heritability were in agreement with those reported previously: 0.59 to 0.82 for NCLB (Hakiza et al. 2004; Welz et al. 1999a) using PLAA; 0.28–0.80 for GLS (Gordon et al. 2004) using PLAA; and 0.62–0.93 for MSV (Welz et al. 1998) using a standard 1–5 scale estimated independently in different genetic backgrounds. Moderate estimates of narrow-sense heritability based on parent–offspring coefficients indicate that resistance to pathogens was heritable and early-generation selection could result in improved germplasm under high disease pressure evaluations. Low initial narrow-sense heritability was observed for NCLB, probably because of the fact that during evaluations conducted in the second year in Ohio, leaf blights were observed late in the season, but blight occurred earlier in evaluations conducted in Uganda. A highly variable estimate of heritability was observed for maize streak. The high variability could be attributed to variable reaction of progenies due to extreme drought during the first season that may have affected virus multiplication, plant growth, and resultant symptom expression.

Predicted and realized genetic gains calculated for this study were for only one cycle. Pooling estimates from successive cycles of selection would probably provide higher estimates of genetic gains. These gains would likely be higher when introgressing disease resistance to susceptible varieties. We also observed that despite variation in reactions of some inbred lines to MSV, the parental inbred line VP31 was moderately resistant. The line VP31 was derived from a South African inbred line, VO613Y, and it is possible that it also contributed resistance to the population. The predicted genetic gains for the methods compared in this study may not have been realized, but the relative values for the different methods were consistent with the trend for actual realized genetic gains and thus provide a useful estimate of the relative effectiveness of the different selection procedures. Results obtained also demonstrated statistical and practical equivalence of phenotypic and genotypic selection for resistance to the three pathogens. Both selection methods produced families that were significantly more resistant than both parents and equaled the resistance of the most resistant check inbred line. This was in contrast to random (control) selection in which the performances of the families were not different from the base population, assuming no selection was practiced in the earlier generation.

Comparison of costs indicated that marker-based selection was relatively cheaper, but equally effective in early generation selection, when compared with phenotypic selection under field conditions for the three foliar disease traits. The cost estimates were simplifications based only on marginal costs but represented realistic estimates during evaluation and selection for disease resistance. The cost-effectiveness and time efficiency obtained using a genotype-based selection scheme compared to phenotype-based selection agrees with reports on other traits comparing the cost of the two selection schemes (Dreher et al. 2003; Moreau et al. 2000). We found marker/phenotypic selection to be highly effective in improving the level of host resistance to multiple foliar pathogens of maize, and potentially cost-effective. Given the rapidly changing field and science of marker technology and reduced cost per marker data point, the cost of genotyping should be even cheaper than that reported, but trends in associated genetic gains are expected to remain the same.

Servin et al. (2004) have suggested an optimal strategy for pyramiding favorable alleles by combining into a single genotype a series of target genes identified in different parents. They proposed an optimal succession of crosses over several pedigree generations until a desired genotype is obtained. This systematic approach may also help to address some of the challenges related to combining multiple resistance genes. In addition to pyramiding several race-specific genes, combining the effects of race-specific genes with QTL that confer partial resistance will potentially enhance durability, as suggested by Carson et al. (2002) and Rodier et al. (1995).

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