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Reaction of Waxy and Opaque-2 Inbreds and their Derived Progenies to Multiple Foliar Diseases of Maize in Uganda

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Abstract

Specialty maize lines possessing important endosperm genes *waxy* and/or *opaque-2* enhance processing and nutritional qualities of the grain. However, production and utilization of specialty maize varieties for food, feeds and various industrial end-uses are constrained by endemic foliar diseases of maize including turcicum leaf blight, gray leaf spot and maize streak virus disease. The purpose of this study was to investigate the reaction of two specialty maize genotypes (waxy and opaque-2) and their derived F_1, F_2 and backcross progenies to multiple foliar diseases of maize. A randomized complete block design was used to evaluate these materials under field conditions in Uganda. Significant differences among populations were observed for susceptibility to turcicum leaf blight and maize streak virus disease. The opaque-2 inbred CML182 did not manifest any maize streak virus disease symptoms during the assessment period. Significant differences were observed for susceptibility to maize streak virus disease between reciprocal crosses but not for turcicum leaf blight suggesting possible maternal effects associated with maize streak virus disease resistance. Susceptibility to turcicum leaf blight and maize streak virus disease was associated with the recessive endosperm genes (waxy and opaque-2). These results show that developing waxy and opaque-2 specialty maize varieties with good agronomic and grain quality attributes is dependent on the choice of parents carrying important resistance as well as endosperm modifying genes.

Keywords: *Maize streak mastrevirus, Exerohilum turcicum*, *Zea-mays,* foliar diseases, Uganda

1. Introduction

Maize (*Zea mays* L.) is an important cereal in sub-Saharan Africa and other maize growing regions worldwide as a food, feed and industrial crop. To meet the increasing demand for industrial, food and agricultural needs, it is imperative that high yielding specialty maize varieties suitable for various end-uses are developed. In maize, the *opaque-2* (*o2*) gene doubles lysine levels in the endosperm enhancing its nutritional value for both food and feed. The *waxy* (*wx*) gene another endosperm modifier, is associated with increased amylopectin content, that improves processing qualities of maize grain. In addition, *wx* pleiotropically influences lysine levels in maize endosperm when present either singly and in combination with *o2* (Tsai, Larkins, & Glover, 1978). Through crossing of maize genotypes carrying important mutant endosperm modifier genes, specialty maize varieties with novel traits, high in nutrition and processing attributes can be developed. Nonetheless, mutants of both w*axy* and *opaque-2* are associated with negative pleiotropic effects that compound their exploitation in maize breeding (Simla, Lertrat, & Suriharn, 2009; Vivek, Krivanek, Palacios-rojas, Twumasi-Afryie, & Diallo, 2008). The negative, pleiotropic effects include susceptibility to biotic stresses such as foliar diseases endemic in sub-Saharan Africa and elsewhere (Pratt & Gordon, 2006). Gray leaf spot (GLS) of maize, caused by *Cercospora zeae-maydis* Tehon & E. Y. Daniels; turcicum leaf blight (TLB), caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (teleomorph = *Setosphaeria turcica* (Luttr.) K. J. Leonard & Suggs; syn. = *Helminthosporium turcicum* Pass.) and maize streak virus disease (MSVD) caused by *Maize streak mastrevirus* are major foliar diseases of maize that critically curtail production in sub-Saharan Africa (Adipala, Lipps, &

Madden, 1993; Okori, Rubaihayo, Adipala, Fahleson, & Dixelius, 2004; Shepherd et al., 2007; Ward, Stromberg, Nowel, & Nutter, 1999). Losses from these diseases may exceed 30% depending on environment and host genotype resistance (Bosque-Perez, 2000; Raymundo & Hooker, 1981; Ward et al., 1999) and can occur simultaneously in a single crop resulting in severe yield losses. For cereals, host plant resistance is considered the most effective control measure in mitigating significant losses due to disease (Pratt & Gordon, 2006). Resistance sources for each of these foliar diseases have been identified and the mechanisms of resistance characterized as quantitative and qualitative (Coates & White, 1998; Welz & Geiger, 2000; Welz, Schechert, Pernet, Pixley, & Geiger, 1998). However, breeding of novel genotypes with multiple resistance, without compromising agronomic attributes remains a challenge especially for quantitative resistance which is preferred over qualitative resistance (Parlevliet, 2002). We are developing specialty maize varieties with improved lysine conditioned by *o2* and high amylopectin content conditioned by *wx* suitable for both food and industry. As part of the adaptability and performance trials, the elite inbred lines and their progeny were evaluated for reaction to endemic major maize foliar diseases. Knowledge of the tolerance levels of parents and progeny will support effective selection of plants and/or lines in the early generation stages and thus guide the breeding process. The aim of this study was to evaluate two inbred lines of waxy and opaque-2 background and their derived F_1, F_2 and backcross progeny for tolerance to multiple foliar diseases of maize in Uganda.

2. Material and Methods

2.1 Plant Materials

The waxy maize inbred line (*wx1*Hi27) is a near-isogenic line 7 backcrosses to inbred Hi27 and was provided by Prof. James Brewbaker (Hawaii Agricultural Research Centre – HARC). The inbred *wx1*Hi27 designated P_1 carries the allele *Ht1* which conditions resistance to turcicum leaf blight (TLB) (Brewbaker, 1997). The QPM inbred CML182 designated P_2 was used as the opaque-2 (*o2*) donor parent and was obtained from the cereals programme of the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda. The CIMMYT inbred line CML202 was used as a resistant check for maize streak virus (MSV) and TLB; CML206 and CML78 were used as susceptible checks for TLB and MSV respectively. For comparative purposes with the waxy by opaque-2 derived progenies, a local hybrid check 'NML1' was included in the experiment. Pedigrees and endosperm characteristics of the maize lines used in the study are presented in Table 1.

Table 1. Pedigrees, endosperm characteristics and source of plant materials used in the study

Genotype	Line	Pedigree	Endosperm characteristic	Source
*wx1*Hi27	Inbred	CM104 (India) (=A Theo 21 (B)#)	Waxy, yellow	Brewaker, HARC
CML182	Inbred	WOMTA1-B-1-1-1-BB	*Opaque-2,* white	NaCRRI
CML078	Inbred	G32C19H32-1-#2-B-###-3-B	Normal, white	NaCRRI
CML202	Inbred	ZSR923S4BULK-5-1-BB	Normal, white	NaCRRI
CML206	Inbred	[EV7992#/EVPO44SRBC3]#BF37SR-2-3SR-2-4-3-BB	Normal, white	NaCRRI
NML1	Hybrid	WL249-27/WL249-16	Normal, white	NaCRRI

HARC = Hawaii Agricultural Research Centre; CML = CIMMYT maize line; NML = Namulonge line; NaCRRI = National Crops Resources Research Institute.

2.2 Population Development and Experimental Design

The first filial generation (F_1) of the cross, waxy (P_1) by opaque-2 (P_2) in the forward direction was developed during the season 2009B (September – December) at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) using the opaque-2 parent (CML182) as female. The F_1 plants from this cross were self-pollinated and backcrossed to each parent to generate the F_2 and backcross (BC) populations BC_1P_1 and BC_1P_2 respectively during the season 2010A (March – June). In all backcrosses, the F_1 was used as the female and parents (P_1 and P_2) were used as male. To determine if reciprocal effects were important, a reciprocal F_1 population was developed using the waxy inbred P_1 as female during the season 2010A (March – June). No F_2 and BC populations were developed from this cross. The parents, forward and reciprocal F_1, F_2, BC_1P_1, and BC_1P_2 populations were evaluated in the field using a randomized complete block design with four replications

during the season 2010B (September – December). Plant rows were 6 meters long, 0.75m apart and 0.30m between plants. Prior to planting, kernels from the F_2 progeny were sorted into four distinct kernel classes or types due to segregation with the aid of visual selection for waxy endosperm and light table analyses for the opaque-2 trait (Vivek et al., 2008). The kernel classes included; non-waxy non-opaque-2 (normal), waxy non-opaque-2 (waxy), opaque-2 non-waxy (opaque-2) and the double recessive waxy-opaque-2 (waxy-opaque-2) endosperm genotypes respectively that approximated the 9:3:3:1 ratio. The BC_1P_1 and BC_1P_2 kernels were sorted into waxy, non-waxy (normal) and opaque-2, non-opaque-2 (normal) in a 1:1 ratio respectively. The kernel classes in the F_2 and BC populations were each planted out in four row plots in the field. The two parents, checks and F_1 populations were each planted in two row plots. To confirm the accuracy of kernels sorted into the different kernel classes, simple sequence repeat (SSR) markers specific to the waxy (Phi027) and opaque-2 (Phi112) locus (www.maizegdb.org) were used in an assay with DNA isolated from randomly tagged plants grown from each kernel class.

2.3 Disease Assessments

To ensure high disease pressure, a field previously under maize production, with debris as source of inoculum for TLB and GLS and surrounded by alternative graminea hosts of MSV and volunteer maize plants was used. Pathogen infection was allowed to occur naturally in the field and data collected from 10 randomly selected plants for each disease observed. For the F_2 and BC progeny planted in four row plots, plants in the two middle rows were used for the disease assessments. Disease data from each of the populations was recorded at 50% flowering because most maize genotypes at this growth stage are susceptible to disease.

2.4 Turcicum Leaf Blight (TLB)

Data on TLB was collected 4 times over a 14 day interval using a quantitative scale of 0-75% based on the percent leaf area affected (PLAA) as a measure of severity (Adipala et al., 1993; Elliott & Jenkins, 1946). Briefly, the scale can be described as 0 = No symptoms; 1 = almost none, few flecks; 3 = 1-3 lesions; 5 = 1-5 lesions; 10 = few lesions visible from a distance (10% blighted); 20 = few lesions on ear leaf and leaf above (20% blighted); 30 = leaves below ear blighted and showing signs of ripping at necrotic spots; 40 = 40% blighted; 50 = leaves below the ear almost blighted; 60 = 60% blighted; 70 = 70% blighted; 75 = all leaves blighted. The four disease ratings were used to calculate the area under disease progress curve (AUDPC) as described by other authors (Adipala et al., 1993; Campbell & Madden, 1990) using the formular

$$\text{AUDPC} = \Sigma[(xi + xi{+}1)/2](ti{+}1 - ti) \quad (1)$$

where *xi* is the disease rating on date *i* and *ti* is the time (in calendar days) on which *xi* was recorded.

2.5 Maize Streak Virus Disease (MSVD)

Maize streak virus severity data was collected only once during the entire experiment, just after pollination because at that stage no more new leaves develop and the symptoms are stable. Ten randomly selected plants from each population were visually assessed on whole plant basis for MSV symptom severity using a modified scale of 0-5 where; 0 = no symptoms (resistant) and 5 = severe streaking with 100% or, all leaves on the plant show streak symptoms (susceptible) (Kyetere, 1996).

2.6 Gray Leaf Spot (GLS)

No data was collected on GLS severity due to extensive blighting from TLB.

2.7 Statistical Analyses

Area under disease progress curves (AUDPC) for TLB was computed in Microsoft Excel 2007 (Microsoft corporation). Analysis of variance was performed for AUDPC and MSV with the statistical software Genstat version 13. (Payne et al., 2010) using the appropriate method for a randomized complete block design.

3. Results

Maize streak virus disease (MSVD) severity and the area under disease progress curve (AUDPC) for turcicum leaf blight (TLB) were significant ($P \leq 0.001$) among the different populations. The waxy parental inbred *wx1*Hi27 had the highest AUDPC score among the inbred lines. The TLB resistant check CML202 expressed a relatively low AUDPC score while the susceptible check CML206 had an AUDPC score higher than the QPM inbred CML182 but lower than the waxy parent *wx1*Hi27 (Table 2). The AUDPC score for the forward and reciprocal F_1 populations were not statistically significant ($P \geq 0.05$) but intermediate between the two parents. Moreover, both F_1 populations were observed to be more susceptible to turcicum leaf blight (TLB) compared with the hybrid check 'NML1' (Table 2). Among the plants derived from kernel types in the F_2 population, the order in decreasing level of susceptibility was waxy, normal, opaque-2 and double recessive kernel class types.

The latter two were not statistically different in their levels of tolerance to TLB. Progeny from backcrosses of F_1 to the waxy parent had higher AUDPC score for TLB than those backcrossed to the QPM parent CML182.

Table 2. Mean AUDPC and MSV severity scores for the inbred *wx1*Hi27 and CML182 and their derived progeny F_1, F_2 and backcross progenies

Population	Endosperm characteristic	AUDPC	MSV
*wx1*Hi27	Waxy	89.0	2.0
CML182	Normal	56.4	0.0
F_1	Normal	69.4	0.8
F_{1R}	Normal	70.6	2.0
BC_1P_1	Normal	83.9	2.2
BC_1P_1	Waxy	86.1	1.8
BC_1P_2	Normal	69.6	0.1
BC_1P_2	Opaque-2	68.5	0.8
F_2	Normal	74.3	0.8
F_2	Waxy	79.5	2.5
F_2	Opaque-2	71.8	0.4
F_2	Double recessive	71.1	0.4
NML1	Normal	38.7	0.0
CML202	Normal	23.4	0.3
CML206	Normal	76.7	0.5
CML78	Normal	64.6	1.7
Mean		68.3^{***}	1.0^{***}
S.E		1.23	0.24

F_1 = First filial generation of forward cross; F_{1R} = First filial generation of the reciprocal cross; F_2 = Second filial generation; BC_1P_1 = Backcross to parent 1; BC_1P_2 = Backcross to parent 2; S.E = Standard error of the means; CML = CIMMYT maize line; NML = Namulonge line; AUDPC = Area under disease progress curve; MSV = Maize streak virus; *** = significant at 0.001.

The QPM parent CML182 did not show any symptoms of MSVD during the growth season while the waxy parent *wx1*Hi27 showed susceptibility for MSVD (Table 2). Both the resistant check CML202 and susceptible check CML78 for MSV had severity scores lower than the waxy parent *wx1*Hi27. Between F_1 populations, the forward cross was lower in MSV severity compared with the reciprocal cross. The hybrid check 'NML1' did not manifest any MSVD and had a severity score of 0. Progeny from the waxy kernel class types in the F_2 generation, had the highest MSVD severity scores, followed by the normal kernel class types. Progeny from the opaque-2 and double recessive kernel types were not statistically different and had the lowest MSVD severity scores in the F_2 population. As with TLB, backcrosses to the waxy parent *wx1*Hi27 resulted in progeny with higher MSVD severity scores compared with backcrosses to the QPM parent CML182.

4. Discussion

The aim of this study was to evaluate two inbred lines of waxy and opaque-2 genetic background and their derived F_1, F_2 and backcross progenies to multiple foliar diseases of maize under field conditions in Uganda. The waxy parent used in this study had been introgressed with the qualitative resistance gene *Ht1* (Brewbaker, 1997), whilst the o2 parent had not been characterized before for reaction to either MSVD or TLB. Our results show that all genotypes tested were susceptible to TLB. The high AUDPC scores for the waxy inbred parent *wx1*Hi27 might indicate breakdown of the qualitative resistance gene *Ht1* or due to the failure of this particular gene to protect against race 0 the predominant *E. turcicum* pathotype in eastern Africa (Adipala et al., 1993; Muiru, Koopmann, Tiedemann, Mutitu, & Kimenju, 2011). Among the progeny, similarity in the AUDPC scores for the

forward and reciprocal F_1 populations suggests the absence of maternal or reciprocal cross effects for TLB resistance (Schechert, Geiger, & Welz, 1997; Sigulas, Hill, & Ayers, 1988). Moreover the reduction in AUDPC scores for backcross progeny to CML182 compared with those to *wx1*Hi27, suggests the possible role of xenia effects in conditioning resistance to TLB. In this study, the differences observed in AUDPC values of progeny derived from all four kernel classes in the F_2 population, are indicative of background effects associated with the mutant endosperm genes *wx* or *o2*. Indeed, earlier mapping studies have demonstrated that quantitative resistance to TLB in maize is associated with recessive endosperm modifying genes such as sugary (*su*) and waxy (*wx*) (Welz & Geiger, 2000). Brewster, Carson, & Wicks III, (1992) also showed that linkage for resistance to TLB is associated with the translocation breakpoint, in normal endosperm (*Wx*), while that for susceptibility co-segregates with the waxy endosperm (*wxwx*). This might explain why plants in this study grown from waxy kernel class types had the highest AUDPC disease score.

The absence of MSVD symptoms in the QPM parent CML182 could be due to either disease escape or the full expression of the major gene to the pathogen. A number of tropical maize germplasm possess this form of resistance with some showing immune responses (Kyetere et al., 1999; Welz et al., 1998). For inbred CML182 there is however need for further investigation into its disease reaction especially under controlled conditions. The reciprocal cross differences in disease reaction for MSVD observed in the F_1 generation suggests possible maternal/ cytoplasmic effects associated with MSV resistance. As observed with TLB, the trend in MSV disease severity among plants grown from all kernels class types in the F_2 population similarly suggests that susceptibility to MSV might be associated with the recessive endosperm gene(s) such as *waxy1*. The implication is that simultaneous selection for important grain quality attributes and disease tolerance can be carried out in the early generation stages during specialty maize breeding. Based on these results, plants grown from the double recessive endosperm kernel class types showed very low severity for MSV and were intermediate between the waxy and QPM parents for TLB. Thus, for selection and advancement in the breeding process plants of the double recessive endosperm genotype are recommended for use in the development of specialty maize varieties.

5. Conclusions

This study evaluated two inbred lines differing in the endosperm genes *waxy* and *opaque-2* and their derived progeny for TLB and MSV resistance. The results showed variation for tolerance to both TLB and MSV. Susceptibility appeared to be associated with the recessive endosperm modifying genes *waxy* and *opaque-2*. The inbred CML182 did not show symptoms of MSV during the assessment period. In addition, reciprocal cross differences were observed for MSV susceptibility in this study but not TLB. To affirm CML182 as a potential source of resistance to MSV more investigation by challenge inoculating plants with viruliferous leafhoppers under controlled conditions needs to be carried out. This should be complemented with genetic studies to provide insight into the mechanism of resistance to MSV in CML182 if present. In addition, the role of reciprocal cross differences in MSV resistance also remains to be investigated. Overall, the results suggest that the success in developing specialty maize varieties with good agronomic and grain quality attributes will depend on the choice of parents carrying important resistance as well as endosperm modifying genes.

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