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Full Length Research Paper

Diallel analysis of cowpea populations for resistance to Cowpea aphid-born mosaic virus disease (CABMV) in Burkina Faso

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Cowpea aphid-born mosaic virus disease (CABMV) is one of the reasons for rejection of cowpea seed by seed inspectors in Burkina Faso. With regard to this, this study was undertaken to analyze the genetic components underlying the resistance of cowpea lines to the cowpea aphid-borne mosaic virus (CABMV) and to determine the mechanism of transmission of the resistance from parents to offspring. Therefore, crosses were made in 5x5 full diallel design. Data analysis was done following Griffing and Hayman method on disease severity and the area under disease progress curve (AUDPC) for five cowpea varieties during the 2015 off-season at Kamboinse research station. The analysis of variance associated with the general and specific combining abilities (GCA and SCA) and reciprocal effect (RCE) showed that the genetic variability was explained by additive effect. The F_1 population showed that there was partial dominance and the narrow sense heritability for severity and AUDPC was high (60%). To improve cowpea for resistance to CABMV, rigorous choice of parents should be made before crosses and there was no maternal effect.

Key words: Cowpea, full diallel, severity, resistance, Cowpea aphid-born mosaic virus disease (CABMV), Burkina Faso.

INTRODUCTION

Cowpea (*Vigna unguiculata*, L. Walp) is a leguminous crop, self-pollinated, grown in all agro-ecological zones of Burkina Faso and has numerous advantages at both agronomical and economical levels. Its grains constitute an important source of protein and income for producers and consumers. Cowpea is also an important fodder.

However, one of the main problems in the genetic improvement of the crop to address is the choice of the parents for hybridization. This choice of parents for hybridization depends, beyond beyond resistance to diverse constraints, heavily on market and consumers' criteria. Tignegre (2010) and Batieno (2014) have

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reported that the market criteria were mainly based on seed size (large) and color (white). Also, the effectiveness of a method of selection depends largely on the number of genes involved in the control of the trait (Zagre et al., 1999).

Within the main constraints for cowpea production, the cowpea aphid-borne mosaic virus (CABMV) is one of the principal reasons for rejection of cowpea seeds by the seed inspectors and also by producers in Burkina Faso. Cultural practices have been used to control the disease but are weak in seed production system. Therefore, there is a need to develop resistant varieties in order to reduce losses due to CABMV.

Thus, the objective of this study was to analyze the genetic nature of resistance of cowpea lines to CABMV in order to formulate hypotheses on the possible ways of using them to improve cowpea for resistance to the disease. For this, a full diallel analysis was used following Hayman (1954) and Griffing (1956) approaches. This method has been already used in cowpea to study the genetics underlying *Striga* resistance (Tignegre, 2010). The Griffing's method is based on the determination of the general and the specific combining abilities. The general combining ability for (GCA) is the average of gametic effects of an individual. It provides information on combining abilities at global and individual level (Griffing, 1956). In other words, it is a measure of the value of the average gametes of a parent (Demarly 1977). It is the ability of both parents to transmit positive or negative characters to their descendants (Allard, 1999). Specific combining ability (SCA) is a deviation from the additivity of general combining. Contrary to GCA, SCA is not linked to a parent, but a cross. Statistically, while GCA appears as a primary effect, SCA is an interaction (Demarly, 1977). GCA varies depending on the additive gene action. It is therefore passed from one generation to another. SCA measures the deviation from the performance of F_1 as compared to the average of the parents.

The method of Hayman (1954) is used to estimate different genetic components for the trait and the various parameters: the additive, dominance, reciprocal effects, heterosis and heritability. It comprises four types of analysis that complement the level of interpretation: the analysis of variance of diallel tables testing the significance of the various terms that are not unlike the specific combining ability, the validity test for the model, the statistical analysis of the genetic components of the total variation and the analysis of relationships between statistical terms.

MATERIALS AND METHODS

Genetic resources

Genetic resources used in this study comprised five released cowpea varieties from Burkina Faso and 20 F_1 hybrids from 5x5 full diallel crosses. Lines used in these crosses were chosen based on their reaction vis-à-vis to CABMV. The five lines involved in the crosses are: KVx396-4-5-2D (resistant), KVx640 (resistant), KVx61-1 (moderately susceptible), KVx30-309-6G (susceptible) and Gorom local (susceptible) all from the long-term storage germplasm of the cowpea breeding program at Kamboinsé Research Station in Burkina Faso.

Methods

Twenty (20) F_1 hybrids and their parents were planted in pots and arranged in randomized complete blocks design (RCBD) with three replications. Each replication comprised 25 entries of one pot per entry containing individual plant. Plants were sprayed to avoid contamination from aphids. The experiment was conducted under screen house at Kamboinsé Research Station (latitude 12°28N, longitude 1°32W and altitude 296m) in Burkina Faso in July 2015. To protect plants, insecticide spray was done using a mixture of PACHA (lambda-cyhalothrin 15 g/l + acetameprid 10 g/l) and TITAN (25 EC Acétamiprid 25 g/l) two weeks after planting at doses of 2 ml per liter of water per product.

Each plant received 45 kg of P_2O_5 per hectare from NPK fertilizer (14-23-14-6S-1B formula). One week after planting, all plants were inoculated using extract of leaves from CABMV serotype D grinded based on weight/volume proportion (p/v) =1/10. The inoculum used was from infected seedlings of Gorom local, a CABMV serotype D susceptible cowpea variety in Burkina Faso. Prior to infestation, the inoculum was homogenized in sodium phosphate buffer (0.01 M, pH 7.4). The extract was filtered through gauze and placed in melting ice. Before inoculation, the leaves of cowpea plants older than a week from the three replications were dusted with the mixture of carborundum 600 mesh, an abrasive product and inoculum using a cotton swab pestle dipped in the extract, the upper leaf surface was rubbed gently (Neya, 2011). The symptoms of CABMV were recorded between the 6^{th} and 21^{st} day after inoculation.

Data collection

Observations were made on:

1. The severity assessment using rating scale 6 classes (0 to 5) which is a strength criterion in CABMV.
2. AUDPC: The area under disease progression curve proposed by Shaner and Finnay (1977) using the following equation AUDPC = $\sum_{i=1}^{n}[(X_i+1 + X_i) / 2][t_i+1 – t_i]$ where n: total number of cases; X_i: the first observation of disease in days; $X_i + 1$: the second observation of disease in days; t_i: time in days from the first observation of disease and $t_i + 1$: time in days for the second observation of the disease. It is a study of a disease development rate of a given crop. This parameter selects the best lines in terms of their ability to slow down the progression of the disease.

Data analyses

Hayman (1954) and Griffing (1956) methods were used for analysis of variance (ANOVA) from DIAL Win 98 software revised 22 September 2002.

The method of Griffing (1956) is based on two models: the fixed pattern and random model. The fixed model is applied to a limited number of lines set for self-pollinated crops and inbred lines of cross-pollinated species.

As for the random model, information may extend to the entire population, provided individuals are the representation of a random mating population in equilibrium. There are four methods for each model according to the use of the parents and crossing type.

a. Reciprocal crosses and parents.

Table 1. Analysis of variance for GCA and SCA and reciprocal using Griffing's method for severity.

Tested effets	Variance (MS)	F
GCA	9.19	3.87 ns
SCA	2.38	3.63**
RCE	0.33	0.15 ns
Variance GCA/Variance SCA	1.29	

**:Highly significant; ns: non-significant; SCA: specific combining ability; GCA: general combining ability; RCE: reciprocal effects.

b. A two-way crossing and parents.
c. Reciprocal crosses without parents.
d. A two-way crossing without parents.

In this experiment, the fixed model and method a were used. The statistical model is:

$Yij = \mu + \lambda i + \lambda j + Sij + eij$

where: μ = population mean; λ (λj) = general combining ability (GCA) of the parent i (j); Sij = specific combining ability of crossing by i j; eij = effect of the environment on the individual ij.

Hayman (1954) used the following symbols for a given character to express the statistics in his model where, VP: variance of a parent; Vr: a variance r parent and his descendants; Wr: r covariance between a parent and his descendants; W'r: covariance between the value of each descendant of r parent and other descendants of that parent; Yr: r value of a parent.

The interpretation by the model of Hayman requires a certain number of conditions: homozygous parents, identical reciprocal crosses, no multi-allelism, diploid parents, absence of epistasis, no maternal effect, independent distribution of the relevant genes of the parents.

The authors can estimate the various genetic components of the change and test their significance from their own variance and the following statistical terms: E: component due to the environment; D: component due to additive effects; H_1: component due to non-additive effects; H_2: component due to unweighted additive effects in terms of a possible asymmetry in the distribution of allele's dominance representative loci; F: covariance between the additive effects and non-additives.
Knowledge of these components allows the following calculations:

$D\text{-}H_1$, in which sign expresses the kind of dominance.

$½ (D + H_1\text{-}H_2\text{-}F) 1/2 (D + H_1\text{-}F) -1 / 4H_2 + E$: Heritability in the narrow sense

The conformity of the model with these restrictions can be rarely achieved in practice. Most of them however, can be checked during the statistical analysis, when the results are consistent with the additive-dominance model Mather and Jinks (1982), although only the interpretation of parental values and F_1 hybrids cannot fully control the factors of non-compliance with the model. Furthermore, the influence of reciprocal effect is erased by working out the average mutual boxes.

RESULTS

Analysis of variance for GCA and SCA and reciprocal using Griffing's method for severity

The results of the variance related to the general combining ability effects (GCA), the specific combining ability (SCA) and the reciprocal effects (RCE) are shown in Table 1.

The analysis of variance was highly significant for the SCA and non-significant for GCA and RCE. SCA effects occur very significantly in expression of severity. The calculated mean value of the GCA/SCA variance ratio is low (1.29).

Analysis of variance for severity by Hayman model

The results of different terms of Hayman variance analysis is presented in Table 2. With regards to the degree of significance of the dominance effects (SCA), the results obtained are consistent with those found using Griffing's method. The results shown in Table 2 are presented based on the different terms described by Hayman. These terms are:

1. The term b_1 is the mean deviation of the first generation F_1 hybrids relative to the average parent which is highly significant for the severity. This result shows that the dominant genes are exerted in a unidirectional manner.
2. The term b_2 which is the average deviation of the F_1 as compared to the average values of each parent is not significant for the severity. This result indicates that there is no asymmetry in the distribution of alleles at loci showing dominance.
3. The term b_3 deviation due to the dominance of own F_1 represents the specific combining ability. This term is highly significant for the severity.
4. The term that tests the differences between reciprocal crosses is not significant for the severity.

Analysis of variance and GCA, SCA and RCE effects by Griffing's method of AUDPC

The results of the variance related to the effect of the general combining ability (GCA), specific combining ability (SCA) and the reciprocity effects (RCE) are shown in Table 3.

The analysis of variance is significant for SCA and not significant for the GCA and RCE. The calculated mean value of the variance ratio GCA / SCA is low (1.24).

Table 2. Analysis of variance for severity in F_1 generation.

Terms of Hayman	Tested effects	Variance (MS)	F
A	additive	16.71	31.36**
B	Dominance	2.04	3.83**
b1	dominance direction	4.32	8.11**
b2	Genes' distribution	1.05	1.97 ns
b3	SCA	2.38	4.46**
C	Maternal Effets	0.12	0.22 ns
D	Reciprocal crosses	0.48	0.9 ns

**: Highly significant; ns: non significant

Table 3. Analysis of variance and AGC, SCA and reciprocal effects by Griffing's method of area under disease progression curve (AUDPC).

Tested effects	Variance (MS)	F
GCA	274.03	3.74 ns
SCA	73.19	3.18*
RCE	11.25	0.49 ns
Variance GCA/variance SCA	1.24	

*: Significant; ns: non-significant; SCA: Specific Combining ability; GCA: general Combining Ability; RCE: reciprocal effects.

Table 4. Analysis of variance for AUDPC in F_1 generation by Hayman's model.

Hayman's terms	Tested effects	Variance (MS)	F
A	additivity	609.42	27.86**
B	Dominance	69.78	3.19**
b1	Direction of dominance	80.08	3.66 ns
b2	Genes' Distribution	62.95	2.88*
b3	SCA	73.19	3.35*
C	Average Maternel Effets	5.42	0.25 ns
D	reciprocal crosses	15.14	0.69 ns

**: Highly significant; ns: non-significant *: significant.

Analysis of variance for AUDPC in F_1 generation by Hayman's model

The results of the different terms are presented in Table 4. The results obtained by the method of Hayman concerning the degree of significance of the dominance effects (SCA) and additive (GCA) are not consistent with those found by Griffing. These results provide the following clarifications:

1. The term b_1 which is the mean deviation of F_1 as compared to the average parent, is highly significant for AUDPC. This result shows that the dominant genes are exerted in a unidirectional manner.

2. The term b_2 which is the average deviation of the F_1 as compared to the average values of each parent is also highly significant for AUDPC.

3. The term b_3 deviation due to the dominance of own F_1 represents the specific combining ability. This term is significant for AUDPC.

4. The term that tests the differences between reciprocal crosses is not significant for AUDPC.

Validity of the assumptions corresponding to the additive-dominance model

The results of the homogeneity of the expression Wr-Vr test are presented in Table 5. The test is not significant for the severity and for the AUDPC, so the model is respected and thus allows further analysis.

Table 5. Analysis of variance homogeneity test (Wr-Vr) attached to each parent according Hayman.

Tested effects	Severity		AUDPC	
	Variance	F	Variance	F
Wr-Vr	0.09	1.34ns	218.77	1.8ns

ns: non-significant; Wr-Vr: degree of dominance.

Table 6. Estimated different genetic characters studied components of F_1 according to Hayman.

Genetic components	Severity		AUDPC	
	Variance	Standard Deviation	Variance	Standard Deviation
E: Environmental variance	0,1776	0,4214	7,2917	2,7003
D: Additive effects	2,678	1,6364	125,486	11,202
H1: No additive effects	1,1094	1,0532	40,1528	6,3366
H2: Unweighted additive effects	1,0199	1,0096	32,5222	5,7028
h2: Dominance heterozygous	0.8079	0.19	10.68	8.15
F: Non-additive x additive covariance	0,6247	0,7903	55,3611	7,4405
D-H1: Type of dominance	1,5686	1,2524	85,3333	9,2376

Table 7. Narrow sense heritability for severity and AUDPC.

Character	Heritability	
	By Griffing	By Hayman
Severity	68.64	63.35
AUDPC	66.99	85.21

Moreover, Vr/Wr regression on the slope of the line for the severity (0.88) and for the AUDPC (1.04) is not significantly different from 1.

Analysis of genetic components

The estimates of the different genetic components of the characters studied for the F_1 are presented in Table 6. These values were used to calculate the narrow sense heritability by Mather and Jinks (1982). The term $D-H_1$ reflects the type of dominance. When this expression is negative, there is super dominance. In that case, the variance of additive effects (D) is smaller than the variance of non-additive effects (H_1). When it is positive, there's partial dominance and this is the case for the severity and AUDPC with respective value of 1.56 and 85.33. When D is equal to H_1, there is a total dominance.

The expression $H_1-H_2 = 0.089$ for severity is low as compared to the H_1 and H_2 estimates of dominance effects. Although, the asymmetry in the distribution of genes is significant (b_2 refers to the analysis of variance), this effect does not play a major role in non-additive effects. The same result was obtained with the area under the disease progression curve (AUDPC); H_1-H_2: 7.63, which is low as compared to the H_1 and H_2 estimates of dominance effects.

Table 7 shows the average values of heritability in the narrow sense obtained by Griffing and Hayman. There is a high heritability strict sense according to Griffing (68.64%) and Hayman (63.35%) for the severity parameter. By cons, it is very high according to Hayman (85.21%) and high according to Griffing (66.99%) for the AUDPC.

Graphical analysis for severity and AUDPC

The graphical representation of Wr (co-variance between a parent r and its progeny) by the Vr (variance of a parent r and its progeny) are given in Figures 1 and 2 for the severity and the AUDPC respectively. Three curves are shown on the graph:

1. A regression line;
2. A dish that cuts the regression line in two points, M and M*
3. A tangent to the parabola is almost confused with the

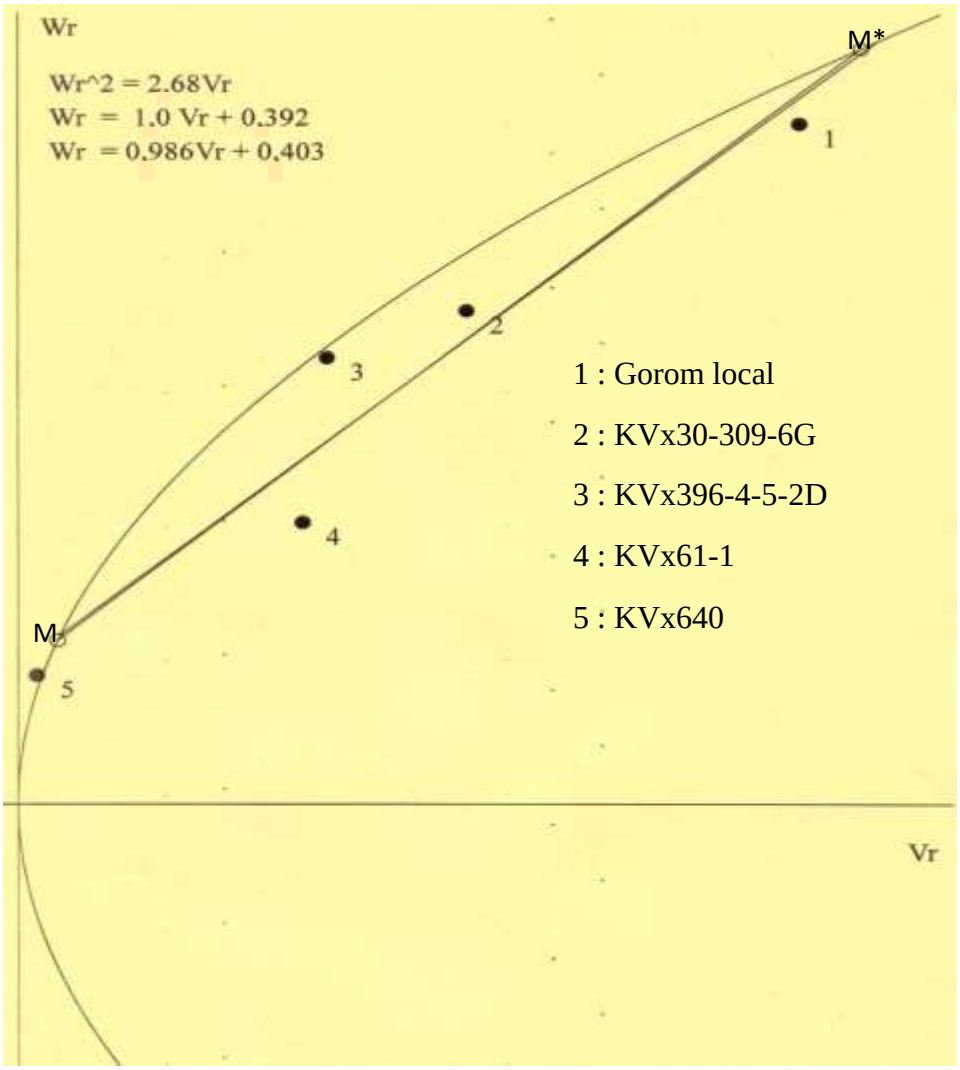

Figure 1. Graphical representation of Wr depending on the severity parameter to Vr. Wr: covariance between a parent r and its progenies; Vr: variance between a parent r and its progenies.

regression line

DISCUSSION

Non-significant GCA was observed for both parameters (severity and AUDPC). This implies that non-additive gene action is operating for these parameters. This result differed from what was observed by Orawu (2007). This author found significant GCA effects in CABMV, suggesting that additive gene action is involved in the resistance of cowpea to the disease. Nevertheless, the ratio of Griffing (1956) between GCA/SCA showed that additive genes were also operating for the resistance of cowpea to CABMV disease. For this author, when the ratio is greater than 1 (one), additive effects are more important than non-additive effects. This is also in agreement with the findings of Singh and Chaudhary (1977). Additive gene action seems to be important in cowpea. Tignegre (2010) also found additive gene action for more than seven parameters under a Striga infestation study.

SCA effects were highly significant for the two parameters studied (severity and AUDPC). This implies that non-additive gene effects involving either dominance or epistasis and in some instances both, were observed for these parameters. However, where non-additive gene effects including epistasis were operative, prediction of the breeding outcome would be difficult as non-additive gene effects are not heritable for pure line cultivars (Tignegre, 2010). Dominance effects (that is, partial dominance, complete dominance or over dominance)

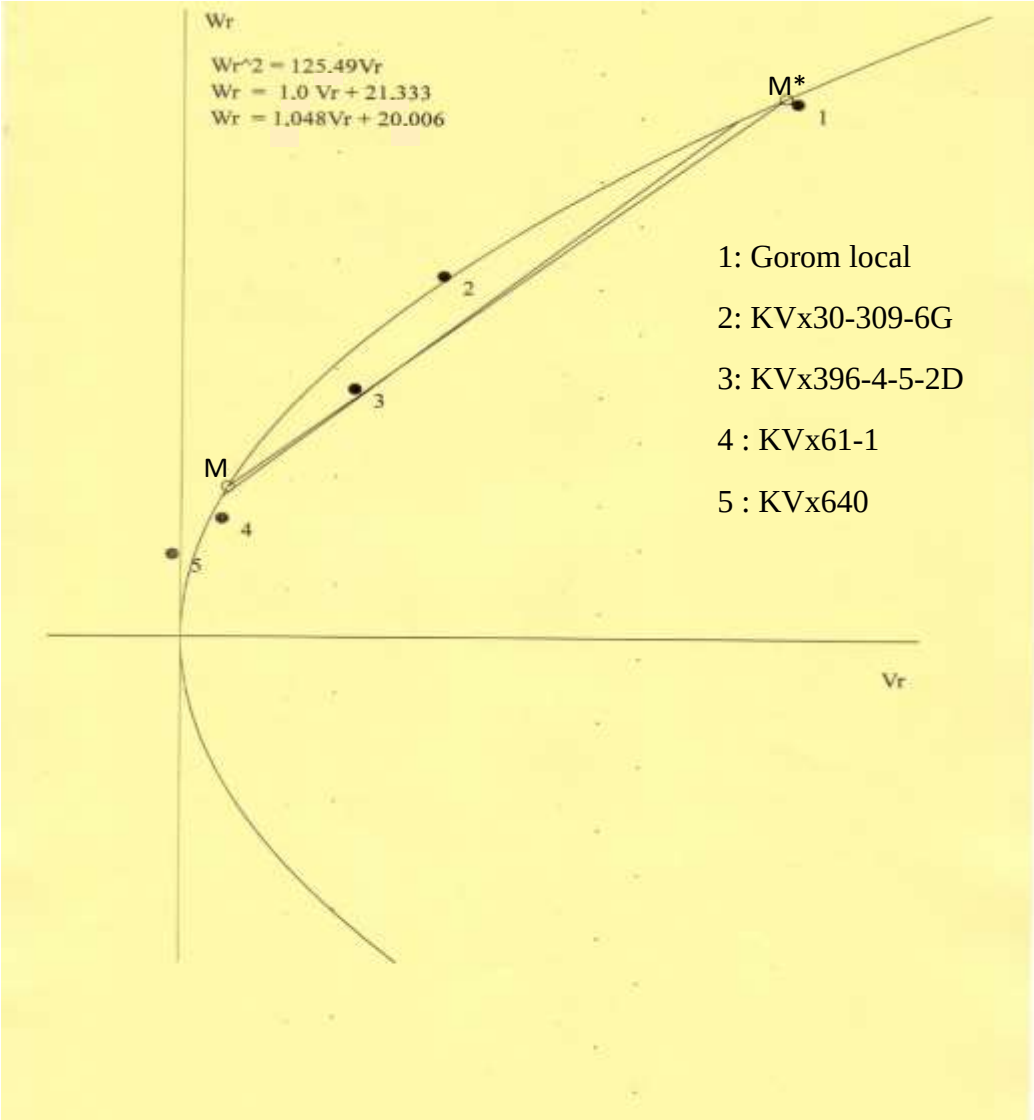

Figure 2. Graphical representation of Wr on Vr function for setting the area under the disease progression curve (AUDPC). Wr: covariance between a parent r and its progeny; Vr: variance between a parent r and its progeny.

cannot be transferred to the progenies and might slow down the progress in selection. However, such gene action would have been useful in hybrid production. Nonetheless, the self-pollinating nature of cultivated cowpea renders difficult the production of hybrid cowpea. However, with some perennial cowpea wild relatives, the occurrence of high rates of cross pollinations (unpublished data) are new fields for hybrid production in cowpea.

There were no maternal and reciprocal effects, suggesting that there were no genetic implications in using a parent as male or female when crossing cowpea for these characters. Therefore, seeds of F_1 and reciprocal crosses can be bulked and used in studying these parameters. These results are in agreement with those of Tignegre (2010). This also implies that no genes originating from the cytoplasm are involved in the inheritance of the characters studied.

Narrow sense heritability measures the breeding value that is passed on to the progenies. Regardless of the method used, high narrow sense heritability was observed in this study. By Griffing's method, the narrow sense heritability was 68.64% for severity and 66.99% for AUDPC. By Hayman's method, the narrow sense heritability was 63.35% for severity and 85.21% for AUDPC. These rates measure the breeding progress that can be expected during selection using the type of protocol employed here.

For all parameters, based on the graphical analysis, with a regression of unit slope b Wr>0.50, a regression coefficient of approximately 50.00% or more indicated

that the additive model was adequate to describe the data (Jinks and Hayman, 1953; Christie and Shattuck, 1992; Dalbholkar, 1992; Sharma, 1995). Considering Figures 1 and 2, two extremes to be taking into account are, M and M* corresponding to the intercepts between the regression line and the parabola. Theoretically, M and M* correspond to the genotypes of the parents that have respectively the parent with dominant genes and parent with recessive genes. All individuals close to M have dominant genes, those close to M* have the recessive genes and intermediate genotypes to the two points have a mixture of dominant and recessive genes. Thus, in both figures, parents 5 and 4 have dominant genes; parents 2 and 3 have both dominant and recessive genes, and parent 1 has the recessive genes for severity and AUDPC parameters. Parents 5 and 4 correspond to resistant genotypes and parent 1 is the susceptible genotype. Parents 2 and 3 are intermediate varieties. The parent 5 is very close to M and parent 1 close to M*. This means that opportunities for transgression are relatively low. The slope of the severity on the regression line is equal to 0.88 and that of the AUDPC is 1.04. These values are not significantly different from 1, showing that there is non-allelic relationship and particularly complementary gene actions between parental combinations. Only additive gene action and partially dominant action exists in the parental combinations. These results are similar to those found in 2012 by Zagre on soybeans.

Conclusion

From this study, it was inferred that from the pot screening, regardless of the method used, non-additive genes were predominant in the inheritance of CABMV resistance with regard to the parameters severity and AUDPC. Only non-allelic interactions (epistasis and failure of some assumption) were present with both parameters (severity and AUDPC).

Narrow sense heritability according to the methods of Griffing and Hayman for severity and area under the disease progress curve is high. This suggests that these resistance parameters are strongly passing from parents to offspring. Hayman's method is more restrictive, the heritability was retain from this model. High values of heritability indicate that additive is the major gene action phenomenon in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Reaction of introduced Korean rice genotypes for resistance to rice blast in Uganda

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Rice blast caused by *Magnaporthe grisea* is an economically important disease which distributed in most rice growing areas of the world. Yield losses up to 100% are attributed to the blast disease in different rice growing regions of Uganda. In order to combat this disease screening of forty-six introduced Korean rice accessions and two checks IR-64 (resistant) and NERICA-1 (susceptible) were done in a 6 by 8 alpha lattice design in two replications under natural infestation in field conditions, and three replications in the screen house at National Crops Resources Research Institute (NaCRRI) of Uganda in 2015, A and B seasons. Final leaf blast severity, lesion size, area under disease progress curve (AUDPC) values, panicle blast and grain yield were highly significant among genotypes. Genotypes SRHB-133, SRHB-93 and SRHB-78 were resistant to rice blast in both field and screenhouse conditions and showed a lower lesion size. Therefore, these genotypes that consistently showed resistance to rice blast disease can be used as a source of resistance gene for rice blast. This leads to conclude that screening in both the field across seasons and confirming their resistance in the screen house helps the breeder to identify the genotypes that are truly resistant for further utilization as resistant sources.

Key words: Rice blast, screening, *Magnaporthe grisea,* Uganda.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important stable foods for more than half of world's population. It provides up to 50% of the dietary caloric supply and a substantial part of the protein intake in Asia (Muthayya et al., 2014). In Sub-Saharan Africa rice consumption among urban dwellers has steadily been grown. From 2002 to 2007, rice production in Africa had increased by an average of 3.2% per year, and from 2007 to 2012 by 8.4% per year (CGIAR, 2013). In Uganda rice production from year 2010 to 2014 increased from 93 to 95 thousand hectares, with a yield increment of 214 to 237 thousand tonnes (FAO, 2014). But, the production and productivity of the crop is hampered by a number of biotic and abiotic factors.

Rice blast, caused by *Magnaporthe grisea*, is one of the most devastating diseases, especially in susceptible

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Table 1. List of selected rice genotypes used for the study in Kampala, Uganda in the two cropping seasons of 2015.

Genotype	Designation	Genotype	Designation	Genotype	Designation
1	SRHB-75	17	SRHB-73	33	SRHB-196
2	SRHB-80	18	SRHB-78	34	SRHB-228
3	SRHB-93	19	SRHB-86	35	SRF3-125
4	SRHB-108	20	SRHB-90	36	SRF3-135
5	SRHB-133	21	SRHB-95	37	SRF3-147
6	SRHB-142	22	SRHB-64	38	SRF3-57
7	SRHB-2	23	SRHB-54	39	SRF3-182
8	SRHB-8	24	SRHB-65	40	SRF3-13
9	SRHB-12	25	SRHB-67	41	SRF3-32
10	SRHB-37	26	SRHB-105	42	SRF3-42
11	SRHB-66	27	SRHB-108	43	SRF3-75
12	SRHB-70	28	SRHB-118	44	SRF3-29
13	SRHB-35	29	SRHB-120	45	SRF3-3
14	SRHB-44	30	SRHB-139	46	SR-7
15	SRHB-56	31	SRHB-170	47	NERICA-1
16	SRHB-71	32	SRHB-182	48	IR-64

susceptible varieties, causing yield losses of 50 to 90% (Hai et al., 2007; Hajano et al., 2011; Chuwa et al., 2015). It is becoming severe under high temperature, high relative humidity (85 to 89%), presence of dew, drought stress and excessive nitrogen fertilization. This disease is a major problem in most of the rice-growing regions of the world (Onasanya et al., 2008). Since the variability of the pathogen from year to year and place to place makes its management difficult, it becomes important to give great attention to resistance breeding (Sharma et al., 2012; Kihoro et al., 2013). It is a serious concern in temperate areas as well as in tropical uplands. Even though the disease affects all the plant parts above ground, seedlings and young or tender tissues are more vulnerable than those of older ones. At optimum temperatures, new blast lesions appear within 4 and 5 days after they fall on the leaf surface. In warm and wet weather conditions, new conidia are produced within hours after the appearance of the lesions, and this continues for several days (Greer and Webster, 2001). Yield reductions due to blast are drastic when panicle itself and the panicle base are infected shortly after heading (Shim et al., 2005).

Geneticaly diversified genotypes play a vital role in any breeding program for resistance to both biotic and abiotic stresses. The use of resistant varieties can not only ensure protection against diseases, but also save the time, energy and money spent on other measures of control (Sharma et al., 2012). The genetics of host-pathogen interactions are of considerable biological interest and great importance in developing disease-control strategies in efforts of resistance breeding (Ribot et al., 2008). Therefore, the present study was conducted to identify rice blast resistant genotypes from a set of introduced Korean rice accessions in Uganda conditions.

MATERIALS AND METHODS

Description of study area and genotypes used

In this study, the first forty-six rice genotypes introduced from South Korea though the Korea-Africa Food and Agriculture Cooperation Initiative (KAFACI) were screened with one resistant (IR-64) and one susceptible (NERICA-1) checks at the National Crops Resources Research Institute (NaCRRI) in Kampala, Uganda during the two rainy seasons of 2015 (Table 1). NaCRRI is located at 0° 31' N, 32° 35' E, with a mean altitude of 1150 m above the sea level. The soils are ferralitic (red sandy and clay loams) and have a pH range of 4.9 to 5.0. The average annual rainfall is 1300 mm and maximum and minimum temperature of 28.5 and 13.0°C, respectively.

Screening under field conditions

A nursery was raised for each genotype and the seedlings were transplanted to the main field. Twenty-one days old seedlings of 48 genotypes were transplanted in the swamp field in a 6 by 8 alpha lattice design with two replications. The spacing of 20 cm between rows and between plants and 40 cm between plots and between blocks with 1 m between replications were used. Four susceptible varieties (NERICA-1, Basimati-370, Sindano and K-85) used as spreader rows were planted between plots two weeks before raising the nursery. This helped to enhance natural infection and to minimize the chance of escape from infection (IRRI, 2014; Vasudevan et al., 2014). In order to promote development of the disease, high humidity was promoted by irrigation twice a day on rain-free days, so that soil of the field experiment was always wet. Other agronomic practices were done as recommended (Asea et al., 2010).

Screening under controlled conditions

Field screened 48 rice genotypes were further evaluated in the screen house using a single isolates of the pathogen to confirm their resistance. Seeds of test lines and the two checks (IR-64 and

NERICA-1) were planted in 25 and 30 cm diameter buckets filled with forest soil (using 4 seeds/pot) in 6 × 8 alpha lattice design in three replications.

Inoculum preparation and inoculation

Blast-infected plants were collected from rice fields at NaCRRI. The infected rice plants were selected by observing the symptoms on the leaves based on the rice blast identification guide (Phadikar et al., 2012). The infected parts were cut into small pieces (0.5-1.0 cm) and then surface sterilized with 2% sodium hypochlorite for three minutes. These pieces were then washed with distilled water and placed on plates of 19.5 g L^{-1} Potato Dextrose Agar (PDA). The PDA plates were then incubated at 25°C for 5 days until sporulation (Hajano et al., 2011). Thereafter, single spores from sporulating lesions were transferred on 4% water agar with the use of an inoculating needle under stereomicroscope for further multiplication for 24 h and the emerging fungus was purified by isolating a single hyphal tip using a sterile needle under a stereo microscope. The resulting pure cultures were incubated at room temperature (25°C) under darkness. After four weeks, the aerial mycelia were slightly washed off by gentle rubbing with a water soaked tooth brush and spore suspension concentration of 1×10^6 spores/ml was prepared using a Neubauer haemacytometer under a compound microscope (Khan et al., 2001). Before inoculation, 0.05% Tween 20 was added to the suspension to increase the adhesion of the spores to the plants. The plants were inoculated with a hand sprayer until run off at the 3 to 4 leaf stage of the plant. High humidity was maintained by covering the area with a white plastic sheet to facilitate infestation. In addition to this, water was sprinkled on the leaves at mid-day for one week, in order to facilitate blast development (Koutroubas et al., 2009).

Data collection

Data on leaf blast severity, lesion size, AUDPC for leaf blast severity and lesion size, panicle blast and yield were collected on five randomly selected plants in the field and on three plants in the screenhouse from each plot according to the standard evaluation system of rice (IRRI, 2014). In addition to these frequency distributions for leaf and panicle blast severity were calculated. Disease evaluations for leaf blast was done four times for each test line at an interval of one week after inoculation in the screenhouse and when the first symptom was observed on the susceptible lines in the field. According to IRRI (2014) standard evaluation system, severity score 0 = no lesions observed, 1 = small brown specks of pin-point size without sporulating center, 3 = small roundish to slightly elongated, necrotic grey spots, 1-2 mm in diameter, 5 = typical susceptible blast lesions 3mm or longer, infecting less than 10% of leaf area, 7 = typical susceptible blast lesions infecting 11-50% of the leaf area and 9 = more than 75% leaf area affected.

$$\text{Blast severity}(\%) = \frac{\text{Sum of all numerical rating}}{\text{Total number of rating x maximum disease rating}} \text{x}100$$

Genotypes were classified according to Shrestha and Misra (1994), for their reaction to leaf blast as 0-15% resistant, 15.1-30% = moderately resistant, 30.1-50% = moderately susceptible and 50.1-100% = susceptible.

To compare relative levels of resistance in the genotypes, weekly assessments of disease severity was done four times. Area under the disease progress curves (AUPDC) was calculated as described by Madden et al. (2008) as; AUDPC = $\sum_{i=1}^{n}\left[\frac{X_{i+1}+X_i}{2}\right]\left[t_{i+1}-t_i\right]$ in which x_i = blast severity at the i^{th} observation, t_i = the time in days after appearance of the disease at the i^{th} day, and n = total number of observations.

Data analysis

The data were subjected to alpha lattice restricted maximum likelihood (ReML) analysis in GenStat 12th edition software package. The genotypes were considered fixed while blocks, replications and season were random effects. However, the randomized complete block analysis was used when the block mean square is greater than the residual mean square. Variance components due to genotypes σ^2_G and genotype by season interactions σ^2_{GXS} and heritability were determined.

The linear model for the across season analysis was as follows:

$$y_{ijkl} = u + s_i + g_j + s/r_{ik} + s/r/b_{ikl} + (s\,x\,g)_{ij} + e_{ijkl}$$

Where, y_{ijkl} = observed value from each experimental unit, u =grand mean, s_i= effect of the i^{th} season, g_j= effect of k^{th} genotype, s/r_{ik} = effect of the k^{th} replication nested within the i^{th} season, $s/r/b_{ikl}$ = effect of r th replication and b th block nested within the i^{th} season, $(s\,x\,g)_{ik}$ = interaction effect of k^{th} genotype and the i^{th} season and e_{ijkl} = the experimental error.

RESULTS

Screening result of genotypes under field conditions

Across season analysis of variance of traits showed significant differences (P≤0.05) among genotypes for final leaf blast severities, lesion size and their respective AUDPC values, panicle blast and yield (Table 2).

The across season analysis result (Table 4) showed that the lowest final leaf blast severity scores (14.3-14.4%) were obtained for three genotypes SRHB-78, SRHB-12 and SRHB-133. Moderately low final leaf blast severities (17.8 - 28.9%) were recorded for ten genotypes which were grouped as moderately resistant. Twenty-four genotypes that had high final leaf blast severities (32.2 - 48.9%) were classified as moderately susceptible. The remaining ten genotypes showed susceptibility levels equal to the susceptible check (Figure 1), NERICA-1 (66.7%) which was followed by SRHB-196 (62.2%).

The genotypes evaluated also showed variation in the AUDPC for leaf blast severity, with seven of them having lower values (120.6 to 182.8%) than the resistant check (IR-64) at 200.3%. Final lesion size ranged from 4.0 mm^2 for genotype SRHB-170 to 63.4 mm^2 for the susceptible check with overall mean of 19.9 mm^2. Low AUDPC values for lesion size were obtained for four genotypes, with mean values ranging from 26.1 to 36.4 mm^2 compared to a value of 43.6 mm^2 recorded on the resistant check (IR-64). The highest lesion size AUDPC was recorded on susceptible check (413.3 mm^2) followed by genotype SRHB-56 (323.8.9 mm^2) (Table 3).

Screening result of genotypes under controlled conditions

The analysis of variance of traits under controlled condition showed significant differences (P≤0.05) among

Table 2. Across season analysis of variance of rice genotypes for leaf and panicle blast severity and lesion under field conditions at NaCRRI, Kampala, Uganda during seasons of 2015A and 2015B.

SOV	df	Severity		Lesion size		PBS	Yield (t/ha)
		FIN	AUDPC	FIN	AUDPC		
Season (S)	1	641.9 ^{ns}	178156**	2276.7**	281720***	441.6**	2.48^{ns}
Rep /Season	2	54.5*	668 ns	21.7**	259^{ns}	4.6 *	0.15^{ns}
Genotype(G)	47	771.9***	158698***	738.1***	35582***	356.5***	1.54***
G x S	47	183.2***	19744 ***	64.2***	5528***	88.2***	0.11^{ns}
Pooled error	76-94	12.3	1157.5	8.5	426	6.6	0.07
Mean		38.8	484	19.9	145	21.9	2.8
GVC		147.2	34738.5	168.5	7513.5	67.1	0.36
VC (G x S)		85.4	9293.3	27.8	2550.9	40.8	0.02
CGD (BH)		0.76	0.88	0.91	0.84	0.77	0.95
CV (%)		9.1	7.0	14.6	14.3	11.7	9.5

*, **, *** significant at 0.05, 0.01 and 0.001 probability respectively, ns = non-significant at > 0.05 probability, SOV = Sources of variation, df = degrees of freedom, Rep = Replication, GVC = Genetic variance component, CGD = Coefficient of genetic determination in broad sense, FIN = Final, AUDPC = Area under disease progress curve, PBS = Panicle blast severity and CV = Coefficient of variation

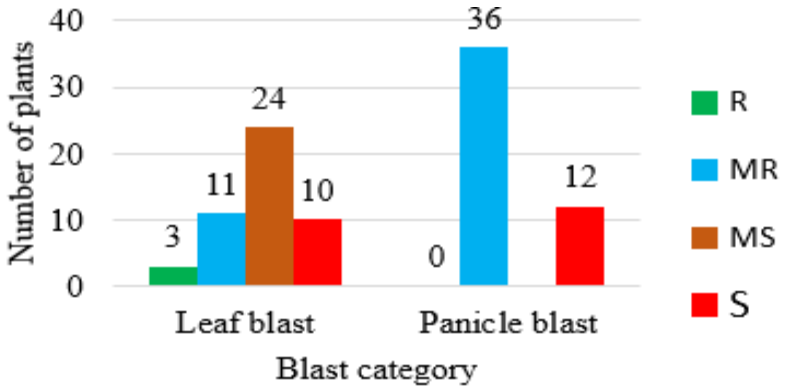

Figure 1. Frequency distribution of rice genotypes for resistance to leaf and panicle blast across seasons under field conditions at NaCRRI, Kampala, Uganda. R = Resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

genotypes for final leaf blast severities, lesion size and their respective AUDPC values, while it showed non-significant for panicle blast and grain yield (Table 4).

The frequency distribution of genotypes for reaction to leaf and panicle blast in the screen house is presented in Figure 2. In this figure five genotypes were resistant, two moderately resistant, twenty-nine moderately susceptible and twelve susceptible. Ten genotypes were resistant to panicle blast, seventeen moderately resistant and 21 were susceptible.

DISCUSSION

Identifying sources of resistance to rice blast has been a major objective for many researchers involved in rice breeding programs (Rama Devi et al., 2015; Biotica et al., 2014; Vasudevan et al., 2014). In this study, 46 introduced genotypes from KAFACI with two checks were evaluated in order to identify resistant sources. The analysis of results revealed that genotypes were significantly different for final leaf blast severity, lesion size, AUDPC values panicle blast severity and yield in both field and screen house conditions. This indicated that genetic variability exists among the screened genotypes, an advantage for improved breeding for blast resistance in rice. Of the genotypes used in this study, none was immune to leaf and panicle blast either in the field or screen house but there were resistant genotypes in these screening conditions.

In the first season's screening for final leaf blast severity under field conditions, four genotypes (SRHB-

Table 3. Disease reaction of rice genotype for blast under field and screen house conditions at NaCRRI Kampala, Uganda during seasons 2015A and B.

Genotype	Field conditions					Screen house condition				
	LBS (%)			Lesion size (mm^2)		LBS (%)			Lesion size (mm^2)	
	Fin	AUDPC	FLBR	Fin	AUDPC	Fin	AUDPC	FLBR	Fin	AUDPC
18	14.4	120.6	R	5.0	26.1	11.1	108.0	R	3.6	19.2
5	13.3	120.6	R	7.1	36.4	11.1	116.7	R	2.4	11.9
3	17.8	143.9	MR	8.7	45.6	11.1	134.0	R	4.2	21.9
9	14.4	147.8	R	9.3	60.1	18.5	142.6	MR	4.0	18.9
12	17.8	147.8	MR	11.6	61.5	11.1	134.0	R	3.0	13.2
31	17.8	182.8	MR	4.5	30.6	35.8	391.0	MS	20.1	119.1
27	17.8	182.8	MR	5.4	34.1	35.8	527.2	MS	19.4	136
13	22.2	210.0	MR	14.7	123.1	38.3	380.2	MS	10.0	55.2
7	21.1	227.5	MR	10.1	63.4	11.1	99.4	R	1.8	8.6.0
39	34.4	276.1	MS	13.9	69.8	35.8	375.9	MS	18.3	108.1
24	27.8	346.1	MR	4.0	36.6	33.3	466.7	MS	11.9	82.9
29	28.9	386.9	MR	9.2	67.8	36.6	430.6	MS	13.4	61.4
26	38.9	394.7	MS	10.3	65.9	51.9	687.0	S	39.8	204.6
23	32.2	408.3	MS	19.8	202.4	30.9	386.7	MS	9.8	72.9
38	38.9	423.9	MS	9.9	62.3	53.1	499.1	S	37.0	170.2
2	38.9	431.7	MS	23.2	130.9	53.1	656.8	S	24.9	156.7
11	34.4	447.2	MS	18.1	126.5	43.2	579.0	MS	17.8	128.6
21	30.0	464.7	MR	12.5	144.9	50.6	527.2	S	21.3	131.7
22	36.7	486.1	MS	16.7	162.0	33.3	276.5	MS	15.3	89.7
43	30.0	497.8	MR	17.3	97.0	33.3	423.5	MS	12.9	109.0
44	37.8	501.7	MS	8.6	62.8	43.2	419.1	MS	18.7	79.6
28	38.9	505.6	MS	9.1	80.3	32.9	534.3	MS	15.5	127.6
40	35.6	507.5	MS	14.1	91.4	45.7	445.1	MS	33.0	147.5
10	48.9	511.4	MS	21.9	169.9	38.3	319.8	MS	11.6	61.0
32	45.6	534.7	MS	11.1	98.1	30.9	276.5	MS	11.4	51.8
36	51.1	550.3	S	20.7	112.7	53.1	587.7	S	40.0	254.8
19	42.2	561.9	MS	25.8	201.1	30.9	350.0	MS	6.4	49.5
17	42.2	573.6	MS	27.1	203.9	55.6	630.9	S	24.7	170.5
41	44.4	585.3	MS	25.7	166.7	43.2	501.2	MS	23.5	155.4
15	45.6	593.1	MS	39.1	318.9	50.6	488.3	S	17.7	125.5
25	51.1	608.6	S	11.3	110.6	44.4	540.1	MS	25.4	159.4
14	51.1	610.6	S	38.9	265.2	33.3	285.2	MS	10.4	66.0
1	46.7	616.4	MS	29.2	196.1	45.7	479.6	MS	19.3	102.1
37	57.8	618.3	S	54.5	323.8	38.3	367.3	MS	23.0	153.4
6	55.6	618.3	S	37.5	243.8	30.9	350.0	MS	8.9	75.3
4	44.4	633.9	MS	39.8	290.7	53.1	760.5	S	30.2	190.3
45	43.3	635.8	MS	11.2	95.3	43.2	445.1	MS	21.0	99.8
30	45.6	649.4	MS	17.7	129.4	45.7	596.3	MS	34.6	204.7
42	51.1	666.9	S	38.9	241.4	43.2	531.5	MS	28.9	169.5
16	44.4	676.7	MS	34.6	316.4	35.8	401.9	MS	8.1	54.6
46	51.1	705.8	S	11.5	115.2	33.3	263.6	MS	6.9	33.1
34	46.7	711.7	MS	13.8	141.6	53.1	522.8	S	34.9	169.0
20	54.4	717.5	MS	33.2	270.5	33.3	337.0	MS	9.7	45.6
8	52.2	731.1	MS	33.9	259.5	45.7	462.3	MS	19.1	119.1
33	62.2	762.2	S	32.1	199.7	50.6	509.9	S	35.2	134.7
35	58.9	764.2	S	9.0	135.0	53.1	626.5	S	40.4	197.9
RC	20.0	200.3	MR	7.9	43.6	27.2	254.9	MR	4.2	21.6
SC	66.7	824.4	S	63.4	413.3	55.6	682.7	S	41.7	258.7

Table 3. Contd.

Mean	38.8	484.0		19.9	145.0	38.2	423.0	18.6	108.0
LSD (P=0.05)	19.3	199.9		11.4	105.8	5.7	71.3	4.1	17.3
CV	9.1	7		14.6	14.3	9.2	10.4	13.3	9.9

RC = Resistant check, SC = Susceptible check, FIN = Final, AUDPC = area under disease progress curve, FLBR = Final leaf blast reaction, LBS = Leaf blast severity, CV = Coefficient of variation, LSD = least significant difference.

Table 4. Analysis of variance of rice genotypes for leaf and panicle blast severity and lesion size in the screen house conditions at NaCRRI, Kampala, Uganda in season 2015A.

SOV	df	Leaf blast severity		Lesion size		PBS	Yield (g/plot)
		FIN	AUDPC	FIN	AUDPC		
Rep	2	60.7 *	24108**	30.7**	840**	8.1^{ns}	85.9^{ns}
Rep/Block	21	12.3^{ns}	2802^{ns}	-	-	-	-
Genotypes	47	489.3***	80917***	404.4***	12655***	433.7***	197.9***
Residual	71 and 92	12.1	1735	6.2	114	4.9	39.1
LEE	73- 77	12.3	1926	-	-	-	-
Mean		38.2	423	18.6	108	22.8	35.8
GVC		159	26330.5	132.8	4180.3	142.9	52.9
CGD(BH)		0.97	0.98	0.98	0.99	0.99	0.80
CV (%)		9.2	10.4	13.3	9.9	9.7	17.5

*, **, *** significant at 0.05, 0.01 and 0.001 probability respectively, ns = non-significant at p> probability, SOV = Sources of variation, df = degrees of freedom, Rep = Replication, LEE = Lattice effective error, GVC = Genetic variance component, CGD = Coefficient of genetic determination in broad sense, CV = Coefficient of variation, FIN= Final, AUDPC = Area under disease progress curve, and PBS = Panicle blast severity.

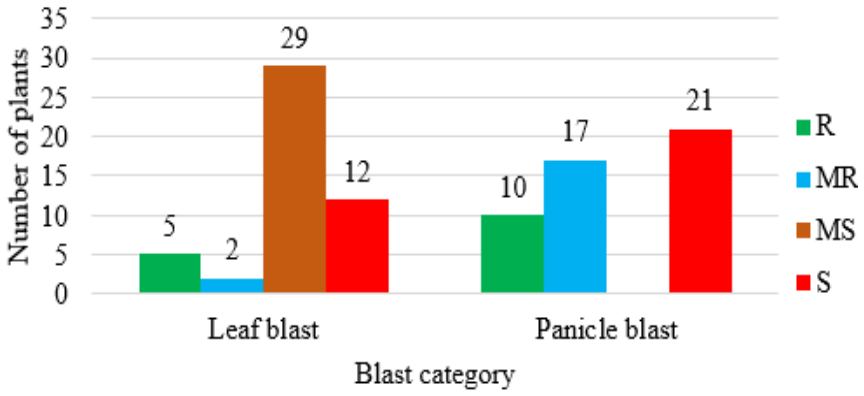

Figure 2. Frequency distribution of rice genotypes for resistance to leaf and panicle blast in the screenhouse at NaCRRI, Kampala, Uganda. R = Resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

133, SRHB-78, SRHB-93 and SRHB-12) were classified as resistant and eight as moderately resistant. In the second season none of the genotypes showed resistance, though 19 that showed moderately resistance were resistant in the first screening. Based on field experiment results across seasons, three genotypes (SRHB-133, SRHB-78 and SRHB-12) showed resistance, and 11 moderate resistances. In the screen house conditions, five genotypes showed resistance (SRHB-93, SRHB-133, SRHB-2, SRHB-70 and SRHB-78) and two moderate resistances. This indicates a difference in performance of the rice genotypes under differing screening conditions and seasons. These results are compatible with the findings of Ghazanfar et al. (2009), Kumar et al. (2012), Pasha et al. (2013a) and Rama Devi et al. (2015) for screening rice genotypes against

resistance to rice blast. Their results revealed that while none of the varieties were immune to blast, genotypes were grouped as resistant, moderately resistant and susceptible. These variations may be attributed variously to genetic difference for resistance to blast, or to variation in environment from season to season and screening conditions. These findings indicate that screening under both field and screen house conditions and in several seasons could be effective for getting genotypes with resistant genes for rice blast disease.

The significant effect of season that produced variation in values for leaf blast, lesion size and their AUDPC values could be due to variable weather conditions. Environmental factors, relative humidity, temperature and amount of rainfall could strongly affect the sporulation, release and germination of blast conidia (Park et al., 2009;Yang et al., 2011).

Variation for panicle blast severity, shown in the analysis of the overall field screening indicates the presence of genetic variation among genotypes. None of the genotypes showed immunity to panicle blast severity, though 36 genotypes were resistant and 12 were found susceptible. However, in the screen house condition 10 genotypes showed resistance, 17 were moderately resistant and the remaining was susceptible. A similar result was reported by Pasha et al. (2013b), Chuwa et al. (2015), Lee et al. (2015). Nagaraju et al. (2008) also reported in screening 265 genotypes, none of them was immune for leaf and panicle blast, eight genotypes were resistant and 138 moderately resistant to leaf blast and 18 genotypes were resistant, and 82 moderately resistant to panicle blast.

Conclusion

In general, this study showed the value of testing the reaction of the introduced Korean rice genotypes to the Ugandan situation, even when they were introduced by the source as being resistant. In this study the across-season field screening results showed that three genotypes were resistant, eleven moderately resistant, 24 moderately susceptible and ten susceptible to rice leaf blast. In the screen house five genotypes were shown to be resistant, two moderately resistant, 29 moderately susceptible and 12 susceptible, again indicating genetic variation among genotypes. Results from the two screening environments showed that genotypes SRHB-133, SRHB-93 and SRHB-78 were more consistent for resistance to rice blast and good performance for yield. So, these genotypes can be either used by farmers after intensive evaluation for production or used to introgress the resistant genes into the locally-adapted elite materials of Uganda. Therefore, genotypes that consistently showed resistance to rice blast disease under both screening conditions can be used as a source for resistance in the rice blast breeding program. From this study, it is possible to conclude that screening in both the field across seasons and in the screen house helps the breeder to identify the genotypes that are truly resistant for further utilization as resistant sources. Additionally, large populations could be screen in the screen house at reduced cost.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Cocoa floral phenology and pollin ation: Implications for productivity in Caribbean Islands

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Cocoa midges [*Forcipomyia sp (Diptera: Cerato-pogonidae)*] are major pollinators of cocoa and it is assumed that the number of fertilized pods and the increase in bean numbers may be the approach to enhancing cocoa yield. An insect survey using suction traps was employed to estimate the midge population dynamics in three Caribbean territories. Separate studies were conducted on the cocoa floral and reproductive phenology in addition to the evaluation of several naturally occurring substrates. The results indicated that the insect population as determined by the suction traps were low (27.1 ± 3.37 to 53.5 ± 8.47 transect site). The trees maintained the floral prolificacy even though the pollination [%] was low for Jamaica (0.91), Trinidad (0.88), and Tobago (0.11). However, it was improved when the midge pollinator population was increased with augmentation of substrates of cacao pods [5660] and banana pseudo-stem (1885). This resulted in significant increases in new pods which increased from < 10 pods/tree in the untreated areas to 49 to 76 pods/tree with substrate augmentation. It was evident that the discarded cocoa pod after harvest was a suitable feeding substrate and breeding site for the midge. This information is to be used to advance further studies in plant-pheromones which can serve as attractants to increase pollination/fertilization in cocoa.

Key words: *Theobroma cacao,* cocoa midges, substrate augmentation, pollinators, floral phenology.

INTRODUCTION

The cacao industry is driven by the major international chocolate manufacturing in Europe and USA. However, all the raw materials are produced in the tropical south and Central America, Africa and the Caribbean (Motamayor et al., 2002). Commercial cacao (*Theobroma cacao* L.; formerly *Sterculiaceae* family; reclassified Malvaceae family] (Alverson et al., 1999) is a tropical tree [3 to 5 m] which is derived from varieties belonging to three major groups *viz*: Criollo, Forastero and Trinitario (Lachenaud et al., 1997).

The varieties and the hybrids exhibit considerable genetic variability in morphological and physiological traits (Cheesman, 1944; Bartley, 2005; Daymond and Hadley, 2004; Maharaj et al., 2011).

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The crop growth is highly influenced by environmental conditions *viz*. temperature (Daymond and Hadley, 2004), flooding (Sena and Kozlowski, 1986), and water stress (Almeida and Valle, 2007). The bi-modal seasons influence the phenological stages of flowering, fruiting and pod growth (Cazorla et al., 1989). The plant produces caulescent flowers with the non-pollinated flowers abscising 24 to 36 h after anthesis (Garcia, 1973). The cacao flower is hermaphrodite and is pollinated by insects, mainly *Forcipomyia* sp. *(*Diptera: Ceratopogonidae (Dias et al., 1997)). The flowers setting to pods are very low [0.5 to 5%] (Aneja et al., 1999).

The quality of pollination can depend on two factors, the degree of pollen compatibility and the number of pollen grains deposited on the stigma (Lanaud et al., 1987). It is assumed that with increased pollen grains pod set is improved (Hasenstein and Zavada, 2001) and more pollinations result from the visit of a single pollinator (Yamada and Guries, 1998). The increase in *Forcipomyia* larvae and pupae associated with rotten banana stems had shown to produce more cocoa flowers (Young, 1986). The pod yield is influenced by photosynthesis and partition of photo-assimilate (Sounigo et al., 2003).

It is assumed that midge population can be a limiting factor in the pollination of cocoa in addition to the environmental conditions. However, populations of insect pollinators are often severely disturbed by hurricanes through flooding of essential habitat and the widespread loss of existing flowers. Small, poor-flight insects such as midges are likely to be swept away by high winds. Climate variation, particularly changes in rainfall leading to sporadic or less rain, may also affect midges which normally thrive in moist humid environments.

Understanding these ecological dynamics can lead to ways of conserving midge populations and mitigating the effects of global climate change and extreme climatic events. The objective of this study is to examine the relationship between the midge population, flower pollination in Trinidad Selected Hybrids (TSH) cacao, and selected weather variables in several different Caribbean cocoa producing islands.

MATERIALS AND METHODS

Characteristics of the study area

A multi-location study during the project period of 2013 to 2016 was conducted on several farms in the islands of Trinidad and Tobago (10.667°N, 61.567°W), and Jamaica (18.1824°N, 77.3218°W) in the Anglo-Caribbean which were previously under natural forest (tropical Montane Crappo-guatecare, fine leaf cocorite, black heart) in altitude 120 to 330 m (Nelson, 2004). The areas experienced annual average temperatures of 26.5 ± 2.09°C, relative humidity of 86.1 ± 12. 6%, and mean monthly rainfall ranging between 19.1 and 235.1 mm (Anon, 2016).

The 4 farms/estates were in Trinidad: Jude Lee Sam Estate (July 2014 - July 2015), San Juan Estate (February 2015 - July 2015), San Antonio Estate (February 2015 - July 2015) in Gran Couva and ECIAF Estate (July 2014 - July 2015) in Centeno. Data was also collected at two (2) sites in Tobago; L'eau Estate (November 2014 - July 2015) and Providence Estate. The 2 estates selected in Jamaica were: Orange River (September 2014 - October 2015) and Richmond (October 2014 – October, 2015).

The cocoa vars. were mainly form the Trinidad Selected Hybrids [TSH] (Maharaj et al., 2011), and the trees were in full reproductive phase. The first flowerings were in early January over a 3 month period, and a second period, depending on the rains, in June. Harvesting usually occurred over a 2 month period around 6 months after the first flowering.

All the islands experienced a bimodal rainfall distribution, with peaks in June and November. The first and second growing seasons typically last from mid-March to mid-July and from mid-August to end of November, respectively. However, this is separated by a short dry spell of about two weeks in September and referred as *petite careme*. The major dry season starts in mid-December and lasts till end of May, and the climate is marked by high incidence of solar radiation and relatively little variation in day length. All data on temperature and relative humidity were measured using the Data Davis Wireless Vantage Weather Pro [Model E14062 Rainfall data, were taken from the meteorological records from the National Water Resources Agency.

Experimental

Four separate studies were conducted during the period 2013 to 2016 in which the European Union COCOAPOP was executed in the following areas:

1. Insect population dynamics.
2. Cocoa floral phenology.
3. Substrate augmentation trials for culture of cocoa midges (Diptera: Ceratopogonidae), and
4. Generalized linear modelling of weather, midge dynamics and floral phenology.

Study 1: *Insect population dynamics*

The cocoa insect population dynamics survey was conducted in the 3 islands on 2 well established and managed farms that cultivated the cacao TSH variety under similar agronomic practices. The selected farms were of similar altitude (120 m) and agronomic conditions. The study was conducted over a minimum of fifteen (15) months duration (2013-2015). However, the data analysis was confined to 2 complete flowering seasons over 1 year period.

Insect suction traps (Arnold and Chittka, 2012) were set up in 9 representatives transects within each cocoa estate of the different territories. These traps were secured onto branches of cocoa tress, powered by 9-volt batteries and insects were sucked into vials containing 90% ethanol. Insect samples were collected for 2 days/month for each sample site, labelled, stored properly for analysis in the insectary for other insects and midge count. Collection was timed to the midge life cycle (Figure 1).

Study 2: Cocoa floral phenology

The cocoa floral phenology was conducted on the same cocoa farms for each island. Over 20 mature cocoa trees95 to 12 m tall] with 5 cushions/ tree were randomly selected and labelled within an experimental area not exceeding 500 m^2. The study ensured that data were collected from a minimum 100 plants over 3 consecutive flowering years (2013 to 2015). The observations were conducted monthly on each tree using the modified BCCH (Bleiholder et al., 1991) on counts of flowers, buds, number of mature flower buds, open flowers, new pods or cherelles, small pods (5 to 10 mm),

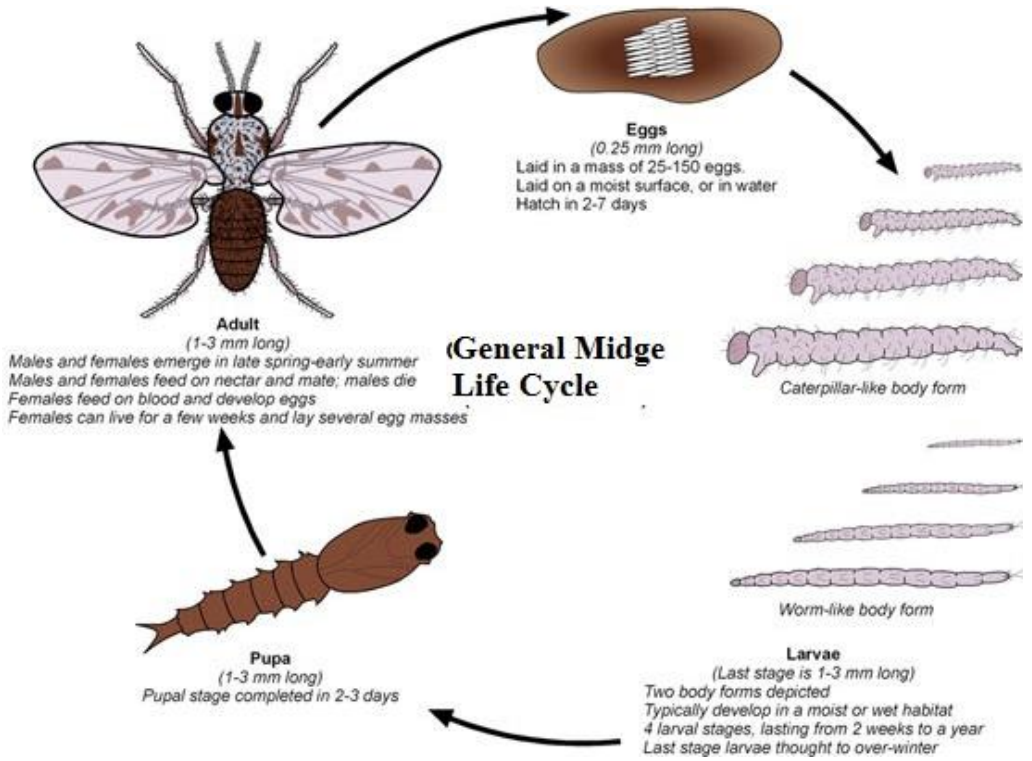

Figure 1. Biting midge life cycle. Illustration by: Scott Charlesworth, Purdue University, based in in part on Pechuman, L.L. and H.J. Teskey, 1981, IN: Manual of Nearctic Diptera, Volume 1.

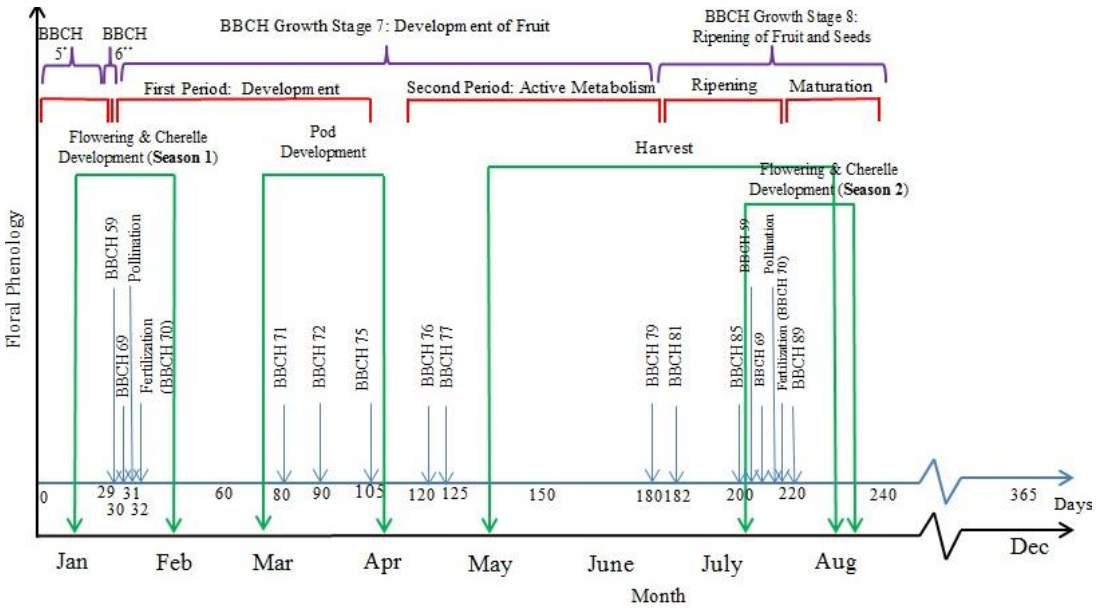

Figure 2. Cocoa floral and reproductive phenology using the modified BBCH model. *BBCH 5, inflorescence emergence; **BBCH 6, flowering.

medium pods (11 to 60 mm), and large pods (>60 mm); numbers of diseased pods, number of aborted pods (cherelle wilt), and fruit-set over both season.

The BBCH scale was amended to include days from the first day buds become visible [FBV] for each stage and was used to compute the length of each reproductive phase (Figure 2).

Table 1. The principal reproductive growth stages 5 to 7 of *T. cacao* var. TSH according to the BBCH (Biologische Bundesantalt, Bundessortenamt and CHemische Industrie, Germany) scale.

Principal growth	Code	Description
Stage 5: Inflorescence emergence	52	Flower buds expanded, emergence of sepal primordia (bud <1 mm long).
	59	Flower bud growth complete (buds 6 mm long and 3 mm large; pedicle 14 mm), buds l closed
Stage 6: Flowering code	61	Beginning of flowering
	69	90% of flowers open
	71	Beginning of fruit growth. Endosperm cellularisation, ovule and pericarp development. Beginning of the cherelle wilt phase. Fruits have reached 10% of final size (zygote dormant)
Stage 7: Development of Fruit	75	End of the cherelle wilt phase. D/L 0.35. Fruits have reached 50% of the final size
	79	Embryos are full-grown, only traces of endosperm remain round the fleshy cotyledons

Figure 3. Chopped banana pseudo-stem as midge substrate.

The BBCH Scale (Bleiholder et al., 1991) and the extended BBCH scale (Hack et al., 1992) covered the 10 principal growth stages numbered 0 to 9 (Table 1). However, for the purpose of this study, only 4 of these stages were considered; namely 'macro stages' numbered from 5 to 7.

Study 3. **Substrate augmentation trials for culture of cocoa midges (Diptera: Ceratopogonidae)**

Two (2) separate studies were conducted on 3 commonly found substrates within the fields to determine if they can augment the midge population as suitable breeding sites (Figures 3 and 4). These studies were confined to Trinidad farms only, as the insectary was located there. The substrates assessed over the 2 crop seasons in 2015 were as follows:

1. Field substrate in-situ assessment, and

2. Field augmentation and insectary evaluation.

Field substrate *in-situ* assessment: During the cropping season of 2015, four (4) cocoa farms were designated for field manipulation to determine if the substrates had any effect on the midge population dynamics. Three substrates were assessed in heaps viz: Rotted cocoa pod (15 kg) (Figure 4), banana pseudo-stem slices (Figure 2) (15 kg) and cocoa leaf litter, all of which replicated three times per farm. All treatments were moistened (5 L water/heap/weekly). The experimental sites (25 m^2/substrate) were laid out as a Latin square (3 × 3) design. During the first 2 months, insect populations were monitored for 2 days per month using a standard suction trap placed in the approximate centre of each area. Cocoa floral phenology was also monitored during the duration of the study which lasted over 6 months.

Field augmentation and insectary evaluation: The field experimentation was conducted at one farm (Gran Couva, Trinidad)

Figure 4. Freshly harvested cocoa pods as midge substrate.

and over a five week period [September to October, 2015]. The treatments were the same three substrate treatments with some variations *viz*., fresh cocoa split pods (35 kg), fresh banana pseudo-stem [35 kg, 10 cm thick slices) and cocoa leaf litter (35 kg) with three replicates of each treatment. The substrates were placed at the base and within the buttress of 15 randomly selected immortelle trees to aid moisture retention. All trees were located within 20 m of one another and from the edges of the field. The substrates samples (2 kg) were evaluated for midge oviposition and larval development from the centre of the piles at 7-day intervals in the insectary. The Ceratopogonid midge larvae after developing in the organic matter were collected using the *Berlese Funnel Traps* (Dietick et al., 1959). The substrates were inspected for larger midge larvae (*Forcipomyia* spp.) which are removed from the substrates and placed in a ball of well-decomposed cocoa pod husk with 100 larvae/vial and adequate air-flow and temperature (26°C).

Study 4. Generalized linear modelling of midge dynamics, floral phenology and weather variable

The approach was to determine the relative role of the midge population dynamics and cocoa floral and reproductive phenology, and its interaction under the prevailing weather variables (rainfall and temperature). This study was conducted over the period 2014 to 2015 in the three countries (Trinidad, Tobago, and Jamaica) on two estates per country. The data was collected from previous midge collection and the floral phenology trials and daily weather data (Table 11) for each location. Best fit generalized linear models were developed to determine the interactions and significance.

Data analysis

The count of flowers and other parameters taken were pooled together on each farm, but separate for each location. All count data were transformed when necessary using the square-root ($\sqrt{x}$ + 0.1) before analysis. Regression analysis were used to determine the relationship between weather variables (temperature, relative humidity, rainfall and light intensity) and flower production, and insect population dynamics using the MINTAB statistical package.

RESULTS AND DISCUSSION

Study 1. Insect population dynamics

There were significant differences between the monthly midge and other insect's population and farms over the 3 territories. There were two distinct and observable high populations May/June and November/January. These periods coincided with the new flushes of cocoa flowers (Figure 4) and the higher rainfall patterns. In Trinidad, the seasonal midge population was 19 ± 3.65 and 53.5 ± 8.47 compared to Tobago which varied between 27.1 ± 3.37 and 22.6 ± 6.47, and Jamaica 21 to 28 ± 4.39/ transect site (Table 3).

In all territories, the low midge population varied between 2 to 6 midges/transect site. Jamaica (82) and Tobago (72) had higher midge populations compared to Trinidad (45). The other insect's population was significantly higher than midges and varied between 1067 and 1547 insects/transect site. This indicated that the midge population was less than 2% of the insect trapped (Table 4).

Study 2. Cocoa floral phenology

The cocoa floral and reproductive phenology followed a similar pattern (Figure 4) as outlined on the modified model developed by Bleiholder et al. (1991). In Trinidad, the mean number of flowers was 33.6 ± 6.1/cushion, with the highest ranging between 40 to 96 flower/cushion

Table 2. Codes and Descriptors used for cocoa phenological cycle in 6 cocoa farms.

Code	Description
F	Flowers
C (0-2)	Cherelles (0" - 2.0")
C (2.1 - 3.9)	Cherelles (2.1" - 3.9")
P (>4)	Pods (>4")
CW	Cherelle Wilt
BP	Black pods
H/T	Harvest/tree
S/P	Seeds/pod

Table 3. *Forcipomyia* sp. *(Diptera: Cerato-pogonidae*) population dynamics in 6 cocoa farms over 3 Caribbean Islands during 1 year.

Month	Trinidad				Jamaica				Tobago			
	Farm 1		Farm 2		Farm 3		Farm 4		Farm 5		Farm 6	
	Other	Midge	Other	Midge	Other	Midge	Other	Midge	Other	Midge	Other	Midge
JAN	537	18	2631	121	1576	35	1017	16	571	28	444	8
FEB	2540	17	1807	58	1048	14	916	14	1500	37	1600	12
MAR	399	2	614	11	1117	9	1360	16	2250	47	2568	19
APR	947	3	1040	15	1159	8	996	26	2723	30	1882	10
MAY	2110	15	1297	50	1431	35	1722	24	2013	15	1127	3
JUN	1459	45	1669	78	730	21	2264	28	496	2	463	6
JUL	1400	45	1337	30	399	7	1303	15	1089	15	1734	34
AUG	773	9	1897	52	950	5	1129	12	1614	24	831	10
SEP	1048	18	1661	38	954	16	2089	46	1500	23	891	60
OCT	1143	14	1839	30	1679	72	1770	41	1121	35	1109	82
NOV	582	17	1480	82	370	3	1111	36	964	43	736	13
DEC	695	25	1301	77	1391	29	2064	67	806	27	521	15
$\bar{x}$ ± **SE**	1136±173	1136±173	1547.**±**133	53.5±8.47	1067**±**112	21.1**±**5.20	1478.4**±**128	28.4**±**4.39	1387.2**±**181	27.1**±**3.37	1158.8**±**181	22.66**±6**.47

[July/Sept, 2015] and lowest [21 to 28] during Jan/March, 2014 (Tables 2 and 5). This represented the 2 major flowering flushes, which corresponded with the early and late wet seasons, respectively.

Tobago experienced a similar weather pattern to Trinidad during that period (Table 11), and the trees in the study exhibited a slightly higher mean flower/cushion (51.1 ± 7.61). The mature cocoa trees displayed 2 distinct flushes, with the first in November/December 2014 (45 to 89), and a second flush (65 to 81) in the beginning of the wet

Table 4. Midge population (%) compared to other insects in cocoa farms over the three locations.

Territory	% Midge to other insect populations	
	Farm 1	Farm 2
Trinidad	1.67	3.42
Jamaica	1.89	1.09
Tobago	1.94	1.95

Table 5. Cocoa phenological cycle in 6 cocoa farms over 3 Caribbean Islands during a one year period [2014/15].

Month	*Trinidad*								Jamaica								Tobago							
	F	*C (0-2)*	*C (2.1-3.9)*	*P (>4)*	*CW*	*BP*	*H/T*	*S/P*	F	C (0-2)	C (2.1-3.9)	P (>4)	CW	BP	H/T	S/P	F	C (0-2)	C (2.1-3.9)	P (>4)	CW	BP	H/T	S/P
July/14	40	0	0	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Aug/14	96	2	0	0	0	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sept/14	48	4	2	0	0	x	x	x	29	0	0	0	1	0	0	0	x	x	x	x	x	x	x	x
Oct/14	33	2	4	1	0	x	x	x	36	2	2	0	1	0	0	0	x	x	x	x	x	x	x	x
Nov/14	32	1	3	3	0	x	x	x	61	3	3	1	1	0	0	0	89	0	1	0	0	x	x	x
Dec/14	19	0	1	3	1	x	x	x	20	3	2	2	0	0	0	0	45	0	0	0	0	x	x	x
Jan/15	21	1	0	1	1	x	x	x	10	1	1	3	3	0	0	0	28	0	0	0	0	x	x	x
Feb/15	19	0	1	0	1	x	x	x	15	1	1	4	3	1	0	10	24	0	0	0	0	x	x	x
Mar/15	28	0	0	0	1	x	x	x	22	0	0	3	3	1	1	30	41	0	0	0	0	x	x	x
Apr/15	28	0	0	1	0	x	x	x	63	1	0	3	1	0	0	10	56	0	0	0	0	x	x	x
May/15	12	0	0	0	0	x	x	x	24	1	0	1	0	0	0	14	65	0	0	0	0	x	x	x
Jun/ 15	48	0	0	0	0	x	x	x	78	0	1	1	0	0	0	12	81	0	0	0	0	x	x	x
July 15	15	0	0	0	0	x	x	x	20	0	0	1	0	0	0	9	36	0	0	0	0	x	x	x
Aug 15	x	x	x	x	x	x	x	x	34	0	0	1	0	0	0	0	x	x	x	x	x	x	x	x
Sept/15	x	x	x	x	x	x	x	x	13	0	0	1	0	0	0	0	x	x	x	x	x	x	x	x
$\bar{x}$	33.8	0.77	0.85	0.69	0.31	x	x	x	32.5	1.05	0.91	1.6	1.0	0.12	0.14	6.53	51.67	0	0.11	0	0	x	x	x
SE.	6.1	0.34	0.37	0.31	0.13	x	x	x	5.98	0.30	0.27	0.33	0.36	0.07	0.05	2.46	7.619	0	0.11	0	0	x	x	x

season (May/June, 2015). The mean flower/ cushion in Jamaica did not vary compared to Trinidad (32 ± 5.98), as the trees were of same variety and age, and also displayed two distinct flusher in Sept/Nov, 2014 (29 to 61) and April/June, 2015 (63 to 78).

The percentage of flowers that were pollinated and successfully fertilized i.e. (Flowers → Cherelles (0" – 2.0")) were higher in Jamaica

Table 6. Population of midges harvested from Cocao field, Centeno (Trinidad).

Months	Cacao leaf litter	Cocoa pods	Banana pseudostems	$\bar{x}$ [SE]
March	4.75	4.25	4.75	4.6±[0.17]
April	5	4.25	2.75	4.0±[0.66]
May	3.5	3.25	1.5	2.8±[0.63]
June	2.75	1.75	2	2.2±[0.30]
July	5	8.25	7	6.8±[0.95]
August	11.5	9.5	11.75	10.9±[0.71]
$\bar{x}$ ± SE	5.4 ± 1.27	5.2 ± 1.23	5.0 ± 1.59	

Table 7. Population of midges harvested from Cocao field, Gran Couva (Trinidad).

Substrate type	Average male	Average female	Average midges	Total midges
Cacao pods	123.9	192.2	316.1	5660
Banana pseudostem	37.61	71.8	109.5	1885
Cacao leaf litter	1.1	2.81	3.7	65
$\bar{x}$ ± SE	54 ± 36.4	88 ± 55.3	143 ± 91.7	2537 ± 1648

(0.91) compared to Trinidad (0.88), and Tobago (0.11). This manifested with a similar pod/cushion yield between countries, with Jamaica (1.5) having a higher pollination/ fertilization, compared to Trinidad (1.0) and Tobago (<1), and was very low for that season (Table 9).

Study 3. Substrate Augmentation trials for culture of cocoa midges (Diptera: Ceratopogonidae)

Trial 1: Field substrate assessment

The field trials (Table 6) indicated that there were no variations between the 3 substrates (5.0 to 5.4 ± 1.27) during the experimental period. However, during the wet months of July/August, 2014, the number of midges caught in the suction traps located in the areas of the banana pseudo-stem, and cocoa pod increased, compared to the litter substrate. Similarly, the cocoa leaf litter was not significantly different from pods or pseudo-stems in August.

The number of midges per suction trap in this trial was consistent to the results obtained in the cocoa insect population dynamics studies (2013/14). The study demonstrated that regardless of the quality of the substrate to improve on the feeding and fecundity of midges, the suction trap appeared to have a determining factor, and may not actually reflect the substrate suitability.

Trial 2: Field manipulative and laboratory evaluations

In this study, no suction traps were used, but samples of the substrate were removed and incubated in the insectary, where the emerging larva were counted, and reared to adult. The results in this study are different from Trial 1, and reflected the potential midge population when interventions of substrates are manipulated in the field.

The fresh cocoa pod (Table 7) left to decay was the preferred substrate for the adult midge to feed and continue its reproductive cycle (Figure 1). The total midge population in the cocoa pod was 3 to 4 times higher than the banana pseudo-stem. The data suggested that increasing the breeding sites with augmentation of cocoa pod substrates can increase the midge population (Table 7) dynamics in the field and new pods development (Table 8). Further, the use of suction traps are not effective or a reliable indicator of the true insect population dynamic in the cocoa estates.

Study 4. Generalized linear modelling of midge dynamics, floral phenology and weather variable

This study involved data transformation and statistical manipulation of observations on the cocoa crop reproductive phenology (Table 9), and midge population dynamics (Table 10) during a one year period, and taking into consideration the prevailing weather variables (Rainfall and Temperature at the different Farm locations) (Table 11).

The generalized linear model revealed that there were variations between farms which influenced the yield of flowers and cherelles (Table 12). Also, the variation in rainfall between months, confirmed the bimodal (wet/dry) season which affected flower emergence and pollination into cherelles. The other main variables in the model;

Table 8. Cocoa pod yield in farms with substrate augmentation.

Substrates	New pod count							
	Cocoa estates							
	San Juan		San Antonio		Jude Lee Sam		Centeno	
	$\bar{x}$ ± SE		$\bar{x}$ ± SE		$\bar{x}$ ± SE		$\bar{x}$ ± SE	
Cocoa litter	12	3.53	22	6.49	29	9.07	20	6.01
Cocoa pods	13	3.93	28	9.02	49	15.86	67	22.01
Pseudo stems	13	3.28	19	6.00	16	5.03	14	4.37

Table 9. Cocoa floral phenology and pod yield in 6 cocoa farms over 3 Caribbean Islands during a one year period [2014/15].

Month/year	Trinidad						Jamaica						Tobago					
	Farm 1			Farm 2			Farm 1			Farm 2			Farm 1			Farm 2		
	Fr	C (2.1-3.9)	Pod (>4)	Fr	C (2.1-3.9)	Pod (>4)	Fl	C (2.1-3.9)	Pod (>4)	Flr	C (2.1-3.9)	Pod (>4)	Flr	C (2.1-3.9)	Pod (>4)	Fl	C (2.1-3.9)	Pod (>4)
July/14	19	1	3	0	0	0	19.5	2.2	1.5	0	0	0	45	0	0	0	0	0
Aug/14	21	0	1	0	0	0	10	1.2	3.1	0	0	0	28	0	0	0	0	0
Sept/14	19	1	0	0	0	0	14.5	0.7	4	0	0	0	24	0	0	0	0	0
Oct/14	28	0	0	0	0	0	21.6	0.4	2.9	0	0	0	41	0	0	0	0	0
Nov/14	28	0	1	0	0	0	62.9	0.2	2.6	0	0	0	56	0	0	0	0	0
Dec/14	12	0	0	0	0	0	24.1	0.2	1.2	0	0	0	65	0	0	0	0	0
Jan/15	48	0	0	0	0	0	78	0.7	1.3	0	0	0	81	0	0	0	0	0
Feb/15	40	0	0	0	0	0	19.7	0.3	1.2	0	0	0	36	0	0	0	0	0
Mar/15	96	0	0	0	0	0	33.8	0.4	1.2	0	0	0	0	0	0	0	0	0
Apr/15	48	2	0	0	0	0	29.4	0.1	0	0	0	0	0	0	0	0	0	0
May/15	33	4	1	0	0	0	35.5	1.9	0	0	0	0	0	0	0	0	0	0
Jun/ 15	32	3	3	0	0	0	61.1	3.3	1.1	0	0	0	89	1	0	0	0	0
$\bar{x}$ ±SE	35±6.4	0.91±.39	91.0±0.39	0.75±0.32	0.0±0.0	0.0	0.0	34±6.25	0.9±0.28	1.67±0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

midge, other insects, and temperature, were not significant and had no impact on flower and pollination. Additionally, the analysis did not reveal any interactions between any of the independent variables on flower and cherelles (Table 13). The analysis showed that the ratio of flowers to cherelle per cushion varied between territories: Jamaica (33:10), Trinidad (33:0.7), and Tobago (18:0.3). However, this data has to be interpreted in the light of the limitations of the suction trap and the true midge population as reported in Study 3. Further, the numbers of flowers were similar

Table 10. Cocoa midge populations in 6 cocoa farms over 3 Caribbean Islands during a one year period [2014/15].

Month	Midge population [SQRT]					
	Trinidad		Jamaica		Tobago	
	Farm 1	Farm 2	Farm3	Farm 4	Farm 5	Farm 6
January	4.4	11.0	6.4	4.1	5.4	3.0
February	4.2	7.7	5.8	3.9	1.0	1.0
March	1.7	3.5	5.9	4.1	6.9	4.5
April	2.0	4.0	5.9	5.2	5.6	3.3
May	4.0	7.1	6.2	5.0	4.0	2.0
June	6.8	8.9	5.3	5.4	1.7	2.6
July	1.0	5.6	4.6	4.0	4.0	5.9
August	3.2	7.3	5.6	3.6	5.0	3.3
September	4.4	6.2	5.6	6.9	1.0	1.0
October	3.9	5.6	6.5	6.5	6.0	9.1
November	4.2	9.1	4.5	6.1	6.6	3.7
December	5.1	8.8	6.2	8.2	5.3	4.0
$\bar{x}$ ± **SE**	3.7 ± 0.45	7.0 ± 0.63	5.7 **±** 0.18	5.2 **±** 0.41	4.3 **±** 0.60	3.7 **±** 0.64

Table 11. Selected weather (Temperature, °C and rainfall, mm) in the cocoa experimental areas during the study.

Month	Trinidad		Tobago		Jamaica	
	Temperature	Rainfall	Temperature	Rainfall	Temperature	Rainfall
January	26.9	281.9	29.4	142.2	23	141.5
February	27.2	293.8	29.4	96.5	23.3	16.6
March	27.2	285.2	29.4	76.4	23.2	26.6
April	28.0	256.	30	105.16	24.2	108.9
May	28.6	247.3	30.5	226.8	24.4	112
June	28.2	251.4	30.5	460.2	26	9.4
July	28.2	275.3	30	431.2	26.7	1.6
August	28.6	293.37	30	329.1	26.5	68.4
September	28.8	305.5	30.5	235.2	25.2	4.2
October	28.7	298.9	30.5	287.0	25.4	0.4
November	28.0	284.4	30.5	389.1	24.5	14
December	27.0	0	30	285.7	23.3	14.6

between territories and treatment substrate, and pollination: fertilization ratio was not affected, regardless of the indicator midge population dynamics.

In the augmentation of substrates, freshly harvested cocoa pod waste was the best medium for midge incubation, and was 3 times more desirable than banana pseudo-stem (Table 7). Similarly in the plots with this substrate, there was significantly improved new pod development in all the locations. There is evidence that pod yield increased with increasing midge population as the substrate improved from decaying cocoa leaf litter, to banana pseudo-stem, to cocoa pod (Equation 1).

$$Y_{\text{New pod}} = \underset{[9.2^{10\text{-}2}]}{10.2} + \underset{[4.2^{10\text{-}3}]}{0.009}\ \text{Midge: } R^2 = 82\%; \quad (1)$$

Generally, the flower emergence per cushion was well within the acceptable expectation for the variety. The midge population was the main pollinator as demonstrated by the substrate study and suggest that it was adequate for the fertilization process, although low. However, the new pod yield was acceptable 12 to 67 (pods/ tree) particularly when the midge was present.

Pound (1933) recommended the minimum yield of pods (50) for a fully grown TSH cocoa tree requiring 7 1/2 pods to 0.5 kg, and yielding 3.5 kg of high class cacao, and 25 for trees 10 to 15 years old. After standardized manual cross-pollination, Bos et al. (2007) obtained 12 fruits/trees and harvested under shade management an average: 27 ± 4% fruits/tree. In Upper Amazon cacao hybrids, 38 to 66% of the trees produced 1 to 10 pods/tree and 7 to 39% had more than 10 pods/tree/year, and high yielding

Table 12. Generalised linear model of Farm x Month x Midge pop x other insect x rain fall x temperature on flower and cherelle production in cocao over 6 farms in three Territories [2014- 2015].

Predictor coefficient	Coefficient	SE	T	P
Constant	53.65	20.20	2.66	0.010
Farm	-7.067	2.901	-2.44	0.018
Month	1.2711	0.9100	1.40	0.167
% Midge/Total	0.940	4.013	0.23	0.816
Insects [SQRT]	-0.2328	0.5306	-0.44	0.662
Midge [SQRT)	-3.416	3.643	-0.94	0.352
Temperature	0.4917	0.4154	1.18	0.241
Rainfall	-1.9317	0.9998	-1.93	0.058

Table 13. Mean farm, month, midge pop, other insect, rain fall, flower and cherelle production in cocoa over 6 farms in three territories (2014 - 2015).

Variable	Mean	SE Mean	St.Dev
Farm	3.500	0.203	1.720
Month	6.500	0.410	3.476
% Midge/total	2.236	0.181	1.538
SQRT(total)	35.068	0.972	8.250
SQRT(Cera)	5.298	0.218	1.852
Temperature	23.44	1.03	8.73
Rainfall	3.362	0.603	5.121
Flower	18.04	2.95	24.99
Cherelle (0-2)	0.3278	0.0953	0.8090

tress and produced up to 180 pods/tree/year (Adomako and Adu-Ampomah, 2003).

According to Mohamed (pers. comm. 2016) the morphology of the cocoa flower does not lend itself easily to insect pollination due to the presence of the staminodes surrounding the style which has a needle-like stigma. The position of the hooded anther opening obtusely from the ovary base makes it difficult to transport the sticky pollen grains downwards. The flower orientation is like a dangling pendulum. The insect will descend directly on the area surrounding the ovary where the nectar glands are located. Per chance if it was crawling out of the flower it passes on the surface of the style, probably depositing pollens on the way out. These pollen will germinate on the surface of the style and affect fertilization. The stigma is no way involved in the fertilization process. The germination of the pollen grains could only occur along the style while the tiny midge is crawling out hence the reason for low pod set.

Krauss and Soberanis (2002) reported that fertilizer improved yields by 11% independent of the disease control measure, but Groeneveld et al. (2010) found that both pollination and resource (shade, fertilizer and water) limitations may cause low fruit : Flower ratios in *T. cacao*. However, none of the resource availability treatments had a significant effect, while number of mature pods and yield increased non-linearly with pollination intensity up to 200% of current yield levels. Despite an increase of fruit abortion with pollination intensity, *T. cacao* yield is determined by the number of flowers pollinated. This suggests pollination deficit in crops can be very large and that a better knowledge of pollen and resource limitation is needed to devise adequate pollinator management strategies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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