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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

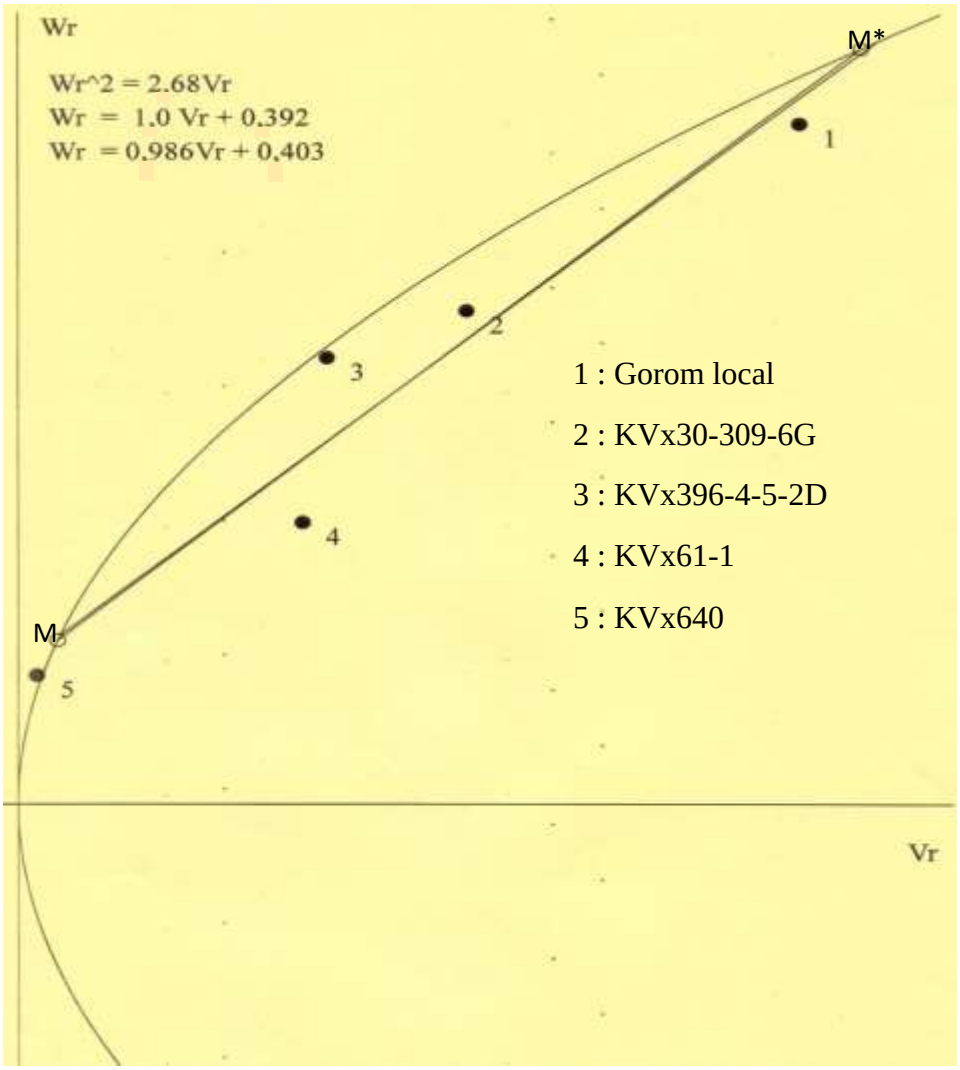

Figure 1. Graphical representation of Wr depending on the severity parameter to Vr. Wr: covariance between a parent r and its progenies; Vr: variance between a parent r and its progenies.

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)

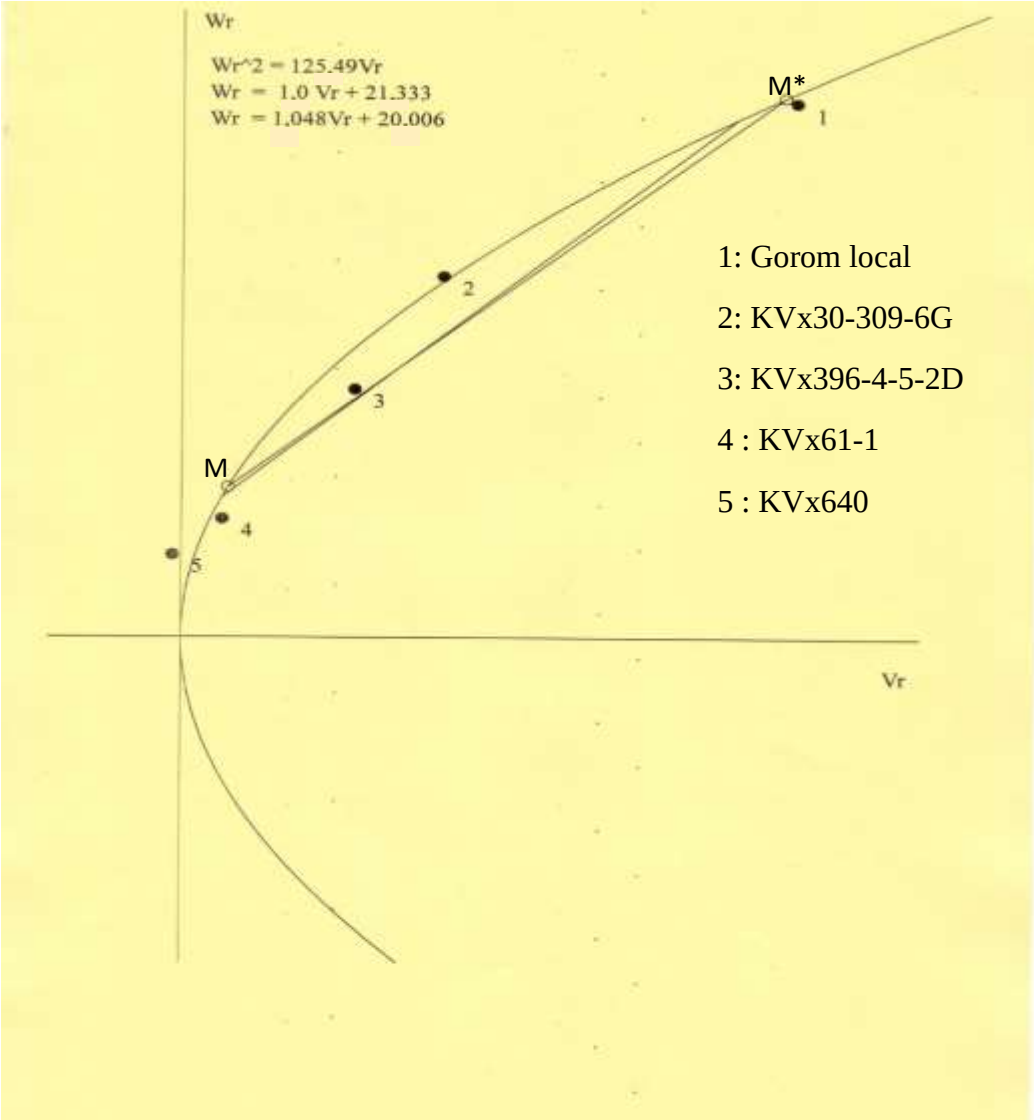

Figure 2. Graphical representation of Wr on Vr function for setting the area under the disease progression curve (AUDPC). Wr: covariance between a parent r and its progeny; Vr: variance between a parent r and its progeny.

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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