

Original Research Article

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**Inheritance and the Allelic Relationship of Resistance to
Cowpea Aphid Borne Mosaic Virus (CABMV) in Two Cowpea Genotypes,
KVX640 and KVX396-4-5-2D, in Burkina Faso**

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A B S T R A C T

Cowpea (*Vigna unguiculata* (L.) Walp.) is a grain legume crop cultivated in all the agro-ecological zones of Burkina Faso. It plays an important role in human nutrition due to its richness in protein and its enormous agronomic, sanitary and economic potentialities. However, several virus originated diseases negatively affect its production, the most important being cowpea yellow mosaic transmitted by aphid or cowpea aphid-borne mosaic virus (CABMV). One of the control measures of this disease, which causes yield losses, is the use of resistant varieties. This study consisted in determining the inheritance and the allelic relationship of the resistance to CABMV in two cowpea genotypes KVx640 and KVx396-4-5-2D. Plants were evaluated in pots under green house at Saria research station in Burkina Faso. The inheritance and allelic relationship studies showed that the resistance is governed by two dominant genes in the two varieties, each variety contributing for a resistant gene. Therefore, the resistance is monogenic. There was not any allelic relationship between the two resistant cowpea varieties, KVx640 and KVx396-4-5-2D, to CABMV. The identification of the nature of the gene governing the resistance to CABMV was done through a screening of F₁ individuals derived from crosses between one resistant parent (KVx396-4-5-2D) and a susceptible check (Gorom local). The F₁ hybrids were uniform; therefore, the resistance is conferred by a dominant gene.

Keywords

Cowpea Aphid Borne Mosaic Virus (CABMV), Cowpea Genotypes, KVX640 and KVX396-4-5-2D.

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Introduction

Cowpea is cultivated in all the agro-ecological zones of Burkina Faso. It plays an important role in human nutrition due to its richness in protein and its enormous agronomic, sanitary and economic potentialities. However, cowpea production

is hampered by several constraints such as insect pests, parasitic weeds and diseases (fungal, bacterial and viral). Among the diseases, *Cowpea Aphid-Born Mosaic Virus* (CABMV) causes enormous yield losses ranged from 60 to 90 % in Burkina Faso

(NEYA, 2011). One of the control measures for resistance to CABMV is the use of resistant varieties. However, the nature of the gene (s) controlling the resistance to CABMV is not well determined yet. Knowing the nature of this (these) gene (s) is very important before undertaking a breeding scheme because it helps developing an appropriated breeding strategy.

The inheritance and the allelic relationship study for the resistance to CABMV could eventually allow to develop resistant varieties to CABMV and respond to farmers' and consumers' needs.

The objective of this study was to determine the inheritance and the allelic relationship of the resistance to this disease in cowpea.

Materials and Methods

Plant materials

The vegetal material used in this study was consisted of two resistant cowpea varieties (KVx640 and KVx396-4-5-2D), and two susceptible (KVx30-309-6G and Gorom local) to CABMV. All these varieties are vulgarized and are originated from Burkina Faso. The inheritance and the allelic relationship study was done using F₂ segregating populations.

Methods

Development of segregating populations

For the inheritance study, the two cowpea varieties KVx640 and KVx396-4-5-2D were sown in pots during the 2013 dry season and were crossed with two susceptible cowpea varieties to CABMV, Gorom local and KVx30-309-6G respectively. The F₁ derived from these crosses were self-pollinated under greenhouse in 2014.

For the allelic relationship study, the two resistant varieties were crossed between them. The F₁ seeds were also self-pollinated.

Assessment for resistance to CABMV

The assessment for cowpea resistance to CABMV was done on:

- 120 F₁ individuals derived from the cross between KVx396-4-5-2D, resistant parent and Gorom local, susceptible parent in order to determine the nature of the gene (s) for resistance;
- 120 F₂ individuals derived from the cross between KVx640 (resistant) and Gorom local (susceptible) and 120 F₂ derived from the cross between KVx396-4-5-2D (resistant) and KVx30-309-6G (susceptible) for the inheritance study and
- 120 F₂ individuals derived from the cross between KVx640 (resistant) and KVx396-4-5-2D (resistant) for the allelic relationship study.

Seeds were sown in pots under greenhouse at Saria research station. A single plant was kept per pot for each cross. Plant was watered every morning. The one week seedlings were inoculated with an extract from young infested plants of the susceptible check (Gorom local). The extract was compress filtered and place into a melting ice. Before inoculation, leaves of one week cowpea plants were sprinkled with carborundum 600 mesh which is an abrasive product. The upper surface of the leaves was delicately scrubbed with a cotton-wool or a pestle soaked into the extract. Inoculated plants were grown under greenhouse free from insects mainly aphids. Symptoms appearance records started on the sixth days after sowing. Symptoms severity which is a parameter of resistance was also scored two

weeks after sowing, this allows to distinguish between resistant and susceptible plants.

Experimental site

The experiment was conducted in March 2015 at Saria research station of the Institute of Environment and Agricultural Research (INERA), located in the central-west part of Burkina Faso. Saria belongs to the North-soudanien district characterized by an annual grasses savanna (FONTES and GUINKO, 1995). The annual rainfall at Saria in 2015 was 920.8 mm for 66 raining days. The F₁ hybrids and the F₂ populations were developed in 2014 at the Center for Environmental and Agricultural Research and Training (CREAF) of Kamboinsé, located at 12 km in the north of Ouagadougou.

Data analysis

Data for the inheritance and the allelic relationship studies were analyzed using the Chi-Square test. So segregation ratios were submitted to the Chi-Square test with Yates correction for continuity using the formula below:

$$X^2 = \sum [(observed\ number - expected\ number) - 0.5]^2 / expected\ number$$

This test allows to calculate the p-value that the difference between observed and expected number is due by chance. The level of rejection of the null hypothesis was fixed at 5%. When the value of the Chi-Square test is less than 5%, the segregation rate is considered improbable.

Results and Discussion

Figure 1 shows different symptoms caused by CABMV on different F₂ populations as well as the susceptible check, Gorom local.

For the different populations tested, symptoms appeared from the sixth to the eleventh day after (DAI) inoculation for individuals derived from cross between KVx396-4-5-2D x KVx30-309-6G; the symptoms appeared from the seventh to the twelfth DAI and from the eighth to the twelfth DAI for populations derived from cross between KVx640 x Gorom local and KVx640 x KVx396-4-5-2D respectively. Most of the individuals were resistant to CABMV. The highest number of diseased plants was observed in the population derived from the cross between KVx640 and Gorom local which is the susceptible check.

Nature of the gene controlling resistance to CABMV

Prior to the inheritance study, it was necessary to know the nature of the gene involved in cowpea resistance to CABMV. Thus over 120 F₁ individuals derived from cross between KVx396-4-5-2D and Gorom local tested through artificial inoculation with CABMV under greenhouse, at INERA/Saria in March 2015, none of them was susceptible. Symptoms appeared from the seventh to the twelfth DAI. Figure 2 illustrates immature F₁ pods and resistant F₁ plants after screening.

Genetic analysis of the resistance in KVx396-4-5-2D and KVx640

Inheritance of the gene for resistance in KVx396-4-5-2D

A F₂ population composed of 120 individuals derived from the cross between KVx396-4-5-2D, a resistant variety and KVx30-309-6G, a susceptible cultivar was screened at INERA/Saria in order to determine the number of gene (s) controlling the resistance in KVx396-4-5-2D. Symptoms appeared on the sixth DAI.

Resistant and susceptible plants were scored taking into account the severity of the symptoms using a scale with five classes starting from 0 to 5. Plants having score 4 and 5 are considered susceptible. Data were submitted to Chi-Square test. Chi-Square test's value was calculated for the two most probable hypothesis which segregation ratios are: 3R: 1S and 15R: 1S. The results are presented in the table I. The value of the Chi-Square test was 0.89 with a p-value of 0.05, significant at 5%. Over 120 F₂ individuals, 115 were resistant and only 5 were susceptible to CABMV. This segregation ratio is only compatible with the ratio 15R: 1S.

Inheritance of the gene for resistance in KVx640

To determine the number of gene (s) controlling the resistance to CABMV in the variety KVx640, 120 F₂ individuals from the cross between KVx640 and Gorom local, the susceptible check, were screened under greenhouse at INEAR/Saria in March 2015.

The results of the Chi-Square test are presented in table II. The value of the test was 0.82 with a p-value of 0.05 that is significant at 5% significance level. Over the 120 individuals, 7 belong to symptoms' severity class 4, therefore susceptible to CABMV. All the plants presented the disease symptoms. The first symptoms appeared on the seventh DAI.

Allelic relationship of the genes controlling the resistance in KVx640 and KVx396-4-5-2D

The objective of this study is to determine whether the genes for resistance to CABMV in the varieties KVx640 and KVx396-4-5-2D are allelic. Thus, a population composed of 120 F₂ from the cross between these two

resistant varieties to CABMV were screened in pots in greenhouse at INERA/Saria in March 2015. All the plants presented the disease symptoms. First symptoms appeared on the eighth DAI. Among the 120 individuals tested, only 4 were susceptible to CABMV. The value of the Chi-Square test was 0.07 with a p-value of 0.05, significant at 5% level of significance. Results are presented in table III.

Nature of the gene controlling the resistance to CABMV

Results of the screening of the 120 F₁ individuals from the cross between the resistance parent, KVx396-4-5-2D and the highly susceptible parent, Gorom local allowed us to know the nature of the gene for resistance to CABMV in the resistant parent. All the 120 individuals were resistant. This suggests that the gene responsible for resistance in KVx396-4-5-2D could be a dominant gene because of the uniformity of the F₁ hybrids. Therefore, the F₁ individuals expressed the phenotype of a dominant allele. The recessive allele will only express itself in the F₂ population. Alleles of each gene segregation independently during gametes formation. ATOKPLE *et al.* (1995), showed that the resistance to race 1 of *Striga gesnerioides* in IT82D-849 is controlled by a single dominant gene, as the resistance to race 3 (Toure *et al.*, 1997). But the resistance to race 3 (Niger) is controlled by a recessive gene (Toure *et al.*, 1997). TAIWO *et al.* (1981); Fisher and Kyle (1994, 1996), in the case of CABMV have also found that the resistance is controlled by a single dominant gene, this confirms our results or by a single recessive gene which is different from our results. For Orawu (2007), resistance to CABMV is controlled by several recessive genes, this do not corroborate with our results. Patel *et al.*, (1982) have shown that a

resistance of cowpea to CABMV is controlled by modifier or minor genes. In fact, several major genes could be responsible to resistance to CABMV.

Inheritance and allelic relationship of the resistance to CABMV in KVx396-4-5-2D

Observed results of the inheritance study from the cross KVx396-4-5-2D x KVx30-309-6G, allowed to identify a resistance governed by two dominant genes. According to the data obtained from this cross, the variety KVx396-4-5-2D possesses one gene for resistance to CABMV. This means that the resistance is determined by a dominant allele of each of the two loci that segregate independently and could be justified by the ratio of segregation observed (15 resistant: 1 sensible). Atokple *et al.*, (1995) have mentioned that the resistance to race 1 of *Striga* is controlled by a single dominant gene in the varieties B301 and IT82D-849.

Resistance to CABMV could be controlled by a single gene in KVx396-4-5-2D because this variety seems to provide a single gene for resistance to CABMV. As regard to the inheritance and the allelic relationship, our results are similar to those of Atokple *et al.* (1995) as for race 1 of *Striga*. Although, resistance to CABV is controlled by two dominant genes. As for the allelic relationship, if the genes for resistance to CABMV from the two parents KVx396-4-5-2D et KVx640 are allelic, all the F₂ progeny will be resistant and so no susceptible plant will be observed. But this is not the case herein. Then we noticed the absence of allelic relationship between the gene for resistance in KVx396-4-5-2D and one in KVx640. Therefore, the variety KVx396-4-5-2D could contribute to the resistance to CABMV with a dominant gene (15 R: 1 S) and then the resistance to CABMV could be controlled by two different dominant genes in these cultivars.

Table.1 segregation ratio of F2 population derived from KVx396-4-5-2D X KVx30-309-6G

Cross	Number of plants assessed	Value				Ratio	X ²	P
		Observed resistant	Observed susceptible	Expected resistant	Expected susceptible			
KVx396-4-5-2D X KVx30-309-6G	120	115	5	112,5	7,5	15 :1	0,89	0,05

Table.2 segregation ratio of F2 population from cross between KVx640 and Gorom local

Cross	Number Of plants assessed	Value				Ratio	X ²	P
		Observed resistant	Observed Susceptible	Expected resistant	Expected susceptible			
KVx640 X Gorom local	120	113	7	112,5	7,5	15 :1	0,82	0,05

Table.3 segregation ratio of F₂ population from cross between KVx640 X KV and 396-4-5-2D

Cross	Number of plants assessed	Value						
		Observed		Expected		Ratio	X ²	P
		resistant	susceptible	resistant	susceptible			
KVx640 X KVx396-4-5-2D	120	116	4	112,5	7,5	15 :1	0,07	0,05

Fig.1 Photograph of different symptoms on cowpea plants



A. Symptoms and their severity class (4) on F₂ infested plants



B. Gorom local, the susceptible check inoculated under greenhouse

Fig.2 Photographs of immature F₁ pods and resistant F₁ plants after screening



Immatures F₁ pods



Resistant F₁ plants after screening

In the opposite, Dubé (2000) noticed the absence of susceptible cowpea plants to *Striga gesnerioides* when crosses were made between Wango-1 and Gorom local and HTR and Wango-1, suggesting that these two cultivars are possessing the same dominant gene. That confirms the existence of allelic relationship between these cultivars. If resistance genes from two parents are not allelic, then we will observe a segregation for the resistance; the ratio of resistant plants over susceptible plant informs about the number of genes involved in the resistance as well as their eventual relationship.

Inheritance of the resistance to CABMV in KVx640

From the allelic relationship study, we noticed that there is not an allelic relationship between the varieties KVx396-4-5-2D and KVx640. Yet, to check the nature of the gene governing the resistance to CABMV, we used F₁ individuals from cross between KVx396-4-5-2D (resistant variety) and Gorom local (susceptible check).

All the individuals showed resistance suggesting a resistance controlled by a dominant gene. From all these results, the resistance in KVx640 could be governed by a dominant (segregation ration, 15 resistant over 1 susceptible). Therefore, two independent and dominant genes could be involved, only one being present in each parent of the cross KVx640 x KVx396-4-5-2D. Since the plants do not possess the same gene, fixed F₁ individuals for this gene could not derived from a cross between them due to the fact that each parent will not provide a homologous allele. Likewise the F₁ individuals will not transmit the same allele to the F₂ individuals. As a consequence all the F₂ individuals will not be resistant.

In conclusion, nowadays in Burkina Faso, CABMV is the most dangerous virus disease on cowpea because it causes enormous yield losses. Control measures have been developed in order to help producers to reach their objectives. Development of cowpea resistant cultivars remains the simplest and the most affordable control measure of this disease. For this reason we interested ourselves to two resistant varieties developed by INERA with aim of determining the genetic characteristic of their resistance to CABMV. So it was important to determine the nature of the gene controlling the resistance. The inheritance and the allelic relationship studies of the gene for resistance are a solution for obtaining varieties possessing good agronomic characters. The resistance in the two varieties could be governed by two dominant genes, each variety contributing for a gene. So the resistance is monogenic. This type of resistance is interesting in a breeding program because it is easy to transfer it in an elite variety, however it could be broken down by the virus. We have also noticed the absence of allelic relationship between the two varieties (KVx640 and KVx396-4-5-2D) for the resistance gene. It could be necessary to confirm our results in rainy season in field condition in order to determine environmental effect. To ease the selection process of selection, it will be interesting to identify molecular markers linked to the gene for resistance in these two varieties. The identification of molecular markers will be useful in gene pyramiding.

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