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Assessment of bambara groundnut landraces for resistance to *Cowpea aphid borne mosaic virus*

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ABSTRACT

Bambara groundnut (*Vigna subterranea)* is an important seed legume in Burkina Faso. It provides a well-balanced diet and many other benefits for populations, especially in rural areas. Despite all these advantages, bambara groundnut cultivation remains subject to many constraints. One of the most important of them is viral diseases. This study was conducted in order to identify from Burkina Faso bambara groundnut landraces, some resistant genotypes to *Cowpea aphid-borne mosaic virus* (CABMV). In this study, 20 bambara groundnut landraces collected from three climatic zone of Burkina Faso were tested. According to their level of sensitivity to the CABMV isolate tested we obtained two groups of genotypes: G1 and G2. After retro-inoculation, only six landraces, namely E119, E117, E105-a from Sahelian zone and E53-a, E103 and E97 from Sudan-sahelian zone, showed a resistance to the CABMV isolate. These results could be used to develop control strategies against viral diseases.

Keywords: *Vigna subterranea*, Burkina Faso, CABMV, viral diseases, susceptibility.

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INTRODUCTION

Bambara groundnut [*Vigna subterranea* (L.) verdc.] is one of the most widely grown seed legumes in sub-Saharan Africa, particularly in Burkina Faso where it is ranked second in terms of consumption (Ouédraogo et al., 2008). In recent years there has been a renewed interest in the cultivation of bambara groundnut (Ouoba et al., 2016). This is partly due to many potentialities available into the plant such as soil improvement through atmospheric nitrogen fixation (Musa et al., 2016), its importance in the food balance for populations, especially in rural areas (Bamshaiye et al., 2011), its well-balanced chemical composition of carbohydrate 63%, protein 19% and fat 6.5% (Mkandawire, 2007; Mbaiogaou et al., 2013), its economic and social importance (Gbaguidi et al., 2015; Nadembèga, 2016).

Despite all efforts made to its improvement, Bambara groundnut production remains under negative influence of many constraints. One of the most important of them is viral diseases. Plant viral diseases are generally difficult to manage because of the occurrence of frequent epidemics and the lack of curative control strategies (Néya, 2011). Several viruses occurring in bambara groundnut have been identified in Africa and four of them were reported in Burkina Faso by previous studies (Néya, 2011; Konaté et al., 2017). Among these viruses, the most widespread on Bambara groundnut in Burkina Faso remains *Cowpea aphid borne mosaic virus* (CABMV) (Konaté et al., 2017; Konaté, 2018).

In order to cope with the damage caused by viruses, genetic control seem to be least demanding, least polluting, least expensive and the best adapted method of control (Mbeyagala et al., 2014). According to Néya et al. (2019), identification of resistant genotypes is one of the prerequisites for undertaking adequate genetic control against plant pathogen, especially viruses. This study aimed to identify, in a population of 20 landraces of Bambara groundnut, genotypes with tolerance or resistance to CABMV in order to undertake an effective fight against this pathogen.

MATERIALS AND METHODS

Plant material

Plant material used in this study consists of 20 Bambara groundnut landraces from germplasm of Environment and Agricultural Research Institute (INERA) of Burkina Faso (Table 1). They have different phenotypic characteristics (Figure 1). Cowpea (*Vigna unguiculata*) variety "Gorom local", most susceptible to CABMV (Neya, 2011; Barro et al., 2016) was used as sensitive control.

Experimental plan

An experiment was carried out in greenhouse condition at temperature ranging from 25 to 30 °C. The experiment was set up in pots using a split plot design with four replications. The main plots consisted of CABMV inoculated Bambara groundnut plants and a plot of non-inoculated plants maintained as control. Each main plot consisted of 20 pots each containing three seeds sown per landrace.

Preparation of inoculum

In order to multiply tested CABMV viral isolate, we carried out the virus inoculation on young "Gorom local" cowpea plants, 10 days old with a CABMV inoculum. Inoculum was prepared by grinding Bambara groundnut CABMV infected leaves on inoculation buffer with a weight / volume ratio of 1/2, (KH_2PO_4 2.04 g/ml, K_2HPO_4) at

5.4 g/ml and pH 7). To the mixture obtained, a pinch of carborundum (600 mesh) was added. Mechanical inoculation was done by rubbing inoculation method to inoculate virus on cowpea plants 10 days old.

CABMV inoculation to Bambara groundnut and cowpea plants

A population of virus-free aphids belonging to the *Aphididae* family (*Aphis craccivora*) were reared on healthy cowpea plants until adult stage. One week after the onset of symptoms, virus-free aphid's populations were then maintained on the symptomatic cowpeas plant leaves for a 1h acquisition feeding after a 1h pre-acquisition period. Cowpea leaves containing aphids were then collected and placed on young leaves of Bambara groundnut at 14 days after sowing at the rate of 5 aphids per pot, except the plants of control (blank). In order to avoid the uncontrolled mixing of aphids, infested plants were covered for 24 h (Figure 2), after which time all plants used in the experiment were sprayed with a mixture of two insecticides deltamethrin and dimethoate.

Presence and absence of symptoms were observed and assessed throughout the trial using a five-grade rating scale adapted from Gumedzoé et al. (1990):

Score 1: No symptoms in plants (no visible mottle or mosaic).

Score 2: Slight mosaic symptom (1 to 25% of plants inoculated were infected).

Score 3: Moderate mosaic symptom (25 to 50% of plants inoculated were infected).

Score 4: Severe symptoms with stunting (50 to 75% of plants inoculated were infected).

Score 5: Highly severe symptoms with stunting (75 to 100% of plants inoculated were infected).

Mean plant severity scores (MSS) for all three replicates were given by the following formula:

$MSS = \text{Mean Severity Rating} = (\Sigma \ 〚 ni * i 〛) / N$

i represents the score obtained on the symptom rating scale; n is the number of samples that received the score i; N represents the total number of samples.

The adapted scale of Gumedzoe et al. (1990) used was modified to take into account the intermediate scores obtained after the calculation of average severity scores. So a landrace is judged:

Resistant or tolerant to the virus isolate used if MSS is 1.

Moderately susceptible to the virus isolate used if $1 < MSS \leq 3$.

Highly susceptible to the virus isolate used if $4 < MSS \leq 5$.

The collected data were entered on Excel 2016.

Table 1: Description of evaluated Bambara groundnut's (*Vigna subterranea*) landraces.

Accessions	Seed colour	Provenance	Climatic Zone
E53-a	Black with cream spots and grey butterfly like eye	Dédougou	Sudan-sahelian
KVS246-1	cream with grey triangular eye	INERA/CREAF	INERA/CREAF
E125	Cream	Titao	Sahelian
KVS235_100GY	Cream	INERA/CREAF	INERA/CREAF
KVS235	Cream	INERA/CREAF	INERA/CREAF
KVS246-3	Black marbled spots on cream background with grey butterfly-like eye	INERA/CREAF	INERA/CREAF
E119	Cream red spotted with grey butterfly-like eye	Nassoumbou	Sahelian
E56-A	Cream with brown dark stripes and black eye	Tougan	Sudan-sahelian
E117	Cream with brown dark stripes and black eye	Baraboulé	Sahelian
E130	Cream with brown dark stripes and black eye	Tikaré	Sudan-sahelian
E105-a	Red brown	Dori	Sahelian
KVS246-2	Cream with triangular gray eye	INERA/CREAF	INERA/CREAF
Nobéré locale	Red dark with brown light stripes and grey butterfly like eye	Nobéré	Sudan-sahelian
E97	Red dark with brown light stripes and grey butterfly like eye	Boulsa	Sudan-sahelian
E63	Red dark with brown light stripes and grey butterfly like eye	Dassa	Sudan-sahelian
E86	Cream with grey triangular eye	Diapaga	Sudan-sahelian
E62	Violet grey and dark with cream background and grey butterfly like eye	Dassa	Sudan-sahelian
E105-b	Red light	Dori	Sahelian
E103	Cream gray with red light spot and gray outline of hilum	Yalgo	Sudan-sahelian
LIFE"141-1"	Red Brown with small black spots	INERA/CREAF	INERA/CREAF

Figure 1: Bambara groundnut (*Vigna subterranea*) landraces used in the study with their phenotypic characteristics.

Figure 2: Bambara groundnut (*Vigna subterranea*) plants covered to avoid aphid's motions.

RESULTS

Bambara groundnut landraces were categorised as highly susceptible, moderately susceptible and resistant according to the severity of symptom induced by the CABMV isolate used (Figure 3). All landraces tested were moderately susceptible or resistant (Table 2).

The calculation of mean severity score of all Bambara groundnuts landraces used allowed us to distinguish two groups of genotypes:

Group G1 composed of landraces having an average note of severity equal to 1. This group contains resistant or tolerant genotypes to the used virus isolate. It is composed of 13 genotypes, including seven from the Sudan-Sahelian zone (E86, E103, E56A, E63, E97, E53 and Nobéré locale), four from the Sahelian zone (E119, E117, E105a and 105b) and two from INERA germplasms named KVS235-100GY and Life 16-141.

Group G2 consists of seven genotypes, including two from Sudan-Sahelian zone (E62 and E130), four from INERA database (KVS235, KVS246-1, KVS246-2, KVS246-3) and only one from Sahelian zone (E125). These genotypes are known as moderately susceptible to the CABMV isolate tested. They have an average note of severity ranging between 2 and 3.

Retro-inoculation was then carried out on cowpea variety "Gorom local" by using extracts of Bambara groundnut leaves, obtained from 13 genotypes, group G1. Retro-inoculation gave a positive response with genotypes E86, E56-A, E63, E105b, Life16-141, Nobéré locale and KVS235-100GY. On the other hand, no visual symptom was observed on the seedlings of cowpea inoculated by the extracts of Bambara groundnut leaves of six other genotypes of G1 group namely E97, E119, E53-a, E117, E105-a, E103.

A: *Cowpea plant infected by CABMV showing severe mosaic symptoms on leaves;*
B: *Bambara groundnut plant showing leaf curling, decreased size and stoppage of growth induced by CABMV infection;*
C: *Bambara groundnut healthy plant.*

Figure 3: Cowpea (*Vigna unguiculata*) and Bambara groundnut (*Vigna subterranea*) plants showing CABMV symptoms on their leaves.

Table 2: Mean severity scores obtained for 20 Bambara groundnut's (*Vigna subterranea*) landraces to *Cowpea aphid-borne mosaic virus* (CABMV).

Samples	MSS to CABMV	Global Score	Level of sensitivity
E125	1,556	2	MS
E119	1,000	1	R/T
E97	1,000	1	R/T
E53-a	1,000	1	R/T
E117	1,000	1	R/T
Nobéré locale	1,444	1	R/T
E130	1,667	2	MS
KVS246-1	2,889	3	MS
E62	2,111	2	MS
E63	1,333	1	R/T
E105-a	1,000	1	R/T
E56-A	1,222	1	R/T

E103	1,000	1	R/T
KVS235	1,556	2	MS
KVS246-3	1,778	2	MS
KVS235-100GY	1,333	1	R/T
E105-b	1,444	1	R/T
Life 16-141-1	1,111	1	R/T
KVS246-2	2,111	2	MS
E86	1,222	1	R/T

MSS= Mean severity score; MS = moderately susceptible; R/T= Resistant or Tolerant.

DISCUSSION

Bambara groundnut susceptibility to viral diseases has been reported by many authors (Brink et al., 2006; Néya, 2011), but the present study aiming to identify resistant genotypes of Bambara groundnut to viral diseases is a first attempt in Burkina Faso. Expression of symptoms depends of specie of virus or viral strain, species and age of the plant but also on its genotype. In this study, 20 landraces of Bambara groundnut susceptibility to CABMV were assessed. According to the severity of symptoms induced by the tested CABMV isolate, we distinguished two groups of genotypes G1 and G2. KVS246-1 genotype belonging to G2 was more susceptible to CABMV with an MSS of 3, which resulted in the expression of severe mosaic symptoms accompanied by leaf curl, reduced size, and stoppage of growth. Studies done by Taiwo et al. (2014) showed a negative correlation between the number of nodules and the CABMV viral infection. This reduction in the number of nodules induced by the viral infection could be at the origin of the symptoms observed.

Individuals of group G1 gave two types of genotypes following retro-inoculation. According to the International seed federation (2012), there are two levels of resistance of a plant to a pest namely high resistance and intermediate resistance. This is the case in the present study for Bambara groundnut landraces E119, E117, and E105-a from Sahelian zone and E53-a, E103, and E97 from the Sudan-Sahelian zone, which showed no viral disease symptoms after three repetitions and retro-inoculation. These genotypes are said to be highly resistant to the CABMV isolate used, compared to other G1 landraces that should be considered intermediate resistant or tolerant.

In this study, deltamethrin and dimethoate mixture used to avoid the uncontrolled mixing of aphids was efficient. The effectiveness of this combination of two insecticides had already been reported by (Néya et al., 2013). However, CABMV is a seed-borne virus transmitted by both seed and aphids in a non-persistent manner, so use of insecticides to control virus transmission is not a recommended measure (Milošević et al., 2012). The best control for viruses from the genus *Potyvirus* should be by resistant varieties and the obtained results in this study should be of great use for the breeder programs for producing resistant cultivars. It is known that if a virus is transmitted by both aphids and seeds, its control and management once in the seed will be very difficult (Bashir et al., 2002). Therefore, the best approach remains in the use of resistant varieties for cultivation (Arif and Hassan, 2002). More studies should be necessary to carry out additional work aimed at determining one or

more genes responsible of natural resistance to CABMV in Bambara groundnut.

Conclusion

The present study was carried out with the objective of assessing the symptomatic behaviour of 20 landraces of Bambara groundnut to CABMV. It highlighted two groups of genotypes that are resistant or tolerant and moderately susceptible to CABMV isolate tested. Sources of resistance were observed with genotypes consisting of landraces E119, E117, E105-a, E53-a, E103 and E97 which could be used as basic material for the development of new CABMV-resistant cultivars. In the context of the fight against viruses of Bambara groundnut, the screening of local varieties by isolated viral strains in Burkina Faso will have to be continued. Particular emphasis should be placed on viral co-infections, which tend to increase the damage caused by viruses. This work should be followed by studies to determine the genes involved in the resistance process of Bambara groundnuts plant.

COMPETING INTERRESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This manuscript deals with one aspect of NMK's thesis done under the supervision of MO and MS. Capture and breeding of aphids was carried out by TO. AO, HN and EP actively participated in the writing and proofreading of the manuscript. All authors read and approved the final manuscript.

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