Varieties’ Response of Vegetable Cowpea to Mechanical Inoculation of Cowpea Aphid-Borne Mosaic Virus in Burkina Faso

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RESEARCH ARTICLE

Abstract
Vegetable cowpea is eaten mainly fresh, in the form of young, immature pods, tender and sweet like the common bean. However, like cowpea with seeds, vegetable cowpea experience yield losses due to the cowpea aphid-borne mosaic virus (CABMV). This study aims to improve yields through the development of vegetable cowpea varieties resistant to CABMV. The study focused on ten varieties of vegetable cowpea, carried out in a greenhouse at the Kamboinsé research station using a randomized complete block design with three replications, all inoculated with CABMV. The data collection concerned resistance parameters. Mechanical inoculation made it possible to observe various symptoms of CABMV, thus highlighting the existence of variability within the varieties tested. Strong correlations were observed between several variables. Thus, the varieties of vegetable cowpea IT85F-2089-5, UG-CP-8, IT85F-2805-5 and Telma were identified as resistant, because belonging to the low severity classes and having a low value of area under the disease progress curve. On the other hand, the varieties RW-CP-5, UG-CP-6, IT83S-911, niébé baguette grimpant possessing a high severity class were judged to be susceptible. These resistant varieties will thus be able to contribute to the improvement of production and the protection of cowpea resources in Burkina.

Keywords: Burkina Faso; CABMV; mechanical inoculation; Response; Vegetable cowpea.

INTRODUCTION
In Burkina Faso, agriculture is the main source of income for the population and is also the pillar of food security. This sector employs more than 80% of the population and contributes about 33% of the Gross Domestic Product (MAHRH, 2011). The cultivation of legumes occupies a place of choice because they generate important foreign exchange for the national economy. Cowpea is one of them which occupies the first place and is very profitable for the country. In Africa, there are mainly two types of cowpeas grown, namely seed and vegetables cowpeas. Cowpea is the preferred dry vegetable in many parts of Africa (Madamba et al. 2006). Cowpeas are consumed at all stages of development. The young leaves and immature pods are used as vegetables while the dry seeds are used in various foods (Nout, 1996). Consumption at the immature pod stage constitutes vegetable cowpea. Vegetable cowpea is consumed at the immature pod stage such as commons beans. It is mainly consumed fresh, in the form of young, tender and sweet immature pods so the common bean. The major interest
vegetable cowpea compared to common bean (*Phaseolus vulgaris* L.) is its heat tolerance and its great adaptation to all growing seasons (Coulibaly et al., 2020). Despite having all this potential, vegetable cowpea remains little known and less popularized in Burkina Faso. Vegetable cowpea cultivation, like seed cowpea, is made difficult by virus diseases that cause considerable yield losses. The major virus disease identified in Burkina Faso is Cowpea Aphid-Borne- Mosaic Virus (CABMV). It is transmissible through seeds, aphid vectors and mechanical inoculation (Nanama et al., 2020). Yield losses ranging from 7% to 60% in ten varieties have been attributed to this virus in Burkina Faso (Néya, 2002). Since its discovery, this virus has been the subject of work on cowpea. However, very little work has been done on the resistance of vegetable cowpea varieties to CABMV. This study is part of the search for a sustainable solution to the decline in vegetable cowpea productivity caused by CABMV. Specifically, the aim is to mechanically inoculate vegetable cowpea varieties and identify those that are tolerant to the disease.

**MATERIALS AND METHODS**

**Plant material**

The trial included ten (10) vegetable cowpea varieties provided by the Asian Vegetable Research and Development Centre (AVRDC) in Mali. Characteristics are shown in Table 1.

**Table 1. Characteristics of the ten (10) vegetable cowpea varieties studied**

<table>
<thead>
<tr>
<th>No.</th>
<th>Varieties</th>
<th>Seed colors</th>
<th>Texture</th>
<th>Resistance to CABMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IT83S-872</td>
<td>Cream</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>2</td>
<td>UG-CP-8</td>
<td>Cream</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>3</td>
<td>UG-CP-6</td>
<td>Cream</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>4</td>
<td>IT83S-911</td>
<td>Red</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>5</td>
<td>Niébé baguette grimpant</td>
<td>Red</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>6</td>
<td>IT85F-2805-5</td>
<td>Cream</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>7</td>
<td>RW-CP-5</td>
<td>Red</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>8</td>
<td>Baguette</td>
<td>Cream</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>9</td>
<td>Telma</td>
<td>Red</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
<tr>
<td>10</td>
<td>IT86F-2089-5</td>
<td>Red</td>
<td>Smooth</td>
<td>Not received</td>
</tr>
</tbody>
</table>

**Experimental site**

The study was conducted at the Formation and Agricultural, Environment Research Center (CREAF) of Kamboinsé of the National Institute for the Environment and agricultural research (INERA). The CREAF is between latitude 12° 28 North and longitude 1°32 West with an altitude of about 296 m. It is located about twelve (12) kilometres (km) north of Ouagadougou on the Ouagadougou-Kongoussi axis. The station is characterized by a Sudano-Sahelian climate with a long dry season from November to May and a short-wet season from June to October (Guinko, 1984). The rainfall recorded during the 2018 agricultural season, was spread from June to November inclusive with 843.5 mm of water in 50 days (Weather CREAF/Kamboinsé 2018).

**Experimental device**

Experimental device is a randomized complete block design with ten (10) varieties in three (3) replications, for example, a total of 30 elementary plots at a rate of 10 per replication. In each elementary plot, only one vegetable cowpea variety is sown in a pot representing the elementary plot.

**Preparation of soil culture**

The soil used for the mixture was first treated by removing debris and mixed with sand to make it looser. The well-moistened mixture was then sterilized over high heat for 1 h 30 min. The sterilized soil was left in a tray until it cooled down and then distributed in 5 L pots.

**Sowing and conduct of the trial**

The seedlings were sown directly, placing 2 to 3 cowpea seeds in each pot at a depth of 2 to 3 cm and covering them lightly with soil. After emergence and inoculation, maintenance work consisted of hoeing as needed to facilitate water infiltration and thus promote plant growth, application of fertilizer and insecticide treatments (with delthamethrin of young plants to reduce the pressure of aphids, a vector of viruses and other insects likely to carry germs in the trial). Watering in the absence of rain was done when necessary.
Mechanical inoculation transmission technique

Inoculum from seedlings of each variety was homogenized in 0.01 M sodium phosphate buffer, pH 7.4 at a grind ratio of 1:10 (w/v). Prior to inoculation, at the 3-4 true leaf stage, a de-matting was carried out to retain the most vigorous plant per pot. The leaves of healthy two-week-old seedlings of these varieties were cleaned with cotton and sprinkled with 600 mesh carborundum. Using a cotton swab or the pestle dipped in the extract, the upper surface of the leaves was gently rubbed (Neya et al. 2019).

Disease development and data collection

The data collection focused on CABMV resistance parameters namely:
- Symptom emergence date (SED) proposed by Barro (2016), noted from the 6th to the 21st day after inoculation (DAI);
- Area Under Disease Progress Curve (AUDPC): which reflects the kinetics of disease progression
- Degree of expression or severity of the disease was recorded on days 21 and 30 after mechanical inoculation

Different symptom classes and their characteristics are observed in Table 2. Indeed, resistant individuals belong to severity classes 0; 1 and 2; those of class 3 are of intermediate resistance and those of classes 4 and 5 are susceptible.

<table>
<thead>
<tr>
<th>Severity classes</th>
<th>Significance of the severity classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1</td>
<td>Mosaic visible only in backlight, light green, dark green, bluish green, no leaf deformation, no decrease in plant size</td>
</tr>
<tr>
<td>2</td>
<td>Mosaic visible only in backlight, leaf curled downwards, possible decrease in size of the plant</td>
</tr>
<tr>
<td>3</td>
<td>Green mosaic, generally in the form of large spots, possible leaf deformation, possible decrease in plant size</td>
</tr>
<tr>
<td>4</td>
<td>Yellow mosaic, no leaf deformation, possible decrease in plant size</td>
</tr>
<tr>
<td>5</td>
<td>Yellow mosaic, leaf curl, decrease in plant size</td>
</tr>
</tbody>
</table>

(Barro et al. 2016)

Statistical analysis

All the data collected for the different parameters were subjected to an analysis of variance (ANOVA) with the XLSTAT statistical software and the means of the variables were compared using the Newman Keuls test at the p= 5% probability threshold. The correlation matrix was performed to study the association of quantitative traits.

RESULTS AND DISCUSSIONS

Figure 1 reports the values of Symptom emergence date (SED) in the different vegetable cowpea varieties inoculated with CABMV. The figure shows that most varieties show symptoms between the 6th and 7th YY.

Figure 1. Symptom emergence date of varieties tested with CABMV
In the table 3 are presented the resistance parameters of cowpea to CABMV.

**Table 3. Resistance parameters of vegetable cowpea to CABMV**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Average</th>
<th>CV</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>SED</td>
<td>6,600</td>
<td>10,969</td>
<td>0.161ns</td>
</tr>
<tr>
<td>SEV21</td>
<td>2,933</td>
<td>39,971</td>
<td>0.004*</td>
</tr>
<tr>
<td>SEV30</td>
<td>3,433</td>
<td>37,224</td>
<td>0.001**</td>
</tr>
<tr>
<td>AUDPC</td>
<td>28,650</td>
<td>36,774</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

ns = not significant (P>0.05), *: significant difference at 5%, **: significant difference at 1%, CV: Coefficient of Variation, SED: Symptom emergence date, SEV21: severity at 21 DAI, SEV30: severity at 30 DAI, AUDPC: Area Under Disease Progress Curve.

Figure 2 shows the disease severity values at 21 and 30 days of age in different cowpea varieties inoculated with CABMV. This figure shows symptom severity in most varieties on a scale of 0 to 5. Vegetable cowpea varieties RW-CP-5, UG-CP-6, IT83S-911, and niébé baguette grimpant (class 4 and 5) recorded the highest severity in the 10-day observation interval (from the 1st to the 2nd observation).

Figure 2. Symptom severity of varieties tested with CABMV

Varieties IT85F-2089-5, UG-CP-8, IT85F-2805-5 and Telma (class 0, 1, and 2) recorded a low severity. They stood out from all other varieties with a low severity class. Baguette and IT83S-B72 are the closest to these varieties, with a lower disease severity (class 3).

Analysis of variance (ANOVA) for the severity of symptoms (Table 3) of the disease was significant at the 21st DAI (P=0.004) and 30th DAI (P=0.001) with a mean severity of 2.93 and 3.43 respectively and a coefficient of variation of 39.971% and 37.224%.

Kinetics of disease progression

Figure 3 clearly shows that the varieties (except for the first four varieties) had a rapid onset of disease severity between the 1st and 2nd observation. Niébé baguette grimpant had a rapid emergence of disease severity with a high AUDPC value (42%). However, the variety UG-CP-8 recorded the lowest AUDPC value (10.5%).

Analysis of variance (Table 3) of Area Under Disease Progress Curve (AUDPC): values revealed a highly significant difference (P=0.002) between the different varieties with a coefficient of variation of 36.77 and a mean of 28.65.
Observations of mechanical inoculation of the 10 vegetable cowpea varieties at the 2-leaf stage induced various generalized mosaic symptoms. These are vegetable and yellow mosaic with leaf curling, yellow mosaic with leaf deformations followed by reduction in plant size. These symptoms are similar to those of (Barro et al., 2016, Nanama et al., 2020) who screened seed and vegetable cowpea varieties with the same virus.

Significant results obtained show that the ten varieties studied contain significant variability with respect to CABMV. These results demonstrate that CABMV is mechanically transmissible and systemic. These results confirm those of Hampton and Tottappilly (2003).

Inoculated vegetable cowpea varieties gave variable severity and ADCPM. This indicates the existence of different levels of resistance in vegetable cowpea depending on the variety, hence the correlations between severity at 21 DAI and severity at 30 DAI an and AUDPC. This result indicates that resistant and tolerant varieties are those with low severity and low AUDPC and susceptible varieties have high severity and high AUDPC. This result is similar to that of Nanama et al. (2020) on vegetable cowpea varieties inoculated with CABMV.

Severity classes 1 and 2 presented by varieties such as IT85F-2089-5, UG-CP-8, IT85F-2805-5 and Telma, show that these varieties would be resistant to CABMV. On the other hand, varieties such as RW-CP-5, UG-CP-6, IT83S-911, Niébé baguette grimpant with the severity rating of 4 and 5 would be the most susceptible. Varieties with low AUDPC values were the least infected by CABMV and these could be qualified as resistant varieties. Therefore, the variety UG-CP-8 with low severity and low AUDPC could be qualified as a resistant variety. The information obtained from the AUDPC evaluation helps breeders to identify the best varieties for their ability to slow down the progression of the disease (Orawu, 2007).

Negative correlation observed between the DAI and the severity at the 21st DAI would show that the delay in the appearance of symptoms evolves in the opposite direction to the severity and vice versa. Indeed, the earlier the symptoms appear on a variety, the higher the severity and vice versa. These results are in agreement with those of Néya (2011) who stated that the first symptoms appear between 6 and 7 DAI for susceptible varieties and that it
was necessary to wait 14 to 21 DAI to observe symptoms on tolerant varieties. This relationship is particularly interesting in plant breeding in that the date of symptom emergence as well as severity can be used to predict the resistance or susceptibility status of vegetable cowpea varieties.

CONCLUSIONS
At the end of this study, it was found that CABMV is responsible for various mosaic symptoms. It is transmissible by mechanical inoculation. The levels of infection varied significantly from one variety to another. The study of resistance through the CABMV resistance parameters showed a high variability among the ten vegetable cowpea varieties studied. Both positive and negative correlations were observed between the severity at 21 DAI and the severity at 30 DAI and AUDPC. On the basis of the analyses concerning the parameters of resistance to CABMV, some varieties stood out from the others. Thus, the resistant varieties UG-CP-8, ITB5F-2805-5 and Telma with low severity and low AUDPC values can be used in a breeding plan for resistant vegetable cowpea varieties.

Author Contributions: A.B. followed the study, collected data and drafted the manuscript; J.N. and Z.C. followed the study, collected data and participated in the correction of the manuscript; Z.D. supervised the work, analyzed the data and corrected the manuscript and M.C. corrected the manuscript.

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Conflicts of Interest
The authors declare that they do not have any conflict of interest.

REFERENCES