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Field Evaluation of the Efficacy of Thermotherapy against Cowpea Mosaic Disease in the Central African Republic

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Abstract

Vigna unguiculata is the main legume grown in Africa. A major constraint to its production is cowpea mosaic disease (CABMV), transmitted by aphids. This disease can cause yield losses of up to 87%. The objectives of this study were to sanitize contaminated cowpea seeds by thermotherapy and to evaluate agro-morphological parameters in the field. Contaminated seeds from a local accession (*Kahkiri*) susceptible to CABMV were heat-treated in water baths of 59˚C, 61˚C, 63˚C, 65˚C and 67˚C for 15 minutes. Agronomic parameters were assessed in the field (Pissa, Central African Republic) over a three-month period up to harvest. Overall, although none of the treatment temperatures tested totally eliminated the virus and resulted in 100% CABMV-free seedlings, heat-treated cowpea seeds produced 87.5% plants with a significantly lower incidence and severity of CABMV symptoms than untreated plants. Our results show that 65˚C represents the optimum treatment temperature, with no negative impact on seed regeneration capacity, and for which we observed the highest seed yield (95.77 kg/ha ± 34.71) and biomass (437.98 kg/ha ± 133.7) equivalent to untreated seeds from asymptomatic plants (95.07 kg/ha ± 27.08 and 441.98 kg/ha ± 132.29, respectively), while the yield of seedlings and biomass in plants from contaminated and untreated seeds was the lowest (43.18 kg/ha ± 27.53 and 236.54 kg/ha ± 92.47, respectively).

Keywords

Vigna unguiculata, Thermotherapy, Incidence, Severity, Cowpea Mosaic, CABMV, Yield

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a legume that plays an important role in the diets of many people, and is grown in all tropical and intertropical zones [1]. Annual global production is estimated at 6.4 million tonnes of dry seeds, over 80% of which are produced in Africa [2]. Annual cultivated area worldwide amounts to over 12.7 million hectares, of which 10.8 million hectares are in Africa [3]. Cowpea is of great economic, social and dietary importance [4]. It contains a high protein content (20% to 25%), vitamins, 64% carbohydrates and mineral elements, giving it the name "poor man's meat" [5], and cowpea improves soil nitrogen content [1].

However, cowpeas are susceptible to numerous diseases caused by bio-aggressors such as fungi, viruses, bacteria and nematodes, but the greatest damage is caused by viruses [1]. More than 20 viruses have been detected on cowpeas in several regions of the world, including more than eight that infect cowpeas in Africa [6]. While some of these viruses occur occasionally and are of local or minor importance, some are widespread and of significant economic importance [6]. *Cowpea aphid-borne mosaic virus* (CABMV) alone can cause yield losses of up to 60% [7]. CABMV, of the Potyviridae family and *Potyvirus* genus, is one of the viruses reported to be the most important cause of cowpea virus disease in Africa [8]. Seed transmission of CABMV is variable, but can reach 80% [9] and is efficiently transmitted by several aphid species [10]. The global nature of CABMV in this instance poses a serious threat of spread through seed shipments [10].

Studies carried out in the Central African Republic (CAR) between 2019 and 2020 showed that the incidence of the disease averaged 80%, with a severity of 2.8 [2]. Cowpea is an essential contributor to food security and poverty reduction. The socio-economic and nutritional importance of cowpeas has led researchers at the Laboratory of Biological and Agricultural Sciences for Development (LaSBAD) to take a growing interest in improving the production of this precious foodstuff by combating cowpea mosaic disease.

The first works carried out by LaSBAD involved treating cassava cuttings for 30 minutes in a water bath at 40˚C, 45˚C, 47˚C, 50˚C, 55˚C and 60˚C, and showed that the best results were obtained with the 47˚C and 50˚C treatments. In the second study, cuttings were treated under the following conditions: 45˚C, 47˚C, 48˚C, 49˚C, 50˚C and 51˚C [11]. In 2012, another experiment was carried out in a water bath at 43˚C, 45˚C, 47˚C, 49˚C and 51˚C in a randomized block design

[11]. The results obtained from the latter confirmed the first two studies, but unfortunately, no sanitization work was ever carried out on plants with contaminated seeds, such as cowpeas. For food security, nutrition and poverty reduction in the CAR, the control of seed-borne pathogens in leguminous crops such as cowpea is of the utmost importance. In this context, the MACOWECA project (Maize and Cowpea for Sustainable Food and Nutrition Security in Western and Central Africa), funded by the African Union, aims to study and build the conditions for greater integration of cowpea into agro-systems, in order to improve and popularize cowpea cultivation throughout the country. With this in mind, this work aims to contribute to improving cowpea production in CAR.

The aim of this work was therefore to carry out experiments on contaminated cowpea seeds using a randomized set-up under natural conditions, with a view to obtaining valid scientific results.

2. Material and Methods

2.1. Experimental Site

The field study was conducted at the LaSBAD experimental site in Pissa, in the Guinean forest zone of the CAR. This climatic zone is characterized by two alternating seasons: a rainy season from May to October and a dry season from November to April. The average annual minimum temperature is 30˚65C, with a moderate difference of 11˚44C between maximum and minimum. Average annual rainfall is around 1600 mm/year. The soil is sandy loam. The village of Pissa 2 is located in the Mbaïki sub-prefecture of the Ombella M'Poko health region. It lies 75 km west of the capital Bangui, between latitude 04˚02'46.4" north and longitude 18˚09'51.6" east.

2.2. Plant Material

The plant material consisted of contaminated seeds collected from diseased plants and healthy seeds from asymptomatic plants of the CABMV-susceptible local accession *kahkiri*. Seed selection criteria are essentially based on their physiological age, the condition of their reserves, and their phytosanitary status. Seeds should not be too mummified, broken or too small, in order to achieve an excellent recovery rate. What's more, large seeds have greater reserves than small ones; this factor helps the plant to settle in better.

2.3. Seed Treatment and Sampling

The bain-marie was filled with a sufficient volume of water and once the equipment had been set to a precise working temperature, the temperature of the bain-marie was checked and recalibrated very regularly using a temperature probe. Once the temperature was stable, the cowpea grains were placed in a hermetically sealed 20 kg freezer bag and soaked in hot water at a precise temperature for 15 min. After treatment, the cowpea grains were removed from the bain-marie and dried at room temperature for 24 hours (see **Table 1**).

Table 1. Seed treatment conditions and parameters.

Treatment	Duration	Severity index	Sample code	Seed/sample weight
59˚C	15 min	3	T_1 59˚C	5 kg
61˚C	15 min	3	T_2 61˚C	5 kg
63˚C	15 min	3	T_3 63˚C	5 kg
65˚C	15 min	3	T_4 65˚C	5 kg
67˚C	15 min	3	T_5 67˚C	5 kg
Untreated contaminated seeds	-	3	Control 1	5 kg
Untreated healthy seeds	-	0	Control 2	5 kg
Total				35 kg

2.4. Experimental Design in Field

A three-repeat Fisher block design was used on the experimental site. The experimental plot covers an area of 2030 m^2. The individual plots have a surface area of 5 m × 5 m = 25 m^2 with a spacing of 1 m between rows and 1 m between bunches on the row in order to achieve a maximum density of 36 plants per individual plot, 756 plants on 21 individual plots. A spacing of 2 m between plots and 2 m between blocks, then 5 m between blocks and the edge. The study plot was installed in the center of the elementary plot, which has a surface area of 4 m × 4 m = 16 m^2 with a maximum density of 16 plants.

2.5. Crop management

Cowpea was sown on 2 August 2022 at Pissa 2. 100 seeds were sown in monoculture and 2 to 3 seeds were planted per pot. Maintenance consisted of weeding and ridging. A total of 3 weeding operations were carried out from the 45th day after sowing (DAS), with an interval of 15 days between each operation. At 15 DAS, weeding was carried out with one plant per stake. The first three young leaves were collected in plastic bags. They were placed in a small cooler and sent to the laboratory for molecular biological analysis. The following agro-morphological parameters were measured: seed regeneration rate, length growth, disease incidence, symptom severity, seed yield and fresh biomass, and average weight of one hundred seeds. During the field observation periods, diseased plants were counted 10 days after sowing in all plots and then counted every 7 days from the tenth day after sowing until 90 days in all plots. Mosaic symptoms were observed, namely vein mosaic, mottled or interveinal mosaic, speckled mosaic and spotted mosaic. The severity of symptoms from 10 to 90 days after sowing was assessed in all plots using the Fargette (1987) scale [12]. This scale has five levels from 0 to 4. The following levels were defined: 0 = no symptoms; 1 = slight mosaics without deformations covering

less than 20% of the leaf surface; 2 = mosaics and chlorosis covering about 50% of the leaf surface with occasional leaf deformations; 3 = mosaics covering most of the leaf accompanied by necrosis and leaf blade deformations; 4 = terminal stage characterised by plant death. The severity was calculated according to the following formula:

$$S = \frac{\sum_{i=1}^{16} ds}{Pt}$$

where:

S is symptom severity or symptom severity index (SSI);

ds is the degree of symptom severity on the leaf surface of diseased leaves;

Pt is the number of diseased plants at the control period.

The prevalence of virus infections was calculated using the following formula:

$$P = \frac{\sum_{i=1}^{16} Pt}{N}$$

where:

P is the Disease incidence prevalence;

Pt is the number of diseased plants at the control period;

N is the total number of plants in the square.

2.6. Detection of CABMV Viruses in Seeds by RT-PCR

The reverse transcription-polymerase chain reaction (RT-PCR) test was used to detect the viruses according to the following protocol.

2.6.1. RNA Extraction Using TRIzol

Mortars and pestles were first sterilised at 150˚C for 2 h and then placed at −80˚C at the same time as the fresh cowpea pods. The cold cowpea seeds were crushed and transferred to a cold 2 mL Eppendorf tube to which 1000 µL TRIzol (stored at 4˚C) and 200 µL cold chloroform were added before homogenisation. The tubes were kept on ice for 5 minutes. Centrifugation at 13,000 rpm for 15 min at 4˚C was performed to separate the aqueous phase. The supernatant was transferred to a new sterile 1.5 mL tube to which 550 µL of cold isopropanol was added and the tubes were placed at −20˚C for approximately 30 min. The mixture was then centrifuged at 13,000 rpm for 10 minutes at 4˚C. The resulting pellet was washed in 500 µL cold 75% ethanol by centrifugation at 12,000 rpm for 5 min, still at 4˚C. After drying, the pellet was resuspended in 30 µL sterile distilled water. RNA extraction with Trizol was followed by treatment with DNase.

2.6.2. Treatment of RNA with DNase

The purpose of DNase is to destroy any residual DNA present in the samples. For this purpose, 10 µg RNA was mixed with 2 µL 10× DNase buffer (*Euromedex*) and 10 µL DNase (1U/µL) Euromedex. This mixture was made up to 20 µL with sterile distilled water and incubated at 37˚C for 30 minutes. 1 µL of 25 mM EDTA was added to the mixture, followed by a second incubation at 65˚C for 10 minutes.

2.6.3. RT-PCR Amplification of CABMV

The RNA obtained had to be reverse transcribed into cDNA (complementary DNA) before amplification. To do this, the first step was to amplify the part coloured red in Figure 1. A reaction medium containing 5 µL of total RNA, 1 µL of Poty-GP1-5'CP-R primer (CAGCTGCGTCAGAGAAGTG) and 10 µL of sterile water was incubated at 70˚C for 10 minutes. After incubation, the reaction medium was immediately cooled on ice for 10 min and then a mixture containing 2 µL RT buffer (10×), 0.5 µL Ribogrip inhibitor, 1 µL FireScript reverse transcription at 200 U, 0.5 µL dNTPs at 20 mM was added to the previous reaction medium to adjust the final volume to 20 µL. Finally, the synthesis of the complementary RNA (cDNA) was performed after incubation of the reaction medium at 50˚C for 30 min and then at 85˚C for 5 min. For the partial amplification of the capsid protein gene, the PCR reaction itself was prepared by mixing (for a final volume of 50 µL) 10 µL of retro transcription product, 1 µL at 10 µM of each of the Poty-GP1-5'CP-R (CAGCTGCGTCAGAGAAGTG) and Poty-GP1-5'CP-F (CGARAAGGART-TRCAAAGG) primers, 0.5 µL at 20 mM dNTPs, 0.5 µL at 1 U Hot start TAQ DNA polymerase, 2.5 µL of MgCl2, 5 µL at 10× Hot start buffer and 29.5 µL of sterile water. Amplification was carried out in the following steps: an initial denaturation for 5 min at 94˚C, followed by 35 cycles (denaturation for 30 sec at 94˚C, hybridisation for 60 sec at 47˚C, elongation for 1 min at 72˚C) and finally a final elongation step for 7 min at 72˚C to complete the amplification reaction.

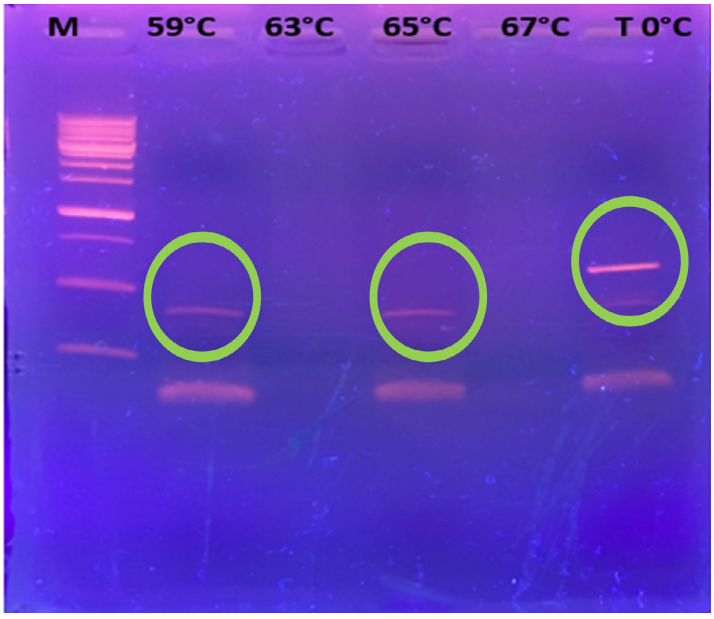

Figure 1. Gel electrophoresis (1%) of partial amplicons of the CABMV Faso capsid protein gene obtained with the Poty-GP1-5'CP-R/Poty-GP1-5'CP-F primers. Lines 1 to 5: CABMV-infected leaves from different samples. 59˚C, 65˚C and T 0˚C: positf; M: 1kb DNA size marker; T0: diseased and untreated seeds.

All RT-PCR reactions were performed on a Gene amp®, PCR system 2700 thermal cycler. At the end of amplification, each PCR product was electrophoresed on

a 1% agarose gel prepared in 1× TAE buffer containing ethidium bromide (BET) at 0.5 µg/ml. Subsequently, 10 µL of PCR product (DNA) mixed with 1 µL of loading buffer (12.5% Ficoll in 5× TAE, bromophenol blue) was added to the gel wells for migration at 100 V for 35 min in the presence of a 1 kb molecular weight marker (Invitrogen™). Gels were visualised using a UV transilluminator.

2.7. Statistical Analysis of Data

The generalized linear model of binomial family was used to compare: 1) seed regeneration rates, 2) CABMV incidences between treatments and 3) proportions of symptom-free plants and those with different levels of CABMV symptom severity within untreated diseased seeds and between treatments. Data on agronomic performance (seed and biomass growth and yield) did not follow a normal distribution according to Shapiro's test ($P < 0.05$). These data were then analyzed using a generalized model with a Poisson distribution. Tests were performed with R software (version 4.3.0) and the probability level for a significant difference was 0.05.

3. Results

3.1. Detection of Viruses Using the RT-PCR Test

The RT-PCR assay is highly effective in detecting CABMV using the Poty-GP1-5'CP-R/Poty-GP1-5'CP-F primer pairs.

3.2. Seed Recovery

Healthy and untreated seeds were totally recovered, whereas diseased and untreated recovered at 79.2%. In treated seeds, the best recovery rate was obtained in samples for treatment at 65˚C (95%), following by the treatment at 61˚C (93%) and at 59˚C (91%). Grain recovery was significantly low for treatment at 67˚C (66.7%; $P < 0.05$) (see Table 2).

Table 2. Recovery rate by sample at the first month.

Treatment	N (out of 48)	Rate of sprouted seeds
Diseased and untreated seeds	38	79.2
Healthy and untreated seeds	**48**	**100**
T_1 (59˚C)	44	91.7
T_2 (61˚C)	45	93.8
T_3 (63˚C)	43	89.6
T_4 (65˚C)	46	95.8
T_5 (67˚C)	**32**	**66.7***

(*) = significantly lower rate according to a GLM with binomial distribution ($P < 0.05$).

3.3. Symptom-Free Plants One Month after Sowing

Seven days after harvesting cowpea seeds, the overall phytosanitary status of the

plants in the field was assessed on the basis of symptoms observed during the vegetative phase. The symptoms observed consisted of simple chlorosis, with a few plants showing symptoms of degrees 1 to 3. Different levels of intensity of CABMV symptoms were observed in the plot on plants resulting from diseased and untreated seeds (Control 1) and a few heat-treated plants. The same plant may present symptoms of very variable intensity (scale 1 to 2) on the surface of trifoliate leaves. The distinct symptoms on the leaves enabled us to classify the infection levels of the plants according to the Fargette scale. The results showed that almost all plants grown from heat-treated seeds showed a low rate of symptom progression (Figure 2). The prevalence of CABMV was low overall, but very high in Control 1, with high severity indices (IS = 1) reaching 45%. The rate of asymptomatic plants was high (80% - 85%) for all the treatments and for healthy and untreated seeds (Control 2), with no statistical difference ($P > 0.05$; Figure 2).

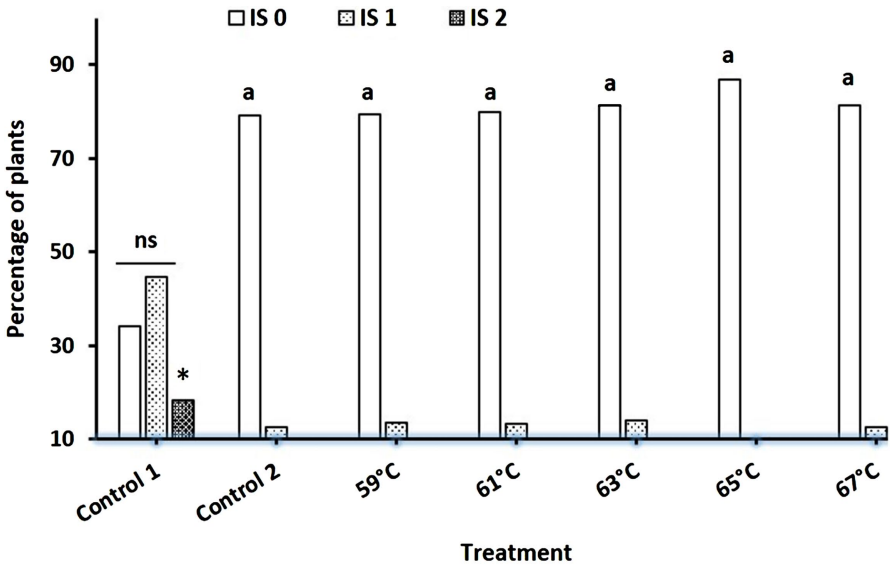

Figure 2. Percentage of plants following index of severity (IS) at month 1 in the experimental field. ns = no statistical difference; (*) = percentage significantly lower. Control 1 = plants emerged from the diseased and untreated seeds; Control 2 = plants emerged from the healthy and untreated seeds.

3.4. Incidence of CABMV

The disease prevalence increased from 15% - 20% at month 1 to 25% - 40% at the second month in all treatments except plants emerged from the diseased and untreated seeds, where the disease prevalence was significantly higher at 60% - 70% ($P < 0.05$; Figure 3). Three months after sowing, the disease prevalence reached 60% and 85% in the treatment at 59˚C and in Control 1, respectively, whereas prevalence remained stable in the other treatments. Control 2 (healthy and untreated seeds) showed a similar pattern of evolution in the disease prevalence as the heat-treated seeds (Figure 3).

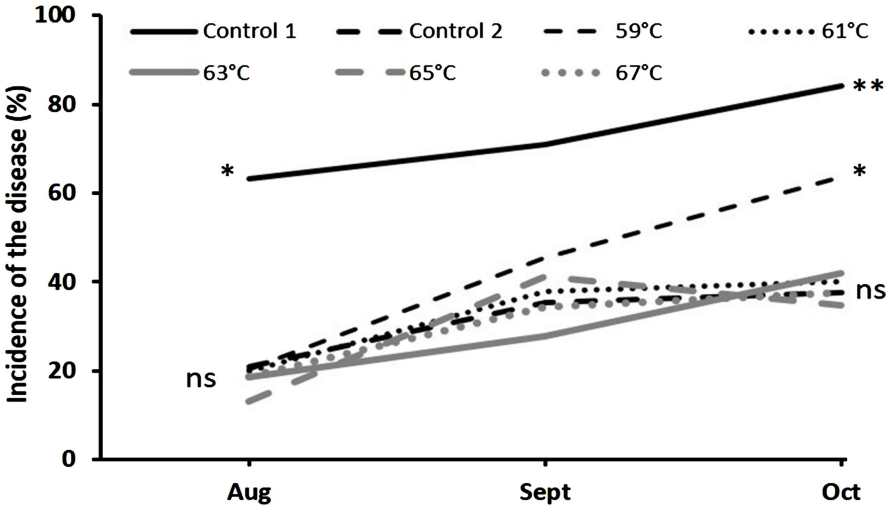

Figure 3. Incidence of CABMV in yield squares over a three-month period. Evolution of the proportion of symptomatic plants as a function of time in relation to the treatments and Controls 1 and 2 in this study. The proportions of diseased plants were compared at the end of the crop cycle, taking Control 1 (*diseased and untreated seeds*) as the reference for these comparisons (Shapiro, *P < 0.05, **P < 0.005, ns = non-significant difference).

3.5. Symptom Severity

The severity of CABMV was assessed on the basis of observation of symptom development on leaf surfaces. Treatment inhibited symptom expression over the 3 months of experimentation, although severity was generally low. Indeed, the disease severity was 0.85 in the diseased and untreated seeds (Control 1) at 1 month, whereas this was significantly lower at around 0.25 for all other treatments ($P < 0.05$). Three months after sowing, the disease severity reached a significant proportion for Control 1, ranging from 1.5 to 2 (1.65 ± 0.3), whereas in the other treatments, the severity index remained inferior to 1. Comparative statistical analysis showed no significant differences in viral disease severity between thermo-treated seedlings and Control 2 ($P > 0.05$) (as shown in Figure 4).

3.6. Agronomic Performance

3.6.1. Height Growth

Heights were compared at the end of the crop cycle (October), taking the untreated diseased plant (Control 1) as the reference for these comparisons. Better growths were recorded for higher temperatures of treatment (63˚C, 65˚C and 67˚C) with stem lengths around 120 ± 9 cm (as shown in Figure 5). The worst stem growth was recorded in Control 1 with an average stem length of 40 ± 4 cm three months after sowing.

3.6.2. Cowpea Seed Yields

The best yield was obtained by plants from infected seeds thermo-treated at 67˚C (114 kg/ha ± 35.96) followed, respectively, by 65˚C (95.77 kg/ha ± 34.71) and the healthy and untreated seeds Control 2 (95.07 kg/ha ± 27.08). However, there was a significant difference between the yield of Control 1 (43.18 kg/ha ± 27.53) and

the others (P < 0.05), except for the treatment at the lowest temperature (59˚C). These results show the impact of the disease on the yield of Control 1, while the 67˚C treatment significantly improved yield (P < 0.005) (as shown in Figure 6).

3.6.3. Cowpea Biomass Yields

The highest biomass yield was observed at 65˚C (437.98 kg/ha ± 133.7), followed by Control 2 (441.98 kg/ha ± 132.29; Figure 7). The lowest yield was obtained by Control 1 (236.54 kg/ha ± 92.47). Biomass yield followed similar results as in grain yield presented above.

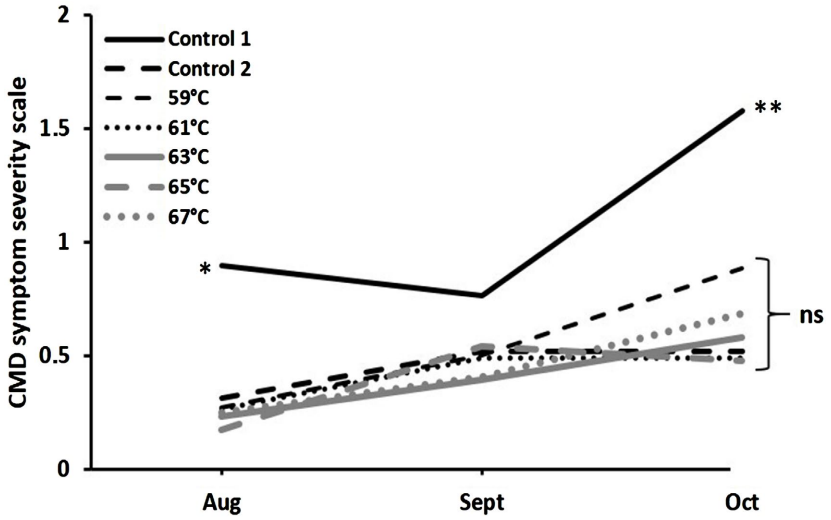

Figure 4. Evolution of CABMV symptom severity index over the time. (*) and (**) indicate P < 0.05 and P < 0.005, respectively.

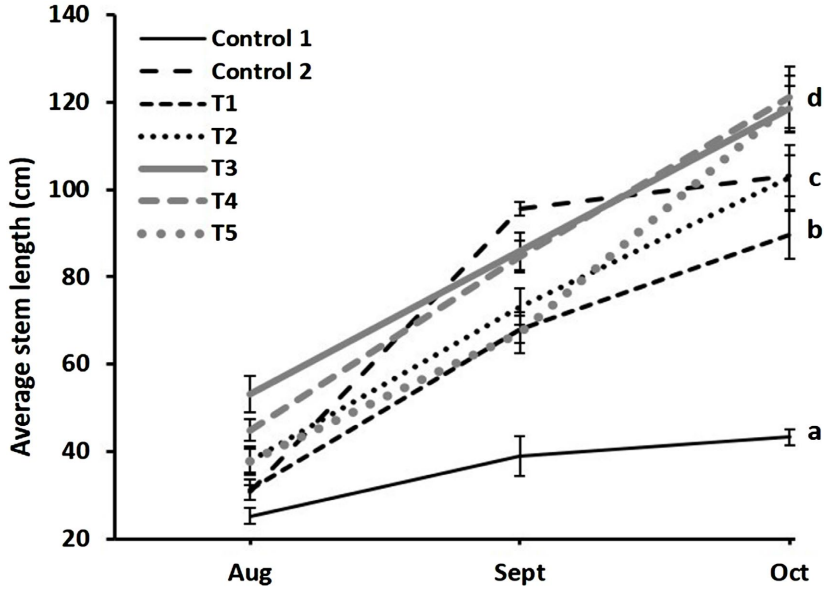

Figure 5. Stem length (cm) of plants over the three months of field observation. Values with different letters are statistically different according to the generalized model with a Poisson family error *distribution* (P < 0.05). T1 (59˚C), T2 (61˚C), T3 (63˚C), T4 (65˚C) and T5 (67˚C).

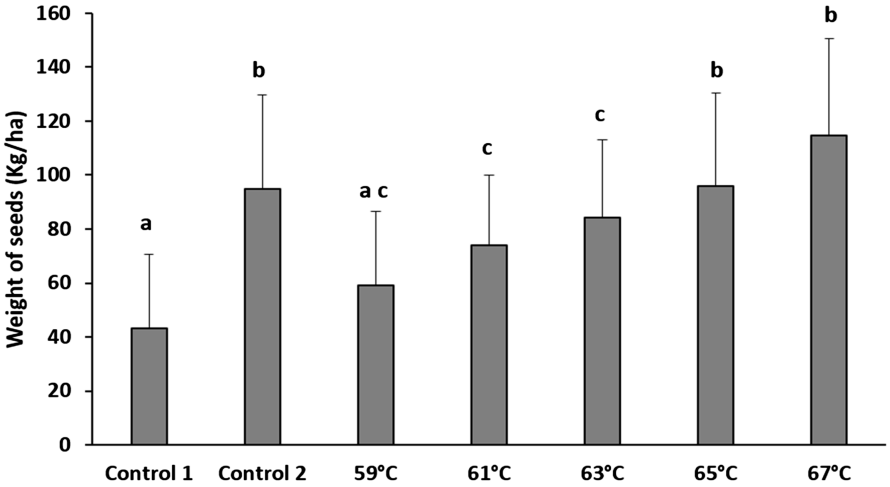

Figure 6. Estimated seed yield in kilograms per hectare (kg/ha). Values with different letters are statistically different according to the generalized model with a Poisson family error distribution ($P < 0.05$).

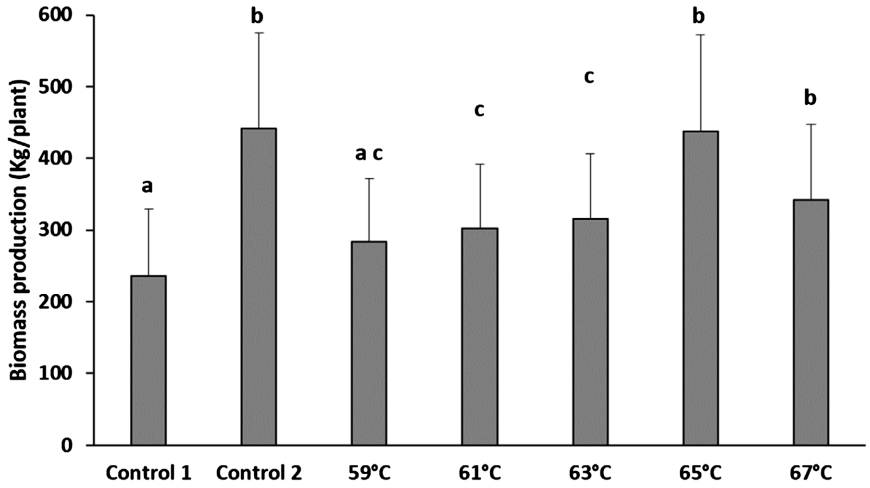

Figure 7. Estimated above-ground biomass yields in the treatments. (kg/ha). Values assigned the same letter are significantly different according to the generalized model with a Binomial error distribution ($P < 0.05$).

3.7. 100-Seed Yield by Weight

The best yield was obtained with seeds from plants thermo-treated at 67˚C, 21.17 g ± 3.08, which is very significantly different from Control 1 (14.01 g ± 3.13; $P < 0.05$). There was no significant difference between treatment at 65˚C (20.75 g ± 3.12), 63˚C (20.53 g ± 3.03) and Control 2 (20.70 g ± 2.95; $P > 0.05$). These results show the impact of the disease on seed enlargement status.

Seed Yield Losses

Yield loss in grain weight occurred in Control 1 (diseased and untreated seeds) and treatments at 59, 61 and 63˚C. Losses in seed yield were statistically more important in Control 1 and in treatment at 59˚C compared to those in treatments at 61 and 63˚C (Table 3). Increasing the temperature of treatment resulted in a decrease in

the loss of seed weight. Treatment at 65˚C was the threshold temperature for the restoration of the grain yield. At 67˚C, a significantly high gain in seed (20.5%) weight was recorded (as shown in Figure 8).

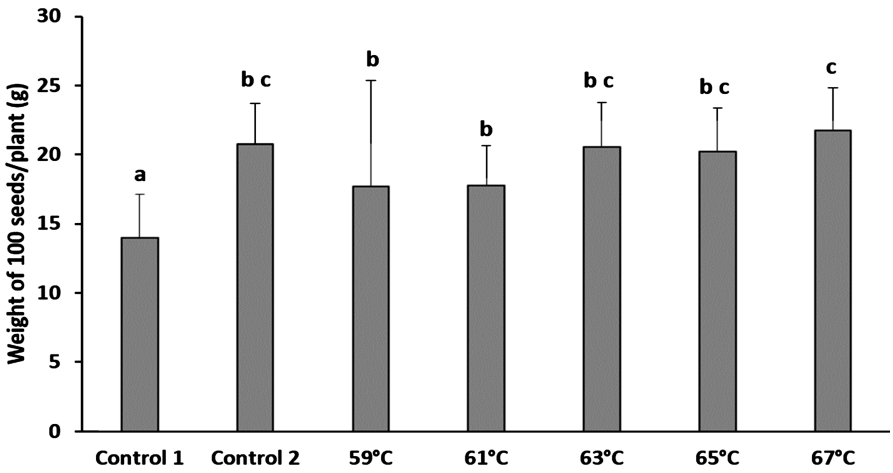

Figure 8. Weight yield of 100 seeds in grams. Values assigned the same letter are not significantly different according to the generalized model with a Poisson error distribution ($P < 0.05$).

Table 3. Yield loss rates by seed weight of plants grown from thermo-treated seeds compared with Control 2 in experiments.

Treatment	Yield (kg/ha)	Loss (%)	Gain (%)
Diseased and untreated seeds	0.7	**54.5**	-
Healthy and untreated seeds	1.5	-	-
59˚C	0.9	37.6	-
61˚C	1.2	22	-
63˚C	1.4	11.3	-
65˚C	**1.5**	**-**	**0.8**
67˚C	**1.8**	**-**	**20.5**

4. Discussion

CABMV has been found in numerous locations throughout the Central African Republic, regardless of ecological zone. The wide distribution of the virus in the country, and probably in other countries of the sub-region, is undoubtedly related to the large production areas of this crop. The results of detection and identification of CABMV in cowpea leaves using the RT-PCR test confirmed the presence of CABMV in leaves collected from plants showing symptoms from diseased seeds and heat-treated seeds. These results are similar to those of Zongo *et al.* (2019), who obtained amplicons of the same size using the same primers to detect CABMV [13]. These results also confirm studies carried out by Neya *et al.* (2008), according to which cowpea seeds transmit CABMV [14].

Studies of seed transmission have mainly been carried out on certain potyvirus-infected seeds and show that this transmission requires infection of embryonic tissues [15] before the suspensor disappears during the early stages of embryonic development [16]. For seed-borne viruses, seed disinfection by hydrotherapy is a technique that can limit or reduce virus density and consequently postpone or extend the time of expression of symptoms during plant recovery and growth during the physiological stages of the plant. The results of our work showed that under natural conditions, the heat-treated sample at 67˚C had a low recovery rate (66.7%) compared to the other treatments, where the recovery rate ranged from 89.6% to 95%. The best recovery rate was obtained by Control 2 at 100%, which was significantly higher compared to treatment at 67˚C ($P < 0.05$). These results show that temperatures above 65˚C damage the seeds (loss of germination capacity). In addition, these results show us that thermotherapy, at reasonable temperature (59˚C - 63˚C), restores the germination capacity of the seeds, giving them a better chance of survival. In the first month after sowing, the impact of cowpea mosaic disease was assessed in the plots. The results showed that the development of cowpea CABMV symptoms was much lower in the heat-treated seed plots than in the control plots. The effect of heat treatment on contaminated seed was very clear when comparing disease development in the plots. Disease spread was much reduced in seedlings from heat-treated seed, indicating a low level of seed-borne transmission of CABMV. However, there is no direct link between the spread of the disease and the ability to transmit the virus by seed [1]. The hypothesis is that hydrotherapy plays an important role in the expression of proteins found in the phloem that are involved in the plant's defence mechanism, particularly in the sieve tubes. These proteins are thought to form part of a structural complex in the phloem involved in protein transport, the conformation of which prevents the viral protein from passing through the phloem to the rest of the plant [17]. This hypothesis therefore confirms the delay or prolongation of symptom expression in plants grown from heat-treated seeds. The statistical analysis did not show a significant difference between the number of asymptomatic plants from heat-treated seeds and Control 2 plots ($P > 0.05$), with the severity indices being zero ($IS = 0$). On the other hand, there was a strong development of symptoms in Control 1 plots, with the statistical test showing no significant difference between the number of symptomatic plants and the number of asymptomatic plants ($P > 0.05$). Similar results were obtained by Zinga (2014) [11].

These results show that heat has a positive effect on reducing the virulence of the virus, which sometimes affects seed germination. This explains the hypothesis that the contamination comes from the seeds. These results confirm the work of Taiwo (2003) [8] and Neya *et al.* (2015) [7] that cowpea seeds are a primary reservoir of viruses. According to Johansen *et al.* (1994) [18], as is the case for most vertically transmitted viruses, seeds contaminated with the virus could be the main source of inoculum for the primary infection. Assessment of disease incidence in the plots based on symptoms showed that over 20% of treated plants were infected in August compared to 63.2% of Control 1 plants. This suggests that the contamination was

probably seed-borne. There was a significant difference in the incidence of CABMV in the thermally treated plants compared to Control 1 ($P < 0.05$). From September to October, there was an exponential increase in the number of plants contaminated by Control 1 to 84.2%. These results therefore show that the hygienic treatment of seeds with hot water inhibits the pathological disorders resulting from the action of viruses on the vegetative organs of the plant and makes the plant resistant to further viral contamination. Disease incidence of over 70% is a major concern for farmers [19]. The results show that CABMV symptoms show a gradient of variable severity depending on the amount of vegetative organs affected by the symptoms. These results confirm the work of Yabi *et al*. (2012) that CABMV symptoms evolve according to the seasons and include symptoms attributed to CABMV [20]. According to Ojuederie *et al*. (2010), climate has a known effect on the distribution and variability of CABMV symptom expression [21]. The highest height growth was observed in the 63˚C, 65% and 67˚C treatments. There was a significant difference ($P > 0.05$) in the 61˚C and Control 2 treatments. The lowest growth was observed in Control 1. These results can be explained on the one hand by the fact that heat stimulates the substances responsible for plant growth and on the other hand, it has an important effect on the activation of substances in the plant that can prevent viruses from migrating to other organs of the plant. The commonly accepted hypothesis is that heat plays a role in the degradation of viral RNA in plant tissues and prevents superinfection by a virus with sufficient sequence homology to CABMV; this technique can activate or increase all the proteolytic activities of protein complexes with multiple catalytic activities that can play a role in the specific degradation of viral proteins and thus lead to the inhibition of viral multiplication. The results could also partly reflect the sensitivity of cowpea to variations in photoperiod [22]. Indeed, numerous studies have shown that the length of the day (circadian rhythm) has variable effects on the vegetative and physiological development of cowpea [23]. The same observation was made by Bonny *et al*. (2011) [24] on voandzou in Côte d'Ivoire. The best seed yields were obtained by treatments 67˚C (114 kg/ha ± 35.96) and 65˚C (95.77 kg/ha ± 34.71), very significantly higher than Control 1 (43.18 kg/ha ± 27.53) ($P < 0.05$). Seed yield results showed no significant difference between 65˚C, 67˚C and Control 2 ($P > 0.05$). The best average biomass yield was obtained by the 65˚C treatment (437.98 kg/ha ± 133.7), which is equivalent to that of Control 2 (441.98 kg/ha ± 132.29), which is much higher than the yield of Control 1 (236.54 kg/ha ± 92.47) ($P < 0.05$). The 100-seed weight results show no significant difference in 100-seed weight between treatments at 67˚C (21.17 g ± 3.08), 65˚C (20.75 g ± 3.12) and Control 2 (20.70 g ± 2.95). On the other hand, the weight of 100 seeds was very low in Control 2 (14.01 g ± 3.13). These results explain the effect of heat by water, which significantly improves seed yields, fresh biomass and average 100-seed weight. This could be explained by the fact that heat treatment has a positive impact on viruses. The application of precise heat for a specific period of time has a positive effect on the genomic organization of the virus sequence and inhibits its synthesis within the plant cell. This also enables all the plant's vegetative apparatus to grow and develop correctly. These results cor-

roborate the studies of Useni *et al*. (2014) that nodulation causes rapid plant growth in cowpea [25] and also acts favorably on seed production. Similar results were obtained on the treatment of cassava cuttings by Zinga (2014) [11]. On the other hand, the yield loss of 1.5 kg/ha recorded in diseased seeds is explained by their disease incidence (94%).

5. Conclusion

This study addresses, for the first time, the thermotherapy treatment of seeds from the traditional cowpea accession grown in the CAR. The results showed that cowpea seeds can be treated with hot water without damaging the germinative capacity of the seed, preventing the viral protein from migrating into all plant organs and substantially increasing cowpea yield. The study showed that the loss of seed yield due to cowpea mosaic can be as high as 54%, depending on the severity of symptoms. Indeed, reduced plant incidence is one of the main causes of high cowpea productivity. The fact that most heat-treated cowpea seeds have a relatively high grain yield is an additional advantage for boosting cowpea production in localities faced with the adverse effects of cowpea virus diseases. This technique can also be considered as a means of combating cowpea mosaic disease.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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