

Screening of widely used accessions of tomato for resistance/tolerance to the *Pepper yellow vein Mali virus* in the central region of Burkina Faso

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Highlights

- Surveys allowed the identification of seven and sixteen tomato accessions from farmers and tomato seed sellers in the central region of Burkina Faso, respectively.
- A negative effect of Pepper yellow vein Mali virus on the production of tomato accessions based on whitefly-mediated inoculation was observed.
- Further research is needed to identify accessions that could be used by farmers for production and breeders in varietal improvement programs.

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Abstract

Tomatoes are a widely consumed fruit, important economically and in terms of food security. Despite this importance, tomato crops are confronted with constraints, including begomoviruses, such as the Pepper yellow vein Mali virus (PepYVMLV). Responsible for leaf deformation and yellowing in cultivated Solanaceae, it is the most virulent begomovirus infecting tomatoes in Burkina Faso. The use of resistant/tolerant accessions would be of great help in finding suitable solutions. This study aims to contribute to the appropriate and effective management of tomato diseases due to PepYVMLV. To achieve this objective, an inventory of tomato accessions sold and produced in the central region of Burkina Faso and a screening of the preferred accessions of tomatoes in semi-control conditions were conducted. Our results showed that sixteen varieties are sold in shops, with Mongal F1, Emerald F1, and Cobra 26 F1 reported to be the most preferred by farmers. Monitoring of the tomato plants inoculated using whitefly-mediated transmission during 32 days showed that Diva F1, a newly introduced accession, was the least susceptible to PepYVMLV, followed by Emerald F1, Cobra 26 F1, and Mongal F1, with yield losses ranging from 25.38 to 359.9 g/plant. Our results suggest that even if the productivity of Diva F1 seems to be slightly impacted by the virus, it is unsuitable for farmers because of its lower yield.

Introduction

Tomatoes (*Solanum lycopersicum* L.) are one of the most important food crops in the world, with more than 36 tons produced per hectare in 2021 (FAOSTAT, 2023). For the same year, in Africa, the total production of this vegetable was estimated at 21 million tons at 13 tons per hectare, while in Burkina Faso, total production was around 291 thousand tons at 17 tons per hectare (FAOSTAT, 2023). Tomato crops contribute to food security by providing vitamins and minerals. They are also a major source of income, particularly for rural and peri-urban communities. Part of the Burkina Faso production is exported to neighboring countries such as Ghana, Côte d'Ivoire, and Togo. The income generated is partly used to buy food such as cereals (Dembélé et al., 2019). Constraints, including fungal, bacterial, and viral infections, induce considerable economic losses. Tomato yellowing and/or leaf curl diseases are among the most important viral constraints on tomato crops in the tropics and subtropics (Leke et al., 2015; Ouattara et al., 2019). These diseases were caused by begomoviruses (family: Geminiviridae) transmitted by the invasive populations of whitefly, Bemisia tabaci (Dogra et al., 2009). One of these begomoviruses was initially characterized in Mali from leaf samples of pepper, showing the typical symptom of yellowing of the leaf vein (Zhou et al., 2008). Based on the symptoms of the disease and the host plant, the virus was named Pepper yellow vein Mali virus (PepYVMLV) (Chen et al., 2009). Subsequent studies have expanded our knowledge of the distribution range of this virus in Burkina Faso (Ouattara et al., 2019). This virus, recently characterized in association with a DNA-B component, was reported to be the most severe and widely distributed virus on tomatoes in Burkina Faso (Ouattara et al., 2019; Ouattara et al., 2022). Given the socio-economic importance of tomato production, it is necessary to develop control methods and strategies based on research on tomato varieties that are resistant/tolerant to this virus. Admittedly, plant breeding efforts have allowed the marketing of improved accessions, but the appreciation of these accessions by farmers and their resistance to PepYVMLV remain unclear. This study aimed to assess the accessions of tomatoes preferred by farmers and evaluate their resistance or tolerance to PepYVMLV in semi-controlled conditions in the central region of Burkina Faso. The availability of such information should play a more critical role in making decisions on the necessity of controlling tomato diseases.

Materials and Methods

Surveys and accession inventory

Surveys were conducted in August 2021 in the central region of Burkina Faso. Thirteen seed sellers and 38 farmers were randomly interviewed. Ten of the 13 seed sellers were from Ouagadougou ($12.363^{\circ}N$, $1.518^{\circ}W$) and the other three were from Loumbila ($12.517^{\circ}N$, $1.385^{\circ}W$). The 38 farmers were located in three sites around Ouagadougou: Boulmiougou (n=11), Nioko (n=19) and Tanghin (n=08). The interviews investigated the tomato accessions available in Burkina Faso, the degree of preference of tomato accessions used by farmers, the production periods, the lifecycle of each accession, and the production company.

Survey and collection of infected entire plants

A second survey was conducted in the vegetable production area of Tabtenga in October 2021. This locality is known to be a hotspot for geminivirus. Entire pepper plants with typical symptoms of begomovirus infection were identified in the field and then pictured, collected, and moved into pots for the whitefly-mediated transmission test described below. First, pots were filled with potting soil. Then symptomatic plants were carefully dug from the ground (not pulled out), so the root systems remained relatively intact. These plants were then transferred into the pots without damaging the roots. This collection process was done before the flowering stage of the plants.



Nursery of tomato accessions

The three preferred accessions identified by the seller and farmer interviews were used for the PepYVMLV resistance test. An accession newly introduced in Burkina Faso, the DIVA, was also used in this study. Based on the first tests carried out by local farmers, this accession has good productivity. Seeds of the four accessions of tomato were sown in a growing medium consisting of a mixture of commercial substrate (2/3, Jardinova, Longué-Jumelles, France) and homemade compost (1/3) in 96-cell seedling starter trays to raise seedlings for transplanting in pots. The cell seedling starter trays were maintained in an insect-proof cage. At the one-leaf growth stage, half of them remained in the insect-proof cage as controls, while the other half was transferred to the cage containing the symptomatic plants of pepper for the whitefly-mediated inoculation experiments described below.

Whitefly-mediated inoculation

A non-viruliferous *B. tabaci* colony was fed on symptomatic pepper (Capsicum frutescens) plants collected as described above. Viruliferous whiteflies were obtained after a 72-hour acquisition access period. The cell seedling starter trays containing healthy tomato seedlings (at the one-leaf growth stage) were transferred into the cage with diseased pepper plants and the viruliferous whiteflies. Then diseased pepper plants were eliminated by water stress to induce the inoculation of healthy tomato seedlings by the viruliferous whiteflies. Inoculated tomato plants were transplanted seven days post-inoculation (dpi) into 20 cm diameter pots containing the growing medium. Three plants per pot and three pots per accession were used. The pots were then arranged in three insect-proof cages (three replicates) based on a complete random block design. Each cage (replicate) contained three plants per tomato accession. Negative controls were healthy tomato plants maintained in insect-proof cages.

Resistance assay and data collection

Symptoms were scored twice a week until 35 dpi. The symptom severity scale ranged from 1 (no symptom) to 10 (plant death), with grades 1-9 corresponding to the scale of Lapidot *et al.* (2006). Apical leaves were collected at 35 dpi for the detection of viral genomes using polymerase chain reaction (PCR), as described below. Fruits from infected and control plants per accession were picked in three harvests. In the first and second harvests, all mature red fruits were collected, while in the last harvest, conducted seven days later, all mature red and immature green fruits were collected. Fresh weights of fruits were recorded for each plant.

Virus detection using polymerase chain reaction

Total DNA was extracted from 20 mg of leaves of all inoculated tomato (symptomatic and asymptomatic plants) and pepper plants (used for virus acquisition process) using the adapted cetyl trimethylammonium bromide method (Doyle and Doyle, 1987), as described elsewhere (Séka *et al.*, 2018). The resulting DNA was stored at -20°C before use. Two sets of primer pairs were used for the specific detection of DNA-A-like (PepYVMLV-A-F 5'GCTCTTGAGTGCGTAATTC3'; PepYVMLV-A-R 5'ATGCAGATTCCGCTGAAG3') and DNA-B (PepYVMLV-B-F (5'GAGATCCAGACAGGTACTG3'; PepYVMLV-B-R 5'GTCG ACCTTCACTACTTCTC 3') components of PepYVMLV. PCR was carried out in 25 μ L volumes containing 5 μ L of 5× buffer, 2.5 μ L of deoxynucleotide triphosphates (2 mM), 1.5 μ L of MgCl₂ (25 mM), 1 μ L of forward and reverse primers (10 mM), and 1 U of GoTaq Flexi DNA polymerase (Promega). After an initial denaturation of 5 minutes at 94°C, 30 cycles consisting of 30 seconds at 94°C, 30 seconds at 56°C,



and 1 minute at 72°C were conducted, followed by a final elongation step for 5 minutes at 72°C. Amplicons were checked by electrophoresis on 1% agarose gels.

Data analysis

All statistical analyses were performed using the R v.3.6.2 (R Core Team, 2020) statistical software. Firstly, based on the seed seller and farmer interview data, the citation frequencies of each accession available in Burkina Faso were calculated as the percentage of accessions of tomatoes cited by a subject among all citations of accessions. Citation frequency differences were examined using the chi-square test based on the contingency table containing the collected data. Secondly, the prevalence of disease and that of virus infection were calculated as the percentage of tomato plants with symptoms of tomato leaf curl disease-tomato yellow leaf curl disease and with positive PCR results, respectively, among all inoculated plants from each cage of the experiment. The differences between accessions were then examined using the chisquare test based on a contingency table containing the collected data. Thirdly, nonlinear regression analyses between the four accessions were performed, testing different linkage functions (Cauchy, cloglog, logistic, logit, loglog, and probit) to fit the progression of disease severity with the gnls function, using the nmle package (Pinheiro et al., 2023). Based on likelihood and using the Akaike information criterion (AIC), the logit function was selected as the best model. In this model, written $y \sim 1+C/(1+exp(A^{*}(x-B)+\log(1))$, disease severity (y) is dependent on the dpi (x)

and three biologically relevant parameters, where A is the slope of the linear phase at the inflection point, 1+C is the disease severity at the plateau phase, and B is the time required to reach 50% of the disease severity at the plateau phase. Fourthly, individual average yields were calculated for each of the four accessions in the infection and control cases based on recorded fresh weights of fruits. Data obtained from infected plants were compared to those of control plants for each accession based on analysis of variance (ANOVA) at the 5% level. The ANOVA was performed after the fitting of the linear model.

Results

Tomato accessions appreciated by producers

To determine the most popular tomato accessions, the surveys allowed the identification of seven and sixteen tomato accessions from farmers and tomato seed sellers, respectively (Table 1). Analysis of citation rates obtained from interviews of farmers showed that the tomato accessions most cultivated by farmers were Mongal F1 (39.5%) and Cobra 26 F1 (21%), compared to Emerald F1 (13.1%), Tropimech (13.1%), Petomech (5.3%), Roma VF (5.3%), and Cerise (2.6%) with a significant difference ($p\leq0.04$). Similarly, data collected from tomato seed shops showed the seven accessions preferred by farmers in addition to nine others (Table 1).

Table 1. Tomato accessions mainly used by farmers and sold in seed shops in the central region of Burkina Faso.

Accessions	Company	Cycle, days	Production period	Citation rates, %	Preference score**			
Data from seed sellers								
Mongal F1	Technisem	65	All seasons	40	4			
Cobra 26 F1	Technisem	65	All seasons	13.3	4			
Tropimech	Technisem	70-75	Cool and dry seasons	3.3	3			
Admiral F1*	Sakata	60-65	Cool and rainy seasons	3.3	3			
Emerald F1	Sakata	60-65	Cool and dry seasons	3.3	3			
UC 82 B*	Green Seeds	70-80	Cool and dry seasons	3.3	3			
Arbra F1*	Sigri seeds	60-65	Cool and rainy seasons	3.3	2			
Martyna F1*	Sakata	60-65	Cool and rainy seasons	3.3	2			
Petomech	Technisem	70-80	Dry and cool seasons	3.3	2			
Roma VF	BS	70-80	Cool and rainy seasons	3.3	2			
Sahara F1*	Sakata	60-65	Cool and rainy seasons	3.3	2			
Sibra F1*	Sigri seeds	60-65	Cool and rainy seasons	3.3	2			
Talato F1*	Sakata	60-65	Cool and rainy seasons	3.3	2			
Tomy F1*	Clause vegetable seeds	60-65	Cool and rainy seasons	3.3	2			
Cerise	Les doigts verts	60-65	Mid-seasons	3.3	1			
TSX-F1*	Tokita	65	Cool and rainy seasons	3.3	1			
Data from farmers								
Mongal F1	Technisem	65	All seasons	39.5	4			
Cobra 26 F1	Technisem	65	All seasons	21	4			
Emerald F1	Sakata	60-65	Cool and dry seasons	13.2	3			
Tropimech	Technisem	70-75	Cool and dry seasons	13.2	3			
Petomesh VF	Technisem	70-80	Dry and cool seasons	5.3	3			
Roma VF	BS	70-80	Cool and rainy seasons	5.3	2			
Cerise	Les doigts verts	60-65	Mid-seasons	2.6	1			

*Accessions cited by seed sellers and not by farmers; ** appreciation of the accessions according to the scale ranging from 1 to 4 with 1 for not preferred, 2 for little preferred, 3 for preferred, and 4 for very preferred.



These were Amiral F1, UC 82 B, Arbra F1, Martyna F1, Sahara F1, Sibra F1, Talato F1, Tomy F1, and Tomato TSX-317 F1 (Table 1). However, the order of preference of tomato accessions is retained only for the two most preferred varieties, namely Mongal F1 and Cobra 26 F1. Taken together, Mongal F1 was significantly preferred by farmers over Cobra 26 F1 (p=0.04).

Effectiveness of whitefly-mediated transmission tests

The most preferred accessions identified above (Mongal F1, Cobra 26 F1, and Emerald F1), in addition to the accession Diva F1, newly introduced in Burkina Faso, were screened for resistance/tolerance to PepYVMLV. All four accessions inoculated using whitefly-mediated transmission developed strikingly distinct symptoms of leaf crumpling with yellowing and stunting (Figure 1). At 32 dpi, Mongal F1 and Diva F1, respectively, showed the highest (88.9%) and lowest (44.4%) prevalence of symptoms in inoculated plants. However, Cobra 26 F1 and Emerald F1 showed symptoms in 55.6% of inoculated plants (Table 2). Based on comparison analysis, a significant difference was observed for Mongal F1 compared to Diva F1 (p=0.04). In contrast, no significant differences were observed between Diva and the two other accessions (p=0.63). In addition, the efficiency of transmission was verified by PCR based on specific primers for PepYVMLV-A and -B DNA molecules. This diagnosis confirmed the presence of PepYVMLV in all plants showing typical symptoms of PepYVMLV infection (Table 2).

Kinetics of estimated symptom severity on tomato accessions

The kinetics of symptom severity in PepYVMLV were compared using the four accessions of tomatoes. Eleven dpi using whitefly-mediated transmission, tomato plants of Mongal F1 and Emerald F1 showed the first symptoms of crumpling, yellowing, and stunting. In contrast, Cobra 26 F1 and Diva F1 exhibited their first symptoms at 14 and 20 dpi (Figure 2). Mongal F1 accession showed quick development of disease with a severity score of 9 at 23 dpi, while a severity score of 5 was recorded for Diva F1 at 30 dpi. The disease progression curves for the other two accessions fall between the above 2, with an early onset of the disease associated with more severe symptoms for Emerald F1 compared to the Cobra 26 F1 accession. Interestingly, logistic growth models of the progression of disease symptom severity in tomato accessions show significant differences (p<0.01) (Table 3).



Figure 1. a) Healthy tomato plant corresponding to control; b) disease symptoms (leaf crumpling with yellowing and stunting) in tomato (*Solanum lycopersicum*) plants inoculated by whitefly-mediation method.

Table 2. Transmission rates of the Pepper yellow vein Mali virus on preferred accessions of tomato using whitefly-mediated transmission35 days after inoculation.

Tomato accessions	Symptoms, %*	PCR diag	nosis, %**	
		DNA-A	DNA-B	
Cobra 26 F1	55.6 (5/9)	55.6 (5/9)	55.6 (5/9)	
Diva F1	44.4 (4/9)	44.4 (4/9)	44.4 (4/9)	
Emerald F1	55.6 (5/9)	66.7 (6/9)	55.6 (5/9)	
Mongal F1	88.9 (8/9)	88.9 (8/9)	88.9 (8/9)	

*Percentage of begomovirus-like symptoms (symptomatic samples/total); **percentage of tomato plants infected by the Pepper yellow vein Mali virus DNA-A or DNA-B (polymerase chain reaction-positive samples/total). PCR, polymerase chain reaction.



Effect of the *Pepper yellow vein Mali* virus on the yield of tomato accessions

To assess the disease effect, tomato fruits were collected per plant and accession in both the infection and control cases, and individual average means and standard deviation of means were calculated (Figure 3). The disease effect was stronger in the Mongal F1 accession, where the infected plants had average yields of 131.2±22.1 g/plant, compared to 491.1±39.4 g/plant for healthy plants (control), a difference of 359.9 g/plant. The second most susceptible accession was Emerald F1 with 364.4±12.3 g/plant and 466.1±10.1 g/plant for infected and healthy plants (control), respectively. It was followed by Cobra 26 F1 with 157.5±13 g/plant and 206.7±12.5 g/plant for infected and healthy plants (control), respectively. Finally, the Diva F1 variety was the least impacted, with average yields of 107.9±8.4 g and 133.3±27.1 g for infected and healthy plants (control), respectively. It only recorded 25.38 g/plant as yield loss. The comparison analysis of yields showed a significant difference for infected plants compared to controls regardless of the tomato accession ($p \le 0.02$) (Figure 3).

Discussion

The importance of tomatoes in Burkina Faso is evident from the numerous accessions developed by seed companies and national research centers. These accessions are distributed to farmers through individual seed shops. This study revealed that the tomato accessions preferred by producers are mostly found in seed shops. This underlines the existence of a correlation between the preference of the farmers, undoubtedly influenced by the appreciation of the consumers, and the availability of the varieties in the shops. Mongal F1 and Cobra 26 F1 accessions were reported to be the most preferred because they were much appreciated by consumers and adapted to all seasons, hence their greater use by farmers. Tropimech and Emerald F1 are considered to be very popular and the most sought-after by foreign buyers because of their long shelf life and resistance during transport. They were reported to be available in seed shops but less adapted to the rainy season, which explains their low use. The low preference for Petomech, Roma VF, and Cerise accessions could be due to their low yield potential, according to the respondents.

The presence of PepYVMLV was revealed by molecular analysis in all varieties, showing typical symptoms of leaf deformation and/or yellowing regardless of the tomato accession tested. This underlines that symptomatology is a good way to detect the presence of PepYVMLV in plant tissues, although it is not sufficient to definitively conclude its presence. Evaluation of the tomato accessions showed that they were all susceptible to PepYVMLV in semi-controlled conditions. However, the degree of



Figure 2. Kinetics of estimated symptom severity of tomato leaf curl disease following whitefly-mediated inoculation of tomato accessions. Each point represents the average for one experiment. The symptom severity scale (y axis) ranges from 1 (no symptoms) to 10 (plant death).



Figure 3. Mean yields of healthy tomato accessions corresponding to control and infected tomato accessions after whitefly-mediated inoculation. For each dot, vertical bars represent 95% confidence intervals. Values on top of the bars indicate the p values obtained from the analysis of variance at the 5% level when data from infected plants were compared to those of the controls.

Table 3. Estimated parameters [95% confidence intervals] of the logistic growth model of the progression of disease symptom severity in plants of tomato accessions inoculated by whitefly-mediated transmission of the *Pepper yellow vein Mali virus* from infected pepper plants. .

Accessions		Parameter estimates for the logit model	
	Α	В	1+C
Cobra 26 F1	0.16 [0.07-0.25]	21.52 [15.26-27.79]	5.79 [3.52-8.07]
Emerald F1	0.27 [0.15-0.38]	16.54 [14.55-18.52]	6.68 [5.77-7.58]
Mongal F1	0.40 [0.35-0.45]	14.72 [14.37-15.08]	7.98 [7.78-8.18]
Diva F1	0.43 [0.25-0.61]	22.51 [21.30-23.72]	4.15 [3.63-4.68]

A, slope of the linear phase at the inflection point of the logistic disease progression curve; B, time to reach 50% of the symptom severity at the plateau phase; 1+C, maximum symptom severity at the final plateau.



Conclusions

The aim of the present study was to identify preferred tomato accessions and to assess their resistance/tolerance to PepYVMLV in semi-controlled conditions using the whitefly-mediated transmission method. The results show that 16 accessions are sold in shops in Burkina Faso, two of which are generally preferred by producers. Whitefly-mediated transmission tests were successful in reproducing infection symptoms. The result of molecular characterization using specific primers confirmed that PepYVMLV is involved in symptom expression in tomato plants. The screening of the preferred accessions allowed the characterization of Diva F1, Emerald F1, Cobra 26 F1, and Mongal F1 as more or less sensitive accessions. This study underlines the need to combine efforts for the identification of accessions resistant/tolerant to PepYVMLV in breeding programs for appropriate and effective management of tomato leaf curl and/or yellowing disease in Burkina Faso and elsewhere.

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