



Prevalence and Occurrence of Various Wilt Pathogens Associated with Tomato (*Solanum lycopersicum* L.) in Togo

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Authors' contributions

*This work was carried out in collaboration among all authors. Author KDA designed the study, directed *R. solanacearum* isolates collection and laboratory analyzes and developed the first draft. Author KS collected *R. solanacearum* isolates, carried out field experiments, managed literature searches and read the manuscript. Author MKA provided general guidance on the study and supervised the work. All authors read and approved the final manuscript.*

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ABSTRACT

In December 2015, wilt symptoms on tomato plants (*Solanum lycopersicum* L.) were observed on vegetable growing perimeters in Sotouboua district in Togo. The disease, manifested by wilting of the youngest leaves followed by wilting and total desiccation of plants who eventually dy, leading to losses of up to 100%, is similar to bacterial wilt. The aim of this study, was to determine the pathogen responsible for the observed symptoms. For this purpose, phytosanitary surveys were carried out on tomato plots in Sotouboua distric, in 2018. During the surveys, Tomato plants infected by wilt and the plots soil samples, were collected on CECODRI project and farmers' plots in the district. Soil samples were analyzed for the detection of nematodes while, tomato leaves, stems and roots were directly observed under binocular loupe and after incubation in Petri dishes containing filter paper moistened with distilled water to encourage sporulation of phytopathogenic fungi. Infected tomato stems and roots were analyzed by stem-streaming and DAS-ELISA tests

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using Agdia inc. *Ralstonia solanacearum* Patho Screen Kit to detect *R. solanacearum*. The results of the phytosanitary surveys showed that the wilt prevalence was 100% in Sotouboua district with incidence rates of up to 100%. Analysis of diseased samples, using stem-streaming and DAS-ELISA tests, revealed that 85.11% of diseased plants were infected by *R. solanacearum*. No nematodes were identified in the roots of the infected plants, but in soil samples only a few nematodes were counted. No fungus was found in the plants with wilt symptoms. It appears, therefore that, the wilt on tomato plants in Sotouboua district was caused by *R. solanacearum*. This, in our knowledge, is the first report on *R. solanacearum* infection on tomato in Togo.

Keywords: Tomato; bacterial wilt; *Ralstonia solanacearum*.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill.) is, after the potato, the most produced and consumed vegetable in the world with an annual production estimated to 177.04 million tones [1,2]. In Togo, with an annual production of 5 582 tons, tomatoes are today one of the most produced vegetables [2]. It is cultivated in the five economic regions of the country, especially in the Maritime region, along the coast, and in the dry season in the lowlands and water points of Central, Kara and Savannah regions [3].

However, the average productivity in the country, which is 4.33 tons of tomato per hectare, remains considerably lower than the average world yield estimated at 33 tons / ha [4]. This low productivity of the tomato crop in Togo is due to phytosanitary constraints, especially, to soil pathogens [5,6].

Since a certain moment, in the district of Sotouboua located in Central region, several cases of wilt of tomato plants have been observed on vegetable growing perimeters and whose causes have remained unknown. On the vegetable sites, losses can go up to 100%, forcing some farmers to abandon their plots (K Simiti, Ecole Supérieure d'Agronomie, Université de Lomé, Togo, unpublished results, 2015).

Wherever tomatoes are produced, symptoms of wilt of tomato plants are frequently reported. This disease, which can cause losses of up to 100%, is often attributed to *R. solanacearum* [7,8]. In West Africa, bacterial wilt, caused by *R. solanacearum*, is reported in several countries including countries bordering Togo: Benin [9], Ghana [10] and Burkina Faso [11]. However, in our knowledge, this disease of tomato plants is not yet reported in Togo.

Several studies have reported that tomato plant wilt symptoms can also be caused by gall

nematodes [12], pathogenic *Fusarium oxysporum* and *Verticillium* fungi [13,14]. To develop effective and sustainable control strategies, the identification of the pathogen is a prerequisite [15].

The general objective of this study was to determine the causes of the wilt of tomato plants on the vegetable sites in Sotouboua district. Specifically, it was to determine the prevalence of the disease in this locality, the incidence of the disease, to identify the pathogens responsible of the wilt of the tomato plants, especially, *R. solanacearum*, *F. oxysporum*, *V. albo-atrum* and nematodes.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in the district of Sotouboua (8 ° 45'N and 0 ° 49'E) located in the Central Region of Togo. The climate is characterized by a single dry season from November to April and a single rainy season from May to October. The annual rainfall is between 1200 and 1500 mm [16]. Soils of the district are ferruginous of sandy type and sandy-loamy, characterized by an acid pH (6), with some deficiencies in organic matter, calcium (Ca) and in phosphorus (P) [17].

2.2 Plant Material

During our phytosanitary surveys, we collected samples with wilt symptoms from three varieties of tomato (Monagl F1, Dangbo and Pola) commonly grown in Sotouboua district. Dangbo and Pola are tomato varieties with determinate growth. Their cycle is short. They have an excellent tolerance to water and heat stress. Dangbo, has an adequate resistance to *F. oxysporum* and viruses [18]. Mongal F1 is a variety with determinate growth. Its cycle is short, varying between 60 and 65 days. It has tolerance

to bacterial wilt and a resistance to drought. It has, also, a resistance to *R. solanacearum*, *F. oxyporum*, *Stemphylium* spp, TMV (Tobacco Mosaic Virus) and root Nematodes [19].

2.3 Phytosanitary Surveys and Prevalence of the Wilt

Phytosanitary surveys were carried out on tomato plantations in Sotouboua district to determine the prevalence and incidence of the tomato wilt in this area. Five tomato production sites were prospected. These sites included a total of 22 plots of tomato including plots of farmers and those of CECODRI project. During the surveys, the tomato fields were traversed along an imaginary Z broken line and on each segment of the line, all the tomato plants were counted. Tomato plants with wilting symptoms were collected on the Z broken line. A total of 188 tomato plants with wilt symptoms were collected on the fields for identification of pathogens (Table 1).

Disease prevalence, according to Nutter et al. [20]. Is the ratio of the number of infected fields in a specific geographical area (country, state, region, prefecture, canton, etc.) to the number of fields surveyed. Disease incidence is the ratio of the number of plants infected by a given pathogen to the number of plants sampled and tested. In this study, wilt prevalence was recorded as the percentage of fields in which the disease is present in the district. The incidence of the disease was determined by the percentage of tomato plant samples infected by the pathogen searched compared to the total number of tomato plants sampled and analyzed.

2.4 Pathogens Identification

In other to identify the exact pathogen responsible of the disease, investigations were carried out on different pathogens known to be responsible of the tomato wilt disease [12,13,14] such as nematodes, *R. solanacearum*, *F.*

oxysporium and *Verticillium* spp, as described below.

2.4.1 Identification of *R. solanacearum* in tomato plants

In tomato plants with wilt symptoms, internal brown spot and whitish exudates characteristic of the presence of *R. solanacearum* [7,21,22], have been investigated.

Stems of infected tomato plants were cut longitudinally and observed for the search of internal brown spot. For the detection of whitish exudates, the stem of tomato plants samples with wilt, were cut at the crown region just above the soil and soaked for a few minutes in water contained in clear bottles.

In addition, serological confirmation was carried out by direct double antibody sandwich ELISA (DAS-ELISA) test, using Agdia inc. *R. solanacearum* PathoScreen Kit, following the manufacturer protocol. This, was performed directly from infected tomato plants stems (the crown region about the soil line) and roots. Saps (100 µl) obtained by soaking 0.2 g of a mixture of tissue samples from the stem and roots of diseased tomato plants in 2 ml of extraction buffer (GEB), were added to microplates coated with monoclonal antibodies to extracellular polysaccharides (EPS) of *R. solanacearum*. After incubation in humid box for 1 hour at room temperature, the plates were washed to remove unbound sample. A monoclonal antibody conjugated to peroxidase, was then added to the plates wells. After incubation, the plates were washed with 1X PBST to remove any unbound conjugate. Then 100 µl of TMB substrate, extemporaneously prepared, was dispensed into each well and then the plates were incubated in a humid box for 15 minutes. According to the protocol of DAS ELISA, wells in which a blue color developed indicated positive results. Wells in which there was no significant color development indicated negative result.

Table 1. Prospected sites and number of tomato plants sampled per site and per variety

Sites	Number of tomato plants sampled by variety			Total
	Dangbo	Pola	Mongol F1	
CECODRI	32	32	32	96
LAKY	11	14	0	25
KAMALA	10	12	0	22
KATAKONA	12	11	0	23
SUCRE	10	12	0	22
Total	75	81	32	188

2.4.2 Detection of phytopathogenic fungi

Leaves collected from withered tomato plants were observed under binocular loupe to search for fungi fructifications. Leaves, stems and roots of plants on which fructifications were not observed, were fragmented and incubated in Petri dishes on filter paper moistened with distilled water to encourage sporulation [23].

After 5 days of incubation, the cultures were observed under binocular loupe to check for the presence of fungi fructifications.

2.4.3 Search for nematodes

Collect of soil and root samples: Soil samples were collected at a depth of 15 cm to 20 cm in the tomato fields. Soils taken from the same tomato plot were mixed to obtain a homogeneous sample characteristic of each plot [24]. In addition, the roots of tomato plants infected with wilt were collected to extract nematodes.

Extraction and counting of nematodes in soil and root samples: The nematodes were extracted according to the protocol described by Sabonga [25]. 5 g of ground root or 100 g of soil taken per sample was deposit on filter paper in a sieve, all placed in a container containing water which lightly covered the soil sample or root sample. Samples were thereafter incubated at 25°C for 24 hours for the soil and 48 hours for the roots. At the end of the incubation, the water of the container was collected in test tubes and then left for a few minutes, a time for the nematodes to fall to the bottom. A volume of 10 ml of the suspension obtained was taken in each test tube, after stirring, and then placed in a squared Petri dish. The Petri dishes were observed under a binocular loupe for the nematodes counting. A total of three aliquots were taken and observed by test tube.

3. RESULTS

3.1 Characteristic Symptoms Observed on Withered Tomato Plants

The characteristic symptoms observed on the tomato plants, during the phytosanitary surveys and on the experimental plots, were a wilting of the younger leaves followed by wilting and yellowing of foliage of the whole plant that dies a few days later. During the fructification of tomato plants, symptoms of wilt followed by desiccation

were also observed. Some wilt was accompanied by crown rot of the plants. A longitudinal section of the stem of some tomato plants showed a brown coloration of tissues of the vascular system.

3.2 Prevalence and Incidence of the Tomato Wilt in Sotouboua District

During phytosanitary surveys, wilt symptoms were present on all the sites surveyed, corresponding to 100% prevalence rate of the district. Diseased tomato plants rates, in the majority cases, were very high up to 100%. More than 60% of the tomato fields surveyed, had totally withered at the tomato plants fructification. Other fields, however, have been partially attacked. In fact, on a plot of KAMALA where the tomato variety Pola was sown, no wilt symptoms were observed; however, this plot is located on the site where plots sown with the same variety of tomato (Pola) have been totally destroyed.

3.3 Detection of *R. solanacearum*

A total of 188 tomato plant samples exhibiting wilt symptoms were analyzed by stem-streaming test and DAS-ELISA test. Of these plant samples analyzed, 141 and 160 were positive for *R. solanacearum*, respectively at stem-streaming test and DAS-ELISA test.

3.3.1 Stem-streaming test

The results on the detection of *R. solanacearum*, in the five tomato production sites of Sotouboua district, are given in Table 2. Of 188 stem samples analyzed, 141 were positive after 2 to 3 minutes soaking in water, an average rate of 75%. However, as shown in Table 2, depending on the collection sites, the average rate of positive samples ranged from 69.57% to 86.36%.

3.3.2 Immunological detection and incidence of *R. solanacearum*

The results on immunological detection of *R. solanacearum* are shown in Table 3. Immunological data were used to calculate the incidence of *R. solanacearum*. The incidence rate per tomato growing site was calculated by dividing the *R. solanacearum* positives samples by the total plants samples analyzed multiplied by hundred. *R. solanacearum* incidence for Sotouboua district was calculated using the average from the sampled sites. According to the data of these analyzes, *R. solanacearum* was

identified in 160 of the 188 tomato plants samples analyzed, corresponding to an average incidence rate of 85.11%. However, as indicated in Table 3, the average incidence rates varied somewhat depending on the collection sites and ranged from 81.82% to 95.45%.

In addition, it should be noted that, among the 160 samples that were positive on DAS-ELISA test, 19 were tested negative at the stem-streaming test, representing 10.11% of the 188 samples analyzed.

Data recorded in Table 4 summarized the incidence rates of *R. solanacearum* in the five sites prospected, based on the results of DAS-ELISA test.

3.4 Detection of Phytopathogenic Fungi

Observations at binocular loupe of leaves of tomato plants with wilt symptoms, revealed no fungus fructification. However, after 4 days of incubation of fragments of diseased tomato plants on moistened filter paper, fungus mycelia were observed in 4% of the Petri dishes, but

there was no fructification. This did not allow the identification of the fungi formed in the Petri dishes.

3.5 Detection of Nematodes in Tomato Roots and in Soil

Results on nematodes detection are shown in Table 5. The observation of withered tomato plants roots suspensions at binocular loupe, showed no presence of nematodes. This could justify the absence of galls on the roots of tomato plants with wilt. However, the results of soil samples analyzes, revealed the presence of nematodes, but at very low population densities on the farmers' and CECODRI project plots. As indicated in Table 5, the average number of nematodes obtained in 100 g of soil varied somewhat depending on the collection sites and ranged from 0.66 to 3.25 nematodes per 100g of soil. The highest average was obtained on CECODRI project site with 3.25 nematodes and the lowest average was registered on KAMALA site, with an average of 0.66 nematode per 100 g of soil.

Table 2. Percentage of tomato plants samples testing positive for *R. solanacearum* on Bacterial streaming test

Sites	Percentage of tomato plants samples positive by variety			Means(%)	Sample size
	Dangbo	Pola	Mongol F1		
CECODRI	75	78,13	71,88	75	96
LAKY	81,82	64,29	0	72	25
KAMALA	70	75	0	72,73	22
KATAKONA	66,67	72,73	0	69,57	23
SUCRE	90	83,33	0	86,36	22
Means (%)	76	75,31	71,88	75,00	-
Sample size	75	81	32	-	188

Table 3. Percentage samples of tomato plants testing positive to *R. solanacearum* on DAS-ELISA test

Sites	Percentage of tomato plants samples positive by variety			Means(%)	Sample size
	Dangbo	Pola	Mongol F1		
CECODRI	81,25	84,38	81,25	82,29	96
LAKY	90,91	78,57	0	84	25
KAMALA	80	83,33	0	81,82	22
KATAKONA	83,33	100	0	91,30	23
SUCRE	100	91,67	0	95,45	22
Means (%)	85,33	86,42	81,25	85,11	-
Sample size	75	81	32	-	188

Table 4. *R. solanacearum* incidence rates (%) in the tomato growing sites prospected Sotouboua

Sites prospected					Mean(%)
CECODRI	LAKY	KAMALA	KATAKONA	SUCRE	
82,29	84	81,82	91,30	95,45	85,11

Table 5. Average number of nematodes counted in soil samples by tomato growing site prospected

Experimental plot & prospected sites	Average number of nematodes in 100 g soil
CECODRI	3,25
LAKY	1,83
KAMALA	0,66
KATAKONA	2
SUCRE	2

4. DISCUSSION

The 100% prevalence of tomato wilting in the localities surveyed suggests that all tomato production perimeters in Sotouboua district are infected with the disease. In addition, the very high incidence rate of wilt on tomato plants, up to 100%, could be explained by a high sensitivity to the disease of tomato varieties grown in the locality. But the variability of the incidence rates according to the sites prospected could be attributed to the agriculture practices adopted by the farmers. In fact, on a plot of KAMALA, where the tomato variety Pola was sown, no wilt symptom was observed; while on plots located on the same site and sown with the tomato variety Pola, incidence rates of up to 100%, were recorded.

According to the explanations given by owner of the plot free from wilt symptoms, this healthy plot was not irrigated with the water of the pond used by other farmers on the site, but he used water of a well. In addition, he practiced crop rotation on this plot for two years. This suggests that the pond water is contaminated and would have spread the disease. In addition, the absence of wilt symptoms on that plot could be also related to the rotation of the cultures practiced during two years. These observations confirmed the role of cultural practices in the spread of the disease. Previous studies have shown that *R. solanacearum*, one of the pathogens responsible of tomato plants wilt, could be disseminated by irrigation water [26]. Other researchers reported that *R. solanacearum* mainly propagates in moist soils, and that crop rotation of 2-5 years can limit the occurrence of the wilt [27,22].

During *R. solanacearum* detection analyzes, between 69.57% and 86.36% of tomato samples were positive at stem-streaming test respectively in the five sites surveyed. These high percentages of positive samples may suggest a bacterial origin of the majority of the wilt symptoms observed on the tomato plants. Indeed, as indicated above, the presence of white filaments of exudates flowing from cut tomato stems in water, is a characteristic sign of the presence of *R. solanacearum* in the tomato plants [7,21,22]. Moreover, *R. solanacearum* confirmatory test carried out by DAS - ELISA tests on tomato diseased samples, showed high level of the bacterium presence in the samples, from 81.82% to 95.45% respectively in the five sites surveyed, with an average of 85.11%. These high incidence rates of *R. solanacearum* in the samples of tomato plants analyzed, suggests that this bacterium is the main cause of the wilt symptoms observed in the tomato fields, confirming the results obtained with stem-streaming test.

With *R. solanacearum* DAS-ELISA test, more samples analyzed gave positive results compared to the stem-streaming test. In fact, the differences between the two results were respectively 7.29%, 12%, 9.09%, 21.73% and 9.09% positive samples for CECODRI project, LAKY, KAMALA, KATAKONA and SUGAR sites. These results could be explained by the fact that in the stem-streaming test, the amount of bacterial exudate emitted by some samples was not sufficient to be detected with the naked eye. Indeed, according to Aloyce et al. [28], citing Alvarez and associates [29], the visibility of bacterial streaming by naked eye depends on bacterial population in the xylem which should

not be low. But, the test DAS-ELISA, interacting directly with the bacteria, made it possible to detect them.

However, despite the effectiveness of the DAS-ELISA test, about 14.89% of the withered samples analyzed were negative. This suggests that some wilting symptoms observed in the fields are related to other diseases such as those caused by fungi including *F. oxysporium* and *Verticillium*, by nematodes, [12,13,14].

Concerning the detection of fungi in diseased tomato plants, the lack of fungus fructifications on both the tomato leaf samples observed under binocular loupe and the samples incubated in Petri dishes, may suggest that, fungi are not the main causal agents of the wilt symptoms observed on the tomato fields.

Results on nematodes detections revealed no presence of nematode in roots suspensions of the withered tomato plants and a very low population densities of nematode in soil sampled taken on tomato sites. Apart from the fact that nematodes could cause wilting symptoms on tomato plants, it has also been reported that fields with a high nematode population are more affected by bacterial wilt [30]. According to Singh and associates [31], root lesions caused by nematodes provide entry routes for *R. solanacearum* leading to massive infection. However, in this study, despite high incidence rates of wilt on the tomato fields, nematode density obtained was negligible. This suggests that nematodes are not the cause of wilt symptoms of tomato plants observed on the sites prospected.

All the results obtained in this study confirmed *R. solanacearum* as the main causative agent for wilt symptoms observed in the tomato fields. This suggests that, since nematodes are excluded, effective control of the disease will consist to the use of resistant tomato varieties and to the practice of crop rotation on plots infected by *R. solanacearum*. However, to be effective, a study of the genetic variability of *R. solanacearum* populations in localities is essential. For this purpose, it will be important to also perform molecular analyzes to determine *R. solanacearum* races in Sotouboua district and to more effectively determine the incidence rates of the pathogen in this locality, since there may be false positives during the DAS ELISA test. This study may also provide information on the agents responsible of wilting symptoms on 14.89% of

tomato plants samples remaining negative for *R. solanacearum* detection tests.

5. CONCLUSION

The results obtained during the present study show that tomato plants wilt is present on all tomato production sites of Sotouboua district with very high incidence rates, up to 100% sometimes. The results of laboratory analyzes concluded that, wilt symptoms observed on tomato plants are bacterial wilt caused by *R. solanacearum*. This, to our knowledge, represents the first report of this disease in Togo. In order to define an efficient and sustainable control method, a study of the genetic variability of *R. solanacearum* populations in the tomato growing localities is important.

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COMPETING INTERESTS

Authors of this manuscript declared that they have not any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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