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Virus Diseases of Some Tomato Cultivars Grown in Buea, Cameroon

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Abstract: Tomato, *Lycopersicon esculentum* (L) Mill is an exotic fruit vegetable which has achieved popularity due to its versatility in fresh and processed forms. Viruses impose serious limitations on tomato cultivation in many countries. Field sampling was done randomly and single leaf samples were collected from 10 randomly selected plants per village. Cultivars observed in the fields were grown in an experimental plot in the university of Buea. Both field and experimental samples collected, were tested for AMV, CMV and PVY by DAS-ELISA. Rio grande, Tropimech, Roma savanna, Roma VF, Carioca and Buea local were the cultivars grown in the study site. Varied virus disease symptoms were observed, ranging from mosaic to leaf curl. AMV, CMV and PVY tested positive in all the locations and on all the cultivars. All cultivars tested positive for the different viruses. The incidence of mixed infection was higher than that of single infection. Generally, the incidence of all the viruses were low ranging from 2.85% to 5.63% in single and mixed infections respectively. The study indicated that tomato in this part of the country is affected by virus diseases and must be taking into consideration for large scale production.

Keywords: Tomato, Viruses, AMV, CMV, PVY, Buea, Cameroon

1. Introduction

Tomato, *Lycopersicon esculentum* (L) Mill is an exotic fruit vegetable which is adapted to an extremely wide range of climatic conditions. It is cultivated throughout Tropical Africa, where cultivars adapted to Tropical conditions are grown [1]. Cameroon is among the major producers of tomatoes in the Central African sub-region with almost 1,068,495 metric tons in 2018 [2]. Tomato is one of the mainstays in the diet of urban households across most of Africa. It is one of the most import fruit vegetables, appearing daily in every household dish either fresh of processed form [3].

In Cameroon, tomato is mainly produced in Foumbot, Bafousam and isolated locations in other agroecological zones. Because of the ubiquitous consumption, large quantities have to be made available to the markets. As such local and peri-urban production is encouraged.

Tomato has achieved popularity due to its versatility in fresh and processed forms [4]. Its production therefore has promising potentials as a source of income and livelihood. Buea is strategically located for the effective exploration of such potentials. Amongst the constraint limiting tomato production in the tropics, diseases are very prominent given the optimum condition for their development. Common challenges of tomato production include cost of hybrid seeds, cost of chemicals and poor yields, pests and diseases [5] Bacteria, viruses, fungi, nematodes, insect pests and weeds are considered to be biotic factors that limit crop production [6]. Viruses impose serious limitations on tomato cultivation in many countries especially in the developing countries [7]. Several viruses, including those already reported elsewhere in Africa, namely PVY, PVX, CMV, ToMV, TSWV, AMV and TYLCV [8] infect tomato in Africa. These viruses could be a problem to tomato growers in some parts of Cameroon. Although various viruses have been reported to be infecting tomato, their proper identification has been inadequate in

many cases. Moreover, due to lack of suitable diagnostic reagents, the incidence, occurrence and effect of these viruses have not been determined in Buea and its environs. Hence, the information on the relative importance of these viruses infecting tomato has been scares. If these viruses are identified and their incidence rate reduced, then tomato production will be optimized to meet as demand by the population.

Many virus strains are known to infect tomato in different parts of the world [9]. These viral differences may be a consequence of genetic and ecological diversity of their host [10]. So far, no survey has been carried out in Buea and it environ s to evaluate the virus problems associated with tomato production. The importance of virus diseases of tomato is obvious. Firstly, there have been importation of seeds from temperate countries where the viruses abound. These seeds accidentally introduced seed- borne viruses or their strains. Secondly, the absence of an organised seed program could lead to a build-up of viruses in the stock. Thirdly, because of the rich tropical flora, various strain of known viruses or new ones may infect tomato with different pathological implications. The problem caused by some of these viruses have been accentuated by the cropping practise adopted [11].

Information on virus incidence, distribution and characterisation is the first line of action in dealing with virus problem, since this sets a working basis for pathologists, agronomists and breeders. Such vital geophytopathological information can only be obtained from a well-designed survey. Therefore, this study seeks to study the ecology of viruses of tomato in Buea and its environs, specifically to determine the incidence of viruses in the study area, to identify the viruses infecting tomato in the study area and to evaluate the impact of viruses on yield under prevailing production systems.

Tomato is infected by a large number of viruses. Some of these viruses have been long associated with the crop such that today they are of world-wide occurrence in tomato producing areas. These viruses impose serious limitations on tomato cultivation in all countries [11]. Among the viruses infecting tomato, the most economically important ones are found in eight virus taxa: Begomo-, Crini-, Poty-, Cucumo-, Tospo-, Tobamo-, Potex viruses and Alfamo viruses.

2. Materials and Methods

2.1. Site Location

This work was carried out along the slope of Mount Cameroon covering Buea and its environs, in the South west region of Cameroon. The area has a humid tropical climate and the climatic pattern is sharply modified by the influence of topography [12]. The mean annual rainfall varies between 2085mm near Ekona to 9086mm at Debunscha. The mean annual temperature at sea level is about 25°C. The soil temperature at sea level, measured at 10cm dept varies from 25°C at 200m through 20°C at 1100 to 15°C at 2200m. The Buea area is characterised by semi-permanent mist and cloudiness. The landscape of the study area is steeply sloping with rocky ridges conforming to individual lava flows separated by numerous ravines. The soil is volcanic and relatively fertile.

2.2. Field Survey and Sampling

The survey was conducted in the early months of the rainy season. During these periods, tomato fields were observed for virus-symptoms. Based on earlier reconnaissance surveys, the following villages were surveyed: Lower Bokova, Molyko, Lower farms, Upper farms, and Mammu. Farms to be sampled were chosen randomly and 10 farms were chosen in each village. Plant sampling was done in a 2 x 2m quadrats spaced 4m apart along the field diagonal or some longest straight line across the field for irregularly shaped fields. In each quadrat, the total number of plants and the number of plants with each virus-like symptoms type were recorded. This method is based on the fact that virus incite morphological changes in the plant. Single leaf samples were collected from 10 randomly selected plants in each quadrat and placed in plastic gripper bags. The samples were labelled to indicate sample number, cultivars and date of collection. These samples were temporarily stored in ice and were later transferred to the Faculty of Science laboratory of the University of Buea for virus testing.

2.3. Serological Detection

The field samples were each tested for Alfafa mosaic virus (AMV), Cucumber mosaic virus (CMV) and Potato virus Y (PVY) by DAS-ELISA [13]. Polystyrene microtiter plates were coated with antisera to respective viruses diluted in the coating buffer as specified by supplier. Coating was at the rate of 100uL per wells. Only the inner well 60 wells of the plates were used to avoid edge effects. The coated plates were placed in polythene bags and incubated at 37°C for 4hours. Batch samples (of ten leaflet each) were collected and triturated in extraction buffer and at the rate of 1g per 5mL buffer and strained through absorbent cotton wool. The sap extract was transferred into a test-tube. Following the incubation of plates with antibodies, the excess antibodies were emptied. The wells were washed with washing buffer. The washing process was repeated three times for each plate. Test samples were then added to duplicate wells at the rate of 100uL per well. The healthy (negative) control consisted of crude sap from a non-symptomatic maize plant. The sample of the healthy control was applied to two wells of each plate. The diseased (positive) control for CMV was from a stock maintained in tomato plant by mechanical inoculation. Those for PVY and AMV were from *Vernonia colorata*. Plates were incubated overnight at a temperature of 4°C. The next morning plated were washed as before. The alkaline phosphatase enzyme conjugate (IgG-AP) of each antivirus antibody appropriately diluted in conjugate buffer was applied to corresponding plates at the rate of 100uL per wells. Plates

were incubated for 4hours at 37°C, washed and p-nitrophenylphosphate substrate dissolved in substrate buffer at the rate of 1mgmL^{-1} was then applied to the plates, observed read in an ELISA reader at 405nm using substrate buffer as blank. A sample was considered positive for a virus when the optical density (OD) value was at least 1.5 times that of the negative control. From the ELISA results, virus incidence was calculated from the formula of [14].

$$Y = \left[1 - \left(\frac{\text{Nt} - \text{Ni}}{\text{Nt}}\right)^{\frac{1}{n}}\right] x\ 100 \quad (1)$$

Y= % Incidence
Nt = No of samples assayed
Ni = No of positive samples for a virus
n= No of leaves in batch of samples.

3. Results

3.1. Cultivars Grown in the Field

The cultivar names of tomatoes given by the farmers during the survey included: Rio grande, Tropimech, Roma savanna, Roma VF, Carioca and Buea local. Most of the field had Rio grande while Buea local was the least grown.

3.2. Virus-like Symptoms Recorded in the Field

Varied viral disease symptoms were observed in all the fields surveyed. These symptoms were common in all the fields and on all the cultivar. Leaf curl, leaf roll, general chlorosis and shoe stringing were very common. All the virus-like symptom types were observed in all the locations in the study area (Table 1).

Table 1. *Incidence of virus-like symptom types in some villages around Buea.*

Location	Symptom types				
	Mosaic/chlorotic mottle	Leaf roll, bronzing and stunting	Leaf curl, chlorosis and stunting	Mixed symptom	% Total symptom
Lower Bokova	13	8	14	4	39
Molyko	10	13	14	3	40
Lower Farms	17	6	17	6	46
Upper farms	25	3	30	5	63
Mammu	15	6	11	2	34

The incidence of all the symptoms types per location varied from 34% in Mammu to 63% in upper farms. However, locations like lower Bokova, Molyko and Lower farms generally had high incidence.

The incidence of leaf curl and mosaic were generally high in all the locations as compared to the other symptom types. Mixed infections were generally lower, ranging from 2% in Mammu to 6% in Lower farms. Mosaic symptoms were between 10% in Molyko to 25% in upper farms. Apart from Molyko with 13% incidence, leaf roll was generally low ranging from 3% in upper farms to 8% in Lower Bokova. Leaf curl incidence in the study area ranged from 11% in Mammu to 30% in Upper farms.

3.3. Incidence of Virus-Like Disease Symptom in Cultivars

All cultivar studied had all the four viral symptoms observed (Table 2).

Table 2. *Incidence of different virus-like symptom types in some tomato cultivars commonly grown in Buea and its environs.*

Cultivar	% Incidence of symptom types				
	Mosaic/ chlorotic mottle	Leaf roll, bronzing and stunting	Leaf curl, chlorosis and stunting	Mixed symptoms	Total infection
Rio-grand	15	14	13	2	44
Tropimech	17	6	17	4	44
Buea local	25	3	24	2	54
Roma savanna	10	4	8	4	26
Carioca	16	11	21	4	52
Roma VF	11	2	9	2	24

Total symptom type ranged from 24% on Roma VF to 54% in Buea Local. Carioca also had a high incidence of 52%. Roma Savanna also had a low incidence of 26%. Both Tropimech and Rio-grande had a high incidence of 50% and 44% respectively. Leaf curl and mosaic symptoms were generally higher in all the cultivars. Mosaic symptom was most common on Buea Local with an incidence of 25% and was lowest in Roma VF with 11% incidence. Leaf curl was widely distributed on all the cultivars, though the incidence was generally below 25%. The highest incidence was 24% on Buea local and the least prevalent on Roma Savanna and Roma VF (8 and 9%) respectively. Mixed symptom was generally low, ranging from 2 to 4%.

3.4. Detection of Viruses by DAS-ELISA

All three viruses tested for by Das-ELISA were detected in the sampled fields. These included *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Alfalfa mosaic virus* (AMV). The incidence of viruses was generally low in the study locations. It ranged from 1.12% to 1.73%. (Table 3). Potato virus Y had the highest incidence of 1.73% for single infection followed by CMV with an incidence of 1.12% incidence. There was no incidence of AMV. Some of the

batches of leaf samples were infected by more than one virus.

The proportion of mixed infection was higher than single infections. For the different virus mixture, PVY and CMV and AMV recorded the highest incidence level of 2.38%.

Table 3. *Incidence of viruses and virus mixture in the study area.*

Viruses/virus mixture	Total number of samples positive	% Incidence
PVY only	9	1.73
CMV only	6	1.12
PVY + CMV	9	1.73
PVY + AMV	6	1.12
PVY + CMV + AMV	12	2.38
Single infection	15	2.85
Mixed infection	27	5.63

Mixed Infections by Viruses was observed. Some of the field samples were found to be infected by more than one virus. It was found that the incidence of leaf sample with mixed infections ranged from 0.0% in upper farms to 11.18% in Lower farms (Table 4).

For the various combinations sorted out, the most common combination was that involving all three viruses. This combination recorded an incidence of 2.58% in lower farms, 3.2% in Lower Bokova, 2.45 in Mammu, and 1.6% in Molyko. This combination was however absent in samples from Upper Farms. No mixed infections were recorded in the latter. Mixed infections by PVY and CMV were also recorded in some locations. However, it was absent at upper farms and Mammu. Some samples infected with mixture of these viruses were found at three locations with incidence level ranging from 1.17% to 2.58%. Mixed infection of PVY and AMV was not common and was found only in two locations; Lower Bokova and lower farms with incidence of 1.8% and 2.58% respectively. However, the combination of CMV and AMV was not detected in any of the batch samples from the locations. The incidence of all viruses in the different locations ranged from 3.61% in Mammu to 22.62% in Lower farms. Incidence of viruses and virus mixtures varied in different locations studied.

Table 4. *Incidence of viruses and virus mixtures in different locations.*

Viruses/virus mixture	Upper farm	Lower farm	Lower Bokova	Molyko	Mammu
PVY	3.9	2.58	1.8	0.79	1.17
CMV	0	0	1.17	2.58	1.17
PVY + CMV	0	2.58	1.17	3.6	0
PVY + AMV	0	2.58	1.8	0	0
PVY + CMV + AMV	0	2.58	3.2	1.6	2.48
Single infections	3.9	2.58	3.2	3.6	2.48
Mixed infections	0	11.18	7.78	6.0	2.48
All viruses	3.9	22.62	16.4	13.6	3.61

4. Discussion

Generally, fields with monocropping had high symptom types recorded compared with those with intercropping. Whereas monocropping provides for host abundance and easy accessibility by vectors while mixed cropping limits vector movement and virus spread to an extent within and between fields [15]. Even though these fields were well managed, virus and virus-like symptoms and viruses assayed for were present in the study area. This should be a course for concern since these viruses are known to be economically important in developing countries.

The incidence of PVY in the study area was much lower than the 28% observed in Ethiopia [16]. Though PVY caused mild infections, this could translate in to yield loss of between 15 -20% [17]. This virus may cause severe disease impact when present in mixed infections with other viruses as observed in the study. Mixed infections are known to cause severe losses in tomato [18]. Although the incidence of PVY was low, it still had the highest incidence as compared to the other viruses tested in the study area suggests that it is not a new pathogen.

Although the incidence of CMV was low relative to PVY, the importance of this virus cannot be over emphasized. With the low incidence, most yield parameters of tomato are affected causing significant yield losses [19]. Most prevalent and economically destructive of tomato are cucumber mosaic virus (CMV) and potato virus Y (PVY). CMV is an important disease of tomatoes in temperate regions. CMV-infected plants can show a broad spectrum of symptoms from mild to severe mosaic, stunting, chlorosis and necrosis depending on viral strain and host [20]. AMV has somewhat limited distribution where it occurred; it did so in combination with PVY. It did not occur singly in this study but have been reported to occur singly in tomato fields in some parts of the world [16].

Mixed infections were common in the study. The incidence of mixed infections was higher than single ones. The results showed that viruses occurring singly or mixed infections are the most important pathogens of tomato in Buea. This agrees with the notion that viruses are very important pathogens of various crops in the Temperate and Tropical area. Mixed infection noted in the survey involved PVY and CMV, PVY and AMV and a complex mixture of PVY, CMV and AMV. This mixed infection has been shown to reduce yield drastically than single infection. Reduction of mild viruses such as PVY and CMV has been magnified in mixed infection. Mixed infections in crops have been shown to increase or decrease the severity of other diseases. The mixed infection induced more severe symptoms and decreased tomato plant height and fresh weight [21]. This demonstrates that the interactions between two tomato viruses in tomato plants are synergistic. Hu, R. et al. [22]

demonstrated that the stem heights and final aboveground fresh weights of ToCV+TYLCV mixed infected plants were significantly less than those of plants infected with ToCV alone. An indication that mixed infection has a serious impact on the growth and development of tomato. Mixed infection resulted in increased accumulations of ToCV and TYLCV, induced more severe symptoms and decreased tomato plant height and fresh weight, demonstrating that the interactions between ToCV and TYLCV in tomato plants are synergistic [23].

The environmental conditions of each location seemed to have appreciable effect on virus incidence which is generally higher in colder locations like Upper farms and Lower farms. This is hardly surprising since more tomato is grown in these locations. The host abundance, couple with increase in farming activities in the field in these locations may lead to greater spread of mechanically transmitted viruses such as PVY which can also be transmitted by vectors. The incidence of tomato virus in Buea is comparable to that occurring in commercial stocks elsewhere with regards to regular seed renewal. This is so despite the fact that tomato has been grown as fruit vegetable in Buea for quite some time without any elaborate seed programme. Although other tomato viruses were not tested for in this area, this does not preclude their existence in these locations for example Tomato leaf curl virus (TLCV), Tobacco mosaic virus (TMV), and Tomato mosaic virus (ToMV) as well as more restricted ones.

5. Conclusion

This study represents the first record of three viruses infecting tomato in Buea and its environs. Related viruses have been recorded in other parts of the world. The three viruses, which may occur singly of in mixed infections, are PVY, CMV and AMV. AMV was never detected singly. At least all three viruses induced symptoms on the sample even though there were some symptomless samples which later tested positive for one of the viruses. PVY recorded the highest incidence, suggesting that this pathogen has long existed in the study area. Mixed cropping or monocropping with two cultivars, coupled with the good field management resulted in the low incidence of viruses in the study area. Though the fields were well managed, virus diseases incidence was still recorded with mosaic symptoms recording the highest. Though seldom lethal, virus diseases can severely reduce the yield, quality and quantity of tomato.

6. Recommendations

More survey should be carried out to monitor disease situation on a routine to basis in the study area and other commonly occurring tomato viruses such as *Tomato mosaic virus* (ToMV), *Tomato mosaic virus* (TMV), Tomato spotted wilt virus (TSWV) as well as more restricted ones should be tested for in the study area.

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