Potyvirus infection analyses in Tamarillo trees in the Northern Province of Rwanda

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ABSTRACT

In Rwanda the tamarillo plant *Cyphomandra betacea* Cav. is mostly grown in the Northern Province by smallholder farmers. They only point out problems of aphid prolonged attacks. However, visual symptoms indicate that tamarillo plant may suffer from both arthropods and phytoviruses. It is important to gather more information on virus infections in tamarillo orchards. We randomly collected samples from plants which had visual symptoms that indicated the presence of phytovirus infections. Samples were analysed in Laboratory of Rwanda Agriculture Board, using Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay. We used antibodies specific to the group of Potyvirus because *C. betacea* plant belongs to the family of Solanaceae, which is mostly infected by potyviruses. Positive samples were also analysed using monoclonal antibodies. Results revealed that tamarillo trees have been infected by Potyviruses. Among positive samples, we additionally identified the *Potato Virus A* and *Potato Virus Y* that are often potato plant viruses. The potyvirus infections in tamarillo orchards must be reconfirmed using both molecular method (Polymerase Chain Reaction) and biological indexing on indicator plants. These results could be very helpful in management of potyvirus infections in tamarillo plants and help smallholder famers choosing alternative control methods instead of using pesticides.

Keywords: Antibody, *Cyphomandra betacea*, DAS-ELISA, Potyvirus

INTRODUCTION

The tamarillo plant *Cyphomandra betacea* Cav. (Solanales: Solanaceae) is native to Andes mountains of South America (Prohens and Nuez, 2001) and was introduced in East Africa around nineteenth century (Gahakwa et al., 2012). It has attractive purple-red or yellow-gold fruit depending on varieties. The fruit is very delicious and has a wide variety of vitamins, minerals, phenolic and carotenoids compounds as well as low carbohydrates content (Waweru et al., 2011). National Agriculture Export Bard of Rwanda a public organization in Rwanda tasked with diversifying export products and is enhancing both quality and quantity of fruits, flowers and vegetables carried out a study to document tamarillo diseases. *C. betacea* is amid fruit trees that are in promotion; it generates income to rural communities of Rwanda. Nevertheless, this crucial crop is facing many biotic challenges most of them are pests and diseases (Waweru et al., 2011). Tamarillo tree hosts a wide range of arthropod pests many of which are aphids that are taken as minor pests for their direct injury but become more serious when they vector plant pathogens such phytoviruses. For instance, when prolonged attacks occur, the production often become poor in terms of quality and quantity and farmers do not particularly afford regional and international horticulture markets. The control method is often the use of pesticides that are expensive and unfordable to all smallholder farmers. In few years ago, farmers acquired pesticides from the Government of Rwanda as credit which was paid back after harvesting. This agricultural input credits were offered for strengthening poor rural communities who were unable, to afford pesticide prices. However, wherever insecticide was applied many problems of pest upset and environment pollution have been documented (Altieri and Nicholls, 2004). The survey carried out in East Africa

(Tanzania) in 2001 showed that farmers in this country suffered from chemical hazardous due to the frequent application of insecticide (Ngowi et al., 2001). Pesticides application often harms the environment; indeed, high concentration of pesticide residues can be found in soil as well as in water. In Portland, research revealed a high concentration of Deldrin in natural environment particular in the water (Robinson and Mansingh 1999) while in Kenya the insecticide application considerably reduced the abundance of natural enemies (Sarwar, 2013). Insecticide application additionally had a negative impact on soil fertility because it delayed the decomposition of the leaf liter (Rasmussen et al., 2012).

In Rwanda, visual symptoms indicate that tamarillo trees suffer from both pests and Phytoviruses. The application of insecticides to control arthropods in tamarillo plants is not promising. Why? First, around the World aphids have shown to develop resistance mechanisms against insecticides (Van Emden and Harrington, 2017). Second, for non-persistent viruses; insecticide application cannot be effective because the needed time to infect the plant is very short. The main aim of this scientific work is to gather more information on virus infections, determine the occurrence of tamarillo diseases and to confirm phytovirus infections reported in the Northern Zone of Rwanda. This study will allow suggesting sustainable control methods that are both friendly to human health and biological diversity.

MATERIALS AND METHODS

Fresh samples were collected from different smallholder farmer plots in Musanze District (Figure 1). Tamarillo plant orchards were separated by more than 10 km from any other tamarillo orchard. The study area faces tropical climate of highlands with mean temperatures of 20°C. The rainfall often ranges between 1400mm and 1800mm. The altitude is 3000m above sea level.

Samples were randomly collected from diseased plants and kept in boxes containing ice. We tally took 17 samples from 4 localities (four samples per locality, except the last locality where we took 5 samples). In laboratory, samples were separately kept in a fridge at 4°C. A laboratory analysis was conducted using the Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay technique (DAS-ELISA). Neither immature nor ripen fruit were sampled. Only fresh leaves with symptoms were collected (Figure 2). We used antibodies specific to potyvirus, because most of the time, solanaceae plants are attacked by potyviruses.

Coating of the Antibody

This step consisted of taking 120 µL of the solution (antibody+buffer) and filling in two repetitions all the wells except the substrate wells. The plate is covered and put in an oven for 2 hours at 37°C. After the incubation period, the plate is washed with washing buffer at a rate of 120 µL per well.

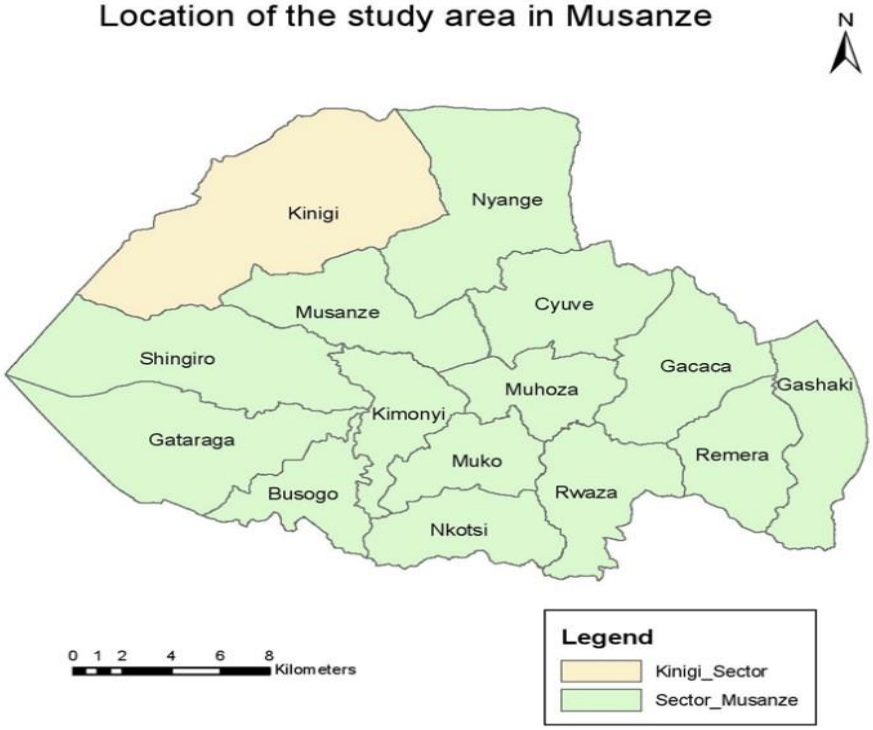

Figure 1. The District in which samples have been collected. Samples were only collected from Kinigi sector which was the most Tamarillo producing region

Figure 2. Smallholder farmer plots where samples have been collected

Preparation and Addition of Antigen

Each sample was crushed and the plant extract (juice) was recovered in eppendorf. At the rate of 120 µL (100 µL of extraction buffer and 20 µL of sample), wells for potyvirus detection were loaded except for the substrate wells and the background wells. The negative controls (healthy plants) were collected in a greenhouse, while the positive controls (infected plants) were purchased from the Swiss company BIOREBA. Charged plates were incubated overnight in the fridge at 4°C. The following day, the plates were washed 3 times with washing buffer at a rate of 120 µL per well.

Addition of the Conjugated Antibody

The Alkaline Phosphatase Conjugated Antibody was diluted 1/1000 in a conjugate buffer and then was added to each of the wells at 120 µL of solution. The incubation lasted 4 hours at 37°C. After the incubation period, the plate was washed three times with the washing buffer with 120 µL for each well.

Substrate addition

A quantity of 120 µL of the substrate solution (para-nitrophenyl phosphate+substrate buffer) was deposited in each of the wells. The substrate reacted with the alkaline phosphatase of the conjugated antibody to give the yellow color in the wells where the virus was present. The incubation was carried out in 15 minutes at room temperature.

Note: The positive sampled were also reanalyzed by monoclonal antibodies (PVA and PVY). The sample which its value of optic density (X) was less than 0.05 was considered as negative (no coloration), 0.05≤ X≤1, was read the following day (tends to yellow) while 0.1 ≤ X indicated the positive sample (yellow).

RESULTS

All sampled tamarillo plants displayed typical virus symptoms. Relied on physical symptoms we have chosen 17 samples for laboratory analysis. Our hypothesis stated that tamarillo tree is infected by potyvirus was confirmed but all samples were not positive (Table 1). The positive control (CP+) has positively reacted while the negative control was free from virus (CN). Thus, all controls were neither contaminated nor damaged. The results revealed that potyviruses infected tamarillo plant. 35% of samples were positive while 65% are free from potyviruses. The monoclonal antibodies revealed that *Potato virus A* and *Potato Virus Y* were present in samples (S16+).

Table 1. Samples and controls in Elisa plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	B	B	B	B	B	B	B	B	B
B	B	S1	BC	S6	S7	S8	BC	S14	CN	BC	CN	B
C	B	S2+	S5	CP+	BC	S11	S13	CN	BC	CN	CN	B
D	B	BC	6	S5	S7	S8	S16+	S14	S16+	CP+	CN	B
E	B	S1	S3+	S4	CP+	BC	S12	S13	S15	CN	CP+	B
F	B	S2+	S4	S9	CN	S10	S17+	S15	CP+	CN	CP+	B
G	B	S4	S3+	S11	S10	S9	S12	S17+	BC	BC	BC	B
H	B	B	B	B	B	B	B	B	B	B	B	B

B: border, BC: blank, CN: negative control, CP: control positive and S1: sample 1

Virus Incidence

Amind 17 samples collected from 4 localities were not all infected by Potyvirus. The most spread virus in our sample was *Potato Virus Y* (2 out of 4 cases: 50%). The sample number 16 was co-infected by both *Potato virus Y* and *Potato Virus A*. There was no correlation between visual symptoms and the presence of potyviruses. Some tamarillo trees that showed symptoms were free from potyvirus. It is mostly difficult to distinguish symptoms of different viruses with necked eyes, they are very similar.

DISCUSSION

Evaluating the infection of a plant to a pathogen using DAS-ELISA is a well-recognized technique (Muniappan and Heinrichs, 2016). This work is the first to show the presence of potyvirus (PVA and PVY) in tamarillo in Rwanda. The infection of potyvirus in tamarillo trees was analyzed on

17 samples. The tamarillo plant hosts many phytoviruses. Around 35% of tested samples were positive while others were negative. The different virus found in Tamarillo trees were also identified in potatoes (Insuasti et al., 2016). The Northern Province of Rwanda is the region where potatoes and tamarillo are mostly produced. Smallholder farmers intercrop tamarillo trees with potatoes in order to diversify their agricultural products, manage weed, keep soil humidity and improve soil fertility without buying any kind of input. We agreed that intercropping has sometimes advantages for example products diversification but in some case when mixed crops share the same pathogens, smallholder farmers get a high loss (Jodha, 1980). The potyvirus found in our samples are pathogens of potatoes and it means that tamarillo trees get pathogens from infected potatoes and vice versa.

It is very crucial to spatially separate tamarillo trees from potatoes to reduce the potyvirus infections. There are many viruses that often infect tamarillo tree and most of them have been identified in New Zealand (Andrews, 2015). The positive sample that is free from both PVY and PVA may be infected by other tomarillo viruses such as Tamarillo Mosaic Virus which is the most viruses that replicates in tamarillo trees. The negative samples did not mean 100% that plants were free from virus. The method sensitivity may influence the results for example; a plant recently infected may be negative because virus particle quantities that are detected by the method are not sufficient. Second, we have used antibodies, which are specific to the genus of potyvirus. The sample may be free from potyvirus but positive for other viruses. It is worthwhile to use other antibodies to verify whether samples were not infected by other virus groups.

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