PCR detection of *Xanthomonas vasicola* pv. *musacearum* in banana: implication for mat management to control BXW wilt

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Introduction

Banana bacterial wilt (BXW) caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) is a devastating disease of banana (Tushemereirwe *et al.,* 2004). Following its identification in Uganda it has rapidly spread across the African Great Lakes Region. One of the most important disease management options has been the use of BXW free suckers. This however is jeopardized by the failure or unwillingness of farmers to completely destroy their infected banana mats (and hence livelihood). This is compounded by limitations in labour; availability and prohibitive costs, with the result that only infected mother plants are destroyed and not whole mats. The apparently healthy suckers from such mats are therefore often planted, with the result that the disease continues to spread. However, in other cases farmers have reported disease control following the destruction of single plants without uprooting the others within the mat. This study was carried out to monitor the movement of insect vector borne *Xvm* along banana pseudo-stem of a mother plant and its associated suckers. Results of this study can be used to guide recommendations on the management of infected banana mats and the use of suckers as planting material. The study employed a polymerase chain reaction (PCR) based molecular tool using primers selected based on their specificity to *Xvm.*

Methodology

Banana plants of cultivars; Kayinja, Kivuuvu and Nfuuka in Mityana and Mubende districts, Uganda were used in this study. These were naturally infected by insects carrying *Xvm*. Infected plants were categorized based on stage of symptom expression as follows: I = the flower bract just started to wilt; II = the flower rachis started to dry; III = one cluster prematurely ripened and IV = two or more clusters prematurely ripened (Figure 1).

I=flower bract just started to wilt

II= the flower rachis started to dry

III= one cluster prematurely ripe

IV= two or more cluster prematurely ripe

Figure 1: Symptom stage of banana infected with *Xvm* used in the study

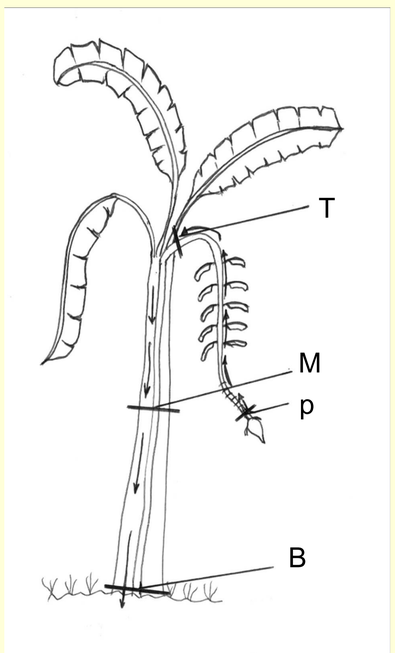

Figure 2: Location of samples taken from mother and suckers.

Four samples per mother plant were taken as follows: (i) just above the male bud (P), (ii) just above the insertion point of the bunch (T), (iii) from the middle of the pseudo-stem (M), and (iv) from the corm (B) (Figure 2). In addition, samples were taken from suckers (S) attached to infected mother plants from the same locations. Sampling tools were disinfected thoroughly between sampling through immersion in 95% ethanol followed by flaming. Each sample was packed separately and taken to the lab for DNA extraction using CTAB method (Zhang *et al*., 1998). PCR reactions were performed using the *Xvm* specific primers Xcm-1 and Xcm-3 that amplified 220bp target DNA sequences following the protocol developed by Lewis Ivey *et al*. (in press).

Results and discussion

Primer pair Xcm-1 and Xcm-3 successfully amplified the expected 220bp fragment from plant tissues carrying *Xvm* (Figure 3). All samples obtained from male bud samples (P) were positive for *Xvm* irrespective of cultivar. *Xvm* was detected in at least 80% of the (T) and (M) samples with the exception of those obtained from the cultivar Kivuuvu where only 50% were positive. In the corm samples (B), the percentage of positive samples ranged from 50-100%. In all these cases the effect of symptom stage and cultivar was not observed. Sucker infection ranged between 25-83%, 75-100%, 67-100% and 75-100% in symptom stage I, II, III, and IV plants, respectively (Table 1). These results imply that: i) this bacterium can migrate between different organs of the mother plant without exhibiting any external symptoms, ii) this bacterium can migrate to apparently healthy suckers. Therefore, the spread of BXW in the field could be due to the use of asymptomatic suckers as planting material.

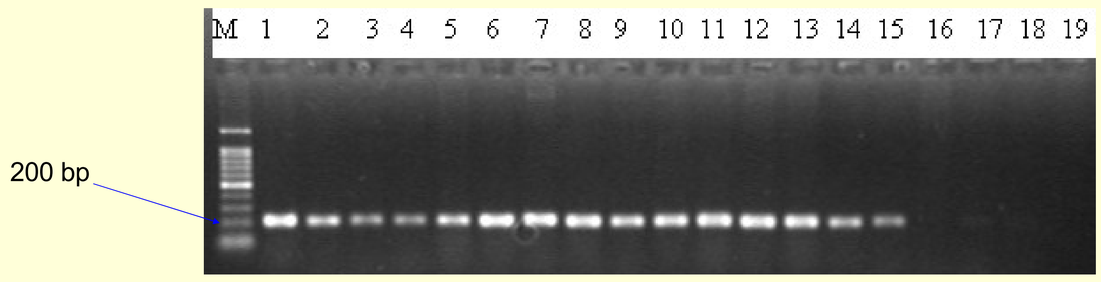

Figure 3: PCR amplification product of *Xvm* from infected banana samples: M-Molecular Marker, 1= positive DNA control, 2 to17 are samples in the order P, T, M and B for four Nfuuka mother plants, 18 =negative control, 19= healthy plant control

Symptom stage	Cultivar	No. of plants sampled	No. of suckers sampled	% plant parts positive for *Xvm*				
				P	T	M	B	S
I	Kayinja	11	12	100	82	91	82	25
	Nfuuka	10	12	100	100	100	90	83
	Kivuuvu	8	12	100	88	50	50	67
II	Kayinja	7	4	100	100	86	71	100
	Kivuuvu	8	8	100	88	88	88	75
III	Kayinja	7	5	100	100	100	57	100
	Nfuuka	3	3	100	100	100	100	67
IV	Kayinja	10	9	100	80	90	90	89
	Nfuuka	7	4	100	100	100	100	75
	Kivuuvu	3	2	100	100	100	67	100

Table 1: Percentage of plant samples that tested positive for *Xvm*

Conclusion

By the time wilt symptoms are expressed and thus recognized, *Xvm* has migrated from the top to the base of the infected mother plant. Furthermore, infection was found to spread into the associated suckers, despite them remaining asymptomatic. Therefore, such suckers pose a serious danger as dissemination vehicles for BXW. Based on our results we therefore recommend that mother plants exhibiting BXW should be destroyed along with all suckers in the mat, however healthy these suckers may appear.

References

Tushemereirwe W, Kangire A, Ssekiwoko F, Offord L.C, Crozier J, Boa E, Rutherford M, Smith J.J, 2004. First report of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. Plant Pathology 53: 802

Zhang Y.-P, Uyemoto Z.K, and Kirkpatrick B.C, 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. J. Virol. Methods, 71: 45–50