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Method Article

Potato Coring Method of Sampling Potato Tubers to detect *Ralstonia solanacearum*

ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* is considered among the most damaging diseases of potato in Sub-Saharan Africa. In Kenya, majority of farmers visually select and save seed from harvested potato tubers and reuse the same tubers for several seasons. Latently infected seed tubers which cannot be identified by visual inspection during certification further compounds the situation compelling the need for laboratory testing. The study evaluated the effectiveness of coring tuber samples to improve sampling efficiency for onward laboratory diagnosis. In this study, the coring method of sampling potato tubers for detection *R. solanacearum* was evaluated. Coring involves taking multiple tuber samples direct from the stolon attachment site into a collection tube containing extraction buffer that provides the extract for further diagnostic tests. Coring was assessed using field samples from different potato growing regions of Kenya including, Koibatek, Molo, Uasin Gishu, Bungoma and Kisii and tested using Nitrocellulose Membrane (NCM) ELISA. These results were compared with other methods of testing. Coring tuber samples was as effective as the standard sampling method used in Kenya to detect *R. solanacearum.* The coring of potato tubers is a reliable and quicker method of sampling that reduces the turnaround time of testing hence improving efficiency.

Key words: Ralstonia solanacearum, potato corer, potato, ELISA

1. INTRODUCTION

Potato (*Solanum tubersum*) belongs to the Solanaceae family and is the second most important food crop after maize in Kenya (MoA, 2008). It is grown by about 800,000 farmers, cultivating 150,000 hectares with an annual production of approximately 1 million tonnes over two growing seasons. National potato production ranges from 4.4 to 15 t/ha with an average of 6.7 t/ha, although yields of 40 t/h have been attained under research conditions (MoA, 2008). Low quality seed, diseases such as bacterial wilt

UNDER PEER REVIEW

and late blight and low soil fertility as well as high cost of inputs such as certified potato tubers have attributed to production constraints resulting into low yield (Gildemacher, P. R., Kaguongo, W., Ortiz, O., Tesfaye, A., Woldegiorgis, G., Wagoire, W. W., et al. 2009).

Ralstonia solanacearum is a pathogen that causes bacterial wilt disease in over 450 plant species (Fig. 1) including many economically important crops such as potato, tomato, eggplant, peanut, pepper, garlic, banana and ginger (Hayward 1991). The disease causes serious problems for potato production in Kenya affecting 77% of potato farms (Gildemacher, P. R., Kaguongo, W., Ortiz, O., Tesfaye, A., Woldegiorgis, G., Wagoire, W. W., et al. 2009). Vegetatively propagated crops, such as potato, are particularly vulnerable to infection due to latent infections. The use of clean certified seed potato and good cultural practices are the most effective methods to manage the disease (Hayward 1991).

Fig. 1. Symptoms of bacterial wilt of potato caused by the bacterium *R. solanacearum* in potato plant (A) and potato tuber (B)

Several sampling methods are used to prepare samples for onward diagnosis. In Kenya, the standard method of sampling seed potato involves taking 400 whole potato tubers per hectare back to the diagnostic facility. The potato tubers are further sub-sampled by pooling batches of 25 tubers to constitute a single sample. The pooled potato tubers are prepared for diagnosis of *Ralstonia solanacearum* by

UNDER PEER REVIEW

cutting through each tuber using a blade and physically ringing out the vascular tissues. This method can be time consuming and labor intensive thus resulting to a prolonged time of testing since whole potato tubers have to be transported back to the testing facility for preparation before laboratory diagnosis is undertaken. To better manage bacterial wilt disease in Kenya, a faster and cheaper method of sampling that can easily be adapted is in demand to improve sampling efficiency.

This manuscript describes the use of potato coring device which has been used in other countries (Weller SA, Elphinstone JG, Smith NC, Boonham N, and Stead DE, 2000) to sample potato tubers to detect *R. solanacearum* directly in the field. The coring device was able to sample each potato tuber at the stolon attachment site with simple press, with direct collection into an extraction tube thus minimizing contamination and extraction processes. Coring can be undertaken in the field as long as testing is undertaken within 24-48 h to prevent deterioration of the potato tuber corers. Coring in the field add efficiency to time as preparations are done in the field and no potato waste is created at the diagnostic facility.

2. MATERIALS & METHODS

Sampling and sample preparation. Potato tubers were randomly collected from 5 farmers from each of the regions: Koibatek, Molo, Uasin Gishu, Mt. Elgon and Kisii. The survey involved collecting 25 potato tubers from 1/8 of a hectare per field which was cored and pooled to constitute a single sample. Sampled tubers were surface sterilized with 70% ethanol and cored at the stolon end using a potato coring device (courtesy of J. Smith, Fera Science Ltd, York, UK; Fig. 2). The corer was disinfected between samples by dipping the metal portion of the tool in 95-100% alcohol and pass through a flame to completely burn off the alcohol before use. One pooled cored sample was placed into a collection tube containing extraction buffer (phosphate buffer pH 7.0; Na2HPO4, KH2PO4 and antioxidant; tetrazolium pyrophosphate). The samples were transported to the laboratory and the mixture transferred to a maceration bag and homogenized with a tissue homogenizer. The potato extract was used for detection of *R. solanacearum* using ELISA (CIP, Lima, Peru). The analysis of *R. solanacearum* using NCM ELISA

UNDER PEER REVIEW

took 4 days to completion including a 48 h incubation step. The results from ELISA were compared to Conventional PCR, qPCR and LAMP.

Fig. 2. Potato coring device (courtesy of J. Smith, **Fera Science Ltd**, York, UK).

3. RESULTS AND DISCUSSION

Positive detection by NCM ELISA was observed in 16 out of 20 pooled cored tuber samples. Negative controls containing healthy potato tubers did not test positive for *R. solanacearum* (Table 1). In addition, a separate control (1 infected tuber in 24 disease- free tubers) tested positive for *R. solanacearum*. All the 20 pooled field samples analyzed by ELISA gave consistent results when compared to conventional PCR, qPCR and LAMP for positive and negative detection (data not shown, manuscript under preparation).

Table 1. Detection of *Ralstonia solanacearum* using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) on field collected potato tubers in Kenya.

Region	Sample ID	Technical Replicate 1	Technical Replicate 2	Technical Replicate 3
Koibatek	Field-1	+	+	+
	Field -2	+	+	+
	Field -3	–	–	–
	Field -4	+	+	+
Bungoma	Field -1	+	+	+

UNDER PEER REVIEW

	Field -2	+	+	+
	Field -3	+	+	+
	Field -4	+	+	+
Uasin Gishu	Field -1	+	+	+
	Field -2	+	+	+
	Field -3	–	–	–
	Field -4	+	+	+
Kisii	Field -1	+	+	+
	Field -2	+	+	+
	Field -3	+	+	+
	Field -4	+	+	+
Molo	Field -1	–	–	–
	Field -2	+	+	+
	Field -3	+	+	+
	Field -4	–	–	–
	Healthy potato tubers	–	–	–
	1 infected in 24 Healthy tubers	–	–	–

Indicates: (1, 2, 3, and 4) Number of field tested in a region
Symbol: + indicate positive test result for field potato samples tested
Symbol: – indicates negative test result for field potato samples tested

The study evaluated the effectiveness of coring tuber samples to assess whether cored samples can provide accurate results for further laboratory diagnosis. Coring leaves waste at the sampling site thus no need to carry bulky tubers back to the testing facility. This eliminated the burden of cumbersome disposal processes of infected waste as tuber waste is left at the sampling site. The current standard practice in Kenya involve taking whole tubers back to the diagnostic facility which is a tedious and cumbersome process. The coring method was efficient as one infected tuber in a pooled sample of 24 healthy tubers tested positive using ELISA, conventional PCR, qPCR and LAMP. Coring resulted in quicker extraction and reduced labor time since coring can be undertaken in the field therefore improving efficiency of the sampling process. Only tuber cores were transported to the lab for homogenization and detection hence significantly reducing the load transported back to the lab. Coring is currently not practiced in Kenya hence can be adapted to increase efficiency of the sample preparation process. Besides the coring method can also be undertaken in the field to reduce the turn-around time of testing by

UNDER PEER REVIEW

doing away with sample preparation in the lab as long as testing is undertaken within 48 h before the cored samples begin to degrade. Coring method can significantly reduce the turnaround time of testing as illustrated in Fig. 3 and 4.

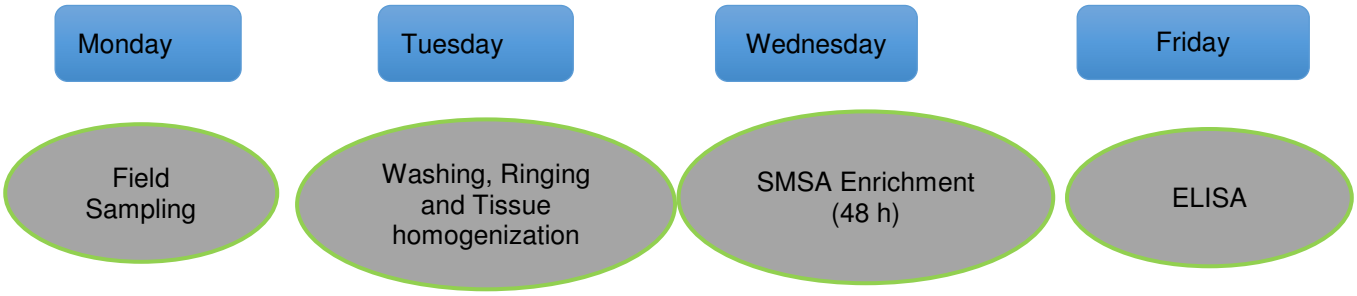

Fig. 3. Turn-around time of testing using Nitrocellulose Membrane (NCM) ELISA when carrying whole potato tubers to the lab for preparation

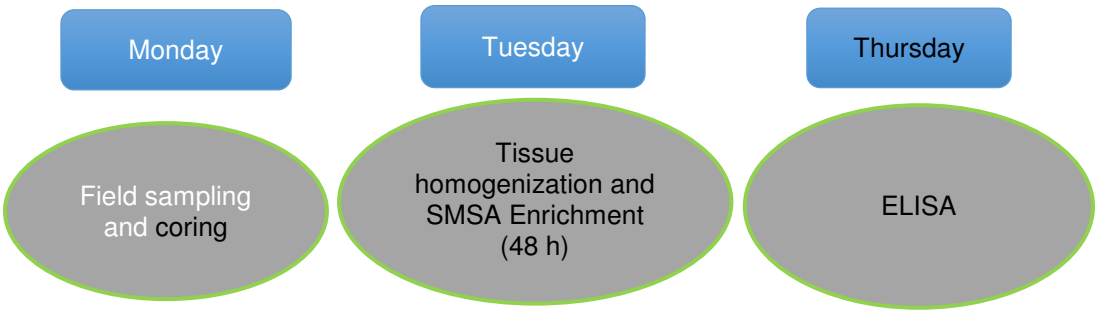

Fig. 4. Turn-around time of testing using Nitrocellulose Membrane (NCM) ELISA when coring potato tubers in the field

4. CONCLUSION

Coring is currently not practiced in Kenya hence can be adapted to increase efficiency of the sample preparation process. Besides the coring method can also be undertaken in the field to reduce the turn-around time of testing by doing away with sample preparation in the lab as long as testing is undertaken within 48 h.

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UNDER PEER REVIEW

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