

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

ABSTRACT

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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.

Keywords: *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 tons 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/ha have been attained under research conditions (MOA, 2008). Low-quality seed, diseases such as bacterial wilt 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 et al. 2009).

Ralstonia solanacearum is a pathogen that causes bacterial wilt disease in over 450 plant species (Hayward 1994) (Fig. 1) including many economically important crops such as potato, tomato, eggplant, peanut, pepper, garlic, banana and ginger (Kelman 1953; Hayward 1991; Elphinstone 2005). The disease causes serious problems for potato production in Kenya affecting 77% of potato farms (Gildemacher et al. 2009). Vegetative 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 the diagnosis of *R. solanacearum* by 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 et al. 2000) to sample potato tubers to detect *R. solanacearum*. The coring device was able to sample each potato tuber at the stolon attachment site with a 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 potato tubers in the field adds 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 passed 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 coring method of sampling was evaluated against the standard sampling method used in Kenya which involved taking 400 tubers from 200 randomly selected potato plants. In this method, potato tubers were transported to the lab, prepared by manual extraction of vascular tissues using a scalpel/knife (25 tubers to constitute a single sample) and transferred into a single bag for crushing and detection of *R. solanacearum* by NCM ELISA.

The potato tubers were tested for *R. solanacearum* by NCM ELISA kit (CIP, Lima, Peru) with the analysis process taking 4 days to completion including a 48 h incubation step (Priou et al. 1997). Briefly, pooled potato tuber containing 25 tubers per sample were cored and macerated in extraction buffer containing 0.1 M citrate buffer (pH 5.6) and incubated in semi-selective broth 48 h with constant agitation. Dot blotting and ELISA was performed on a nitrocellulose membrane with positive and negative field samples for *R. solanacearum* identified through color reactions on nitrocellulose membrane.

The results from ELISA were compared to Conventional PCR (Fegan and Prior, 2006), qPCR (Chen et al. 2010) and LAMP. The LAMP assay was performed in a 25 µl reaction according to published protocol by Wastling et al. 2010. The LAMP reaction was carried out at 65°C for 1 h (Manuscript under preparation).



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

103		Field -4	+	+	+
104	Uasin	Field -1	+	+	+
	Gishu	Field -2	+	+	+
105		Field -3	-	-	-
		Field -4	+	+	+
106	Kisii	Field -1	+	+	+
107		Field -2	+	+	+
108		Field -3	+	+	+
109		Field -4	+	+	+
110	Molo	Field -1	-	-	-
111		Field -2	+	+	+
112		Field -3	+	+	+
113		Field -4	-	-	-
114		Healthy potato	-	-	-
115		tubers	-	-	-
116		1 infected in 24	+	+	+
117		Healthy tubers			
118					
119					
120					
121					
122					
123					
124					
125					
126					
127					
128					
129					
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Indicates: (1, 2, 3, and 4) Number of fields 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. The results indicated that the coring method worked perfectly just as the standard sampling method in Kenya which is a proven sampling tool (Mwangi et al. 2008; Prior, 1997 and Elphinstone, 1996) which involves cutting a thin slice of the tuber from around the stolon end with a scalpel, and removing a strip of about 3 x 3 mm along the vascular tissues for detection of *R. solanacearum*. The results indicated that the standard practice of sampling potato tubers in Kenya for detection of *R. solanacearum* was reliable as the coring method of sampling with a p-value

1.000 observed when the sampled using both methods. Since the p-value was greater than the 0.05 level of significance, the coring and the standard practice of sampling potato tubers in Kenya were absolutely similar in terms of reliability for detection of *R. solanacearum*.

Previous studies have illustrated that coring method of sampling was reliable and efficient for detection of *R. solanacearum* (Weller et al. 2000 and Pastrok and Maiss, 2000) using conventional PCR and qPCR. The current standard practice in Kenya entails taking whole tubers back to the diagnostic facility before sample processing which is tedious, labor intensive and cumbersome process. Coring method of sampling potato tubers 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 coring method was as efficient as the standard method of sampling potato tubers in Kenya as it was able to detect *R. solanacearum* in field collected potato tubers including one infected tuber in a pooled sample of 24 healthy tubers using ELISA, conventional PCR, qPCR and LAMP (data not shown, manuscript in preparation). Previous studies have indicated the physical removal of vascular tissues after dissection using a scalpel also resulted into positive detection of *R. solanacearum* in potentially infected field collected potato tubers (Pastrok and Maiss, 2000).

During the experimental work, it was evident that coring resulted in quicker extraction and reduced labor time as opposed to manual extraction of vascular tissue as no dissection of potato tubers was required during the extraction process. Coring can be undertaken in the field, therefore, improving the 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 method of sampling potato tubers is currently not practiced in Kenya hence can be adapted to increase the 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 before the cored samples begin to degrade. Coring method can significantly reduce the turnaround time of testing as illustrated in Fig. 3 and 4.

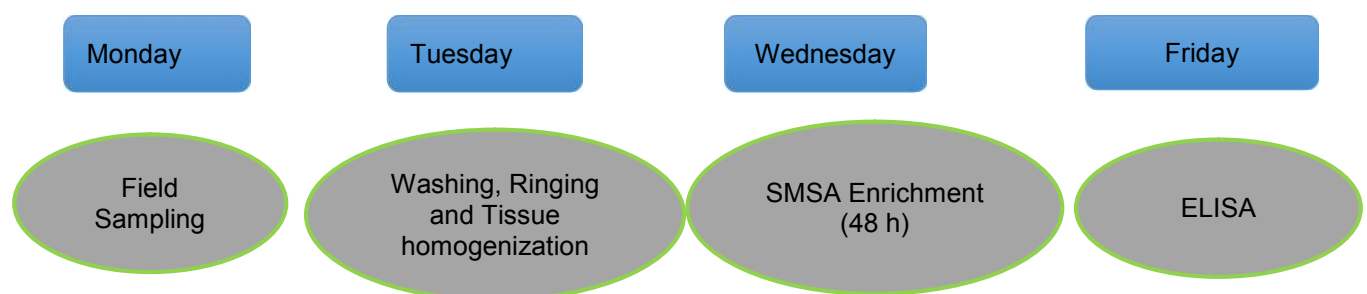


Fig. 3. Turn-around time of testing using Nitrocellulose Membrane (NCM) ELISA when carrying whole potato tubers to the lab for preparation

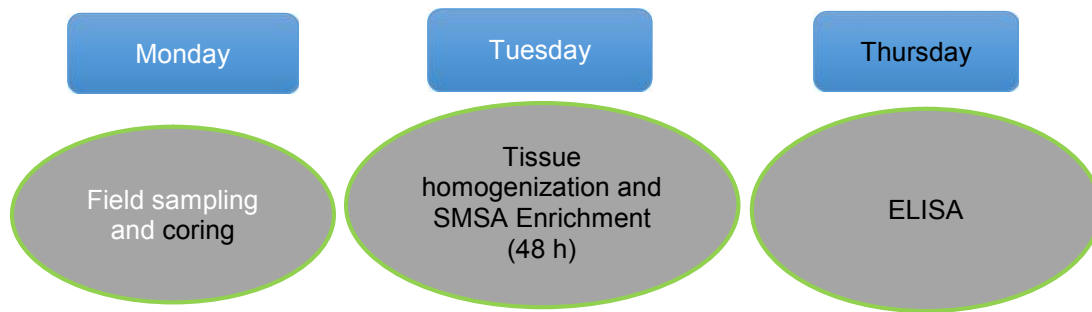


Fig. 4. Turn-around time of testing using Nitrocellulose Membrane (NCM) ELISA when coring potato tubers in the field

4. CONCLUSION

Coring is currently not practiced in Kenya hence can be adapted to increase the 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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AUTHORS' CONTRIBUTIONS

Author A, performed the analysis, wrote the protocol and the manuscript. Author B reviewed the manuscript. Author C performed the analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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