

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

2 **ABSTRACT**

3 Bacterial wilt caused by *Ralstonia solanacearum* is considered among the most damaging
4 diseases of potato in Sub-Saharan Africa. In Kenya, majority of farmers visually select and save seed
5 from harvested potato tubers and reuse the same tubers for several seasons. Latently infected seed
6 tubers which cannot be identified by visual inspection during certification further compounds the situation
7 compelling the need for laboratory testing. The study evaluated the effectiveness of coring tuber samples
8 to improve sampling efficiency for onward laboratory diagnosis. In this study, the coring method of
9 sampling potato tubers for detection *R. solanacearum* was evaluated. Coring involves taking multiple
10 tuber samples direct from the stolon attachment site into a collection tube containing extraction buffer that
11 provides the extract for further diagnostic tests. Coring was assessed using field samples from different
12 potato growing regions of Kenya including, Koibatek, Molo, Uasin Gishu, Bungoma and Kisii and tested
13 using Nitrocellulose Membrane (NCM) ELISA. These results were compared to PCR, qPCR and LAMP.
14 Coring method was statistically reliable ($p>0.05$) when compared to the standard sampling method used
15 in Kenya to detect *R. solanacearum*. The coring of potato tubers is a reliable and quicker method of
16 sampling that reduces the turnaround time of testing hence improving efficiency.

17 *Keywords: Ralstonia solanacearum, Coring method, potato, ELISA*

19 **1. INTRODUCTION**

20 Potato (*Solanum tubersum*) belongs to the Solanaceae family and is the second most important food
21 crop after maize in Kenya (MOA, 2008). It is grown by about 800,000 farmers, cultivating 150,000
22 hectares with an annual production of approximately 1 million tonnes over two growing seasons. National
23 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
24 been attained under research conditions (MOA, 2008). Low-quality seed, diseases such as bacterial wilt
25 and late blight and low soil fertility as well as high cost of inputs such as certified potato tubers have
26 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) including many economically important crops such as potato, tomato, eggplant, peanut, pepper, garlic, banana and ginger (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).



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

In Kenya, the standard sampling method of seed potato involves taking 400 whole potato tubers per hectare for diagnosis. 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 *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 in a prolonged time of testing since whole potato tubers have to be transported back to the testing facility for preparation before laboratory diagnosis is

commenced. 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 work report use of potato coring device (Weller et al. 2000) to sample potato tubers to detect *R. solanacearum* directly in the field.

2. MATERIALS & METHODS

Sampling and sample preparation.

Potato tubers were randomly collected from 5 farms from 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 coring method of sampling was evaluated against the standard sampling method which involved taking 400 tubers from 200 randomly selected potato plants. Manual extraction of the tuber vascular tissues was done using a scalpel/knife (25 tubers to constitute a single sample) and transferred into a single bag for crushing.

The potato tubers were tested for *R. solanacearum* by NCM ELISA kit (CIP, Lima, Peru) (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 (Wastling et al. 2010). Briefly, conventional PCR was carried out in 20 µl reactions 4 pmol of primers 759/760 and 2 µl of DNA extracted from field collected potato tubers. Reactions were heated to 96°C for 5 minutes and then cycled through 30 cycles of 94°C for 15 seconds, 59°C for 30 seconds and 72°C for 30 seconds. Samples (5 µl) of reaction mixtures were examined by electrophoresis through 2% agarose gels and bands were revealed by staining in 0.5 µg mL⁻¹ gel red. Quantitative PCR reactions were performed using the following parameters: Initial preheat for 10 minutes at 95°C, 40 cycles at 95°C for 20 seconds, 62°C for 25 seconds, 72°C for 35 seconds, and 85°C for 3 seconds. LAMP reactions were performed in 25 µl volume containing: 0.2 µM each of external primers (F3 and B3), 2.0 µM each of internal primers (FIP and BIP), 0.8 µM each of loop primers (LF and LB) (Integrated DNA Technologies, USA), 0.8 M betaine (Sigma-Aldrich, Germany), 120 µM of hydroxy naphthol blue (Sigma-Aldrich), 1.40 mM of each dNTP (New England Biolabs, Ipswich, MA), 6 mM of MgSO₄, 1X isothermal DNA buffer (New England Biolabs), 8 U of *Bacillus stearothermophilus* DNA polymerase (New England Biolabs), and 2 µl of bacterial DNA (10-20 ng).

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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*. The results of the ELISA assay for all the 20 pooled field samples that tested either positive or negative gave similar results when compared to conventional PCR, qPCR and LAMP.

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	+	+	+
	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 fields tested in a region

Symbol: + indicate positive test result for field potato samples tested

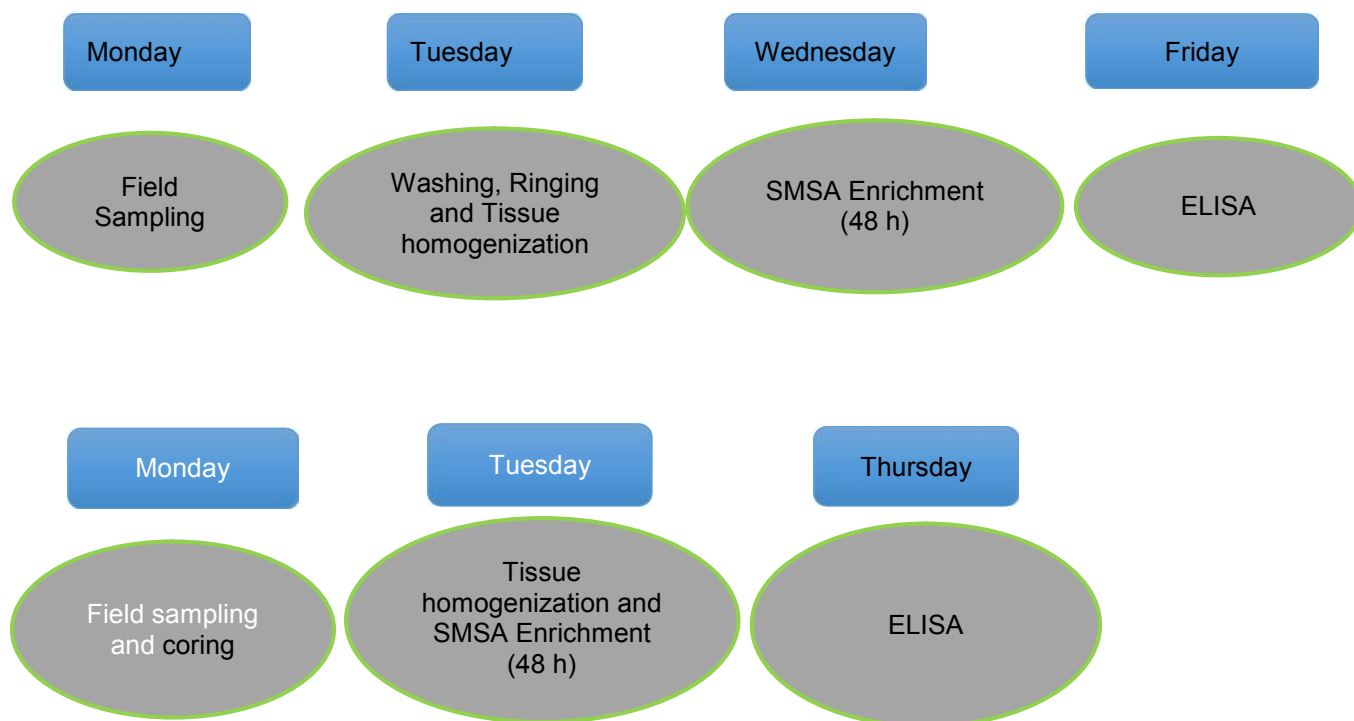
Symbol: – indicates negative test result for field potato samples tested

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 currently used in Kenya (Mwangi et al. 2008; Prior, 1997 and Elphinstone, 1996). The procedure 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 statistically reliable ($p>0.05$) when compared to coring method. Thus, 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 method entails taking whole tubers back to the diagnostic facility before sample processing which is tedious, labor intensive and cumbersome process. 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 coring method compared to the standard method of sampling potato tubers was efficient in detection *R. solanacearum* in field collected potato tubers. 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 and Prior, 1997). During the extraction process, 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. Coring can be undertaken in the field, therefore, improving the efficiency of the sampling process. 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

161 turn-around time of testing by doing away with sample preparation in the lab as long as testing is
162 undertaken within 48 h before the cored samples begin to degrade.

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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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