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1 **Rapid and specific detection of *Yam mosaic virus* by reverse-transcription recombinase**
2 **polymerase amplification**
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5 Gonçalo Silva[1*], Moritz Bömer[1], Chukwuemeka Nkere[2], P. Lava Kumar[2], Susan E. Seal[1]
6
7 [1]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK
8 [2]International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320, Ibadan, Nigeria
9
10
11
12
13
14
15
16 [*] Corresponding author
17 Tel.: +44 (0) 1634 883158
18 E-mail address: g.silva@gre.ac.uk
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28 **Abstract**

29 *Yam mosaic virus* (YMV; genus *Potyvirus*) is considered to cause the most economically important
30 viral disease of yams (*Dioscorea* spp.) in West Africa which is the dominant region for yam
31 production globally.
32 Yams are a vegetatively propagated crop and the use of virus-free planting material forms an essential
33 component of disease control. Current serological and PCR-based diagnostic methods for YMV are
34 time consuming involving a succession of target detection steps.
35 In this study, a novel assay for specific YMV detection is described that is based on isothermal
36 reverse transcription-recombinase polymerase amplification (RT-exoRPA). This test has been shown
37 to be reproducible and able to detect as little as 14 pg/µl of purified RNA obtained from an YMV-
38 infected plant, a sensitivity equivalent to that obtained with the reverse transcription-polymerase chain
39 reaction (RT-PCR) in current general use. The RT-exoRPA assay has, however, several advantages
40 over the RT-PCR; positive samples can be detected in less than 30 min, and amplification only
41 requires a single incubation temperature (optimum 37°C). These features make the RT-exoRPA assay
42 a promising candidate for adapting into a field test format to be used by yam breeding programmes or
43 certification laboratories.

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47 Keywords: Yam; *Dioscorea* spp.; YMV; Recombinase polymerase amplification; Diagnosis

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61 **1. Introduction**
62 Yams (*Dioscorea* spp.) are an important food crop in tropical and sub-tropical countries, particularly
63 in West Africa which contributes over 93% of global yam production (Maroya et al., 2014). Although
64 several yam species are present in this region, the white Guinea yam *D. rotundata* is the most widely
65 cultivated (Asiedu and Sartie, 2010; Kenyon et al., 2008). Yams are generally propagated vegetatively
66 through their tubers, which allows the perpetuation and accumulation of tuber-borne pathogens,
67 particularly viruses (Kenyon et al., 2001). To date, several virus species belonging to at least six
68 different genera have been reported in yams from West Africa (Kenyon et al., 2001; Mambole et al.,
69 2014; Menzel et al., 2014) and some of these can have a significant impact on tuber yield and quality
70 as well as impede yam germplasm movement. Another factor contributing to the decline of yam
71 productivity is the scarcity and affordability of 'clean' (virus-free) seed yams (Maroya et al., 2014;
72 Seal et al., 2014).
73 Of the many viruses identified, *Yam mosaic virus* (YMV; genus *Potyvirus*), *Yam mild mosaic virus*
74 (YMMV; genus *Potyvirus*), *Cucumber mosaic virus* (CMV; genus *Cucumovirus*) and several species
75 of *Dioscorea*-infecting badnaviruses are widespread across the numerous yam growing regions in
76 West Africa (Ampofo et al., 2010; Asala et al., 2012; Eni et al., 2010; Eni et al., 2008; Njukeng et al.,
77 2014; Seal et al., 2014). YMV is in these reports often described as the most economically important
78 of all the viruses.
79 YMV has a single-stranded, positive-sense RNA genome of 9608 nucleotides encapsidated in
80 flexuous filamentous ~785 nm long particles (Aleman et al., 1996; Thouvenel and Fauquet, 1979).
81 The virus is dispersed into new areas by the planting of infected plant material and can also be spread
82 locally by several aphid species in a non-persistent manner (Aleman-Verdaguer et al., 1997). YMV
83 infection can generate a range of symptoms including mosaic, mottling, green vein banding and leaf
84 deformation, and stunted growth leading to reduced tuber yield.
85 The development of a rapid and sensitive diagnostic method to verify that planting material to be
86 distributed is virus-free is of paramount importance to control the spread of YMV. Several serological
87 and nucleic acid-based methods have been reported for the detection and characterization of plant
88 viruses. For YMV, specific detection methods include enzyme-linked immunosorbent assay (ELISA),
89 a dot blot immunoassay (DBI), a direct tissue blotting immunoassay (DTBI), immunosorbent electron
90 microscopy (ISEM) and immunocapture reverse transcription-polymerase chain reaction (IC-RT-
91 PCR) (Eni et al., 2012; Mumford and Seal, 1997; Njukeng et al., 2005). These methods, however, are
92 time consuming, requiring lengthy set-up and detection procedures by trained personnel, and are
93 hence not easily adapted to a format suitable for on-site application.
94 In recent years isothermal amplification methods, such as recombinase polymerase amplification
95 (RPA) (Piepenburg et al., 2006) and loop-mediated isothermal amplification (LAMP) (Notomi et al.,
96 2000) have been developed for routine detection of pathogens. These methods offer simple and rapid

97 detection systems, with high specificity and sensitivity and are suitable for field diagnosis and poorly
98 equipped laboratories (Amer et al., 2013; Boyle et al., 2013; Escadafal et al., 2014).
99 RPA tests have been described recently for the diagnosis of important plant viruses such as *Little*
100 *cherry virus* 2 (Mekuria et al., 2014) and *Plum pox virus* (Zhang et al., 2014). One reason this
101 sequence-specific isothermal nucleic acid amplification method has emerged as a novel technology in
102 molecular diagnosis, is that it offers quick and sensitive detection without the need for thermal cycling
103 as required for PCR (Zanoli and Spoto, 2013). Exponential amplification in a RPA reaction depends
104 on specific enzymes and proteins such as recombinase, single strand binding protein and a strand
105 displacing DNA polymerase that can operate at a low and constant temperature (optimal temperature
106 is 37°C) (Piepenburg et al., 2006). In the case of RNA targets, such as YMV, a reverse transcriptase
107 enzyme can be included in the RPA reaction (Abd El Wahed et al., 2013; Euler et al., 2013).
108 In this study, the application of reverse transcription-recombinase polymerase amplification (RT-
109 exoRPA) as a robust, sensitive, and quick method for YMV detection is described. This assay offers
110 advantages that could assist it to become the method of choice for West Africa for rapid selection of
111 YMV-free planting material for further propagation and distribution to smallholder farmers.
112
113
114 **2. Materials and Methods**
115 2.1 Plant Samples
116 Yam tubers from *D. alata* and *D. rotundata* were imported from the International Institute of Tropical
117 Agriculture (IITA, Ibadan, Nigeria) and were grown in a quarantine aphid-proof glasshouse at the
118 Natural Resources Institute (NRI, UK) as described by Mumford and Seal (1997). Individual leaf
119 samples were collected in small polythene bags (10 x 15 cm) and used immediately, or stored at 4°C
120 for no more than 24 hours until further processing.
121
122 2.2 RNA Extraction, cDNA synthesis and PCR assays
123 Total nucleic acids were extracted from 100 mg leaf tissue using a modified CTAB method previously
124 described by Abarshi et al. (2010). Extracted nucleic acids were resuspended in 50 µl of RNase-free
125 water and stored at -80°C until use. After extraction, genomic DNA contamination was removed by
126 digestion with DNase I (from "Isolate II RNA Mini Kit", Bioline, UK) for 10 min at 37°C followed
127 by RNA repurification following the kit instructions.
128 Single-stranded cDNA was synthesised using a QuantiTect Reverse Transcription kit (Qiagen,
129 Germany) according to manufacturer's recommendations. The presence of YMV was confirmed using
130 the primer pair YMV CP 1F (5'-ATCCGGGATGTGGACAATGA-3') and YMV UTR 1R (5'-
131 TGGTCCTCCGCCACATCAAA-3') designed by Mumford and Seal (1997), which amplifies a 586
132 bp region comprising the coat protein (CP) gene and the 3' UTR region.

133 The quality of synthesised cDNAs and purified RNAs as templates for PCR and RPA assays, was
134 verified by amplification of an internal control gene with primers Dz_actin_F (5'-
135 TGCTGGATTCTGGTGATGGT-3') and Dz_actin_R (5'-AGCAGCTTCCATGCCTATCA-3')
136 designed from *D. zingiberensis* actin gene sequence (Genbank accession JN693499) which amplify a
137 362 bp fragment.
138 The PCR protocol was set up in 20 µl reactions containing 1x DreamTaq buffer (Thermo Scientific,
139 UK), 0.25 mM of each dNTP (Thermo Scientific, UK), 0.2 µM of each primer, 1 U DreamTaq DNA
140 polymerase (Thermo Scientific, UK) and 1 µl of 1/10 dilution of cDNA as template. The following
141 PCR program was used: 94ºC for 2 min, followed by 30 cycles of 94ºC for 30 s, 55ºC for 30 s, and
142 72ºC for 30 s and one final cycle of 72°C for 10 min.
143 For one-step RT-PCR the conditions were adapted from those recommended by Mumford and Seal
144 (1997). To the PCR reaction mix described above, 2.5 U AMV Reverse Transcriptase (Promega, UK)
145 were added and 1 µl of purified RNA was used as template. Tubes were then subjected to thermal
146 cycling consisting of a RT step at 50°C for 10 min, followed by 95ºC for 4.5 min and 35 cycles of
147 95ºC for 30 s, 55ºC for 1 min and 72ºC for 1 min and one final cycle of 72°C for 10 min.
148
149 2.3 RPA Assays
150 RPA primers were designed to the YMV CP gene by performing a multiple sequence alignment of
151 127 CP nucleotide sequences obtained from Genbank. Primers and exo-probe design followed the
152 manufacturer's (TwistDx, UK) specific parameters. According to a YMV complete genome sequence
153 (accession NC_004752) primers YMV RPA 3F (forward) and YMV RPA 3R (reverse) anneal to
154 positions 9019 and 9139, respectively, generating a 121 bp product (Table 1). RPA exo-probe (YMV
155 exo 3F/3R) was synthesised by Eurogentec S.A (Belgium) and contains a tetrahydrofuran residue
156 (THF), which replaces a nucleotide in the target sequence, flanked by a dT-fluorophore and a dT-
157 quencher group and a 3'-modification group (C3-spacer) blocking any polymerase extension (Table
158 1).
159
160 **Table 1**. RT-exoRPA primers and probe designed for YMV detection.

Name	Sequence (5' – 3')	Genome Location (NC004752)	Product size (bp)
YMV RPA 3F	CAAATTTATCCGGRATGTGGACRATGATGGAC	9019 - 9049	121
YMV RPA 3R	GCGTCACTRAAATGCATCATTATYTGACGAAA	9139 - 9108	
YMV exo 3F/3R	TGTGGGTTTGGCATTTTCTATGATCGGTT(F)C(Z)A(Q) GGATATTCCACTT-Spacer C3	9107 - 9061	

161 F = FAM-dT: thymidine nucleotide carrying fluorescein; Z = tetrahydrofuran residue; Q = BHQ1-dT: thymidine nucleotide carrying
162 Blackhole Quencher-1
163

164 RPA was performed using the materials and protocols in the TwistAmp exo kit (TwistDx, UK). The
165 same cDNAs analysed by PCR were used as templates in RPA reactions, which were performed in 10
166 µl total volumes containing 420 nM of both RPA primers, 120 nM of RPA exo probe, 14 mM
167 magnesium acetate and TwistAmp rehydration buffer. All reagents except for the magnesium acetate
168 and template were prepared in a mastermix which was used to rehydrate the dried reaction pellets.
169 This solution was then distributed into 0.2 ml PCR tubes and 1 µl of 1/10 dilution of cDNA was
170 added. To initiate the reaction, magnesium acetate was pipetted into the cap of each tube.
171 Subsequently, tubes were recapped and centrifuged briefly. Fluorescence measurements in the FAM
172 channel were performed in a real-time PCR instrument (Mastercycler® ep realplex, Eppendorf,
173 Germany) at 37°C (the optimal reaction temperature) every 1 min for 30 min. Fluorescence data was
174 normalised and baseline adjusted using Mastercycler® ep realplex software.
175 For one-step RT-exoRPA, 25U of M-MuLV Reverse Transcriptase (New England Biolabs, UK) and
176 6U of RiboLock RNase Inhibitor (Thermo Scientific, UK) were added to the mastermix described
177 above and 1 µl of purified RNA was used as template in 10 µl reactions.
178
179 2.4 Comparison of RT-exoRPA and RT-PCR
180 Relative detection sensitivities of one-step RT-exoRPA and one-step RT-PCR protocols were
181 evaluated by using serial dilutions of a RNA obtained from an YMV-infected plant. RNA was
182 extracted as described above and quantified spectrophotometrically using a Nanodrop 2000 (Thermo
183 Scientific, UK). Dilutions were made with RNase-free water in a 10-fold series down to 10^{-6}
184 (equivalent to 140 fg/µl). Each dilution was tested in four replicates.
185
186
187 **3. Results**
188 3.1 Detection of YMV by RT-PCR and RT-exoRPA
189 To evaluate the suitability of RPA for the detection of YMV, cDNAs synthesised from total RNAs
190 from leaf preparations of 25 yam plants were used as templates in a two-step RT-exoRPA assay
191 performed in a real-time thermal cycler set at 37°C for 30 min (Fig. 1).
192 YMV amplification occurred in 14 yam samples, all representing *D. rotundata* samples. Furthermore,
193 some of these samples showed very rapid amplification signals of just 3 – 5 min, suggesting that they
194 contained a high virus titre. The same cDNAs were analysed by PCR using primers YMV CP 1F and
195 YMV UTR 1R (Mumford and Seal, 1997), and results from this assay revealed that the same 14
196 samples gave positive results (Table 2).
197 In a second experiment, purified total RNAs were directly analysed by both one-step RT-exoRPA (by
198 adding M-MuLV reverse transcriptase to the RPA mastermix) and by one-step RT-PCR following the
199 Mumford and Seal (1997) methodology. Results showed that the same 14 samples were detected as
200 YMV-positive by both methods (see Table 2).

201 In all reactions only specific products of the desired size were generated and there was no
202 amplification in the negative controls (Fig. 1, Fig. 2). Results of the one-step and two-step RT-PCR
203 and RT-exoRPA reactions were in agreement. In both one-step and two-step RT-exoRPA reactions
204 positive amplification signals above the threshold were obtained in less than 26 min (Table 2).
205
206 **Table 2**. Detection of YMV by one-step and two-step RT-exoRPA and RT-PCR assays. Threshold
207 time (min) are shown for RPA assays and positive (+) and negative (-) results obtained for RT-PCR
208 reactions.

			Two-step		One-step	
Sample Name	**IITA accession number**	**Yam Species**	**RT-RPA (min)**	**RT-PCR**	**RT-RPA (min)**	**RT-PCR**
IV1	TDr 00/00362 H8_M	*D. rotundata*	4.50	+	7.13	+
IV2	TDr 94/01108 M6_2	*D. rotundata*	7.00	+	12.83	+
IV7	TDr 03/00196 H3_B	*D. rotundata*	7.03	+	13.80	+
IV8	TDr 00/00403 H3_M	*D. rotundata*	4.89	+	8.76	+
IV10	TDr 07/00033 B4_2	*D. rotundata*	3.79	+	7.82	+
IV5-1	TDr 03/00058 B5-2	*D. rotundata*	5.05	+	10.13	+
IV8-1	TDr 00/00403 H4-H1	*D. rotundata*	5.13	+	10.20	+
LR3	Aloshi M5-B	*D. rotundata*	8.29	+	18.15	+
LR5	Adaka H5-4	*D. rotundata*	7.88	+	15.91	+
LR5-1	Adaka B10-2	*D. rotundata*	17.52	+	25.34	+
G1	TDa 85/00250	*D. alata*	-	-	-	-
G2	TDa 95/310	*D. alata*	-	-	-	-
G3	TDa 98/150	*D. alata*	-	-	-	-
G4	TDa 98/159	*D. alata*	-	-	-	-
G5	TDa 98/01166	*D. alata*	-	-	-	-
G6	TDa 99/00240	*D. alata*	-	-	-	-
G7	TDa 00/00005	*D. alata*	-	-	-	-
G8	TDa 00/00194	*D. alata*	-	-	-	-
G10	TDa 01/00039	*D. alata*	-	-	-	-
G11	TDa 02/00012	*D. alata*	-	-	-	-
G12	TDa 03/00059	*D. alata*	-	-	-	-
G13	TDr 89/02475	*D. rotundata*	11.02	+	19.50	+
G15	TDr 95/18544	*D. rotundata*	3.85	+	7.63	+
G21	TDr 99/02607	*D. rotundata*	4.91	+	9.47	+
G23	TDr 1892	*D. rotundata*	5.18	+	10.20	+

209
210 The actin housekeeping gene was targeted by RT-PCR to act as an internal control for the reaction.
211 This was to ensure that a negative result could be attributed to a lack of viral RNA rather than
212 inhibition of the assay caused by the presence of inhibitors found in yams, such as polysaccharides
213 and polyphenols (Mumford and Seal, 1997). In all reactions, the amplification of the actin gene using
214 specific primers resulted in a product of 362 bp confirming the good quality of the templates used in
215 RT-PCR and RT-exoRPA (Fig. 2). The same result occurred for all samples described in Table 2.
216

217 Next, the temperature range of the one-step RT-exoRPA assay for the detection of YMV using RNA
218 as a template was assessed. Reaction temperatures from 30°C to 42°C were tested using one YMV-
219 negative (G4) and three YMV-positive (IV1, IV10, G23) samples (Fig. 3). It was found that the
220 reactions performed were reproducible over the full temperature range tested, even when the assays
221 were performed at 30°C. However, it became apparent that the relative fluorescence intensities
222 decreased with reduced reaction temperatures while demanding more reaction time to cross the
223 fluorescence threshold.
224
225 3.2 RT-exoRPA specificity
226 The specificity of the RT-RPA assay was confirmed by cross reactions assays using RNA from *Yam*
227 *mild mosaic virus* (YMMV), a distinct but closely related member of the genus *Potyvirus* that is often
228 present in *D. alata*. Some of the *D. alata* samples tested, e.g. G4, G7, G8 and G10, are known to be
229 YMMV-positive by PCR and sequencing (results not shown). No cross reactions occurred for these
230 samples, indicating a high specificity of the primers used (Table 2).
231
232 3.3 RT-exoRPA sensitivity
233 The detection limit of the RT-exoRPA assay was ascertained by testing 10-fold serial dilutions
234 (starting from 140 ng/µl) of a purified RNA obtained from an YMV-infected *D. rotundata* plant and
235 by comparing results with RT-PCR. Each dilution was tested in four independent assays (Table 3). A
236 representative assay is shown in Fig. 4.
237 For both RT-exoRPA and RT-PCR assays, consistent positive results were obtained for samples
238 containing at least 14 pg/µl of RNA (10^{-4} dilution). For lower concentrations, 1 positive result in 4
239 replicates was obtained for samples with RNA concentrations of 1.4 pg/µl and none of the samples
240 with a concentration of 140 fg/µl was detected as positive.
241
242 **Table 3**. Sensitivity of the one-step RT-exoRPA and one-step RT-PCR assays for the detection of
243 YMV. Total RNA extracted from an YMV-infected *D. rotundata* leaf was 10-fold serial diluted from
244 140 ng/µl to 140 fg/µl, each concentration tested four times. The number of positive test results is
245 presented in relation to the total number of tests performed at each concentration.

	number of positive replicates / total tested	
RNA concentration	**RT-exoRPA**	**RT-PCR**
140 ng/µl	4 / 4	4 / 4
14 ng/µl	4 / 4	4 / 4
1.4 ng/µl	4 / 4	4 / 4
140 pg/µl	4 / 4	4 / 4
14 pg/µl	4 / 4	4 / 4
1.4 pg/µl	1 / 4	1 / 4
140 fg/µl	0 / 4	0/ 4

246

247 As shown in Fig. 4A, at the lowest concentration of 14 pg/μl the time required for the onset of
248 amplification was just under 17 minutes suggesting that 30 minutes for data collection period is more
249 than sufficient to detect YMV-positive samples. These results revealed that both RT-exoRPA and RT-
250 PCR methods possess comparable sensitivities in their ability to detect YMV.
251 The impact of the reduced reaction temperature on sensitivity was also evaluated by comparing the
252 sensitivity of the RT-exoRPA assay at 37°C (the optimal reaction temperature) and 30°C. A decrease
253 in sensitivity by a factor of 10 was observed when the reaction was performed at 30°C (data not
254 shown).
255
256
257 **4. Discussion**
258 In this study the application of RT-exoRPA for the detection of YMV from yam plants was analysed
259 and compared to the standard RT-PCR method for YMV molecular diagnosis. The RT-exoRPA assay
260 was developed to target the CP region of the YMV genome which has been the region of choice for
261 detecting yam potyviruses (Bousalem et al., 2000; Mumford and Seal, 1997). This assay demonstrated
262 a similar performance to RT-PCR with a detection limit of 14 pg/μl of purified RNA and no cross-
263 reactivity with a closely related potyvirus. Previous studies concerning the development of RPA
264 assays for the detection of several pathogens, such as the zoonotic pathogen *Francisella tularensis*
265 (Euler et al., 2012), biothreat agents (Euler et al., 2013), dengue virus (Teoh et al., 2015) and White
266 spot syndrome virus (Xia et al., 2014), also reported comparable sensitivities between RPA and PCR-
267 based methods. However, the time and man-power required to perform a RPA assay is considerably
268 reduced. In fact, in this study the reaction time of the RT-exoRPA was less than 30 min compared to
269 >150 min required in the RT-PCR to reach a similar detection sensitivity.
270 Both RT-exoRPA and RT-PCR methods were also shown to be highly reliable in detecting YMV in
271 several yam samples. All samples were analysed separately by RT-PCR with the Dz_actin_F and
272 Dz_actin_R primers designed in this study from a *D. zingiberensis* actin gene sequence. This enabled
273 confirmation that a negative result was because of the lack of viral RNA rather than the presence of
274 reaction inhibitors in the template, avoiding false-negative results which commonly have a negative
275 impact in routine YMV diagnosis (Mekuria et al., 2014; Mumford and Seal, 1997).
276 The short reaction time combined with the requirement of a single temperature in the region of 37°C,
277 means that the RT-exoRPA assay developed in this study has several advantages over RT-PCR and
278 ELISA currently used for YMV diagnosis. One advantage is that the RT-exoRPA does not require the
279 reaction temperature to be precisely controlled. Furthermore, although in this study the amplification
280 signal of the RT-exoRPA was analysed using a laboratory real-time PCR instrument, in principle only
281 a simple heating device is needed to achieve a temperature around 37°C (Craw and Balachandran,
282 2012; Lillis et al., 2014). This simple heating instrument is a major advantage of the RPA assay in
283 places experiencing power failures as the reaction could proceed although at a slower pace. Only a

ten-fold drop in sensitivity was found when RT-exoRPA reactions were performed at 30°C, which is in the region of common ambient temperatures experienced in West Africa (Lillis et al., 2014). It should be noted that a ten-fold reduction in the sensitivity at a lower temperature could lead to the appearance of false-negative results. However, this could be avoided by the devices for fluorescence detection being fitted with rechargeable batteries heating the reaction to 37°C. Currently there are already battery-operated portable instruments available for reading the fluorescence generated in RPA reactions, such as the Twista fluorometer (TwistDx, U.K.) or ESEquant tubescanner (Qiagen, Germany) (Euler et al., 2013; Teoh et al., 2015; Zhang et al., 2014), adding further protection from equipment failure due to irregular power supplies commonly experienced in many yam growing regions of the world including in West Africa.

In contrast to RPA, RT-PCR needs more stringent conditions such as thermal cycling steps at higher temperatures. Thus, besides requiring more energy, the RT-PCR could be affected in places were reliable electrical power is intermittent because sub-optimal temperatures for each reaction step would result in it being necessary to repeat any affected runs.

Future studies will investigate procedures to adapt the RT-exoRPA assay developed here to a format suitable for on-site applications. For this technique to be adopted as the routine test for YMV, it will also be necessary for the cost of reagents to be reduced. The RT-exoRPA assay currently costs ~US $6 per test for reagents alone, compared to ~US $1.5 per RT-PCR reaction. The difference in cost of the two tests is, however, less marked as increased labour is needed for the setup of the RT-PCR and agarose gel visualisation of resulting products. It is expected that the cost of RPA consumables will decrease as this becomes less of a novel technique and increases in use globally (Escadafal et al., 2014).

YMV is one of the major limiting factors for yam production (Maroya et al., 2014) and has been shown to be widespread across several regions in West Africa (Asiedu and Sartie, 2010). Since yams are generally propagated vegetatively through their tubers and aphid spread is believed to be low, the use of virus-free seed yams is the most important way to limit the spread and incidence of YMV (Eni et al., 2010; Seal et al., 2014). Therefore, the development of a sensitive diagnostic test is of foremost importance to enable yam planting material to be indexed for YMV (Kenyon et al., 2008). The RT-exoRPA assay developed in this study has the potential to be a promising tool for that purpose.

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321 **Figure captions**

322 **Fig. 1**. Amplification plot of real-time two-step RT-exoRPA amplification of YMV. Normalised
323 fluorescence intensities were plotted against time in minutes. The solid bar corresponds to the
324 threshold line (graph generated by Mastercycler® ep realplex software). NTC refers to the non-
325 template control (water control). Sample details are given in Table 2.

326

327 **Fig. 2**. Resolution of one-step RT-PCR amplification products of YMV (left) and the actin
328 housekeeping gene (right). MW: 1kb Plus DNA Ladder; NTC: non-template (water) control.

329

330 **Fig. 3**. Efficacy of the one-step RT-exoRPA assay at temperatures from 30 - 42°C. RPA reproducibly
331 detects YMV even at 30°C, but requires increased reaction incubation times.

332

333 **Fig. 4**. Sensitivity of the RT-exoRPA (A) and RT-PCR (B) assays for the detection of YMV in 10-
334 fold serial diluted total RNA from an YMV-infected plant. A) Normalised fluorescence intensities
335 were plotted against time in minutes (graph generated by Mastercycler® ep realplex software). B)
336 MW: 1kb Plus DNA Ladder; NTC: non-template (water) control.

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