First Loop-mediated isothermal amplification (LAMP) diagnostic method to detect the potato pale cyst nematode, *Globodera pallida*

Camacho M.J.[1,2*], Inácio M.L.[1,3], Mota M.[2] & de Andrade E.[1,3]

1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.)
2 NemaLab, MED – Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Univ. Évora
3 GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal
*mjoao.camacho@iniav.pt

The potato cyst nematode (PCN) ***Globodera pallida*** has acquired significant importance throughout Europe due to its nefarious effects on potato production. Early, rapid and reliable diagnosis of PCN is critical during the surveillance programs and for the implementation of control measures. Molecular DNA-based methods are available, but require expensive laboratory facilities, equipment and trained technicians. As result, a **loop-mediated isothermal amplification method (LAMP)** has been developed by Notomi (2000). **LAMP** is a single tube technique for the amplification of nucleic acid, using 4 to 6 primers that target 6 to 8 locations within a given DNA sequence under isothermal conditions (60–65 ºC), yielding large amounts of products in a short time (30 to 60 min). Amplified products can be visualized by gel electrophoresis, by a visible by-product (colorimetric detection) or by measuring the fluorescence emitted by DNA intercalating dyes such as SYBRGreen (Subbotin, 2013).

In this work, we developed and validated the first **LAMP assay to detect *Globodera pallida*.** This diagnostic method uses 4 primers (Table 1) recognizing 6 distinct regions of the "3'end18S-ITS1-5.8S-ITS2-5'end28S" rDNA region. LAMP assay for *G. pallida* detection should be performed according to the protocol in Table 2. The reaction mixtures prepared with master mix ISO-004 (OptiGene, London, UK) should be incubated at 64 °C, for 20 min and terminated by incubation at 95–85 °C, 0.05 °C/s or for 60 min if using the isothermal master mix ISO-001 (OptiGene, London , UK).

Table 1. Set of primers for *Globodera pallida* LAMP assay.

Primers	Set
FIP (F1c + F2)	ACA CTC ATG TGC CCA CAG GGT GGG CTG GCA CAT TGA T
BIP (B1c + B2)	TGG GGT GTA ACC GAT GTT GGT GAG CGA CCC GAC GAC AA
F3	ACA CAT GCC CGC TAT GTT
B3(a)	CCC TGT GGG CGT GCC A

Table 2. Preparation of LAMP reaction master mix for *Globodera pallida* positive amplification control.

Component	Initial Concentration	Vol/Reaction (µL)
ISO-004 (or 001) master mix	-	15
Primers FIP and BIP	50 µM	0.80
F3 and B3a	50 µM	0.15
Molecular grade water	-	3.1
DNA template	≥5 pg	5

The **primers used for the LAMP amplification** (Table 1) specifically detected *G. pallida.* No false positives were observed either with other closely related species (Figure 1) or non-related species (Figure 2). In a single situation, the DNA of one *Heterodera* sp. amplified but the melting temperature of the product was different from the expected for *G. pallida* (Figure 1). **G. pallida LAMP assays** detected *G. pallida* in DNA extracts with concentrations, at least, equal or above 5 pg/µL (Figure 3), even when using pooled samples with 1 *G. pallida* J2 mixed with 40 *G. rostochiensis* J2 (Figure 4).

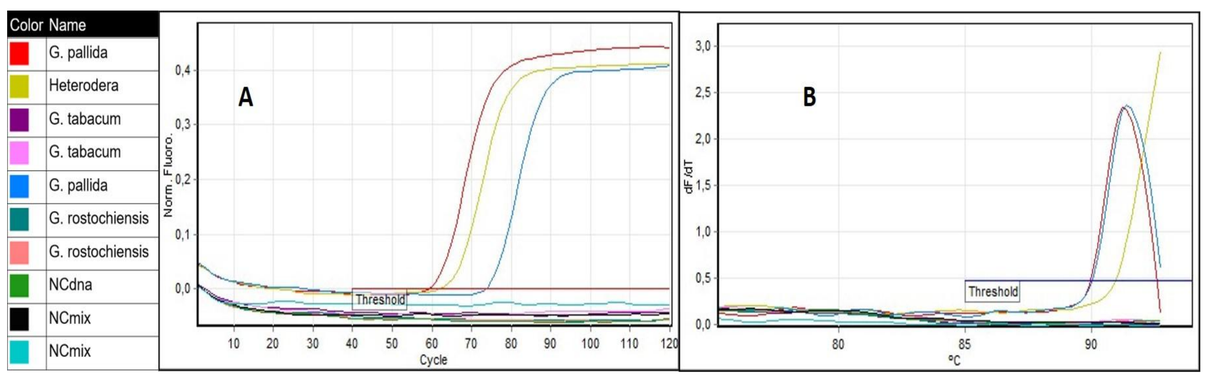

Figure 1 . LAMP assay run on a rt-PCR instrument using genomic DNA from *Globodera pallida, G. rostochiensis, G. tabacum, Globodera* n. sp and *Heterodera* sp.: (**A**) amplification curves, (**B**) derivative of the melting temperature curve.

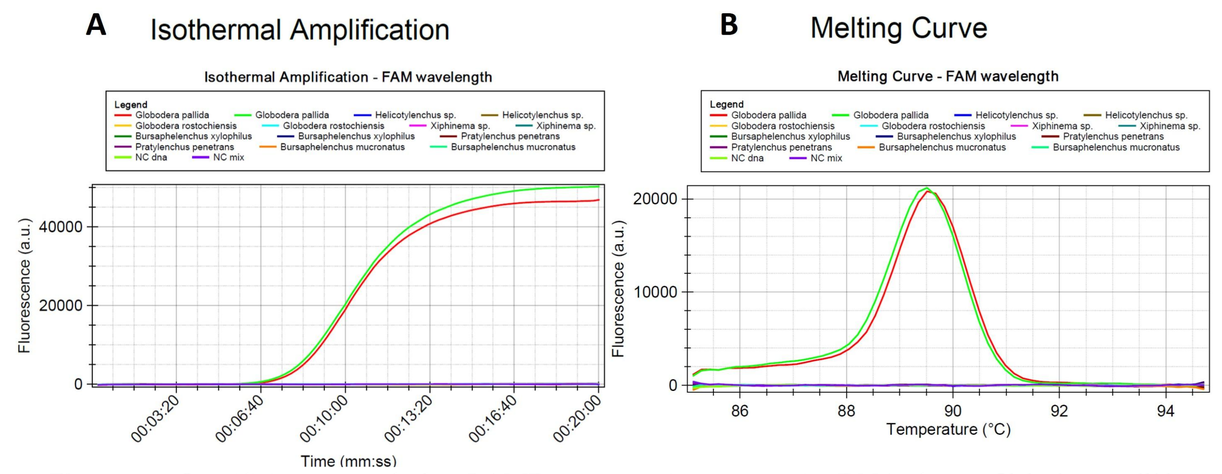

Figure 2. Specificity test of the LAMP assay using genomic DNA from *Globodera pallida, Pratylenchus penetrans, Xiphinema* sp., *Helicotylenchus* sp., *Bursaphelencus* (*B. xylophilus* and *B. mucronatus*): (**A**) amplification curves and (**B**) derivative of the melting temperature curve.

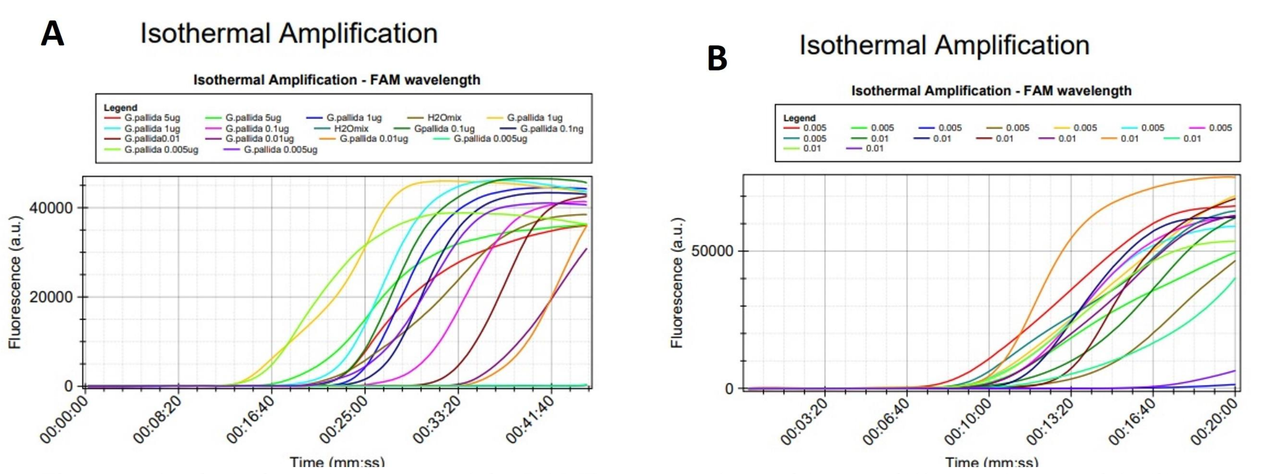

Figure 3. Analytical sensitivity test of the LAMP assay performed in two different times and facilities: (**A**) Laboratory of Molecular Biology at INIAV; (**B**) NemaLab-Laboratory of Nematology in Évora.

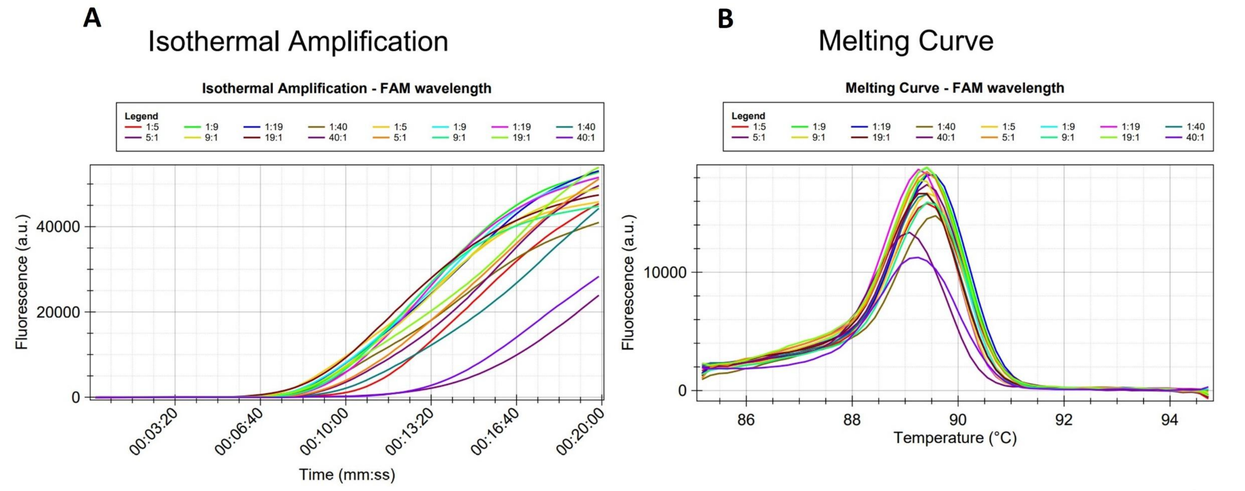

Figure 4 . Diagnostic sensitivity test of the LAMP assay performed in the Laboratory of Nematology in Évora. Amplification of DNA extracts from pools having different proportions of *G. rostochiensis*: *G. pallida* J2

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Our method can be used on-site what may help increasing early detections. This will have significant impact for the official control services and for producers because the time and costs with the transport of samples to the NRL may be reduced.

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