Detection and quantification of cyst forming nematodes using Real-Time PCR with SYBR Green I Dye

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DNA technology provides several methods for quantification of pests. A real time PCR method using SYBR Green I Dye and an ABI 7900 HT sequence detection system (Fig. 1) was developed to detect and quantify juveniles of the potato cyst nematode (*Globodera pallida* and *Globodera rostochiensis*) and sugar beet cyst nematode (*Heterodera schachtii*) (Fig. 2), which are considered as major nematode pests in potato and sugar beet production world-wide.

Fig. 1. ABI Prism 7900HT sequence detection system.

Fig. 1. Cyst of *Heterodera schachtii* and a sugar beet field infected by this nematode.

The PCR quantification technique measures nematode numbers indirectly by assuming that, the number of target DNA copies in the sample is proportional to the number of target nematodes. The quantitative information in a PCR comes from those few cycles where the amount of DNA grows logarithmically from barely above background to the plateau (Fig. 3). The real-time instrument allows continuously monitoring of samples during PCR using double stranded SYBR Green I Dye. The log-linear region can easily be identified as the fluorescence data appear on the computer screen and then compared with the standard (Fig. 4).

SYBR Green I Dye has no sequence specificity and can be used to detect any PCR product as it binds to any the double-stranded DNA. However, this virtue has a drawback, *i.e*. the dye binds also to any non-specific product including primer dimers. To overcome this problem, the melting curve analysis can be employed. PCR products can be melted by increasing the temperature of the sample. At the melting temperature (Tm) of the product, a sharp reduction is seen in the level of measured fluorescence. Non-specific products tend to melt at a much lower temperature than the longer specific products. As both the shape and position of the DNA melting curve are function of the GC/AT ratio and the length of sequences, they can be used to differentiate amplification products (Fig. 5).

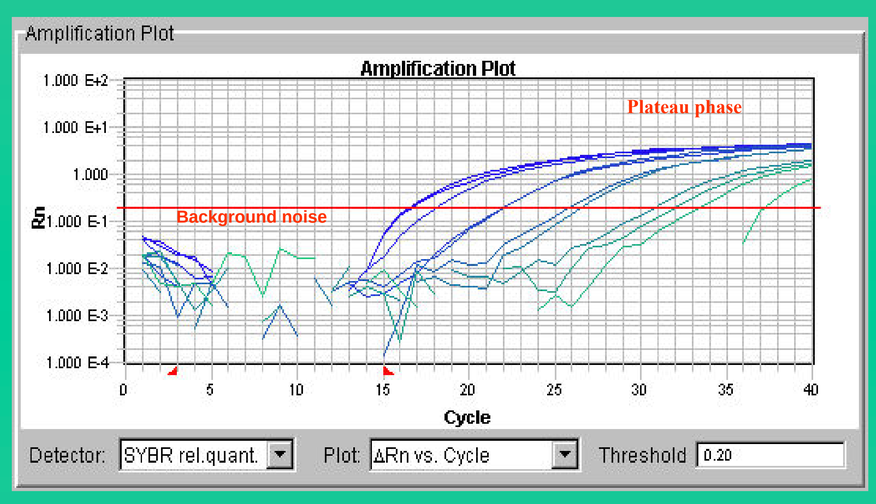

Fig. 3. Monitoring amplification plot in Real time PCR with specific primer for *Heterodera schachtii*. Flouresent signal is proportional to starting DNA amplification.

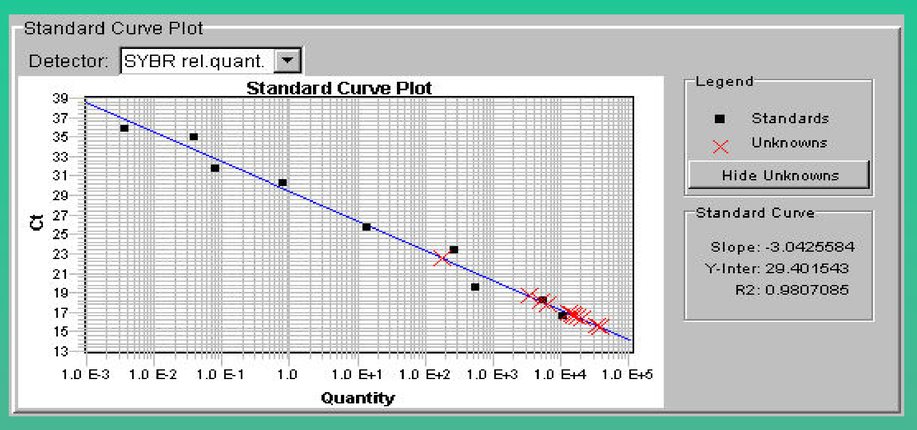

Fig. 4. Standard curve ploted with known numbers of *Heterodera schachtii* juveniles.

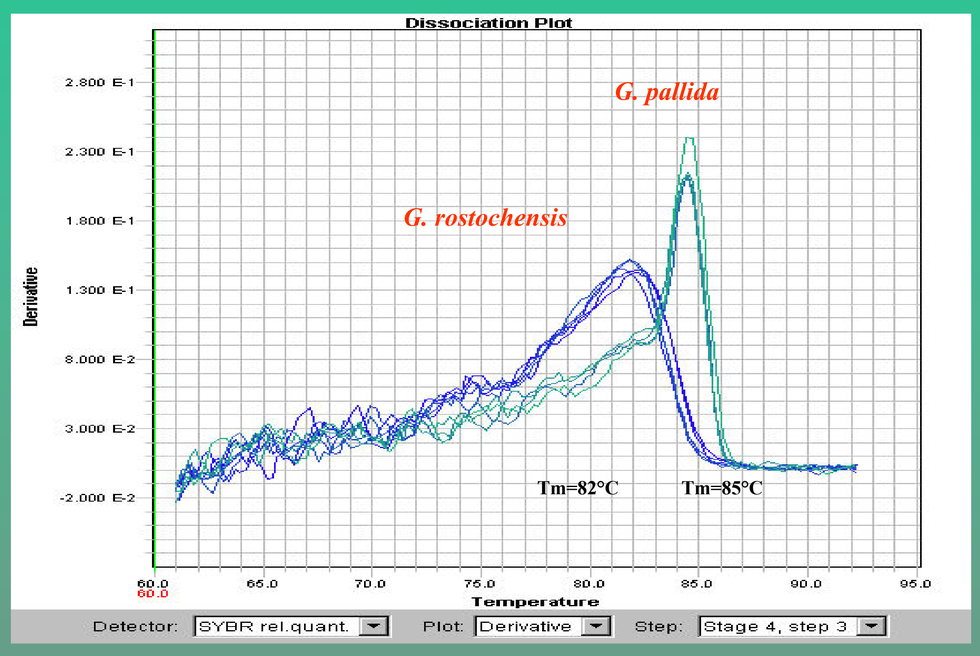

Fig. 5. The melting curve analysis of PCR products obtained with multiplex PCR using species specific primers for *G. pallida* and *G. rostochiensis.*

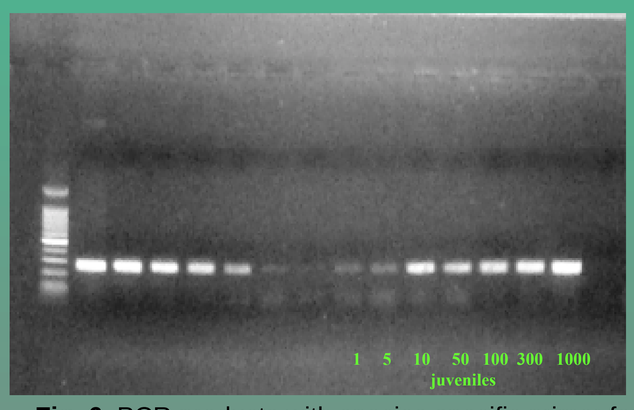

Fig. 6. PCR products with species specific primer for *Heterodera schachtii* obtained from samples with different nematode numbers.

Compared with the traditional PCR methods (Fig. 6), real-time PCR with SYBR Green I Dye has several advantages. It is faster, more sensitive and allows the quantification of target DNA. An automated system using the melting curve analysis overcomes the laborious process of estimation of PCR product with gel electrophoresis.