Christian-Albrechts-University of Kiel
Faculty of Agricultural and Nutritional Sciences
Plant Breeding Institute http://www.plantbreeding.uni-kiel.de/

Quantification of root lesion nematodes by RT-qPCR in the roots of cereal plants

Fatemi, Ehsan[1]; Shah, Smit[1]; Hachtel, Viola[2]; Hallmann, Johannes[2]; Jung, Christian[1].
[1]Plant Breeding Institute, University of Kiel
[2]Institute for Epidemiology and Pathogen Diagnostics, JKI Münster

1. Introduction

Root lesion nematodes (RLN), *Pratylenchus neglectus* is causing severe damage in German barley production. Since the assessment of RLN in the field as well as in the greenhouse is cumbersome and time-consuming, this pest has been largely disregarded by European cereal breeders so far. Therefore, a rapid method to quantify the root lesions nematodes in the infected roots is needed. In this study, we are aiming to develop a sensitive and reliable diagnostic method for species-specific quantification of *P. neglectus* in infected roots.

2. Methods

With the aim to develop **an SYBR Green-based RT-qPCR** quantification method for RLN, we first checked the primer efficiency and specificity in amplifying RLN. For *P. neglectus* quantification, we used **NEM91** and **NEM92** primers that bind to the inter transcribed spacer region (**ITS1**) of *P. neglectus* because it is known to be species-specific. To check the specificity of the primers, we used the DNA from *P. neglectus*, *P. crenatus*, *P. penetrans*, *P. thornei* as templates.

In order to compare the RT-qPCR method with the microscopic method, we used the DNA of 20 barley plants (10 Beysehir (Resistant) and 10 Valentina (Susceptible)). From half of the plants (5 Beysehir and 5 Valentina), DNA was extracted and was used for SYBR Green-based RT-qPCR analysis (Mokrini et al., 2013). While the roots of the remaining plants were kept under the misting chamber for counting RLN via microscopic counting (Keil et al., 2009).

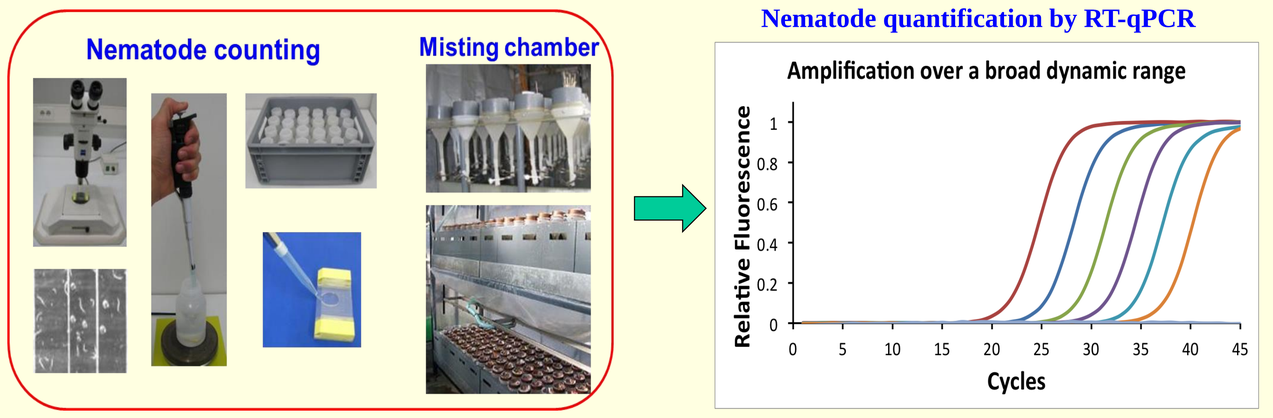

Fig. 1: Two different methods for counting the nematodes in the plant's root (Misting chamber method was mentioned by Keil et al. 2009).

3. Results

3.1 Primer efficiency and specificity to quantify *P. neglectus*

For *P. neglectus*, the average Ct value was 22, while the melt curve peak was at 87.5°C

For other *Pratylenchus* species, the average Ct value was ranging between 34-37, while the melt curve peak was at 80°C → Unspecific amplification

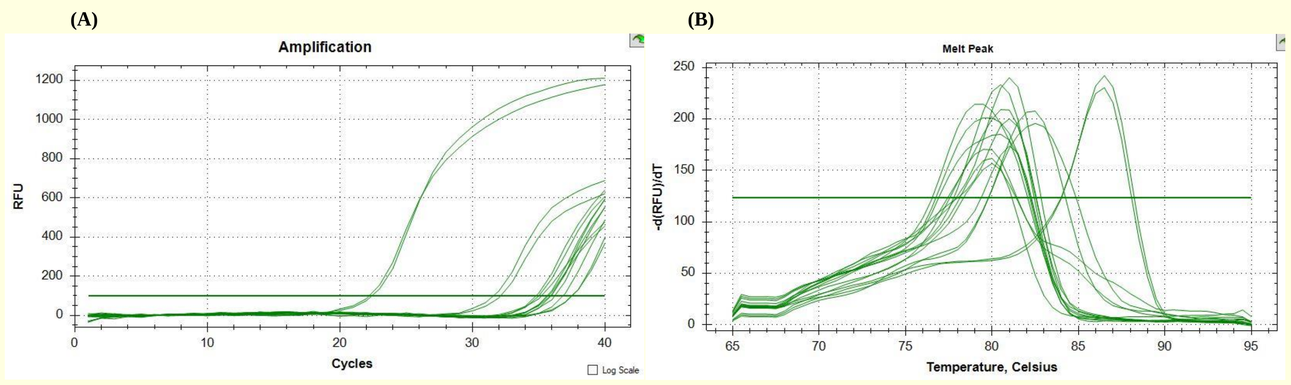

Fig.2: (A) The amplification curve from RT-qPCR analysis using NEM91 + NEM92 primers (two technical replicates). **(B)** The melt curve from RT-qPCR analysis using NEM91 + NEM92 primers.

3.2 Analyzing the sensitivity of RT-qPCR to quantify *P. neglectus*

The current RT-qPCR is sensitive to detect 78.125 pg/µl concentration of nematode DNA in the root.

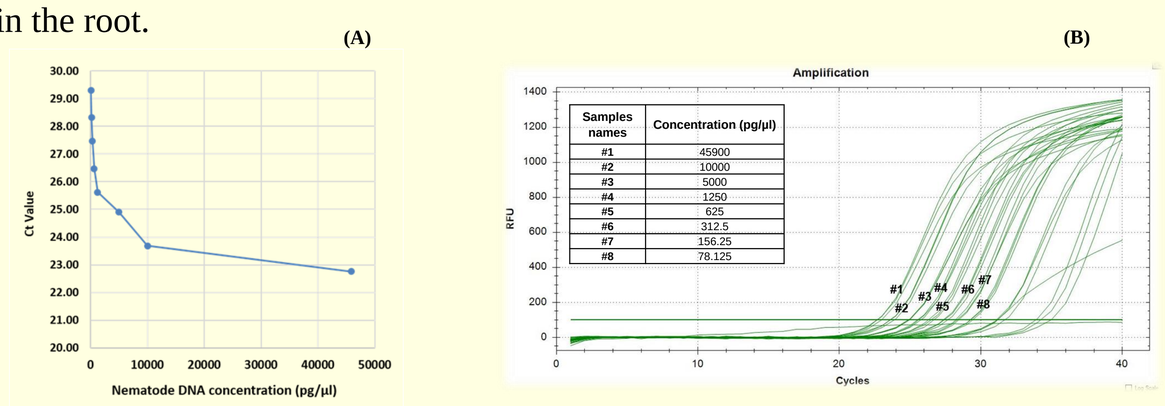

Fig.3: (A) Regression curve showing correlation between the Ct. values and nematode DNA concentration.
(B) The amplification curve from RT-qPCR using serial dilutions of nematode DNA (three technical replicates).

3.3 Comparison between RT-qPCR method and microscopic method for nematode quantification

The correlation between RT-qPCR and the microscopic method was 73% (Pearson correlation).

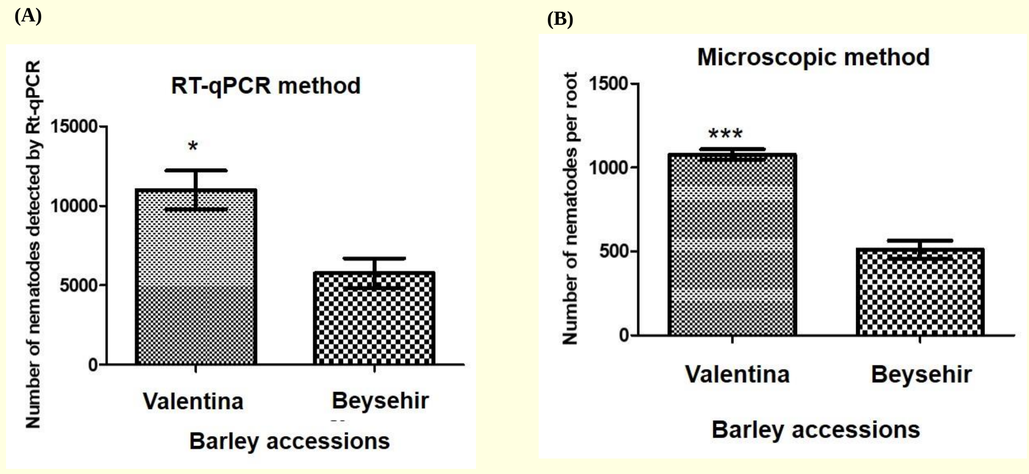

Fig.4: (A) Counting the nematodes by RT-qPCR method **(B)** Counting the nematodes by misting chamber method. The total number of plants is 20 plants. 5 Beysehir and 5 Valentina for each method. T-test performed (*P < 0.05,***P < 0.001).

- In the microscopic method, only alive and mobile nematodes that can go through the sieve in the misting chamber were counted.
- While, RT-qPCR can detect all stages of nematodes in the root such as eggs and death nematodes.

4. Conclusions and future prospects

Based on the initial experiments, it was evident that the primer pair NEM91 and NEM92 specifically amplify *P. neglectus* DNA. Moreover, using RT-qPCR, we could detect as low as 78 pg/µl concentration of nematode DNA in the infected barley roots.

We will perform a greenhouse experiment with 50 DH lines from the Beysehir-Valentina population with contrasting resistances infected with *P. neglectus* to validate the RT-qPCR protocol. We aim to measure RLN infections only by RT-qPCR in the future which will enable routine measurements to select resistant plants among large segregating populations.

Acknowledgements

This research was funded by the German Federal Ministry of Education and Research under the grant number 031B0186D . The authors gratefully acknowledge the technical help of Bettina Rohardt, Brigitte Neidhardt-Olf and Monika Bruisch.

Bundesministerium für Bildung und Forschung

Literature

Keil, T., Laubach, E., Sharma, S., Jung, C. (2009). Screening for resistance in the primary and secondary gene pool of barley against the root-lesion nematode *Pratylenchus neglectus*. Plant Breeding 128: 436-442.

Mokrini, F., L. Waeyenberge, et al., (2013). "Quantitative detection of the root-lesion nematode, *Pratylenchus penetrans*, using qPCR." European Journal of Plant Pathology 137(2): 403-413.