# DNA-based soil analysis of *Aphanomyces euteiches* increases sustainable production of legume-based foods

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Pea root rot, caused by the plant pathogenic oomycete *Aphanomyces euteiches*, is the major problem in pea cultivation in Sweden. The dormant oospores survive up to 20 years in soil. Therefore, it is important for the grower to cultivate the peas in fields with healthy soil. **The aim** of this study is to provide growers with a sensitive molecular-based method for analyzing *A. euteiches* in soil.

## Materials and Methods

Risk assessment of pea root rot was investigated in 24 soil samples collected from different regions in Sweden (Figure 1a). Bioassays were conducted for each field soil in a greenhouse with a susceptible pea cultivar. The plants were uprooted and assessed for disease symptoms after 3 weeks. DNA was extracted from the pea roots and analyzed by PCR and gel electrophoresis. Soil DNA was analyzed by Droplet digital PCR (ddPCR) (Figure 1b).

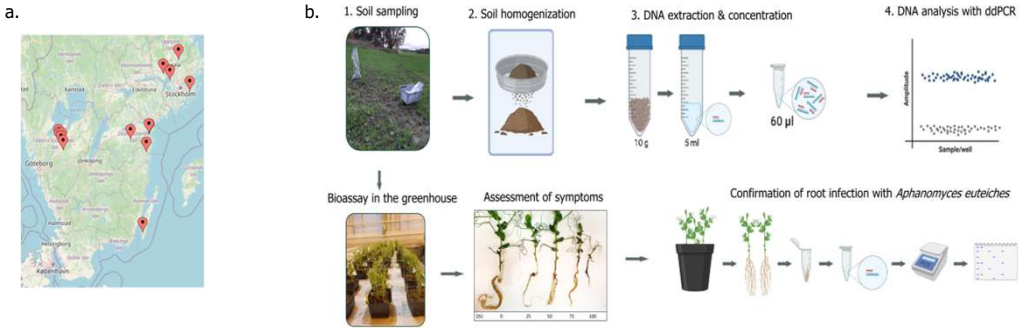


Figure 1. Workflow describing soil sampling from 24 Swedish pea fields, 1a: field locations, 1b: soil sampling and processing followed by analysis in a greenhouse bioassay as well as DNA extraction and analysis using Droplet digital PCR (ddPCR).

## Results

- Occurrence of *A. euteiches* DNA in pea roots was confirmed in 10 of the 15 soil samples tested in the first bioassay (Figure 2), and in 5 of the 9 samples tested in the second bioassay.
- *Aphanomyces euteiches* DNA was detected in 21 of 24 soil samples. The number of gene copies varied across the samples, ranging from 1 gene copy $g^{-1}$ soil to 25 974 gene copies $g^{-1}$ soil (Figure 3).
- The disease severity index (DSI) was significantly and positively correlated with the number of gene copies $g^{-1}$ soil in the second bioassay (Figure 4b), but not in the first bioassay (Figure 4a).

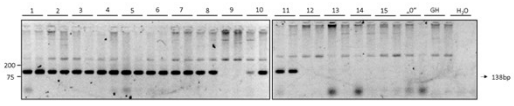


Figure 2. Identification of *Aphanomyces euteiches* in pea roots grown in soil sample number 1-15, soil "0" is a healthy field soil, and soil "GH" is a greenhouse soil. A 75 bp DNA bad amplified in sample no. 1-8 and sample no. 10-11.

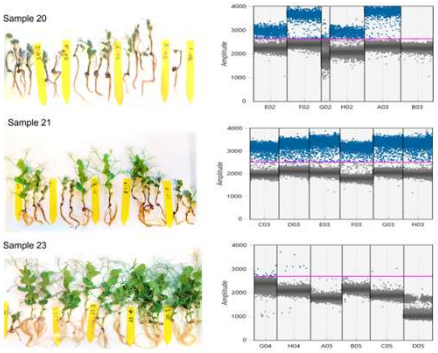


Figure 3. Symptoms of pea rot in pea plants grown in soil samples no. 20, 21 or 23 (left), and the respective ddPCR analysis of occurrence of *Aphanomyces euteiches* (amplitudes showing positive DNA amplification in form of blue droplets, right).

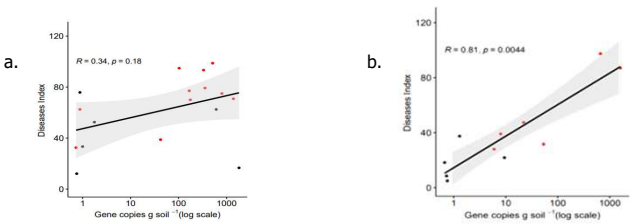


Figure 4. Correlation between number of gene copies (ddPCR) and disease severity index (bioassay). 4a: correlation in bioassay 1 (soil sample 1-15), and bioassay 2 (soil sample 16-24).

## Conclusion

- Risk assessment of *A. euteiches* in field soils was successfully achieved by ddPCR. The non-significant correlation between DSI and respective number of gene copies in bioassay 1 was probably due to presence of other soil-borne pathogens causing symptoms on the pea roots.

EKHAGASTIFTELSEN
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