METHOD FOR DETECTING *MACROPHOMINA PHASEOLINA*

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PATENT STATUS: GRANTED

PRIORITY NUMBER: 102017000057466

PRIORITY DATE: 26/05/2017

PUBLISHED AS: EP3406738

Invention

The invention relates to a molecular diagnostic method for detecting the presence of the phytopathogenic fungus *Macrophomina phaseolina* in infected soil, seed and plant samples.

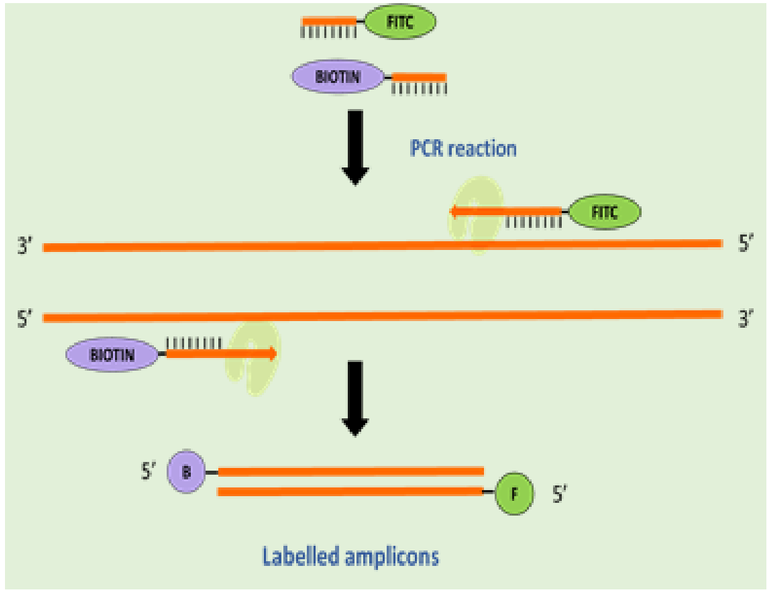
The diagnosis is carried out in a very short time (about 2 hours), possibly also *in situ*, and includes in brief: 1) the preparation of the sample, 2) the extraction of nucleic acids, 3) the PCR-NALFIA reaction.

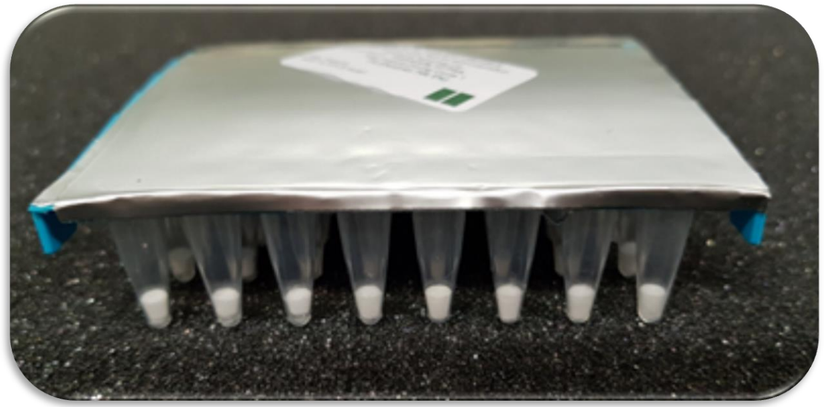
In the current diagnostic kit, all the PCR components, including the patented species-specific primers, which are the strength of the prototype, are lyophilized. This allows to have a diagnostic tool ready for use at room temperature and to be able to perform analyses not only in the laboratory but also directly in the field. The freeze-dried ready-to-use format is simply reconstitute by adding PCR-grade water along with the DNA template. This saves time and reduces the risk of contaminations.

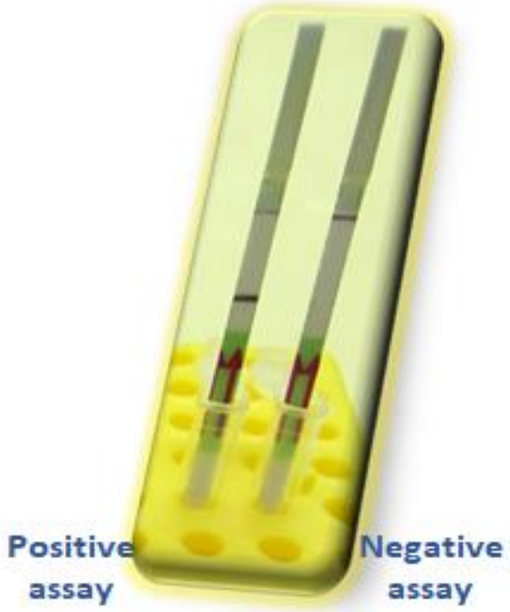
The lyophilized tube content is a unique product, not available on the market, which will allow the phytopathogenic fungus *M. phaseolina* to be detected quickly and specifically.

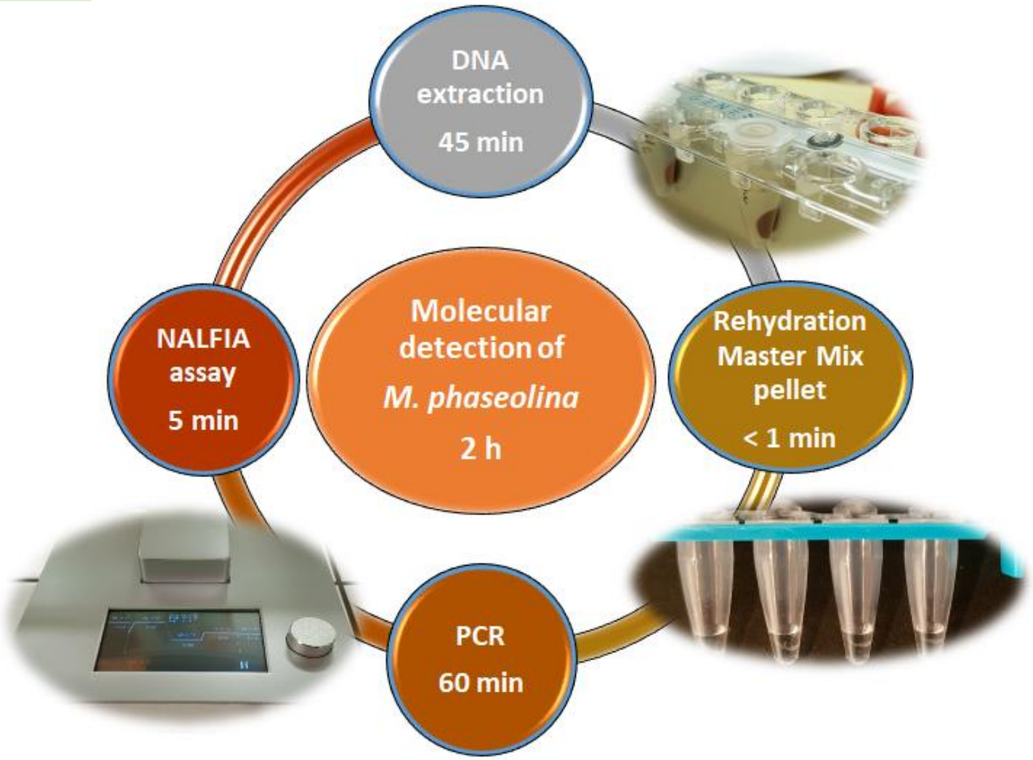
The use of the kit is very simple, unskilled people can easily perform the test after minimal training, and the results are clear and easy to understand.

Drawings & pictures









Industrial applications

- Agricultural sector: farms engaged in monoculture,
  farms producing cereals, vegetables, oil seeds and fibres;
- Companies that produce diagnostic kits all over the world can use the proposed technology to produce and market a new specific product for the identification of *M. phaseolina*;
- Public and private diagnostic laboratories and the laboratories of the Plant Health Inspection Services, which process a very large number of samples;
- Laboratory of Human clinical pathology, as the fungus is also an opportunistic human pathogen in immunosuppressed patients.

The plant pathogen *Macrophomina phaseolina* is distributed worldwide and has a host range of more than 500 plant species. The fungus attacks crops that are the source of staple foods, like maize and sorghum, as well as sunflower and soybean. Other crops affected include common beans, canola, cotton, tobacco, strawberries and some vegetables. The disease can cause yield losses of up to 90% in sunflower, 30-60% in strawberry, 50% in soybean, 70% in maize. However, when conditions are favorable for the development of *M. phaseolina*, infections can result in total crop failure.

Early diagnosis and screening of *M. phaseolina* requires a simple, rapid and inexpensive test, without the use of sophisticated and expensive equipment and reagents that are not always available in normal laboratories. The prototype diagnostic kit meets the need both to detect the pathogen in real time and to simplify the diagnosis technique in order to export the technologies developed in research laboratories to those of analysis, where there are many samples to process and diagnosis times must be quickly to meet farmers' needs.

Possible developments

A first prototype of the diagnostic kit has already been developed and validated in the laboratory and in the field thanks to research carried out with UNIPI funding (acronym MP102-LABINABAG). The project also envisaged the setting up of a portable mini-laboratory for phytopathological diagnosis, able to carry out DNA analyses directly 'in the field'.

The industrial development of the commercial prototype kit will be carried out through the improvement of the already existing prototype (TRL 4/5) by investigating the following aspects:

- refinement of the formulation and validation of the final product with laboratory and field samples;
- refinement of the prototype packaging for a practical and efficient use by the end users and to ensure an excellent preservation of the product at room temperature;
- use of the kit by a group of selected diagnostic laboratories (target customers) and analysis of the feedback requested on the product sent.

A collaboration will be undertaken with a specialist freeze-drying company with proven experience in developing products for molecular diagnostics. The research team is open to consider licensing the patented technology for its use by interested companies.

Watch a **technology video demonstration** at the following link: https://www.knowledge-share.eu/brevetto/metodo-per-rilevare-macrophomina-phaseolina/

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