Rapid detection strategy for pathogens causing blackleg of *Brassica napus*

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Introduction

The fungus *Leptosphaeria maculans* leading to Phoma stem canker (blackleg) of *Brassica napus* (oilseed rape, canola) produces the phytotoxin sirodesmin PL, which is responsible for major yield losses of oilseed rape worldwide. Due to the importance of *L. maculans* disease in global trade, rapid and simple detection of *L. maculans* at port and field in China is pivotal to control the spread of this disease and guarantee the quality of oilseed rape seeds.

In our study, we adopted isothermal nucleic acid amplification technique to develop potable detection of *L. maculans* and its approximate species *L. biglobosa*.

Results

1. Duplex real-time RPA

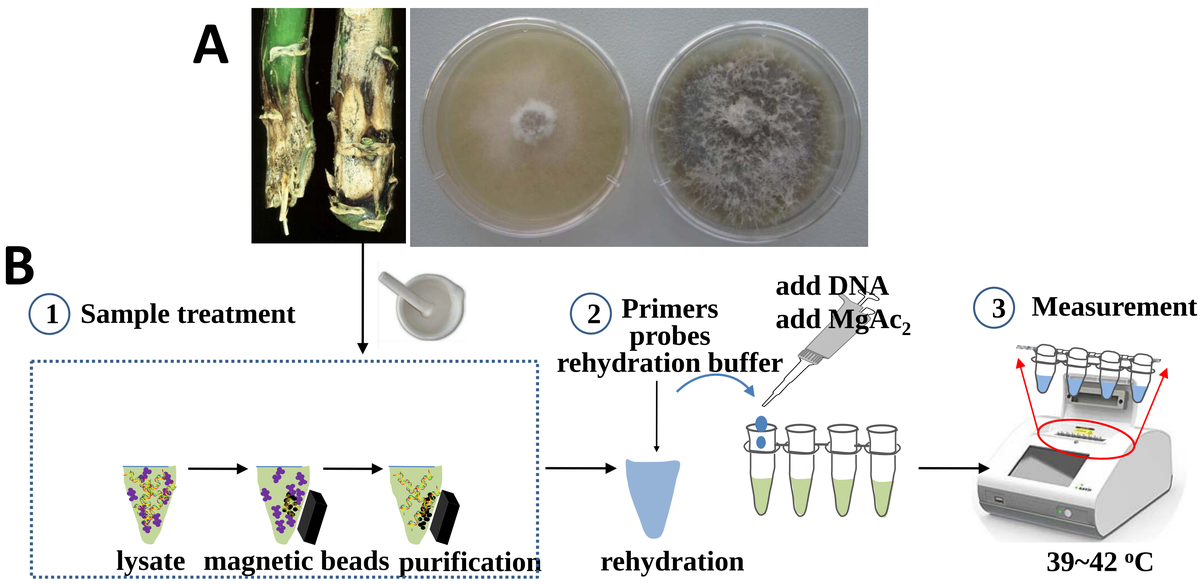

Fig. 1. Symptoms of *L. maculans* infected *Brassica napus* (A, left) and, hypha of cultured *L. maculans* (A, right), strategy of rapid genomic DNA identification (B).

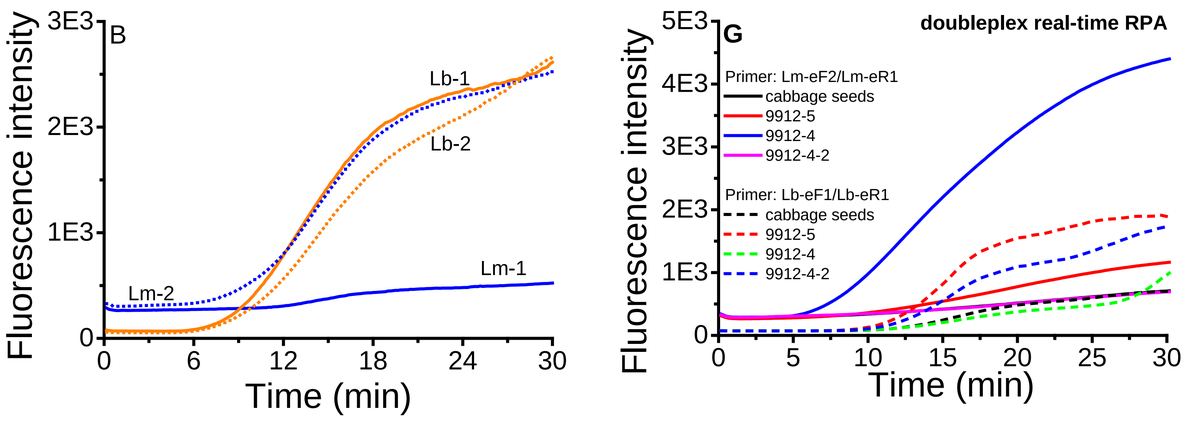

Fig. 2 A. The effect of primers and probes concentration on the doubleplex assay of *L. maculans* and *L. biglobosa.* Blue solid lines indicate 200 nM primers and 60 nM probe of *L. maculans*, blue dotted lines indicate 400 nM primers and 120 nM probe of *L. maculan*. Orange solid lines indicate 400 nM primers and 120 nM probe of *L. biglobosa,* orange dotted lines indicate 200 nM primers and 60 nM probe of *L. maculans*. B. The doubleplex real-time RPA assay of fungi containing DNA of *L. maculans* and *L. biglobosa.*

Conclusions

Three RPA-based methods have been developed to detect fungi leading to Phoma stem canker (blackleg) of *Brassica napus* (oilseed rape, canola) . Real-time RPA with duplex probes can detect *L. maculans* and *L.biglogsa* in one reaction for 30 min. Laterial flow strips detection of RPA products can distinguish *L. maculans* from *L. biglobosa*, and no complex equipment was required. To assay the low amount of *L. maculans* in oilseed rape seeds, an ultrasensitive detection with combined microcantilever sensor and RPA was developed.

References

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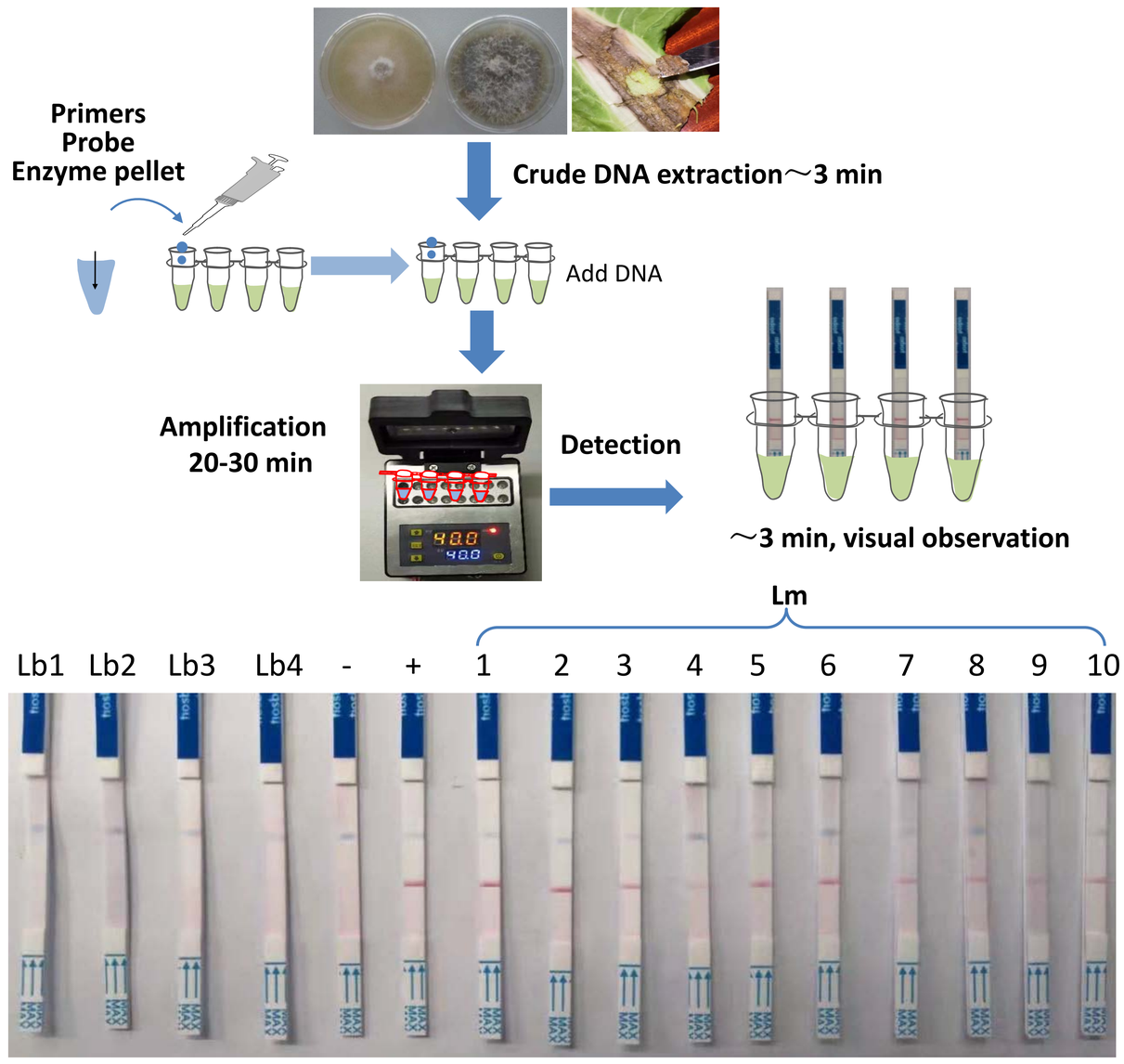
2. RPA with Laterial flow strips



3. Ultrasensitive detection with microcantilever sensor

A sensitive diagnostic platform for plant pathogen detection using a synergistic combination of RPA and an AuNP-enhanced dynamic MCL biosensor (RPA-MCL) was developed. This RPA-MCL was validated to be a highly sensitive and reliable diagnostic tool for detecting *L. maculans* in oilseed rape seeds with a genomic DNA concentration of 57 ppm. Based on our efforts toward the development of the AuNP-enhanced RPA-MCL, we believe that this rapid and sensitive assay is suitable for field applications, particularly during inspection of bulk goods at ports that require rapid clearance.

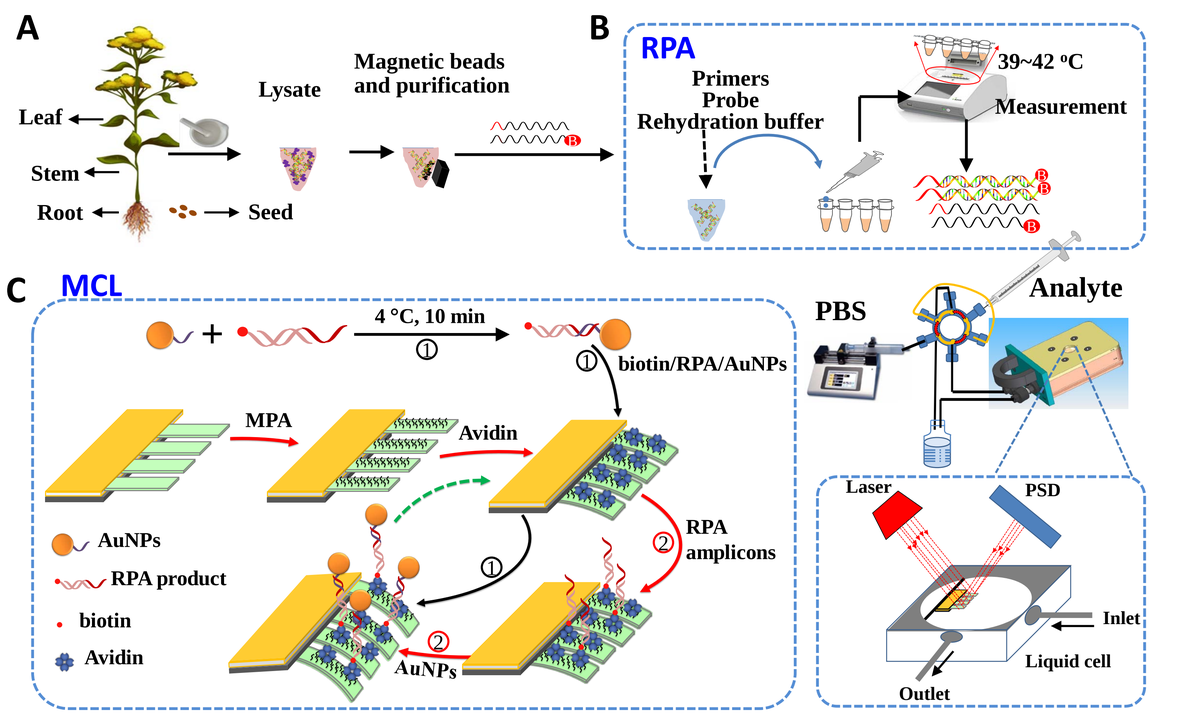


Fig. 3 Schematic illustration of the combined RPA and microcantilever (MCL) assay strategy.

Acknowledgments

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