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開發甜瓜萎凋病菌帶菌種子之自動檢測技術

Development of an automated detection system for melon seeds infected by *Fusarium oxysporum* f. sp. *melonis*

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本研究將隔絕式恆溫聚合酵素連鎖反應 (insulated isothermal PCR, iiPCR) 技術搭配自動核酸萃取技術導入甜瓜萎凋病菌之帶菌種子 (苗) 檢測流程上，利用甜瓜萎凋病菌具檢測專一性之增幅引子與螢光探針搭配快速核酸分析儀，開發出甜瓜萎凋病菌之自動檢測技術平台，並以人工置備帶菌種子檢體測試此甜瓜萎凋病菌檢測技術平台之可應用性。本研究所開發之甜瓜萎凋病帶菌種子 (苗) 檢測技術平台，未來將此平台導入協助監測健康種苗管理，協助檢防疫工作，或應用於甜瓜抗性品種選育及防治製劑篩選，或可縮短其品種或藥劑開發所需花費的時間，如以一來，應有助於甜瓜病害綜合管理，減少或避免甜瓜受到萎凋病菌危害時所造成經濟的損失。

This study developed a automatic, efficient diagnosis method based on the TaqMan probe-based insulated isothermal PCR method for rapid detection of *F. oxysporum* f. sp. *melonis*, which is currently among the most serious fungal vascular diseases on melon worldwide. By using the POCKIT™ central nucleic acid analyzer with a set of patterned primer and probe, the nucleic acid extraction and iiPCR assay (including DNA amplification and signal monitoring) can be completed automatically with a single default program within 85 minutes. A artificial samples detection evaluation was performed to determine whether the nucleic acid extraction and iiPCR assay was suitable for the detection of Fom in asymptom infected seeds and seedlings samples. The fully automated molecular detection assay could potentially be a useful tool for routine quarantine detection of *F. oxysporum* to avoid further dissemination.

參考表一

利用四種分子檢測技術，輔助鑑定供試菌株之部分鑑定結果。(僅列出此表做為示意參考表)

Table 1. Isolates of plant pathogens used in this study and their PCR amplification results with PCR-based identification methods.

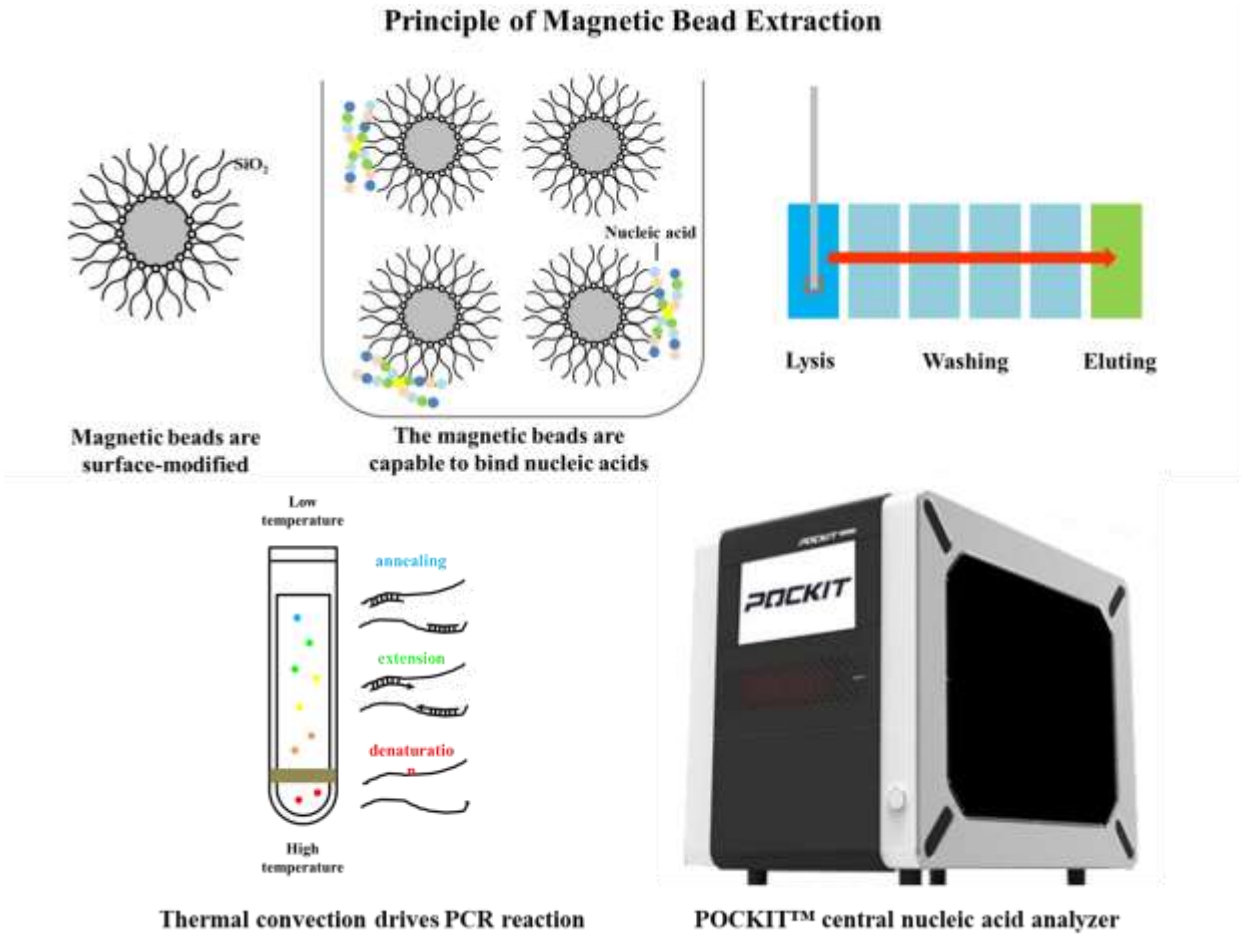
Organisms	Isolates	Original host	Result of specificity assay using PCR[a]		
			ITS1/ITS4[b]	Fa15F/Fa15R[c]	TDCP2F/TDCP2R[d]
Fusarium oxysporum					
Fusarium oxysporum* f. sp. *melonis	8	Melon (*Cucumis melo* L.)	+	+	+
F. oxysporum* f. sp. *tracheiphilum	1	Asparagus bean	+	-	-
F. oxysporum* f. sp. *gladioli	1	Gladiolus	+	-	-
F. oxysporum* f. sp. *lactucae	1	Lettuce	+	-	-
F. oxysporum* f. sp. *lilii	1	Lily	+	-	-
F. oxysporum* f. sp. *lycopersici	1	Tomato	+	-	-
F. oxysporum* f. sp. *niveum	1	Watermelon	+	-	-
F. oxysporum* f. sp. *cubense	1	Banana	+	-	-
Fusarium oxysporum	1	Anoectochilus	+	-	-
***Fusarium* spp.**					
Fusarium solani	1	Melon	+	-	-
F. Acuminatum	1	Bermuda grass	+	-	-
F. Verticilliodes	1	Rice	+	-	-
Non-*Fusarium*					
Colletotrichum gloeosporioides	2	Melon	+	-	-
***Alternaria* sp.**	1	Melon	+	-	-

[a]The molecular detection methods were used for confirming the identification of the pathogens used in this study.

[b]The conserved primer set ITS1/ITS4 was used to amplify and sequence the ~500-bp rDNA region for identifying the internal transcribed spacers 1 (ITS1), 5.8S rDNA and ITS2 of the pathogens listed in Table 1.

[c]The F. *oxysporum* f. sp. *melonis* race *2*-specific primer set Fa15F/Fa15R designed by Luongo *et al*. (2012) was used to confirm the identification of the pathogens listed in Table 1.

[d]The *F. oxysporum* f. sp. *melonis* race 2-specific primer set TDCP2F/TDCP2R designed by this study was used to confirm the identification of the pathogens listed in Table 1.



參考圖一

自動核酸萃取 (Automatic Nucleic Acid Extraction System)、隔絕式恆溫聚合酵素連鎖反應 (insulated isothermal PCR, iiPCR) 與全自動核酸分析儀 (POCKIT™ Central Nucleic Acid Analyzer) 之圖解樣式圖。

Fig 1. Diagrammatic representations of the Automatic Nucleic Acid Extraction System, the insulated isothermal PCR (iiPCR) assay, the POCKIT™ Central Nucleic Acid Analyzer.

參考表二

本研究所開發之隔絕式恆溫聚合酵素連鎖反應 (insulated isothermal PCR, iiPCR) 技術對帶菌種子之檢測應用性。

Table 2. Molecular detection of *Fusarium oxysporum* f. sp. *melonis* (Fom)-contaminated seeds extracted by the insulated isothermal PCR assay developed in this study

Primer sets	Fom-contaminated ratio (%)[a]						P[b]	N[c]
	25%	10%	5%	2.5%	1%	0%		
SYBR green-based real-time PCR								
Fa15F/Fa15R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-
TDCP2F/TDCP2R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-
TaqMan probe-based real-time PCR								
Fa15F/Fa15R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-
TDCP2F/TDCP2R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-
Insulated isothermal PCR (iiPCR)								
Fa15F/Fa15R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-
TDCP2F/TDCP2R	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	+ (3/3)	- (0/3)	+	-

[a]Four hundred Fom-contaminated seeds were prepared by mixing proper amounts of Fom-contaminated and Fom-free seeds.

[b]For the positive control, 20 ng of Fom (PM-TDC-F009) genomic DNA was used as a DNA template in the molecular detection assays.

[c]For negative control, sterile ddH_2O was used instead of DNA template.