2015分子標誌輔助育種及種傳病害檢測技術研討會

茄科作物抗病分子標誌技術產業應用性





104 年 11 月 11 日

孫永偉

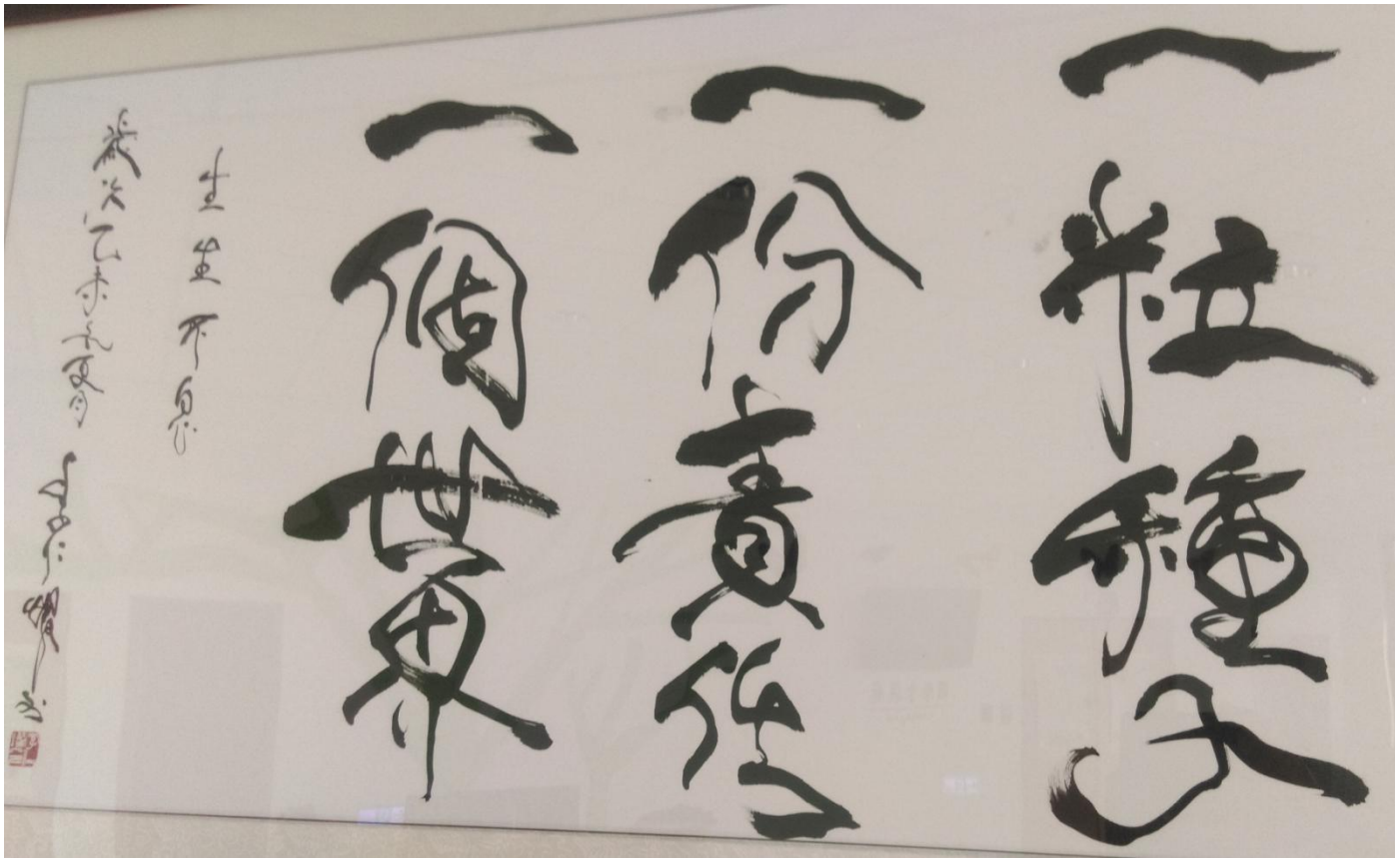
農委會種苗改良繁殖場

茄科分子標誌應用

- 獲得70萬組番茄SNP標誌，126組已實際應用於遺傳分析(臺灣大學)。
- 番椒設計16000組SSR分子標誌(320組已驗證)；已開發番椒細胞質雄不稔之稔性恢復基因SSR(臺灣大學)。

◆擁有種子(=食物)就可以掌握世界??

◆一粒種子　一份責任　一個世界（生生不息)VV
一粒種子，一個責任，無限希望(陳文郁董事長)



何謂好種子(好品種)??
(抗病、高產、風味、...)



http://he.aliexpress.com/store/product/200-seeds-High-Quality-Vegetable-seed-tree-tomato-seeds-of-tomato-greenhouse-seeds-of-tomato-tree/1395511_2021171182.html?storeId=1395511



- 以番茄為例，種子售價約新台幣0.5元/粒(不抗病)或1.5～3元/粒(抗病)；千粒重約1.5~3公克，推估種子需求量年約需1060公噸，種子產值非常驚人。
- 世界各種子公司急需抗病品種。



◆ **抗病品種優勢：**

- 可減少農藥施用(減少50~90%用量)
- 種子苗售價高(提升5~10倍)且國際競爭力強
- 確保產量(農民收益高)



王百祿先生提供



王宏偉先生提供

番茄主要病害



黃化捲葉病毒



萎凋病



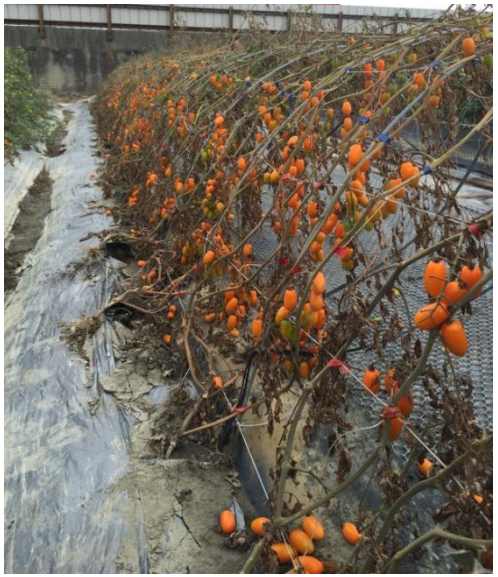
根瘤線蟲



青枯病



斑點萎凋病毒



晚疫病

陳迪偉博士提供

如何在眾多植株中篩選抗病好品種??
（自然淘汰、病害接種、分子標誌、…）



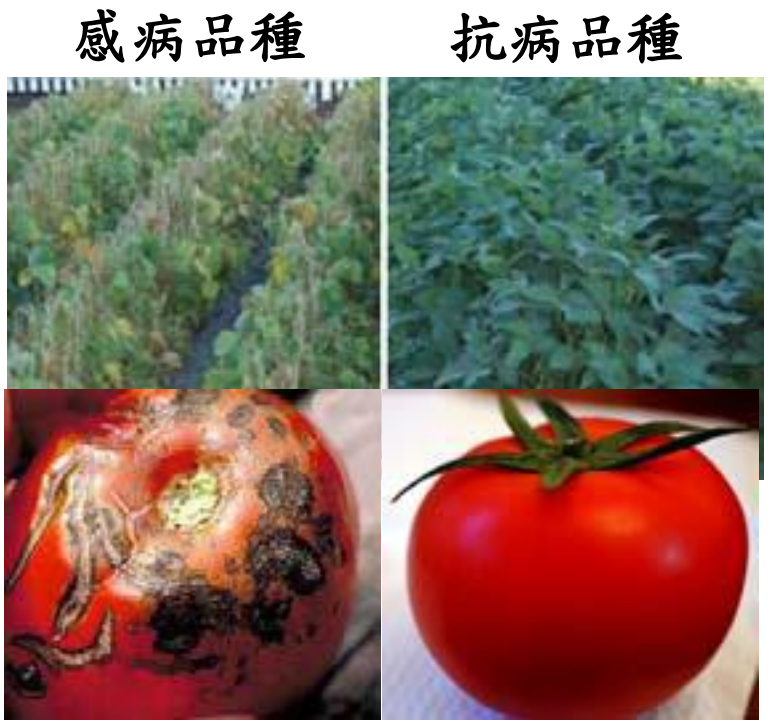
http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_for_disease_resistance_Blight.htm

分子標誌用途

- 品種鑑定
- 種子純度
- 轉基因作物
- 抗病分子標誌輔助育種
- …

抗病分子標誌輔助育種(Marker-assisted selection，MAS)優點

- 種子或幼苗期分子檢測結果不受環境影響。
- 鑑定作物抗感病基因型(R/R、R/S、S/S)。
- 確認自交系(R/R、S/S)與F1(R/S)基因型。
- 縮短育種年限。
- 避免病原接種造成病害蔓延。
- 篩選優良抗病F1品種。



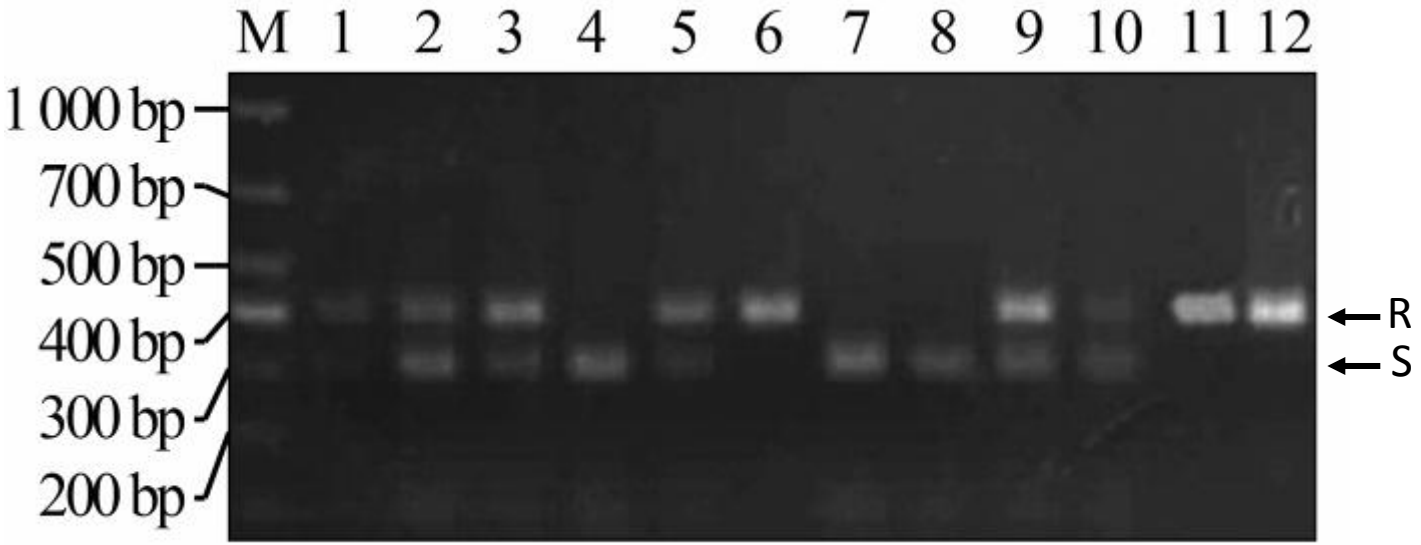
(朱鴻鈞, 2010)

分子標誌輔助育種方法

◆利用PCR技術：

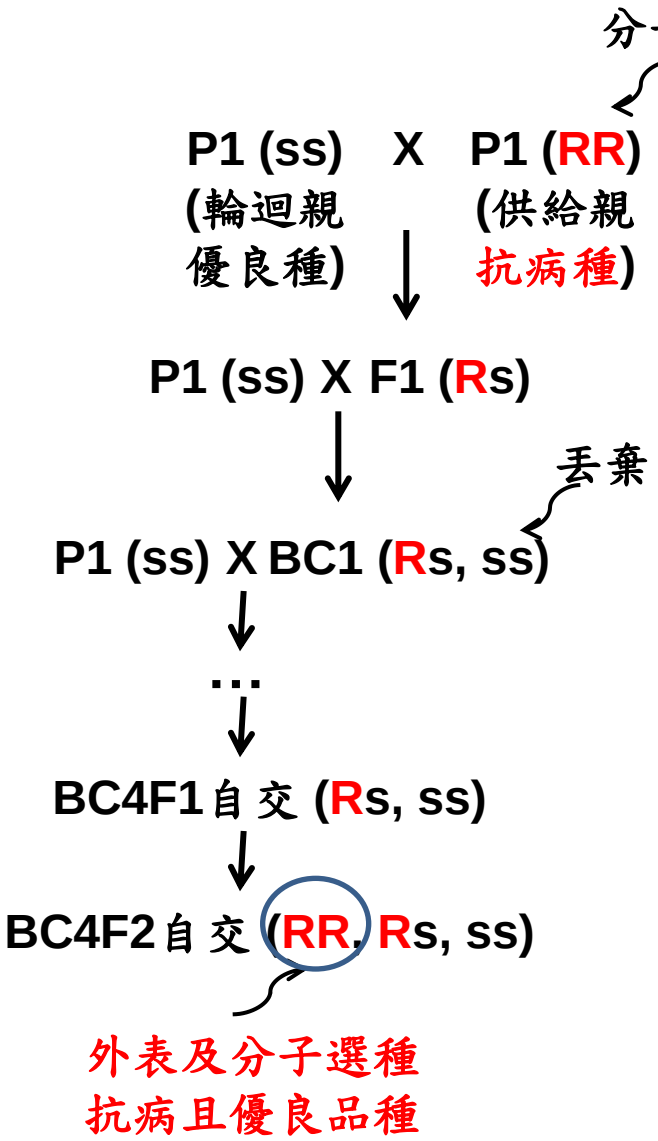
- RAPD(Random Amplified Polymorphic DNA)
- AFLP(Amplification Fragment Length Polymorphism)
- SNP(Single nucleotide Polymorphism)
- CAPS(Cleaved Amplified Polymorphic Sequence)
- **SCAR(Sequence Characterized Amplified Region)**
- SNP(Single nucleotide Polymorphism)
- …

◆可判別抗病或感病基因型(R/R、R/S、S/S)(1對基因控制)。



http://chinese.sophiapublisher.com/html-511-1-mpb

回交育種流程



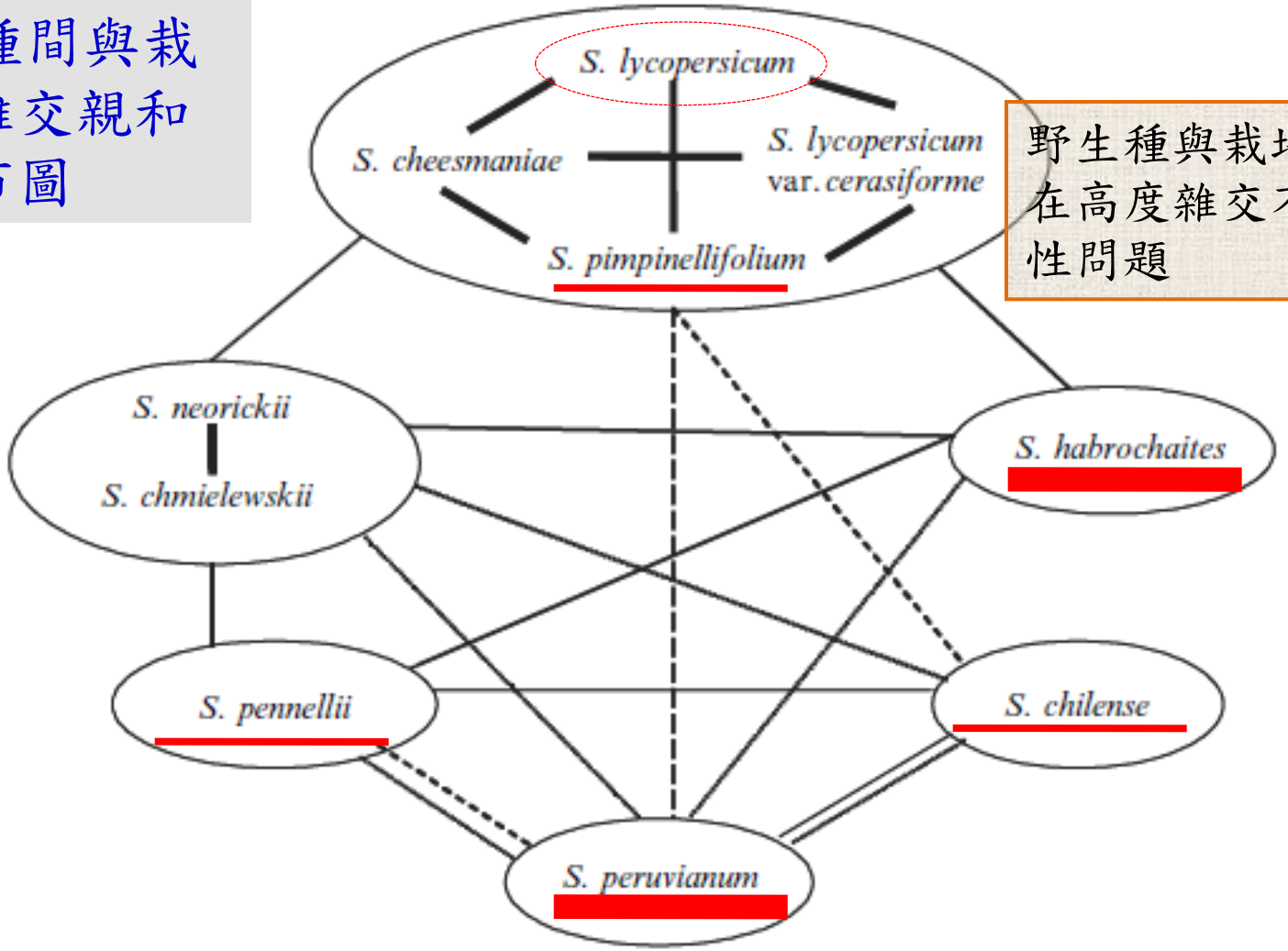
回交代數	與輪迴親相似度
BC1	75%
BC2	87%
BC3	94%
BC4	97%



開發抗病分子標誌準備工作

- 抗病與感病確定品種。
- 病原菌與接種技術。
- 搜尋相關基因或分子標誌序列。
- 設計專一性引子組。

◆野生種間與栽培種雜交親和性分布圖



野生種與栽培種存在高度雜交不親合性問題

——— and ——— solid interconnecting lines designate compatible crosses and the thickness of the lines indicates the degree of crossability; – – – – – – – indicates crosses that can be retrieved by embryo culture; and, ... indicates cross failures

Fig. 4. Diagram of crossability of tomato and its wild relatives (modified from Stevens and Rick, 1986).

番茄黃化捲葉病毒 TYLCV

- 新葉變小、褶皺、黃化、簇狀、邊緣上卷等，為番茄三大病毒之首，被稱為**番茄癌症**，主要靠銀葉粉蝨(世界級**超級害蟲**)傳毒。
- 臺灣已發現有4種，AYVHuV、ToLCHsV、ToLCTWV(台灣種)、及TYLCTHV(泰國種)，其中以台灣種及泰國種為最主要。



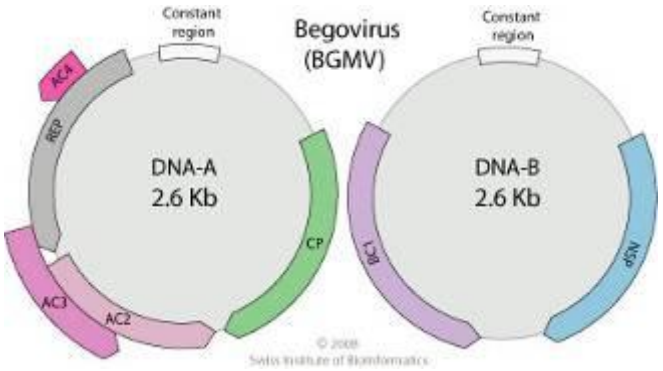


陳迪偉博士提供

TYLCV

台灣主要TYLCV種類

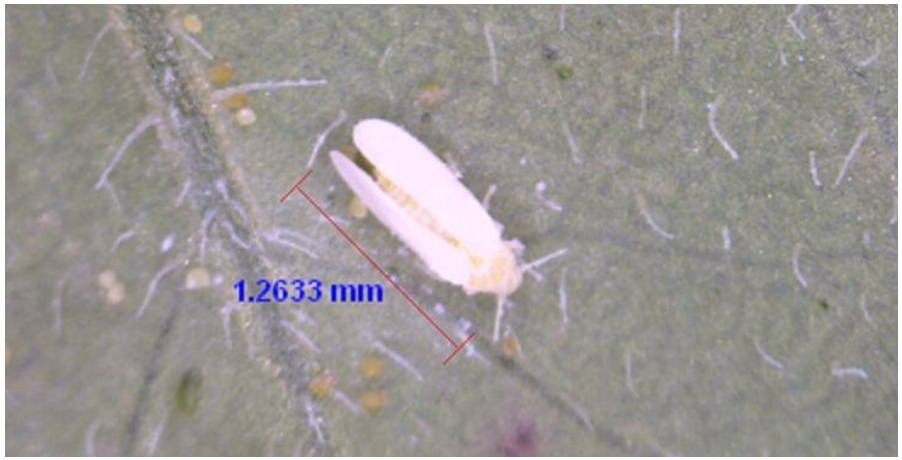
- 一條環狀單股DNA (Monopartite)：只含DNA-A，所謂台灣種病毒，致病力較弱，一般抗病基因(*Ty*)對其均具有抗性，近年極少單獨存在。
- 二條環狀單股DNA (Bipartite)：包含DNA-A及DNA-B，所謂泰國種病毒，致病力極強，發生機率有愈來愈高趨勢，一般單一抗病基因(*Ty-1*、*Ty-2*、*Ty-3*、*Ty-4*)對其多不具抗性。



http://viralzone.expasy.org/all_by_species/111.html

TYLCV主要靠銀葉粉蝨(Silver leaf whitefly)傳播

- 成蟲體長約1mm；需50目以上隔離網。
- 每一雌蟲可產卵200~500粒，經2-3星期幾乎所有的卵均可發育為成蟲，往往於短期內密度驟增，至無法控制局面。
- 1隻吸食病毒株汁液1 hr.後即能傳毒。1隻粉蝨傳毒率為29%，10隻傳毒率可達100%。(蘇,2009)



粉蝨接種法

- 可大面積接種及篩選，但不易控制病毒接種量(壓力)。
- 粉蝨接種室：世界蔬菜中心

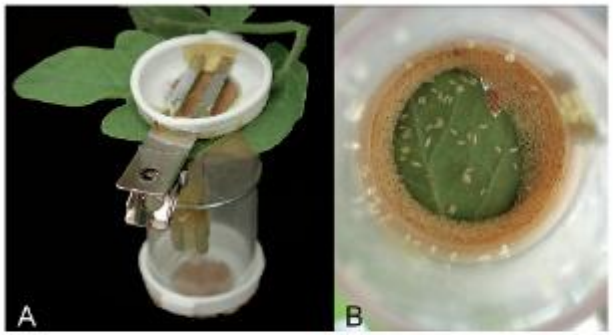


Figure 2. Inoculation of a tomato plant using a clip cage. Viruliferous whiteflies were aspirated into a clip cage, and the cage was attached to a tomato leaf. (A) Side view of the clip cage attached to a tomato leaf. (B) Bottom view of the clip cage, showing the caged whiteflies feeding from the tomato leaf abaxial side.

Czosnek, 2007

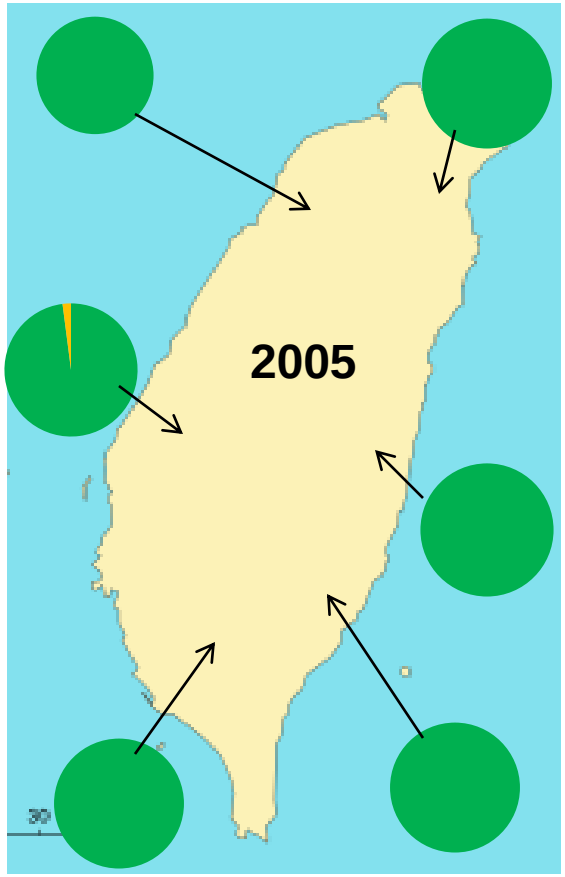
粉蝨接種室

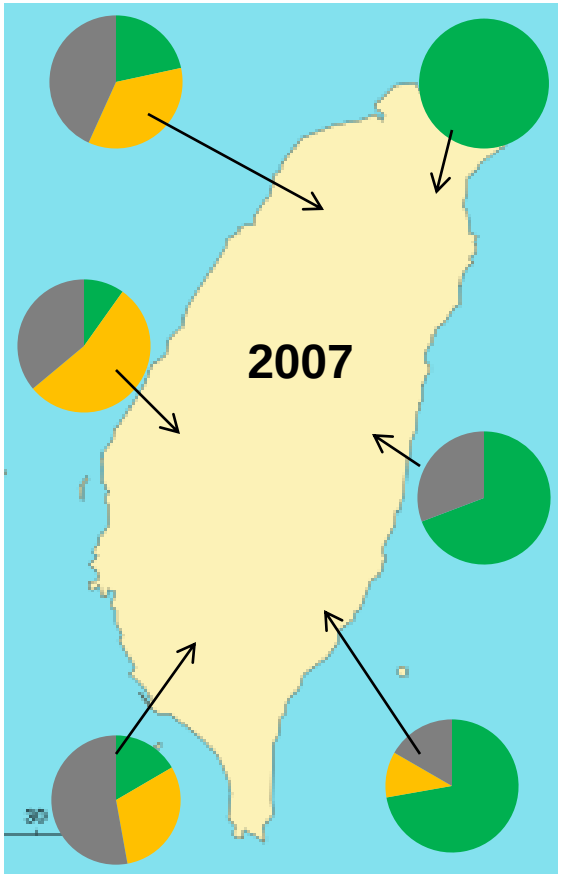


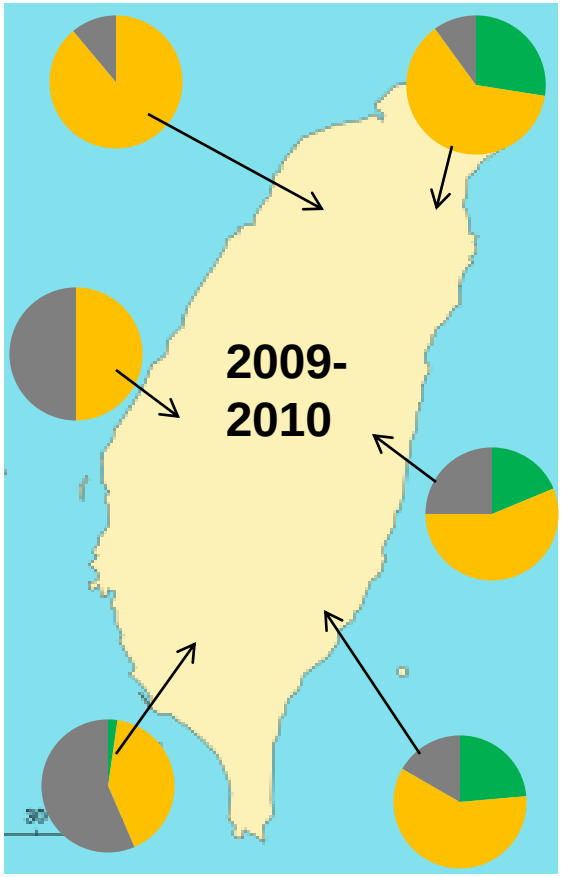


世界蔬菜中心提供

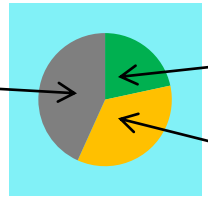
The changing of tomato BGVs in Taiwan







Mixed infection of ToLCTWV and TYLCTHV



ToLCTWV (Ty-2 effective)

TYLCTHV (Ty-2 not effective)

Tsai W. S. *et al*., (2011) *Plant Pathology,* 60, 787-799.



病毒(ToLCTWV, TYLCTHV)接種與檢測

Entry code	*Ty1*	*Ty2*	*Ty3*	*ty5*	ToLCTWV infection			TYLCTHV infection		
					Symptomatic plants	PCR + / symptomless plants	Virus infec.	Symptomatic plants	PCR + / symptomless plants	Virus infec.
TY52	R	S	S	S	24/24	NT	24/24	24/24	NT	24/24
CLN2498D	S	R	S	S	2/21	3/19	5/21	18/23	4/5	22/23
CLN3024A	R	R	S	S	0/24	0/24	0/24	2/24	7/22	9/24
CLN3078A	S	R	R	S	0/24	0/24	0/24	0/23	1/23	1/23
CLN3078I	S	R	R	S	0/24	0/24	0/24	0/20	0/20	0/20
CLN3109F	S	R	S	S	0/23	0/23	0/23	0/24	0/24	0/24
CLN3126A-7	S	R	S	S	0/23	0/23	0/23	0/23	1/23	1/23
CLN3125E	R	R	R	S	1/24	8/23	9/24	1/24	3/23	4/24
CLN3022E	S	S	S	S	24/24	NT	24/24	24/24	NT	24/24
ANT22 (Susceptible)					24/24	NT	24/24	24/24	NT	24/24

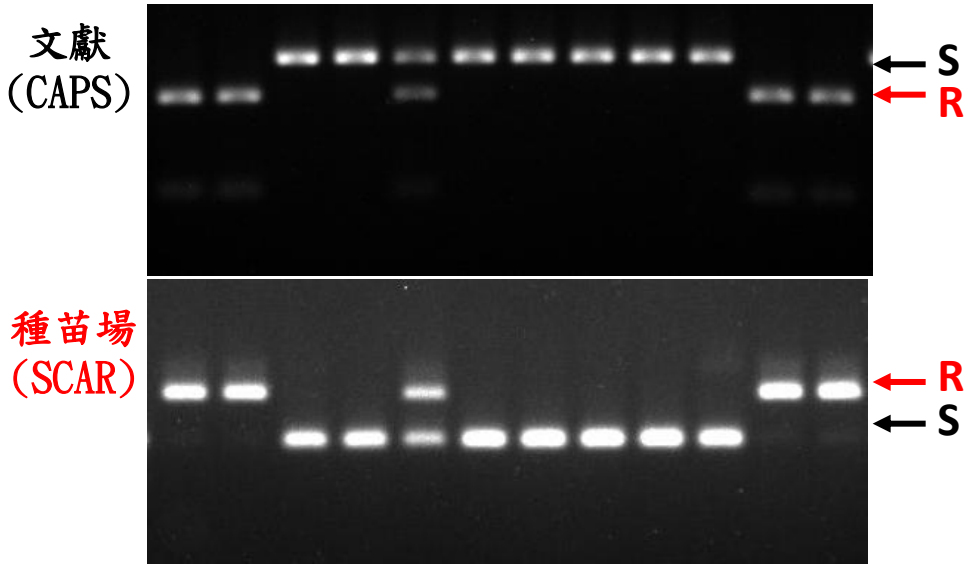
番茄抗黄化捲葉病毒

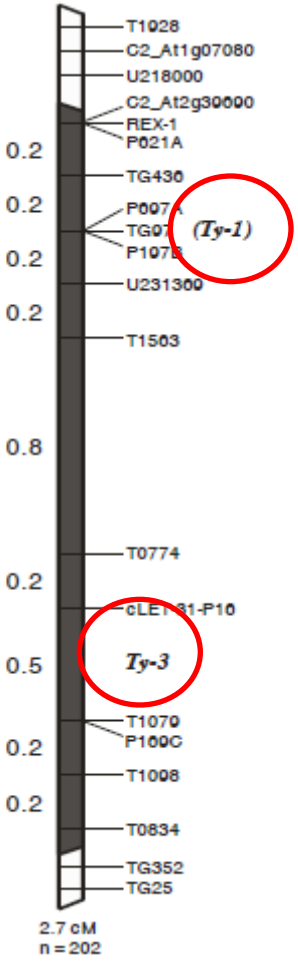
Diseases	Gene	Marker	Varties	Chr.	Reference
TYLCV	Ty-1	TG97, TG25, JB-1, REX-1, Aps-1	LA1969, LA3473, LA3474	6	de Castro et al., 2007
	Ty-2	TG36	B6013	11	Garcia et al., 2007
	Ty-3, Ty-3a	TG153, CT83, FLUW-25	LA1932, LA2779, LA4440	6	Ji et al., 2007
	ty-5	SINAC1	LA0441, PI126926, PI126930	4	Hutton et al., 2011
	Ty-6		LA2779	10	Hutton and Scott, 2013

野生番茄抗粉蝨品系

- 秘魯番茄(*S. peruvianum*)：PI126926、PI126930、PI390681、LA441
- 多毛番茄(*S. habrochaites*)：PI134417、LA1353、LA1777(高抗)、LA2329
- 潘那利番茄(*S. pennellii*)：PI246502、LA1340、LA2560、LA716

抗黃化捲葉病毒基因 (*Ty-1*)



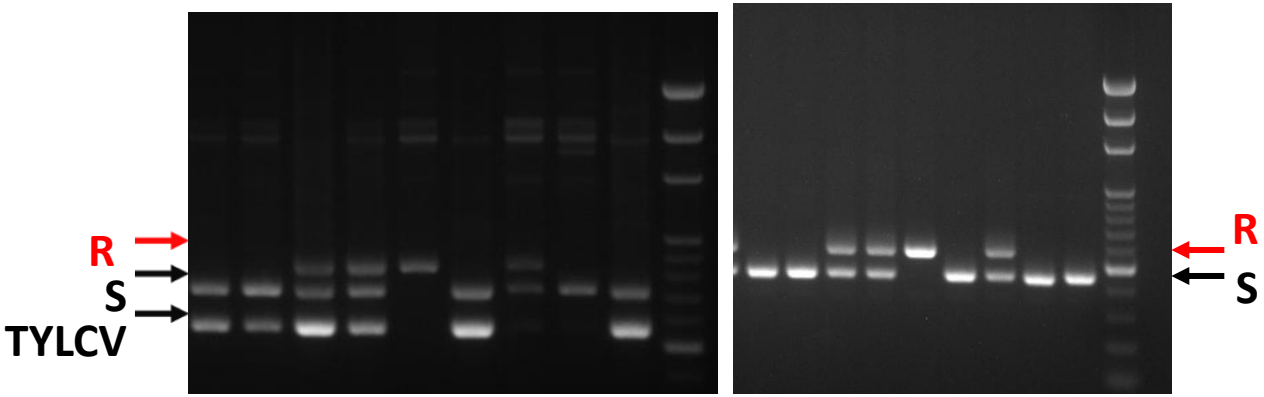


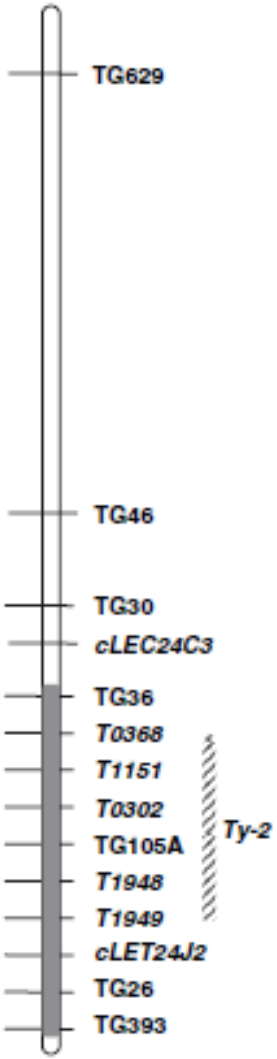
●Ty-1 標誌：國外為CAPS；本研究為SCAR(省時省成本)。

抗黃化捲葉病毒基因

Ty-2/TYLCV

種苗場(SCAR)　　國外文獻(SCAR)



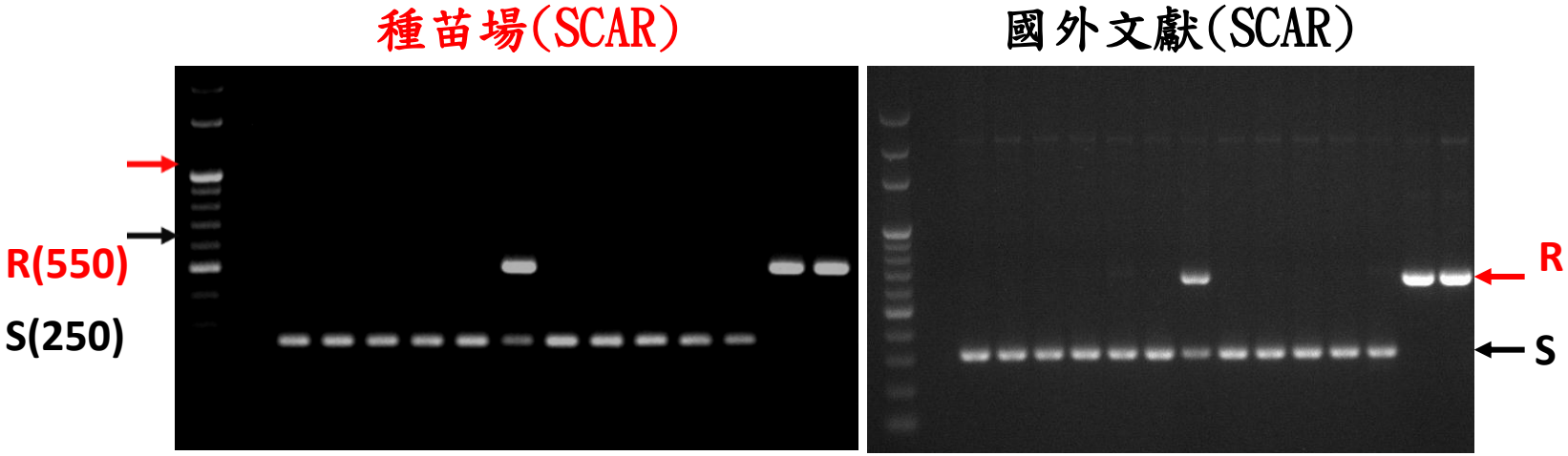


Czosnek, 2007

●Ty-2 標誌：國外為SCAR；本研究為SCAR(含TYLCV病毒)。

抗黃化捲葉病毒基因

Ty-3

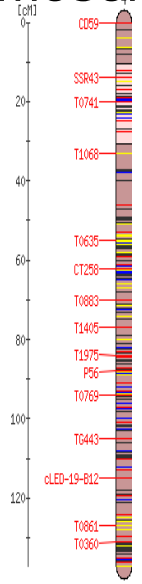


●Ty-3 標誌：國外為SCAR；本研究亦為SCAR (Ty-3a)。

抗黃化捲葉病毒基因

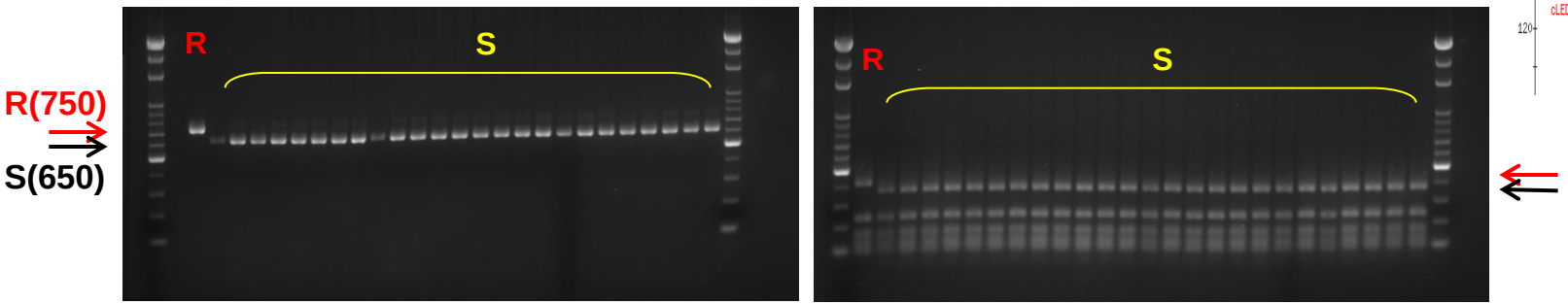
ty-5

Chromosome 4



種苗場(SCAR)

國外文獻(CAPS)



- 已建立TYLCV、*Ty-1*、*Ty-2*、*Ty-3*及*ty-5*專一性分子標誌。
- 目前台灣多高致病力泰國種病毒(TYLCTHV)，單一抗病基因抗病毒力差，多抗病基因抗病毒力佳，傳統以外表型表現方式不易篩選抗病株。

Theor Appl Genet (2009) 119:519–530
DOI 10.1007/s00122-009-1060-z

ORIGINAL PAPER

Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*

Abstract *Tomato yellow leaf curl virus* (TYLCV) is devastating to tomato (*Solanum lycopersicum*) crops and resistant cultivars are highly effective in controlling the disease. The breeding line TY172, originating from *Solanum peruvianum*, is highly resistant to TYLCV. To map quantitative trait loci (QTLs) controlling TYLCV resistance in TY172, appropriate segregating populations were analyzed using 69 polymorphic DNA markers spanning the entire tomato genome. Results show that TYLCV resistance in TY172 is controlled by a previously unknown major QTL, originating from the resistant line, and four additional minor QTLs. The major QTL, we term *Ty-5*, maps to chromosome 4 and accounts for 39.7–46.6% of the variation in symptom severity among segregating plants (LOD score 33–35). The minor QTLs, originated either from the resistant or susceptible parents, were mapped to chromosomes 1, 7, 9 and 11, and contributed 12% to the variation in symptom severity in addition to *Ty-5*.

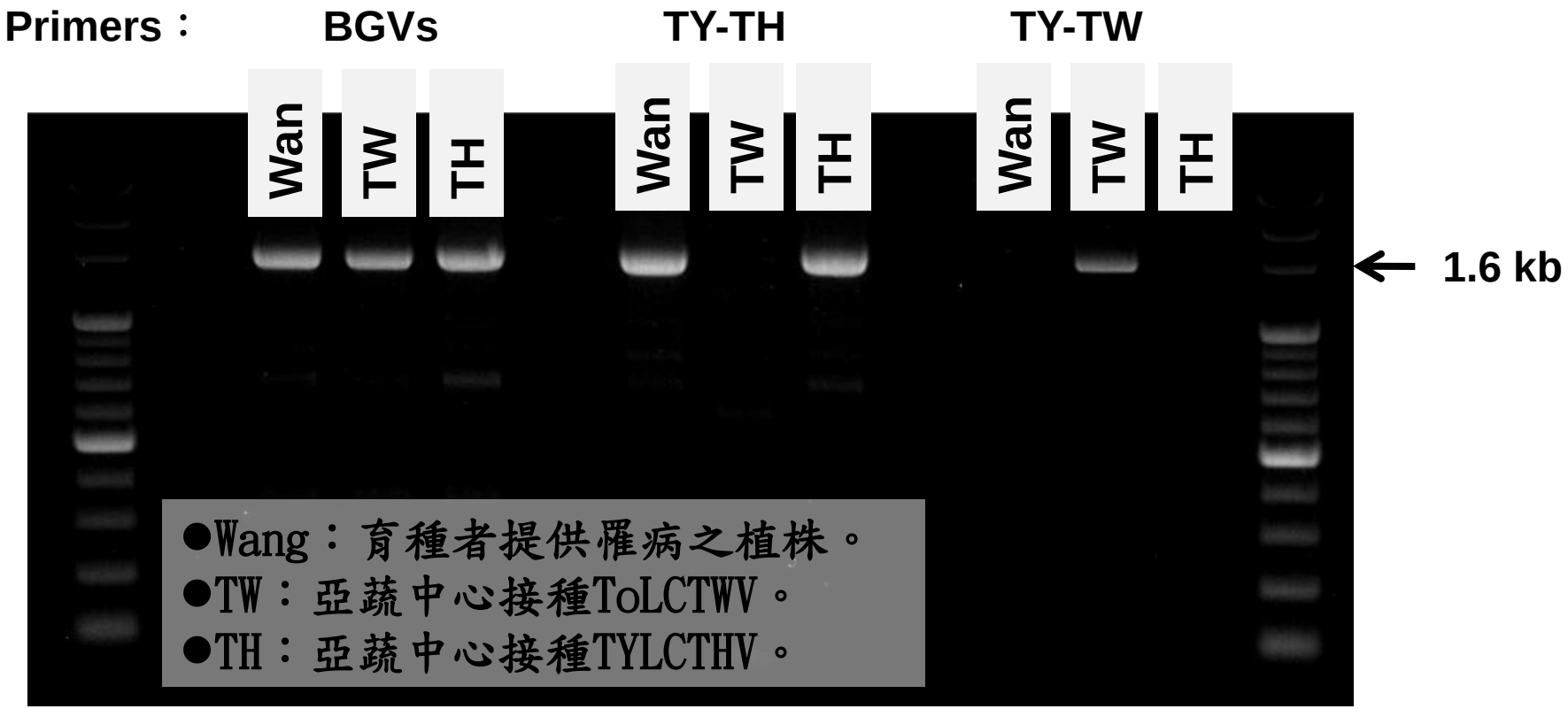
- TY172（*ty-5*）：以色列由秘魯番茄導入。
- ty-5：隱性基因?

Table 2 Average disease severity index (DSI) of parental lines and their F_1 hybrids

Line	DSI first inoculation	DSI second inoculation
LA1589	$3.3^{A} \pm 0.0$ ($n = 40$)	$3.3^{A} \pm 0.1$ ($n = 40$)
TY172 × LA1589	$2.1^{B} \pm 0.1$ ($n = 40$)	$2.3^{B} \pm 0.1$ ($n = 40$)
TY172	$0.1^{C} \pm 0.0$ ($n = 40$)	$0.1^{C} \pm 0.0$ ($n = 40$)
M-82	$3.3^{A} \pm 0.0$ ($n = 28$)	
TY172 × M-82	$1.7^{B} \pm 0.1$ ($n = 31$)	
TY172	$0.0^{C} \pm 0.0$ ($n = 19$)	

Results are displayed as mean ± SE; *n* number of plants in each accession; *DSI* disease severity index as described in the "Materials and methods" and previously in Friedmann et al. (1998) and in Lapidot and Friedmann (2002); different superscript letters represent statistically significant mean values ($P < 0.05$) based on Tukey–Kramer HSD test (Kramer 1956)

病毒(Begomovirus、ToLCTWV-臺灣種、TYLCTHV-泰國種)分子標誌



- 已建立TYLCV、*Ty-1*、*Ty-2*、*Ty-3*及*ty-5*及TYLCV專一性分子標誌。
- 番茄品種需具備2個以上抗病基因，方有機會呈現較明顯抗性。

吉林农业科学 2004,29(2)：45-48 Journal of Jilin Agricultural Sciences

文章编号：1003-8701(2004)02-0045-04

番茄远缘杂交不亲和性的研究

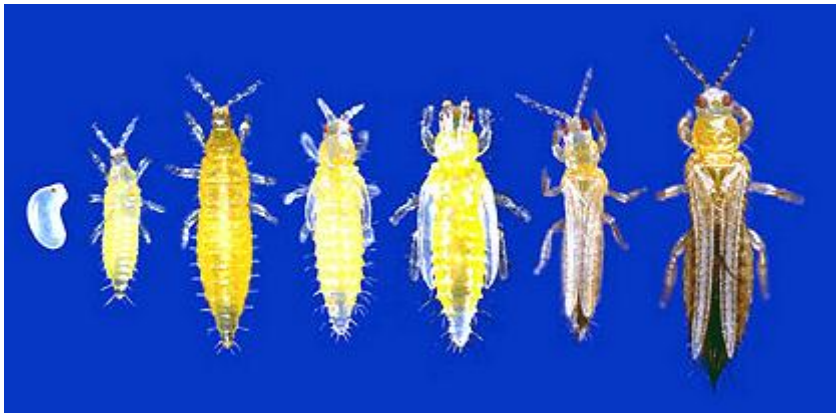
马光恕，廉 华，景艳丽

(黑龙江八一农垦大学植物科技学院园艺系，黑龙江 大庆 163000)

摘 要：目前番茄种质资源日趋匮乏，种质资源的研究和创新对番茄育种来说具有重大意义。与之相反，野生种番茄及番茄近缘野生种(SL)却拥有丰富的遗传资源，它们对番茄遗传资源改良具有不可估量的价值，远缘杂交无疑是改良番茄遗传资源的最佳途径，但远缘杂交不亲和性是影响其成功与否的关键因素。因此，主要论述了番茄远缘杂交不亲和性的研究情况。

抗斑點萎凋病毒(*Tomato spotted wilt virus*；TSWV)

- 斑點、斑駁、矮化、萎凋、壞疽等，番茄由薊馬傳播最嚴重的世界性病毒病害，番茄三大病毒之一。
- *Sw-1*、*sw-2*、*sw-3*、*sw-4*：存在栽培種番茄(*S. lycopersicum*)，對TSWV抗性較差。
- *Sw-5*：來自於祕魯種番茄(*S. peruvianum*)，抗性最強，另對*Groundnut ringspot virus* (GRSV)、*Tomato chlorotic spot virus* (TCSV)、及*Chrysanthemum stem necrosis virus* (CSNV)亦有明顯抗性。
- *Sw-6*：抗性低於*Sw-5*(Rosello *et al*., 1998)。
- *Sw-7*：抗性情形尚不清楚(Dianese *et al*., 2011) 。



http://www.apsnet.org/edcenter/intropp/lessons/viruses/Pages/TomatoSpottedWilt.aspx

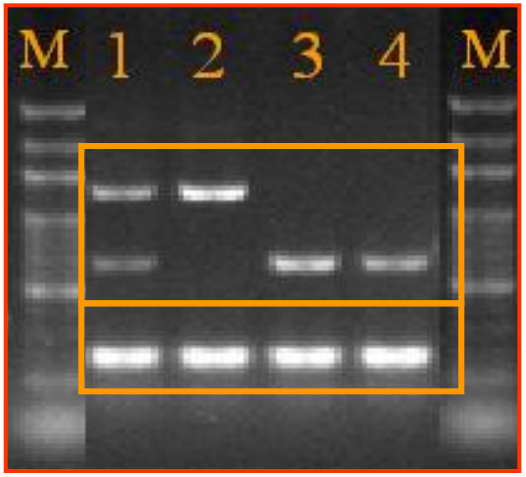


陳迪偉博士提供

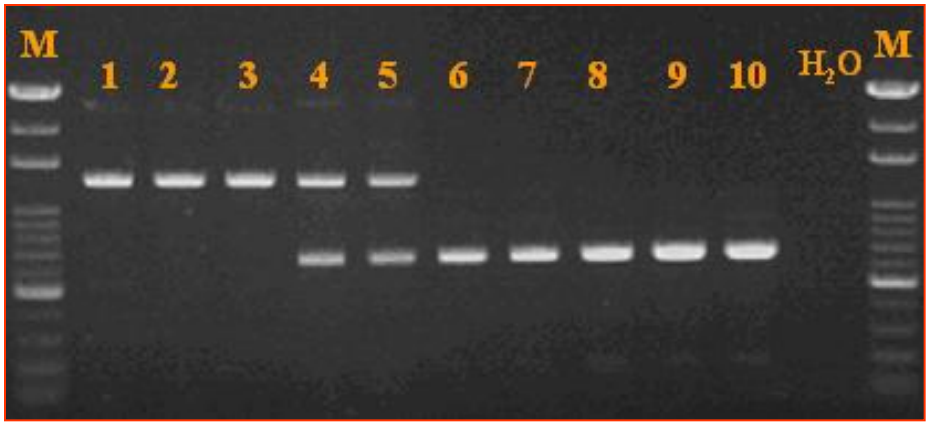
抗斑點萎凋病毒

Diseases	Gene	Marker	Varties	Chr.	Reference
TSWV	Sw-5	Sw5-2, CT71, G5, K16, S12, CT220, ZUP641	L00893, PI128654, PI128660, LA3667	9	Dianese et al., 2010; Langella et al., 2004; Garland et al., 2005

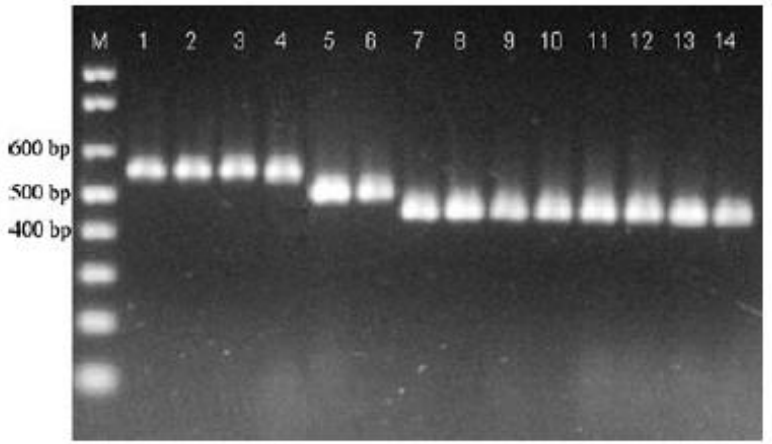
抗斑點萎凋病毒分子標誌



種苗場(SCAR)



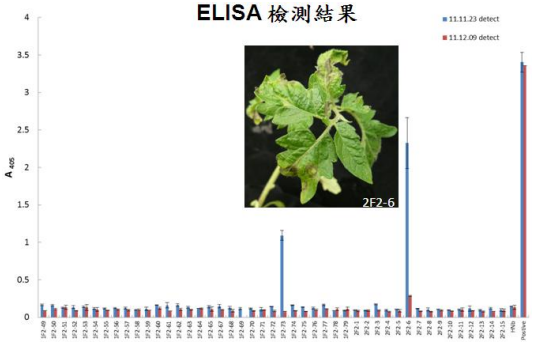
國外文獻(SCAR)

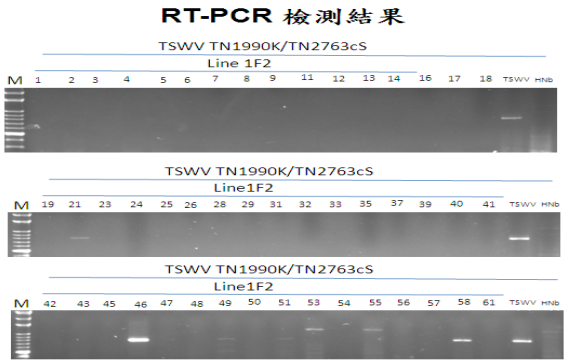


- 已建立抗病基因(Sw-5)及TSWV病毒專一性分子標誌。

抗感病能力檢測：F1 (Sw5/ sw5)
F2(79單株)

	F2分離比: ~1 : 2 : 1		
基因型	*Sw5/ Sw5*	*Sw5/ sw5*	*sw5/ sw5*
F2族群	0/13株(0%) 0/20株(0%)	1/46株(0%) 0/33株(0%)	5/20株(25%) 11/20株(55%)
病癥	•接種葉檢出TSWV，接種葉黃化後枯萎掉落。後續新葉無檢出TSWV及無病癥出現。 •唯1F2-21檢出TSWV但無出現病癥。		•接種葉檢出TSWV，後續新葉亦檢出TSWV並出現黃化、黃斑及壞疽病癥或死亡。







抗番茄嵌紋病毒
(*Tomato mosaicvirus*, ToMV)

◆在全世界可感染超過150種植物，機械傷口傳播，為番茄三大病毒之一。主要病毒株有3種，ToMV-0、ToMV-1、ToMV-2等。

◆目前已知番茄存在3種抗嵌紋病毒基因，*Tm-1* (strains 0, 2)、*Tm-2* (strains 0, 1)、*Tm-2*^{2} (strains 0, 1, 2)。

Susceptible：ToMV-1



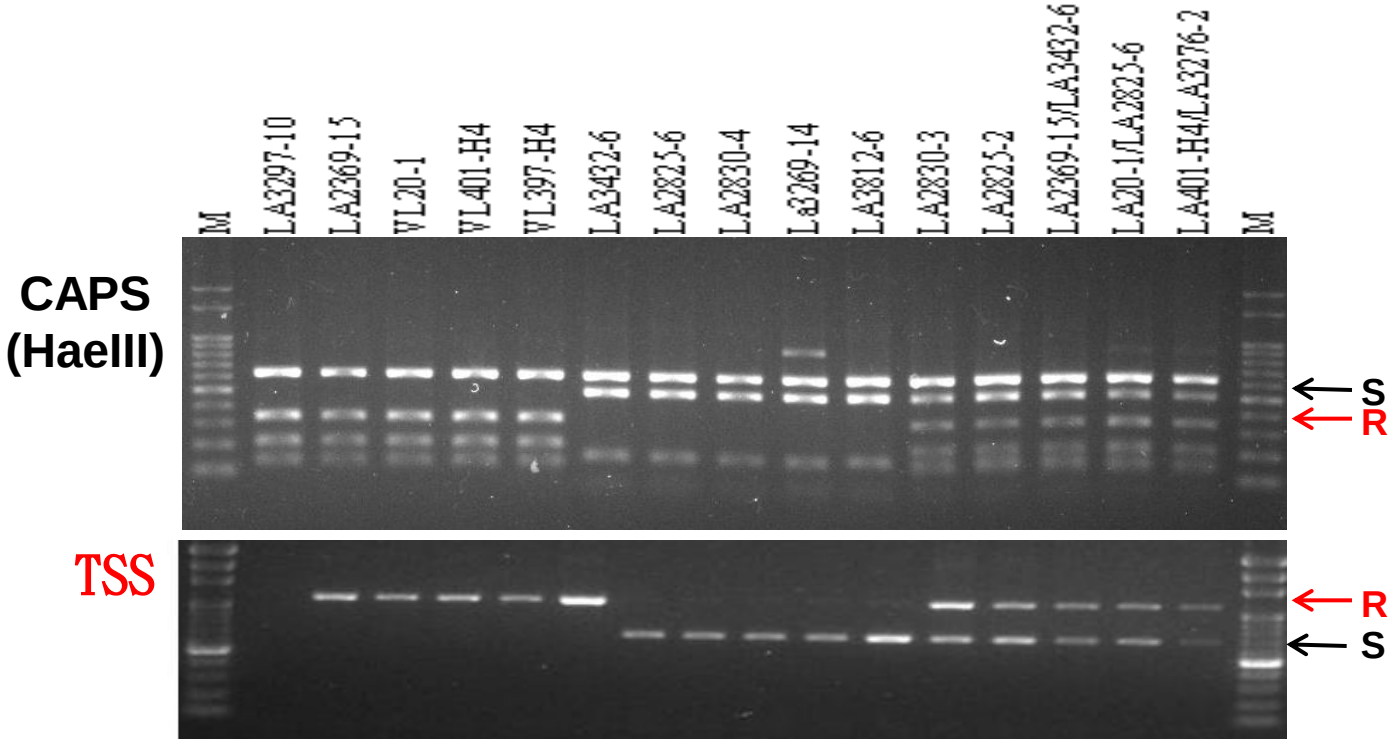
Resistant



抗嵌紋病毒

Diseases	Gene	Marker	Varties	Chr.	Reference
TMV	Tm-1	PK12, SCN20, TG101	GCR237, LA2369, LA2825, LA2826, LA2830, LA3269, LA3271, LA3276, LA3297, LA3432, LA3812	2	Arens et al., 2010
	Tm-2	OPK6, OPA-12, SCB12, SCE16, SCG09, SCI18, SCN13, TG79, TG291, TG207, SNP901, TM2-748, SNP2494, TMV-2262	PI128650, LA3432, LA1024, LA1221, LA1791, LA2088, LA2399, LA2531A, LA2531C, LA2827, LA2828, LA2829, LA2830, LA2912, LA2939, LA2968, LA3027, LA3028, LA3029, LA326, LA3273, LA3274, LA3275, LA3292, LA3297, LA3309, LA3310, LA3348, LA3349, LA3432, LA3433, LA3471, LA3549, LA3769, LA3812, LA4285, LA4286	9	Sobir et al., 2000
	$Tm\text{-}2^2$	ToMV	LA2830, LA3769, LA3471, LA3349, LA3348, LA3310	9	Dax et al., 1998

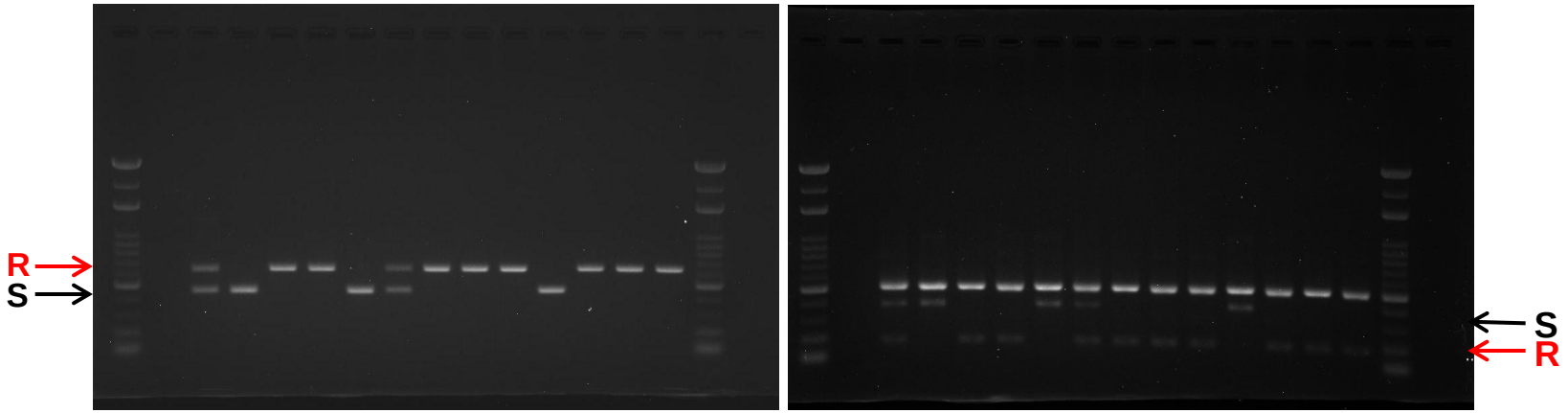
抗番茄嵌紋病毒基因
Tm-1



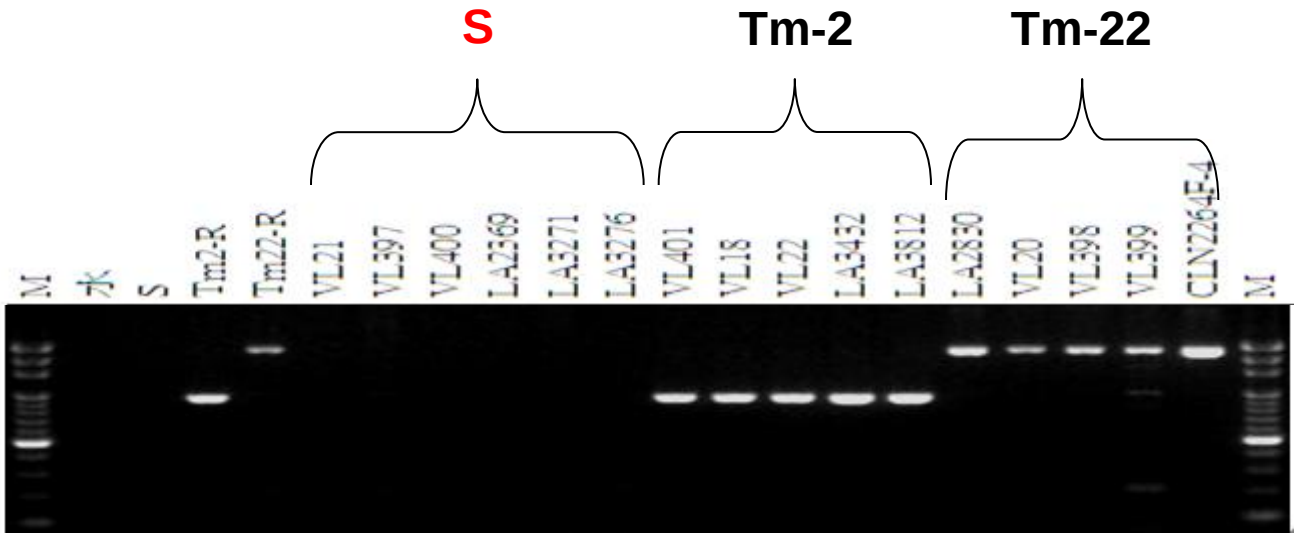
抗番茄嵌紋病毒基因
Tm-2/Tm-22

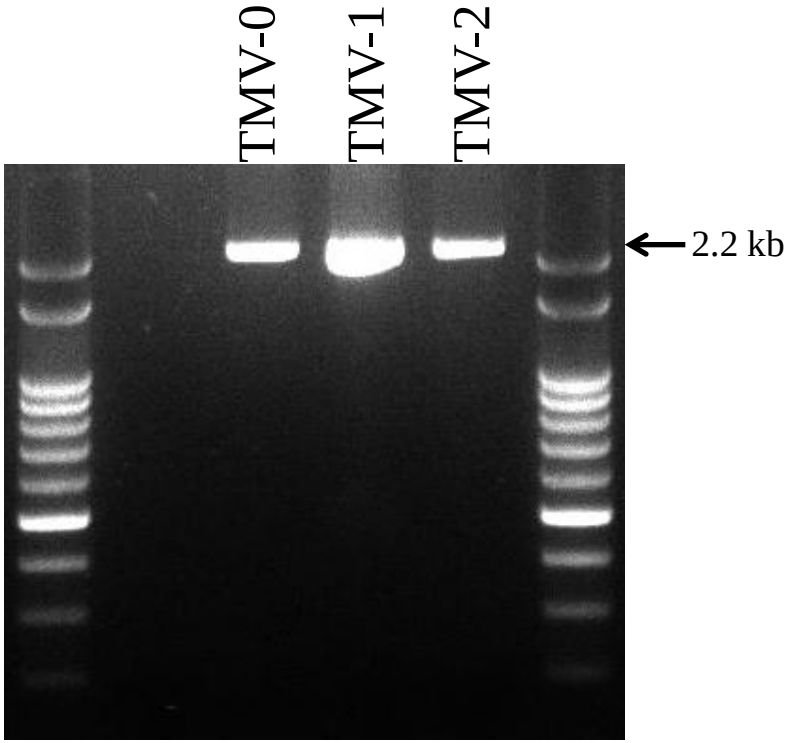
TSS（SCAR）

國外文獻(SCAR)



抗番茄嵌紋病毒基因
Tm-2、Tm-22





- 已建立Tm-1、Tm-2（Tm2/Tm22、S）、Tm-22（Tm2、Tm22）、ToMV專一性分子標誌。

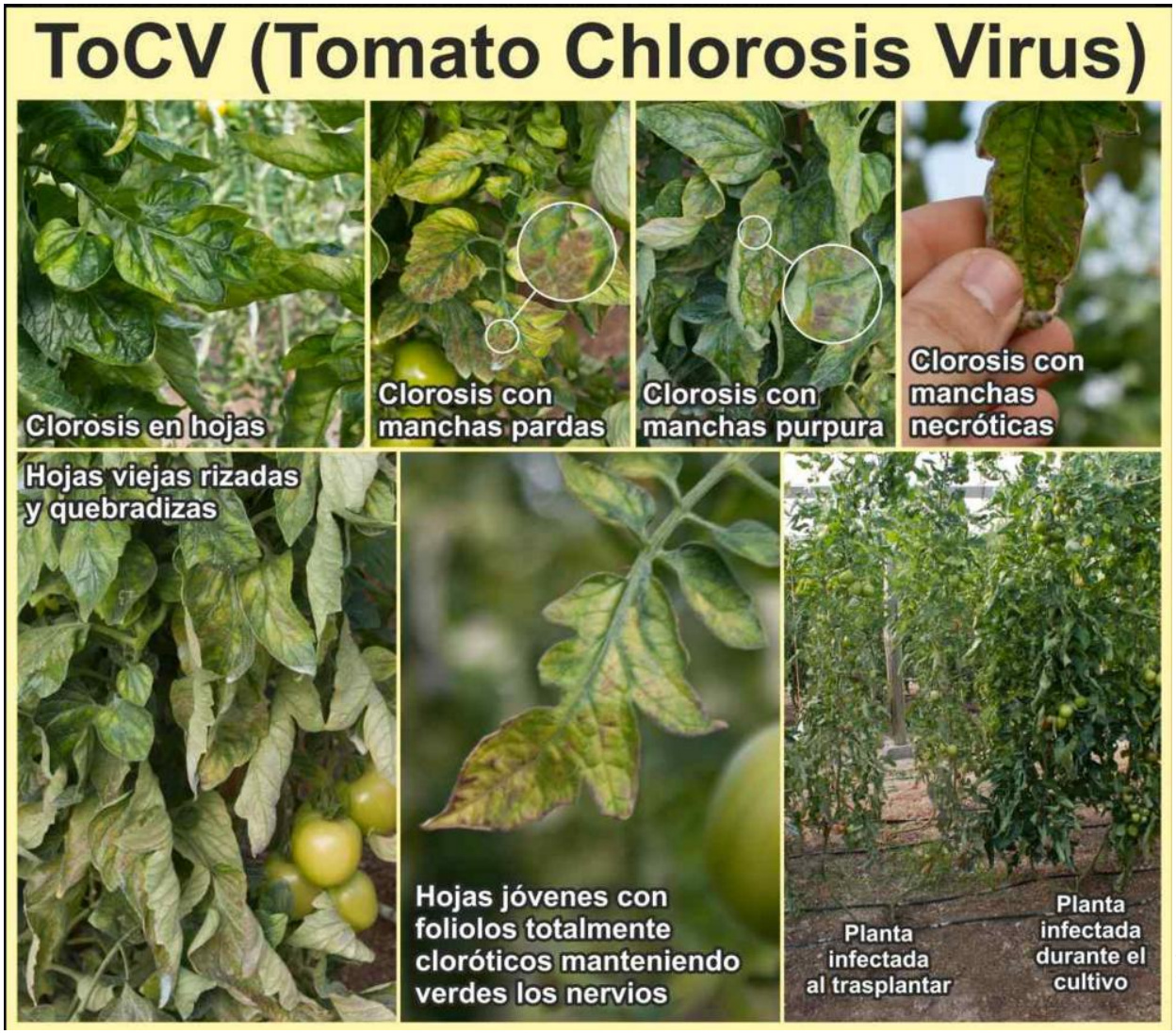
39



抗退綠病毒 (Tomato chlorosis virus；ToCV)

- 1998 年在美國佛羅里達州首次報導發現，是唯一能通過不同屬的粉蝨(B, Q, A型煙粉蝨、溫室白粉蝨、銀葉粉蝨和紋翅粉蝨)傳播的病毒。
- 可感染茄科（番茄、甜椒和煙草等）、番杏科、莧科、夾竹桃科、藜科、菊科和藍雪科等植物，ToCV不能通過摩擦接種傳播。
- 主要抗病品種LA0444 (Solanum peruvianum)、LA1028 (S. chmielewskii)；目前絕大多數栽培品種均不具抗性。
- 未來中國大陸可能大爆發(已請亞蔬中心、蔡文錫老師開發病毒接種與檢測技術)。

退綠病毒



http://elhocino-adra.blogspot.tw/2012/02/tomato-chlorosis-virus.html



抗晚疫病(Late blight)
(Phytophthora infestans)

- 1840年代發生馬鈴薯晚疫病，造成愛爾蘭大饑荒後，學者開始重視馬鈴薯與番茄晚疫病研究。
- 冬季低溫高濕最嚴重真菌性病害，常導致100%植株死亡。
- 主要菌株為T-1。
- 一般番茄抗晚疫病性狀由2類基因控制：質基因(Ph-1、Ph-2、Ph-3)；數量基因。
- 植株同時具備Ph-2與Ph-3基因，抗性極強。

抗晚疫病



穴盤苗接種後封於塑膠袋內



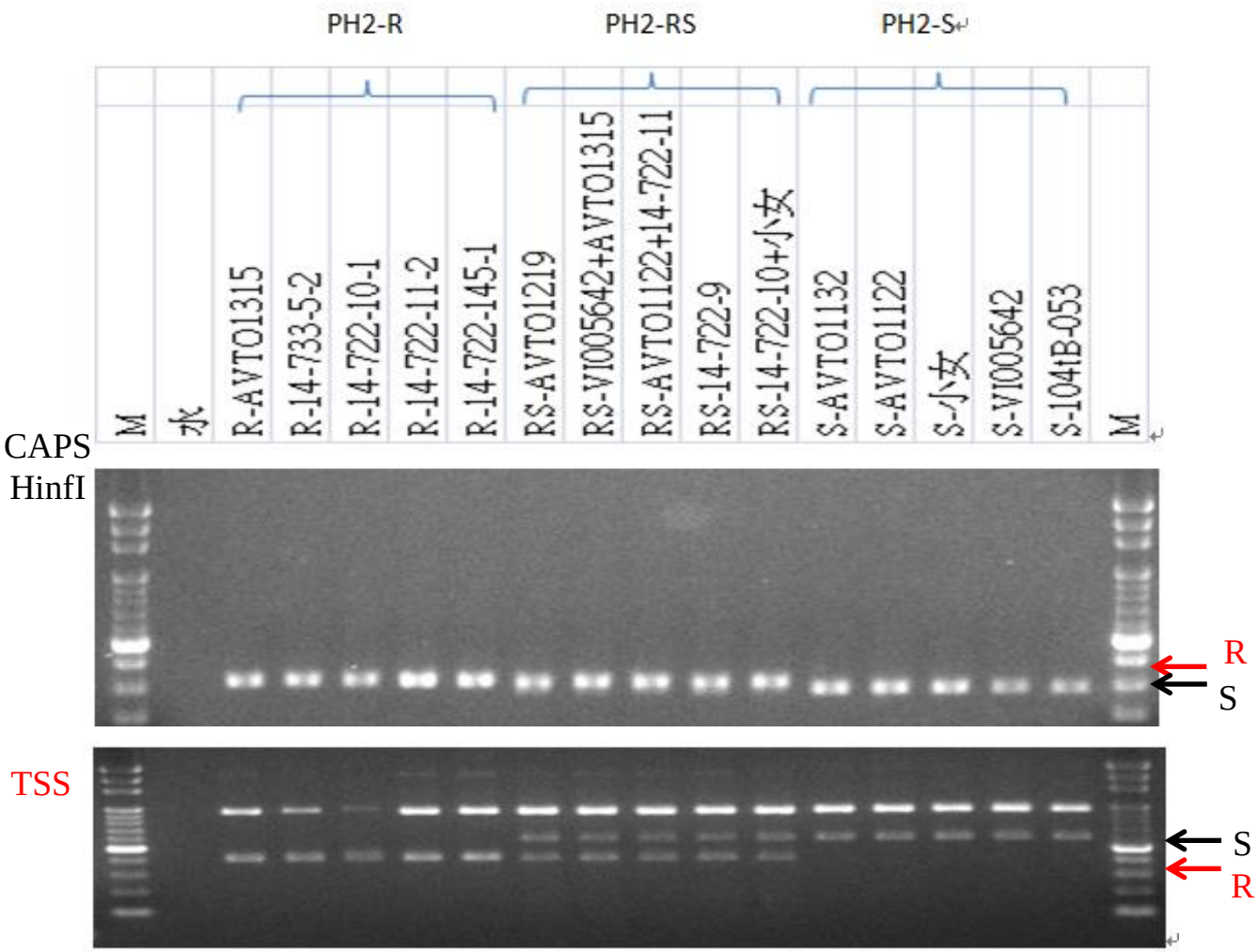
穴盤苗接種 1 周後植株外觀

- 接種晚疫病菌1周，感病種(VI005642)全死；抗病種(AVTO1219 、AVTO1311、AVTO1315；Ph-2/Ph-3)全活且無任何病徵。

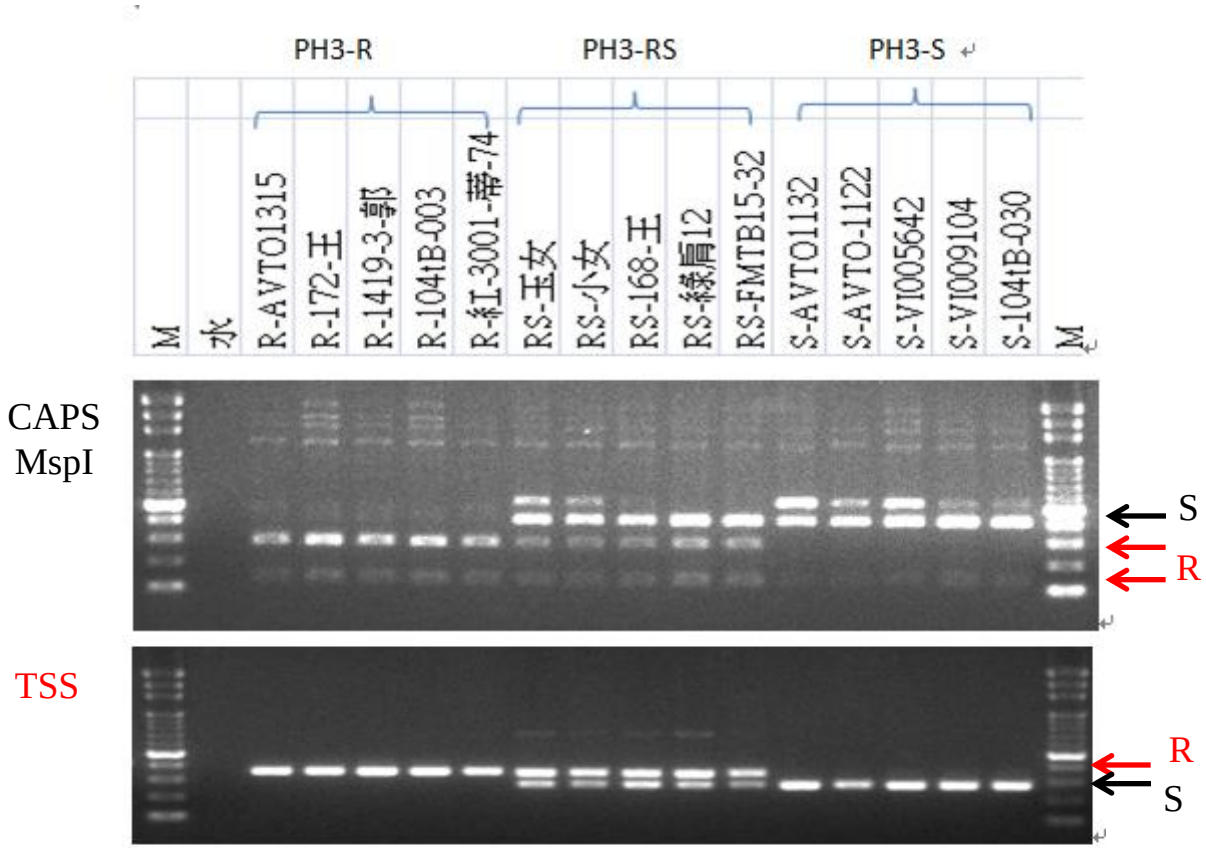
抗晚疫病

Diseases	Gene	Marker	Varties	Chr.	Reference
Late blight (*Phytophthora infestans*)	Ph-2	dTG63, dTG422, TG233, TG403	L3708, LA3151, LA3152, AVTO1219, AVTO1311, AVTO1315	10	ICAR Annual Report 2014-15
	Ph-3	TG591, TG328	L3708, AVTO1219, AVTO1311, AVTO1315	9	Robbins et al., 2010

抗晚疫病(Ph-2)


PH2-R
PH2-RS
PH2-S
M
水
R-AVT01315
R-14-733-5-2
R-14-722-10-1
R-14-722-11-2
R-14-722-145-1
RS-AVT01219
RS-VI005642+AVT01315
RS-AVT01122+14-722-11
RS-14-722-9
RS-14-722-10+小女
S-AVT01132
S-AVT01122
S-小女
S-VI005642
S-1041B-053
M
CAPS
HinfI
R
S
TSS
S
R

抗晚疫病(Ph-3)


PH3-R
PH3-RS
PH3-S
M
水
R-AVTO1315
R-172-王
R-1419-3-郭
R-104tB-003
R-紅-3001-蒂-74
RS-玉女
RS-小女
RS-168-王
RS-綠肩12
RS-FMTB15-32
S-AVTO1132
S-AVTO-1122
S-VI005642
S-VI009104
S-104tB-030
M
CAPS
MspI
S
R
TSS
R
S

抗晚疫病

抗病種(Ph2、Ph3)

感病種

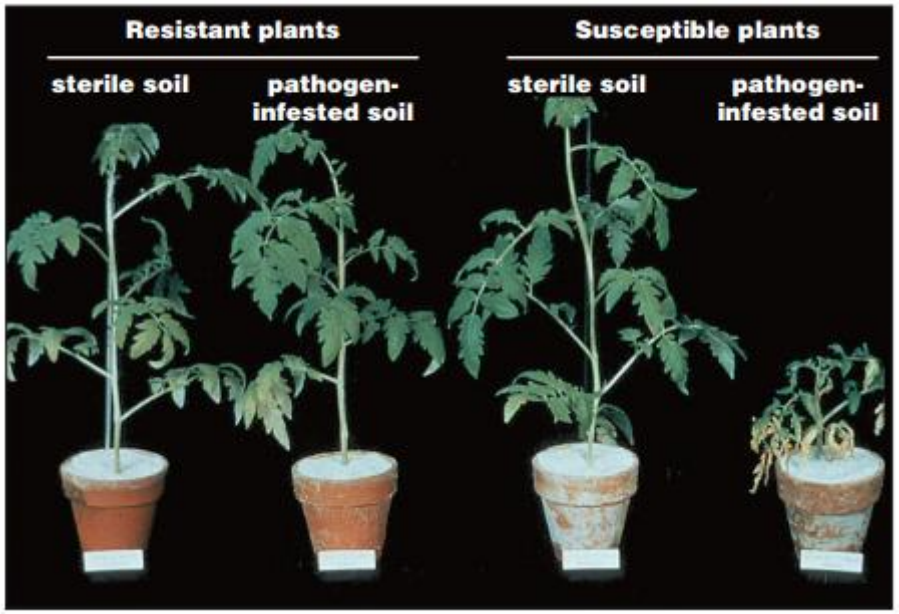


王百祿先生提供

抗萎凋病(Fusarium wilt)
(*Fusarium oxysporum* f. sp. Lycopersici)

◆為土壤及種傳非常嚴重真菌性病害，植株莖部縱切面，維管束呈現褐變；置於清水中，不會有白色煙霧狀菌液滲出。生理小種有race1、race2 (臺灣主要)、race3 (國外主要)。

◆主要抗病基因I-1 (抗race1)、I-2 (抗race2)、I-3 (抗race3)，分別來自野生番茄*S. pimpinellifolium*及*S. pennellii* 。

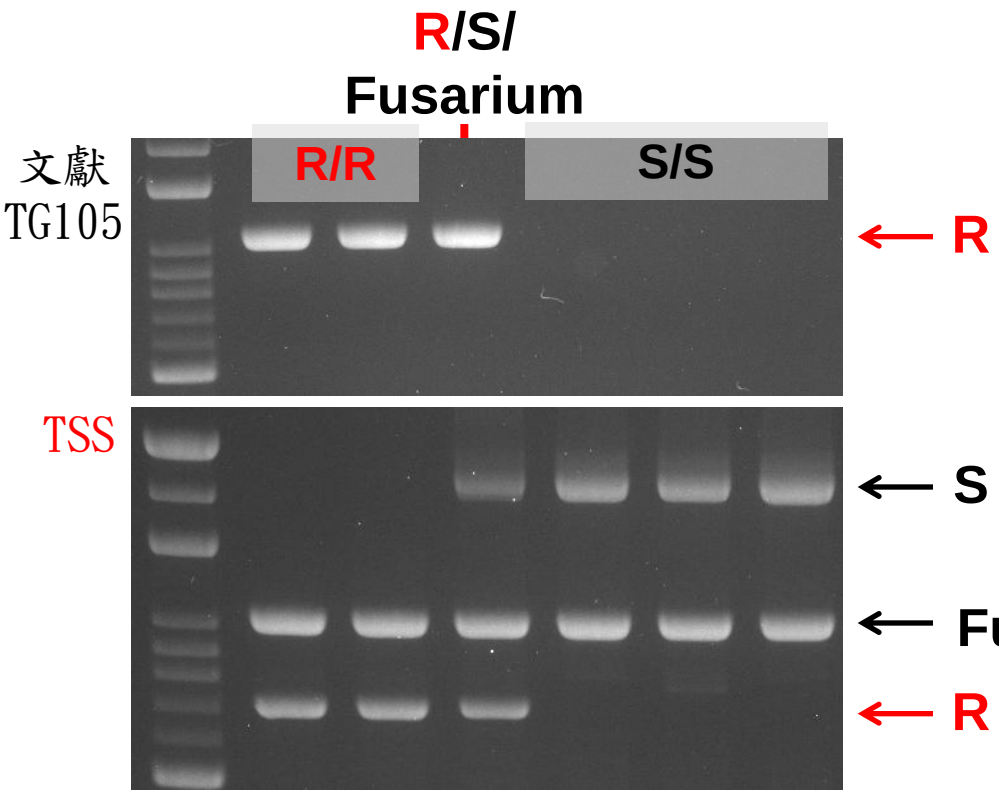


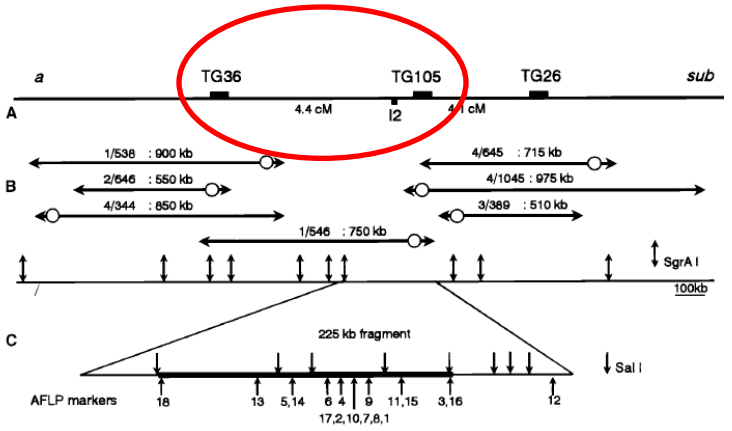
(http://learningstore.uwex.edu/assets/pdfs/A2617.pdf)

抗萎凋病

Diseases	Gene	Marker	Varties	Chr.	Reference
Fusarium wild (*Fusarium oxysporum* f.sp. lycopersici)	I-1	At2, TG523	PI 370093, PI126915, LA0276, LA0490, LA0743, LA0744, LA1022, LA1097, LA1221, LA2088, LA2399, LA2414, LA2821, LA2822, LA2823, LA2912, LA2937, LA2938, LA2939, LA2940, LA2941, LA3025, LA3026, LA3029, LA3038, LA3040, LA3041, LA3042, LA3130, LA3254, LA3428, LA3465, LA3471, LA3472, LA3475, LA3527, LA3528, LA3540, LA3847, LA3911, LA4025, LA4026, LA4285, LA4286, LA4345, LA4354, LA4504	7	Arens et al., 2010
	I-2	TFus, TG36, TG105, Z1063	PI 126915, LA2821, LA2823, LA3130, LA3465, LA3471, LA3527, LA3528, LA3847, LA4025, LA4026, LA4285, LA4286, LA4354, LA4504	11	El Mohtar et al., 2007
	I-3	P7-43B, P7-43DF, PTG-190, RGA332, TG20, TG128, Got-2	PI414773, LA0716, LA4025, LA4026, LA4065, LA4066, LA4067	7	Barillas et al., 2008

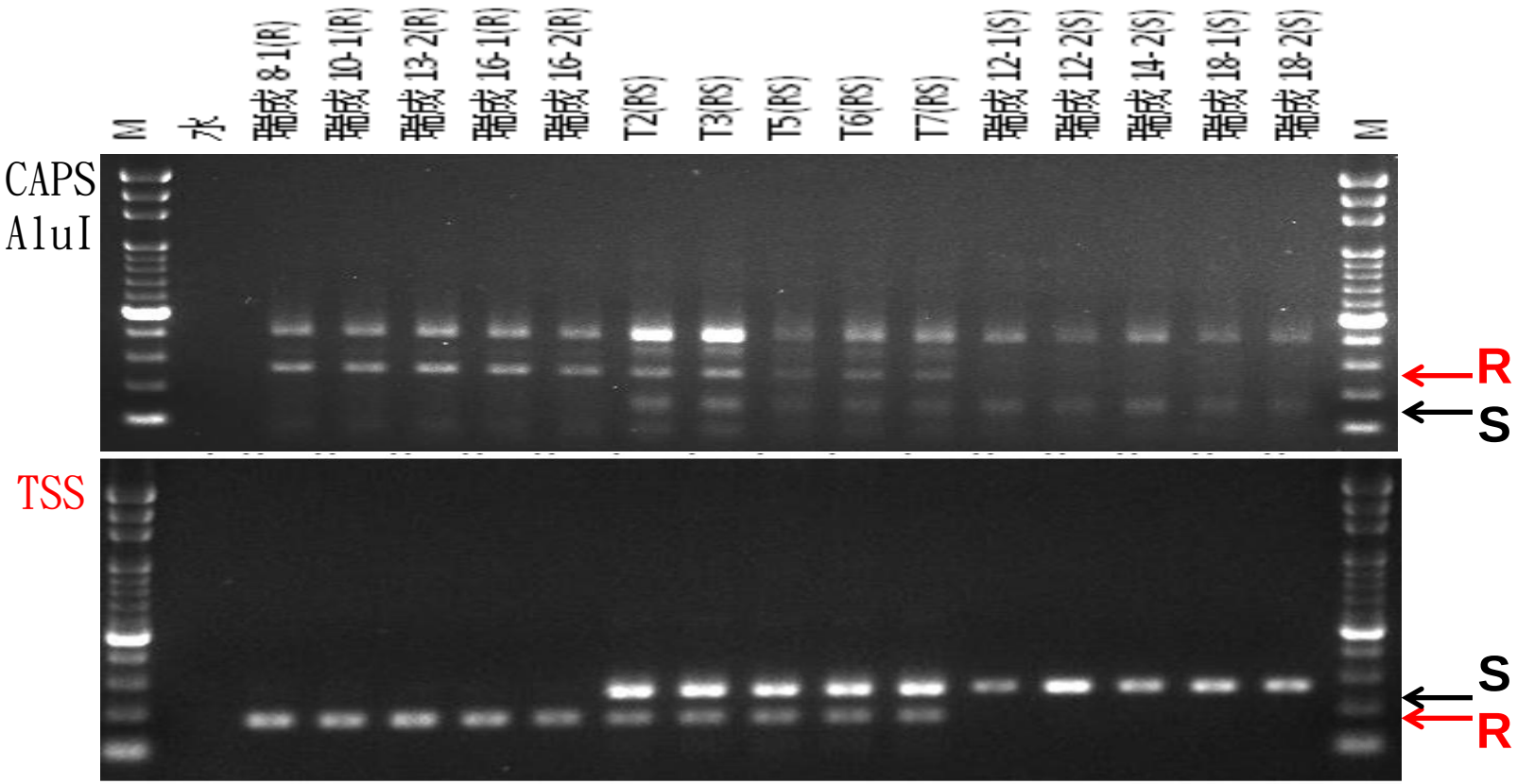
I-2/Fusarium spp.





- 目前已初步開發可同時檢測番茄抗萎凋病基因(I-2)及病源菌 Fusarium spp.之專一性分子標誌。

I-3


M
水
稲成 8-1(R)
稲成 10-1(R)
稲成 13-2(R)
稲成 16-1(R)
稲成 16-2(R)
T2(RS)
T3(RS)
T5(RS)
T6(RS)
T7(RS)
稲成 12-1(S)
稲成 12-2(S)
稲成 14-2(S)
稲成 18-1(S)
稲成 18-2(S)
M
CAPS
AluI
R
S
TSS
S
R



抗根腐病(Fusarium crown and root rot)
(*Fusarium oxysporium* f. sp. *radicis-lycopersici*)

- 好發於冷涼地區番茄幼苗莖基部，初期成水浸狀，之後呈褐色至腐爛，為嚴重土傳病害之一。
- 雖由鐮孢菌引起病害，但與萎凋病菌株不同，抗病基因(Frl)亦完全不同。
- 中國大陸已嚴重。

根腐病





http://www.semena.org/agro/diseases/fusarium-crown-root-rot-e.htm

抗根腐病

Diseases	Gene	Marker	Varties	Chr.	Reference
Fusarium crown and root rot (*Fusarium oxysporum* f.sp. radicis-lycopersici)	Frl	UBC116, UBC194, UBC655	LA1791, LA2827, LA2828, LA2829, LA2830, LA3273, LA3292, LA3471	9	Fazio et al., 1999

抗黃萎病(Verticillium wilt)
(*Verticillium dahliae* Kieb.或*V. albo-atrum*)

- 可感染600餘種植物，為番茄重要土壤傳播維管束病害，病徵類似萎凋病，於中國大陸發生情形嚴重。
- 生理小種有race1、race2。
- 主要抗病基因Ve-1與Ve-2(抗race1)緊密連鎖；極少數品種對race2具部分抗性。



(http://vegetablemdonline.ppath.cornell.edu/factsheets/...)

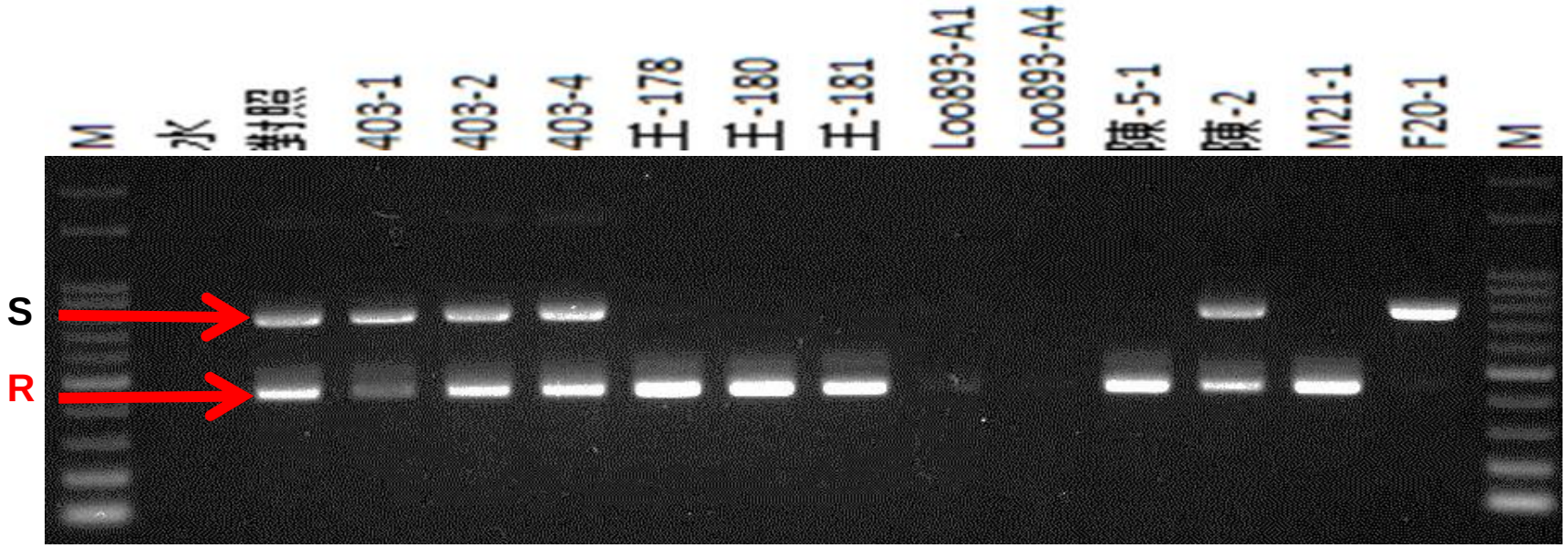


(Diwan *et al*., 1998)

抗黃萎病

Diseases	Gene	Marker	Varties	Chr.	Reference
Verticillium dahlia, V. albo-atrum	Ve-1	V1LeO, Ve1_2072, GP39	VFN8(LA1022), LA2823, LA3428	9	Acciarri et al., 2007
	Ve-2	V2LeO, Ve2_2720, Ve2_SNP	VFN8(LA1022), LA2823, LA3428	9	Arens et al., 2010

抗黃萎病基因
Ve-1



- 已開發可檢測番茄黃萎病抗性與感病基因(Ve-1)之共顯性專一性分子標誌。



抗葉霉病(Leaf mould)
(*Fulvia fulva* Cooke Cuferri (新菌名稱), *Cladosporium fulvum* Cooke (舊菌名稱))

- 好發於低溫高濕的設施栽培內，分生孢子隨氣流或雨水接觸或種子傳播。
- 抗病基因來自野生多毛番茄(*S. habrochaites*)及醋栗番茄(*S. pimpinellifolium*)，於1986年雜交導入栽培種番茄內。
- 已確認Cf-1～Cf-24共24個抗病基因，Cf-4與Cf-9基因位於相同基因座緊密連鎖；Cf-2與Cf-5基因位於相同基因座緊密連鎖，此2群基因極難區分，亦為目前研究重點。

葉霉病



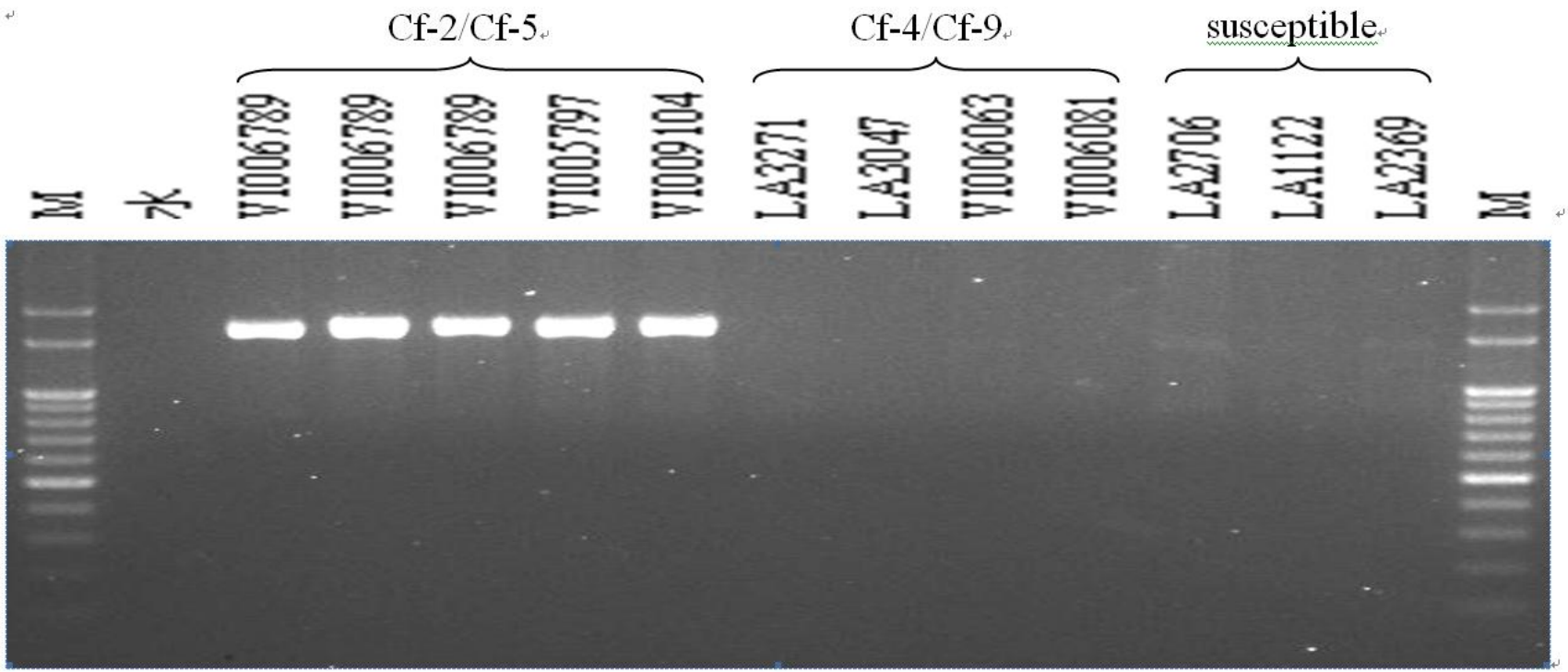


https://www.ppdl.purdue.edu/PPDL/weeklypics/4-2-12.html

抗葉霉病

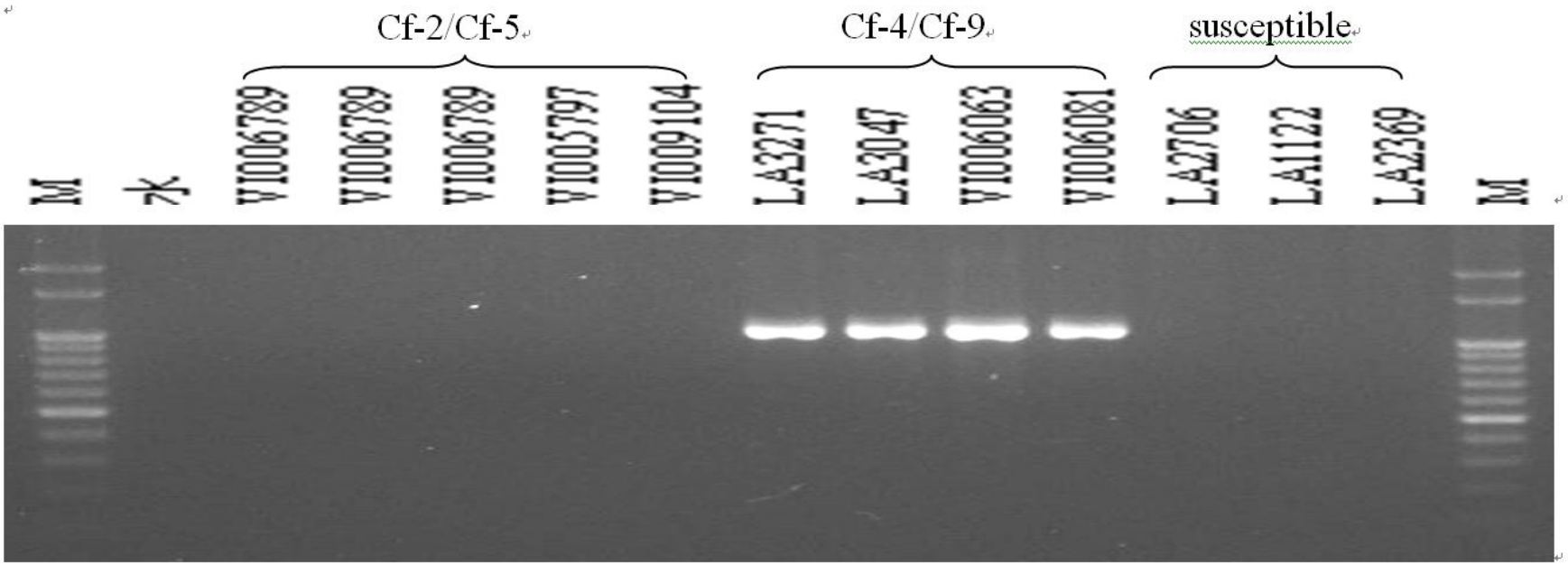
Diseases	Gene	Marker	Varties	Chr.	Reference
Leaf mould (*Cladosporium fulvum*)	Cf-2, Cf-5	Cf5, 2-5C, TG232	PI 187002, LA0917, LA1190, LA3043, LA2444	6	Grushets-kaya et al., 2007
	Cf-4, Cf-9	CF8/C10R, TG236	PI 126915, PI 126933, PI 370085, LA1186, LA2446, LA3045, LA3047, LA3051, LA3267	1	Truong et al., 2011

抗葉霉病



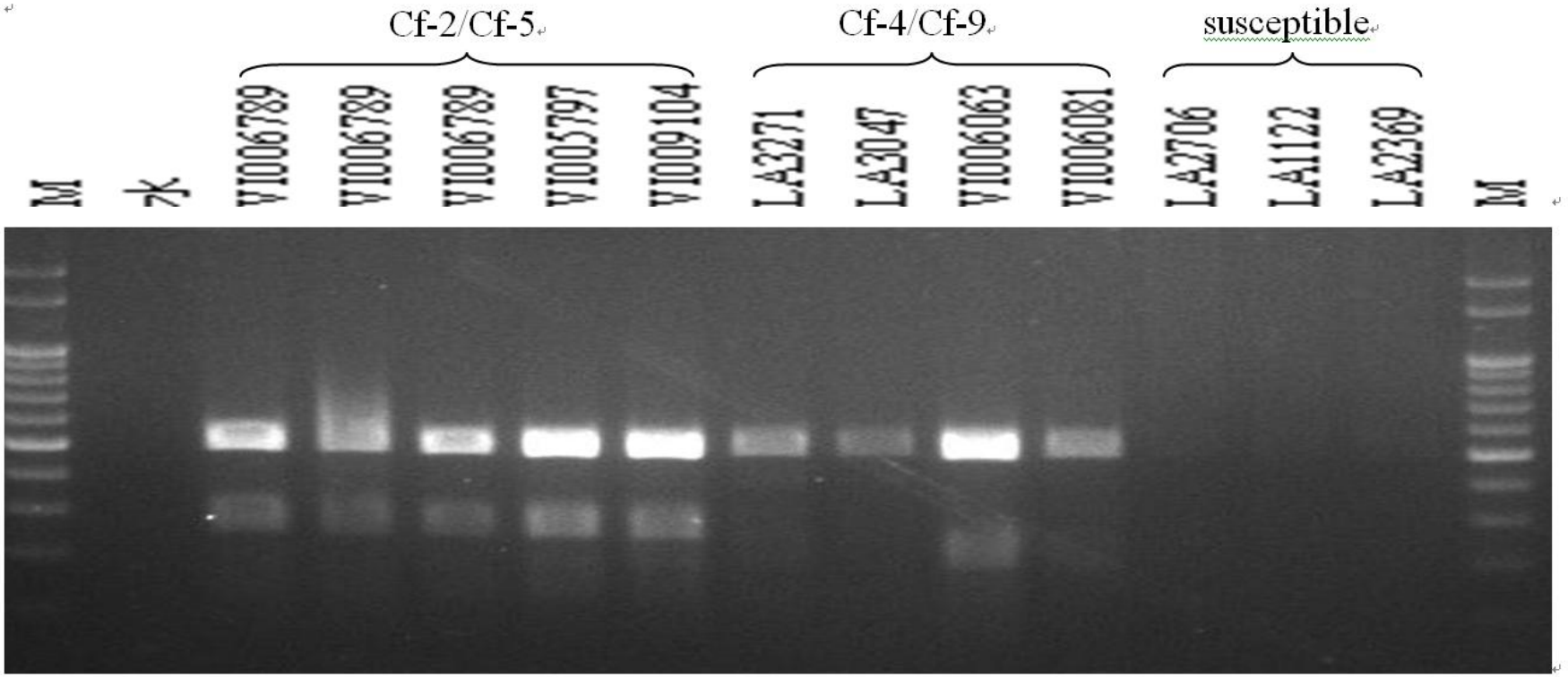
利用分子標誌Cf2/5-1.5k檢測番茄抗感病(*Cf-2*、*Cf-4*、*Cf-5*、*Cf-9*、susceptible)品種之PCR結果。

抗葉霉病



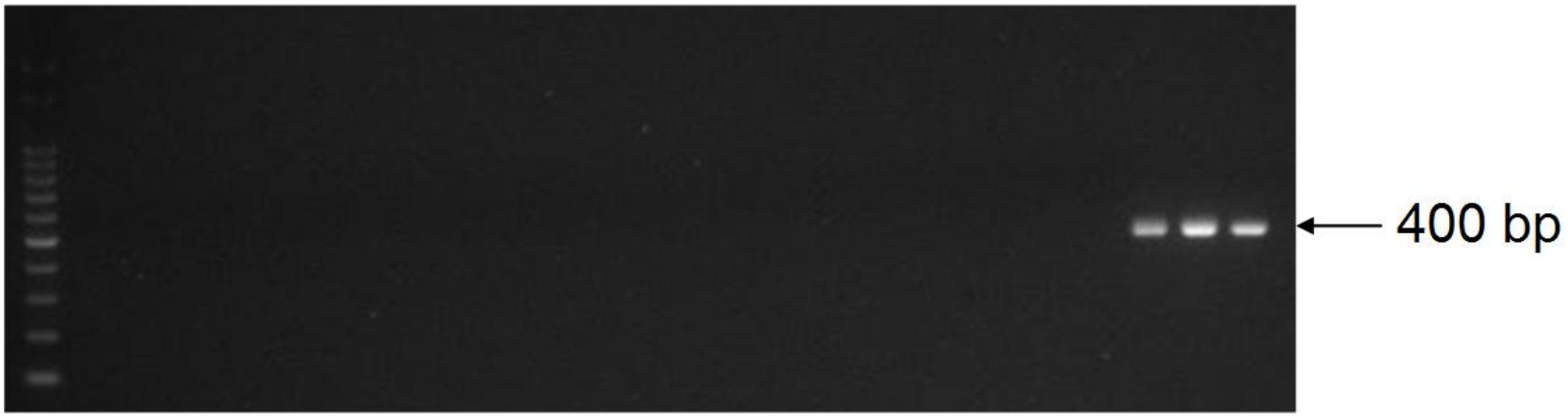
利用分子標誌Cf4/9-1k檢測番茄抗感病(*Cf-2*、*Cf-4*、*Cf-5*、*Cf-9*、susceptible)品種之PCR結果。

抗葉霉病



利用分子標誌Cf5/9-CAPS檢測番茄抗感病(*Cf-2*、*Cf-4*、*Cf-5*、*Cf-9*、susceptible)品種之PCR結果。

抗葉霉病



番茄葉霉病AVR2-KC132845分子標誌之PCR檢測結果。



抗白粉病(Powdery mildew)
(*Leveillula taurina, Oidium lycopersici, O. neolycopersici*)

- 好發於溫室內，偶爾亦會出現於大田。
- 抗病基因有質與數量性狀二類共同作用。
- *Lv*基因野生智利番茄對*L. taurina*病原菌為單一顯性抗病基因。
- 有3個基因*Ol-1*、*ol-2*及*Ol-3*對病原菌*O. neolycopersici*呈現抗性。

白粉病



http://www.grownorthwest.com/wp-content/uploads/2013/08/powdery-mildew-web.jpg

抗白粉病

Tomato

Diseases	Gene	Marker	Varties	Chr.	Reference
Powdery mildew (*Oidium lycopersicum*)	Ol-1	SCAE16, SCAG11, SCAK16	G1.1560	6	Huang et al., 2000
powdery mildew (*Leveuillula taurica*)	Lv	CT211, CT219	LA0458, LA3118, LA3119	12	Chunwong-se et al., 1994



抗細菌性斑點病(Bacterial spot)
(*Xanthomonas* spp.)

- 含*X. euvesicatoria* (race T1)、*X. vesicatoria* (race T2)、*X. perforans* (races T3, T4, T5)及*X. gardneri* (race T2)，其中*X. perforans*是引起病害的主要菌種。
- 普遍發生於世界各地(尤其是熱帶與亞熱帶低區)種傳細菌性病害。
- 番茄S. lycopersicum var. cerasiforme (PI 114490，黃色小番茄)對於多菌株(T1、T2、T3及T4)呈現抗性。
- 抗病基因*Rx-1*、*Rx-2* 與*Rx-3* 抗T1菌株； *Rx-4* (=Xv-3)抗T3菌株。

細菌性斑點病



http://agronomyday.cropsci.illinois.edu/2009/tours/b4diagnos/

抗細菌性斑點病

Diseases	Gene	Marker	Varties	Chr.	Reference
Bacterial spot (*Xanthomonas* spp.)	Rx-1	L1, R1	LA0716	1	Tai et al., 1999
	Rx-3	Rx3-L1	Hawaii 7998		Yang and Francis, 2005
	Rx-4	CosOH57, SL20181	PI 128216	11	Robins et al., 2009

抗青枯病(Bacterial wilt)
(*Ralstonia solanacearum*)

- 經由植物氣孔或傷口侵入體內，並快速蔓延維管束內，堵塞水分輸送，造成植株萎凋枯死。
- 番茄種植於熱帶與亞熱帶地區發生最嚴重的土傳細菌性病害，即使溫帶地區亦是常見，屬於世界性病害。
- 抗青枯病基因以數量性狀基因為主，品種需堆疊較多抗病基因方能獲得較佳抗病效果。
- 由抗病品種Hawaii 7996與Hawaii 7998發現存在單一顯性抗病基因。

青枯病



陳迪偉博士提供



https://www.clemson.edu/public/regulatory/plant_industry/pest_nursery_programs/plant_prob_clinic/fact_sheet_folder/bac_wilt_tomato.html



抗青枯病

Diseases	Gene	Marker	Varties	Chr.	Reference
Bacterial wilt (*Ralstonia solanacearum*)	Bw	$TSCAR_{AAT/CGA'}$ $TSCAR_{AAG/CAT'}$ TG230, TG564	Hawaii 7996, Hawaii 7998	6	Miao et al., 2009；Wang et al., 2000；Danesh et al., 1994

抗根瘤線蟲(Root knot nematodes)
(*Meloidogyne* spp.)

◆為內寄生型害蟲，嚴重影響根系發育與吸收、萎凋及容易感染其他病原菌、果實產量品質低落。主要抗性育種材料存在於野生種秘魯番茄。

◆已發現9個抗根瘤線蟲基因，Mi-1、Mi-2、Mi-3、Mi-4、Mi-5、Mi-6、Mi-7、Mi-8、Mi-9等基因。

◆Mi-9基因即使生長於32℃高溫環境，仍有明顯抗性，對產業應用價值將非常大。





- *Mi*基因(含*Mi-1*及*Mi-1.2*，來自野生種*S. peruvianum*)，同時抗根瘤線蟲、粉蝨、蚜蟲。

The Root-Knot Nematode Resistance Gene *Mi-1.2* of Tomato Is Responsible for Resistance Against the Whitefly *Bemisia tabaci*

Gloria Nombela,[1] Valerie M. Williamson,[2] and Mariano Muñiz[1]

[1]Centro de Ciencias Medioambientales (CSIC). Serrano 115 Dpdo. 28006 Madrid, Spain; [2]Department of Nematology, University of California, Davis 95616, U.S.A.

Submitted 23 December 2002. Accepted 21 February 2003.

The tomato gene *Mi-1.2* confers resistance against root-knot nematodes and some isolates of potato aphid. Resistance to the whitefly *Bemisia tabaci* previously has been observed in *Mi*-bearing commercial tomato cultivars, suggesting that *Mi*, or a closely linked gene, is responsible for the resistance. The response of two biotypes of *B. tabaci* to tomato carrying the cloned *Mi* was compared with that of the isogenic untransformed tomato line Moneymaker. Our results indicate that *Mi-1.2* is responsible for the resistance in tomato plants to both B- and Q- biotypes. *Mi-1.2* is unique among characterized resistance genes in its activity against three very different organisms (root-knot nematodes, aphids, and whiteflies). These pests are among the most important on tomato crops worldwide, making *Mi* a valuable resource in integrated pest management programs.

The utilization of resistant plants to control *B. tabaci* recently has increased because whiteflies appear to be resistant to many of the chemicals employed in their control (Russel 1978). A number of commercial cultivars of tomato contain the *Mi* gene, which provides resistance to three species of root-knot nematodes (*Meloidogyne* spp.) (Roberts and Thomason 1986) and some isolates of the potato aphid *Macrosiphum euphorbiae* (Thomas) (Goggin et al. 2001; Rossi et al. 1998). Studies carried out in our laboratory detected a level of resistance to both B- and Q-biotypes of *B. tabaci* in several *Mi*-bearing commercial tomato cultivars, suggesting that *Mi*, or a closely linked gene, was implicated in the resistance (Nombela et al. 2000, 2001).

The *Mi* locus has been cloned and found to contain two highly homologous genes, *Mi-1.1* and *Mi-1.2* (Milligan et al.

Jablonska et al. 2007

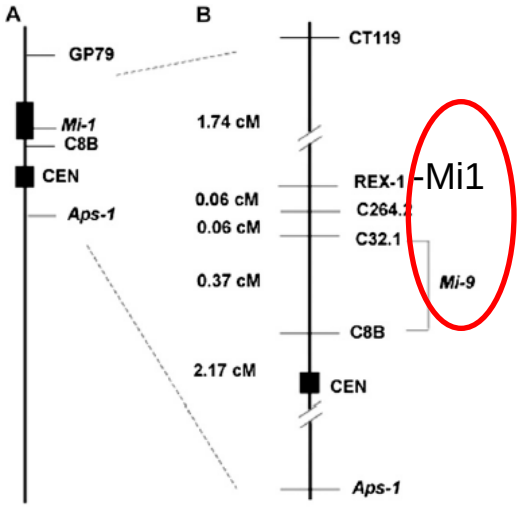
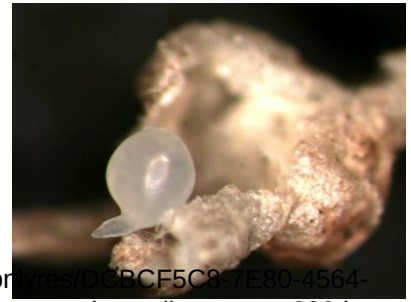


Figure 1. Genetic map of *Mi-1* and *Mi-9* on the short arm of tomato chromosome 6. A, *Mi-1* location and flanking markers. Thick bar represents the introgressed region from *S. peruvianum* in the tomato cv Motelle. B, *Mi-9* linkage map on the short arm of chromosome 6 generated by the mapping population *S. arcanum* accessions LA2157 × LA392. Numbers on the left of the vertical line indicate genetic distances (centimorgans).





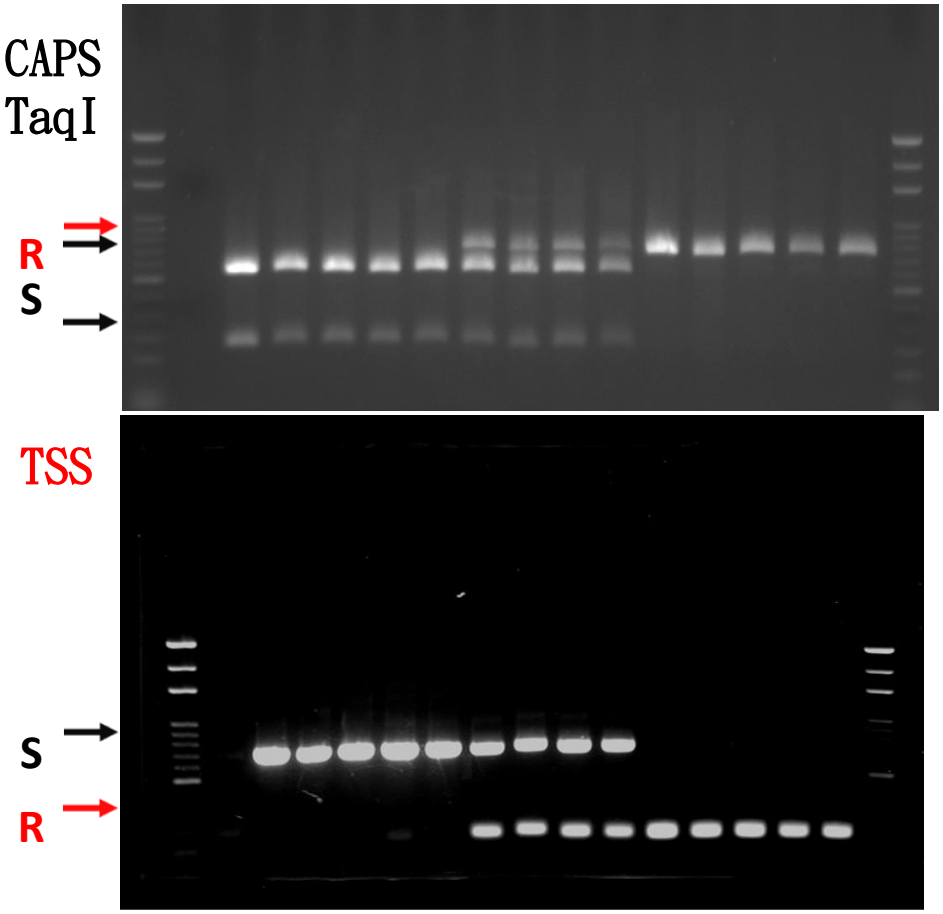
抗根瘤線蟲



http://www.lsuagcenter.com/NR/rdonlyres/DCBCF5C8-7E80-4564-8BEF-2088A0AEE974/4938/cukeonrootresizeandincreaseto200.jpg

Diseases	Gene	Marker	Varties	Chr.	Reference
Root knot nematodes (*Meloidogyne* spp.)	Mi-1	Mi23, PMi, REX-1, Aps C8B, CT119	PI 128657, LA0656, LA1022, LA1221, LA2399, LA2819, LA2820, LA2822, LA2823, LA2826, LA2912, LA2937, LA2938, LA2939, LA2940, LA2941, LA3471, LA3806	6	Williamson et al., 1994; Arens et al., 2010; Seah et al., 2007
	Mi-3	TG180, TG263	PI 126443, LA3858	12	Yaghoobi et al., 2005
	Mi-9		LA2157	6	

抗根瘤線蟲基因(Mi-1)



- 傳統鑑定抗根瘤線蟲基因：養線蟲→接種→2個月→洗根算根瘤數。
  缺點：病害易擴散、耗時、耗人力、誤差大(環境影響線蟲生長)。

- *Mi*基因(含*Mi-1*及*Mi-1.2*，來自野生種*S. peruvianum*)，同時抗根瘤線蟲、粉蝨、蚜蟲。

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The *Mi* locus has been cloned and found to contain two highly homologous genes, *Mi-1.1* and *Mi-1.2* (Milligan et al.





結論

- 已建立抗黃化捲葉病毒基因(Ty1、Ty2、Ty3、ty5)(含TYLCV病毒臺灣種及泰國種)標誌
- 抗斑點萎凋病毒基因(SW5及TSWV病毒)標誌
- 抗嵌紋病毒基因(Tm1、Tm2、$Tm2^2$)標誌
- 抗晚疫病基因(Ph2、Ph3)標誌
- 已建立抗萎凋病基因(I2/Fusarium、I3)標誌
- 已建立抗黃萎病基因(Ve1)標誌
- 已建立抗根瘤線蟲基因(Mi1)標誌
- 部分技術已技轉給國內業者，業者反應分子標誌技術對協助育種者助益相當大。

茄科抗嵌紋病毒

Diseases	Gene	Marker	Varties	Chr.	Reference
TMV (Pepper)	Tm-1, Tm-2				Lefebvre et al., 1995
	L1(P0), L2(P0,P1), L3(P0,P1,P1,2), L4(P0,P1,P1,2,P1,2,3)		PI 159236, PI 260429		Tomita et al., 2011

茄科抗斑點萎凋病毒

Diseases	Gene	Marker	Varties	Chr.	Reference
TSWV (Pepper)	R	CD72, CT124, GC082		10	Lefebvre et al., 2002

茄科抗Potyvirus屬病毒

Diseases	Gene	Marker	Varties	Chr.	Reference
Potyvirus (Pepper)	pvr1, pvr2, pvr5			4	
Potyvirus (Potato)	Ry, Rx1, Rx2	1Rx1, 5Rx1, 10Rx2, 106Rx2	GP122718, Cara, Bzura, Lorctt	12, 12, 5	Bogdan et al., 2005; Ahmadvand et al., 2013

茄科抗晚疫病

Diseases	Gene	Marker	Varties	Chr.	Reference
Late blight (Potato)	QTL	Pi_QTL		3,4,5,6, 8,9,11	Bradshaw et al., 2006；Gabriel et al., 2007

抗白粉病

Diseases	Gene	Marker	Varties	Chr.	Reference
Powdery mildew (Eggplant)	MLO (感病)	SmMLO1			Appiano et al., 2015
Powdery mildew (Potato)	MLO (感病)	StMLO1			

茄科抗根瘤線蟲

Diseases	Gene	Marker	Varties	Chr.	Reference
Root knot nematodes (Pepper)	Me3, Me4	CT135		9	Djian-Caporalino et al., 2001



未來規劃

- 繼續建立抗病(Mi-9、Ty-6、Frl、細菌性斑點病、青枯病、…)共顯性SCAR分子標誌。
- 開發OpenArray：16 genes(RR, RS, SS) X 144 samples (NT 15-/gene/sample) 。
- 結合抗病分子標誌與病害接種技術之高效率選種。
- 利用分子標誌與病害接種技術快速篩選F2 (或雙F1雜交)多抗病基因型同質結合植株(半成品)。
- 利用組培技術克服種間雜交不親合問題。
- 雙單倍體(Double Haploid)選種技術開發。

分子標誌產業應用
F2多抗病同質結合基因型選種

F1 (AaBbCcDdEe…)自交

↓

F2分離出現各種基因型
(AA, Aa, aa/BB, Bb, bb/CC, Cc, cc/DD, Dd, dd/…)

↓

分子標誌與病害接種
篩選**F2**多抗病同質結合基因型單株
(AABBCCDDEE…)

↓

確認**F3**優良單株
(AABBCCDDEE…)



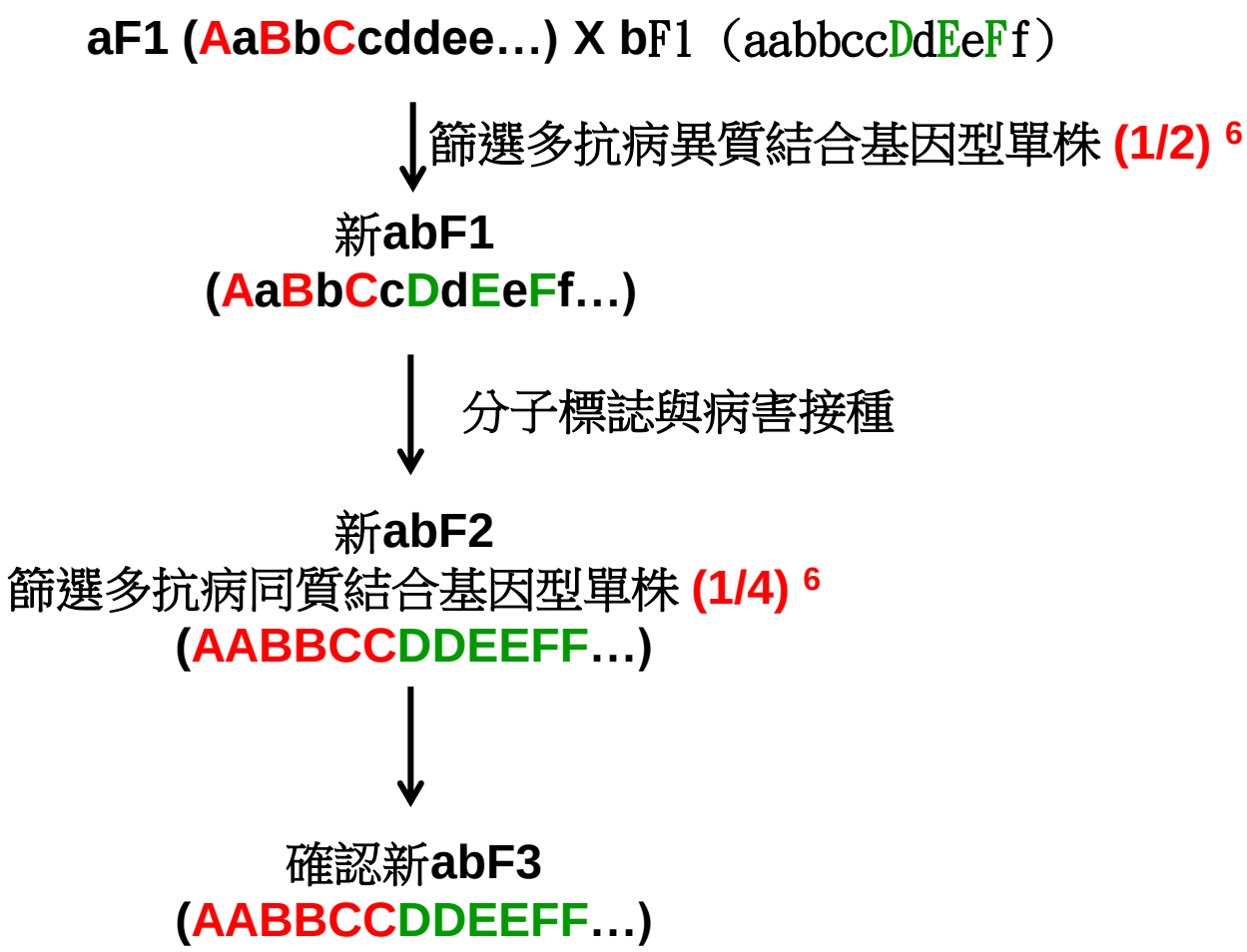


F2多抗病基因選種已獲得單株(田間青枯病嚴選)：

Ty1(R/S)、Ty2(R)、Ty3a(R/S)、Tm22(R)、

I2(R)、I3(R/S)、Ve1(R/S)、Ph3(R)

分子標誌產業應用
雙F1多抗病同質結合基因型選種



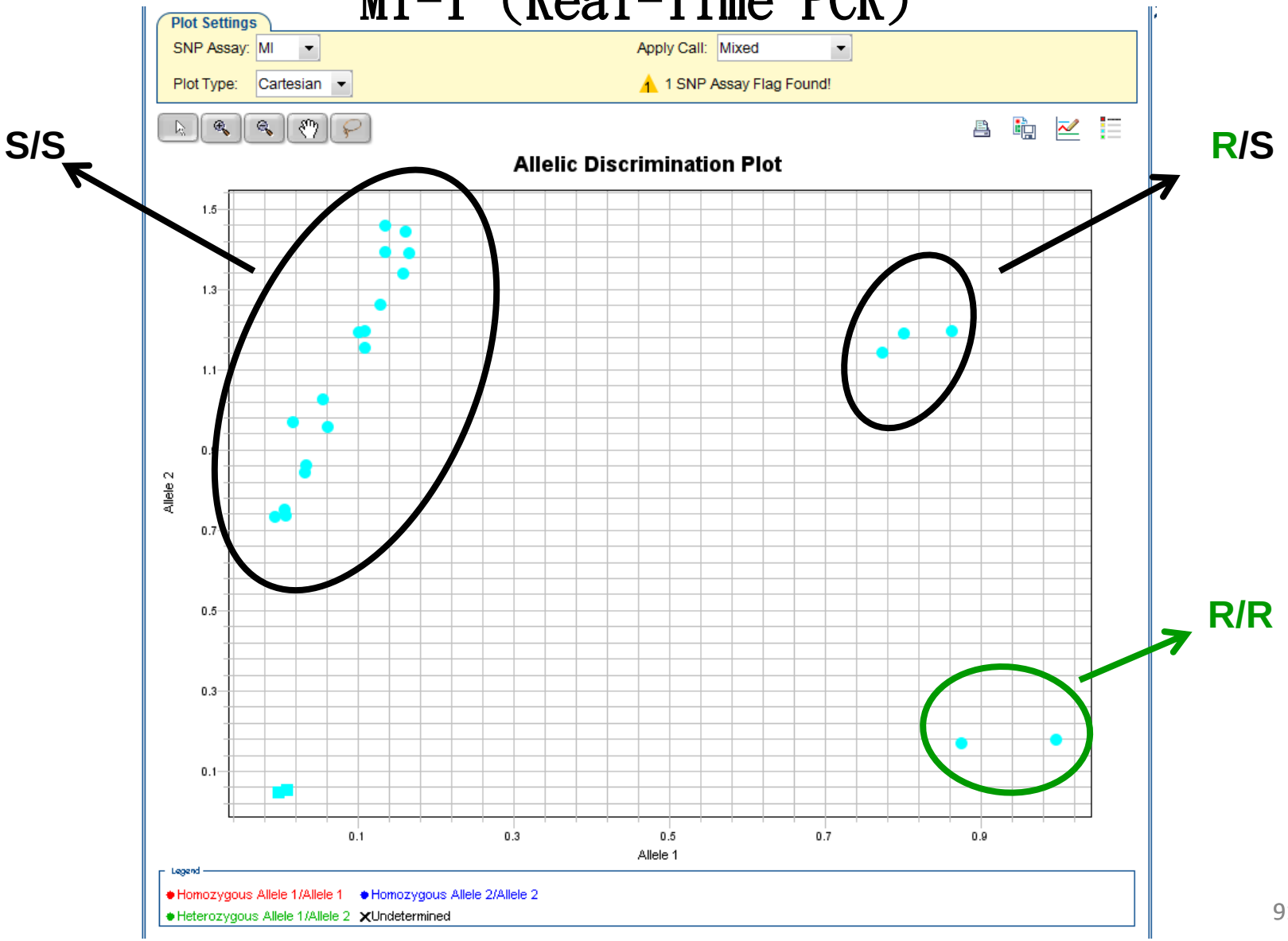
已檢測F1好品種

✓A：Ty1(R/S)、Mi1(R/S)、Tm22(R/S)、Sw5(R/S)、I2(R)、I3(R/S)、Ve1(R/S)、Ph2(R/S)、Ph3(R/S)

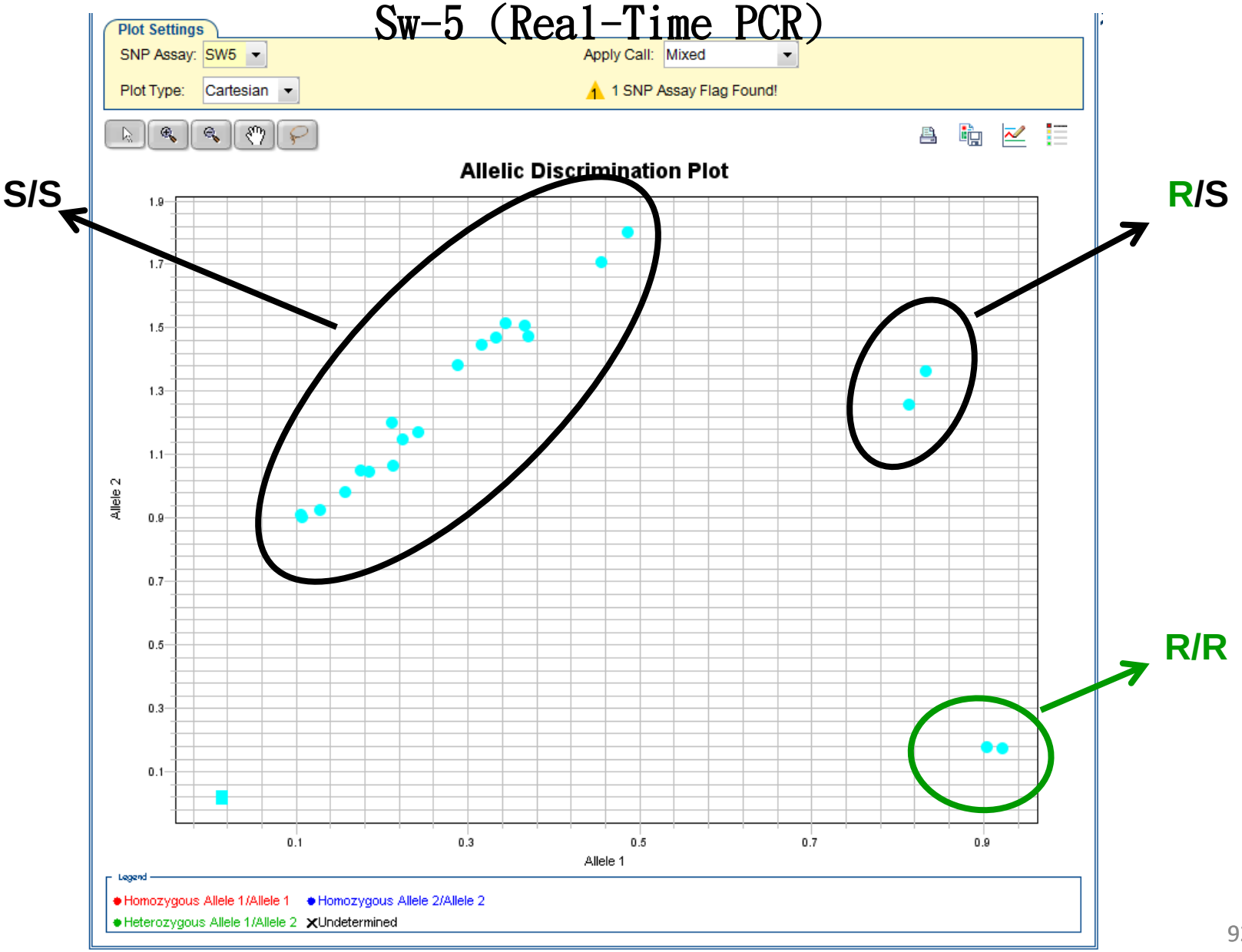
✓B：Ty1(R/S)、Ty3a(R/S)、Tm22(R/S)、I2(R)、Ve1(R)、Ph2(R/S)、Ph3(R/S)

✓C：Ty1(R/S)、ty5(R/S)、Mi1(R/S)、Tm22(R/S)、Sw5(R/S)、I2(R)、I3(R/S)、Ve1(R/S)、I3(R/S)

分子標誌產業應用
Mi-1 (Real-Time PCR)



分子標誌產業應用
Sw-5 (Real-Time PCR)



希　望

- 未來結合育種、病蟲害、栽培、分子、組培等專家，成立跨領域研究團隊，快速育成具國際競爭力優良半自交系(半成品)，授權給國內種子公司。
- 農委會經費支持。
- 本國種子公司使用新技術與新優良品種於育種，提升產品國際競爭力。

http://www.naturesdry.com/

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