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葡萄次病毒之瓜苗檢定[1] (簡報)

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摘要：農試所及臺中地區葡萄產地之巨峰等6個品種之57個葡萄葉片材料，經製作葡萄次病毒接種源 (viroid inoculum)，並於春夏季間進行葡萄次病毒 (grapevine viroid, GV) 之瓜苗檢定 (cucumber assay) 結果，供試57個葡萄材料中，有23個材料在接種之指示植物 (*Cucumis sativus* L. cv. Sūyō) 呈現 GV 典型病徵反應，該罹病瓜苗在接種後5～7週呈現捲葉 (leaf curling) 及葉脈透化 (vein clearing) 等初期病徵，在後期呈現頂端黃化 (top yellowing) 及矮化 (stunt) 等特異性病徵，此項 GV 瓜苗檢定之病徵反應結果與日本佐野等報告之葡萄潛伏性次病毒 (grapevine latent viroid) 相近似。

關鍵詞：葡萄，次病毒，瓜苗檢定法，次病毒檢定。

1984年四方等[9]在日本首先報告葡萄核醣核酸型次病毒 (grapevine viroid RNA)。佐野等[5,6,7]報告日本之葡萄潛伏性次病毒 (grapevine latent viroid) 爲蛇麻矮化次病毒之葡萄分離系統 (grapevine isolate of hop stunt viroid, HSV-g)，並報告法國、德國、澳洲、美國及匈牙利等國家引進日本之葡萄品種材料與日本本土保存者皆多數潛存 HSV-g。繼後 Semancik 等多數學者[3,4,8,10]相繼報告歐洲，澳洲及美國等地區之主要葡萄品種保存材料大部份潛存各種葡萄次病毒 (grapevine viroid isolates, GV)。葡萄爲無性繁殖作物，其品種材料之擴散造成 GV 潛存蔓延於世界各葡萄產地。本省葡萄品種材料係由日本及歐美等國外引進，是否有 GV 潛存有待探究。本試驗初步沿用佐野等[7]之 HSV-g 之瓜苗檢定法 (cucumber assay) 測試本省葡萄品種材料之 GV 潛存情形，供爲今後本省健康葡萄種苗研究繁殖之參考。

向日本大和種苗公司購用檢驗合格之健全胡瓜種子 (*Cucumis sativus* L. CV. Sūyō)，以消毒土壤播種育苗，供爲 GV 之瓜苗檢定 (cucumber assay) 之指示植物。

79年春夏季間，採集農試所試區之 Kyoho, Itali IP65，Concord，鉢栽之 Cabernate franc，Rupestris St. George，LN-33，中部葡萄產地之 Kyoho 幼葉，合計6個品種57個材料凍存之(表1)。將葡萄葉片凍存材料，按佐野等[7]之 GV 接種源製作及瓜苗檢定 (cucumber assay) 方法製成 GV 接種源並進行瓜苗檢定試驗。

葡萄葉片冷凍材料之 GV 接種源製作過程如次：

Frozen tissue (100g).

↓ 200ml 1M K_2HPO_4 (autoclaved)

↓ 2g SDS

↓ 10ml 0.05% bentonite suspension (autoclaved)

1. 臺灣省農業試驗所　研究報告第1578號。本計畫承行政院國家科學委員會補助 (NSC79-0409-B055-16)，謹致謝意。

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↓ blended in mixer for 5 min at room temp.
↓ 100 ml phenol : chloroform (1 : 1)
↓ blended in mixer for 3 min at room temp.
↓ centrifuge 6500 rpm, 10 min.
aqueous phase
↓ 50 ml phenol : chloroform (1 : 1)
↓ blended in mixer for 3 min at room temp.
↓ centrifuge 6500 rpm, 10 min.
aqueous phase
↓ 2.5 vol Et-OH
↓ dry ice 1 hr.
↓ centrifuge 6500 rpm, 10 min.
ppt (interphase)
↓ 100 ml DW
↓ 2 vol (200 ml) Et-OH
↓ dry ice 1 hr.
↓ centrifuge 6500 rpm, 10 min.
ppt (interphase)
dissolved in
1 ml 0.1M Tris-HCl, 0.01M EDTA, 0.1M NaCl. pH 7.5
↓ 0.05% bentonite
Inoculum for mechenical inoculation

表1. 葡萄次病毒之瓜苗檢定

Table 1. Detection of grapevine viroids by cucumber assay

地區 Location	品種 Cultivar	瓜苗檢定結果(2) Result of cucumber assay	病徵(1) Symptoms
TARI	Concord	3/8	LC, VC, LY, S
	Kyoho	4/8	LC, VC, LY, S
	Itali IP65	2/8	LC, VC, LY, S
	Cabernet franc	2/3	LC, VC, LY, S
	Rupestris St. George	1/3	LC, VC, LY, S
	LN-33	2/3	LC, VC, LY, S
Tungshu	Kyoho	5/12	LC, VC, LY, S
Tsuolan	Kyoho	4/12	LC, VC, LY, S

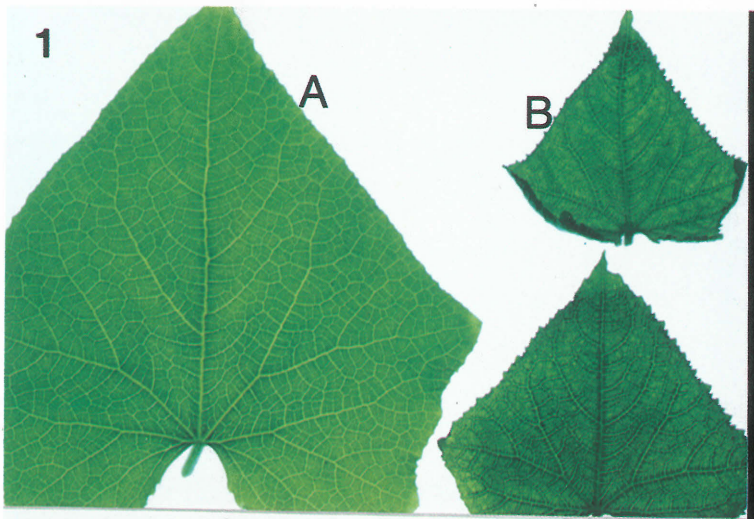
(1)LC: leaf curling. VC: Vein clearing
LY: leaf yellowing. S: stunt.
(2)Numerator/denominator=Infected plants/inoculated plants.

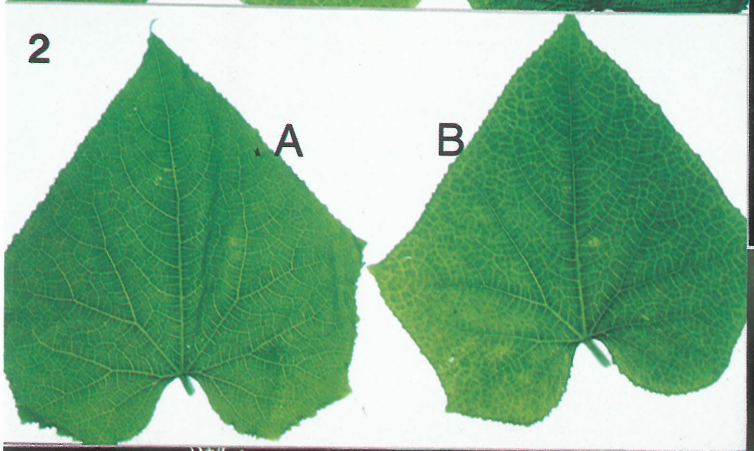
在79年春夏季間，將上述 GV 接種源以汁液接種方法接種在缽栽4～6天之瓜苗乳葉（CV.Sūyō），放置在25～35°C溫室約9～12週，定期施用農藥，另以無接種瓜苗爲對照，觀察記錄瓜苗核定植物之生育及病徵出現情形。另在79年冬季（低溫季節），將上述瓜苗檢定得到之 GV 罹病瓜葉材料分別回接健全瓜苗乳葉（CV. Sūyō），觀察低溫季節 (20°C以下) 環境下之 GV 在瓜苗之病徵反應。

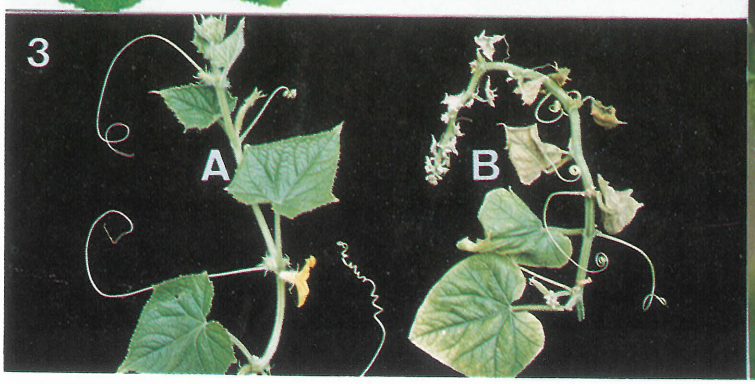
春夏季間，以供試6個品種57個葡萄葉片材料之 GV 之瓜苗檢定結果，有23個材料在瓜苗指示植物（CV. Sūyō）呈現 GV 之特殊病徵反應（表1.）。瓜苗經接種5—7週後，初期在幼葉呈現捲葉（leaf curling），葉脈透化（vein clearing）及葉片暗化（leaf color darkening），後期呈現頂端黃化（top yellowing），葉片黃化（leaf yellowing）及矮化（stunt）等病徵（圖1～5）。另在冬季低溫季節，將上述 GV 罹病瓜葉材料回接到瓜苗結果，皆未有病徵反應。上述葡萄材料之瓜苗檢定結果與佐野等[5,7]報告之 HSV-g 之瓜苗檢定結果相近似，亦與過去已 GV 等 viroids 僅適宜於高溫環境（25—35°C）始能感染發病之報告相符[1,2,7,9]。又上述 GV 之瓜苗檢定結果，其檢定植物之病徵反應有嚴重矮化及輕度捲葉型，嚴重型者其病徵發展快速，全株極端矮化且頂端芽葉捲屈變小而黃化，輕型者其病徵進展緩慢，輕度矮化及捲葉，依此項檢定植物之病徵反應之差異結果推測供試各種葡萄品種材料間似存有病原性差異之分離株（grapevine viroid isolates）存在。

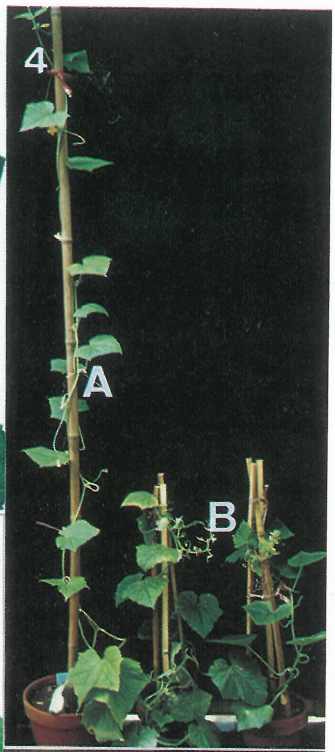
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1
A
B


2
A
B


3
A
B


4
A
B


5

圖片說明：

Explanation of plates:

Fig.1. The grapevine viroid infected cucumber leaves (*Cucumis sativus* L. cv. Sūyō) showing leaf curling and darkening (B) and the healthy (A).

Fig.2. The viroid infected cucumber leaf showing vein clearing (B) and the healthy (A).

Fig.3. The viroid infected cucumber plant showing top necrosis and leaf yellowing (B) and the healthy (A).

Fig.4. The viroid infected cucumber plants showing stunting (B) and the healthy (A).

Fig.5. The viroid infected cucumber apical bine showing leaf curling, leaf yellowing, leaf rugosity and internode schortening.

Detection of grapevine viroids by cucumber assay[1] (Research Note)

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Summary

Fifty seven grapevine inocula were extracted from leaf collections of six grapevine varieties cultivated in Taiwan agricultural research institute and Taichung district. The grapevine viroids were detected in 23 out of 57 grapevine inoculum extractions tested by the viroid indexing method of cucumber assay during spring to summer season.

The grapevine viroid induced typical viroid symptoms on cucumber indicator plants (*Cucumis sativus* L. cv. Sūyō) 5-7 weeks after inoculation. The viroid infected cucumber plants showed specific symptoms of leaf curling, top yellowing and stunt, and the results of cucumber assay were similar to those of grapevine latent viroid (HSV-g) which had been described by Sano *et al.* in Japan.

Key words : grapevine, viroid, cucumber assay, viroid detection.

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