

夜來香微嵌紋病毒在植株內之分布 及其對病毒檢測之影響

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摘 要

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夜來香微嵌紋病毒(Tuberose mild mosaic potyvirus, TMMV) 為感染夜來香 (*Polianthes tuberosa* L.) 造成植株葉片及花莖上產生輕微嵌紋病徵之致病病毒。利用 TMMV 之抗血清以酵素連結雙抗體包夾法(double antibody sandwiched ELISA, DAS-ELISA) 比較植株中葉片、花莖、花朵、球根及根部各組織中病毒濃度之差異時發現 TMMV 之分布極不均勻，各部位中以花莖鞘葉，花莖及新葉之病毒濃度較高。不同葉序之葉片間以內層之新葉病毒濃度較高，外層老葉則逐漸遞減。花莖上不同部位之鞘葉中，以下位較成熟之鞘葉病毒濃度較高。不同成熟期花莖之花瓣組織則以愈成熟者之病毒含量較高。另外 TMMV 在夜來香球根中之分布亦不均勻，比較側芽，鱗葉及基盤組織等三部位後發現側芽及鱗葉之病毒濃度較高，其病毒檢出率約為檢查基盤組織之三倍。故以球根為檢測對象時，應以前二者為取樣標的之檢測結果準確度較高。又取樣時若將每一球根取一側芽提升至取三個側芽混合檢測時，其病毒檢出率可由 93% 提升至 100%。本研究結果可作為檢測夜來香植株或貯藏期球根之 TMMV 帶毒率之採樣依據，而且證實正確之取樣可增加檢測 TMMV 病毒之準確性。

(關鍵詞：夜來香、夜來香微嵌紋病毒、病毒分布、病毒檢定)

緒 言

夜來香，又名晚香玉，為原產於墨西哥之龍舌蘭科球根花卉，引進國內栽培已有三

百多年歷史，主要栽培於嘉義、虎尾及屏東地區，其花朵潔白清麗頗受日本市場喜愛，部份品種可供提煉香精，種球亦有部份外銷，因此近年來被視為具有發展潛力之本土化球

根花卉^(3, 11, 13)。有關夜來香病毒病害的研究資料極端缺乏，僅 Horner and Person⁽⁹⁾ 於 1988 年報告曾經發現由馬鈴薯 Y 屬病毒 (*Potyvirus*) 所引起之病毒病，然而其只記載該病毒部份純化之顆粒形態及電顯觀察組織超薄切片中圓柱狀內含體之結果，對該病毒之血清特性及其分類地位並無確切之結論。本研究室近年之調查亦證實本省栽培之夜來香幾乎已全面受到一種 *potyvirus* 之感染，經比較不同地區分離株之特性後發現其彼此間並無差異應屬相同之病毒，而根據血清類緣及寄主反應特性比對後證實此種發生於夜來香之 *potyvirus* 應屬一未經完整鑑定之新病毒，因此將其命名為夜來香微嵌紋病毒 (*tuberosa mild mosaic potyvirus*，簡稱 TMMV)^(2, 5, 9)。而本研究之目的在探討 TMMV 於夜來香植株中之分布，藉以提供檢定 TMMV 時正確之採樣部位與方式之依據，以提高病毒檢定之準確性，建立檢定 TMMV 之標準模式。

材料與方法

供試夜來香來源

本研究所使用之夜來香病株乃採自嘉義地區，經血清檢定確定感染 TMMV 者，將其種球種植於本所試驗田中，以二年生植株供作病毒檢測試驗用。另採收直徑達 2-2.5cm 之種球，經風乾後取室溫下保存一個月內之球根進行病毒檢測。

病毒抗血清來源

本試驗所使用之 TMMV 抗血清乃以純化之病毒顆粒進行免疫注射於家兔體內後所製作而成，病毒純化之步驟與抗血清製作之方法已於過去之報告中述明⁽⁶⁾。經免疫注射之家兔每週進行靜脈採血一次，每次約可獲得 50ml 血液，經紅血球分離後約可獲得 25ml 之血清。血清儲存於 -20°C 冷凍櫃備用。酵素連結抗體反應法 (ELISA)

由於過去之試驗結果發現以本研究室所

製作之 TMMV 抗體進行病毒檢測時，酵素連結雙抗體包夾法 (double antibody sandwiched ELISA, DAS-ELISA)⁽⁷⁾ 之專一性遠高於間接式 (antigen-coating indirect ELISA)，且對健康植株之背景值較低⁽⁵⁾，故本試驗所有病毒檢測均以 DAS-ELISA 行之。進行 DAS-ELISA 時乃將待檢之夜來香組織以 50 mM 之磷酸鉀緩衝液 (pH 7.5) 研磨，並稀釋為 30 倍 (30 ml/g) 後進行檢測。首先將 TMMV 抗體之免疫球蛋白 (IgG, 1 mg/ml) 以 15 mM 碳酸鈉緩衝液 (sodium carbonate buffer, pH 9.6) 稀釋 1200 倍加入 EIA 反應盤內 (100 μ l/well)，經 37°C 反應 3-4 小時，再以 1X PBST 水洗三次，爾後分別加入供測之夜來香汁液 (100 μ l/well)，於 25°C 下反應 3-4 小時或置於 4°C 定溫箱中隔夜，經 PBST 水洗三次後，加入已結合鹼性磷酸酶之 TMMV IgG (稀釋 1200 倍使用)，於 25°C 下反應 3-4 小時或置於 4°C 定溫箱中隔夜，反應盤以 PBST 水洗四次後加入鹼性磷酸酶基質 (1 mg/ml) 於 25°C 下進行呈色反應，經 2 小時後以 ELISA 判讀機 (Molecular Devices, E-Max)，讀取 405 nm 波長下之吸光值。

檢測不同部位組織內病毒濃度之採樣方法

夜來香不同部位包括植株之花穗、葉叢、根系等及採收風乾後的球根上之側芽、鱗葉及基盤等處 TMMV 濃度分布之檢測試驗，分別採取各部位之組織 0.1 g 進行研磨 (組織未足 0.1 g 者以採得之實重作檢測)，再以 50 mM 之磷酸鉀緩衝液 (pH 7.5) 稀釋為 30 倍 (30 ml/g)，每一樣品汁液加入二個 EIA 盤孔穴中進行 DAS-ELISA 測定。本研究各項病毒檢測均重複兩次試驗，並以無病毒之夜來香實生苗葉片為健康對照。

結 果

TMMV 在夜來香植株上不同部位組織中濃度之差異

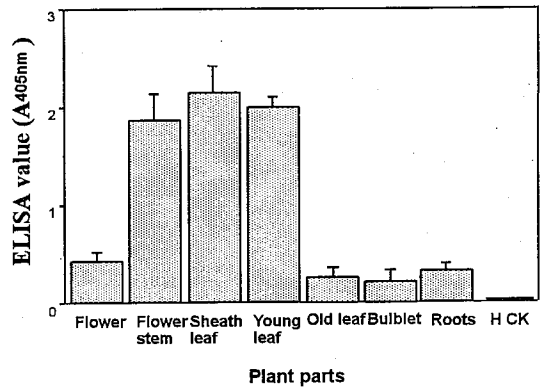
以田間生長二年生之夜來香植株為對象，將植株區分成花穗(包括花朵、花莖表皮及花莖鞘葉)、葉叢(包括老葉及新葉)、根系、及球根等部位，以 DAS-ELISA 法比較八棵不同植株之上述各部位中所含 TMMV 濃度之高低，結果發現 TMMV 之分布極不均勻，其中以花莖鞘葉，花莖表皮組織及新葉等處之病毒濃度較高，而花朵、老葉、球根及根部所含之病毒濃度較低(圖一)。夜來香全株含 TMMV 濃度最高之部位為花莖鞘葉，其 ELISA 反應值平均為 2.15 ± 0.21 。

TMMV 在夜來香不同葉序之葉片上濃度之差異

夜來香之葉片細長線形，自球根頂端抽生，葉序由內而外漸次成熟，為了解病毒在不同成熟度葉片上之濃度差異，將葉片由內層之新葉往外層之老葉分成五組不同葉序之葉片，依序採樣進行 DAS-ELISA 以比較病毒濃度之差異。經比較八棵植株之葉序，結果發現新葉之 ELISA 反應值平均為 1.13 ± 0.18 ，高於老葉之 0.22 ± 0.08 ，顯示新葉組織中所含之 TMMV 濃度較高，且隨葉齡增加而病毒濃度逐漸遞減(圖二)。

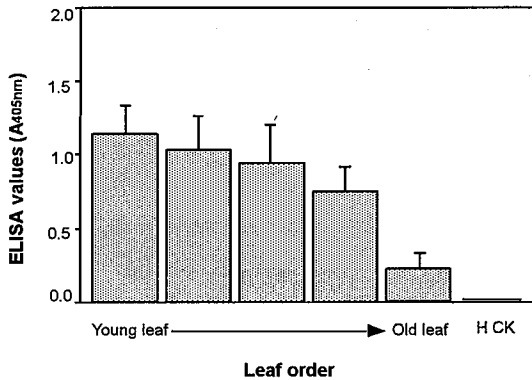
TMMV 在夜來香花朵及花莖鞘葉上濃度之差異

將夜來香花莖依分化成熟度區分為幼期(花穗仍包被於花莖鞘葉內)、中期(花朵已分化且伸出鞘葉但仍呈綠色)及成熟採收期(花朵分化成熟且最下位第一對花已轉白)。於各期花莖上之花朵由上而下依序採取花瓣組織進行 DAS-ELISA，以了解不同成熟度之花朵內所含病毒濃度之差異。經比較六支切花之結果發現所區分之三種時期花莖之花瓣組織中，已達成熟採收期之花朵不管位置高低其 ELISA 反應值均較高(約 0.25 ± 0.04 至 0.26 ± 0.02)，而初期花莖上之花朵 ELISA 值普遍偏低(約 0.09 ± 0.02 至 0.11 ± 0.02)(圖三)。另外將夜來香花莖上之鞘葉由上而下依序採



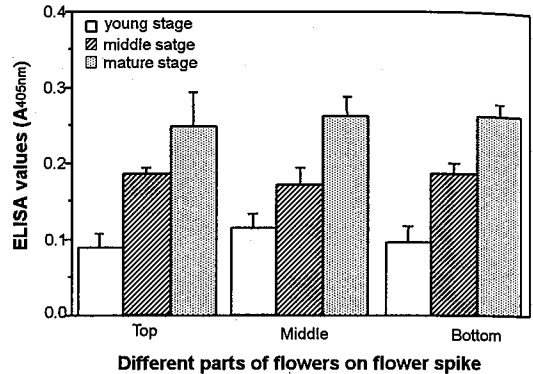
圖一、夜來香微嵌紋病毒在夜來香植株不同部位組織內濃度之差異。將田間二年生之夜來香植株區分為花穗(包括花朵、花莖及花莖鞘葉)、葉叢(包括新葉及老葉)、球根及根系等部位，分別採取其組織，以 50 mM, pH 7.5 磷酸鉀緩衝液充分研磨後稀釋為 30 倍(30 ml/g)組織液，再以 DAS-ELISA 測定其病毒濃度。圖中所示結果乃八棵植株之不同部位組織所測得之 ELISA 反應平均值，反應值乃加入酵素基質 120 分鐘後於 405 nm 下所測得之吸光度。

Fig. 1. Comparison of tuberose mild mosaic potyvirus (TMMV) concentrations in different parts of tuberose plants. Two-year-old tuberose plants were collected from the field and tissues from flower petals, flower stems, flower stem sheath leaves, leaves including youngest and oldest, bulblets and roots were sampled and ground (30 ml/g) in 50 mM phosphate buffer (pH 7.5). Virus concentrations in individual samples were determined by DAS-ELISA as described in the text and indicated as absorbance values at 405 nm taken 120 min after the addition of enzyme substrate. The data shown in the figure are the averages of absorbance values taken from eight different tuberose plants. Vertical lines represent standard error.



圖二、夜來香微嵌紋病毒在夜來香植株不同葉序之葉片組織中濃度之差異。將田間二年生之夜來香植株葉片由內往外分成五組不同葉序之葉片，以 50 mM, pH 7.5 磷酸鉀緩衝液充分研磨後稀釋為 30 倍(30 ml/g)組織液，再以 DAS-ELISA 測定其病毒濃度。圖中所示結果乃八棵取自田間之夜來香之不同葉位組織所測得之 ELISA 反應平均值，反應值乃加入酵素基質 120 分鐘後於 405 nm 下所測得之吸光度。

Fig. 2. Comparison of tuberose mild mosaic potyvirus (TMMV) concentrations in leaf tissues of different leaf orders of tuberose plants. Two-year-old tuberose plants were collected from the field. Five groups of leaf tissues of different leaf orders from the youngest (most inner one) to the oldest (most outside one) were sampled, ground in 30x volume (30 ml/g) of 50 mM phosphate buffer (pH 7.5) and determined by DAS-ELISA as described in the text for TMMV concentrations. The data shown in the figure are the averages of absorbance values at 405 nm taken from leaf tissues of eight different tuberose plants. Vertical lines represent standard error.

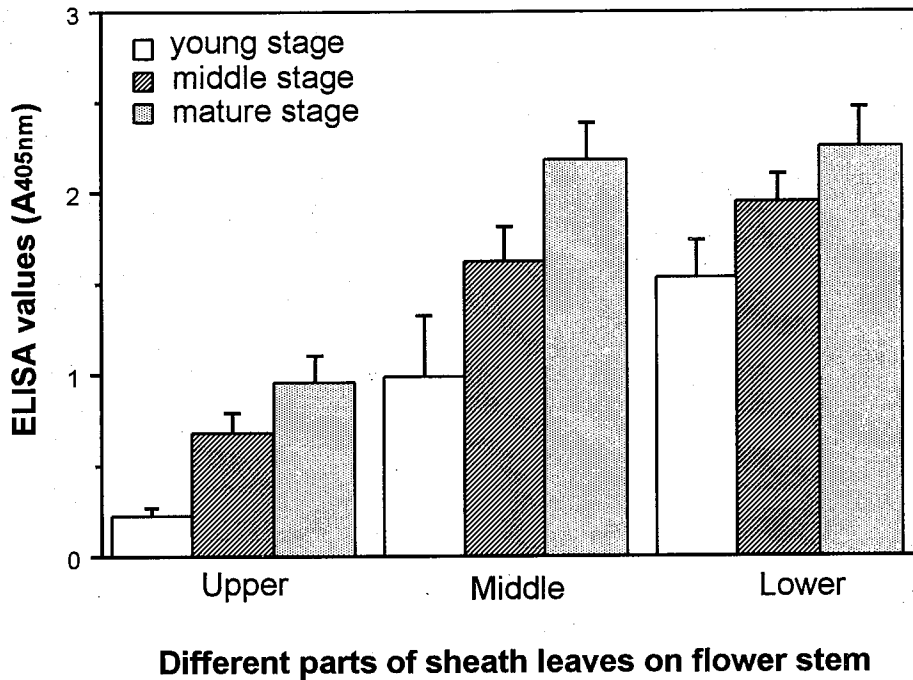


圖三、夜來香微嵌紋病毒在不同成熟度花朵之花瓣組織中濃度之差異。將夜來香之花莖分成三個不同之成熟期，□代表幼期花莖（花穗仍包被於花莖鞘葉內）；▨代表中期花莖（花朵已分化且伸出鞘葉但仍呈綠色）；▩代表達成熟採收期之花莖（花朵分化成熟且最下位第一對花已轉白）。分別採取各期花莖最頂端 (Top)、中段 (middle) 及底端 (bottom) 處花朵之花瓣，以 50 mM 磷酸鉀緩衝液 (pH 7.5) 充分研磨後稀釋為 30 倍 (30 ml/g) 組織液，再以 DAS-ELISA 測定其病毒濃度。圖中所示結果為六支花莖之花瓣組織所測得之 ELISA 反應平均值，反應值為加入酵素基質 120 分鐘後於 405 nm 下所測得之吸光度。

Fig. 3. Comparison of tuberose mild mosaic potyvirus (TMMV) concentrations in flower petal tissues of tuberose plants at different ages. Tuberose flower stems were divided into young (□), middle (▨) and mature (▩) stages, in which the flower buds were further divided into top, middle and bottom parts and the petal tissues were separately sampled, ground in 30x volume (30 ml/g) of 50 mM phosphate buffer (pH 7.5) and determined by DAS-ELISA as described in the text for TMMV concentrations. The data shown in the figure are the averages of absorbance values at 405 nm taken from the petal tissues of flower buds of six different flower stems. Vertical lines represent standard error.

樣進行 DAS-ELISA。結果發現不管任何時期之花莖，其下位鞘葉組織之 ELISA 反應值介於 1.5 ± 0.18 至 2.23 ± 0.20 間，而較上位未成

熟者 ELISA 值則介於 0.22 ± 0.03 至 0.95 ± 0.10 間，此結果顯示花莖上之下位較成熟鞘葉內之病毒濃度高於上位未成熟者(圖四)。



圖四、夜來香微嵌紋病毒在不同成熟度之花莖鞘葉組織中濃度之差異。將夜來香之花莖分成三個不同之成熟期；□ 代表幼期花莖（花穗仍包被於花莖鞘葉內）；▨ 代表中期花莖（花朵已分化且伸出鞘葉但仍呈綠色）。▩ 代表達成熟採收期之花莖（花朵分化成熟且最下位第一對花已轉白）。由不同成熟期之花莖由上而下間隔採取 9 片鞘葉分別以 50 mM, pH 7.5 磷酸鉀緩衝液充分研磨後稀釋為 30 倍(30 ml/g)組織液，再以 DAS-ELISA 測定其病毒濃度，將所得結果依序以每三片鞘葉為一組分成上位(upper part)，中位(middle part)，及下位(lower part)等三組，取其 ELISA 反應平均值，以代表三種不同葉位鞘葉組織之病毒濃度。圖中所示結果為六支花莖之鞘葉組織所測得之平均值。ELISA 反應值為加入酵素基質 120 分鐘後於 405 nm 下所測得之吸光度。

Fig. 4. Comparison of tuberose mild mosaic potyvirus concentrations in sheath leaf tissues of tuberose flower stems. Tuberose flower stems were divided into young (□), middle (▨) and mature (▩) stages, in which nine sheath leaves were sampled, ground in 30x volume (30 ml/g) of 50 mM phosphate buffer (pH 7.5) and determined by DAS-ELISA as described in the text for TMMV concentrations. ELISA readings of these nine leaves were averaged into three parts (i.e. the upper, middle and lower portions), in which the lower portions were the oldest while the upper ones were the youngest. The data shown in the figure are the averages of absorbance values at 405 nm taken from the sheath leaves of six flower stems. Vertical lines represent standard error.

TMMV 在夜來香球根上之分布

採取 27 個夜來香球根上之側芽、鱗葉、及基盤等處之組織進行 DAS-ELISA, 其 ELISA 反應值平均分別為 0.90 ± 0.34 、 0.80 ± 0.39 、 0.50 ± 0.23 ; 病毒檢出率分別為 93、93、33%, 顯示球根中側芽及鱗葉之病毒濃度及病毒檢出率均較基盤者高 (表一)。

側芽取樣數對檢測夜來香球根之帶病毒率之影響

由於上述實驗中發現檢測球根之側芽與

鱗葉所得之病毒濃度及病毒檢出率均比基盤組織高, 因此較適合做為病毒檢測之對象。但是在初步之試驗中發現同一球根中不同側芽之病毒濃度高低差異大(結果未出示), 部份側芽甚至無法測出病毒存在。為避免採樣所造成之誤差, 本實驗比較由同一球根分別採取一個、二個或三個側芽混合之取樣對病毒檢出率之影響, 經比較 57 個球根之結果發現採取三個側芽混合檢測方式可將病毒檢出率由只採一個側芽之 93% 提升至 100% (表二)。

表一、夜來香微嵌紋病毒在夜來香球根之不同部位內濃度之差異

Table 1. Comparison of tuberose mild mosaic potyvirus (TMMV) concentrations among different parts of tuberose bulb tissue by DAS-ELISA ¹⁾

Tissue part ²⁾	Av. ELISA reading ³⁾	% of detection ⁴⁾
Lateral bud	0.90 ± 0.34	93
Bulb leaf	0.80 ± 0.39	93
Core tissue	0.50 ± 0.23	33
Healthy control	0.01 ± 0.01	—

¹⁾ DAS-ELISA as described in the text was used to determine the virus concentration in bulb tissues. Absorbance readings (405 nm) were taken 120 min after the addition of enzyme substrate solution.

²⁾ Three different parts of tissues were sampled from 27 bulbs, ground in 30x volume (30 ml/g) of 50 mM phosphate buffer (pH 7.5) and tested for TMMV concentrations by DAS-ELISA.

³⁾ Only those samples with positive reactions (ELISA readings exceed 0.15) were averaged.

⁴⁾ (No. of bulbs positively detected with TMMV infection divided by the total number of bulbs tested) x 100%.

表二、側芽取樣數對偵測夜來香球根中之夜來香微嵌紋病毒之影響

Table 2. Effect of the sampling numbers of lateral buds per bulb on the detection of tuberose mild mosaic potyvirus (TMMV) by DAS-ELISA ¹⁾

Sampling no. of lateral buds per bulb ²⁾	Av. ELISA reading ³⁾	% of detection ⁴⁾
1	1.17 ± 0.49	93
2	1.27 ± 0.02	97
3	1.23 ± 0.12	100
Healthy control	0.02 ± 0.02	—

¹⁾ DAS-ELISA as described in the text was used to determine the virus concentration in lateral buds of tuberose bulbs. Absorbance readings (405 nm) were taken 120 min after the addition of enzyme substrate solution.

²⁾ A total of 57 tuberose bulbs were used in this test. Each bulb was sampled by taking 1, 2, or 3 separate lateral buds, ground in 0.5, 1 and 1 ml of 50 mM phosphate buffer (pH 7.5), respectively, and testing for TMMV concentrations by DAS-ELISA.

³⁾ Only those samples with positive reactions (ELISA readings exceed 0.15) were averaged.

⁴⁾ (No. of bulbs positively detected with TMMV infection divided by the total number of bulbs tested) x 100%.

討 論

絕大多數的植物病毒侵入其寄主後常造成系統性感染⁽⁸⁾，使寄主全身各部位均有病毒存在。然而過去之經驗顯示病毒在寄主植物各部位之分布並不均勻，濃度差異頗大^(4, 10, 12, 14)。本研究結果亦顯示 TMMV 在夜來香全株中之濃度呈不均勻分布狀態，雖然由根系、球根、葉片乃至花朵上均可檢測到病毒，但濃度高低卻有所差異，部份組織之檢測結果其 ELISA 反應值甚至與健康對照者無異。此種病毒濃度在寄主植物內分布不均之現象，勢必會影響病毒檢定結果之準確性。以本實驗之夜來香所得結果為例，TMMV 不僅在植株各部位之分布不均，且在不同葉序之葉片、不同成熟度花莖之花朵及花莖鞘葉上亦有顯著差異，因此在進行病毒檢定時，若任意採取部份組織作檢測，也會因採樣部位之不同而獲得不同結果，甚至造成檢定結果之誤判。根據本試驗檢測 TMMV 在夜來香各部位分布之結果，含較高病毒濃度之部位若作為檢定取樣時之依據，則病毒檢測之正確性必可相對提高。實驗結果證實若檢定對象為田間生長株時，可就下位之花莖鞘葉或新葉進行採樣；若對象為切花時，可就下位花莖鞘葉或花莖表皮組織進行採樣；而檢定的對象若為貯藏期之球根時，則可就側芽或鱗葉進行採樣。由於 TMMV 之濃度在球根不同側芽上的分布亦相當不均勻，因此若針對球根側芽進行採樣檢定時，應同時採取至少三個側芽混合進行檢測，以降低因病毒濃度不均而僅只採一個側芽造成部份檢定結果之誤判。

抗血清檢定法為病毒診斷與檢定的主要技術，其中又以 ELISA 被廣泛應用於大量樣品的病毒篩檢⁽¹⁾。本研究室過去已發現利用 DAS-ELISA 進行 TMMV 之偵測不僅具有高度敏感性⁽⁵⁾，且具有不與其他二十餘種不同 potyvirus 抗原反應之高度專一性⁽⁶⁾，如今再配合本研究結論指出之正確取樣方式，可進一步提高檢測 TMMV 結果之可信度。而本研

究所獲知之病毒分布差異現象，亦可作為其他作物病毒檢測之參考。

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ABSTRACT

Chen, C. C., Chiang, F. L., and Chang, C. A. 1998. Distribution of tuberose mild mosaic potyvirus in tuberose (*Polianthes tuberosa* L.) and its influence on virus detection. Plant Prot. Bull. 40: 199 - 207. (Department of Plant Pathology, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan, R. O. C.)

Tuberose mild mosaic potyvirus (TMMV) causes mild mosaic symptoms on leaves and flower stems of tuberose (*Polianthes tuberosa* L.). Detection of TMMV by DAS-ELISA in different parts of tuberose plants, including leaves, flower stems, flower petals, bulbs and roots, revealed that TMMV was unevenly distributed throughout the plant. The highest concentration of TMMV was found in sheath leaves of flower stems, followed by flower stem tissue and young leaves. Concentrations of TMMV were found higher in internal younger leaves and gradually decreased in external older ones. When sheath leaves on flower stems were compared, the larger and more mature sheath leaves consistently demonstrated higher TMMV concentrations than those of the smaller and younger ones. When flower buds were tested, TMMV concentrations in petal tissues increased with the age of flower stems. In bulb tissue of tuberose, TMMV was also found distributed unevenly. The virus concentrations in lateral buds and apex scale leaves were significantly higher than those in the core tissue of tuberose bulb, as reflected in the results that successful detection rates of TMMV by sampling from the former two tissues were three times as that from the last one. Although TMMV distributed in different lateral buds unevenly, a detection rate of 100% could be accomplished by sampling and combining tissues of 3 lateral buds from a indexed bulb. These results provide useful information on correct sampling for successful detection of TMMV in tuberose plants or bulbs.

(Key words: tuberose, tuberose mild mosaic virus, distribution, virus indexing)