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The Occurrence of Grapevine Leafroll Disease Among the Main Grapevine Cultivars and Breeding Stocks in Taiwan

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ABSTRACT

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A preliminary survey of the occurrence of leafroll disease among the grapevine collections in Taiwan was conducted. A total of 50 grapevine cultivars or breeding lines showing suspicious leafroll symptoms were examined by chip bud grafting field indexing and enzyme linked immunosorbent assay (ELISA) for the identity of the causal agents. The results indicated generally a high incidence of leafroll-associated virus infection among these tested grapevines including the main commercial cultivars like Kyoho, Italia, Black Queen and Golden Muscate. By electron microscopy, the long flexuous closterovirus particles were detected from affected grapevine tissues. The performed ELISA further indicated the high proportion of complex infection by Type III and Type IV leafroll viruses among the test grapevines. In biological indexing analysis, symptom development on indicator grapevines LN-33, and Cabernet Franc, reflected a higher proportion of leafroll infection as compared to that detected by ELISA, indicating the possible existence of other serotypes. The main symptoms of leafroll associated virus infection on tested grapevines included delayed bud break and fruit maturation, reduced cluster size, uneven fruit maturation, leaf rolling, and altered leaf colors-typically leaf reddening among red grapes and the yellowing among white grapes. Kyoho, the main red table grapevine, was an exception in that leaf yellowing in stead of reddening was generally observed. Impact of the disease was most evident by the substantially deteriorated growth development and fruit yield. The high proportion of disease incidence clearly indicated the potential threat of the disease and the great attention it deserved.

Key words: Chip-bud grafting field indexing, closterovirus, disease occurrence, ELISA, grapevine leafroll disease, serotype.

INTRODUCTION

Grapevine is a perennial fruit tree; the cultivation and breeding of grapevine are both long time process. The history of grapevine cultivation in Taiwan is fairly short as compared to that in Europe or America. During the developmental stage of grapevine cultivation industry, with the attempt to achieve rapidly the goal of high yield and quality, a great number of cultivars, breeding lines and rootstock materials were introduced by breeders or growers from the main grapevine growing countries all over the world. According to the available records, more than one thousand grapevine cultivars have been introduced to Taiwan during the past two decades(4,16,22). Among them, quite a few adapted and performed well to the local environmental conditions and hence became the major extensively growing cultivars(4). The mass introduction of breeding as well as cultivation materials from various sources implicated the potential threat of virus diseases.

In grapevine cultivation, a lot of virus diseases have been reported. The disease severity and the economical impact both depended greatly on the host-virus combinations. Leafroll, corky bark and rupestris stem pitting are among the known virus diseases which had imposed the greatest damages on quality and yield of grape production worldwide(3,9). In Taiwan, due to the great improvement of cultivation techniques, the

increasing needs of fresh market, as well the establishment and prosperous development of wine-making factory, viticulture has become an important part in the agriculture system recently. However, the information of occurrence of virus diseases and their potential impacts were still lacking. The main objective of this study was to explore the potentially existing problem. By chip-bud grafting field indexing using the appropriate grapevine virus indicator varieties developed in the University of California at Davis (U. C. Davis)(10), enzyme linked immunosorbent assay (ELISA) with available antisera, and transmittence electron microscopy, the existence of virus diseases among the main grapevine cultivation or breeding sources on the island were surveyed. A high incidence of grapevine leafroll (GLR) disease was detected in the surveyed samples. The symptomology, the field occurrence, and the potential effects of this disease on grapevine production are herein discussed.

MATERIALS AND METHODS

Field indexing

A total of 50 grapevine cultivars or breeding lines with suspecious virus-infected symptoms were tested. The field indexing study was conducted in an experimental field of Taiwan Tobacco Research Institute (TTRI) located at Tsao-Hu, Taichung County during the year 1986 to 1989. Virus free indicator grapevine varieties LN-33 (Couderc 1613 X Thompson Seedless), Cabernet Franc (*Vitis vinifera*), Mission (*V. vinifera*), and St. George (*V. rupestris*) were introduced from the Foundation Plant Material Service of U. C. Davis (with the courtesy of Dr. A. C. Goheen) in 1981 and were propagated and maintained in an isolated field in TTRI since then. From the year 1985 to 1988, dormant cuttings from these indicator plants were collected every year in January. For field indexing, dormant cuttings were collected from the fields of cultivar collection and breeding in TTRI at the same season. By chip-bud grafting technique(8), dormant buds from collected samples were grafted onto the indicator cuttings which were pre-rooted in sands and later transferred to soil containing plastic bags in a greenhouse. Approximately one month after grafting, they were then transplanted to indexing field. Development of disease symptoms among these indicator cuttings in relation to leafroll, corky bark or rupestris stem pitting infections were surveyed periodically for 18 months as previously reported (10,23).

ELISA test

Grapevine leafroll associated virus (GLRaV) was purified from naturally infected Black Queen, Kyoho, and Golden Muscate by differential centrifugation with 20% sucrose cushion followed by two times of Cs_2SO_4 density gradient centrifugation as that described by Zee et al.(24). Antisera against the NY-1 isolate (Type III) and CA-4 isolate (Type IV) of GLRaV, a gift from Dr. D. Gonsalves of Cornell University, were used in the tests for disease survey. The γ-globulins were purified from these available antisera and conjugated to alkaline phosphatase as that described by Clark and Adams in 1977 (5). For ELISA, antiserum was preabsorbed with tissue extract from healthy LN-33 as that by Zee et al.(24). Existence of leafroll virus infection in field-grown grapes was performed by direct ELISA technique. About 20 petioles from mature leaves were collected from each surveyed grapevines at the late growing season (November to December) and used for the ELISA test. Petioles obtained from healthy grapevine virus indicator plants Cabernet Franc or LN-33 were used as the control.

Electron microscopy

Plant materials obtained from the grapevine virus indexing field in TTRI were used for the electron microscopic examination. The midrib portion of grapevine leaves were collected during the late growing season from grapevines that showed characteristic disease symptoms of virus infection and plants which appeared to be healthy. To reveal the morphological characteristics of the associated virus, the virus was partially purified from an apparently diseased grapevine according to the method described by Hu et al. (14) and negatively stained by 1% uranyl acetate for electron microscopic observation. For ultrastructural studies of the infected cells, the test samples were cut into approximately 2 × 5 mm pieces, fixed in 2% glutaraldehyde, post fixed in 2% osmium tetroxide, dehydrated in a graded alcohol series, and finally embedded in LR White (17). Ultrathin sections about 60 to 90 nm in thickness were then obtained and stained with uranyl acetate and lead citrate for electron microscopic examination. For immuno-electron microscopic studies, partially purified virus samples were coated on Formvar-film grids, decorated with diluted antiserum at room temperature and then stained with 1% uranyl acetate. The grids were rinsed with distilled water after each step except after staining. A JEOL model JEM-200CX electron microscope was used for the ultrastructural examination.

RESULTS

Electron microscopy of the disease-associated viral agent

The long flexuous closterovirus virus-like particles were consistently observed in either partially purified virus preparations (Fig. 1) or in the ultrathin sections of

the disease tissue (Fig. 2). The estimated particle length of the virus from diseased Black Queen was about 2200 nm, similar to that reported for grapevine leafroll associated virus. The electron microscopy of ultrathin sections of GLRaV infected samples further revealed the presence of at least two kinds of virus particles. In Cabernet Franc which was graft-inoculated with apparently diseased Black Queen, it was about 10 nm in width (Fig. 2A). Whereas in that graft-inoculated with diseased Missouri Riesling, it was about 8 nm (Fig. 2B). The ultrastructural characteristics of the diseased tissue resembled that of typical closterovirus infection (1). The performed immuno-electron microscopy using the NY-1 and CA-4 antisera obtained from Dr. Gonsalves further confirmed the identity of the test virus (data not shown).

Occurrence of GLRV and the serological characteristics

The field indexing assay indicated that great proportion of the main commercially grown cultivars in Taiwan including Kyoho, Italia, Black Queen and Golden Mustcate were all affected by GLRaV (Table 1). Among the samples surveyed, infection by corky bark

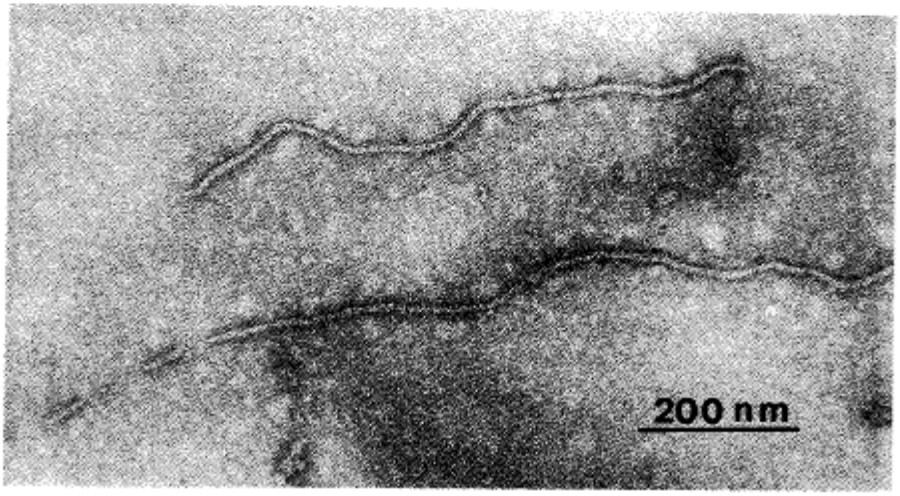

Fig. 1. Electron microscopy of the grapevine leafroll associated virus (GLRaV) partially purified from the stem cortex tissue of diseased Black Queen. The virus particles were negative stained with 1% uranyl acetate.

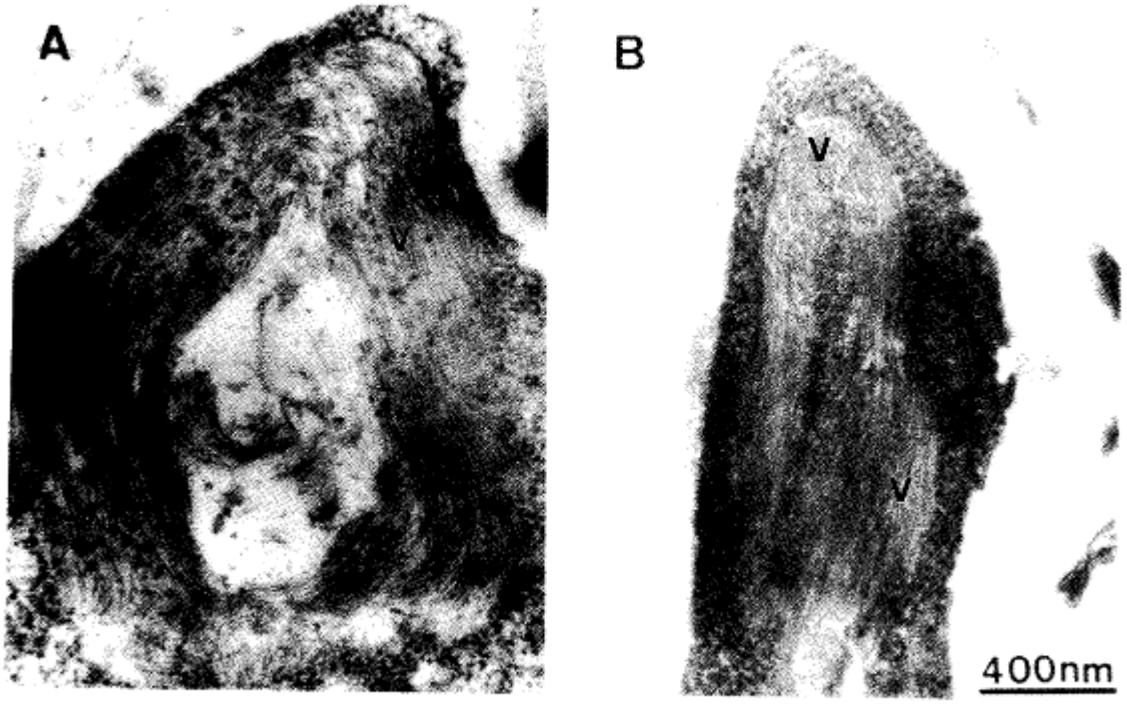

Fig. 2. Detection by electron microscopy the existence of GLRaV particles at 10 nm (A) and 8 nm (B) of width, respectively in ultrathin cross-section of diseased Cabernet Franc petiolar tissues. The virus particles were in aggregates which were restricted only in phloem tissues. (V, virus particles; and bar indicated an estimated length of 400 nm).

TABLE 1. Detection by chip-bud grafting field indexing and enzyme linked immunosorbent assay (ELISA) of the closterovirus infection among the main grapevine cultivars and breeding lines grown in a cultivar preserving field in Taiwan Tobacco Research Institute (TTRI). The indicator grapevines used for the field indexing test included LN-33, Cabernet Franc, St. George and Mission introduced from U. C. Davis; whereas antisera (AS) used for the ELISA included NY-1 (Serotype III) and CA-4 (Serotype IV) were generously given by Dr. Gonsalves of Cornell University. Field indexing results were surveyed 18 months after survived grafting seedlings were transplanted to test field as that routinely practiced in U. C. Davis.

TTRI No.	Cultivars or lines	Indicator Grapevines			ELISA	
		LN-33	Cabernet Franc	Mission	NY-1 AS	CA-4 AS
0108	A301	–	–	nd	–	–
0204	Royalty	R	R	nd	+	–
0205	Ruby Cabernet	–	–	–	–	–
0213	Thompson Seedless	–	–	–	–	–
0301	Rulander	R	R	nd	–	+
0304	Prof. Meiertraube	–	–	–	–	–
0307	Trollinger Klone	nd	–	nd	–	–
0312	Barbera	R	R	nd	–	+
0401	Missouri Riesling	nd	R	R	+	+
0406	Muscate Saint-Vallier	nd	R	nd	–	+
0409	Lemberger	nd	R	nd	–	–
0410	Clairette Mazel	R	R	R	+	+
0505	Seibel 100	–	R	R	–	+
0508	Cabernet Sauvignon	R	R	nd	+	+
0513	Dutchess	nd	–	–	–	–
0601	Black Queen	R	R	R	+	–
0603	Campbell Early	R	nd	nd	–	–
0604	Calzin	R	–	–	–	–
0605	Golden Muscate	nd	R	nd	–	+
0607	Niagara	nd	–	nd	–	–
0611	Chenin Blanc	–	–	nd	–	–
0613	Champion	R	R	R	+	–
0709	Buffalo	nd	R	nd	+	+
0713	D × K151	nd	nd	R	–	–
0803	Gros Guillanme	R	R	nd	–	+
0807	Himrod Seedless	nd	R	R	+	–
0809	Igawa 17	R	R	nd	+	+
0810	Grand Noir	R	R	nd	+	+
0901	Mills	R	R	nd	+	+
0906	Helena	R	R	nd	–	–
0909	Lagrein	R	R	R	+	–
0912	Super Hamburg	nd	R	nd	+	–
1004	Royal Red	nd	R	R	–	+
1005	Kyoho	nd	R	nd	+	+
1007	Muscate Bailey A	–	–	nd	–	–
1107	Petite Boushet	R	R	nd	+	–
1109	Rose D Italia	–	–	–	–	–
1113	Rubired	R	R	nd	–	+
1202	Seibel	nd	nd	R	–	+
1203	Cabernet Sauvignon	R	R	nd	+	–
1205	Chasselas	R	R	R	+	–
1207	Flame Tokay	R	R	nd	+	–
1302	Seibel 13053	nd	–	nd	–	–
1303	Golden Coin	R	R	R	+	–
1305	Italia	nd	R	R	+	+
1403	Koshu	R	R	nd	+	+
1501	Rozaki	R	R	nd	+	–
1601	Neo Muscate/5BB	R	R	nd	+	+
1602	New York Muscate	nd	–	nd	–	–
	Delaware##	nd	R	nd	+	–
	LN-33 (Healthy)	–	–	R or –	–	–

##, Acquired from Grapevine Center of National Chung Hsing University; +, ELISA positive; R, Development of leaf reddening symptoms; –, No symptom development during the test, or ELISA negative; and nd, Not determined.

or Rupestris stem-pitting was not observed. The disease seemed to be also common among the less cultivated varieties like Himrod Seedless, Super Hamburg, Neo Muscate, Champion, Royal Red, Buffalo, and Delaware. During the course of this investigation, the occurrence of GLR was also investigated by ELISA techniques as above described. The ELISA results were generally in accordance to that of field indexing assay in regard to the disease occurrence, although a considerably higher proportion of the disease incidence was observed in the biological indexing analysis using LN-33 and Cabernet Franc as indicator hosts. The discrepancy of detected disease incidence may reflect the presence of serotypes other than that of types III and IV. The serological reactivity of tested samples revealed that GLRaV present on Black Queen grapevine reacted positively with NY-1 antiserum, but had no reactivity with the CA-4 antiserum. In contrast to this, virus present on Golden Muscate reacted positively only with CA-4 while not with NY-1 antiserum. And virus present on Kyoho and Italia reacted strongly with either NY-1 or CA-4 antisera.

Field symptoms

In field, the symptom development on diseased grapevines depended greatly on the cultivation environment and cultivars. The disease symptoms generally become prominent at late growing season. On red grapevine cultivars, diseased leaves with typical leafroll symptoms generally turned reddish. In this investigation, this type of symptoms were commonly observed among the indicator varieties such as Cabernet Franc, LN-33 or Mission (Fig. 3). The reddish rolling leaves were quite distinct as compared to the greenish ones on healthy vines. In the grapevine cultivar collection field at TTRI, the reddish leaf rolling symptoms were also noted on some red grapevine varieties like Koshu, Chassel and Cabernet Sauvignon (Fig. 4, upper picture). In contrast to that on most red grapes, leafroll disease symptoms on white grapes appeared to be fairly inconspicuous. On Himrod Seedless, leaf rolling was generally accompanied with interveinal yellowing symptoms (Fig. 4, lower picture). The main commercial table grape Kyoho was an exception to this among the red cultivars. On Kyoho, the leaf rolling symptoms seemed to be fairly mild; and in stead of turning red, leaf yellowing was sometimes observed at late growing season (Fig. 5). In addition to the leaf symptoms, substantial decline of vine growth was also typical among these red grapevines. On Kyoho, Pinot Noir, Cabernet Sauvignon, Koshu and Chassel, less fruit coloration, retarded and uneven fruit maturation, reduced yield, low sugar content and poor taste were all noticed as deleterious effects during the harvesting period. Among red grapevines tested, Koshu, Chassel, Flame Tokay, and Cabernet Sauvignon seemed to be fairly sensitive to leafroll infection in that even spring bud-break were significantly delayed (Fig. 6).

The impact of leafroll infection appeared to be very prominent for the two-cropping system grapevines in Taiwan (12). Quick decline of the vine growth, obvious reductions of both yield and quality, or in some cases even inability of fruit set, were frequently observed. In a grapevine cultivar collection field in TTRI where the two-cropping system was generally performed, a few Pinot Noir and Cabernet Sauvignon vines were found to be infected by leafroll virus by immunoassay. As compared to that on healthy control, the fruit set on these diseased plants were generally substantially smaller, and the fruit maturation was not even and was generally 2 to 3 weeks late (Fig. 7).

The sensitivity of grapevines to GLRaV infection appeared to vary greatly among different cultivars. As mentioned, the leaf rolling symptoms on Kyoho were fairly mild although substantial deleterious effect on fruit quality and yield were observed (Fig. 8). On the main red wine grapevine cultivar Black Queen, both leaf rolling and reddening were quite evident at the late growing season (Fig. 9); and the disease was also noted by the uneven fruit maturation. Whereas on the main white wine grapevine cultivar Golden Muscate, leafroll associated virus infection resulted no distinct symptoms on leaves; and the growth of the vines remained fairly vigorous.

DISCUSSION

Leafroll associated virus infection was a well known determinative limiting factor for grapevine cultivation (14). The disease occurred worldwide; it affected greatly both quality and yield of grape production and thus was one of the prime target diseases for research activities and quarantine inspections among the main grapevine growing countries. The phloem-limited closterovirus association of GLR was well recognized (14,21). Because of the great difficulty in virus purification, the availability of high titer polyclonal antibodies was in general very limited. The detection of the disease still depended greatly on the time and labor consuming field indexing techniques (11,19).

By the Chip-bud grafting field indexing technique, ELISA and electron microscopy, we have demonstrated in this investigation the wide occurrence of leafroll virus infection among the main commercially grown grapevine cultivars as well as the breeding or rootstock materials. The widespread and the great severity of the disease among the main commercially growing cultivars clearly indicated the urgent need of a suitable and efficient control strategy. Mealybug has been recently reported to be a potential insect vector for the GLRaV transmission (7,18,20). However, in Taiwan, direct

Fig. 3. Detection of symptom expression among tested indicator grapevines Cabernet Franc (upper left), LN-33 (upper right) and Mission (lower picture) in a field indexing trial performed at Taiwan Tobacco Research Institute. The pictures were taken approximately 18 months after chip-bud grafting inoculation. On both Cabernet Franc and LN-33, the typical leaf reddening symptom was observed only on diseased vines; however, on Mission, such symptom sometimes appeared even on the healthy vines.

Fig. 4. A typical symptom expression of GLRaV infection on a red grape variety Cabernet Sauvignon (upper picture) as compared to that on a white grape variety Himrod Seedless (lower picture). The interveinal redding on Cabernet Sauvignon was commonly observed on most red grape varieties. Whereas on most white grape varieties, premature yellowing as that of Himrod Seedless was in stead a commonly observed symptom.

evidence of disease spread in the field awaits to be explored. The widespread of the disease among the grapevines in the tested cultivar and breeding line preserving field was apparently due to the use of infected cuttings as the propagating material and the mixed planting of healthy and diseased grapevines in the same field plot. Reestablishment of vineyard with cuttings from virus free vines appeared to be the main resolution for the control of this disease.

The need of virus free propagating material emphasized the great importance of disease indexing and quarantine measures. The chip-bud grafting field

Fig. 5. The symptoms of GLRaV infection on Kyoho grapevine during a late growing season. The leaf rolling symptom was much less conspicuous as compared to that on Cabernet Franc or LN-33. Moreover, leaf interveinal yellowing was common, while leaf redding typical of that on a red grape variety was never observed.

Fig. 6. A delayed spring bud break on Flame Tokay grapevine due to GLRaV infection. Note that quite substantial canopy has already developed on the vines behind the affected plant.

Fig. 7. Effect of GLRaV infection on the fruit set of Pinot Noir. The reduced yield, and delayed and uneven maturation were both common for the diseased plants. Upper picture, healthy control; and Lower picture, GLRaV affected.

indexing technique and ELISA were the two methods most commonly applied for this purpose (19). In practical application, field indexing was laborious and time consuming; and the development of characteristic disease symptoms was greatly dependent on the cultural conditions (6,7,15). As a comparison, the ELISA technique was apparently a more desirable method in terms of efficiency and precision. However, a prerequisite for this technique application was the availability of suitable antisera (2,13). We have shown in this study the presence of at least two serologically and morphologically distinct isolates of GLRaV among the commercially grown grapevines. The virus has been purified from Kyoho, Golden Muscate, and Black Queen cultivars respectively for polyclonal as well as monoclonal antibody preparation. The reactivity of polyclonal antibody against viruses from Black Queen and Golden Muscate resembled respectively to that

Fig. 8. Effect of GLRaV infection on the fruit quality and yield on Kyoho grapevines. The two berry clusters shown on the left were from GLRaV affected vines; shown on their right were fruits from the compared healthy vines.

Fig. 9. The leaf-reddening symptoms of leafroll infection observed on Black Queen-- the main red wine grape cultivar in Taiwan.

obtained from NY-1 and CA-4 antisera. Whereas the reactivity of that from Kyoho was strongly to NY-1 and weakly to CA-4, implicating the possibility of complexed infection in this test cultivar (unpublished results). Preparation and characterization of monoclonal and polyclonal antibodies against these different virus sources are currently in process.

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摘　　要

陳慧璘[1]、陳脉紀[2]、曾德賜[2]. 1994. 葡萄捲葉毒素病於臺灣葡萄栽培品種與育種材料上之發生概況調查. 植病會刊3:156-167. (1. 臺中縣草湖　菸類試驗所病蟲害系，2. 臺中市　國立中興大學植物病理學系)

爲瞭解葡萄捲葉病毒感染在本省葡萄種源上之發生概況，本研究一則利用葡萄毒素病指示品種行田間芽接檢診，另則利用兔子抗血清行酵素聯結抗體免疫吸附測定法(ELISA)血清檢診，總計調查菸類試驗所品種保存區中50個具疑似捲葉病毒感染病徵之葡萄品種或品系；檢測結果發現其中葡萄捲葉病之罹病率相當高，目前田間大面積推廣栽培之葡萄品種巨峰、義大利、黑后、與金香等均受到感染。這些罹病葡萄，其組織之超薄切片經電顯鏡檢並可發現有長絲狀典型的Closterovirus病毒粒子之存在。ELISA血清檢測結果，進一步顯示第三、第四型捲葉病毒於供試葡萄材料上，發生相當普遍。比較芽接田間檢診及血清檢診結果更發現：部份葡萄材料，經嫁接檢診出現罹病正反應者，卻無法與第三型或第四型捲葉病毒抗血清起反應，顯示本省葡萄材料上，還有其他血清型之捲葉病毒。供試葡萄品種中，爲捲葉病毒感染所表現之典型病徵包括植株春季萌芽延遲，果實成熟延遲，果串變小，成熟不一致，葉片捲曲、變色—白色品種葉片黃化、紅色葡萄品種葉片紅化，紅色鮮食葡萄品種巨峰爲一例外，其罹病植株爲葉片黃化之病徵。此一病害對葡萄生產最顯著之影響爲罹病植株生育明顯退化及產量低降；由本調查結果顯見捲葉病於本省葡萄之發生爲害相當普遍，其潛在威脅甚值得有關單位重視。

關鍵詞：芽接田間病害檢診、Closterovirus病毒、病害發生、ELISA檢定、葡萄捲葉病、血清