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Researches on Virus Diseases of Ornamental Plants in Korea

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韓國에서 花卉植物의 바이러스病 硏究現況

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INTRODUCTION

During the past 15 years, flower and bulb crop roduction has expanded to geographic areas hat allow growing and marketing at less cost than n traditional growing areas. Central and South merica, Africa, the Middle East, and the Far East row a diverse range of species that are vegetatively ropagated as cuttings or bulbs and grown into a inished product that is then exported to traditional onsumer markets in Europe or the United States. lants of more than 60 genera in 30 families are hipped to more than 40 countries through distribuion centers like the United Aalsmeer Flower Auction in the Netherlands (21).

Rapid vegetative propagation of diseased plant as increased the risk of spreading viruses in cutngs, bulbs, and corms. The role of vegetative rnamentals in the occurrence and spread of plant irus diseases is an integral part of the ecological spect of virus transmission. The epidemiology of virus disease in a given area may be a highly omplex phenomenon involving a number of wild osts, commercial crops, and different insects. he epidemiology of cucumber mosaic virus is ood example. A number of elements are associated ith cucumber mosaic epidemics in tomato, pepper, cucumber, lettuce, and other crops (10). Winged aphids acquire virus in late winter and early spring from overwintering virus sources such as garden ornamentals. In the spring, cucumber mosaic virus is transmitted from infected crops to new ornamental plantings and weeds where the virus may remain until fall. During late fall and winter, aphids gradually increase, and the cycle resumes.

Twelve ornamental crops and 50 of the most economically important viruses, viroids, are listed in Table 1. Many of the viruses listed in Table 1 include many of our most important viruses. Therefore, vegetative ornamentals may serve as reservoirs of both viruses and insect vectors.

Lee and La (25) seems to be the first who identified *cymbidium mosaic virus in ornamentals* in Korea. Thirty one viruses in 12 ornamental crops occurring in Korea are listed in Table 1. Many of the viruses listed in Table 1 affect foliage and flower size, number, and color. Improved plant quality has been associated with eliminating one or more viruses from many ornamental species, and selecting healthy plants for vegetative propagation is an important approach to control.

Some virus diseases described as symptomless are improperly named. For example, lily symptomless virus reduces vegetative growth and both flower size and number compared with virus-free

plants derived from tissue culture (1).

Control of virus diseases depends on establishing and maintaining healthy propagation stock. Methods of virus indexing of bioassay, serology, and electron microscopy in research diagnosis or routine indexing are described in this paper. Other approaches to control include meristem-tip culture and reducing spread of insect-borne.

ORNAMENTAL VIRUS SPREAD

There are three classes of viruses of ornamentals based on ecological and phytosanitary considerations(14). The first class includes viruses that have wide host ranges, usually have efficient vectors, and are already widespread. Examples are the cucumoviruses (cucumber mosaic virus group), including strains of cucumber mosaic virus and related viruses, and the nepoviruses (nematode-transmitted group), including tomato and tobacco ringspot viruses. Although viruses in these classes are sap-transmitted, the greatest danger results from aphid transmission of the cucumoviruses and nematode transmission of the nepoviruses. Viruses in the second class infect only one or a few ornamental species and are restricted to the geographic areas where the crop is grown. Some carla-viruses (carnation latent virus group) and poty-viruses (potato virus Y group) and in this category; other examples are carnation mottle virus, carnation ringspot virus, and chrysanthemum stunt viroid. The third class includes viruses that are host-specific and cause symptomless infections. They are not efficiently spread may be geographically restricted.

DIAGNOSIS AND INDEXING

In virus disease control, distinguishing between research diagnosis and routine indexing is important. Research diagnosis is used to determine if a virus is present and to identify it. This often involves laboratory in virus purification, identification, and strain differentiation in small number of samples. Routine indexing is the detection of known viruses and usually involves testing a large numb of samples. Indexing methods should be rapi inexpensive, and easy to perform but still be re able, sensitive, and specific. The choice of indexi procedures may depend on the type of ornament crop and the stage of growth when a test is perfc med.

VIRUS INDEXING PROCEDURES

Bioassay is the primary method used in routi indexing for several of the economically importa viruses in ornamentals. The choice of a particul test procedure is determined by both the vir and the host. One host may have inhibitors th prevent reliable transmission without the use c chemical additives. The same virus may be mo readily transmitted from another host.

Some other factors that influence results of bioassay are the effects of light and temperature o symptom expression and virus content of th naturally infected host and on the susceptibility o the bioassay species. Sap inoculation procedure are used to detect chrysanthemum aspermy, chry santhemum B, carnation mottle, ringspot, latent etched ring, vein mottle, cymbidium mosaic, an orchid tobacco mosaic viruses. Improvements i bioassay procedures include addition of polyethy lene glycol to geranium extracts and 2-mercaptoe thanol to rose leaf extracts. Sampling the species tc be indexed when new growth first appears in th spring is usually desirable and sometimes essential

Serological test procedures for research diagnosis have been used for many years to detect and identify several viruses in ornamentals. Serological tests have the advantage of speed and specificity in disaese diagnosis. Chloroplast agglutination and microprecipitin tests have been employed to detect flexuous rod-shaped viruses, such as carnation latent and vein mottle in carnation and B virus in chrysanthemum. Lily symptomless and tulip breaking viruses have been diagnosed with microprecipitin tests. Immunodiffusion and the enzyme linked immunosorbent assay(ELISA) tests are now being

ied, hower. Immunodiffusion tests were first lapted for detection of small polyhedral viruses. arnation mottle virus can be successfully detected crude sap of carnation using agar double diffusion sts. Narcissus tip necrosis virus can be detected rectly in crude sap from narcissus in agar diffusion sts. Other polyhedral viruses, such as carnation ngspot, cucumber mosaic, tomato and tobacco ngspot, apple mosaic, can usually be detected in ;ar diffusion tests only after the virus is concenated from the naturally infected host. The single mmunodiffusion drop(IDD) test was first deveped for detection of lily symptomless virus (32). he ELISA procedure has significantly extended the ie of routine serological indexing. This method fers great promise for detection of viruses in bulb ssue as well as in leaves. It would be an advantage the routine indexing carried out with the IDD test n lily leaf samples in the summer could be substited by testing the bulbs during the storage period 2). The ELISA test is also used to detect cucumer mosaic and bean yellow mosaic viruses in adiolus (33), narcissus mosaic and tip necrosis iruses in narcissus (2), nerine latent virus in nerine, nd freesia mosaic and bean yellow mosaic viruses n freesia. Routine indexing by ELISA is now perormed with viruses in several flower crops, includng aspermy and chrysanthemum B viruses in chryanthemum (20,27), carnation cryptic and carnation leck viruses in carnation (27), and cymbidium osaic and orchid tobacco mosaic viruses in orchids.

By electron microscopy, virus particles can be bserved in crude sap extracts from some infected pecies. The virus can be recognized and tentatively dentified on the basis of its size and shape. This rocedure, known as negative staining, has been sed to detect cymbidium mosaic virus in orchid 22). The method was slightly more sensitive than he bioassay procedure for detecting this virus.

A recent advance combines serology and electron icroscopy using the technique of immune electron icroscopy(IEM) (28). This method greatly increaes the sensitivity of electron microscopy detection. he technique involves observation of specific binding of antigen and antibody in the electron microscope. This procedure has been applied in several ways. The simplest method is to apply a drop of antiserum to an electron microscope grid and place the freshly cut surface of a leaf in the drop for 1 or 2 seconds. The antiserum clumps the virus particles and coats them with antibody if the antibody in the antiserum is related to the virus. The virus particles are visualized by negatively staining the preparation with an electron-dense stain before the sample is examined. These procedures have been used to detect chrysanthemum virus B, carnation vein mottle and carnation necrotic fleck viruses, orchid tobacco mosaic and cymbidium mosaic viruses, and narcissus latent virus (2). Flexuous rod-shaped viruses can be detected in bulbous iris with direct negative staining of the sap, and the IEM method should be applicable for detecting and identifying iris mild and severe mosaic, bean yellow mosaic, and other rod-shaped viruses in iris, gladiolus, tulip, lily, and other bulb species and to many rod-shaped viruses in flower crops. The IEM method was nearly twice as sensitive as ELISA in detecting arabis mosaic, prunus necrotic ringspot, and strawberry latent ringspot viruses in extracts from rose (34). Clump and the modified Derrick procedure can trap low concentration of virus and be used to detect virus in crude sap preparations. Application of the IEM procedure may be most useful in research diagnosis involving specific virus and strain identification and in routine indexing where a limited number of samples are tested from nuclear stock or plants from tissue culture where very high sensitivity is required.

VIRUS-FREE PLANT

The technique of virus elimination by meristem culture is based on the uneven distribution of viruses in plants as originally described by Limmasset and Cornuet (26). By 1984, over 50 species had been freed of viral infections by meristem culture techniques (15,29,37). In spite of the remarkable success of meristem culture, it still

Table 1. A list of the most economically important 50 viruses or viroids in 12 ornamental crops

Host plant	Virus	Particle size	Geographic distribution
Carnation	Carnation mottle	28nm	Worldwide (Korea, Lee 1981)
	Carnation necrotic fleck	1300nm	Worldwide (Korea, unpubl.)
	Carnation vein mottle	760nm	Worldwide
	Carnation latent	650nm	Worldwide (Korea, unpubl.)
	Carnation etched ring	45nm	Worldwide (Korea, unpubl.)
	Carnation ringspot	30nm	Europe, North America
	Carnation Italian ringspot	34nm	Italy
	Carnation yellow stripe	30nm	
	Carnation cryptic		
	Carnation mottle		
Chrysanthemum	Chrysanthemum aspermy	28nm	Worldwide
	Chrysanthemum virus B	680nm	Worldwide (Korea, unpubl.)
	Chrysanthemum chlorotic mottle		Worldwide
	Chrysanthemum stunt		Worldwide
Dahlia	Dahlia mosaic	50nm	Worldwide (korea, unpubl.)
	Cucumber mosaic	28nm	Worldwide (Korea, unpubl.)
	Tomato spotted wilt	85nm	Worldwide
	Tobacco streak	28nm	Europe, North America, Japan
Freesia	Freesia mosaic	28nm	Europe, North America
	Bean yellow mosaic	750nm	Worldwide (Korea, unpubl.)
	Cucumber mosaic	28nm	Japan
Gladiolus	Bean yellow mosaic	750nm	Worldwide (Korea, Lee 1983)
	Broad bean wilt	28nm	Europe, North America
	Cucumber mosaic	28nm	Worldwide (Korea, Lee 1983)
	Tobacco ringspot	28nm	Worldwide
	Tobacco mosaic	300nm	Worldwide
	Tobacco rattle	80-180nm	Europe, North America
	Tomato ringspot	28nm	Europe, North America
	Tomato spotted wilt	85nm	Europe, North America
Hippeastrum	Hippeastrum mosaic	750nm	Worldwide (Korea, unpubl.)
	Cucumber mosaic	28nm	Worldwide (Korea, Lee 1981)
	Tobacco mosaic	300nm	Europe
	Tomato spotted wilt	85nm	Europe
	Hippeastrum latent	520nm	
	Hippeastrum mosaic	650nm	
Iris (bulbous)	Iris mild mosaic	760nm	Worldwide
	Iris severe mosaic	760nm	Worldwide (Korea, Chang 1982)
	Bean yellow mosaic	750nm	Worldwide
	Turnip mosaic	750nm	Worldwide
	Narcissus latent	650nm	English, Netherlands
	Broad bean wilt	28nm	Worldwide
	Tobacco ringspot	28nm	North America
	Iris fulva mosaic	800nm	
	Iris	Rhabdo	
Lily	Lily symptomless	640nm	Worldwide (Korea, unpubl.)
	Lily virus X	520nm	Netherlands (Korea, unpubl.)
	Citrus tatter leaf	650nm	Japan
	Tulip breaking	760nm	Worldwide (Korea, unpubl.)
	Tobacco rattle	80-180nm	Europe, North America
	Arabis mosaic	30nm	Europe, North America
	Broad bean wilt	28nm	Europe, North America
	Cucumber mosaic	28nm	Worldwide (Korea, unpubl.)

Host plant	Virus	Particle size	Geographic distribution
Lily	Tobacco ringspot	28nm	Europe, North America
	Tomato aspermy	28nm	Worldwide
Narcissus	Narcissus latent	650nm	English, Netherlands
	Narcissus mild mosaic	650nm	Japan
	Narcissus mosaic	550nm	Worldwide (Korea, unpubl.)
	Narcissus yellow stripe	755nm	Worldwide (Korea, unpubl.)
	Narcissus tip necrosis	30nm	Netherlands
	Arabis mosaic	30nm	Worldwide
	Broad bean wilt	28nm	Japan
	Cucumber mosaic	28nm	Worldwide
	Raspberry ringspot	28nm	Europe
	Strawberry latent ringspot	30nm	Europe
	Tobacco rattle	80-180nm	Worldwide
	Tomato blackring	30nm	Europe, Japan
	Tomato ringspot	28nm	North America, Japan
	Narcissus degeneration		Netherlands
	Narcissus season yellow		Netherlands
Orchid	Orchid fleck	150nm	Korea, Japan (Chang 1977)
	Cymbidium mild mosaic	28nm	Korea, Japan (Chang 1978)
	Cymbidium mosaic	475nm	Worldwide (Korea, La 1976)
	Cymbidium ringspot	28nm	English
	Dendrobium mosaic	750nm	Japan, Korea (Chang 1984)
	Habenaria mosaic	750nm	Japan
	Odontoglossum ringspot	300nm	Worldwide (Korea, Chang 1984)
	Spiranthus mosaic	760nm	Korea, Japan (Chang 1985)
	Bean yellow mosaic	750nm	Worldwide (Korea, unpubl.)
	Cucumber mosaic	28nm	Worldwide (Korea, unpubl.)
	Tomato ringspot	28nm	Japan
Rose	Apple mosaic	28nm	Worldwide (Korea, unpubl.)
	Arabis mosaic	30nm	Worldwide
	Prunus line pattern	28nm	Worldwide (Korea, unpubl.)
	Prunus necrotic ringspot	23nm	Worldwide
	Strawberry latent ringspot	30nm	Worldwide
Tulip	Tulip breaking	760nm	Worldwide (Korea, unpubl.)
	Arabis mosaic	30nm	Worldwide
	Cucumber mosaic	28nm	Worldwide (Korea, unpubl.)
	Lily symptomless	640nm	Worldwide
	Tobacco mosaic	300nm	Worldwide
	Tobacco necrosis	26nm	Worldwide
	Tobacco rattle	80-180nm	Europe
	Tomato blackring	30nm	Europe
	Tomato bushy stunt	30nm	Europe, North America
	Tulip virus X	520nm	

remains unclear as to why the shoot apical meristems cotain very little or no virus. Recent evidence suggests that in certain host-virus combinations, viruses are present in high concentrations in the apical meristem (16,35,36). Therefore, a combination of thermotherapy with meristem culture can increase the yield of virus-free plants over meristem culture applied alone. Virus-infected stock plants are treated at 35-40°C constant temperature, and meristem tips are removed from vegetative branches after a few weeks or several months (30). The tips are transferred to a sterile medium where some of the resulting plantlets are usually virus-free.

Recent studies have suggested the suppression of

virus in cultured plant tissues may be effected by the incorporation of the nucleoside analogue ribavirin into the culture medium. This broad spectrum antiviral agent has been shown to act against animal viruses and plant viruses (11,13,19), and was effective in eradicating PVM, PVS, PVX, PVY, CMV, and CLSV (3,4,12,18,31). From these studies, it may be concluded that antiviral chemicals could be used to eliminate viruses during routine propagation of plants through in vitro culture procedures.

Recently, ornamental plant crops have higher unit value than many field crops, and the decorative value and longevity of the finished product are of primary importance for successful marketing in Korea. Many of the fungus and bacterial diseases of bulb and flower crops can be controlled with fungicides and sanitary practices. Virus disease control, based on indexing and nuclear stock propagation, is more difficult to achieve. Virus-free plant programs require large investments of money and personnel.

Controlling virus diseases in vegetatively propagated ornamentals has involved the development and use of a variety of techniques, including bioassay, serology, and electron microscopy. Rapid advances have recently been made in developing the new and more sensitive detection procedures discussed in this paper. These procedures are being applied by major virus-free stock propagations in the world, but not yet in Korea.

The success of virus-free stock programs for ornamental plant crops can be attributed to the close cooperation of industry, scientists, and plant protection officials.

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