Effective Methods for Controlling Chrysanthemum Stunt Viroid©

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INTRODUCTION

The disease caused by chrysanthemum stunt viroid (CSVd) is the most important viral disease in chrysanthemum plants. It causes stunting of growth and reduced flower quality (Fig. 1). Damage caused by CSVd has been increasing since its discovery in Japan in 1977.

A viroid is the minimum pathogen composed of only RNA. Chemical treatments are ineffective against the solid molecular structure. Growers must improve production by preventing infection in plants, thus, maintaining the concentration of CSVd at low level in the environment. Viroid-free plants and resistant cultivars are required for the study.

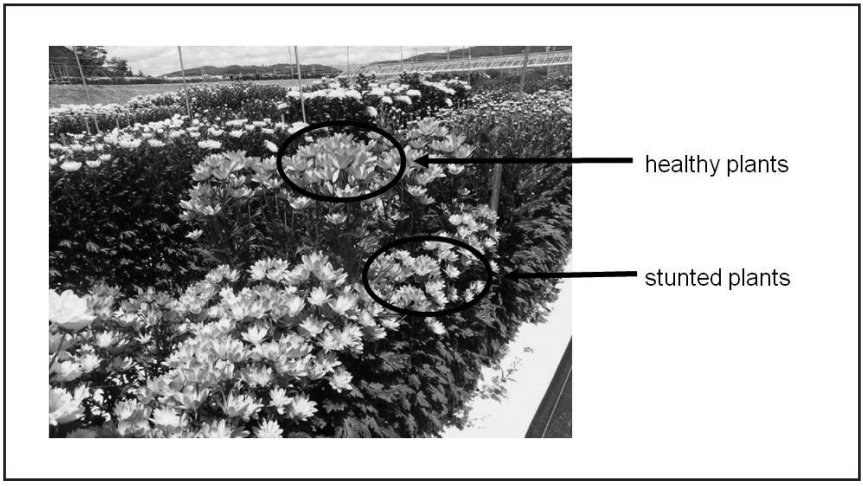

Figure 1. Typical symptom of disease caused by *Chrysanthemum* stunt viroid.

DETECTION OF CHRYSANTHEMUM STUNT VIROID

The symptoms of the disease caused by CSVd in chrysanthemum plants include stunted growth, chlorosis in the leaves, and a change in the time of flowering (early in many case). Chrysanthemum stunt viroid can be detected to identify the disease caused by it. Methods of detection include a bioassay using a test cultivar, return-gel electrophoresis of CSVd RNA, Dot-blot hybridization, reverse transcription polymerase chain reaction (RT-PCR), and reverse transcription loop-mediated isothermal amplification (RT-LAMP). The author of this review has developed a

method of wooden toothpick/direct RT-PCR, RT-LAMP. This method obviates RNA extraction and decreases the risk of contamination with CSVd. As shown in Fig. 2, a wooden toothpick is used to prick the leaf blade or petiole several times and is then soaked in a reaction buffer contained in a microtube. Then RT-PCR or RT-LAMP was performed directly. Thus, easy and sensitive detection of CSVd is possible.

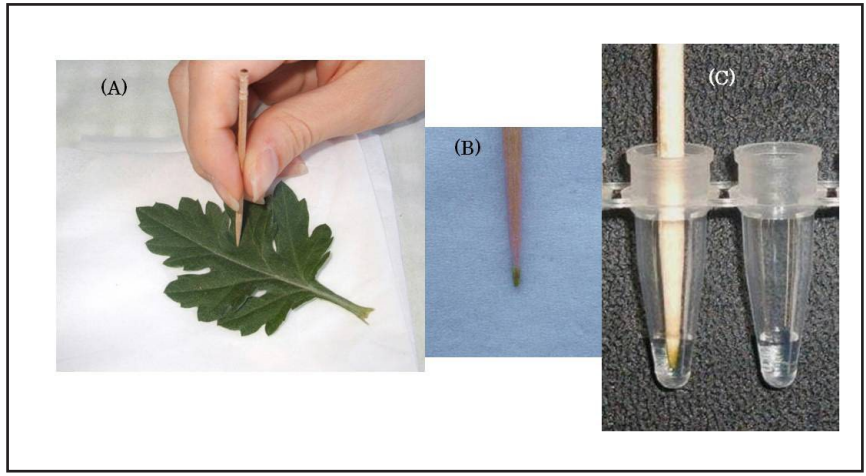

Figure 2. Method of 'wooden toothpick/direct RT-PCR, RT-LAMP' (Ohishi et al., 2005). (A) Pricking leaf blade or petiole several times. (B) Toothpick stained with sap. (C) RT-PCR or RT-LAMP is performed directly in a reaction buffer contained in a microtube.

LATENT INFECTION OF CHRYSANTHEMUM STUNT VIROID

Some cultivars infected with CSVd express no symptoms. The author investigated 79 symptomless plants around 10 years ago, and CSVd was detected in about 90% of samples by RT-PCR/nested PCR, which was the most sensitive detection method at the time. The data thus obtained suggests that CSVd was widespread in Japan.

CONTROLLING CHRYSANTHEMUM STUNT VIROID

Results obtained in the author's experiments until now have indicated that the concentration of CSVd was either very high or very low, in many cases. The concentration of CSVd in susceptible cultivars mostly remains at a low level for some time after infection, but certain factors triggered an increase in the concentration of CSVd and the cultivars begin to show symptoms (Fig. 3). Once the concentration of CSVd increases, the diseased plants never recover. Controlling the factors that raise the concentration of CSVd is difficult. Resistant cultivars, in which the CSVd concentration rarely increases after infection, have been found recently. In the future, resistant cultivars will contribute immensely to reducing the damage of the disease.

Effective Methods for Controlling CSVd Are as Follows.

Application of Resistant Cultivars. All cultivars are likely to be infected with CSVd. However, some cultivars in which the concentration of CSVd dose not increase have been found (Omori et al., 2009). It is possible to estimate resistance to

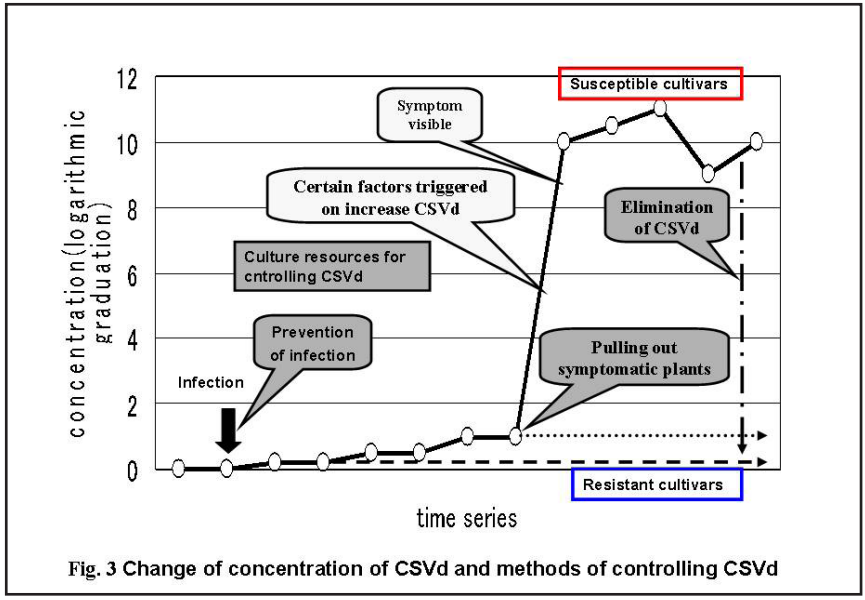

Figure 3. Change of concentration of CSVd and method of controlling CSVd.

CSVd by grafting the assay cultivars onto viruliferous stocks (Fig. 4). Plant breeding companies should inform growers about the resistance of certain plants to CSVd by printing relevant information in a catalog, coupled especially with cultivars that show strong resistance. Improving resistance to CSVd by crossbreeding is a valid approach because the resistance is hereditary (Omori et al., 2009).

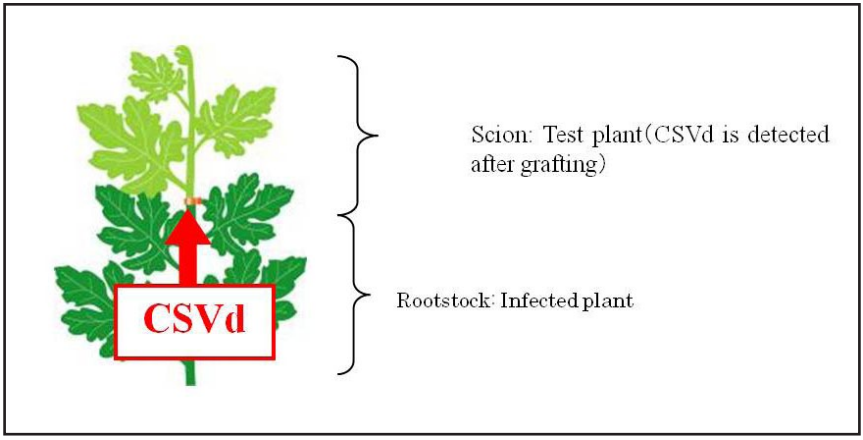

Figure 4. Estimate of resistance to CSVd by grafting (Omori et al., 2009.

Prevention of Infection. It is very important to disrupt the routes of infection to prevent the spread of CSVd. Although it is believed that CSVd is transmitted only by sap, seed transmission should also be considered (Fig. 5). Because CSVd is likely to be transmitted through ovules and pollen, symptomatic plants should never be used as parents. Tools such as pruning scissors should be sterilized thoroughly to prevent infection. Chrysanthemum stunt viroid RNA is heat resistance and can be detected after boiling (Ohishi et al., 2003). For complete sterilization, it is necessary to heat tools until they become red hot. Effective chemicals for sterilization include sodium chlorate, sodium chlorate, sodium hydroxide, and formalin. Among the above mentioned chemicals, sodium chlorate is the easiest to obtain and use; for example, a solution of 5% of active chlorine is used to prepare a bleaching agent.

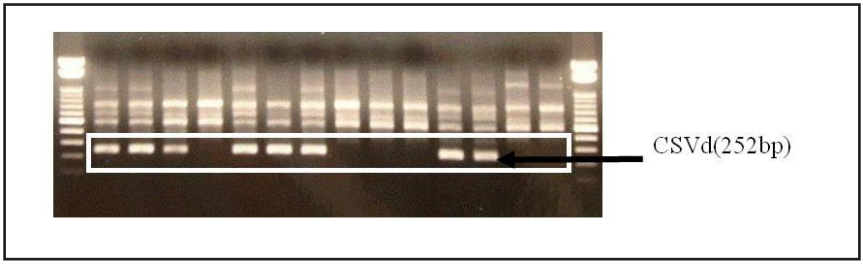

Figure 5. Seed transmission of CSVd (Ohishi et al., 2001). RT-PCR analysis of CSVd in seedlings of infected plants with high concentration crossed with infected plants with low concentration.

Culture Resources for Controlling Chrysanthemum Stunt Viroid. As shown in Fig. 3, the concentration of CSVd in susceptible cultivars increases rapidly and the symptoms become visible thereafter. Although the major factors responsible for the disease caused by CSVd are high temperature, solar radiation, and pinching, the details are unclear. It is important to investigate the factors causing the disease and find the culture resources necessary to control it.

Reduction of Damage Caused by CSVD Pulling Out Symptomatic Plants. Radical steps for controlling CSVd include improvement of resistance of the plants to CSVd and cultivation of CSVd-free plants. Pulling out symptomatic plants is recommended. The damage caused by CSVd can be reduced by pulling out the stunted plants with small-sized leaves in a field or nursery bed as soon as the symptomatic plants are spotted. It is recommended that the symptomatic plants should be burned. Spraying herbicide on the stunted plants and burying them are also considered effective methods.

Elimination of CSVD from Infected Plants. Raising CSVd-free plants from susceptible cultivars is the most effective way of controlling the disease. It is difficult to eliminate CSVd by a shoot apical meristem culture. Recently, Hosokawa et al. (2004) reported that CSVd-free chrysanthemum can be obtained by shoot regeneration from a leaf primordium-free shoot apical meristem dome attached to a root tip of cabbage (Fig. 6).

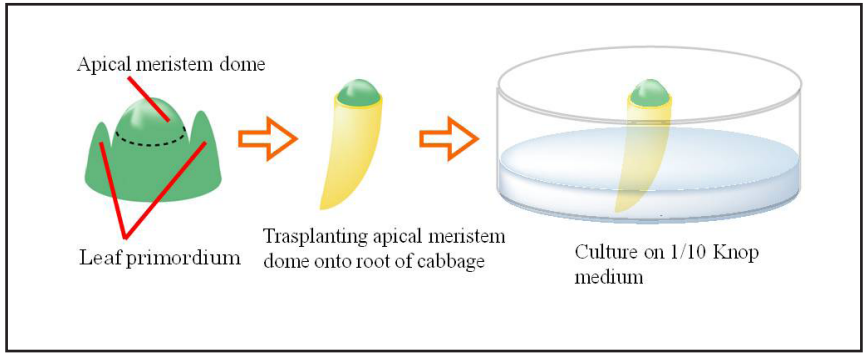

Figure 6. Elimination of chrysanthemum stunt viroid from an infected chrysanthemum cultivars (Hosokawa et al., 2004).

CONCLUSION

Damage due to CSVd has spread all over Japan and it is feared to increase if effective measures are not taken. It is recommended that growers and nurseries sterilize their tools, pull out symptomatic plants, and use resistant cultivars.

LITERATURE CITED

Hosokawa, M., A. Otake, K. Ohishi, E. Ueda, T. Hayashi, and **S. Yazawa.** 2004. Elimination of chrysanthemum stunt viroid from an infected chrysanthemum cultivars by shoot regeneration from a leaf primordium-free shoot apical meristem dome attached to a root tip. Plant Cell Rep. 22:859–863.

Ohishi, K., Y. Okumura, and **K. Morioka.** 2001. Transmission of chrysanthemum stunt viroid through seed of *Chrysanthemum*. Jour. Japan. Soc. Hort. Sci. 70 (Suppl.2):192.

Ohishi, K., Y. Okumura, and **K. Morioka.** 2003. Extraction of CSVd-RNA by boiling for Dot blot hybridization. Hort. Res. 2:51–54.

Ohishi, K., S. Fukuta, and **T. Ohya.** 2005. Detection of viruses and viroids from *Chrysanthemum* by RT-PCR and RT-LAMP using tooth-pick. J. Japan. Soc. Hort. Sci. 74(Suppul.1):466.

Omori, H., M. Hosokawa, H. Shiba, N. Shitsukawa, K. Murai, and **S. Yazawa.** 2009. Screening of *Chrysanthemum* plants with strong resistance to chrysanthemum stunt viroid. J. Japan. Soc. Hort. Sci. 78(3):350–355.