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Effect of Temperature on the Concentration of *Chrysanthemum stunt viroid* in CSVd-infected Chrysanthemum

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CSVd-infected chrysanthemum plants grown under 10°C or 15°C growth chamber for 2 months resulted a higher dilution endpoint of template RNA for Reverse transcription and polymerase chain reaction (RT-PCR) than those grown for 1 month: 10^{-4} (1.35×10^{-2} ug/ml) for 1 month, and 10^{-3} (1.35×10^{-1} ug/ml) for 2 months. Independent experiment, shoots cut from CSVd (*Chrysanthemum stunt viroid*)-infected chrysanthemum plants grown under 10°C or 20°C growth chamber for 2 months showed the same CSVd concentration as control (30°C) at 8 weeks after moving them to normal green-house condition (30°C). From those results, it was concluded that even though the concentration of CSVd was reduced in plants grown at low temperatures, when they were moved to normal glass-house temperature CSVd concentration increased to that of untreated plants in 8 weeks. This conclusion was supported by the rapid replication of CSVd in chrysanthemum after infection.

Keywords **:** Chrysanthemum, CSVd, low temperature

Chrysanthemum is an important cut-flower world-wide. They are grown in all Provinces of Korea for cut-flowers or as pot-flowers: approximately 766 ha of chrysanthemum plants are grown for cut-flowers and 64 ha for pot-flowers in Korea (Ministry of Agriculture and Forestry, 2004). Part of the production has been exported to Japan. The amount of export has been increasing every year. It was totaled $9.3 million in 2004 (Ministry of Agriculture and Forestry, 2004).

Chrysanthemum stunt viroid (CSVd) is the most important viral disease in chrysanthemum worldwide. It causes yellowing, early flowering, shortening of plant height by 2/3 to 1/2, reduction of leaf size and flower size (Chung et al., 2005; Dusi, 1990; Kusunoki et al., 1993). Around 0~66.8% of chrysanthemum plants are infected with CSVd in Korea (Chung et al., 2005).

Low temperature was effective in eliminating viroids from *Potato spindle tuber viroid* (PSTVd)-infected potatos and CSVd-infected chrysanthemum plants (Cichal and Kryczyński, 1987). CSVd or *Chrysanthemum chlorotic mottle viroid* (CChMVd) became inactivated increasingly with the prolonged low-temperature treatment at 5°C combined with meristem tip culture (Paludan, 1985).

In this study possibilities of reducing damages caused by CSVd by growing CSVd-infected chrysanthemum plants in low temperature before preparing cuttings from them have been examined.

Materials and Methods

Plant materials. CSVd-infected chrysanthemum cultivars Sharotte and Kasandra were collected from commercial green-house in Goyang, Gyeonggi Province and Yesan, Chungnam Province, respectively. They were maintained in green-house and propagated by cutting.

Low temperature treatment. Growth chamber condition: Light intensity was 10 klux, humidity 75~80% and light period 16 hours. CSVd-infected chrysanthemum plants were grown at 10, 15, 25, 35°C for 1 month or 2 months in growth chamber and then CSVd dilution end point of template RNA for Reverse transcription polymerase chain reaction (RT-PCR) was compared between them. Independent experiment, CSVd-infected chrysanthemum plants were grown under 10°C and 20°C for 2 months. Shoot tips (1.5~2 cm) cut from them were immediately grafted on healthy chrysanthemum after finishing low temperature therapy and them grew them under normal glass-house (30°C). Twelve plants per cultivar were grafted. Eight weeks after grafting, the concentration of CSVd was compared with untreated plants using RT-PCR.

Dissemination by CSVd-sap contaminated knife. To examine how rapidly CSVd replicate in plants after infection, days required for detection of CSVd by RT-PCR from chrysanthemum after dissemination with contaminated knife was investigated. Dissemination of CSVd on chrysanthemum by knife was as follows: Cutting CSVd-infected plants by knife, and then healthy chrysanthemum

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plants were cut using the knife. Transmission rate by contaminated knife was average of 40 events.

Extraction of RNA and RT-PCR conditions. RNA was prepared with 0.1 g of leaves using CF11 cellulose (Whatman) according to a method described previously (Shiwaku et al., 1996). Pellet was resuspended in 50 ul of nuclease-free water. Primers and RT-PCR conditions were described in the previous report (Chung et al., 2005).

Results

Concentration of CSVd in chrysanthemum grown under low temperature. Dilution end point of template RNA, extracted from CSVd-infected chrysanthemum grown under 10, 15, 25 and 35°C in 1 month, for RT-PCR was $10^{-4} \sim 10^{-5}$ (Table 1, Fig. 1). In prolonged treatment of 2 months, dilution end point of template RNA became lowered to 10^{-3} for 10°C or 15°C, whereas 10^{-4} in 25°C or 35°C treatment (Table 1). In cv. Sharotte, CSVd concentration was lowered in 35°C compared with 25°C (Fig. 1).

Concentration of CSVd after low temperature treatment. Dilution end point of template RNA for RT-PCR was similar to that of untreated plants in 8 weeks after finishing low temperature treatment (Table 2), indicating presence of similar level of CSVd in those plants.

Table 1. Dilution end point of template RNA for RT-PCR with CSVd-infected chrysanthemum according to temperatures treated and the duration

Cultivar	Duration (mon)	Temperature (°C)	Dilution end point of RNA		
			10^{-3}	10^{-4}	10^{-5}
Kasandra	1	10	3/3[a]	3/3	2/3
		15	3/3	3/3	2/3
		25	3/3	3/3	2/3
		35	3/3	3/3	3/3
	2	10	3/3	0/3	0/3
		15	3/3	0/3	0/3
		25	3/3	3/3	0/3
		35	3/3	3/3	0/3
Sharotte	1	10	3/3	0/3	1/3
		15	3/3	0/3	2/3
		25	3/3	3/3	3/3
		35	3/3	3/3	1/3
	2	10	3/3	0/3	0/3
		15	3/3	0/3	0/3
		25	3/3	3/3	0/3
		35	3/3	3/3	0/3

[a] Number of positive samples/Number of tested samples.

Table 2. Concentration of CSVd in shoot tip-grafted chrysanthemum cut from CSVd-infected chrysanthemum plants cultivated under 10°C and 20°C for 2 months followed by growing in green-house (30°C) for 8 weeks[a]

Cultivar	Temperature (°C) treated	Dilution end point of template RNA for RT-PCR
Sharotte	10±1	10^{-4}
	20±1	10^{-4}
	30±1 (green-house)	10^{-4}
Delmonte	10±1	10^{-4}
	20±1	10^{-4}
	30±1 (green-house)	10^{-4}

[a] Size of shoot tip grafted was 1.5~2 cm.

Table 3. Weeks required for detection of CSVd by RT-PCR from chrysanthemum after dissemination with CSVd-contaminated knife, and transmission rate of CSVd by knife

Weeks after dissemination	Transmission rate (%)
2	0.0
3	0.0
4	85.7

Days required for detection of CSVd after infection. CSVd was detectable by RT-PCR in 4 weeks after dissemination by contaminated knife (Table 3). Transmission rate of CSVd by knife was 85.7%.

Discussion

It has been known that high temperature favors the replication of viroid RNA (Kassanis, 1954; Mühlbach Sänger, 1977; Nyland et al., 1969). Viroid free potato and chrysanthemum plants were obtained from meristem-tips excised from *Potato spindle tuber viroid*-infected potato plants and from chrysanthemum plants infected with CSVd or CChMVd after 6 months therapy in a growth chamber at 5°C (Cichal and Kryczyński, 1987). We examined whether low temperature treatment of CSVd-infected chrysanthemum before planting would be effective in reducing damages by CSVd.

According to increasing the duration of low temperature treatment, dilution end point of template RNA for RT-PCR became lowered (Table 1). Meanwhile, shoots grown at normal green-house temperature followed by 2 months of low temperature treatment at 10°C or 20°C showed similar level of CSVd was detected as untreated ones, indicating that low temperature treatment of CSVd-infected chrysanthemum before planting was not effective for diminishing damages caused by CSVd. Above result concluded that although the concentration of CSVd might be reduced with prolonged low temperature treatment, CSVd would

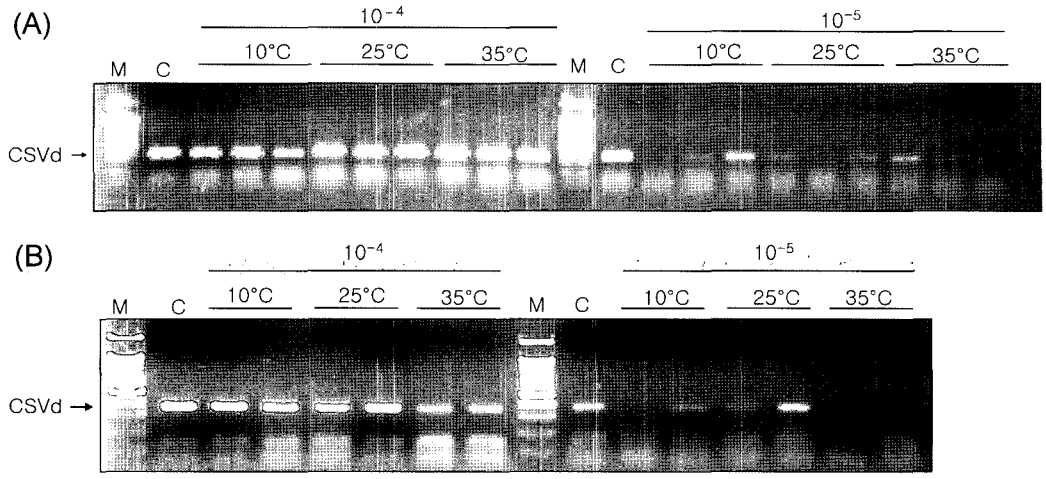

Fig. 1. Concentration of CSVd in infected chrysanthemum plants (A, 'Kasandra'; B, 'Sharotte') cultivated under respective temperature for 1 month. Letters above the upper bars indicate dilution degree of template RNA for RT-PCR.

replicate abruptly after back to normal temperature. This assumption was supported by the rapid replication of CSVd as to be detectable in 4 weeks by RT-PCR after infection.

Growing conditions at 25~28°C reduced days required for symptom development of CSVd-infected chrysanthemum than that grown under 30°C (Bachelier et al., 1976). In the present study lower concentration of CSVd in 'Sharotte' treated at 35°C than at 25°C (Fig. 1) was accordance with the above report, suggesting that exceed high temperature unfavorable for the replication of CSVd. Also response to high temperature was cultivar dependent.

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