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Incidence of *Coleus blumei viroid* 1 in Seeds of Commercial *Coleus* in Korea

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A viroid was detected from symptomless *Coleus blumei* cultivar 'Kong Scarlet'. It consisted of 249 nucleotides (GenBank accession no. EU410620), which was 100% identical to a *Coleus blumei viroid* 1 (CbVd 1) reported from China (GenBank accession no. DQ178399), indicating that the viroid was an isolate of CbVd 1. Attempts were made to determine if commercial *Coleus* seeds were infected with CbVd. Infection rates in seedlings of the 14 commercial cultivars of *Coleus* ranged from 0 to 100%. CbVd 1 caused discoloration and growth retardation in some cultivars, but is symptomless in others. These results indicated that *Coleus* in commercial markets in Korea is highly infected with CbVd.

Keywords **:** *Coleus blumei viroid* 1, seed transmission

Coleus plants that have ornamental leaves are descended from *Coleus blumei*, which has nettle-like, bronze-colored leaves. These plants are natives of Indonesia and Africa. They can be raised from seed or stem cuttings. The colors of their foliage range through yellow, red, crimson, and pink.

Viroids infecting *Coleus* have been found in *Coleus* in Brazil (Fonseca et al., 1989), Germany (Spieker et al., 1996a), Canada (Singh and Boucher, 1991), Japan (Ishiguro et al., 1996) and China (Li et al., 2006). Four kinds of viroid has been reported: *Coleus blumei viroid* 1 (CbVd 1) (Ishiguro et al., 1996; Spieker et al., 1990), CbVd 2 (Spieker, 1996b), CbVd 3 (Spieker, 1996b) and Coleus yellow viroid (CYVd) (Fonseca et al., 1989, 1994).

Since *Coleus* is a common ornamental plant in Korea and it originated from seeds produced from Japan and the United States, attempts were made to determine if commercial *Coleus* seeds being sold in Korea are infected with CbVd.

Materials and Methods

Source of *Coleus*. A *Coleus* cultivar 'Kong Scarlet' was collected from a flower shop (Fig. 1). The plant was a source *Coleus* of a CbVd 1-K identified in this study. Seeds of 14 commercial *Coleus* cultivars were bought from a seed company in Korea. Those seeds were produced from Japan or United States. They were used for the survey of infection rate of seeds by CbVd.

Preparation of RNA for reverse transcription and polymerase chain reaction (RT-PCR). RNA was prepared with 0.1 g of leaves using CF11 cellulose according to a method described previously (Shiwaku et al., 1996). Pellet was resuspended in 40 μl of nuclease-free water.

Primers and RT-PCR conditions. A pair of primer (CbVd-chi F: 5'-TGGATCCAGCGCTGCAACGGAATC-CA-3'; CbVd-chi R: 5'-TTGGATCCGCCAGGGAACCC-AGGTAAG-3') was used for amplification of *Coleus blumei viroid* 1 Korean isolate. To confirm the sequence of the primer regions, an additional set of primer, forward (CbVd-K F: 5'-TGGCTCGAACTGACTAGAACGGT-3') and reverse (CbVd-K R: 5'-AGCTCGTTTAAGCTGA-ACTAGGGT-3') was used for RT-PCR.

Complementary DNA (cDNA) synthesis was accomplished as follows: Two μl of RNA (100 ng/μl) and 2 μl of 10 pm reverse primer was heated at 70°C for 5 min followed by adding 1×PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 5 mM $MgCl_2$, 1 mM each dNTP, 1 μl of reverse transcriptase (Promega, USA) and 1 μl of RNase inhibitor (1 U/μl) on ice and incubated at 37°C for 1 hr.

The PCR reaction mixture contains 20 μl of cDNA, 1×PCR buffer, 0.5 mM of each dNTP, 2 μl of 10 pm each primer, 4 mM $MgCl_2$ and 2.5 U GoTaq DNA polymerase (Promega, USA). Forty PCR cycles were conducted in PTC-0220 Perlitier Thermal Cycler (MJ Research, USA). The thermal conditions were as follows: denaturation at 94°C for 30 sec (2 min for the first cycle), annealing at 50°C for 1min and extension at 72°C for 40 sec and additional extension at 72°C for 10 min.

Determination of nucleotide sequences. Nucleotide sequences of CbVd 1-K were determined according to a method described previously (Chung et al., 2006).

Determination of infection rate of seeds by CbVd.

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Infection rate of commercial seeds by CbVd was determined from seedlings using RT-PCR. RT-PCR was conducted using a primer pair CbVd-K F/CbVd-K R.

Infectivity of *in vitro* RNA transcripts. To test the infectivity of the CbVd 1-K, RNA transcripts were generated by *in vitro* transcription of a plasmid DNA containing a full length CbVd 1-K cDNA insert. Template plasmid DNA was linealized by digestion with *Sal* I restriction enzyme followed by phenol extraction. RNA transcripts were prepared using mMESSAGE mMACHINE T7 according to manufactures' instruction (Ambion, USA). Five of healthy Coleus cv. 'Highway magic' plants were inoculated with 20 μl of RNA transcripts per plant after wounding stems using a blazer.

RNA was prepared from the inoculated plants 1 month after inoculation, and RT-PCR was conducted using a primer pair CbVd-K F/CbVd-K R. PCR products were cloned and nucleotide sequences were determined according to a method described previously (Chung et al., 2006).

Damages of Coleus cultivars by CbVd 1-K. The leaf size of 10 *Coleus* cultivars including 'Highway Rose' was measured at flowering stage. Eighteen to twenty plants per cultivar were used.

Results

Nucleotide sequences of CbVd1-K. Full length nucleotide sequences of a CbVd isolate was determined from *Coleus* cv. 'Kong Scarlet' (Fig. 1) and was submitted to the GenBank under the accession number of EU 410620. The CbVd isolate was designated as CbVd 1-K in this study. It consisted of 259 nt and showed 100% identical to a CbVd 1 from China (GenBank accession no. DQ178399).

Fig. 1. Coleus cultivar 'Kong Scarlet', source plant of *Coleus blumei viroid* 1 Korean isolate (CbVd 1-K).

Table 1. Infection rate of *Coleus* seedlings by *Coleus blumei viroid* 1-K

Cultivars	No. of seedlings tested	No. of seedlings Infected	Infection rate[a] (%)
Highway Rose	32	16	50.0
Highway Magic	32	0	0.0
Highway mixture	16	7	43.8
Highway Lemon	18	1	5.6
Highway Red Velvet	20	1	5.0
Wizard Golden	20	10	50.0
Wizard Scarlet	24	24	100.0
Wizard Rose	40	40	100.0
Wizard Velvet Red	20	6	30.0
Wizard Jade	20	15	75.0
Kong Green	20	5	25.0
Kong Red	20	15	75.0
Kong Mosaic	20	12	60.0
Kong Scarlet	20	9	45.0

[a]Detection of CbVd1-K was conducted using RT-PCR with a primer pair CbVd-K F/CbVd-K R.

Infection rate of seedlings with CbVd. CbVd infection was surveyed from seedlings of 14 commercial coleus cultivars (Table 1). About 47.5% of seedlings tested were infected. Meanwhile all seedlings of 'Highway magic' tested were not infected. Nucleotide sequences determined from 14 cultivars of seedlings were 100% identical to CbVd 1-K (EU 410620).

Infectivity of *in vitro* RNA transcripts. Infectivity of RNA transcripts on 'Highway magic' was determined by RT-PCR at 1 month after inoculation. One out of five plants was infected by CbVd 1-K RNA transcripts. Nucleotide sequences were 100% identical to CbVd 1-K.

Damages of Coleus cultivars by CbVd. CbVd 1-K faded color and reduced size of leaves in 'Highway Rose' seedlings, but had no effect in others (Table 2, Fig. 2).

Discussion

CbVd caused chlorosis or purple pigmentation in leaves of some *Coleus blumei* cultivars, but was symptomless in some other plants (Fonseca et al., 1989). As have been proposed by Fonseca et al. (1989), faded color was associated with CbVd 1-K in pink colored 'Highway Rose', meanwhile yellow spots in leaves of yellowish green cultivars was irrelevant to CbVd 1-K in this study. Most cultivars examined were not affected by CbVd 1-K in growth, as was a previous report (Fonseca et al., 1989; Singh and Boucher, 1991). Only cultivar 'Highway Rose'

Fig. 2. Growth of *Coleus* cultivar 'Highway Rose', as affected by *Coleus blumei viroid* 1-K (CbVd 1-K). A: CbVd 1-K- infected plants; B: healthy plants.

Table 2. Growth of Coleus cultivars as affected by *Coleus blumei viroid* 1-K

Cultivars	Division	Leaf color	Leaf size[a]
Highway Rose	Healthy	Dark pink	9.5
	Diseased	Faded light pink	8.7*
Highway Lemon	Healthy	Yellow	8.9
	Diseased	Yellow	8.9 ns
Wizard Golden	Healthy	Yellowish green	10.5
	Diseased	Yellowish green	10.5 ns
Wizard Rose	Healthy	Dark pink	9.5
	Diseased	Faded light pink	9.5 ns
Wizard Velvet Red	Healthy	Dark purple	8.1
	Diseased	Dark purple	8.5 ns
Wizard Jade	Healthy	Yellowish green	8.7
	Diseased	Yellowish green	8.9 ns
Kong Green	Healthy	Yellowish green	21.5
	Diseased	Yellowish green	21.0 ns
Kong Red	Healthy	Purple/Red	9.1
	Diseased	Purple/Red	9.2 ns
Kong Mosaic	Healthy	Red/Yellow/Green	15.5
	Diseased	Red/Yellow/Green	15.8 ns
Kong Scarlet	Healthy	Dark Purple	9.5
	Diseased	Light purple	9.5 ns

[a]*, not significantly different at $p \leq 0.05$; ns, not significant

was dwarfed by CbVd 1-K. In 'Wizard Rose', all seedlings examined were infected with CbVd. But there was a variance in leaf color. Some of them were normal dark pink, but the others were faded, assuming that leaf color was affected by CbVd 1-K in case of pink colored cultivar.

Percentage of seed infection varied with cultivars. This result was consistent with the findings of Singh and Boucher (1991) where infection rates in seed lots from 4 commercial sources of *Coleus* ranged from 16 to 68%. In this study, all seedlings of 'Highway magic' tested were CbVd free, meanwhile 100% of seedlings of both 'Wizard Scarlet' and 'Wizard Rose' was infected. One possible explanation for the variable infection rate of 0 to 100% is infection status of the parental combinations used for crossing. In previous reports (Chung and Pak, 2008; Singh, 1970), a relatively high incidence of seed transmission occurred when both parents were infected. When the male parent was infected the incidence of infected progeny was higher than when the female parent was infected.

We tried to examine if 'Highway Magic' is resistance to CbVd 1-K. Five plants of the cultivar were mechanically inoculated with sap of CbVd 1-K-infected *Coleus*. 'Highway Rose' was used as a susceptible control. When they were inoculated with CbVd 1-K, 1 out of 5 'Highway Magic' and 5 out of 5 'Highway Rose' were infected in 45 days post inoculation (data not shown). This result indicated that 'Highway Magic' is resistance to CbVd 1-K. From the results we assumed that in case of 'Highway Magic', parental plants used for crossing might not be infected with CbVd, giving 0% infection of seedlings (Table 1).

Infection of commercial seeds with CbVd 1-K was high in Korea. Sequence comparison revealed that all 13 cultivars tested in this study were infected with CbVd 1-K which is 100% identical to a Chinese isolate CbVd 1 (Li et al., 2006). This result was probably caused by the fact that China also used the Wizard series cultivars as we did in this study. These results showed that new viroid has been spread through imported seeds that it needs for a certification

program to protect the spread of CbVd in Korea.

References

Chung, B. N., Kim, D. C., Kim, J. S. and Cho, J. D. 2006. Occurrence of Chrysanthemum chlorotic mottle viroid in chrysanthemum (*Dendranthema grandiflorum*) in Korea. *Plant Pathol. J.* 23:334-338.

Chung, B. N. and Pak, H. S. 2008. Seed transmission of Chrysanthemum stunt viroid in Chrysanthemum. *Plant Pathol. J.* 24:31-35.

Fonseca, M. E. N., Boiteux, L. S., Singh, R. P. and Kitajima, E. W. 1989. A small viroid in Coleus species from Brazil. *Fitopatol. Braz.* 14:94-96.

Fonseca, M. E. N., Marcellino, L. H., Kitajima, E. W. and Boiteux, L. S. 1994. Nucleotide sequence of the original Brazilian isolate of coleus yellow viroid from *Solenostemon scutellarioides* and infectivity of its complementary DNA. *J. Gen. Virol.* 75:1447-1449.

Ishiguro, A., Sano, T. and Harada, Y. 1996. Nucleotide sequence and host range of coleus viroid isolated from coleus (*Coleus blumei* Benth.) in Japan. *Ann. Phytopathol. Soc. Jpn.* 62:84-86.

Li, S. F., Su, Q. Guo, R., Tsuji, M. and Sano, T. 2006. First report of *Coleus blumei* viroid from coleus in China. *P. Pathol.* 55:565.

Shiwaku, K., Iwai, T. and Yamamoto, Y. 1996. Cloning and nucleotide sequence of *Chrysanthemum stunt viroid. Hyogo Pre. Agric. Inst.* (*Agriculture*) 44:1-4.

Singh, R. P. 1970. Seed transmission of potato spindle tuber virus in tomato and potato. *Amer. Potato. J.* 47:225-227.

Singh, R. R. and Boucher, A. 1991. High incidence of transmission and occurrence of a viroid in commercial seeds of Coleus in Canada. *Plant Dis.* 75:184-187.

Spieker, R. L. 1996a. A new sequence variant of *Coleus blumei viroid* 1 from the *Coleus blumei* cultivar 'Rainbow Gold'. *Arch. Virol.* 141:2153-2161.

Spieker, R. L. 1996b. *In vitro*-generated 'inverse' chimeric *Coleus blumei viroids* evolve in vivo into infectious RNA replicons. *J. Gene. Virol.* 77:2839-2846.

Spieker, R. L., Haas, B., Charng, Y., Freimuller, K. and Sänger, H. L. 1990. Primary and secondary structure of a new viroid species (CbVd 1) present in the *Coleus blumei* cultivar 'Bienvenue'. *Nucl. Acids Res.* 18:3998.