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Occurrence of Cherry Necrotic Rusty Mottle virus (CNRMV) and Cherry Green Ring Mottle Virus (CGRMV) on Sweet Cherry

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From 2010 to 2012, abnormal ring spot symptoms had been observed on the pericarps of sweet cherry cv. 'Sato Nishiki' cultivated in Gyeongbuk Province, Korea. The abnormal cherries were diagnosed with CNRMV and CGRMV using RT–PCR and sequencing analysis. Homology analysis revealed that CNRMV isolates had the highest homology (97.4–98.9%) with a CNRMV isolate from Japan (EU188439), while CGRMV isolates had the highest homology (97.8–98.9%) with a CGRMV isolate from Canada (FJ402843). This is the first report of the occurrence of CNRMV and CGRMV on sweet cherry in Korea.

Key words: sweet cherry, virus, RT–PCR, homology

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is widely cultivated worldwide and the cultivation area has recently increased in Korea (Wünsch and Hormaza, 2004; Kang and Cho, 2007). During the cultivation periods, sweet cherry is susceptible to infection by several viral agents including cherry green ring mottle virus (CGRMV), cherry necrotic rusty mottle virus (CNRMV), Little cherry virus–1 (LChV–1), Little cherry virus–2 (LChV–2) and cherry virus A (CVA) (Keim–Konrad and Jelkmann, 1996; Parker *et al*., 1976; Rott and Jelkmann, 2001; Wadley and Nyland, 1976). CNRMV is a severe viral disease on sweet cherry that has been reported in North America, Europe, New Zealand and Japan (Wadley and Nyland, 1976; Isogai *et al*., 2004). CNRMV produces brown angular necrotic spots, rusty chlorotic and shot holes symptoms on leaves and blisters, gum pockets and general necrosis on bark (Rott and Jelkmann, 2001). In addition, CGRMV infects several *Prunus* species including sour cherry (*Prunus cerasus* L.), flowering cherry (*P. serrulata* Lindl.), peach (*P. persica* Batsch), sweet cherry (*P. avium* L.) and apricot (*P. armenaca* L.) and has been reported in Europe, North America, Japan and China (Parker *et al*., 1976; Isogai *et al*., 2004; Zhou *et al*., 2011). CGRMV produces yellow mottling and ring–like band symptoms on leaves, as well as misshapen, bitter and unmarketable fruit on Montmorency sour cherry (Parker *et al*., 1976); however, sweet cherry, apricot and peach are known to be symptomless hosts of CGRMV (Zhang *et al*., 1998).

To date, ring spot symptoms on the leaf or pericarp of stone fruit species have primarily been reported in association with plum pox virus (PPV), prune dwarf virus (PDV), prunus necrotic ringspot virus (PNRSV) and CGRMV (Karayiannis, 1989; Cropley *et al*., 1964; Uyemoto and Scott, 1992; Parker *et al*., 1976). Moreover, since cherry trees were first introduced in Korea, there have been no reports of the viral disease on cherry to date. In May 2010, we observed ring–like symptoms on cherry pericarp while surveying the major cherry cultivation regions in Daegu, Gyeongsan and Gyeongju. RT–PCR assay of the pericarp of abnormal fruits revealed that collected abnormal cherries were co–infected with CNRMV and CGRMV. Therefore, we report here the first occurrence of CNRMV and CGRMV in Korea and phylogenetic analysis based on its coat protein gene sequences.

MATERIALS AND METHODS

Sample collection

During the harvest of sweet cherries from May to July of 2010 to 2012, we surveyed cherry orchards located in Daegu (DG), Gyeongsan (GS) and Gyeongju (GJ) region. In the course of our investigation, we collected over 50 abnormal cherries which appeared ring spot symptoms on pericarp, locally. In addition, all investigated cherry trees were confirmed to be cv. 'Sato Nishiki' (*P. avium* cv. Sato nishiki).

Reverse transcription–polymerase chain reaction (RT–PCR) assays for the diagnosis of ring spot symptoms

Total RNA was extracted from abnormal pericarps found to have ring spot symptoms using an RNeasy mini kit (Qiagen, USA) according to the manufacturer's protocol. RT–PCR was then conducted to detect PPV, PDV, CLRV and CNRMV/CGRMV using specific primer pairs and conditions were adjusted according to previous reports (Wetzel *et al*., 1991; Parakh *et al*., 1995; Shin,

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2009; Park *et al.*, 2004). The RT–PCR was conducted using an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, USA) with AccuPower® RT–PCR Premix (Solgent, Daejeon, Korea). The RT–PCR products were then electrophoresed on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light.

Determination of coat protein gene of CNRMV and CGRMV

To determine the entire coat protein (CP) gene sequences of detected CNRMV or CGRMV, RT–PCR was conducted using the CGRMV 1 / CGRMV 2 primer pair with AccuPower® RT–PCR Premix (Solgent, Daejeon, Korea). The RT–PCR conditions were the same as previously described (Li and Mock, 2005) and the procedure was conducted using an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, USA). Each of the amplified fragments was then cloned by T–Blunt vector using T–Blunt™ (Solgent, Daejeon, Korea) and sequenced.

Comparative analysis of coat protein gene and phylogenetic relationship

All obtained CP gene sequences of CNRMV and CGRMV were compared with those of other isolates from different countries using the Genetyx program (Genetyx Corporation, Tokyo, Japan). In addition, phylogenetic analysis was conducted to analyze the relationship between CNRMV and CGRMV from Korean isolates and those from different countries.

RESULTS AND DISCUSSION

Abnormal cherries with ring spot symptoms were observed in Daegu, Gyeongsan and Gyeongju (Fig. 1). The symptoms on the abnormal pericarp appeared as ring shaped or ring spot areas; however, necrosis was not observed around the symptoms. Previous studies have reported that CGRMV can induce ring–shaped symptoms with necrosis on the pericarp of sour cherries (Parker *et al.*, 1976), and ring–shaped symptoms were clearly observed on sweet cherries in the present study (Fig.

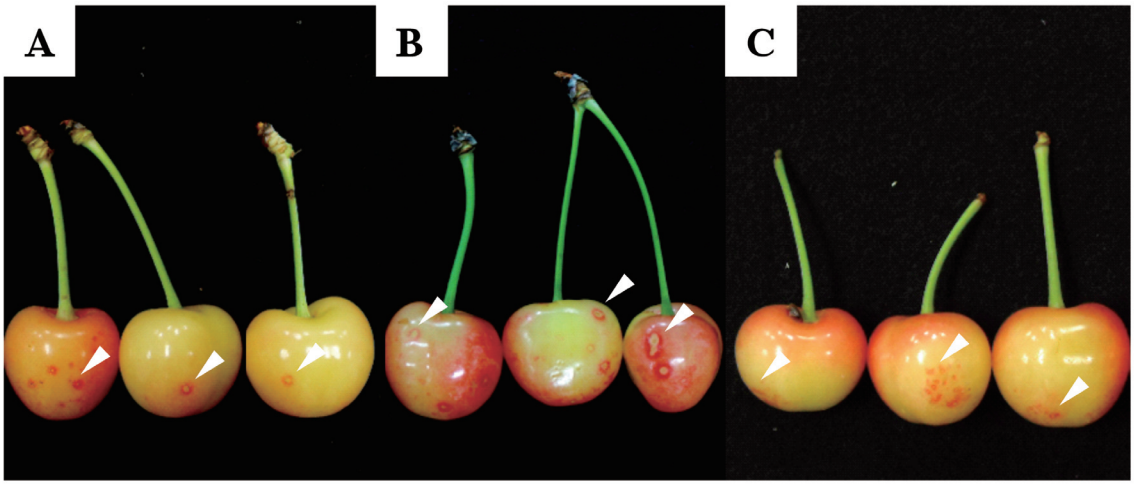

Fig. 1. Abnormal cv. 'Sato Nishiki' cherries with ring spot symptoms on the pericarp. A: Daegu isolates, B: Gyeongsan isolates, C: Gyeongju isolates. Arrows indicate abnormal ring spots on the pericarp.

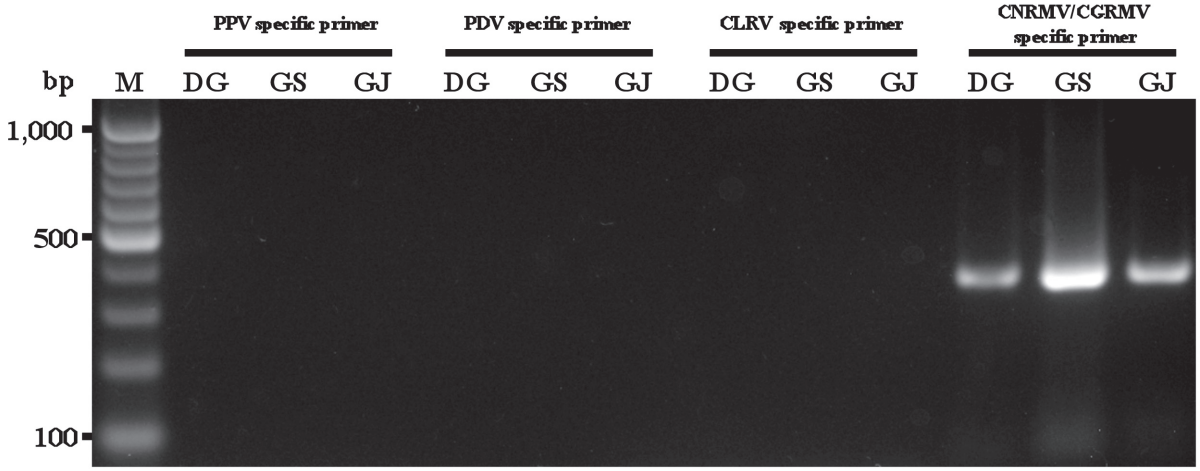

Fig. 2. RT–PCR was conducted using PPV (P1 / P2), PDV (PDV–f / PDV–r), CLRV (CLR–C10 / CLR–N60) and CNRMV/CGRMV (prCN4f / prCN4r) specific primer pairs. Amplified DNA fragments were observed using the CNRMV/CGRMV specific primer pair following electrophoresis on 1.5% agarose gel. M: 100bp DNA ladder, DG: Daegu isolate, GS: Gyeongsan isolate, GJ: Gyeongju isolate.

1A, B and C, arrow head). During the survey period, over 50 abnormal cherries were collected from each of the three cities; however, typical viral symptoms; such as yellowing, necrotic leaf spots and shot–holes were not observed on any of the investigated leaves.

RT–PCR was conducted to analyze collected abnormal cherries for the presence of the virus. RT–PCR using the prCN4f / prCN4r primer pair amplified DNA fragments of the expected size (approximate 400 bp) from all the samples collected from Daegu, Gyeongsan and Gyeongju (Fig. 2). Therefore, it was assumed that abnormal cherries were infected with CNRMV or CGRMV. To identify whether the two viruses were co–infected and determine the entire CP gene of CNRMV or CGRMV, RT–PCR was conducted using the CGRMV 1 / CGRMV 2 primer pair (Li and Mock, 2005). The results of this analysis revealed the presence of DNA fragments of the expected size (approximate 960 bp) in all samples with abnormal pericarps collected from all three cities (Fig. 3). Sequencing of the amplicons confirmed that all the abnormal cherries were co–infected with CNRMV and CGRMV. Moreover, sequencing revealed 804 bp (267 deduced amino acids) and 807 bp (268 deduced amino acids) of the CNRMV and CGRMV CP gene sequences and that three, five and three strains of CNRMV–DG, GS and GJ and two, three and ten strains of CGRMV– DG, GS and GJ isolates were present, respectively. In the case of CNRMV–Korean isolates, obtained deduced amino acid sequences of the CP gene from Daegu, Gyeongsan and Gyeongju strains showed 97–100% homology with each other. Moreover, CNRMV–Korea isolates showed the highest homology (97.4–98.9%) with the CNRMV isolate from Japan (EU188439). Phylogenetic analysis of the deduced amino acid sequences of the CNRMV CP gene present in the GenBank database revealed that CNRMV–DG, CNRMV–GS and CNRMV–GJ strains formed a cluster. As shown in figure 4A, the Korean CNRMV–DG isolate clustered with the Japan isolate (EU188439), the CNRMV–GJ isolates clustered with the India isolate (FN546178) and the CNRMV–GS isolates clustered separately with CNRMV–DG and GJ isolates

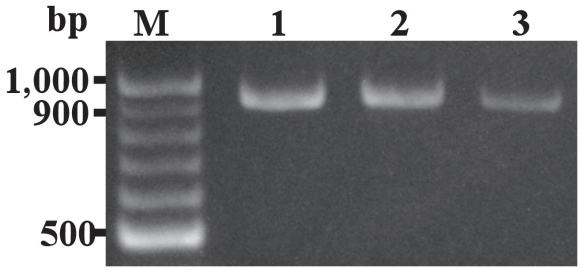

Fig. 3. Expected DNA fragments were observed using the CGRMV 1 / CGRMV 2 primer pair. Amplified DNA fragments were observed by electrophoresis on 1.5% agarose gel. M: 100bp DNA ladder. 1: Daegu isolate, 2: Gyeongsan isolate, 3: Gyeongju isolate.

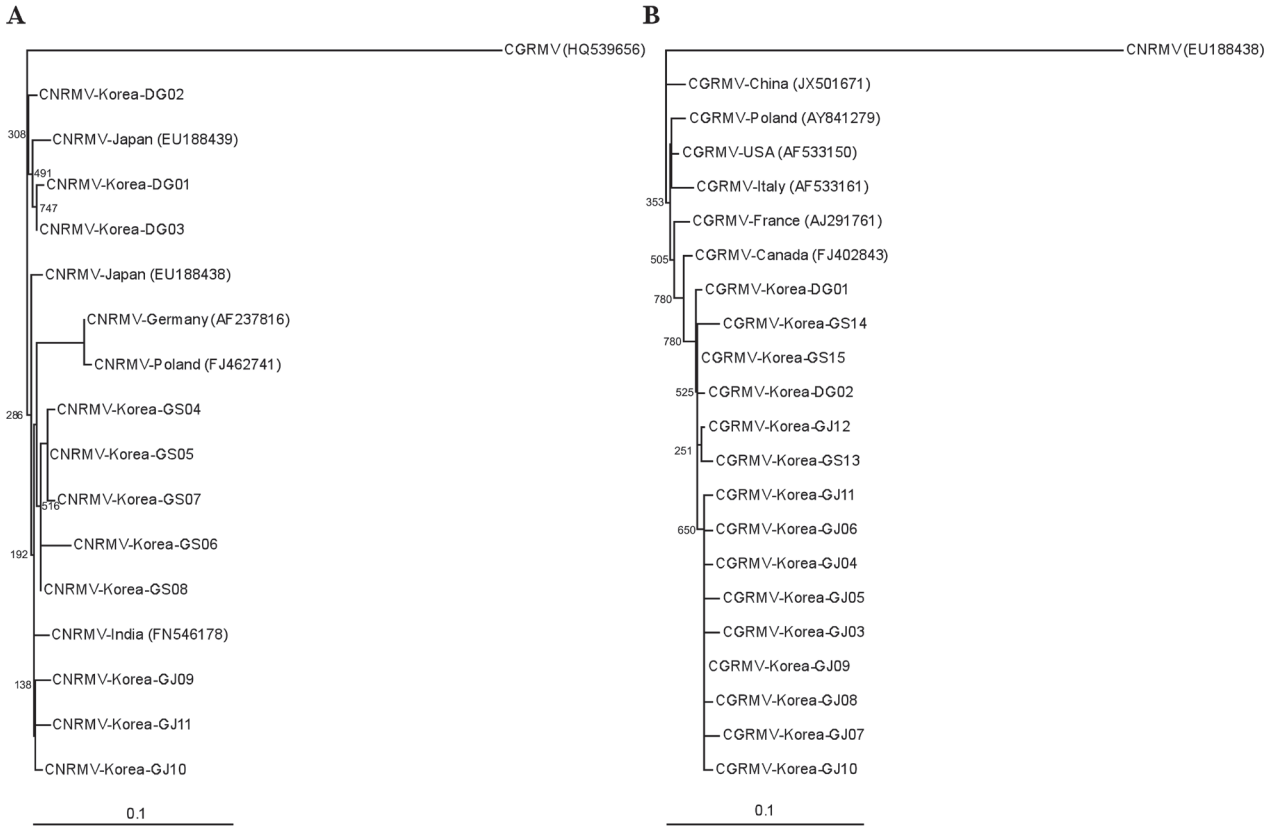

Fig. 4. Phylogenetic trees derived from aligned coat protein (CP) deduced amino acid sequences of CNRMV and CGRMV. A: Comparison of CNRMV–DG, GS and GJ isolates with amino acid sequences from other countries registered in GenBank, B: Comparison of CGRMV–DG, GS and GJ isolates with amino acid sequences from other countries registered in GenBank. DG: Daegu, GS: Gyeongsan, GJ: Gyeongju.

(Fig. 4A).

Moreover, all CGRMV–Korean isolates from Daegu, Gyeongsan and Gyeongju showed 98.1–100% homology with each other at the deduced amino acid level. Finally, all CGRMV–Korea isolates showed the highest homology (97.8–98.9%) with the CGRMV isolate from Canada (FJ402843). Phylogenetic analysis based on the deduced amino acid level revealed that even though CGRMV–Korean strains showed some variation, they all clustered in the same group (Fig. 4B).

We also carefully observed cherry leaves during cultivation periods for 2 years; however, we found no viral symptoms on the leaves of co–infected trees. Furthermore CNRMV infects sweet cherry, the appearance of viral symptoms on leaves is influenced by cultivar or environmental conditions (Li and Mock, 2008), and sweet cherry is known to be a symptomless host of CGRMV (Zhang *et al*., 1998). Accordingly, it is assumed that typical virus symptoms may not be observed on leaves of sweet cherry in Korea.

Further studies are still needed to survey the distribution of viral disease on cherry trees in Korea and to identify the appearance of viral symptoms on leaves using woody indicators such as 'Sam' (*P. avium* cv. 'Sam') for CNRMV and 'Kwanzan' (*P. avium* cv. 'Kwanzan') for CGRMV. Overall, this is the first report of CNRMV and CGRMV infected sweet cherry in Korea.

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