

Review Article**Research Progress on Carnation Etched Ring Virus****Wang Jiaying^{1,2}, Cui Junxia^{1,2}, Zhao Xiuling^{1,2}, Zhang Jihong^{1,2}, Chen Xianfeng^{1,2,*}**¹Technical Centre, Ningbo Customs, Ningbo, China²Institute of Inspection and Quarantine Science and Technology, Ningbo, China**Email address:**

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To cite this article:Wang Jiaying, Cui Junxia, Zhao Xiuling, Zhang Jihong, Chen Xianfeng. Research Progress on Carnation Etched Ring Virus. *Journal of Plant Sciences*. Vol. 9, No. 5, 2021, pp. 272-275. doi: 10.11648/j.jps.20210905.16**Received:** August 24, 2021; **Accepted:** September 14, 2021; **Published:** October 16, 2021

Abstract: Carnation (*Dianthus caryophyllinus*) is one of the most popular ornamental flowers worldwide. *Carnation etched ring virus* (CERV), belonging to the Caulimovirus genus, is the second most significant virus on carnation crops around the world. CERV particles are isometric, with a diameter of 47 nm, which contains one double-stranded, circular DNA at the length of about 7932 kbp as genome. CERV causes necrotic flecks, rings and line patterns on carnation leaves. Infected carnations reduce the number of lateral shoots and flowers. Flower quality is also affected and flower production can be delayed by up to one week. CERV is transmitted from infected to healthy plants by *Myzus persicae* in a semi-persistent manner. In addition, vegetative propagation, changed cultural practices, international trade, and movement of planting material to newer areas may lead to virus spread and disease outbreaks. To identify CERV, multiple methods are available including biological (inoculation onto indicator hosts), serological (ELISA), and molecular (real-time PCR) ones. In order to eliminate CERV, virus-free stocks has been applied and protected from possible re-infection. Chemical treatment as sixty seconds with commercial bleach at 7% (v/v) or NaOH at 0.5% (w/v) in a systemic *Saponaria vaccaria* bioassay has been proved to serve best in inactivating CERV. Widespread dissemination of this virus may be a result of international trade in carnation crops before CERV had been well inspected. This paper sheds light upon recent research progress on CERV in hope of taking this virus under control.

Keywords: *Carnation etched ring virus*, Ornamental Flowers, Virus Control, Quarantine

1. Introduction

Carnation (*Dianthus caryophyllinus*) is one of the most popular ornamental flowers in many countries [1, 2], which takes up an indispensable part in the floriculture industry. Viruses, viroids and phytoplasma are persistent threats to the propagation of ornamental crops. *Carnation etched ring virus* (CERV, also called *Carnation etched ring caulimovirus*) is the second most significant virus on carnation crops around the world [3-5]. This virus has been so far reported to exist wherever carnations are grown extensively in both glasshouses and fields. CERV was first reported in the UK, yet the origin is unknown. It is listed as a quarantine plant pest by both Madagascar and Peru [6]. Taxonomically, CERV belongs to the Caulimovirus genus, the Caulimoviridae family [7]. Widespread dissemination of this virus is inferred to be a result of flourishing international trade in carnation crops before

CERV had been recognized and methods been established for detection and identification. Luckily, the viral incidence has been significantly reduced since the introduction of virus-tested stocks and the plant quarantine policy.

2. Viral Particles

Viral particles of CERV are isometric, with a diameter of ca 47 nm. They sediment as a single component with a sedimentation coefficient of 206 S. CERV genome consists of one double-stranded, circular DNA at the length of about 7932 kbp [8, 9], with a GC content at 37%. Among the genes encoded by CERV, those are most conserved with respect to polyprotein region (37-65% amino acid similarity), but are least conserved with respect to inclusion body matrix protein (5-37% amino acid similarity). CERV isolates from India and Netherland show 96-99% homology based on different genes

at the amino acid level. Phylogenetic analysis indicates that CERV has independently evolved among Caulimoviruses, but is closely related to Cauliflower mosaic virus [10].

CERV particles accumulate usually in cytoplasm, within ovoid or spherical inclusion bodies in diameter of nearly 10 µm occurring relatively abundantly in leaf palisade, mesophyll and epidermal cells. Inclusion bodies are readily detected in epidermal cells, especially those of inoculated *Saponaria vaccaria*. Those inclusions are moderately stable, thus can be purified from infected plant tissues [11]. Viral particles have also been observed in nuclei of *Dianthus barbatus* (sweet william) and *S. vaccaria*, but not in carnations.

3. Host and Symptoms

Carnation is the only known natural host of CERV. Yet, it can be experimentally transmitted to some species in the Caryophyllaceae, notably *D. barbatus* (sweet william), *Silene armeria* and *S. vaccaria* [12]. CERV affects both flowering and vegetative growing stages of carnations.

In the carnation leaves, CERV infection causes necrotic flecks, rings and line patterns which sometimes enlarge to form blotches. In mixed infection with other carnation viruses, symptom aggravates, including leaf chlorosis, necrotic spots, rings, streaking and flecking of stems. CERV-infected carnations become less vigorous, and reduce the number of lateral shoots and flowers. Flower quality is also affected and flower production can be delayed by up to one week. Thus, CERV would be a big potential threat to the carnation crop production industry.

CERV accumulates mainly in leaf, petal, stamen, pistil, and ovary tissues. Viral particles are irregularly distributed over the host plant, with high titre in leaf, petal, stamen, pistil, and ovary tissues. Thus leaves or petals are preferred tissues for routine diagnosis.

4. Transmission

CERV can do vector transmission, which means it is transmitted from infected to healthy plants by *Myzus persicae* in a semi-persistent manner. Vegetative propagation, changed cultural practices, international trade, and movement of planting material to newer areas can lead to spread of CERV and disease outbreaks. Yet CERV is not seed-borne. Long distance spread of this virus taking place mainly during international trade should be eliminated in order to slow down the world-wide diffusion.

Since virus-infected carnations become less vigorous, and reduce the number of lateral shoots and flowers. Flower quality is also affected and flower production can be delayed by up to one week. Meanwhile possibility exists that CERV can be disseminated inadvertently in the course of international trade of carnation crops. CERV has been listed as a quarantine plant pest by both Madagascar and Peru. Certain measures must be taken to ensure the absence of CERV before carnations enter another country.

5. Detection and Identification

To combat viral diseases, it is important to diagnose the viruses efficiently and effectively. As symptom severity varies with seasons, risks exist that CERV may be disseminated in infected plants showing inconspicuous or no symptoms. However, this risk is now immensely reduced with intensified use of sensitive methods for virus detection and identification and the availability of elite virus-tested stocks.

Since infected plants can not be inspected reliably via vision because symptom variability may lead to visual difficulty at some period. It is necessary to use one or more sensitive methods for virus detection. Leaves and petals are preferred tissues to sample for routine diagnosis.

5.1. Herbaceous Indicator Plants

Symptoms induced by viral infection in herbaceous indicator species can indicate the identity of CERV. Graft- or mechanically-inoculated *D. caryophyllus* cv. Joker develops typical etched ring leaf symptoms. Symptoms are intensified in mixed infection with other viruses. Systemically infected *S. armeria* develops necrotic line patterns and blotches in leaves. Mechanically-inoculated *S. vaccaria* cv. Pink Beauty is stunted with leaf symptoms like concentric red rings, necrotic or yellow spots, lines and rings [13].

Symptom development is largely affected by temperature, day length and light intensity. The optimum conditions are 27°C with a 16-hour light period at ca 2000 foot-candles.

Furthermore, serological and molecular methods are required for unequivocal identification.

5.2. Serological Methods

Traditional diagnosis requires bio-assay including an indicator plant, determination of host range and symptomatology, which is quite time-consuming. Progress in biochemistry and immunology has led to the development of many new, accurate, rapid and less labour-intensive methods. The serological methods for diagnosis, detection and identification of plant viruses play a vital role. They are the most widely used nowadays besides molecular ones. Immunosorbent electron microscopy (ISEM), enzyme-linked immunosorbent assay (ELISA), gel diffusion and microprecipitin tests have long been applied in CERV detection and identification [14].

Among those above, ELISA is the preferred method which is both faster and more sensitive. ELISA is well documented and proved to be a valuable detection tool for plant viruses. In addition, the specificity of ELISA can preclude confusion of even closely related virus strains [15].

5.3. Molecular Methods

Advances in molecular techniques have evolved immensely, leading to reliable detection of targeted pathogens. Molecular hybridization and polymerase chain reaction (PCR) in all forms are the most sensitive and fastest methods for virus identification recently. Among them conventional PCR (or

RT-PCR for RNA viruses) and real-time PCR (or RT-PCR) are used most frequently. Real-time PCR (or RT-PCR) has gained wide acceptance as a sensitive method for nucleic acids detection because it allows a large dynamic range of target quantitation, while conventional PCR (or RT-PCR) can only provide qualitative results. The sensitivity afforded by real-time PCR (or RT-PCR) is reported to be 1000 times higher than DAS-ELISA or conventional PCR (or RT-PCR) [16]. But molecular methods often require precise instruments in labs [17].

6. Control and Elimination

After introduction of a hitherto uncommon virus from a crop, it is necessary to do the next three things. That is, to identify the target, to manage spread, and to eliminate it as thoroughly as possible.

6.1. Cultural Control

Like other plant viruses disseminated mainly via vegetative propagation, CERV is best controlled during the process of production, propagation and subsequent distribution of carnation crops. According to reports, a greater percentage of virus-free plants were obtained when meristems were collected from donor plants which had been first treated at 37 °C for 60 days [18]. Virus-free stocks should always be propagated under certain conditions that minimise the possibility of re-infection. Corresponding measures can be isolation from possible sources of reinfection, minimal handling of plants and, if necessary, the application of insecticide to control the aphid vector of CERV. In vitro techniques have been standardized to produce tested ornamental crops to ensure the absence of CERV. What's more, farmers are supposed to be trained for production and maintenance of virus-free quality planting material [19].

6.2. Chemical Inactivity

Sixty seconds treatment with commercial bleach at 7% (v/v) or NaOH at 0.5% (w/v) in a systemic *S. vaccaria* bioassay has been proved to serve best in inactivating CERV [11].

7. Conclusion

CERV, belonging to the Caulimovirus genus, is the second most significant virus on carnation crops around the world. CERV particles are isometric, with a diameter of 47 nm, which contains one double-stranded, circular DNA at the length of about 7932 kbp as genome. CERV causes necrotic flecks, rings and line patterns on carnation leaves. Infected carnations reduce the number of lateral shoots and flowers. Flower quality is also affected and flower production can be delayed by up to one week. CERV is transmitted from infected to healthy plants by *Myzus persicae* in a semi-persistent manner. In addition, vegetative propagation, changed cultural practices, international trade practices, and movement of planting material to newer areas

may lead to spread of CERV and disease outbreaks. To identify CERV, multiple methods are available including biological (inoculation onto indicator hosts), serological (ELISA), and molecular (real-time PCR) ones. In order to eliminate CERV, virus-free stocks has been applied and protected from possible re-infection. Chemical treatment as sixty seconds with commercial bleach at 7% (v/v) or NaOH at 0.5% (w/v) in a systemic *S. vaccaria* bioassay has been proved to serve best in inactivating CERV.

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