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Short Communication
Transmission Efficiency of Cucumber Mosaic Virus by *Myzus persicae* According to Virus Strain and Aphid Clone from China

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Abstract

Background: Cucumber mosaic virus (CMV) is one of the most important viruses infecting vegetables in the fields throughout the world. Transmission efficiency of CMV could depend on the variability of virus strain but also aphid vector species and/or clones. **Materials and Methods:** By sequence analysis, the coat protein gene of CMV strains from different regions revealed that the CMV isolates used for this study belong to the same group. Both CMV strains and *Myzus persicae* (Sulzer) aphid clones were investigated for their role in viral dispersion by reciprocal tests on *Nicotiana tabacum* (L.) using the same clone of *Myzus persicae* towards different CMV strains or using one CMV strain on different *Myzus persicae* clones. **Results:** Virus transmission efficiency was found to be significantly influenced by selected CMV strains (from 5-30% of transmission rate for identical aphid clone) and also by the selected aphid clones (variation from 15-70% of transmission rate for identical virus strain). **Conclusion:** The CMV transmission efficiency depends on the variability of virus strain but also aphid vector clones. Combining the variability of CMV transmission rates for both aphid and virus sides, the prediction and modeling of virus spreading seems to be difficult to organize and are closely dependent on the variability of each protagonist-aphid and virus.

Key words: Cucumber mosaic virus, *Myzus persicae*, transmission efficiency, gene cloning, aphid species

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aphids are known to transmit more than 200 phytoviruses in a non-circulative manner[1]. Among them, *Myzus persicae* (Sulzer) is a polyphagous species that was found on hundreds of host plants, able to transmit cucumber mosaic virus (CMV, the genus *Cucumovirus*, the family Bromoviridae) which is one of the most ubiquitous virus found in a broad diversity of plants[2,3]. Transmitted by a non-circulative way, plant virus particles bind to aphid receptors on the maxillary stylets cuticle. Coat Protein (CP) strategy used by *Cucumovirus*, typically CMV was proposed by Chen *et al.*[4] suggesting that viruses bind to the aphid stylets receptors via the domain of their capsid protein. Changes in abundance and/or composition of both aphid receptors and virus CP could then influence the virus transmission efficiency. Indeed, some amino acid modifications in the coat protein of CMV were found to change the virus transmission by *Aphis gossypii* Glover[5,6]. Amino acid determinants for CMV transmission have been mapped by Liu *et al.*[7]. It is known that the transmission efficiency is affected by selected aphid species[8-11], but there is a lack of accurate information on the impact of aphid clones, such as from *M. persicae* and CMV strains on transmission efficiency of this virus.

The first objective of the current study was to assess intraspecific variation in the transmission of CMV among *M. persicae* clones and virus strains collected from several geographic areas. Second, by alignment of amino acid sequence of coat protein of CMV strains, the impact of differences on virus transmission efficiency was evaluated.

MATERIALS AND METHODS

Chinese virus strains were collected directly on plants showing evident symptoms in the fields in China, while Belgium CMV strains were provided by the applied Microbiology-Phytopathology Department (UCL) in Belgium (Table 1). Virus strains were used to infect *Nicotiana tabacum* (L.) plants to be further selected as CMV sources for virus transmission assays.

Myzus persicae clones were collected from several host plants and geographic areas in China (Table 2) and reared in a controlled condition incubator (20±1°C and 16 h light photoperiod).

CMV gene cloning and analysis: Samples were processed with RNA extraction (Invitrogen, Carlsbad, USA) then with two steps RT-PCR program. The genome sense primer 5′-YASYTTTDRGGTTCAATTCC-3′ and the antisense primer 5′-GACTGACCATTTTAGCCG-3′ were used to prime the reaction for CMV detection[12]. The primers flank the coat protein gene and allow therefore the amplification of a 933-966 bp, according to the different isolates considered. The DNA synthesis supermix (TransGen Biotech, Beijing, China) was used for first strand cDNA synthesis. The program of RT step consisted of 50°C for 30 min, followed by 85°C for 5 min. Eppendorf tubes contained 2.5 µL RT product and 22.5 µL mix PCR containing PCR reaction buffer (Biomed-tech Beijing China) primers and ddH_2O. The cyclic conditions were: Initial denaturation at 94°C for 30 sec, followed by 40 cycles of 52°C for 30 sec, extension at 72°C for 1 min with a final extension of 72°C for 10 min.

The PCR products were analysed by electrophoresis in 1.2% agarose gel stained with ethidium bromide. The PCR amplicons were then purified (Qiaquick PCR purification kit, Qiagen, Germany) before sequencing using a BigDye terminator sequencing kit (Applied Biosystems). The purified product was subjected to sequencing (Beijing Sunbiotech Co., Ltd.) and sequences were analysed on BLAST and also MEGA5.1 software and DNA star program for phylogenetic tree building.

Virus transmission efficiency assays and CMV detection: To initiate virus acquisition, aphids were removed from the host plant species and starved for 2 h. The third-instar nymphs of *M. persicae* were then fed on artificial diet (CMV-infected tobacco tissue crushed in a 15% sucrose-containing solution) through a stretched Parafilm® membrane[13]. To test the transmission of the various aphid clones, the same virus solution was used. However for comparison of the transmission of different CMV strains, the virus contained in the plants was previously quantified by ELISA. After acquisition access period, aphids were transferred onto healthy tobacco seedlings overnight (1 aphid per plant) to assess the transmission capacity of the virus. For each treatment, 5 replicates of 10 plants were performed for a total of 50 plants. After transmission assays, plants were sprayed with insecticide (pyrethroid) and set in a greenhouse for 3 weeks before CMV detection by DAS-ELISA according to provider instructions (DSMZ, Braunschweig, Germany). For each experiment the number of infected plants was calculated as a proportion of the number of plants tested. Data were analysed by a one-way ANOVA. Prior to the analyses, a data arcsin√n transformation was applied to normalize distributions. All analyses were performed using MINITAB® 17 software.

Table 1: CMV strains collected from several geographic areas

Virus strain abbreviation	Region
2012.2	Louvain-la-Neuve Belgium
1022	Roulers Belgium
1024	Roulers Belgium
BeCh	Beijing China
1766	Shouguang China
1769	Taian China
1770	Taian China
1772	Shouguang China

Table 2: *Myzus persicae* clones collected from several Chinese geographic areas and host plants

Aphid clone abbreviation	Crop source	Region
BJp	Turnip	Beijing
BJe	Cabbage	Beijing
BJo	Tobacco	Beijing
STp	Turnip	Shandong, Taian
STe	Cabbage	Shandong, Taian
STo	Tobacco	Shandong, Taian
SJp	Turnip	Shandong, Jinan

To assess the effect of CMV strains on virus transmission efficiency, 8 virus strains (Table 1) were used with *M. persicae* (BJp clone). According to the results obtained in the 1st experiment, 1772 virus strain was selected to test the efficiency of transmission of seven aphid clones (Table 2).

RESULTS AND DISCUSSION

Transmission rate of CMV according to virus strains: Significant variation of virus transmission efficiency was observed from 4-30% transmission rates according to the considered CMV strain ($F = 8.8$, $p<0.001$) (Fig. 1) revealing that 1772 was transmitted much better than other CMV strains (7 times more transmitted that 1024 and 2012.2 virus strains).

The alignment of amino acid sequence of coat protein of CMV strains (Fig. 2) and phylogenetic analysis (Fig. 3) illustrated some differences: In position 13, serine for 2012.2, in position 15 and 147, proline for 1770 but also for this strain in position 33 and 208, isoleucine and lysine in position 25 and 71, proline and threonine for BeCh. These CMV strains belong to the same subgroup.

Transmission rate of CMV according to the aphid clones: Significant differences of CMV transmission efficiency were found between *M. persicae* clones ($F = 4.8$, $p<0.01$). Two clones collected in Taian, Shandong province (STp and STe) were found to be best CMV vectors with 70 and 50% rates, respectively. Other clones transmitted with low efficiency rates (5-20%) (Fig. 4).

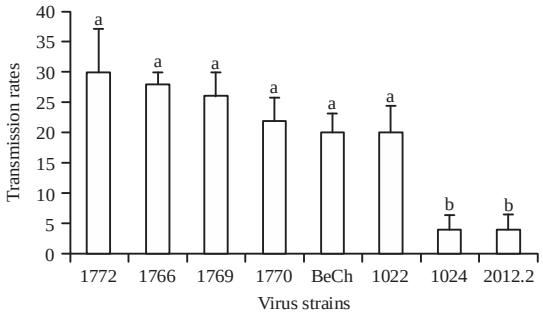

Fig. 1: Mean transmission efficiency rates of CMV strains collected from China and Europe by *Myzus persicae* single clone. Bars represent standard errors of means. Letters show significant differences as indicated by Tukey's test

No clear CMV transmission ability was found from original host plant collection. Cucumber mosaic virus has a worldwide distribution with one of the broadest host ranges of any known viruses[14] and *Myzus persicae* (Sulzer) is one of the most important aphid species that feed and reproduce on tobacco, so it should be essential players in the transmission of different CMV strains[15]. A large number of parameters was already found to have an impact on virus transmission: Virus strains, aphid species, source and host plant species on which aphid was collected and/or maintained[1,10].

In this study, the interest has been focused on both virus strains and aphid clones diversity for CMV-*M. persicae* model from different locations.

Foremost, the capacity of CMV to be transmitted by one aphid clone was found to significantly vary depending on the virus strain. Previous studies indicate similar results concerning other viruses[16]. Another model, cauliflower mosaic virus (CaMV, the genus *Caulimovirus*, the family caulimoviridae), was studied and found to be transmitted according to specific interactions involving 2 particular viral proteins (named P2 and P3) to form a transmissible viral complex[17]. Variability in coat protein of viruses can also have a very major impact in the viral transmission since it acts in the binding between virus and internal mouthparts of aphid vector. Here, the nucleotide alignment using phylogenetic analysis revealed that our CMV isolates belong to the same subgroup and some amino acids changed at seven different positions. Although these amino acid changes, no significant difference of viral transmission rate was observed to be related to particular amino acid variation. Nevertheless, virus strains collected from Europe (2012.2 and 1024 CMV strains) were determined to be less transmitted by a Chinese aphid clone indicating that some

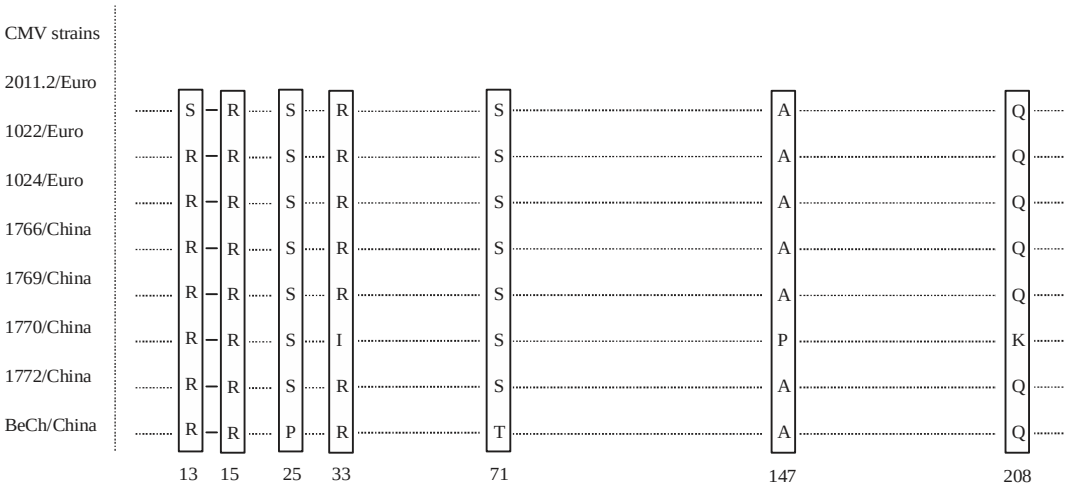

Fig. 2: Amino acid sequence comparison of the Coat Protein (CP) of CMV-2011.2, 1022, 1024, 1766, 1769, 1770 and 1772, BeCh. Differences between sequences are indicated using the one letter code for amino acids. Numbers denote residue position within the CP

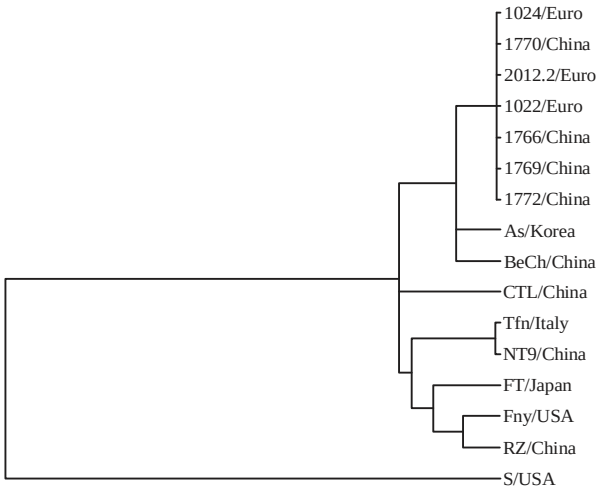

Fig. 3: Phylogenetic analysis of collected CMV strains in this study and other CMV strains based on the nucleotide alignment using MEGA v5.1. Virus strain names were followed by original country names

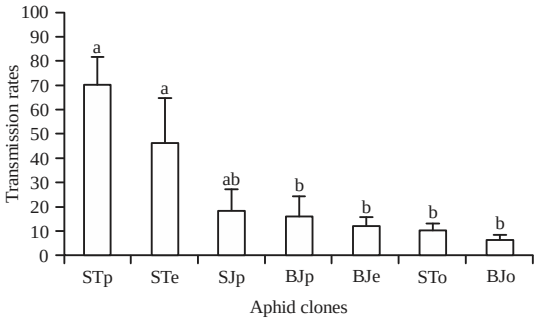

Fig. 4: Mean transmission efficiency rates of CMV from China (1772 strain) by a range of Chinese *Myzus persicae* clones. Bars represent standard errors of means. Letters show significant differences as indicated by Tukey's test

synchrony for geographical region for both virus and aphid vector should be further investigated[18]. Even if virus strain influences the transmission rate, the aphid clone is also an important point to consider.

Second part of this study was focused on transmission efficiency of several geographical populations of *M. persicae* from China. This aphid species being selected as one of the best CMV vectors[5]. In the present study, it is clearly shown that aphids have a very different virus transmission capacity from one clone to another, the ones collected from Shandong area being mostly more efficient than others from Beijing region. Associating both virus strain (1772) and aphid clones (STp and STe) collected from similar location, in Shandong area was found to correspond to highest efficiency of CMV transmission rates (50-70%). It indicates that there should have double considerations related to geographic origin for both aphid and virus partners when studying virus transmission[19]. Also, host plant of collected aphids should also be integrated in our thinking. In this study, both aphid clones from tobacco plants (STo and Bjo) were demonstrated to be the least efficient vector. Kanavaki *et al.*[20] observed a significantly lower propensity of PVY^N transmission with *M. persicae nicotianae* (a tobacco specialist) than with *Myzus persicae* s.str. (a generalist) under arena conditions. *M. persicae* clones able to grow and develop on tobacco plants were previously

considered as specific and particular clones displaying different biological parameters when compared to more generalist clone found on other plants. More than strictly plant-aphid interactions, these tobaccos related insect clones should perhaps be also considered as particular for virus transmission. Host plant should influence the aphid feeding behavior and so the virus transmission. Morphs of aphids, such as green or pink *Acyrthosiphon pisum* (Harris) were also found to differentially transmit CMV[21]. One suggestion to explain changes in virus ability in aphids for non-circulative models was to consider the diversity and abundance of receptors on insect sucking mouthparts. Particularly in the extreme tip area named the acrostyle region of aphid stylets. Blanc *et al.*[22] reported evidence for the existence and precise location of a first receptor for the CaMV at the tip of aphids maxillary stylets.

Several differences in the transmissibility of virus strains by aphids clones have been previously reported for CMV[23,24] but also for other non-circulative viruses[25,26]. Despite these various studies, the aphid-virus interaction is still a mystery. Only accurate data on aphid receptor was elucidated for CaMV. Further study are under progress to identify receptors in aphid vector for different virus model. Recent trends in the field are opening questions on the diversity and sophistication of viral adaptations that optimize transmission from the manipulation of plants and vectors ultimately increasing the chances of acquisition and inoculation[22].

CONCLUSION

It was known that aphid species influenced the CMV transmission but in this study, it was identified that the variability of CMV strain and *Myzus persicae* clone are also both involved in the transmission efficiency. However, differences in the amino acid sequences of the coat protein between virus strains have no impact on virus transmission. Contrariwise, host plant and geographical situation of aphid clones and virus strains affect the viral transmission efficiency. Further information on aphid and virus interactions in viral transmission is needed. A better understanding of CMV transmission efficiency in aphids may change predictions of virus spreading and epidemiological models including the determined variability of each protagonist in regions and improving control strategies for aphid-virus associations.

SIGNIFICANCE STATEMENT

This study highlights the importance of interaction between virus strains and aphid clones in the transmission efficiency. Data will help to think further about this interaction and so to better understand the mechanisms of virus transmission, limit it and reduce the use of pesticides, harmful for human health and environment.

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