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Study of CMV - plant - aphid interactions focusing on *Myzus persicae* in vegetable crops

Rongling YIN

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Promoteurs: Prof. Frédéric Francis

Prof. Liu Yong

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Abstract: *Myzus persicae* aphid is a polyphagous pest found on hundreds of host plants including several vegetable crops and plays a role as virus potential vector. Transmission efficiency of *Cucumber mosaic virus* by *M. persicae* was selected as a model. In a non-persistent manner, virus particles bind on the top of aphid stylet and transmitted in a few minutes. Transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained. The aim of this study was to investigate the complex dynamics and interactions of host plants, vectors and viruses. Here, effects of virus strains, aphids from different places, and lectin competitors on virus transmission have been studied. Firstly, molecular characterization of CMV coat protein and transmission efficiency were studied. The D1772 CMV strain was found to be the best transmitted by *M. persicae* and selected to develop further experiments to compare efficient/bad vector among chinese aphid strains from different places. Transmission efficiency was different and related to changes in coat amino acid sequence. Moreover, *M. persicae* clones collected from China and Belgium displayed discriminant vector efficiency indicating that geographical diversity also affects on virus transmission. Secondly, aphid from turnip was most efficient than other clones to transmit CMV. Two clones from Jinan and Shandong displayed lower transmission efficiency. In conclusion here, aphid clone factor had more effects on virus transmission than virus strain factor. Finally, *Galanthus nivalis agglutinin* (GNA), *Wheat germ agglutinin* (WGA) and *Pisum sativum lectin* (PSL) were found to have competition effect with virus when aphids were fed on artificial diet including these lectins before virus transmission assays. Inhibition rates of vector efficiency were all above 50%. According to this study, we investigated and discussed different strategies for virus transmission understanding and potential regulation in order to later promote sustainable control of virus in vegetable crops in China.

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General introduction

Myzus persicae aphid is a very polyphagous pest found on hundreds of host plants including several vegetable crops. Its role as virus potential vector was cited for many viruses found including vegetable crops. Transmission efficiency of virus，such as Cucumber mosaic virus, transmitted by M. persicae to vegetable crops is the most common model used in many researches. In a non-persistent manner, virus particles bind on the top of aphid stylet and transmitted in a few minutes, and transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained

Management of vector-borne plant diseases has presented a challenge because of complex dynamics and interactions of host plants, vectors and viruses within natural environment. Lectins as defense proteins in plants are present in large quantities in storage organs and seeds that are especially vulnerable to pathogens or pest insects(Peumans and Van Damme, 1995). Numerous reports in recent years have shown that lectins are toxic to various pest insects belonging to economically important insects such as *Lepidoptera, Coleoptera, Diptera* or *Hemiptera* in genetic engineered plants or artificial diets with lectins, which is negatively affect the performance of pest insects. In the last decades, some plant lectins were shown to be toxic to several aphids.

Plant-aphid-virus interactions have been researched for several decades, and there are some important questions studied, and still being in process. Although there

are reports on virus transmission, we focus on transmission efficiency affected by geographic aphid species, virus strains and plant lectins, and finally we hope to get a better understanding of the virus-aphid interactions and to propose new insight of lectins in non-persistent virus transmission control in crop protection.

Chapter Ⅰ: Plant -aphid -*Cucumber mosaic virus* interactions focusing on aphids in a non-persistent manner

Plant -aphid -*Cucumber mosaic virus* interactions focusing on aphids in a non-persistent manner

Rongling Yin[1, 2], Frédéric Francis[1], Claude Bragard[2], Yong Liu[3], Julian Chen[4]

(soumis à *Biotechnology*, *Agriculture*, *Society* & *Environment*; BASE)

[1]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.

[2]Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

[3]Plant protection, Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[4]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

Summary

Plant-aphid-virus interactions have been researched for several decades, including aphids transmit viruses to plant and plant defense to viruses and aphids, and there are some important questions studied, and still being in process. Some researches have been studied on interactions of virus-aphid, interactions of plant-virus and interactions of plant-aphid. Here we present researches of process of *Cucumber mosaic virus* (CMV) transmission and transmission efficiency in order to more understanding on interactions of plant-aphid-virus.

Keywords: interaction, transmission, non-persistent, bottleneck, *Cucumber mosaic virus*.

Introduction

Efficient virus transmission from host plant to another plant by vectors is very important. Arthropods could transmit most of plant viruses, especially aphids in Hemiptera. Actually, aphids could transmit over 200 plant viruses in a non-persistent manner(Nault, 1997), such as *Myzus persicae* and *Cucumber mosaic virus* (CMV) which is the most common model used in many researches. In a non-persistent manner, virus particles bind on the top of aphid stylet and transmitted in a few minutes, and transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained (Simons, 1957), some of them have been discussed (Perry Keith L. et al., 1998; Betancourt et al., 2008; Canto et al., 2009; Mello et al., 2011).

The control of virus diseases transmitted in a non-persistent manner by aphids should be one task to avoid prophylactic pesticide treatments to prevent virus spread, importantly continuous threat. And there are some reports on vector transmission associated bottlenecks showed it's very important among the interactions of virus-aphid-plant (Ali et al., 2006; Moury et al., 2007; Betancourt et al., 2008; Desbiez et al., 2011).

This review will focus on the process of virus transmission in a non-persistent manner especially model of CMV and *M. persicae* in order to get a better understanding of interactions of virus-aphid-plant.

Relationship of plant, vector and virus

Because viruses cannot penetrate the intact plant cuticle and the cellulose cell wall, plants have a barrier to infection. This problem is overcome either by avoiding the need to penetrate the intact outer surface (e.g., in seed transmission or by vegetative propagation) or by some method involving penetration through a wound in the surface layers, such as in mechanical inoculation and transmission by insects. There is considerable specificity in the mechanism by which any one virus is naturally transmitted. And about transmission via plant material, they are mechanical transmission, seed transmission, pollen transmission, vegetative propagation and grafting.

But many plant viruses, over 400 plant viruses in addition to 697 virus species that have been reported but are not yet officially recognized by the ICTV, are transmitted from plant to plant in nature by invertebrate vectors, members of the Insecta and Arachnida classes of the Arthropada, and the Dorylaimida order of the Nematoda (Roger, 2009). And particularly important vectors are Hemipteran insects transmitting the majority of the vectored viruses (55%) (Nault, 1997; Van Emden et al., 2007; Hogenhout et al., 2008). The most important family among these vectors is the aphids (Aphididae), which transmit many more viruses than whiteflies (Aleyrodidae) or leafhoppers (Cicadellidae) (Nault, 1997).

Virus transmission by aphids (or other vector) involves the transfer of virions from infected to healthy plants. It shows interest from two points of view. One of

them is that aphids or other vectors spread many viruses in the field causing biological economic loss, in Europe, it has been suggested that losses due to viruses in field crops are 10-15%, and even higher in vegetables and fruits(Carr and Loebenstein, 2010). Also it would be even worse if the control methods were not already in place, using pesticide, which would double world losses to 70% of total production (Oerke et al., 1994). The other view is the interest of relationship between vectors and viruses, especially as some viruses have been shown to multiply in the vector. Also viruses can be regarded as both plant and animal viruses. Transmission by vectors is usually a complex phenomenon involving interactions within the virus, the vector, and the host plant, combined with the effects of environmental conditions (Hogenhout, 2008).

Aphids are the main vector in most of the detailed studies on virus transmission and virus-vector relationships. And according to passing to the vector's interior, the type of transmission divided into two parts, non-persistent transmission, including stylet-borne and foregut-borne, and persistent which contains circulative and propagative (Nault, 1997; Matthews and Hull, 2002). Of the over 300 known aphid-borne viruses, most are non-persistent, and *M. persicae* is known to be able to transmit a large number of non-persistent viruses, whereas other aphids transmit only one virus.

There are many generations per year in the lifecycle of aphids. And a sexual phase happen from autumn with males and females produced, then males and females will mate and produce some special eggs which are able to survive after

a freezing winter. About the asexual phase, eggs will hatch in spring, producing wingless aphids that soon begin parthenogenetically producing new wingless females. Then generation and generation will be produced in hot weather, and they can produce up to 12offspring a day which are called nymphs, approximately 4 times before becoming adult aphids. Obviously, the summer cycle is the active phase in the aphid lifecycle. Also viruses are transmitted by wingless and winged aphids in this phase more effective, and there is no significant difference of relative transmission rates for both morphological states in the same species (Verbeek, 2009; Boukhris-Bouhachem, 2011).

Behavior of aphids on virus plants

Host plant selection by aphids occurs as a series of steps to search and find their host plants and identify feeding sites. It has been defined for aphids in the following way (Powell et al., 2006; Fereres and Moreno, 2009):

- Pre-alighting behavior (before landing)
- Plant contact and assessment of surface cues after landing
- Probing on superficial tissues
- Location and insertion of stylets at the appropriate feeding site
- Salivation followed by committed sap ingestion

Pre-alighting behavior

And also plant selection maybe extended to other homopterans and sap-sucking

insects. It is known that insects have ocular photoreceptors responding in a bandwidth of ultraviolet (200-400nm), visible or photosynthetically active radiation (PAR) (400-700nm) and the far red (700-800nm) (Fereres and Moreno, 2009), and for example, *M. persicae* have three types of photoreceptors that were sensitive to the green region (c. 530nm), the blue-green region (490nm) and near UV (330-340nm) (Kirchner et al., 2005). During flight, aphids respond to visual factor stronger than others, like sound, odor and learning, and locate host plants from the contrast between soil background and green yellow colour of plant (Kring, 1972; Döring, 2004).

Also different volatile compounds released from plants will be responsed by aphids to recognize their host plants, which have been widely accepted that olfaction plays a role in many aphid species (Jones, 1944; Van Emden and Harrington, 2007). However the presence of host plant odours did not affect the targeted flight in flight chamber bioassays (Nottingham, 1993), expect for the carrot aphid, *Cavariella aegopodii*, which was caught in water traps with carvone compound (Chapman, 1981). It appears that the difference maybe found in plant contact after landing.

Plant contact and selection

When aphids land on a plant, they walk and move their antennal sensilla to detect olfactory cues which released by host plant. A study showed that odour volatilized by host and non-host plants influenced the behavior of M. persicae,

which was that odour of the host plant sweet pepper Capsicum annuum L. (Solanaceae) was significantly attractive, while odour of non-host plants was significantly repellent(Amarawardana et al., 2007). And it’s certain that volatiles from host plant may act a negative role on aphids.

Also trichiomes as a feature of plant surfaces, in some cases, provide resistance to aphids, not only mechanical means, for example, deterring aphids to move and probe, but also chemicals released by “heads” of glandular trichomes is sticky and/or toxic to aphids. There are some reports that tomato species with different density trichomes have effects on population of the green peach aphid (M. persicae) (Simmons, 2003), and high density of glandular trichomes and chemicals secreted by Cucumis melo L. deter A. gossypii settling on (Bukovinszky, 2005).

Probing behavior of aphids

When viruses are transmitted by aphids, there are three phases in transmission cycle for non-persistent viruses, including acquisition, retention and inoculation. When probing and feeding behavior occurring after very brief probes, it is detailed by DC-EPG signals which were distinguished into three specific and distinct sub phases: II-1, II-2, II-3(Fig.2, Martín et al., 1997). Acquisition of stylet-borne viruses is associated to the third subphase (II-3) of the potential drop (pd) (Powell et al., 1995; Martín et al., 1997). During this period, acquisition is not only restricted to typical non-persistent viruses like CMV, which retained on

the stylet tips, but also affect acquisition efficiency. It means if sub phase II-3 is not long enough, virus acquisition will be reduced and it will influence virus transmission (Collar and Fereres, 1998).

It also reported that the sub phase II-1 was related to the inoculation of CMV and PVY by their vectors *A. gossypii* and *M. persicae*, respectively. And the ingestion-salivation hypothesis was proposed which suggested that watery salivation was the mechanism mediating the release of virions from the stylet tips (Martín et al., 1997). It's also one reason to influence virus transmission (see section 4). Retention sites of non-persistant viruses are within the common food/salivary canal located at the tip of the aphid maxillary stylets. Two molecular strategies have been reported: the capsid strategy, which is the way virions directly bind the receptor via a domain of their capsid protein (for example, the genus of Cudumoviurs), and the helper strategy, which is the way virion-receptor binding is mediated by viral protein "helper components" as a bridge, the genera Potyvirus and Caulimovirus are the best konwed.

Feeding behavior of virus transmission by aphids

About examples of pathogen-induced effects on host odor cues are the induction of characteristic volatile emissions by Potato leaf roll virus (PLRV) and Barley yellow dwarf virus (BYDV) are more attractive to aphid vectors than emissions from healthy plants (Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004; Srinivasan et al., 2006; Ngumbi et al., 2007). But in contrast, study on

CMV-infected squash plants shows that virus plants are poor hosts for two species of aphid vectors, M. persicae and Aphis gossypii, and population growth of aphid vectors reduced when forced to feed on infected plants and have a higher rates to emigrate when given the opportunity(Mauck et al., 2010). So volatiles emitted from infected plants with different viruses may affect different behaviors of aphids on virus plants which depend on different mode of virus transmission.

There is one study (Fereres and Moreno, 2009) mentioned that positive effects often occur when Homopteran insects feed on plants infected with non-circulative viruses. In interactions of aphid-virus-plant, complex connections exist. From viruses point, for non-persistent viruses, because aphids will lose transmission ability in few minutes to hours, residence time will be reduced in order to spread virus. But from aphids point, they like stay for long time in order to develop and reproduce. And for plants, there could be a conflict of interest between the virus and the aphid.

Vector transmission associated bottlenecks during horizontal transmission

When aphids transmit viruses from one infected plant to healthy plant in horizontal transmission mode (virus are transmitted from one plant to another plant), there are two steps between infected plant and healthy plant: from infected plant to vector and from viruliferous vector to healthy plant. Virus transmission rate can be influenced during these two steps. For non-persistent viruses,

acquisition and inoculation access periods are brief, usually just a few minutes. Non-persistent viruses are not transmitted by aphid simply, like CMV as a “generalist” plant virus can infect more than 1000 species, but in generalists, some strain have a high degree of specificity usually observed (Ng and Falk, 2006; Blanc et al., 2011).

The molecular determinants for retained and inoculated by vectors have been well studied (Ng and Falk, 2006; Hogenhout et al., 2008). There are two strategies for non-persistent viruses, capsid protein and helper proteins (see section 5). Few viral particles are retained and inoculated by vectors in the case of non-circulating viruses, and population bottlenecks during horizontal transmission of plant viruses by aphids have been postulated to occur for a long time, indicating that the virus has to develop trade-offs between vector transmissibility and other fitness traits. But positive selection was detected at amino-acid positions involved in aphid transmission of CMV (Moury, 2004). Reports on non-persistent viruses have showed that as low as 1–2 infectious virus particles are transmitted on average by a single aphid (Moury et al., 2007; Betancourt et al., 2008; Desbiez et al., 2011). Results from Ali et al showed that the 12 CMV mutants were readily acquired from the source plants by both aphid species, M. persicae and A. gossypii, but the number of mutants decreased significantly when the aphids transmitted the population to test plants, indicting that the bottleneck event occurred during the inoculation period (or infection event) rather than the acquisition access period (Ali et al., 2006). So more

importance of transmission will depend on the number of transmission events related to the aphid population density for aphid-transmitted viruses, and the results that Betancourt et al present strongly suggest that during horizontal transmission, the occurrence of severe bottlenecks is general for viruses non-persistently transmitted by aphids (Betancourt et al., 2008) which are the largest group of plant viruses (Ng and Perry, 2004; Ng and Falk, 2006).

Vector transmission

In a non-persistent manner, plant virus particles attach directly to aphid receptors on the maxillary stylet cuticle within the common food/salivary canal, where viruses directly bind the receptors via a domain of their capsid protein, that is CP strategy used by Cucumoviruses, typically CMV. And helper strategy is that via an additional viral compound referred to as helper component (HC). What influence transmission efficiency of virus are amino acid determinants of CP. Five amino acid changes in the coat protein (positions 25, 129, 162, 168, and 214) of CMV were required to restore efficient transmission by M. persicae and a construct with modified amino acids 129, 162 and 168 was efficiently transmitted by A. gossypii, but poorly for M. persicae (Perry Keith L. et al., 1998), and amino acid determinants for virus transmission have been mapped (Liu et al., 2002).

Transmission efficiency is not only affected by virus strains, but also aphid species, source and recipient plant species, and plant species on which the aphid is maintained(Simons, 1957), Different species of aphids, also different

biological of same aphid species, transmit CMV with varying efficiencies (Simons, 1959; Normand and Pirone, 1968; Basky and Nasser, 1989). M. persicae and A.gosspii are two important vectors to transmit plant viruses in a non-persistent manner, also used most commonly in studies of non-persistently transmitted viruses. It's a polyphagous nature for two aphid species that allows them to feed on a wide range of plant hosts. So it's one important property for viruses like CMV that infect a large number of plant species. In laboratory assays, reports showed that A. gossypii appeared to be the more efficient one in two vectors transmitting CMV (Perry Keith L. et al., 1998; Pinto et al., 2008). But few reports showed effects of geographic differences of same aphid species on transmission efficiency of CMV.

Also climate changes such as increased CO2 and/or temperature might affect the spread of plant viruses via changing geographical distribution range, their densities, migration potential and phenology of plants and vectors (Canto et al., 2009). Understanding factors of virus transmission mechanism is very important to develop effective strategies to block interactions between viruses and aphids in aphid-virus-plant interaction.

Conclusion

Plant-aphid-virus interactions have been researched for several decades, and there are some important questions studied, and still being in process (Figure 3). Although there are reports on virus transmission, here we focus on transmission

efficiency affected by geographic aphid species, virus strains, and finally we hope to get a better understanding of the virus-aphid interactions and to propose new insight in non-persistent virus transmission control in crop protection.

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Figuers and tables

Table 1 Manner of virus transmission transmitted by different aphids

Manner of transmission*	Plant virus			Main aphids of vector
	Family, Genus	Type Species	Number of species	
NP	Betaflexiviridae Carlavirus	CLV	43	*Myzus persicae*
NP	Bromoviridae Alfamovirus	AMV	1	*Myzus persicae*
NP	Bromoviridae Cucumovirus	CMV	4	*Myzus persicae* *Aphis gossypii*
NP	Potyviridae Potyvirus	PVY	146	*Myzus persicae* *Aphis gossypii*
NP	Potyviridae Macluravirus	MacMV	6	*Myzus persicae*
NP	Rhabdoviridae Nucleorhabdovirus	SYNV, SYVV	10	*Aphis coreopsidis* *Hyperomyzus lactucae* or leafhhopper
NP	Secoviridae/Comovirinae Fabavirus	BBWV-1	4	*Myzus persicae* [B]
SP	Caulimoviridae Caulimovirus	CaMV	9	*Acyrthosiphon pisum*
SP	Caulimoviridae Badnavirus	RYNV	25	*Amphorophora agathonica*
SP	Closteroviridae Closterovirus	BYV	11	*Myzus persicae* *Aphis fabae*
SP	Secoviridae Sequivirus	PYFV	3	*Cavariella aegopodii* *Cavariella pastinacae*
P	Luteoviridae Luteovirus	BYDV SbDV BLRV	6	*Sitobion avenae* *Schizaphis graminum* *Acyrthosiphon pisum* *Myzu persicae*
P	Luteoviridae Polerovirus	PLRV BWYV	13	*Myzus persicae*

P	Luteoviridae Enamovirus	PEMV	1	*Acyrthosiphon pisum* *Myzus persicae*
P	Nanoviridae Nanovirus	SCSV	5	*Myzus persicae* *Aphis craccivora* *Aphis gossypii*
P	No family Umbravirus	CMoV	7	*Cavariella aegopodii* *Aphis craccivora*
P	Rhabdoviridae Cytorhabdovirus	LNYV, SCV, BNYV	9	*Hyperomyzus lactucae* *Hyperomyzus carduellinus*
Not sure [A]	Alphaflexiviridae Lolavirus	LLV	1	Aphid
Not sure [A]	Nanoviridae Babuvirus	BBTV	3	Aphid
Not sure [A]	Secoviridae Waikavirus	RTSV	3	Aphid or leafhopper
Not sure [A]	Secoviridae Sadwavirus	SDV	1	Nematode or aphid, seed
Not sure [AB]	Pospiviroidae Pospiviroid	PSTVd	10	Aphid, hopper, beetle

Data from International Committee on Taxonomy of Viruses (ICTV)-report of 2011, Descriptions of Plant viruses (DPV) and Desbiez *et al.*, 2011

* Manner of aphid transmission: non-persistent (NP), semi-persistent (SP), persistent (P)

[A] mentioned in Desbiez *et al.*, 2011

[B] mentioned in DPV

Viruses mentioned in this table (from top) are Carnation latent virus, Alfalfa mosaic virus, Cucumber mosaic virus, Potato virus Y, Maclura mosaic virus, Sonchus yellow net virus, Sowthistle yellow vein virus, Broad bean wilt virus 1, Cauliflower mosaic virus, Rubus yellow net virus, Beet yellows virus, Parsnip yellow fleck virus, Barley yellow dwarf virus, Soybean dwarf virus, Bean leafroll virus, Potato leafroll virus, Beet western yellows virus, Pea enation mosaic virus, Subterranean clover stunt virus, Carrot mottle virus, Lettuce necrotic yellows virus, Strawberry crinkle virus, Broccoli necrotic yellows virus, Lolium latent virus, Banana bunchy top virus, Rice tungro spherical virus, Satsuma dwarf virus, Potato spindle tuber viroid.

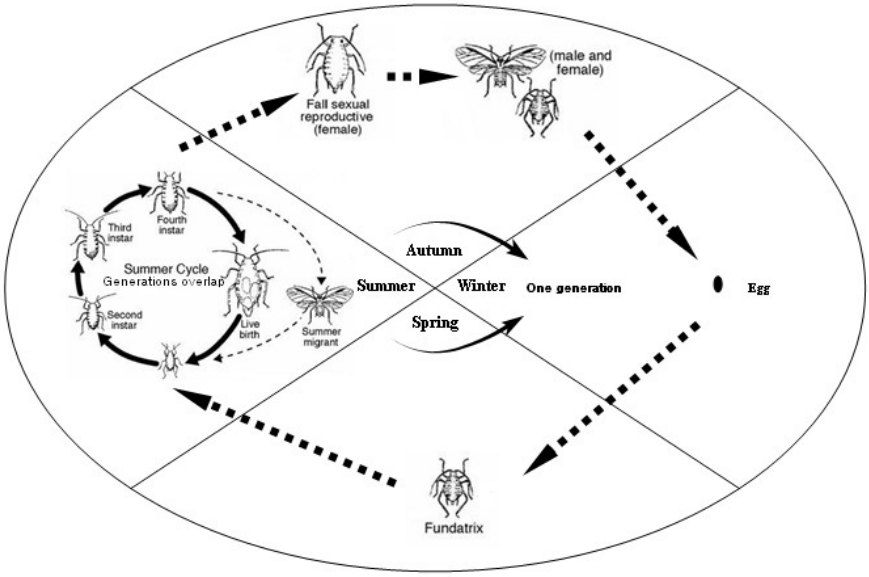

Fig.1 Life cycle of *Myzus persicae*

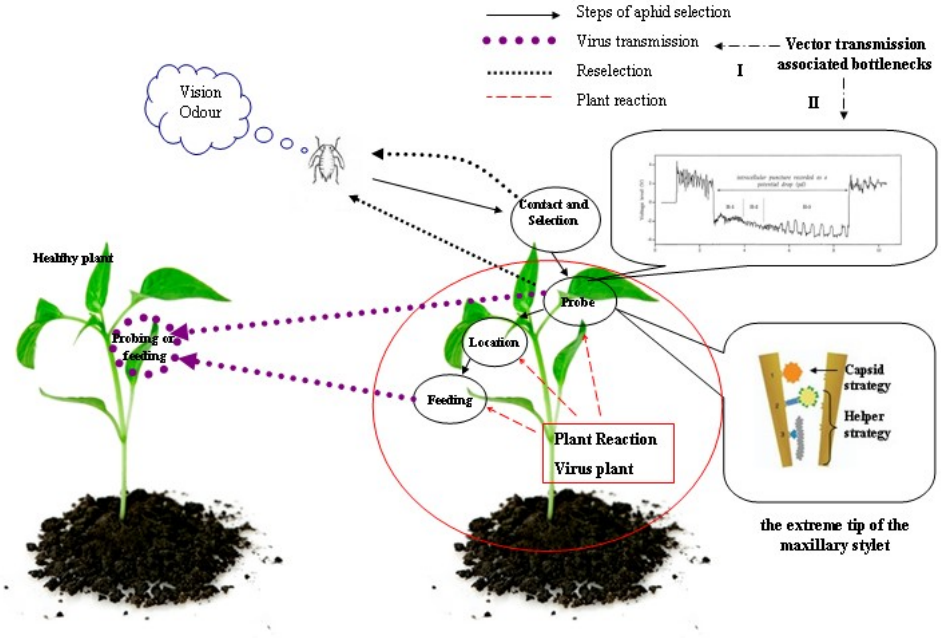

Fig.2 Host plant selection and virus transmission with aphids. Aphids respond visual and odor cues from different sources to find their host plants and identify sites. In order to settle on, probing behavior by aphids will be processed. And according to response from host plants and population, aphids will decide to move on new plants. I: From viruliferous vector to healthy plant, II: From infected plant to vector.

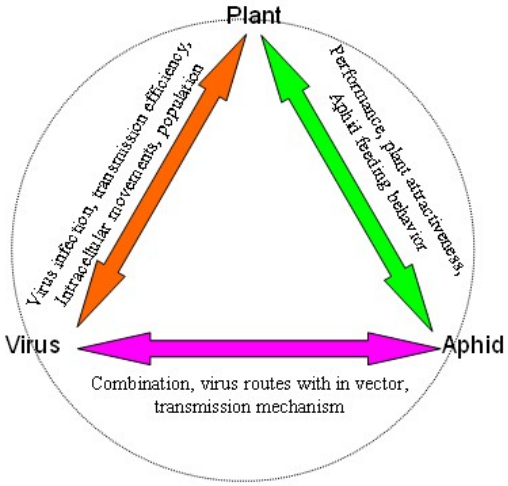

Fig.3 Interactions of plant virus transmitted by aphids. This figure presents some aspects of plant-virus-aphid interactions. The mechanisms of virus transmission by aphids, acquisition, inoculation and role of saliva, are being studied (red). The aspect of aphid feeding behaviors is discussed in this review (green).

General introduction

Many plant viruses, over 400 plant viruses in addition to 697 virus species that have been reported but are not yet officially recognized by the ICTV, are transmitted from plant to plant in nature by invertebrate vectors, members of the Insecta and Arachnida classes of the Arthropada, and the Dorylaimida order of the Nematoda. Virus transmission by aphids (or other vector) involves the transfer of virions from infected to healthy plants. It shows interest from two points of view. One of them is that aphids or other vectors spread many viruses in the field causing biological economic loss, in Europe, it has been suggested that losses due to viruses in field crops are 10-15%, and even higher in vegetables and fruits. Also it would be even worse if the control methods were not already in place. The other view is the interest of relationship between vectors and viruses, especially as some viruses have been shown to multiply in the vector. Also viruses can be regarded as both plant and animal viruses.

China as a very large country has a wide diversity of crops, vegetables, wheat and so on, also viruses damage plants severely. So more and more studies on plant viruses have been published on international journals, but some researches on plant viruses are not always readily available in the literature written in English. We present on plant viruses infecting *Solanaceae*, *Cruciferae*, *Cucurbitaceae*, *Leguminosae* sp. and wheat in China in order to provide more information about viruses in China

Chapter Ⅱ: Bibliographic review on vegetable and wheat virus in China

Bibliographic review on vegetable and wheat virus in China

Rongling Yin[1, 2], Yong Liu [1], Julian Chen [2], Frédéric Francis [3], Claude Bragard [4]

[1]Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

[3]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.Plant protection,

[4] Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

Summary

China is very large country with a wide diversity of crops. The data on plant viruses is not always readily available in the literature written in English. This review presents on plant viruses infecting *Solanaceae, Cruciferae, Cucurbitaceae, Leguminosae sp.* and wheat in China. Additional information, yet unpublished, is also proposed to the reader. Common viruses and references about viruses in China have been gathered to provide information for researchers.

Keyword: vector, species, aphid, coat protein.

Introduction

Shandong is the largest province to produce vegetables in China. Output of vegetables and wheat is very important, but plant viruses damage vegetables and wheat, including mosaic, ring spot, necrosis, shrinking and dwarf. It makes production of vegetables and wheat reduced. For plant viruses, in 1991, the 5th ICTV report acknowledged less than 380 virus species, whereas in the 8th report in 2004, more than 900 species were defined. In China, many viruses have been reported, including major viruses infecting vegetables and wheat. Here, we present informations on virus infecting *Solanaceae*, *Cruciferae*, *Cucurbitaceae*, *Leguminosae sp*. and wheat in China to provide basic information for researchers and further perspectives on aphid-virus investigations.

Associated vegetable and virus species

Solanaceae virus

Many researches were performed on TuMV in China. Chen et al (2002) [1] researched the 3 'end partial sequence variation of 10 TuMV isolates in different host plants from Zhejiang province, China. According to gene phylogenetic tree of coat protein (CP), most of TuMV isolates could be assigned to two evolutionary distant affinity groups. The isolate RS exist in the CP genes from the reorganization of group 1. Song et al (2005) [2] analyzed CP gene sequence of 6 isolates of TuMV from Shandong province, which belong to the same world-B

group. Shi et al (2002, 2005 and 2007) [3-5] analyzed variation of CP and HC-Pro gene of TuMV isolates from vegetables, such as *Brassica narinosa*, *Brassica chinensis*, *potherb mustard* and so on. All of TuMV isolates in China belong to the Asian-BR group and world-B group.

Tian et al (2007)[6] found that Chinese TuMV isolates could be divided into three subgroups, and named Asian-BR group, world-B group and basal- BR group which appeared in 2005. Isolates within Chinese basal- BR group were firstly found in 2007, Wang et al (2009) [7] got the entire sequence TuMV Chinese isolate of basal- BR group for the first time.

In group, Li et al [8] researched molecular population genetics of Chinese TuMV. Phylogenetic analysis results of these 180 isolates of Chinese TuMV were statistically found a basal- BR composition of a sudden outbreak of sub-state, the maximum haplotype diversity value of 1.000, which also showed that diffusion was evident. Isolates of basal-BR subgroup were only found in Taian, Shandong Province. And basal-BR from radish in Taian was in a state of sudden expansion [9].

Turnip mosaic virus resistance is also been studied. There are studies of SSR marker, gene expression analysis of systemic acquired resistance response on TuMV resistance to Chinese cabbage, analysis of AFLP molecular markers of TuMV resistance of Chinese cabbage and inheritance of disease resistance mechanisms [10-12].

Plant induced resistance is an active defense responses of plant. Under action of inducing factor, resisting pathogen infection by activating plant's own resistance mechanisms. It is an important form of plant disease resistance. Now in the process of crop production, characteristics of plants and environment control factors are more and more used for controlling plant pests and diseases. Plants can produce disease resistance after being stimulated by a number of external factors; the resistance is named plant induced resistance. It opens a new way to control of plant diseases, thus becoming hot spot in the field of plant pathology and plant physiology research.

Proteins such as activator are a class one which selected, isolated and purified from a variety of fungi. It improved that immune system of plants by mains of activation of the molecules immune system in plants, and promote the growth of plant roots and increase chlorophyll content of leaf by mains of inspired a series of metabolic regulation in plants. In this way, they can achieve the objective of improving crop yields [13]. Dewen Qiu et al (2005) [14] studied plant activator protein of tobacco mosaic virus induced resistance pot and field effect and on the growth and quality of tobacco. Results showed that plants activation can significantly affect incidence and development of tobacco mosaic virus. Plants activation has a suppression effect to RNA and TMV CP [15].

Fusion protein is one of artificial proteins structured by using genetic engineering techniques to put two or more to gene segment encoding protein connected together destination and express them. Fusion protein is used widely, include in

plant diseases. Here is a report, which used a synthetic method for synthesis of the full-length harpin gene, and expressed Harpin protein in *E. coli* [16]. The results showed that Harpin protein can induced allergic reactions of tobacco and pepper, and induced activity of plant resistance to TMV.

The incidence of CMV is common in China, scholars have isolated CMV from 38 families in 120 kinds of plants, such as *Cruciferae*, *Solanaceae*, *Leguminosae sp.* and *Cucurbitaceae*. Xu et al [17] divided other existing CMV strains or isolates into two sub-groups, CMV subgroup Ⅰ and Ⅱ, according to host reactions, serological relationships, virus coat protein peptide mapping analysis, dsRNA analysis, nucleic acid hybridization, RT-PCR products of enzymatic analysis and DNA sequencing analysis and other methods. This distinction reflects evolutionary relationship between them. Determination of similarity rate of partial sequences from more than 50 strains of CMV CP gene nucleotide sequence, similarity rates of different isolates in same subgroup is more than 90%, while isolates in different subgroups only 70% -80% [18].

Professor Cao and Qin from Department of Plant Protection, Nanjing Agricultural University got a TNV which isolated from soybean and did some preliminary works on biology, morphology and serological of this isolate [19, 20]. The isolate has wide host range, can infect 34 species plants in 9 families. Symptoms are usually localized dry spots, after this isolate system infecting

soybean, necrosis spot will appear in upper leaves.

The challenge of transgenic plants with *potato virus X Potexvirus* (PVX) revealed that expression products of PVY-C HC-Pro mutants in transgenic plants grestly abolished functions of HC-Pro in enhancing accumulation and pathogenicity of PVX, indicating that CCCT and PTK motifs of HC-Pro were required for PVX/ PVY synergism. Meanwhile, results demonstrated that PVY-C HC-Pro had a function in accelerating long-distance movement of PVX in these transgenic plants for the first time [21]. And some studies on sequence analysis of CP and HC-pro gene of Potato Virus Y O, C, N strain [22].

Occurrence and distribution, detection and prokaryotic expression of *Tomato spotted wilt virus* have been studied in several reports [23, 24, 25, 26].

Studies on detection of *Tobacco rattle virus* (TRV) and *Tobacco ringspot virus* (TRSV) [27, 28], identification of *Tobacco necrosis virus* [29], clone of TEV CP, HC-Pro and cross protection between *Tobacco etch virus* and *Sugarcane mosaic virus* [30] have been showed. And also characterization of *Tomato mosaic virus*[31] and *Tobacco leafcurl yunnan virus*[32-34] of Chinese isolate and its nucleotide sequence, isolation and identification of *Alfalfa mosaic virus* strains and disease resistance of transgenic plants [35, 36] have been studied.

In China, Xiang benchun first reported *Pepper mild mottle virus* which was

founded in Xinjiang chili pepper in 1994 [37]. Later in Qingdao, Baoding, Huinong and other areas, there are reports of this virus occurred. In recent years, with introduction varieties of foreign sweet pepper, pepper mild mottle disease also appears in greenhouse in vicinity of Beijing [38-40]. In 2006, this virus had been isolated, and genome sequence has been analyzed, named PMMoV isolates in China (PMMoV-CN) [41].

Cruciferae virus

The expression profile of CaMV 35s promoter was clearly described using the GUS as a reporter gene in transgenic cotton from results of GUS gene expression in cotton callus, somatic embryogenesis, cotton root, stem, leaf, flower organs and developing embryo [42].

It is also clear that small differences in sequence must account for the observed differences in host range and symptoms. It seems like that distinction between RMV and YoMV will be difficult to maintain, but further sequences of biologically-characterized isolates are needed before drawing a firm conclusion on their nomenclature and taxonomy [43]. Sequence analysis shows that *Ribgrass mosaic virus* Shanghai isolate (RMV-Sh) is closely related to Youcai mosaic virus.

In China, viruses of sugar beet were noticed by some researchers, and BtMV was

considered the causal agent, and some viruses infecting sugar beet also were studied including *Beet western yellows virus* [44], *Beet necrotic yellow vein virus*[45],*Beet black scorch virus*[46],*Beet soilborne virus*[47], *Beet western yellows virus*[48], and also some results of detection of Broad bean wilt virus (BBWV) [49] and identification and characterization of Oilseed rape mosaic virus (ORMV) [50]. For RaMV, there is no report only mentioned in book [51].

***Cucurbitaceae* virus**

A seed- borne virus of *Cucurbitaceae* was discovered recently in main land of China, which is dangerous disease potentially [52]. In 1987, Xu et al isolated and identified the virus from watermelon, melon and cucumber in Taiwan [53]. There are reports about identification and detection of *Cucumber green mottle mosaic virus*, cloning and sequence analysis of the CP gene [54].

Watermelon mosaic virus (WMV) widespread occurrence in China, researchers have obtained different isolates from Shanxi, Shandong, Yunnan, Liaoning, Shanxi, Xinjiang, Henan and Heilongjiang province and so on[55-58]. Wu et al reported genome sequence of Chinese WMV, differences between France and China WMV strains in 2006, indicating that the WMV genome has diversity around the world [59]. Others reports also showed isolation and identification receptor of WMV in aphids [60].

Here reported identification of Watermelon mosaic virus 2 isolate (WMV-2) and its coat protein gene sequence [61]. In the survey of virus diseases of

Cucurbitaceae during the 1955-1965 years in China, researchers reported that the Melon mosaic virus (MMV) is the main drug of its. But now researches prove that the MMV is WMV-2 [62].

Studies on Chinese squash leaf curl virus: biological and serological properties and molecular hybridization [63]. Now, scholars have do some researches on construction of Papaya ringspot virus Hunan isolate [64], cloning expression of its HC-pro gene [65], and transgenic papayas in China [66] .

Some works about identification, sequence analysis of ZYMV's coat protein and Chinese strain for resisting to ZYMV in watermelon [67-69] have been studied, also genomic sequence of a Chinese isolate of SqMV[70].

Fabaceae virus

Researchers have reported partition of Soybean mosaic virus (SMV) strains in China, but classification conclusions are not unified. Considering the geographical distribution and genetic mutations and other factors, researchers unified partition the national SMV strains, in order to facilitate further research in United States, Japan and other countries. There is not having uniform standard to demarcation SMV strains in China, which will create difficulties for future in-depth study and molecular detection and genomic of SMV [71, 72].

As fellows, Nucleotide Sequence Analyses of RNA3 of Peanut stunt virus [73],

Cloning and sequencing of peanut stripe virus Coat Protein gene [74,75], sequence determination of small coat protein gene of Broad bean stain virus and the development of RT-PCR detection method [76,77]. And researchers identified and studied broad bean true mosaic virus (BBTMV), from the biological, physical and chemical characteristics and serological characteristics and others sides [78,79]. Coat Protein of Bean yellow mosaic virus isolates from Faba bean in Yunnan, Chinahave been analyzed [80]. And some works have done about the serology prime identification of pea mosaic virus [81], SRNA sequences in *Tospovirus*[82], first Report of Broad bean wilt virus 2 in Echinacea purpurea in China[83].

Poaceae virus

In China, Zhou et al isolate barley yellow dwarf virus into GAV, GPV, PAGV and RMV [84]. Because of the different geography sources, the variability of BYDVs may exist. The main objective of the experiments was to identify the molecular variability, strains type and phylogenetic relationship of different BYDVs Cp genes using the materials of Chinese wheat isolates which were infected by virus [85].

It have wide distribution in China, the disease appeared in Rongcheng in Shandong in 1958, Sichuan Yaan in the sixty years, and gradually spread to many other regions. The Yangtze River and Huaihe River in the provinces and other regions in Henan, Shaanxi, Shandong and other provinces have also occurred on

and it is serious harm to growth and yield of wheat [86-88].An experimental system using cDNA clones suitable for Production of infectious RNA transcripts in vitro， and to inoculate these to cultured wheat cell lines and tobacco protoplasts. Primary research on the function of the PI Protein encoded by WYMV RNA2 was carried out in this infectious system [89].

A single open reading frame of 891 nucleotides and a no translated region of 258 nucleotides at the 3'-end excluding the poly (A)-tail. The nucleotide sequence shares homology of 67.6%and 69.9% with barley yellow mosaic virus (BaYMV) RNA 1 and wheat spindle streak mosaic virus RNA1 reported in France (WSSMV-F) with in the same length of the 3'-terminals [90].

The result s indicated that none of all thirteen isolates was identical each other at the molecular level. Differences in RNA2 were greater than those in RNA1. The variations were very complicated so that none of modern molecular techniques could simply correlate the variations at nucleotide level with pathogenicity or strain differentiation [91]. Molecular cloning, sequencing and expression of CP gene of Barley stripe mosaic virus China strain (BSMV-CH) in *E.coli* and its antiserum preparation [92].

Coat Protein gene of the virus was amplified by RT-PCR, cloned into PUC19

vector and sequenced. It was identical in length (753 nucleotide; 251 amino acids) to those of isolate from Japan, Korea, Germany, France and the UK. The Results confirmed that the virus detected in China was indeed BaMMV and suggested that Chinese strain of BaMMV has also long established [93].

Other viruses

Transformation of *Soil-Borne Mosaic Virus* [94], and molecular detection and identification of wheat rosette stunt disease have been studied[95].

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Tables

Table 1 Viruses infecting *Solanaceae* plants

Species, Acronym	Family, Genus	Vectors or mode of transmission	Related references
Turnip mosaic virus, TuMV	*Potyviridae Potyvirus*	Aphid	1-12
Tobacco mosaic virus, TMV	*Virgaviridae Tobamovirus*	Mechanical, seed	13-16
Cucumber mosaic virus, CMV	*Bromoviridae Cucumovirus*	Aphid, seed	17-18
Tobacco necrosis virus, TNV	*Tombusviridae Necrovirus*	Fungus	19
Potato virus X/Y, PVX/Y	*Potyviridae Potyvirus*	Aphid	20, 21
Tomato spotted wilt virus, TSWV	*Bunyaviridae Tospovirus*	Thrip	22, 23
Watermelon silvery mottle virus, WSMoV	*Bunyaviridae Tospovirus*	Thrip	24
Peanut yellow spot virus, PYSV	*Bunyaviridae Tospovirus*	Thrip	25
Peanut chlorotic fan -spot virus, PCFV	*Bunyaviridae Tospovirus*	Thrip	26
Tobacco rattle virus, TRV	*Virgaviridae Tobravirus*	Nematode	27
Tobacco ringspot virus, TRSV	*Secoviridae Nepovirus*	Nematode, seed	28
Tobacco etch virus, TEV	*Potyviridae Potyvirus*	Aphid	30
Tomato mosaic virus, ToMV	*Virgaviridae Tobamovirus*	Mechanical, seed	31
Tobacco leafcurl yunnan virus,	*Geminiviridae*	Whitefly	32-34

TbLCYNV	*Begomovirus*		
Alfalfa mosaic virus, AMV	*Bromoviridae* *Alfamovirus*	Aphid	35, 36
Pepper mild mottle virus, PMMoV	*Virgaviridae* *Tobamovirus*	Mechanical, seed	37-40
Tomato ringsopt virus, ToRSV	*Secoviridae Nepovirus*	Nematode, seed	41

N: Mentioned but there is no paper about it.

Table 2 Viruses infecting *Cruciferae* plants

Species, Acronym	Family, Genus	Vector or mode of transmission	Related references
Turnip mosaic virus, TuMV	*Potyviridae Potyvirus*	Aphid	*
Cucumber mosaic virus, CMV	*Bromoviridae Cucumovirus*	Aphid, seed	*
Tobacco mosaic virus, TMV	*Virgaviridae Tobamovirus*	Mechanical, seed	*
Tobacco ringspot virus, TRSV	*Secoviridae Nepovirus*	Nematode, seed	*
Alfalfa mosaic virus, AMV	*Bromoviridae Alfamovirus*	Aphid	*
Cauliflower mosaic virus, CaMV	*Caulimoviridae Caulimovirus*	Aphid	42
Ribgrass mosaic virus, RMV	*Virgaviridae Tobamovirus*	Not sure	43
Beet mosaic virus, BtMV	*Closteroviridae Closterovirus*	Aphid	44
Beet necrotic yellow vein virus, BNYVV	*Benyvirus*	Fungus	45
Beet black scorch virus, BBSV	*Necrovirus*	Fungus	46
Beet soilborne virus, BSBV	*Pomovirus*	Fungus	47
Beet western yellows virus, BWYV	*Polerovirus*	Aphid	48
Broad bean wilt virus, BBWV	*Secoviridae Fabavirus*	Aphid	49
Youcai mosaic virus, YMoV	*Virgaviridae Tobamovirus*	Mechanical, seed	50
Radish mosaic virus, RaMV	*Secoviridae Comovirus*	Beetle	51

* Mentioned above in this paper

N: Mentioned but there is no paper about it.

Table 3 Viruses infected *Cucurbitaceae* plants

Species, Acronym	Family, Genus	Vector or mode of transmission	Related references
Cucumber mosaic virus, CMV	*Bromoviridae Cucumovirus*	Aphid	*
Tobacco mosaic virus, TMV	*Virgaviridae Tobamovirus*	Mechanical, seed	*
Turnip mosaic virus, TuMV	*Potyviridae Potyvirus*	Aphid	*
Cucumber green mottle mosaic virus, CGMMV	*Virgaviridae Tobamovirus*	Mechanical, seed	52-54
Watermelon mosaic virus, WMV	*Potyviridae Potyvirus*	Aphid	55-62
Squash leaf curl virus, SLCuV	*Geminiviridae Begomovirus*	Whitefly	63
Papaya ringspot virus, PRSV	*Potyviridae Potyvirus*	Aphid	64-66
Zucchini yellow mosaic virus, ZYMV	*Potyviridae Potyvirus*	Aphid	67-69
Squash mosaic virus, SqMV	*Secoviridae Comovirus*	Beetle, seed	70

* Mentioned above in this paper

N: Mentioned but there is no paper about it.

Table 4 Viruses infected *Leguminosae sp.* plants

Species, Acronym	Family, Genus	Vector	Related references
Cucumber mosaic virus, CMV	*Bromoviridae Cucumovirus*	Aphid	*

Turnip mosaic virus,TuMV	*Potyviridae Potyvirus*	Aphid	*
Alfalfa mosaic virus, AMV	*Bromoviridae Alfamovirus*	Aphid	*
Soybean mosaic virus, SMV	*Potyviridae Potyvirus*	Aphid	71,72
Peanut stunt virus, PSV	*Bromoviridae Cucumovirus*	Aphid	73
Peanut stripe virus	*Potyviridae Potyvirus*	Aphid	74,75
Broad bean stain virus, BBSV	*Secoviridae Comovirus*	Seed	76,77
Broad bean true mosaic virus, BBTMV	*Secoviridae Comovirus*	Seed	78,79
Bean yellow mosaic virus, BYMV	*Potyviridae Potyvirus*	Aphid	80
Pea mosaic Virus	*Potyviridae Potyvirus*	Aphid	81
Tomato spotted wilt virus, TSWV	*Bunaviridae Tospovirus*	Thrip	82
Broad bean wilt virus, BBWV	*Secoviridae Fabavirus*	Aphid	83

* Mentioned above in this paper

N: Mentioned but there is no paper about it.

Table 5 Viruses infected wheat plants

Species, Acronym	Family, Genus	Vector	Related references
Barley yellow dwarf virus, BYDV	*Luteoviridae Luteovirus*	Aphid	84,85
Wheat yellow mosaic virus, WYMV	*Potyviridae Bymovirus*	Fungus	86-89
Wheat spindle streak mosaic virus, WSSMV	*Potyviridae Bymovirus*	Soil	90
Barley stripe mosaic virus, BSMV	*Virgaviridae Hordeivirus*	Seed	91, 92
Barley mild mosaic virus, BaMMV	*Potyviridae Bymovirus*	Fungus	93
Wheat soil-borne mosaic virus, WSBMV	*Virgaviridae Furovirus*	Soil, fungus	94
Northern cereal mosaic virus, NCMV	*Rhabdoviridae Cytorhabdovirus*	Planthopper	95

N: Mentioned but there is no paper about it.

Chapter Ⅲ: Objectives

This thesis focussed on the process of virus transmission in a non-persistent manner especially the CMV and *M. persicae* model.

The first part was the analysis of molecular characterization of coat protein of CMV. Virus strain with high transmission efficiency by *M. persicae* was selected.

The second part was the investigation of the transmission efficiency of different virus strains by green peach aphid collected from different plants and places in China.

The third part was the evaluation of the effects of plant lectins when ingested before transmission assays on virus transmission by aphids.

All of these parts provided more information's to better understand the virus-aphid interactions and to propose new insight in non persistent virus transmission control in crop protection.

In the fourth chapter, coat protein of CMV from different places and plants in China, and molecular characterization of coat protein have been analyzed and discussed.

Virus strains from China and Belgium have been tested to determine transmission efficiency by *M. persicae*. Discussion including transmission efficiency related to CMV coat protein characters in order to understand relation between aphids and

virus was performed.

In the fifth chapter, aphids from different places in China and plant was used to determine CMV transmission efficiency according to aphid clone diversity.

In the last chapter, plant lectins (Galanthus Nivallis Agglutinin, Wheat Germ Agglutinin, Pisum Sativum Lectin) have been tested to evaluate competition effects on virus transmission, and to improve the role of plant lectins in virus-aphid interaction process.

In conclusions, CMV transmission efficiency according to virus strains and aphid clones diversity from different places in China were investigated. Lectins in CMV - plant - aphid interactions focusing on *M. persicae* in vegetable crops was studied and finally got a better understanding of the virus-aphid interactions and to propose new insight in non-persistent virus transmission control in crop protection.

Chapter Ⅳ: Effects of virus strains on transmission efficiency of CMV transmitted by Myzus persicae

Ⅳ.1 Molecular characterization of coat protein of Cucumber Mosaic Virus from Shandong and Beijing in China

(soumis à Plant Pathology)

Rongling Yin[1, 2], Frédéric Francis[1],Claude Bragard[2],Yong Liu[3], Julian Chen[4], Dengfa Cheng[4]

[1]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.

[2]Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

[3]Plant protection, Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[4]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

Abstract

Many new vegetable varieties are introduced from abroad in China, and as the type species in the genus *Cucumovirus* of family *Bromoviridae*, *Cucumber mosaic virus* (CMV) is infecting large number of plant species using vector transmission by aphids in a non-persistent manner with capsid strategy. In this study, total RNA was extracted from leaves of infected plants collected from Beijing, Shandong Province in China and amplified in reverse transcriptase polymerase chain reaction with primer set designed in the coat protein region of CMV. Bands of expected size (~657bp) were visualized in agarose gel. The results of comparison and phylogenetic tree revealed that 5 CMV isolates belong to subgroup IB, not subgroup II for low identity. And the comparison of amino acid sequences showed different at some positions (25, 31, 33, 65, 71, and 207). Also coat protein amino acid changes may show effects on transmission by aphids.

***Key words*:** subgroup, identity, transmission, isolates

Introduction

Cucumber mosaic virus (CMV) is the type species in the genus Cucumovirus, family Bromoviridae. CMV has one of the largest host ranges of any virus. Numerous reports show that the number of plant species identified as hosts for CMV has increased steadily as new host surveys are conducted. The host range of CMV today exceeds 800 species, more than 85 families. CMV hosts include solanaceous crops, such as tobacco, tomato, and pepper, along with cucurbitaceous crops and many ornamentals (Palukaitis et al., 1992). In China, there are lots of vegetables bases in Shandong province and in northern of Beijing there are also many bases providing green vegetables especially during the Olympic Games in 2008.

The genome of CMV consists of three positive sense, single-stranded RNAs (RNA 1, RNA 2 and RNA 3) and a subgenomic RNA (RNA 4) encoded by RNA3 that is involved in encapsidation(Suzuki et al., 1991, Palukaitis et al., 1992). Several CMV isolates reported from all over the world have been placed into two subgroups I and II, CMV subgroup I has been recently divided into IA and IB on the basis of gene sequences available for CMV strains and phylogenetic analysis (Palukaitis and Zaitlin, 1997, Roossinck et al., 1999, Roossinck, 2002). Recent phylogenetic analysis of CMV by use of CP ORF and 5’non-translated region (NTR) sequences confirmed the grouping and also led to further subdivision of subgroup I into IA and IB(Roossinck et al., 1999), and recombination between

subgroups IA and IB was reported(Chen et al., 2007).

The Cucumovirus use vector transmission by aphids (such as Myzus. persicae, Aphis. gossypii) in a non-persistent manner with capsid strategy (Brault et al., 2010, Foster et al., 2008). Coat protein determined virus transmission efficiency that was demonstrated by two independent research groups using different Cucumoviurs species (Chen and Francki, 1990, Gera et al., 1979). In addition to affecting transmission, the known roles of the CMV coat proteins have been shown to play important roles in encapsidation, systemic movement (Suzuki et al., 1991), host range (Shintaku and Palukaitis, 1990), and aphid transmission (Gera et al., 1979, Chen and Francki, 1990, Perry et al., 1994). Small changes of coat protein in the virus can dramatically influence transmission and some changes in the transmission phenotype can differ radically depending on the species of aphid-vector. Amino acid of coat protein changes may condition a differential interaction with some specific factors of vector, host, or viral origin, and only one (limiting) part of the transmission process may be differentially affected(Perry et al., 1998).

With the development of economy, the quality and demand of varieties is higher and higher, farmers has introduced many new vegetable varieties from abroad, especially after 2008. So in this study, molecular characterization of the coat protein of these isolates from Shandong and Beijing and their exact identification were reported. The aim is to find if there is some differences among isolates and also if some changes happened in some isolates. We also studied phylogenetic

relationship of CP with other CMV isolates, and changes in virus coat protein that may influence aphid transmission.

Materials and methods

Materials

Infected plants leaves (*Brassicaceae*, *Cucurbitaceae* and *Solanaceae* families) collected in the field from two different districts, Shandong province and Beijing in China, divided each of them into two pieces then inoculating with tobacco（*Nicotiana tabacum*）and putting them into low temperature freezer (-70 ℃) after taking back to laboratory.

RNA extraction and RT-PCR

TRIZOL Reagent (Invitrogen) was used for RNA extraction. We used two-step RT-PCR. The genome sense primer 5’-ATGGACAAATCTGAAT CAAC-3’ derived from the beginning of the first 20bases of the coding region and the antisense primer 5’-TCAAACTGGGAGCACCC-3’ representing last 17 bases of the coding region of the CP gene were used to prime the reaction for CMV detection (Siju et al., 2007). DNA Synthesis Supermix (TransGen Biotech, Beijing, China) was used for first strand cDNA synthesis. The program of RT step consisted of 50℃ (30min), followed 85℃ (5min). Per eppendorf tube contained 2.5µl RT product and 22.5 µl mix PCR (PCR reaction buffer (Biomed-tech Beijing China), primers, ddH2O). Samples were amplified in a thermocycler and the program followed 40 cycle reaction profile involving

denaturation at 94℃(30 s), primer annealing at 50℃ (30s), extension at 72℃ (1min) with a final extension of 72℃(10min).The amplified products were analyzed on 1.2% agarose gel electrophoresis in TAE buffer, stained with ethidium bromide. The product of PCR fragment was cut from gel and eluted in order to clone.

Cloning and analysis of CP

The purified product was cloned in pEasy-T3 vector (TransGen Biotech, Beijing, China), then the ligated vector was transformed into *E.coli* (Trans-T1) provided by the cloning kit. Positive colonies were selected to do PCR and were subjected to sequencing (Beijing Sunbiotech Co., Ltd.), using primer M13. Sequences data were fed for BLAST analysis, which also were analyzed using MEGA v4.1（http://www.megasoftware.net/）and DNAstar program to structuring phylogenetic tree with other sequences that had been present already in the NCBI database worldwide and from the same vicinity by accounting standard reference of CMV from each subgroup.

Results and analysis

RT-PCR and cloning

Total RNA was extracted from infected leaves of plants. cDNA was made from total RNA by downstream primer application. RT-PCR on isolates of cucumber, tobacco in Taian(TA), cucumber in Shouguang,(SG) and tomato in Beijing(BJ) amplified successfully the coat protein gene of virus and a product of

expected size (~650bp) was observed (Fig. 1). The product of PCR-amplified fragment was cut from gel and eluted in order to clone. The transformed cells were plated on selection media containing ampicillin, X-gal and IPTG. Positive colonies were selected to do PCR and then were subjected to sequencing.

Phylogenetic analysis of 5 CMV isolates with others based on nucleotide sequence

Phylogenetic relationship of 5 CMV isolates with the strains of *Cucumber mosaic virus* coat protein (657bp) subgroups Ⅰ (A and B), II present in GenBank (Table. 1), including parts of CMV isolates from Shandong and Beijing in China, based on the nucleotide alignment using MEGA v4.1.

The alignment files created by MEGA v4.1 were bootstrapped 1000 times for generating neighbor-joining phylogenetic tree using Tree Explorer (Fig. 2). Tobacco mosaic virus (TMV) CP gene (accession No. AY313136) was used as outsource. Sequence analysis of CP of CMV isolates revealed that 5 CMV isolates belong to subgroup I, and all of them (BJ-tomato, TA-tobacco, SG, TA-cucumber, TA) belong to subgroup IB. The sequences were compared to equivalent sequences from a range of other cucumber mosaic virus coat protein gene present in GenBank. Only one of them (SD丨EF159146) from Shandong and Beijing belongs to subgroup IA, the rest of them belong to subgroup IB.

Multiple alignments based on complete coat protein gene

Manual multiple sequence alignment is performed at deduced amino acid

levels by taking into account a standard reference from each subgroup IB (D42079, Y16926), IA(D10538, D00462, D28487) and subgroup II (AF063610, L15336, M21464).

The results of coat protein amino acid changes revealed that at position 12, Serine(S) is observed in TA-tobacco. At position 26 (25, exactly the 25 amino acid) and 66（65）, TA-CP shows proline (P) and arginine (R) is different with other 4 CMV isolates (S). At position 32（31）, 34（33）, 208（207）, TA-cucumber CP shows lysine (K), threonine (T) and leucine (L), which is the same (valine, V) to subgroup II but is different from D28487 (FT, IA). At position 72（71）, Serine (S) was observed in SG-CP, TA-tobacco-CP, also in D42079 (C7-2, IB).

Comparison of percent nucleotide sequence and the deduced amino acid identities

A comparison was made of percent nucleotide sequence and the deduced amino acid sequence of the coat protein gene of 5 CMV isolates with other CMV coat protein gene sequences reported from the same geographical region with standard sequence of subgroup IB(Y16926, Tfn), as well as sequences from subgroup IA and II. Sequence comparisons were made with the DNAstar program. The result showed that the standard CMV strain (Y16926, Tfn) showed high sequence identity (97%) with 5 CMV isolates at the nucleotide level, also at the amino acid level (Maximum 99.5). On the other hand, CMV from subgroup IA (D10538, Fny) showed also high sequence identity (nucleotide level maximum 95%, amino acid level higher than96%). But subgroup II stains

showed maximum 83% sequence identity indicting that the sequences do not belong to subgroup II.

Discussion

Molecular characterization is considered to aid in better understanding of the genetic composition, variation caused by mutation and recombination, and taxology of the virus that helps in finding out how the strain under study relates to other strains of CMV reported from all over the world, including those reported from the same local geographical region. There also is knowledge of how new strains evolve or adapt to new hosts and geographic conditions provided by molecular characterization.

RT-PCR can be used to rapidly and sensitively detect plant viruses. Actually, RT-PCR method has proven to be more sensitive than enzyme linked immunosorbent assay and dot-blot hybridization (Hu et al., 1995). Various methods have been used to analyze the diversities among different CMV isolates. Most of these studies were phylogenetically oriented and allowed the subdivision of CMV isolates from all over the world into three subgroups: IA, IB and II (Palukaitis et al., 1992, Roossinck et al., 1999, Roossinck, 2002).

Phylogenetic and diversity studies have shown that there are three subgroups of CMV (Palukaitis et al., 1992, Palukaitis and Zaitlin, 1997, Roossinck et al., 1999). Subgroups I and II are quite distantly related, and their genomes have approximately 75% nucleotide identity. Subgroup I can be further divided into

subgroups IA and IB that are more closely related (92–95% nucleotide identity) (Roossinck, 2002). The overall pairwise sequence identity of 5 isolates with members of subgroup II is lower at both nucleotide and amino acid sequence levels than subgroup I, as well as their position generated in the phylogenetic trees, revealed that the 5 isolates belongs to subgroup I (Fig.2 and Table 2). The overall higher degree of homology exhibited at the amino acid level between all the strains might indicate the constraints imposed on the virus: variation in the coat protein to maintain the structural and functional role presumably for virion stability, transmission by aphids and the movement within the host plants (Wikoff et al., 1997, Perry et al., 1998, James et al., 2000).

Strains of cucumber mosaic virus vary with respect to the efficiency by which they can be transmitted by different species of aphids and there is specificity in that not all aphid species can function as vectors (Bhargava, 1951, Kennedy et al., 1962). Genetic analyses of CMV have provided important information relevant to transmission. The primary determinant for the aphid transmissibility of CMV has been shown to be the coat protein (Gera et al., 1979, Chen and Francki, 1990).

Alteration in single amino acid position induces altered symptom expression in host plants (Suzuki et al., 1991, Shintaku et al., 1992, Suzuki et al., 1995). Aphid transmissibility is affected by amino acid changes in the coat protein (positions 25, 129, 162, 168, and 214) (Perry et al., 1994, Perry et al., 1998). Sequence analysis of CP at the amino acid level reveal that there are many

positions where amino acid shows diverse effect compared to others isolates used in the study, especially amino acid 25（Pro to Ser）may make different from strains in aphid transmission. The other positions are N32(31)T, R34(34)K, I206(205)V, H208(207)L of TA-cucumber, N12S, T72(71)S of TA-tobacco and T72(71)S of SG (Fig.3). These positions may have some impact on the coat protein orientation, symptom expression, transmission etc. which need to be further studied.

The virus was able to be transferred mechanically injured tissues from infected plants to non-infected plant and produced the systemic and local symptoms characteristic of CMV. Plant-feeding arthropods, especially the aphid species (*A. gossypii* and *M. persicae*) transmitted in non-persistent manner that have been described as efficient vectors in plant-to-plant transmission of viruses. Studies on genetic structure and diversity would be important to help in better understanding the evolutionary mechanisms that generate and/or maintain variation in viral populations and their evolution, and also can be used to develop transgenic geraniums that will be useful for the growers. Thus, such studies may help in the development of strategies for the control of viral diseases.

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The authors express their gratitude to Dr Frederic FRANCIS, Gembloux Agro-Bio-Tech, Universite de Liege and financial assistance from project of Développement et valorisation de nouvelles stratégies de lutte contre les

ravageurs, vecteurs de maladies virales, en milieu rural dans la Province de Shandong (P.R. Chine).

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Figures and tables

Table 1 Coat protein gene sequences of various *CMV* strains used for comparison

Accession no.（abbreviations）	Geographic origin	Group	Accession no.（abbreviations）	Geographic origin	Group
DQ916111	Shandong（SD）		AB004780（KM）	Japan	IA
EF122499	SD		D10538（Fny）	USA (NY)	IA
EF159146	SD		D00462（C）	USA (NY)	IA
EF409974	SD		L36251（Kor）	Korea	IA
EU429567	SD		U66094（Sny）	Israel	IA
FJ403473	SD		U22821（Ny）	Australia	IA
FJ403474	SD		D28487（FT）	Japan	IA
DQ302714	Beijing（BJ）		D10544（FC）	USA	IA
DQ302715	BJ		AJ890464（OL）	India	IA
DQ302716	BJ		AJ831578（L1）	India	IA
DQ302717	BJ		AJ890465（Lt）	India	IA
L15336（Trk7）	Hungary	II	D42079（C7-2）	Japan	IB
M21464（Q）	Australia	II	AJ271416（2A1-A）	USA	IB
AF063610（S）	USA	II	AF013291（As）	Korea	IB
AF127976（LS）	USA	II	Y16926（Tfn）	Italy	IB
U10923（SP103）	USA	II	AB042294（IA-3a）	Japan	IB
AB006813（m2）	Japan	II	D28780（NT9）	Taiwan	IB
U22822（Sn）	Australia	II	U31220（Oahu）	USA	IB
L40953（Wem）	Unknown	II	X89652（Phym）	India	IB
AJ585086（AL）	India	II	AF281864（D）	India	IB
AF350450（H）	India	IB			

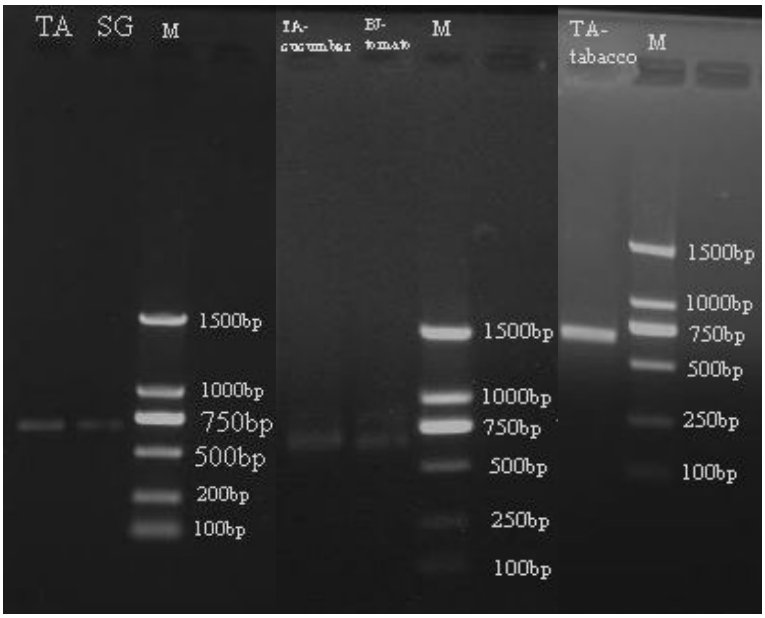

Fig. 1 RT-PCR amplification of CMV coat protein gene

TA: cucumber leaves, SG: cucumber leaves, TA-cucumber: cucumber leaves

(different plant from TA), BJ- tomato: tomato leaves, TA-tobacco: tobacco leaves.

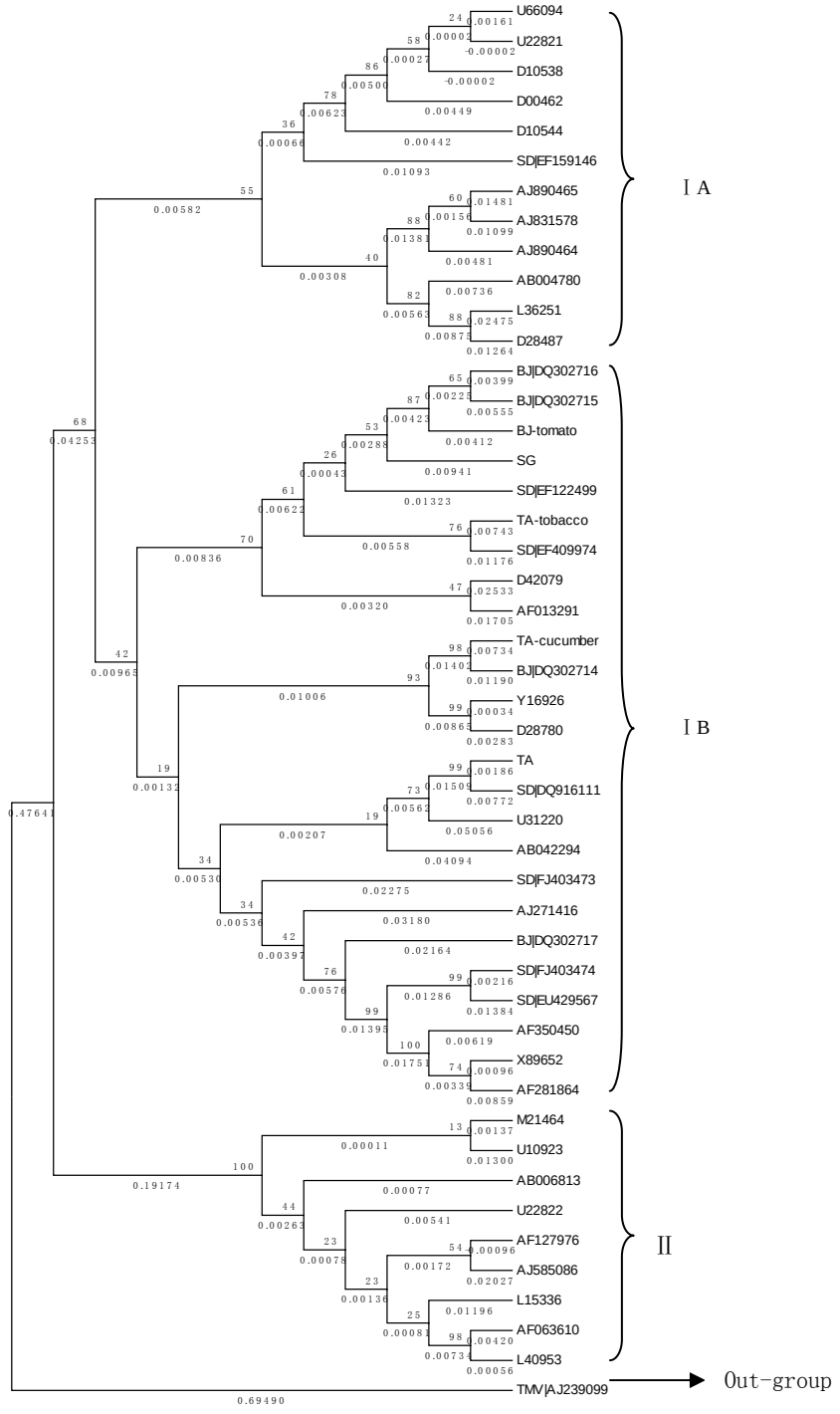

Fig. 2 Phylogenetic relationship of 5 CMV isolates with the strains of CMV subgroups I (A and B), II based on the nucleotide alignment using MEGA v4.1. *Tobacco mosaic virus* (TMV) (Acc. No. AY313136) was used as an outgroup. The bootstrapping and branch length values are above and below the joining lines.

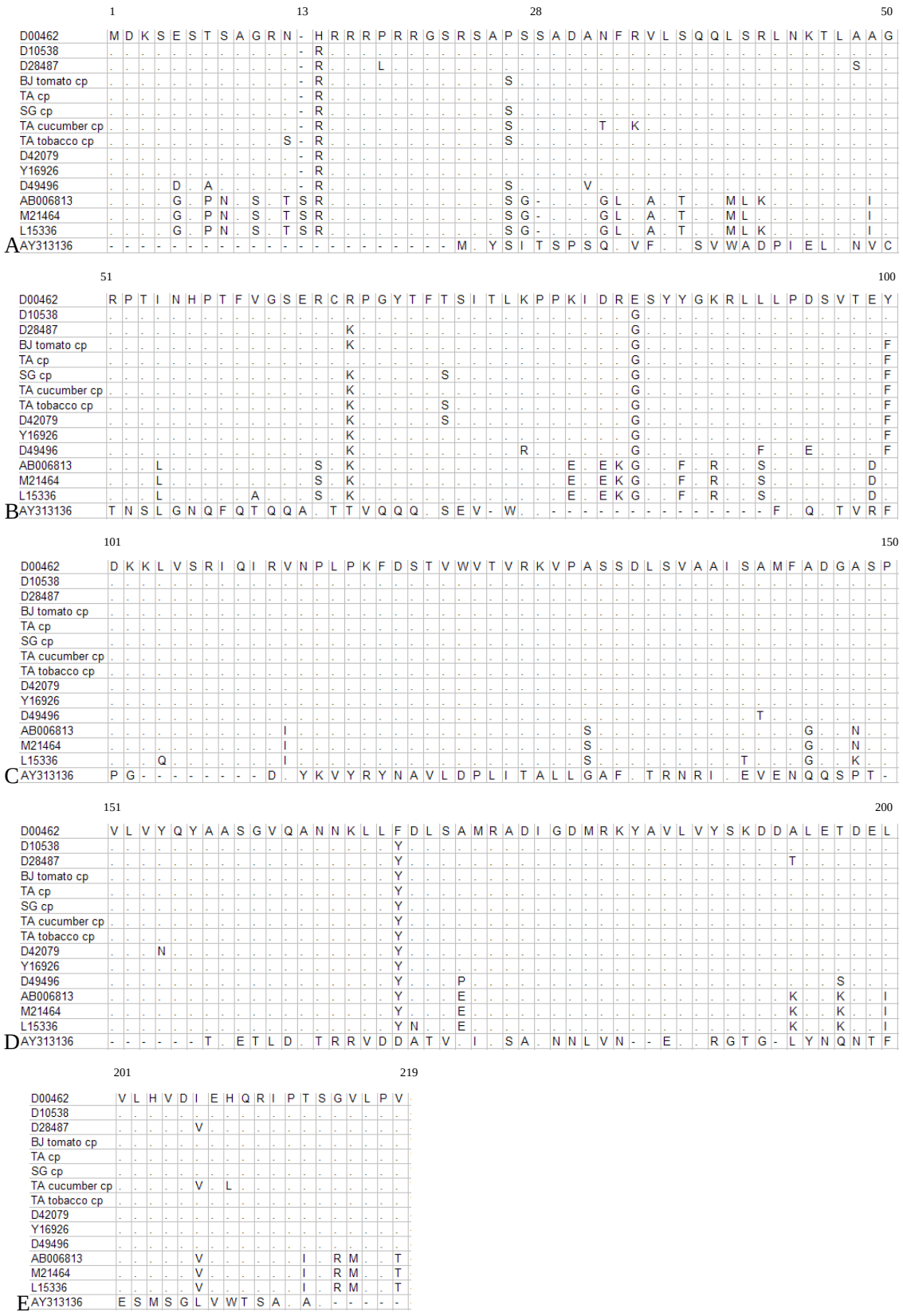

Fig.3 Amino acid sequence alignment(A, B, C, D, E block) of coat protein of 5 CMV isolates with that of CMV strains of subgroups I (A and B), II using MEGA v4.1.

Table 2 Comparison of percent nucleotide sequence(below diagonal),the deduced amino acid identities(above diagonal) and sequence identities of the coat protein gene of CMV with sequences of other CMV(sequence comparisons were performed with DNAstar program)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D00462	1	*	98.6	96.3	97.2	98.2	96.8	95.4	96.3	96.8	97.7	93.1	81.2	81.7	79.4	17.6
D10538	2	98.7	*	97.7	98.6	99.5	98.2	96.8	97.7	98.2	99.1	94.5	82.6	83	80.7	17.6
D28487	3	96.3	96.7	*	97.2	97.2	96.8	96.3	96.3	96.8	97.7	93.1	82.6	83	80.7	17.6
BJ tomato	4	94.4	94.8	94.1	*	99.1	99.5	98.2	99.1	98.6	99.5	95.9	82.6	83	80.7	17.6
TA	5	94.5	95	92.4	93.6	*	98.6	97.3	98.2	98.6	99.5	95.0	82.1	82.6	80.3	17.6
SG	6	94.1	94.5	93.8	98	93.6	*	97.7	99.5	99.1	99.1	95.4	82.1	82.6	80.3	17.6
TA cucumber	7	93.8	94.2	93.2	94.2	94.5	94.2	*	97.3	96.8	97.7	94.0	82.1	82.6	80.3	17.6
TA tobacco	8	93.9	94.4	93.6	97.4	93.8	97.6	93.8	*	98.6	98.6	95.0	82.1	82.6	80.3	17.6
D42079	9	92.2	92.5	92.6	94.8	93.9	95.1	94.2	95	*	99.1	94.5	81.7	82.1	79.8	17.6
Y16926	10	91.7	92.8	92.2	95	96.3	95	97.1	94.8	92.4	*	95.4	82.6	83	80.7	17.6
D49496	11	90.2	91.4	91.0	94.4	91.9	94.1	90.9	94.4	90.9	92.2	*	80.7	81.2	78.9	15.1
AB006813	12	73.5	75.3	74.9	78.2	77	77.8	77.5	77.5	75.3	75.7	74.4	*	99.5	97.7	15.7
M21464	13	73.5	75.3	74.6	78.5	77.2	78.1	77.6	77.8	74.6	74.9	73.7	98.6	*	97.2	15.7
L15336	14	72.9	74.6	74	77.5	76.1	77	76.6	76.7	74.1	75	74.3	98.7	98.4	*	13.8
AY313136	15	46.2	47	46.2	39.6	41.8	40.4	41.6	43.8	47.5	46.8	43.5	42.4	45.6	43.4	*

Ⅳ.2: Effects of virus strains on transmission efficiency of CMV transmitted by Myzus persicae

(soumis à European Journal of Phytopathology)

Rongling Yin[1, 2], Frédéric Francis[1], Claude Bragard[2], Yong Liu[3], Julian Chen[4]

[1]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.

[2]Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

[3]Plant protection, Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[4]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

Summary

Cucumber mosaic virus is one of the most important viruses infected vegetables in the field, and is transmitted by aphids in a non-persistent manner in nature. *Myzus persicae*, as the main aphid, can transmit CMV effectively, but transmission efficiency is not only affected by aphid, but also virus strains. Here we found transmission efficiency is different, although virus strains are not affecting on transmission efficiency.

Keyword: aphid; vegetable; non-persisitent; effective; strain; vector

Introduction

Efficient virus transmission from host plant to another plant by vectors is very important. Arthropods could transmit most of plant viruses, especially aphids in Hemiptera. Actually, aphids could transmit over 200 plant viruses in a non-persistent manner(Nault, 1997), such as *Myzus persicae* and *Cucumber mosaic virus* (CMV) which is the most common model used in many researches(Ali et al., 2006, Akhtar et al., 2010). In a non-persistent manner, plant virus particles attach directly to aphid receptors on the maxillary stylet cuticle within the common food/salivary canal, where viruses directly bind the receptors via a domain of their capsid protein, that is CP strategy used by *Cucumoviruses*, typically CMV(Blanc et al., 2011).

What influence transmission efficiency of virus are amino acid determinants of CP. Five amino acid changes in the coat protein (positions 25, 129, 162, 168, and 214) of CMV were required to restore efficient transmission by *M. persicae* and a construct with modified amino acids 129, 162 and 168 was efficiently transmitted by *A. gossypii*, but poorly for *M. persicae* (Perry Keith L. et al., 1998), and amino acid determinants for virus transmission have been mapped (Liu et al., 2002).

Transmission efficiency is not only affected by virus strains, but also aphid species, source and recipient plant species, and plant species on which the aphid is maintained(Simons, 1957), Different species of aphids transmit CMV with

varying efficiencies (Simons, 1959, Normand and Pirone, 1968, Basky and Nasser, 1989). But few reports showed effects of geographic differences of same aphid species on transmission efficiency of CMV.

Although there are reports on virus transmission, here we focus on transmission efficiency affected by geographic aphid species, virus strains, and finally we hope to get a better understanding of the virus-aphid interactions and to propose new insight in non-persistent virus transmission control in crop protection.

Material and methods

Material

Virus isolates are from Applied microbiology – Phytopathology, Earth & Life Institute, provided by Professor Claude BRAGARD (Table 1). Infected plants, *Nicotiana tabacum*, will be virus source for experiment.

Myzus persicae are collected from different places in China (Table 1), and raised in illuminating incubator (*Pisum sativum* L， 22℃±1℃， L:D=16:8), Agro-Bio-Tech, Universite de Liege.

Virus Abbreviation	Region
2012.2	European
1022	European
1024	European
BJ-P	Beijing China

D1766	Shouguang China
D1769	Taian China
D1770	Taian China
D1772	Shouguang China

Method

Gene clone

Samples were processed with RNA extraction (Invitrogen, RNA extraction kit), then with two-step 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(Choi et al., 1999). DNA Synthesis Supermix (TransGen Biotech, Beijing, China) was used for first strand cDNA synthesis. The program of RT step consisted of 50℃ (30min), followed 85℃ (5min). Per eppendorf tube contained 2.5μl RT product and 22.5 μl mix PCR (PCR reaction buffer (Biomed-tech Beijing China), primers, ddH2O). Samples were amplified in a thermocycler and the program followed 40 cycle reaction profile involving denaturation at 94℃(30 s), primer annealing at 52℃ (30s), extension at 72℃ (1min) with a final extension of 72℃(10min).The amplified products were analyzed on 1.2% agarose gel electrophoresis in TAE buffer, stained with ethidium bromide. The product of PCR fragment was cut from gel and eluted in order to clone and gel extraction, then the purified product was subjected to

sequencing (Beijing Sunbiotech Co., Ltd.). Sequences data were fed for BLAST analysis, which also were analyzed using MEGA v4.1 （ http://www.megasoftware.net/ ） and DNAstar program to structuring phylogenetic tree with other sequences that had been present already in the NCBI database worldwide and from the same vicinity by accounting standard reference of CMV from each subgroup.

Virus transmission efficiency

To initiate virus acquisition, aphids are removed from their normal host plant species and starved for 2-3 h. Third or fourth-instar nymphs or adults are given a 5-6 hours - acquisition access period on virus suspension (virus solution + 15% sucrose) through a stretched parafilm membrane. After acquisition access period (AAP), aphids will be transferred onto virus-free plant seedlings to assess their capacity to transmit the virus for overnight. (For each treatment, 10 seedlings are infested for a total of 50 seedlings over five replicated experiments.) After inoculation, the seedlings will be sprayed with pesticide, placed in a greenhouse, and observed for CMV symptoms. The plants are then tested for CMV infection 3 weeks later using ELISA. The data is analyzed by One-Way ANOVA / Duncan's multiple range tests with SPSS.

Results

Transmission efficiency of different viruses

The result (Fig 1) showed that D1772 was transmitted by aphids (*M. persicae*)

better than other viruses, so D1772 is chosen to do next part that is to choose good and bad vector from 7 aphids from different places.

Analysis and discussion

Transmission efficiency of virus strains

The sequences of Coat Protein show no special positions (Fig 2), and CMV isolates based on the nucleotide alignment using MEGA v5.1 phylogenetic analysis (Neighboring Joining Analysis) show that they belong to subgroup IB (Fig 3). Although some reports about transmission efficiency of different CMV strains transmitted by aphids showed that CMV strains had an effect on aphid transmissibility (Ali et al., 2006, Ng et al., 2005, Gildow et al., 2008), it shows there are no significant differences among virus strains from Fig. 1. Transmission efficiency is different, but there are no significant differences among them.

And for CMV, D1772 is collected from Shouguang Shandong. And also ST clones collected in Taian Shandong near Shouguang have higher transmission efficiency than BJ clones collected in Beijing far away from middle of Shandong area. It indicates that there are geographic differences between Shandong clones and Beijing clones.

To sum up, we will see that in this study, virus strains have less effects on virus transmission. Even transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained (Simons, 1957), but virus strains in

virus transmission are very important, which combined with aphid receptors, especially in natural environment for non-persistent virus and the contact between them could be the most important part for virus transmission.

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Figures and tables

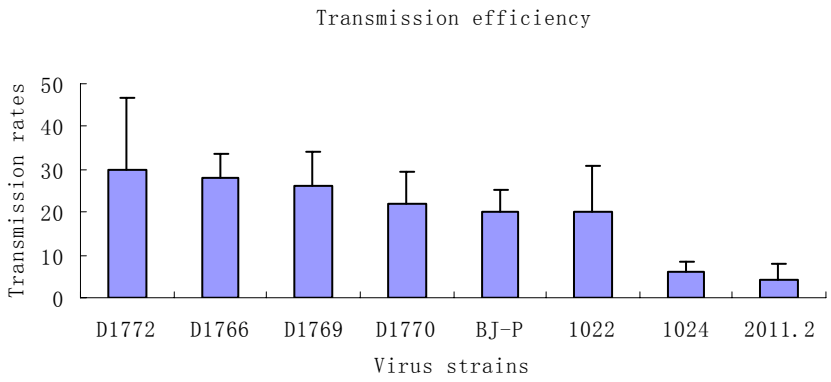

Fig 1 Transmission efficiency of different virus isolates

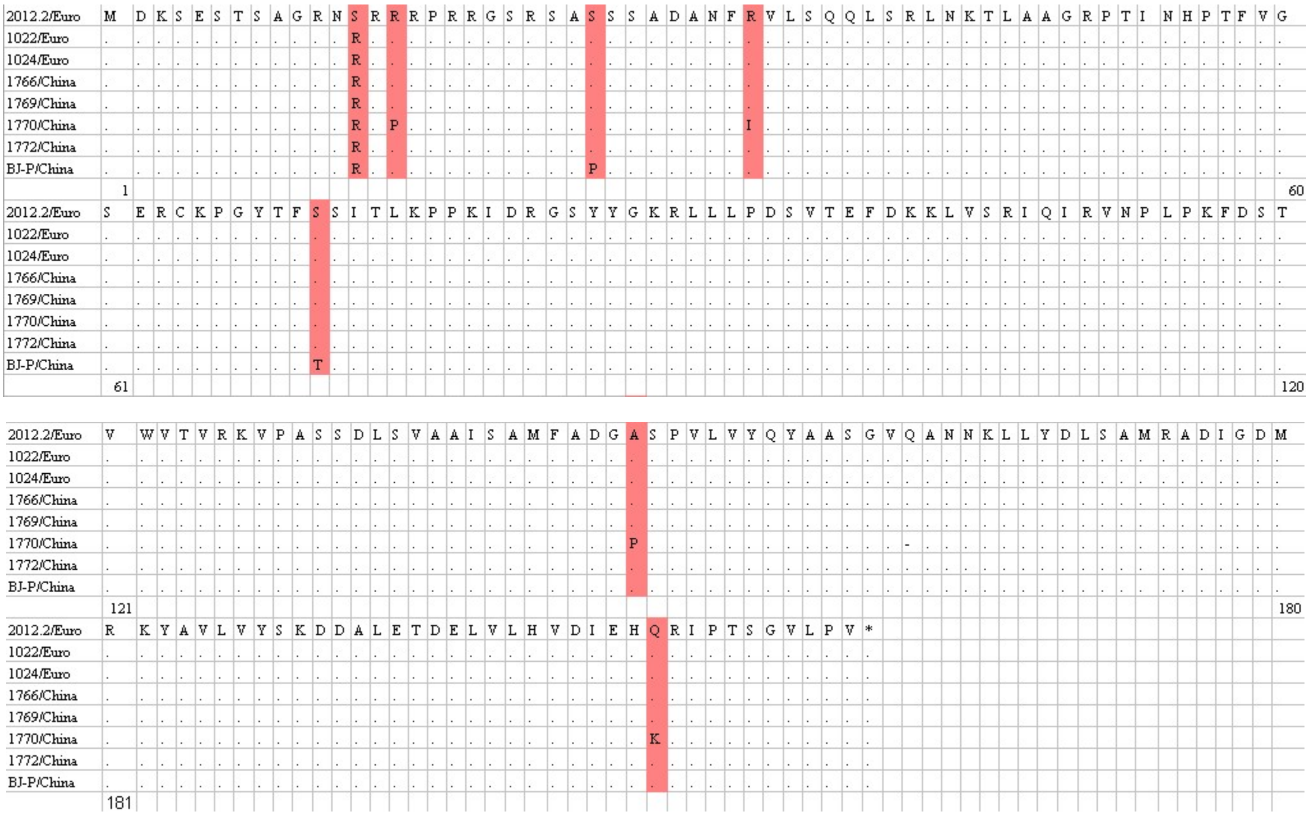

Fig 2 Alignment of 8 CMV capsid protein gene

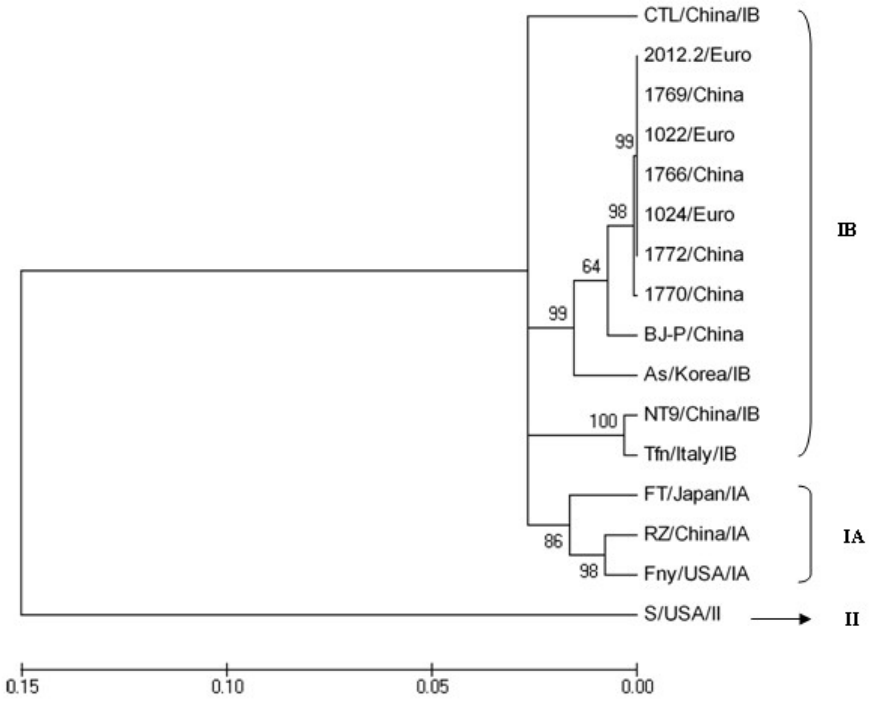

Fig 3 Phylogenetic analysis of CMV and other CMV isolates based on the nucleotide alignment using MEGA v4.1.

The bootstrapping and branch length values are above and below the joining lines.

General introduction to chapter V

Aphids are the main vector in most of the detailed studies on virus transmission and virus-vector relationships. And according to passing to the vector’s interior, the type of transmission divided into two parts, non-persistent transmission, including stylet-borne and foregut-borne, and persitant which contains circulative and propagetive. Of the over 300 known aphid-borne viruses, most are non-persistent, and *M. persicae* is known to be able to transmit a large number of non-persistent viruses, whereas other aphids transmit only one virus.

Transmission by vectors is usually a complex phenomenon involving interactions within the virus, the vector, and the host plant, combined with the effects of environmental conditions. In a non-persistent manner, virus particles bind on the top of aphid stylet and transmitted in a few minutes, and transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained

Chapter Ⅴ: Effects of aphids from different places on transmission efficiency of CMV transmitted by *Myzus persicae*

(soumis à Journal of Insect Behavior)

Rongling Yin[1, 2], Yong Liu [1], Julian Chen [2], Frédéric Francis [3], Claude Bragard [4]

[1]Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

[3]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.Plant protection,

[4] Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

Summary

Cucumber mosaic virus is one of the most important viruses infected vegetables in the field, and is transmitted by aphids in a non-persistent manner in nature. *Myzus persicae*, as the main aphid, can transmit CMV effectively, but transmission efficiency is not only affected by aphid, but also virus strains. Here we found transmission efficiency is different. Different aphids have an effect on it. Aphid from peper, Taian Shandong, shows significant difference with other aphids (F=5.915, df=8, $P < 0.05$).

Keyword: aphid; vegetable; noncirculative; effective; strain; vector

Introduction

Efficient virus transmission from host plant to another plant by vectors is very important. Arthropods could transmit most of plant viruses, especially aphids in Hemiptera. Actually, aphids could transmit over 200 plant viruses in a noncirculative (nonpersistent) manner(Nault, 1997, Bragard et al., 2013), such as *Myzus persicae* and Cucumber mosaic virus (CMV) which is the most common model used in many researches(Ali et al., 2006, Akhtar et al., 2010). In a noncirculative manner, plant virus particles attach directly to aphid receptors on the maxillary stylet cuticle within the common food/salivary canal, where viruses directly bind the receptors via a domain of their capsid protein, that is CP strategy used by Cucumoviruses, typically CMV(Blanc et al., 2011), and make a loss of vegetable crops production.

Transmission efficiency is not only affected by virus strains, but also aphid species, source and recipient plant species, and plant species on which the aphid is maintained(Simons, 1957), Different species of aphids transmit CMV with varying efficiencies (Simons, 1959, Normand and Pirone, 1968, Basky and Nasser, 1989). But few reports showed effects of geographic differences of same aphid species on transmission efficiency of CMV.

Here we focus on transmission efficiency affected by geographic aphid species, virus strains, and finally we hope to get a better understanding of the virus-aphid interactions and to propose new insight in non-persistent virus transmission

control in crop protection.

Material and methods

Material

Virus isolates are from Applied microbiology – Phytopathology, Earth & Life Institute, provided by Professor Claude BRAGARD (Below). Infected plants, *Nicotiana tabacum*, will be virus source for experiment. D1772 is collected from Shouguang, Shandong, China.

Myzus persicae are collected from different places in China, and raised in illuminating incubator (*Pisum sativum* L，22℃±1℃，L:D=16:8), Agro-Bio-Tech, Universite de Liege.

Aphid Abbreviations	Sources	Region
BJp	turnip	Beijing
BJe	cabbage	Beijing
STo	tobacco	Shandong Taian
BJo	tobacco	Beijing
SJp	turnip	Shandong Jinan
STp	turnip	Shandong Taian
STe	cabbage	Shandong Taian

Method

Virus transmission efficiency

To initiate virus acquisition, aphids are removed from their normal host plant species and starved for 2-3 h. Third or fourth-instar nymphs or adults are given a 5-6 hours - acquisition access period on virus suspension (virus solution + 15% sucrose) through a stretched parafilm membrane. After acquisition access period (AAP), aphids will be transferred onto virus-free plant seedlings to assess their capacity to transmit the virus for overnight. (For each treatment, 10 seedlings are infested for a total of 50 seedlings over five replicated experiments.) After inoculation, the seedlings will be sprayed with pesticide, placed in a greenhouse, and observed for CMV symptoms. The plants are then tested for CMV infection 3 weeks later using ELISA. The data is analyzed by One-Way ANOVA / Duncan's multiple range tests with SPSS.

Detection of endosymbiotic bacteria in *Myzus persicae*.

DNA will be extracted from *M. persicae* with kits (*Promega* DNA extract kit), and then will be amplified with PCR program (Tsuchida *et al*). Finally, we will see clear bands on gel.

Results

ST turnip (STp) is higher than other clones of aphids (Fig 1), and also it showed significant differences between STp and other clones of aphids expect ST cabbage (STe) from results of statistical analysis (F=5.915, df=8, $P < 0.05$). No significant differences were detected among other clones (F=3.226, df=5, $P > 0.05$). Base on the same host plant from different geographic areas, the result

shows that STp is the highest aphid from turnip to transmit CMV, and other two aphids, BJp and SJp, have lower transmission efficiency. And for cabbage and tobacco, although Ste has higher transmission efficiency than BJe, there are no significant differences (BJe and Ste, BJo and STo). Base on the same geographic area, aphid from turnip transmit virus more effective than other vegetables.

The essential intracellular symbiotic bacterium *Buchnera* was detected in all the strains examined, also *Rickettsia* (16SrDNA), while others bacteria were not detected at all. *Spiroplasma* is not found only in BJ-tobacco. PAUS in ST-turnip, JN-turnip, BJ-turnip, BJ-cabbage and PASS (16SrDNA) ST-turnip, BJ-tobacco, JN-turnip, ST-tobacco are not found. *Rickettsia* (Citrate) just exists in BJ-turnip. There is no connection between virus transmission with endosymbionts.

Discussion and conclusions

Even reports have proved that *M. persicae* is one of the best vector to transmit CMV (Perry Keith L. et al., 1998), but few study is on transmission efficiency of geographical population of aphid. Here we found transmission efficiency is different. Aphids from different places have an effect on it. Aphid from peper, Taian Shandong, shows significant difference with other aphids (F=5.915, df=8, $P < 0.05$)

For transmission efficiency of different aphids, it was divided into two parts because it is hard to manage numbers of plants. BJp, BJe and STo are in first part, and others are in next part. All parts had been done in one week. From the results,

it showed that aphids from Shandong area had high efficient transmission than others, expect STo. But this clone of aphid was raised in Beijing collected from tobacco in Shandong in 2010. Maybe it changed in two years and just was similar with BJo.

And for CMV, D1772 is collected from Shouguang Shandong. And also ST clones collected in Taian Shandong near Shouguang have higher transmission efficiency than BJ clones collected in Beijing far away from middle of Shandong area. It indicates that there are geographic differences between Shandong clones and Beijing clones.

To sum up, we will see that in this study, aphids have more effects on virus transmission than virus strains. Even transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant species on which the aphid is maintained (Simons, 1957), but vectors in virus transmission are very important, which can transmit virus helpfully, especially in natural environment for non-persistent virus and the contact between them could be the most important part for virus transmission.

Acknowledgments

The authors express their gratitude to financial assistance from project of Développement et valorisation de nouvelles stratégies de lutte contre les ravageurs, vecteurs de maladies virales, en milieu rural dans la Province de Shandong (P.R. Chine).

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Figure and tables

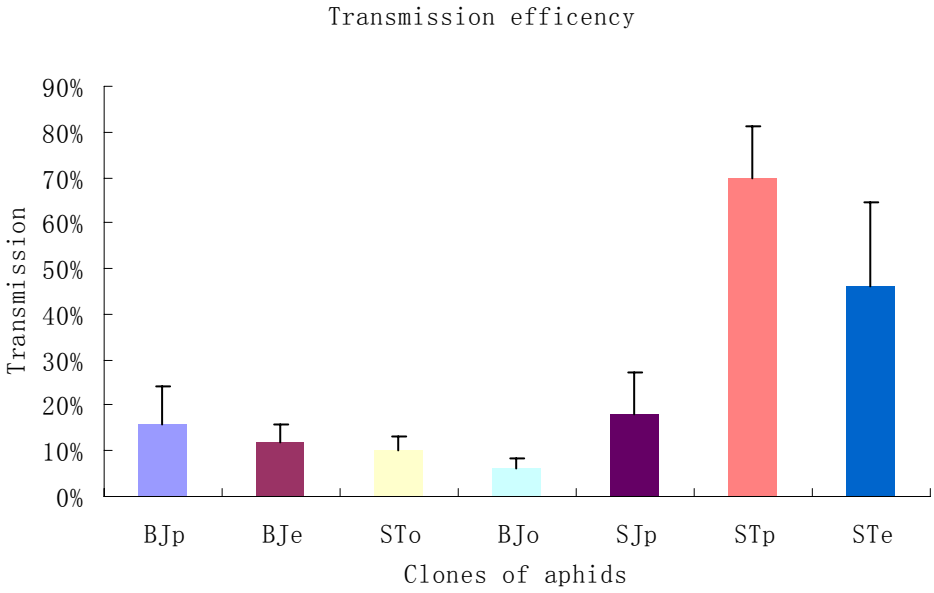

Fig 1 Transmission efficiency of aphid clones. The data in the table are mean ± *SE*. Different letters in the same measuring parameters mean significant difference at 0.05 leve1 detected by One-Way ANOVA / Duncan's multiple range test.

Table 3 detection of symbiotic bacteria

Target symbionts	Target gene	*Myzus persicae* from different places						
		ST-turnip	BJ-tobacco	JN-turnip	BJ-turnip	BJ-cabbage	ST-cabbage	ST-tobacco
Buchnera	16SrDNA	1	1	1	1	1	1	1
PASS	16SrDNA	-	-	-	1	1	1	-
	groEL	-	-	-	-	-	-	-
PAUS	16SrDNA	-	1	-	-	-	1	1
PABS	16SrDNA	-	-	-	-	-	-	-
Rickettsia	16SrDNA	1	1	1	1	1	1	1
	Citrate	-	-	-	1	-	-	-
Spiroplasma	16SrDNA	1	-	1	1	1	1	1
	dnaA	-	-	-	-	-	-	-
Wolbachia sp.	wsp	-	-	-	-	-	-	-
Arsenophonus sp.	16SrDNA	-	-	-	-	-	-	-

General introduction to chapter VI

Management of vector-borne plant diseases has presented a challenge because of complex dynamics and interactions of host plants, vectors and viruses within natural environment. As we know, viruses are transmitted by invertebrate vectors especially by kinds of aphids in nature more than other ways and as generally with insecticides used to control pest population, greater knowledge of the modes of action and activity profiles of insecticides will improve opportunities for controlling vector populations and mitigating virus transmission from one plant to another. In recent years, selecting a particular insecticide treatment has expanded considerably which as more selective modes of action have been developed. Plant lectins have been known for a longer time. Lectins as defense proteins in plants are present in large quantities in storage organs and seeds that are especially vulnerable to pathogens or pest insects. Numerous reports in recent years have shown that lectins are toxic to various pest insects belonging to economically important insects such as Lepidoptera, Coleoptera, Diptera or Hemiptera in genetic engineered plants or artificial diets with lectins, which is negatively affect the performance of pest insects.

Chapter Ⅵ: Study on effect of lectins to virus transmission

Study on effect of lectins to virus transmission

Rongling Yin[1,2], Frédéric Francis[1], Claude Bragard[2], Yong Liu[3], Julian Chen[4]

[1]Unite d'Entomologie fonctionnelle et evolutive, Functional & evolutionary Entomology Universite de Liege, Gembloux Agro-Bio-Tech, Passage des Deportes 2, 5030 Gembloux, Belgium.

[2]Applied microbiology – Phytopathology, Earth & Life Institute, Faculté d'ingénierie biologique, agronomique et environnementale UCL, Croix du Sud 2, Bte 3, B-1348, Louvain-la-Neuve, Belgium.

[3]Plant protection, Shandong agricultural university, No. 61, Daizong Road, Taian, 271018, Shandong, China.

[4]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No.2 West Yuan Ming Yuan Road, Beijing 100193, China.

Abstract

Plant lectins have been known to be defense proteins in plants to pest insects. Artificial diet of virus solution with lectins has been provide to *Myzus persicae* in order to test virus transmission, which shows that lectins have an effect on virus transmission by aphids, lower than control. And the inhibition rates are all above 50%, which means it is negative for virus transmission.

Keyword: lectin; negative; virus transmission; aphid

Introduction

Management of vector-borne plant diseases has presented a challenge because of complex dynamics and interactions of host plants, vectors and viruses within natural environment. As we know viruses are transmitted by invertebrate vectors especially by kinds of aphids in nature more than other ways and as generally with insecticides used to control pest population, greater knowledge of the modes of action and activity profiles of insecticides will improve opportunities for controlling vector populations and mitigating virus transmission from one plant to another. In recent years, selecting a particular insecticide treatment has expanded considerably which as more selective modes of action have been developed.

Plant lectins have been known for a longer time. Lectins as defense proteins in plants are present in large quantities in storage organs and seeds that are especially vulnerable to pathogens or pest insects (Peumans and Van Damme, 1995). Numerous reports in recent years have shown that lectins are toxic to various pest insects belonging to economically important insects such as Lepidoptera, Coleoptera, Diptera or Hemiptera in genetic engineered plants or artificial diets with lectins, which are negatively affect the performance of pest insects. In the last decades, some plant lectins were shown to be toxic to several aphids. There are several lectin families, as follows.

GNA-related lectins

Galanthus Nivalis Agglutinin (GNA) purified from snowdrop bulbs is probably the best studied plant lectin that specifically binds to terminal mannose residues from highmannose N-glycans which were shown to occur very frequently on insect glycoproteins (Schachter, 2009). It has shown to be toxic to pest insects in Hemiptera, and has been successfully engineered into a variety of crops including sugarcane, rice, wheat, potatoes and tobacco to make them have higher resistance against pest insects. Report showed that transgenic wheat plants were shown to be severe entomotoxic on development and survival of the grain aphid (*Sitobion avenae*) (Stoger et al., 1999). Also, a phloem-specific GNA expression enhanced resistance to corn leaf aphid under greenhouse conditions and in field evaluation (Wang et al., 2005).

Legume lectins

The legume lectins are a large family of homologous carbohydrate binding proteins that are found in the seeds of most legume plants (Sharon and Lis, 1990, Loris et al., 1998). The mannose-binding legume lectin from jackbean concanavalin A (ConA) was shown to be toxic to *M. persicae* (Gatehouse et al., 1999), and also *A. pisum* (Sauvion et al., 2004). And when ConA and *Pisum sativum* agglutinin (PSA) were studied towards the Hemipteran planthopper, ConA showed significant antimetabolic effects towards nymphs of taro planthopper (*Tarophagous proserpina* Kirkaldy) whilst PSA showed no significant effects toward the insect (Powell, 2001), although lectins have a similar binding specificity. It shows that a specific plant lectin maybe not active

against a given pest insect or a non-target insect (Vandenborre et al., 2011). Although PSA has been reported to be toxic to *A. pisum*(Rahbé et al., 1995), there are no reports that PSA is not toxic to other aphids, so maybe it also works on other aphids or not.

Hevein-like lectins

Hevein-like plant lectins have been studied for their entomotoxic properties. Wheat germ agglutinin (WGA) is an N-acetyl glucosamine-specific lectin known to have insecticidal activity in this family. Although WGA was shown to be very active against insects of Lepidopteran and Coleoptera, there are few reports about hevein-like proteins towards Hemipteran insects. There is Hessian fly responsive 3 (HFR-3) which has sequence similarity and similar chitin-binding activity to WGA, and it showed to be toxic to *Sitobion avenae*, but WGA almost not (Pyati et al., 2012).

To sum up, there are few reports on effects of GNA, PLA and WGA on virus transmission by aphid. So we put three lectins into Cucumber mosaic virus - *M. persicae* - plant model, to show effects of these three lectins on transmission.

Material and methods

Virus isolates are from Applied microbiology – Phytopathology, Earth & Life Institute, provided by Professor Claude BRAGARD (Table 1). Infected plants, *Nicotiana tabacum*, will be virus source for experiment.

Myzus persicae are collected from different places in China (Table 1), and raised in illuminating incubator (*Pisum sativum* L， 22℃±1℃， L:D=16:8), Agro-Bio-Tech, Universite de Liege. And also lectins are provided, *Galanthus Nivalis Agglutinin* (GNA), *Pisum sativum* agglutinin (PSA), Wheat germ agglutinin (WGA).

	Aphid		Virus	
Abbreviations	Sources	Region	Abbreviation	Region
BJo	tobacco	Beijing	D1772	Shouguang China
STp	turnip	Shandong Taian		

Method

Virus transmission efficiency

To initiate virus acquisition, aphids are removed from their normal host plant species and starved for 2-3 h. Third or fourth-instar nymphs or adults are given a 5-6 hours - acquisition access period on virus suspension with lectins (GNA, WGA, PSA) (virus solution + 15% sucrose + 0.05% lectin) through a stretched parafilm membrane. After acquisition access period (AAP), aphids will be transferred onto virus-free plant seedlings to assess their capacity to transmit the virus for overnight. (For each treatment, 10 seedlings are infested for a total of 50 seedlings over five replicated experiments.) After inoculation, the seedlings will be sprayed with pesticide, placed in a greenhouse, and observed for CMV symptoms. The plants are then tested for CMV infection 3 weeks later using

ELISA. Virus suspension without lectins is for the control. The data is analyzed by One-Way ANOVA / Duncan's multiple range tests with SPSS.

Inhibition rate of different lectins

According to inhibition rate, (control - treat)/control, the results will be analysed.

Results

Controls of percentage (24±4.00%, 12±4.90%) are higher than treatments of antificial diet with lectins (GNA, WGA and PSA). And the inhibition rate of each lectin is more than 50%. For each aphid, treatment of lectins is different. Percentage of aphid ST turnip is higher than aphid BJ tobacco (Fig 1), so the inhibition is opposite (Fig 2).

Discussion

Feeding with artificial diets clearly showed that GNA, WGA and PSA had a negative effect on the development of aphid. Also result showed that aphids fed on artificial diet with these three lectins had an effect on virus transmission. It means lectins reduced the transmission efficiency of virus transmitted by aphids. Inhibition rate are all above 50%. Although many lectins are showed to have clear entomotoxic properties, at this moment it remains very difficult to predict whether a specific plant lectin will be active against a given pest insect and/or non-target insect. Such as WGA which belongs to Hevein-related lectins will bind to carbohydrate structures such as the chitin-microfibrils in the peritrophic

membrane (PM)(Vandenborre et al., 2011), and *Hemipteran* insects lack a functional PM in their midgut in contrast to insect species belonging to the order of *Lepidoptera* or *Coleoptera*, but the result showed the effect is negative. It may be due to different reasons.

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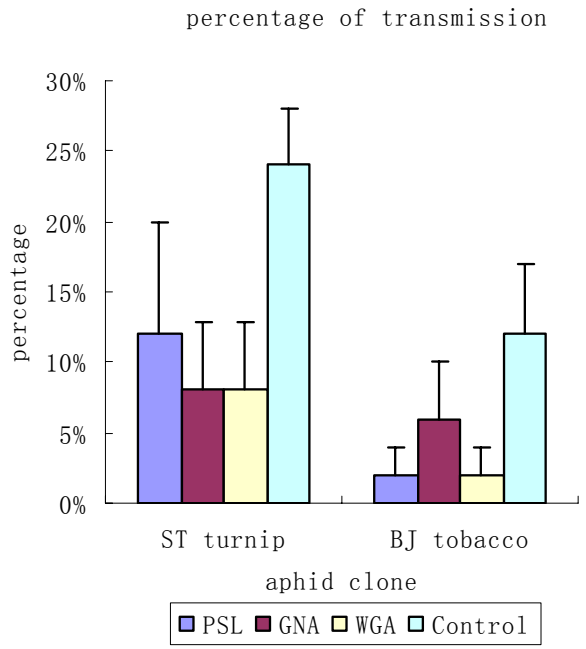

Fig.1 Percentage of virus transmission by aphid clones, ST turnip and BJ tobacco.

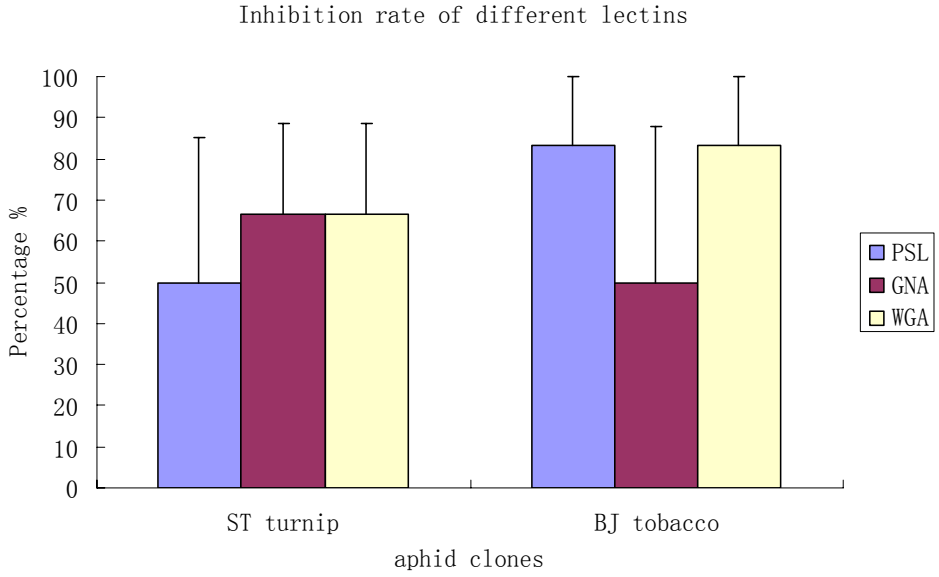

Fig.2 Inhibition rate of different lectins, PSA, GNA and WGA

Chapter VII: General conclusions

The control of virus diseases transmitted in a non-persistent manner by aphids should be one task to avoid prophylactic pesticide treatments to prevent virus spread, importantly continuous threat. We focus on transmission efficiency affected by geographic aphid species, virus strains, and effects of lectins, and finally we hope to get a better understanding of the virus-aphid interactions and to improve virus and aphid control in non-persistent virus transmission in crop protection.

1) **Molecular characterization of coat protein of Cucumber Mosaic Virus (CMV)**

Conclusion：CMV isolates based on the nucleotide alignment using MEGA v5.1 phylogenetic analysis (Neighboring Joining Analysis) show that they belong to subgroup IB. Studies on genetic structure and diversity would be important to help in better understanding the evolutionary mechanisms that generate and/or maintain variation in viral populations and their evolution, and also can be used to develop transgenic geraniums that will be useful for the growers

2) **Transmission efficiency of different strains of CMV by *Myzus persicae***

Conclusion：D1772 (CMV) is transmitted by aphids (*M. persicae*) better than other viruses, so D1772 is chosen to do next part that is to choose good and bad vector from 7 aphids from different places. Virus strains have less effect on virus transmission. Even transmission efficiency is affected by a number of factors, like virus strains, aphid species, source and recipient plant species, and plant

species on which the aphid is maintained, but virus strains in virus transmission are very important, which combined with aphid receptors, especially in natural environment for non-persistent virus and the contact between them could be the most important part for virus transmission.

3) **Transmission efficiency of D1772 by different clones of aphids**

Conclusion: ST turnip (STp) is higher than other clones of aphids, and also it shows significant differences between STp and other clones of aphids expect ST cabbage (STe) from results of statistical analysis (F=5.915, df=8, $P < 0.05$). No significant differences are detected among other clones (F=3.226, df=5, $P > 0.05$). For transmission efficiency of different aphids, it was divided into two parts because it is hard to manage numbers of plants. BJp, BJe and STo are in first part, and others are in next part. All parts had been done in one week. From the results, it showed that aphids from Shandong area had high efficient transmission than others, expect STo. But this clone of aphid was raised in Beijing collected from tobacco in Shandong in 2010. Maybe it changed in two years and just was similar with BJo. And compare with conclusion 2, we will see aphids have more effects on virus transmission than virus strains.

4) **Detection of endosymbiotic bacteria in *Myzus persicae*.**

Conclusion： The essential intracellular symbiotic bacterium Buchnera was detected in all the strains examined, also *Rickettsia* (16SrDNA), while others bacteria were not detected at all. *Spiroplasma* is not found only in BJ-tobacco.

PAUS in ST-turnip, JN-turnip, BJ-turnip, BJ-cabbage and PASS (16SrDNA) ST-turnip, BJ-tobacco, JN-turnip, ST-tobacco are not found. *Rickettsia* (Citrate) just exists in BJ-turnip.

5) **Effects of lectins on virus transmission**

Conclusion：*Galanthus nivalis agglutinin* (GNA), *Wheat germ agglutinin* (WGA) and *Pisum sativum lectin* (PSL) had a negative effect on the development of aphid. Also result showed that artificial diet with these three lectins to feed aphids had an effect on virus transmission. It means lectins reduced the transmission efficiency of virus transmitted by aphids. Inhibition rate are all above 50%.

Indeed, according to this study, we could partly and reasonably combine those strategies of strains diversity, aphids on virus transmission and effects of lectins to regulate and control the population of vegetable aphids in order to regralute the stability of agricultural system, and will be useful and convenient for farmers.

Chapter VIII: List of publications, oral presentations and posters

- Rongling Yin, Yong Liu, Julian Chen, Dengfa Cheng, Jingrui Sun. Identification of virus species transmitted by aphid. China Agricultural Science and Technology Press. 2010. P759
- RonglingYin, Yong Liu, Julian Chen, Dengfa Cheng, Claude Bragard, Frédéric Francis**.** Summary of molecular characterization of coat protein of Cucumber Mosaic Virus from Shandong and Beijing in China. China Agricultural Science and Technology Press. 2011. P815
- YIN Rongling，CHEN Julian,LIU Yong,CHENG Dengfa,SUN Jingrui,Claude BRAGRAD, Frédéric FRANCIS. Dection of symbiotic bactrica from Myzus persicae. China plant protection society conference in 2012, Beijing, China Agriculture Science and Technology Press, 2012. 578
- Rongling YIN, Frédéric FRANCIS, Claude BRAGARD, Yong LIU, Julian CHEN. Study on transmission efficiency of CMV transmitted by *Myzus persicae* from different places. 9th International Symposium on Aphids, Beijing, China. 2^{nd} – 4^{th} June.2013. S4.5, 49-50.
- Rongling YIN, Frédéric FRANCIS, Claude BRAGARD, Yong LIU, Julian CHEN. Effects of aphids from geographic areas on transmission efficiency of CMV transmitted by *Myzus persicae*- submitted to *Journal of Insect Behavior* (under review)
- Rongling YIN, Frédéric FRANCIS, Claude BRAGARD, Yong LIU, Julian CHEN. Plant -aphid -Cucumber mosaic virus interactions focusing on aphids

in a noncirculative manner. *Biotechnology, Agronomy, Society and Environment*. (under review).

- Rongling YIN, Frédéric FRANCIS, Claude BRAGARD, Yong LIU, Julian CHEN. Effects of virus strains on transmission efficiency of CMV transmitted by Myzus persicae. Journal of insect science (under review).

Oral Presentations

Study of transmission efficiency of CMV on virus strains and *Myzus persicae* clones. Workshop and exhibition of international collaboration projects on wheat and vegetable pests control between China and Belgium. Tai'an, China, 15th-17th May. 2013.

Poster

Study on transmission efficiency of CMV transmitted by *Myzus persicae* from different places.9th International Symposium on Aphids, Beijing, China. 2nd – 6th June. 2013.