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Comparative transmission of two cucumber mosaic virus isolates by two color morphs of *Acyrthosiphon pisum* (Harris)

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Summary. – Cucumber mosaic virus (CMV) is one of the most important legume-infecting viruses, which is transmitted effectively by pea aphid *Acyrthosiphon pisum* (Harris) (Hem: *Aphididae*). Transmission efficiency of two CMV isolates (As and Kh from cowpea and bean hosts, resp.) by red and green color morphs of pea aphid were evaluated on bean plants. Triple-antibody sandwich ELISA (TAS-ELISA) using CMV-specific monoclonal antibodies revealed that both CMV isolates belonged to the serotype II. Bean plants inoculated by viruliferous aphids were assayed by double-antibody sandwich ELISA (DAS-ELISA) at 16 days post inoculation (dpi). The results showed that the transmission rate of CMV-As by the red morph was significantly higher than by the green morph, resulting in significantly higher transmission rate of the virus (As + Kh) by the red morph than by the green morph, with $p \leq 0.1$. Similarly, the efficiency of CMV transmission by the red morph of *A. pisum* was higher than the efficiency of transmission by the green morph. The higher transmission rate and efficiency of CMV by red pea aphid would be important in the epidemiology. Based on these results, we hypothesize that the transmission efficiency of CMV is affected more by the difference in transmission determinants of *A. pisum* color morphs than by the sequence of virus coat protein determinants.

Keywords: Aphididae; Bromoviridae; color polymorphism; transmission efficiency

Introduction

Cucumber mosaic virus (CMV, the genus *Cucumovirus*, the family *Bromoviridae*) is one of the most common viruses with broad host range, including more than 1200 plant species in over 100 families of dicotyledons and monocotyledons. The virus is destructive due to rapid spread and causes great losses in vegetables, ornamentals, and fruits (Palukaitis and Garcia-Arenal, 2003). CMV is transmitted in a non-persistent manner by more than 80 species of aphids in 33 genera with varying degrees of efficiency (Edwardson and Christie, 1991). Pea aphid *Acyrthosiphon pisum* (Harris), which itself transmits more than 30 plant viruses (Blackman and Eastop, 2000), is one of the effective CMV-vectors in snap bean (Gildow *et al.,* 2008). This vector is a suitable model for different studies, due to its special features such as color polymorphism with red and green colors. Body color is genetically determined, with red being dominant over green (Caillaud and Losey, 2010). The color morphs of aphids may vary in growth rate, host range, defensive behavior, susceptibility to natural enemies, and some other aspects (Markkula, 1963; Tomanović *et al.,* 1996; Losey *et al.,* 1997; Braendle and Weisser, 2001; Caillaud and Losey, 2010; Tsuchida *et al.,* 2010). Many strains of CMV have been described differing in host range, symptoms, and aphid vectors. Transmission efficiency of CMV by aphids could be affected by virus coat protein (CP) determinants (Perry *et al.,* 1998).

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Abbreviations: CMV = cucumber mosaic virus; CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA; dpi = days post inoculation; DSMZ = Deutsche Sammlung von Mikroorganismen und Zellkulturen; MAb = monoclonal antibodies; PBST = phosphate buffer saline-Tween; PMV = pea mosaic virus; PVP = polyvinyl pyrolidone; TAS-ELISA = triple-antibody sandwich ELISA

Transmission efficiency has been defined as the probability of infection with a specific virus isolate and vector species under a set of environmental conditions (Sylvester, 1954). The capacity of a given virus to be transmitted by an aphid species may vary depending on the virus isolate or strain (Antignus *et al.,* 1989). Differences in the transmissibility of strains of stylet-borne viruses by aphids have been reported previously (Hollings, 1955; Badami, 1958; Swenson *et al.,* 1964; Normand and Pirone, 1968). On the other hand, the variability between clones of the same aphid species in the transmission ability of a particular virus has been previously reported for CMV (Simons, 1959).

The role of *A. pisum* as a vector of several non-persistently transmitted legume-infecting viruses is well documented. French bean (*Phaseolus vulgaris*), as a natural host plant for CMV and pea aphid, is an important legume crops in Iran. However, less is known about the role of pea aphid color morphs in transmission of legume isolates of CMV. This experiment was performed to compare the transmission efficiency of two CMV isolates from different hosts by two color morphs of pea aphid.

Materials and Methods

Plants. French bean plants were grown in pots containing sawdust under controlled conditions (23–25°C and 16:8, L:D) in insect-free chambers and used for virus transmission experiments at two-leaf stage.

Virus isolates. Two field isolates of CMV, Kh and As, were originally isolated from bean fields of Markazi province and cowpea (*Vigna unguiculata*) fields of Guilan province, Iran, respectively. They were biologically purified by serial single local lesion passage on *Chenopodium quinoa* and propagated on bean plants by mechanical inoculation in 0.05 mol/l phosphate buffer, pH 7, in an insect-free greenhouse. Systemically infected plants were used as source of each of the CMV isolates in transmission tests**.**

TAS-ELISA. The reactivity of CMV isolates with 11 CMV-specific monoclonal antibodies (MAb, Table 1) prepared by DSMZ were tested TAS-ELISA as described previously by Thomas *et al.* (1986). CMV polyclonal antibody (DSMZ AS-0929) was used for coating the plates, followed by loading of systemically infected bean plants leaf extract in ELISA extraction buffer (PBST, phosphate buffer saline-Tween, containing 2% Polyvinyl pyrolidone). The absorbance value of each well was measured at 405 nm by ELISA reader (Beckman AD 340). The reaction was considered positive when absorbance values reached more than twice the mean value of the healthy controls.

Aphid colonies. Red and green color morphs of *A. pisum* were collected from alfalfa fields in Karaj, Iran and reared for several generations on bean plants under controlled conditions (25 ± 1°C and 16:8, L:D).

Transmission experiments. Five-day old aphids of each color morph, which were in second or third nymphal stage, were fasted for two hours before the acquisition feeding in order to increase the probing probability. Aphids were then placed on newly expanded CMV-infected bean leaves, considering aphid color morphs and virus isolates, and allowed to feed during three minutes acquisition access period. After acquisition feeding, five aphids were transferred onto each healthy bean plant for a 24 hrs inoculation access period. The following day, plants were sprayed with metasystox insecticide to kill all the aphids. 20 bean plants were inoculated for each CMV isolate-aphid morph combination. Aphids fed on healthy bean plants were used to mock inoculation on healthy bean plants as negative control for each treatment.

DAS-ELISA. Infection status of all inoculated bean plants was tested at 16 dpi by DAS-ELISA as described previously by Clark and Adams (1977), using CMV-specific polyclonal antibody (DSMZ, AS-0929) and plants leaf extracts in ELISA extraction buffer (1:10). Mechanically inoculated symptomatic and mock-inoculated bean plants were used as positive and negative controls. Plants were considered infected when the absorbance values at 405 nm reached more than twice the mean value of the negative controls. Virus transmission rate was calculated as the number of infected plants divided by the number of inoculated plants. Comparisons between transmission rates of each virus isolate/aphid morph combinations were made using t-test. Differences were considered significant when $p \leq 0.1$. The transmission rate of a single aphid (P^*) was calculated by using the formula suggested by Gibbs and Grower (1960):

$$P^* = 1-(1-T)^{1/I}$$

where T = transmission rate as T = R/N; R = number of infected plants; N = number of inoculated plants: I = number of aphids per inoculated plant.

Results

Serotype of CMV isolates

Both isolates, CMV-As and CMV-Kh, showed positive reaction with serotype II-specific MAb (AS-490/1) in TAS-ELISA and no reaction with AS-489/1 serotype I-specific antibody (Table 1). Thus, both CMV isolates (from different hosts) used in this study belong to the serotype II (Anonymous, 1998).

Transmission rate and efficiency of CMV isolates by pea aphid color morphs

Based on the results of DAS-ELISA at 16 dpi, the transmission rates for each CMV isolate/ pea aphid color morph combination were determined and compared with each other (Table 2). The transmission rate of CMV-As by red morph was significantly higher than that by green

Table 1. Reactivity of two CMV isolates with CMV specific monoclonal antibodies in TAS-ELISA[a]

CMV isolates	CMV specific MAb										
	486/1	487/1	488/1	489/1	490/1	491/1	494/1	655/5	656/1	656/3	656/7
CMV-As	–	–	+	–	++++	+	+	+	–	–	++
CMV-Kh	–	–	+++	–	+++++	+	++	+++	–	–	++

[a]TAS-ELISA reactions were scored based on the mean actual absorbance value of three wells of each sample at 405 nm. (–) = not different from values obtained from mock-inoculated controls with OD <0.1; (+) = weak reaction; (+) = 0.1<OD<0.3; (++) = 0.3<OD<0.6; (+++) = 0.6<OD<1; (++++) = 1<OD<2; (+++++) = OD >2.

Table 2. Comparison of CMV isolates transmission by two color morphs of *Acyrthosiphon pisum*

A. pisum color morphs	CMV isolates		
	As	Kh	As + Kh
Red	5/20[a] (25%)	5/20 (25%)	10/40 (25%)
Green	1/20 (5%)	3/20 (15%)	4/40 (10%)

[a]Transmission rate = number of infected plants to inoculated plants. Percentage of infected plants is in parentheses.

morph (p = 0.03), while there was no significant difference between the transmission rate of CMV-Kh by two pea aphid color morphs (p = 0.22). The green morph transmitted CMV-Kh isolates better than CMV-As (with no significant difference), while the red morph transmitted both virus isolates with equal rate. Considering both isolates, transmission rate of CMV by red and green morphs of *A. pisum* was 25% and 10%, respectively, with significant difference (p = 0.1). In control sets, which were inoculated by aphids fed on healthy bean plants, no CMV infection was detected.

In addition, the transmission rate of a single aphid (P*) was calculated for each color morph of *A. pisum*. Based on the P* value, we concluded that the red morph of this aphid transmitted CMV with a higher efficiency than the green morph (Table 3).

Discussion

This is the first study on CMV transmission efficiency by pea aphid with special reference to the color morphs. Results revealed that geographical isolates of CMV, which were isolated from bean and cowpea, were transmissible by pea aphid, similar to results of Gildow *et al.* (2008), who reported a successful transmission of CMV to snap bean plants by *A. pisum*. Our results showed that red morph of *A. pisum* is a more efficient vector of CMV than green morph in greenhouse condition. These results need to be further studied and confirmed in field condition. These results are similar to those of (Kvicala, 1967), who detected more efficient transmission of pea mosaic virus (PMV) by red morph of pea aphid compared to green morph. Red and green morphs of pea aphid have no difference in body size or morphological features, while a variety of behavioral differences have been reported before (Lowe and Taylor, 1964; Lambers, 1966; Losey *et al.,* 1997; Braendle and Weisser, 2001). Various transmission rates of CMV isolates by two pea aphid color morphs can be related to different behaviors such as different feeding habits. According to our knowledge, there is no study on feeding habits of this aphid with respect to different color morphs.

Specific interactions between virus isolates and aphid morphs can also cause different efficiencies of virus transmission by different morphs. From the epidemiological perspective, the awareness of vector transmission efficiency is critical for complete understanding of pathogen spread and for developing and implementing pest and disease management models and control strategies. Life stage of the vector, temperature and other variables may affect transmission rate and efficiency, all of which should be further studied. Transmission efficiency varies also with the source and recipient plant species, and plant species, on which the aphid is maintained (Simons, 1957). Although red morph of pea aphid transmitted the two studied CMV isolates with equal rate, green morph transmitted the Kh isolate better than the As isolate (15% versus 5%), without a significant difference. It seems that the higher transmission rate of CMV-Kh could be related to similarity between original source plant of this isolate and recipient plant species (French bean) in this study. It was previously described that the transmission efficiency of the two virus isolates could be affected by differences in the amino acid sequences of the coat protein. CMV CP interacts directly with components of the aphid stylet to influence transmission efficiency (Chen and Francki, 1990; Perry *et al.,* 1998). As to the CMV isolates in this study, the transmission rate of CMV-As by red morph was significantly different than that of green morph, while the transmission rate of CMV-Kh by the two morphs was comparable. This suggests that the transmission rate and efficiency of CMV isolates by the two color morphs of pea aphid is more likely affected by the hypothetical differences in the determinants

Table 3. Transmission rate for a single aphid calculated for different CMV isolates, aphid species (color morphs) and plant hosts

Origin	Host	Vector	[a]Virus (isolate)	[b]P*	Reference
Iran	*Phaseolus vulgaris*	*Acyrthosi phonpisum* (red morph)	CMV (Kh)	**0.16**	Our results
Iran	″	*A. pisum* (red morph)	CMV (As)	**0.16**	″
Iran	″	*A. pisum* (green morph)	CMV (Kh)	0.03	″
Iran	″	*A. pisum* (green morph)	CMV (As)	0.01	″
Spain	*Cucumis melo*	*Aphis gossypii*	CMV (M6)	0.41	(Garzo *et al.,* 2004)
Spain	″	*A. gossypii*	CMV (B20)	0.26	″
Brazil	*Cucurbita pepo*	*A. gossypii*	CMV (Brazilian)	0.14	(Pinto *et al.,* 2008)
Brazil	″	*Myzus persicae*	CMV (Brazilian)	0.09	″
USA	*Nicotiana tabacum*	*M. persicae*	CMV (Wis. 102)	0.20	(Normand and Pirone, 1968)
USA	″	*M. persicae*	CMV (Wis. 14)	0.03	″
USA	″	*M. persicae*	CMV (MCJ)	0.007	″
USA	″	*M. persicae*	CMV (C1)	0.19	″
USA	*P. vulgaris*	*A. gossypii*	CMV-Le	0.13	(Gildow *et al.,* 2008)
USA	″	*Aphis glycines*	CMV-Le	0.09	″
USA	″	*A. pisum*	CMV-Le	**0.05**	″
USA	″	*Aphis craccivora*	CMV-Le	0.03	″
USA	″	*M. persicae*	CMV-Le	0.006	″
USA	″	*Aphis fabae*	CMV-Le	0.002	″

[a]Different isolates of CMV have been mentioned along with the name conferred by the authors studying them. [b]P* = transmission rates for single aphids according to the formula of Gibbs and Gower (1960).

of CMV transmission in the vector than by differences in the sequence of the CMV CP amino acid determinants.

Comparison of our data with previous studies is difficult, because the transmission rate and efficiency vary with different factors, such as the developmental stage of the aphid and the plant, acquisition time, temperature, genetic variation of virus isolates, hosts and aphids. To facilitate the comparison, the transmission rate for a single aphid (P*) was calculated from our data and from data of previous studies (Table 3). The transmission efficiency of CMV by *A. pisum* red morph was higher than for green morph in this study, which was similar to the results obtained previously for this virus by *Aphis gossypii* (Pinto *et al.,* 2008; Gildow *et al.,* 2008; Garzo *et al.,* 2004). On the other hand, the transmission efficiency for single green morph of *A. pisum* in this study was determined as 0.01–0.03, similar to the results of Gildow *et al.* (2008) on *A. pisum* and *Aphis craccivora* transmission efficiency for CMV-Le isolate on *P. vulgaris*. Surprisingly, only green morph of *A. pisum* was used in their experiments (Gildow, E., personal communications). Although the prevalence of red over green morph in nature described before (Caillaud and Losey, 2010), population changes of pea aphid color morphs have not yet been studied in Iran. It is clear that the evaluation of virus transmission efficiency by different morphs of aphids is fundamental for epidemiological assessments and application of suitable management strategies in given areas.

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References

Anonymous (1998): Detection and biodiversity of Cucumber mosaic cucumovirus. Conclusions from a ringtest of European Union COST 823 (New Technologies to improve Phytodiagnosis). J. Plant Pathol. 80, 133–149.

Antignus Y, Raccah B, Gal-on A, Cohen S (1989): Biological and serological characterization of Zucchini yellow mosaic and Watermelon virus-2 isolates in Israel. Phytoparasitica 17, 289–298. http://dx.doi.org/10.1007/BF02980758

Badami RS (1958): Changes in the transmissibility by aphids of a strain of Cucumber mosaic virus. Ann. Appl. Biol. 46, 554–562. http://dx.doi.org/10.1111/j.1744-7348.1958.tb02239.x

Blackman RL, Eastop VF (2000): Aphids on the World's Crops: An Identification and Information Guide. John Wiley and Sons Ltd, Chichester, 466 pp.

Braendle C, Weisser WW (2001): Variation in escape behavior of red and green clones of the pea aphid. J. Insect Behav. 14, 497–509. http://dx.doi.org/10.1023/A:1011124122873

Caillaud MC, Losey JE (2010): Genetics of color polymorphism in the pea aphid, Acyrthosiphon pisum. J. Insect Behav. 10, 1–13.

Chen B, Francki RIB (1990): Cucumovirus transmission by the aphid Myzus persicae is determined solely by the viral coat protein. J. Gen. Virol. 71, 939–944. http://dx.doi.org/10.1099/0022-1317-71-4-939

Clark MF, Adams AN (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34, 475–483. http://dx.doi.org/10.1099/0022-1317-34-3-475

Edwardson JR, Christie RG (1991): Cucumoviruses. In: Edwardson JR, Christie RG (Eds): CRC Handbook of Viruses Infecting Legumes. CRC Press, Boca Raton, Florida, pp. 293–319.

Garzo EI, Duque M, Fereres A (2004): Transmission efficiency of different non-persistent viruses infecting melon by four aphid species. Spanish J. Agr. Res. 2, 369–376.

Gibbs AG, Gower JC (1960): the use of a multiple-transfer method in plant virus transmission studies: some statistical points arising in the analysis of results. Ann. Appl. Biol. 48, 75–83. http://dx.doi.org/10.1111/j.1744-7348.1960.tb03506.x

Gildow FE, Shah DA, Sackett WM, Butzler T, Nault BA, Fleischer SJ (2008): Transmission efficiency of Cucumber mosaic virus by aphids associated with virus epidemics in snap bean. Phytopathology 98, 1233–1241. http://dx.doi.org/10.1094/PHYTO-98-11-1233

Hollings M (1955): Investigation of chrysanthemum viruses. I. Aspermy flower distortion. Ann. Appl. Biol. 43, 86–102. http://dx.doi.org/10.1111/j.1744-7348.1955.tb02455.x

Kvicala BA (1967): Comparative transmission of pea mosaic virus by one strain of Myzus persicae Sulz and two strains of Acyrthosiphon pisum (Harris). Biol. Plantarum 10, 51–57. http://dx.doi.org/10.1007/BF02920744

Lambers DHR (1966): Polymorphism in the Aphididae. Annu. Rev. Entomol. 11, 47–78. http://dx.doi.org/10.1146/annurev.en.11.010166.000403

Losey JE, Ives AR, Harmon J, Ballentyne F, Brown C (1997): A polymorphism maintained by opposite patterns of parasitism and predation. Nature 388, 269–272. http://dx.doi.org/10.1038/40849

Lowe HJB, Taylor LR (1964): Population parameters, wing production and behaviour in red and green Acyrthosiphon pisum (Harris) (Homoptera: Aphididae). Entomol. Exp. Appl. 7, 287–295. http://dx.doi.org/10.1111/j.1570-7458.1964.tb00730.x

Markkula M (1963): Studies on the pea aphid Acyrthosiphon pisum (Hom., Aphididae), with special reference to the differences in the biology of the green and red forms. Ann. Agric. Fenn. 2, 1–30.

Normand RA, Pirone TP (1968): Differential transmission of strains of cucumber mosaic virus by aphids. Virology 36, 538–544. http://dx.doi.org/10.1016/0042-6822(68)90185-2

Palukaitis P, Garcia-Arenal F (2003): Cucumoviruses. Adv. Virus Res. 62, 241–323. http://dx.doi.org/10.1016/S0065-3527(03)62005-1

Perry KL, Zhang L, Palukaitis P (1998): Amino acid changes in the coat protein of Cucumber mosaic virus differentially affects transmission by the aphids Myzus persicae and Aphis gossypii. Virology 242, 204–210. http://dx.doi.org/10.1006/viro.1998.8991

Pinto ZV, Rezende JAM, Yuki VA, Piedade SMS (2008): Ability of Aphis gossypii and Myzus persicae to transmit Cucumber mosaic virus in single and mixed infection with two potyviruses to zucchini squash. Summa Phytopathol. 34, 183–185. http://dx.doi.org/10.1590/S0100-54052008000200016

Simons JN (1957): Three strains of Cucumber mosaic virus affection bell peppers in the Everglades area of south Florida. Phytopathology 47,145–150.

Simons JN (1959): Variation in efficiency of aphid transmission of southern Cucumber mosaic virus and Potato virus Y in pepper. Virology 9, 612–623. http://dx.doi.org/10.1016/0042-6822(59)90152-7

Swenson DG, Sohi SS, Welton RE (1964): Loss of transmissibility by aphids of Bean yellow mosaic virus. Ann. Entomol. Soc. Am. 57, 378–382.

Sylvester ES (1954): Aphid transmission of non-persistent plant viruses with special reference to the Brassica nigra virus. Hilgardia 23, 53–89.

Thomas JE, Massalski PR, Harrison BD (1986): Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivities with other whitefly transmitted geminiviruses. J. Gen. Virol. 67, 2739–2748. http://dx.doi.org/10.1099/0022-1317-67-12-2739

Tomanović Ž, Brajković M, Krunić M, Stanisavljević L (1996): Seasonal dynamics, parasitization and color polymorphism of the pea aphid, Acyrthosiphon pisum (Harris) (Aphididae: Homoptera) on alfalfa in the south part of the Pannonian area. Tiscia 30, 45–48.

Tsuchida T, Koga R, Horikawa M, Tsunoda T, Maoka T, Matsumoto S, Simon JC, Fukatsu T (2010): Symbiotic bacterium modifies aphid body color. Science 330, 1102–1104. http://dx.doi.org/10.1126/science.1195463