Sovinska, R., Dunich, A., Mishchenko, L . (2021): Phylogenetic analysis of two Ukrainian isolates of cucumber mosaic virus from gladioli grown under different agroecological conditions. Agriculture and Forestry, 67 (4): 51-59. doi:10.17707/AgricultForest.67.4.05

DOI: 10.17707/AgricultForest.67.4.05

Roksolana SOVINSKA*, Alina DUNICH, Lidiya MISHCHENKO[1]

PHYLOGENETIC ANALYSIS OF TWO UKRAINIAN ISOLATES OF CUCUMBER MOSAIC VIRUS FROM GLADIOLI GROWN UNDER DIFFERENT AGROECOLOGICAL CONDITIONS

SUMMARY

Cucumber mosaic virus (CMV) is one of the most widespread and harmful viruses infecting gladiolus plants worldwide. The aim of the study was to perform a phylogenetic analysis of two CMV isolates from gladioli grown in different regions of Ukraine. For the first time, 443 nt sequences of the capsid protein (*CP*) gene of gladiolus isolates CMV-Gl-Skv-20 (MW847710) and CMV-Gl-SkvP-20 (MW847714) from Kyiv and Poltava regions, respectively, were submitted to the NCBI GeneBank. Phylogenetic analysis showed that isolates clustered with different phylogenetic subgroups. CMV-Gl-Skv-20 belongs to subgroup IA, and has nucleotide (nt) sequence identity 81.9%-99.27% and amino acid (aa) identity 82.3%-97.6% with isolates from this group. The highest identity of the CMV-Gl-Skv-20 was found to be with Turkish CMVs from *Rapistrum rugosum* TUR83, TUR86 and *Brassica* TUR4 (98.8%-99.3% nt and 97,6% aa), as well as with Australian isolates Ny and 207 from tomato, and banana isolate Cameroon (98.9%-99% nt and 97,6% aa). CMV-Gl-SkvP-20 belongs to subgroup IB and shares 95.8%-100% nt and 96%-100% aa identity with the members of this subgroup. CMV-Gl-SkvP-20 has the highest identity with Ukrainian isolates from cucumber Ukr-1409 and *Echinacea* P-EP-Ukr-19 (99.5%-100% nt and 99.2-100% aa), Chinese pumpkin isolates ZBR, WHR, isolate lu-17-14 from sweet potato and SXCH from *Bupleurum sp.* (98.3-99% nt and 98.4%-99.2% aa), as well as with gladiolus South Korean isolate ABI (98.3% nt and 97.7% aa). The results support the fact that gladioli are affected by CMV not only through the corms, but by vector insects circulating in growing areas.

Keywords: cucumber mosaic virus, gladiolus, coat protein gene, phylogenetic analysis, Ukraine.

[1]Roksolana Sovinska *(Corresponding author: roksolana1@meta.ua), Alina Dunich, Lidiya Mishchenko, Virology Department, ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv, UKRAINE

Paper presented at the 12th International Scientific Agricultural Symposium "AGROSYM 2021".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Recieved:07/08/2021 *Accepted:19/11/2021*

INTRODUCTION

Cucumber mosaic virus (CMV) is one of the most dangerous and widespread plant viruses in the world, which can infect more than 1,200 species of plants from more than 100 families, including economically important crops. Cucumber mosaic virus belongs to the *Bromoviridae* family, *Cucumovirus* genus. Despite that cucumber mosaic virus is characterized by seed transmission for some plant species (Montes *et al.,* 2021), vector transmission is considered more efficient. More than 70 species of aphids (*Aphidoidea*) are able to transmit the virus non-persistently (Tungadi *et al.,* 2017). CMV isolates differ in host range and pathogenicity and divided into three main subgroups: IA, IB and II. Isolates of these subgroups are characterized by uneven distribution in the world. Thus, IA and IB CMVs have tropical and subtropical origin, causing severe disease and significant economic losses. Group II isolates predominate in temperate regions and cause milder symptoms in plants (Berniak *et al.* 2009). Numerous phylogenetic studies of CMV show that subgroup IB mainly includes isolates from Asia and the Middle East, in contrast to subgroups IA and II, which include isolates from different countries and continents, mostly from Europe. Detailed phylogenetic analysis of CMV isolates revealed that subgroup IA originated from subgroup IB. The only obvious differences between the phylogenetic trees based on *CP* and *3a* gene sequences are: i) the degree of branching, which is slightly higher in *3a* tree; ii) the branch lengths, which are longer in the *CP*-based tree, especially for branches leading to subgroups I and II (Roossinck, 2002). Genetic analysis of CMV subpopulations indicates heterogeneity of genetic variation. Asian subpopulations have significantly higher genetic diversity, compared to American ones, and the difference within subgroup I and originating from Asia differ by 7– 12% in sequence arrangement from other subgroup I strains (Shahmohammadi *et al.,* 2019).

Cucumber mosaic virus in gladiolus plantations was found in Argentina (Arneodo *et al.* 2005), the United States (May *et al.,* 1963), India (Dubey *et al.,* 2010), Israel (Gera *et al.* 1990), the Netherlands (Asjes *et al.,* 1997), the Czech Republic (Pokorny *et al.,* 2009), South Korea (Park *et al.* 1998). The virus is revealed on *Gladiolus* plants in Ukraine. Symptoms of CMV infection on gladioli are characterized by leaf chlorotic stripes, mosaics, growth retardation, and 'color break' of the flower (Sovinska *et al.,* 2020). Infection can lead to the loss of cultivar, destruction of gladiolus collections in botanical gardens, as well as to complication of the selection and creation of gladiolus cultivars.

In Ukraine, the cucumber mosaic virus has been detected in agricultural plantations since 1970. However, the study of the prevalence and detailed analysis of cucumber mosaic virus in gladiolus plantations, its molecular characteristics has not been conducted in Ukraine before. The aim of the study was to perform a phylogenetic analysis of two CMV isolates from gladiolus plants grown in different regions of Ukraine.

MATERIAL AND METHODS

Samples collection and visual diagnostics

Initially, sampling of *Gladiolus sp*. variety Victoria Skvyrska was conducted in the autumn of 2019 in the Kyiv region, northern Ukraine. Visual diagnostics revealed symptoms of viral infection on leaves, flowers and corms. Fifteen samples of gladiolus with both virus-specific symptoms and visually healthy plants were selected. Healthy plants that were confirmed by ELISA and RT-PCR have been planted next year in field conditions in the Poltava region (central Ukraine) in the agrocenosis where CMV circulation was revealed (Mishchenko *et al.*, 2021).

Enzyme-linked immunosorbent assay

To determine the presence of viral antigens, the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used. The analysis was performed using commercial antibodies against cucumber mosaic virus manufactured by Loewe (Germany) in three replicates. Samples of healthy gladioli were used as negative control. Commercial CMV positive control was used (Loewe, Germany). The reaction results were recorded on the Termo Labsystems Opsis MR (USA) reader with Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive.

RNA extraction, RT-PCR and sequencing

GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, USA) was used to extract total RNA from gladiolus leaves. Samples of healthy gladioli were used as negative control. RevertAid Reverse Transcriptase (Thermo Scientific, USA) was used for cDNA synthesis. The amplification steps were performed using a Genetic Research Instrumentation LTD thermocycler (United Kingdom). The amplification reactions were set up as follows: initial denaturation for 3 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. The final extension was at 72 °C for 5 min. The primers are expected to amplify DNA product of 500 bp. PCR products were separated on a 1.5% agarose gel with DNA markers CSL-MDNA-100bp (Cleaver Scientific, United Kingdom), and visualized under UV light. The PCR products were purified from the agarose gel using Zymoclean Gel DNA Recovery Kit (Zymo research, USA).

The PCR products were sequenced on a 3130 Genetic analyzer (Applied Biosystems HITACHI) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Sample analysis was performed using Sequencing Analysis Software v5.2.0. The obtained sequences of capsid protein (*CP*) gene fragments of gladiolus isolates CMV-Gl-Skv-20 from Kyiv region and CMV-Gl-SkvP-20 from Poltava region were submitted to the NCBI GenBank under accession numbers MW847710 and MW847714, respectively.

Phylogenetic analysis

The sequences of Ukrainian cucumber mosaic virus isolates isolated from gladiolus CMV-Gl-Skv-20 and CMV-Gl-SkvP-20 were compared with sequences of 64 CMVs from different countries available in the NCBI GenBank using the

BLAST program. Nucleotide and amino acid sequences were aligned using Clustal W. Phylogenetic tree for the 443 bp fragment of coat protein gene of CMV isolates was constructed in MEGA 10 by the Neighbor Joining method using the Jukes–Cantor model with 1000 bootstrap replicates to estimate the statistical significance of each node. Peanut stunt virus, isolate ER (Ac No NC_002040) was taken as an outgroup. The pairwise nucleotide sequence identity scores between every isolate with each other represented as color-coded blocks using SDT v.1 software (Sequence Demarcation Tool Version 1.1). Multiple amino acid sequences alignment of the CMV isolates coat protein was performed with BioEdit 7.2.5 program.

RESULTS AND DISCUSSION

Gladiolus samples of the Ukrainian variety Victoria Skvyrska were selected in the summer-autumn period of 2019 in the Kyiv region. Gladioli with symptoms of viral infection and visually healthy plants were used in the study (Fig. 1A, 1B, 1C). DAS-ELISA showed the presence of CMV antigens in plants with chlorotic stripes on the leaves and flower ‘color break’. Visually healthy gladioli did not contain CMV antigens.

Fig.1. Symptoms of CMV infection on gladioli var. Victoria Skvyrska: A – ‘color break’ of the flower and chlorotic stripes on the leaves (Kyiv region, 2019); B – flower ‘color break’ (Kyiv region, 2019); C – visually healthy plants (Kyiv region, 2019); D – chlorotic stripes on the leaves (Poltava region, 2020);

In 2020, corms from healthy gladioli were planted in field conditions in the Poltava region, where the circulation of cucumber mosaic virus of IB subgroup was previously revealed on other plants (Mishchenko *et al.*, 2021). In gladioli planted in 2020, symptoms of stripes on the leaves were observed (Fig. 1D). DAS-ELISA and RT-PCR confirmed presence of CMV in these plants. The results of RT-PCR are consistent with the data obtained by DAS-ELISA and demonstrate the presence of CMV in the studied samples of gladiolus leaves

The sowing season of 2020 in Poltava region was characterized by harsh summer with high average daily temperatures, low soil moisture and the predominance of sunny weather. The beginning of the autumn season was

influenced by a severe drought, which lasted until October. Climate changes associated with global warming are closely related to the level of losses from plant diseases because the environment significantly affects plants, pathogens and their vectors (Mishchenko *et al.*, 2017). Such weather conditions are favorable for aphids' reproduction and CMV distribution.

Next step was to compare molecular characteristics of two CMV isolates from gladioli grown in 2019 (Kyiv region) and in 2020 (Poltava region). For the first time, 443 nt fragments of the *CP* gene of gladiolus CMV isolates CMV-Gl-Skv-20 (Ac. No. MW847710) and CMV-Gl-SkvP-20 (Ac. No. MW847714) from Kyiv and Poltava region, respectively, were sequenced and deposited to NCBI GenBank. Phylogenetic analysis showed that isolates of CMV-Gl-SkvP-20 and CMV-Gl-Skv-20 belong to different phylogenetic groups. Isolate CMV-Gl-Skv-20 belongs to subgroup IA (Fig. 2). It has an identity of 81.9%–99.27% by nucleotide sequence (nt) and 82.3% –97.6% by amino acid (aa) with isolates of this subgroup (Fig. 3). CMV-Gl-Skv-20 has the highest identity of 98.8% – 99.3% nt and 97.6% aa with Turkish isolates from *Rapistrum rugosum* TUR83 (LC066509), TUR86 (LC066515) and from *Brassica* TUR4 (LC066500); 99% nt and 97.6% aa identity with banana Cameroon isolate (EU428827); and 98.8 – 98.9% nt and 97.6% aa with Australian tomato isolates 207 (AJ585517) and Ny (CMU22821). Sequences of gladioli CMV isolates from other countries were also included to the phylogenetic analysis. Analysis showed that they are belonging to subgroup IA. The studied fragment of the *CP* gene of the CMV-Gl-Skv-20 isolate does not have a high identity with the gladiolus CMV isolates available in the NCBI GenBank database. Thus, CMV-Gl-Skv-20 has an identity 94.6% nt and 92.1% aa with the gladiolus isolate GPP (AJ131623) from the Netherlands and identity of 81.9% – 82.9% nt and 83%-84.7% aa with isolates from *Gladiolus dalenii* from India Glad-NBRI-10 (KP713797), Glad-NBRI-4 (KP713798).

Phylogenetic analysis of the CMV-Gl-SkvP-20 isolate showed that the isolate belongs to subgroup IB and have the identity 95.8% – 100% nt and 96% – 100% aa with isolates of this subgroup (Fig. 2, 3). CMV-Gl-SkvP-20 has the highest identity of 99.5% –100% nt and 99.2 – 100% aa with Ukrainian isolates from cucumber Ukr-1409 (KT199741) and purple coneflower P-EP-Ukr-19 (MT978189), the identity of 98.3–99% nt and 98.4%–99.2% aa with Chinese pumpkin isolates ZBR (KP710850), WHR (KP710851), isolate lu-17-14 (MK778781) from sweet potato and SXCH (JX993913) from *Bupleurum sp.*, as well as identity of 98.3% nt and 97.7% aa with gladiolus South Korean isolate ABI (L36525).

Comparative analysis of the amino acid sequences of gladiolus isolates CMV-Gl-Skv-20 and CMV-Gl-SkvP-20 with 64 isolates from different countries and hosts was performed. The unique amino acid substitution in the studied sequence of the *CP* gene of the CMV-Gl-Skv-20 isolate was revealed, namely: G → R at the position 96 (Fig. 4).

The analysis of the 443 bp *CP* gene sequence of the CMV-Gl-SkvP-20 isolate has not detected any aa substitutions compared to the sequences available in the NCBI GenBank database (Fig. 4).

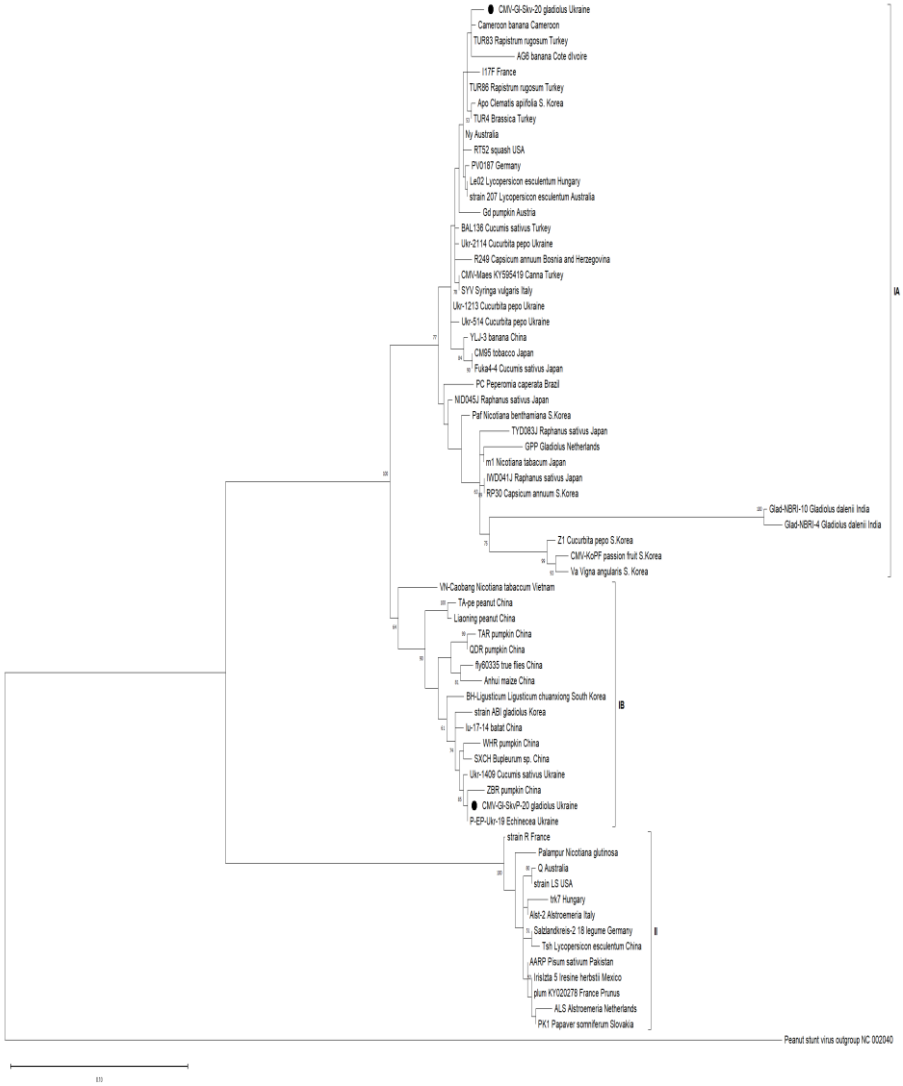

Fig. 2. Neighbor-Joining tree based on nucleotide sequences of 443 bp *CP* gene region of CMV isolates. Jukes-Cantor model was performed. The scale bar shows the number of substitutions per base. Peanut stunt virus (Ac. No NC002040) used as an outgroup. The studied Ukrainian isolates were marked with a dot.

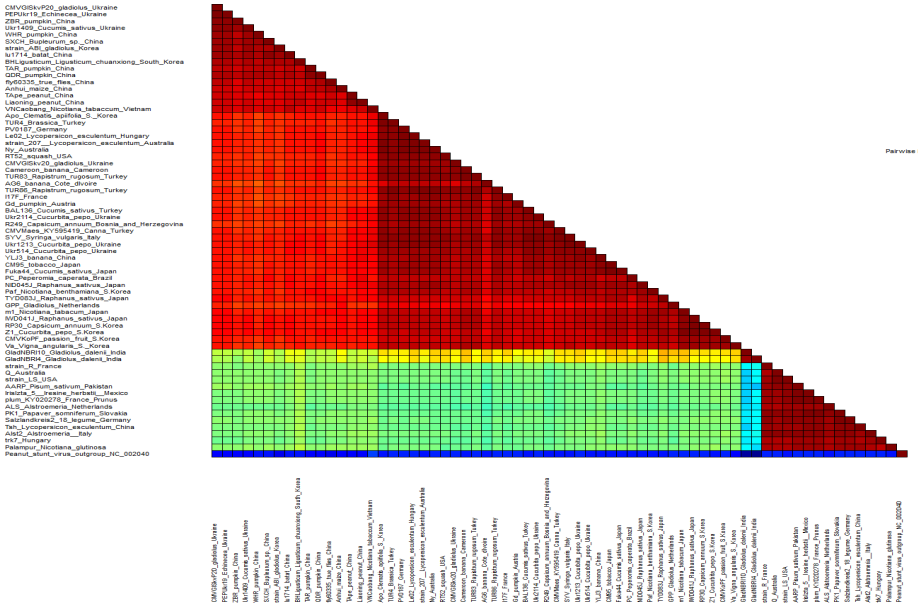

Fig. 3. Graphical representation of pairwise nucleotide identity of 443 bp *CP* region of 64 CMV isolates (with percentage identity scale).

Fig. 4. Comparative analysis of amino acid sequences of CMV-Gl-Skv-20, with other CMV isolates. Numbers on top represent the deduced CP amino acid position. Only the differences are shown.

CONCLUSIONS

Our investigations revealed that CMV-Gl-SkvP-20, belongs to subgroup IB and has the highest percentages of identity with Ukrainian isolates from cucumber Ukr-1409 and purple coneflower P-EP-Ukr-19, the last was studied earlier and originates from the same agrocenosis. In contrast, the isolate CMV-Gl-Skv-20 (Kyiv region, 2019) belongs to subgroup IA, has the greatest identity with the Turkish, Cameroonian and Australian isolates from vegetable plants and bananas. Despite that CMV-Gl-Skv-20 was initially identified as a gladiolus isolate, a comparison with other CMV gladiolus isolates of subgroup IA available in the NCBI GenBank indicates a low similarity with them inside the subgroup. This can be explained by different origin of isolates or/and aa substitution revealed in the CP gene.

Thus, the studied CMV isolates from gladiolus of the same variety, but grown in different regions of the country, belong to different phylogenetic subgroups and therefore have different origin. The isolate from Kyiv region is most similar to foreign CMV isolates, while the isolate from Poltava region is most similar to Ukrainian ones, and the most similar to purple coneflower isolate, which grew in the same agrocenosis with the studied gladioli. These results suggest that gladioli in the Poltava region were infected not by affected corms, but by aphids which transmitted the CMV from vegetable or other crops growing in this agrocenosis.

REFERENCES

Arneodo J.D., Breuil S.D., Lenardon S.L. (2005). Detection of *Bean yellow mosaic virus* and *Cucumber mosaic virus* infecting gladiolus in Argentina. Agriscientia, No. 22, pp.87–89.

Asjes C.J. (1997). Incidence and control of viruses in gladiolus in the Netherlands. VII International Symposium on Flowerbulbs. doi: 10.17660/ActaHortic.1997.430.111.

Berniak H., Malinowski T. (2009). Comparison of ELISA and RT-PCR assays for detection and identification of cucumber mosaic virus (CMV) isolates infecting horticultural crops in Poland. Journal of Fruit and Ornamental Plant Research, Vol. 17 (No. 2), pp. 5–20.

Dubey V.K., Singh V. P. (2010). Molecular characterization of *Cucumber mosaic virus* infecting *Gladiolus*, revealing its phylogeny distinct from the Indian isolate and alike the Fny strain of CMV. Virus Genes, Vol. 41, pp. 126–134.

Gera A., Cohen J. (1990). The natural occurrence of bean yellow mosaic, cucumber mosaic and tobacco mosaic viruses in lisianthus in Israel. Plant Pathology, Vol. 39, pp. 561–564.

May C., Miller P.R., Clellan W. D. (1963). Plant Diseases, the Yearbook of Agriculture. United States. Department of Agriculture. Washington D.C.

Mishchenko L., Dunich A., Mishchenko I., Berlizov V., Petrenkova V., Molchanets O. (2017). Influence of climate changes on wheat viruses variability in Ukraine. Agriculture & Forestry, Vol. 63 (No. 4), pp. 43–50.

Mishchenko L.T., Dunich A.A., Dashchenko A.V., Bondus R.O. (2021). Molecular characterization of cucumber mosaic virus infecting *Echinacea purpurea* in Ukraine. Archives of Phytopathology and Plant Protection, Vol. 23, pp. 1–16.

Montes N., Cobos A., Gil-Valle M., Caro E., Pagán I. (2021). *Arabidopsis thaliana* genes associated with cucumber mosaic virus virulence and their link to virus seed transmission. Microorganisms, Vol. 9 (No. 4), p. 692.

Park I.S., Weon K.K. (1998). The viruses in *Gladiolus hybribus* cultivated in Korea 2. *Broad bean wilt virus*, *Cucumber mosaic virus* and *Tobacco rattle virus*. Korean Journal Plant Pathology, Vol. 14, pp. 83–91.

Pokorny R., Selvaraj D. G. (2009). Survey of viral pathogens in gladiolus, iris, and tulip in the Czech Republic. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, Vol. 57(No. 5), pp. 79–86.

Roossinck M. (2002). Evolutionary history of *Cucumber mosaic virus* deduced by phylogenetic analyses. Journal of Virology, Vol. 76 (No. 7), pp. 3382–3387.

Shahmohammadi N., Dizadji A., Habibi M. K. (2019). Differentiation and phylogeny of Cucumber mosaic virus isolates originating from ornamentals in Iran; concerning genetic structure of virus. EcoPersia, Vol. 8, pp. 162–178.

Sovinska R., Dunich A., Mishchenko L. (2020). Affection of *Gladiolus* plants with bean yellow mosaic virus, cucumber mosaic virus and tobacco ringspot virus in some Northern and Central regions of Ukraine. Bulletin of Taras Shevchenko National University of Kyiv. Biology, Vol. 81 (No. 2), pp. 36–42. (In Ukrainian)

Tungadi T., Groen S., Murphy A., Pate A., Iqbal J., Bruce T., Cunniffe N., Carr J. (2017). Cucumber mosaic virus and its 2b protein alter emission of host volatile organic compounds but not aphid vector settling in tobacco. Virology Journal, Vol. 14.