Transcriptome responses of virus-transmitting aphid vectors are shaped by the host plant and the virus mode of transmission

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Abstract

Background: Numerous studies document modifications in vector orientation behavior, settling and feeding behavior, and/or performance due to virus infection in host plants. These alterations are often expected to enhance virus transmission, which has led to the hypothesis that such effects are manipulations caused by virus adaptations. However, until now, the genetic basis of these effects on vectors that can be direct (effects that occur following acquisition and retention of virions) or indirect (plant-mediated effects) are mostly unknown.

Results: Transcriptome profiling of *Myzus persicae* aphids feeding on turnip yellows virus (TuYV) and cauliflower mosaic virus (CaMV) infected *Arabidopsis thaliana* and *Camelina sativa* revealed a substantial proportion of commonly deregulated genes, revealing general players in plant-virus-aphid interactions. We identified also aphid genes specifically deregulated by CaMV or TuYV infection, which might be related to the viral transmission mode. Furthermore, we observed strong host-specific differences in the gene expression patterns with plant virus infection causing more deregulations of aphid genes on Arabidopsis than on Camelina, likely related to the differences in susceptibility of the plant hosts to these viruses. Finally, stress-related aphid genes were downregulated in Myzus on both infected plants, regardless of the virus.

Conclusions: TuYV – relying on the circulative persistent mode of transmission – tends to affect developmental genes, which could increase the proportion of alate aphids in TuYV viruliferous aphids, but also contribute to their locomotion, neuronal activity, and lifespan, while CaMV – using the non-circulative non-persistent mode of transmission – had a strong impact on feeding-related genes and in particular those related to salivary proteins. In general, these transcriptome alterations target pathways that seem to be particularly adapted to the transmission mode of the corresponding virus and could be evidence of manipulation caused by viral adaptation.

Keywords: Caulimovirus, polerovirus, aphid vector, insect-plant interactions, transmission, transcriptome profiling, RNA-seq

Introduction

Aphids are major pests not only because they deprive plants of nutrient resources when feeding on phloem sap but also because they transmit many plant-pathogenic viruses. Indeed, most plant viruses rely on vectors for transmission to a new susceptible host (for example Dietzgen et al., 2016). As phloem-feeders, aphids play a preponderant role in plant virus transmission, because their particular feeding behavior allows direct delivery of virus particles into the cytoplasm of cells of the epidermis, mesophyll, vascular tissue and/or the phloem sap of a new host. Their hypodermic needle-like mouthparts, the stylets, can penetrate cuticle and cell walls and enter into plant cells and sieve tubes without inflicting any major damage. More precisely, aphids alighting on a new plant will initiate probing phases (i.e. test the potential host for suitability) consisting of extracellular pathways and exploratory intracellular punctures into the epidermis and underlying tissues and, if accepted, plunge then their stylets into the sieve cells whose sap constitutes their principal food source (Tjallingii and Hogen Esch, 1993). During the probing and feeding phases, aphids secrete different saliva types that contain amongst other compounds effector molecules modulating interactions with the plant immune system and susceptibility (Rodriguez and Bos, 2013).

Viral infection often modifies plant phenotypical traits such as leaf color, morphology, surface properties, composition and quantity of volatile organic compounds (VOCs) and metabolites (Matthews, 2014). This may impact vector behavior and performance, i.e. attract/deter vectors, modify their feeding behavior and incite/discourage colonization (reviewed by Fereres and Moreno, 2009). There is evidence that such virus-mediated modifications can facilitate virus transmission, a concept known as 'pathogen manipulation'. The modifications depend on the virus mode of transmission (Mauck et al., 2012; Mauck et al., 2018). So-called non-persistent/non-circulative viruses have fast transmission kinetics and are acquired and inoculated, but also lost from the vectors, within seconds to minutes (Day and Irzykiewicz, 1954). The non-circulative viruses rely on other parameters for optimal transmission than so-called persistent/circulative viruses that have slow transmission kinetics (hours to days) as the virus is injected as a saliva component into a new host, following the passage of viral particles from the intestine to the salivary glands. Consequently, vectors retain and transmit the circulative viruses for weeks or lifelong (reviewed by Gray and Banerjee, 1999).

How virus-mediated plant modifications translate into changes in vector behavior and performance, is largely unknown (reviewed by Dáder et al., 2017; Fereres and Moreno, 2009; Mauck et al., 2019). It is assumed that most of the modifications are indirect, i.e. aphids and other vectors react to virus-induced changes in the plant. For example, yellowing symptoms induced by virus infection may attract and encourage the settling of insect vectors (for example Chesnais et al., 2022b; Johnston and Martini, 2020). A well-characterized example of such plant modifications by non-persistent, non-circulative viruses is the cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*). VOCs emitted by CMV-infected squash attract the green peach aphid (*Myzus persicae*, hereafter Myzus), but once landed on the infected squash, the poor palatability of the plant incites the aphids to leave fast (Mauck et al., 2010; Mauck et al., 2014). This aphid behavior is perfectly adapted to an efficient acquisition and transmission of CMV which relies on a short acquisition time and a rapid dispersal for propagation (Bhargava, 1951). Therefore, this example might be considered as 'host manipulation'. Although there are more examples in the literature (reviewed by Dáder et al., 2017; Fereres and Moreno, 2009), host-induced vector manipulation by non-persistent/non-circulative viruses is rather under-explored. The non-circulative virus studied here – cauliflower mosaic virus (CaMV, genus *Caulimovirus*, family *Caulimoviridae*) – follows the same transmission kinetics as CMV

except that it is retained longer (hours range) in its aphid vectors (Markham et al., 1987) and therefore its transmission mode has been classified also as 'semi-persistent', a term coined by Sylvester (1956). Previous work (Chesnais et al., 2019) showed that Myzus vectors did not show any preference for *Camelina sativa* (hereafter Camelina) plants infected with the severe CaMV isolate B-JI, but the number of intracellular probing punctures was increased and phloem ingestion and fecundity reduced on infected plants. Using CaMV-infected *Arabidopsis thaliana* (hereafter Arabidopsis) as virus host, Myzus spent less time in the pathway phase and more time feeding on phloem and aphid fecundity was lowered, compared to healthy control plants (Chesnais et al., 2021). A similar feeding behavior was observed for Myzus feeding on Arabidopsis infected with the milder CaMV isolate Cm1841r but in contrast to plants infected with the B-JI isolate, fecundity was not affected (Chesnais et al., 2021). Thus, there are contrasting results on possible manipulation of host plants by CaMV that might depend on the virus isolate and host plant species.

Quite a body of evidence for 'manipulation' by persistent/circulative viruses has been collected for poleroviruses (genus *Polerovirus*, family *Solemoviridae*). Most work on poleroviruses shows that virus-infected plants are more attractive to aphids than healthy plants and that aphid feeding is improved and fecundity higher on infected plants (reviewed by Bosque-Pérez and Eigenbrode, 2011; Dáder et al., 2017; Mauck et al., 2018). Curiously, aphid preference changed after polerovirus acquisition, and aphids carrying poleroviruses preferred healthy plants over virus-infected plants (for example Alvarez et al., 2007; Carmo-Sousa et al., 2016). There is evidence that purified virus particles can bring along this preference change (Ingwell et al., 2012), indicating that for persistent/circulative viruses, not only host plant-mediated changes but also direct virus-mediated changes in aphids are to be considered. The circulative virus studied here – turnip yellows virus (TuYV, genus *Polerovirus*, family *Solemoviridae*) – increases emission of VOCs in two host plants – Arabidopsis and Camelina – but only TuYV-infected Camelina, and not TuYV-infected Arabidopsis, attracted Myzus more than did healthy control plants (Claudel et al., 2018). Aphids feed longer from the phloem of TuYV-infected Camelina than from that of healthy Camelina, which might favor the acquisition of phloem-limited TuYV (Chesnais et al., 2019). Recently, a post-acquisition effect of TuYV was observed: virus-carrying Myzus aphids showed increased vector locomotory and fecundity as well as prolonged phloem feeding behavior. However, in this study, the authors did not distinguish between direct effects of the virus on the vector and indirect effects mediated by the infected host plant (Chesnais et al., 2020).

While virus-mediated effects on aphids and other hemipteran vectors are well documented, knowledge of how these effects are accomplished and which aphid genes are involved is scarce. Published examples indicate that deregulation of aphid genes related to stress, cuticle, development and nucleic factors is a common feature of aphids feeding on plants infected with poleroviruses or luteoviruses (Brault et al., 2010; Li et al., 2020; Patton et al., 2021). For non-circulative viruses, the effect of viral infection of plants on aphids seems more variable. CMV acquisition by Myzus from infected tobacco changed the expression of vector genes related to metabolism, stress, and cuticle (Liang et al., 2021), whereas a study on the soybean aphid *Aphis glycines* fed on soybean plants infected with soybean mosaic virus (SMW, genus *Potyvirus,* family *Potyviridae*) revealed only minor changes in aphid gene expression (Cassone et al., 2014).

In this paper, we explored how infection of plants with circulative versus non-circulative virus affects the transcriptome of viruliferous aphids. We specifically addressed whether the transmission mode influences the aphid transcriptome profiles and whether alterations in the aphid transcriptome correlate with distinct behaviors of viruliferous aphids. We identified common and virus-specific deregulated genes as well as plant host-specific effects on aphids. The aphid *M. persicae* was

selected for this study because it is an excellent vector for both the circulative, persistent TuYV and the non-circulative, semi-persistent CaMV. On the plant side, we chose two species, *A. thaliana* and *C. sativa* that are suitable hosts for both viruses.

Material and methods

Aphids

A Dutch green peach aphid clone (*Myzus persicae* Sulzer, 1776) was used for the experiments. It was reared on Chinese cabbage (*Brassica rapa* L. pekinensis var. Granaat) in a growth chamber at 20±1 °C and a 16 h photoperiod. Only wingless forms were used in assays. For synchronization, adults were placed on detached Chinese cabbage leaves that were laid on 1 % agarose in a Petri dish. The adults were removed 24 h later and the newborn larvae used in transcriptomic experiments 5 days later.

Viruses

CaMV isolate Cm1841r (Chesnais et al., 2021), which is a transmissible derivative of isolate Cm1841 (Tsuge et al., 1994), and TuYV isolate TuYV-FL1 (Veidt et al., 1988) were maintained in Arabidopsis Col-0 and propagated by aphid inoculation of 2-week-old plants. Growth conditions were as described below.

Virus infection and aphid infestation

Seeds of *Arabidopsis thaliana* Col-0 or *Camelina sativa* var. Celine were germinated in TS 3 fine substrate (Klasmann-Deilmann) in 7*7 cm pots and watered with tap water. Growth conditions were 14-h day 10-h night with LED illumination and a constant temperature of 21±1 °C. Two-week-old plants were inoculated with 3-5 wingless Myzus aphids that had been allowed a 24-h acquisition access period on Arabidopsis infected with TuYV or CaMV or on healthy Arabidopsis. Plants were individually wrapped in clear plastic vented bread bags to prevent cross contamination. Aphids were manually removed after a 48-h inoculation period. Eighteen days post-inoculation (dpi), 25 to 30 synchronized 5-day-old non-viruliferous aphids were placed for infestation on the rosette (Arabidopsis) or the apical leaves (Camelina) of CaMV- or TuYV-infected or mock-inoculated plants. After 72 h infestation (= 21 dpi), aphids were collected with a brush. Three biological replicates were used for analysis. For Arabidopsis, one biological replicate consisted of 4 plants, from which 25-30 aphids were collected (total of 100-120 aphids). For Camelina, one replicate was 3 plants from which 30 aphids were collected (total of 90-100 aphids). Aphid samples were conserved at -80 °C until processing.

RNA purification and Illumina sequencing

Total RNA was extracted from aphid cells with TRI Reagent (Molecular research center, USA) and chloroform followed by isopropanol precipitation step. Briefly, frozen aphids (10-50 mg) were homogenized in 1 ml TRI Reagent and incubated for 2 hours at room temperature. Subsequent phase separation by addition of 200 µl cold chloroform and centrifugation for 15 min at 12,000 g was followed by RNA precipitation with 500 µl cold isopropanol. After 10 min centrifugation at 12,000 g, the RNA pellet was washed twice with 1 ml of 75 % ethanol, air-dried and resuspended in 30 µl RNase-free water.

Illumina sequencing and quality control of 18 aphid total RNA samples were performed at Fasteris (www.fasteris.com) using a standard stranded mRNA library preparation protocol. All the libraries (3 biological replicates per each of the six conditions) were multiplexed in one NovaSeq flowcell SP-200

with 2x75 nt paired-end customized run mode. The resulting 75 nt reads from each library were used for Myzus transcriptome profiling.

RT-qPCR

For RT-qPCR analysis of Myzus gene expression, 10 µg total RNA was converted into cDNA using AMV Reverse Transcriptase (Promega) and oligo-dT primer. Real-time qPCR reactions (10 µl) including 3 µl of cDNA and 0.5 µl of 10 µM primers were processed in the LightCycler® 480 instrument (Roche) using the SybrGreen master mix (Roche) following the recommended protocol. The thermocycler conditions were as follows: pre-incubation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 58-60 °C for 20 s and 72 °C for 20 s. The gene expression was normalized to the *Myzus persicae* internal reference gene EF1 (Table S2).

Raw data processing and quality control for transcriptome profiling

Processing was carried out on the Galaxy France platform (https://usegalaxy.fr/) (Afgan et al., 2016). Raw reads quality was checked with FastQC (v0.11.8) and the results were then aggregated with MultiQC (v1.9). For aphids on Arabidopsis, between 64.6 and 88.8 million 75 nt paired-end reads were obtained with a mean phred score >30 for all bases. For aphids on Camelina, between 61.8 and 82.4 million 75 nt paired-end reads were obtained with a mean phred score >30 for all bases. In all samples, there were no overrepresented sequences and only a few adapter-containing reads (0.20 % reads with adapter sequence at the last bases). Reads were aligned on the *Myzus persicae* O reference genome ('Myzus_persicae_O_v2.0.scaffolds.fa' (annotations : 'Myzus_persicae_O_v2.0.scaffolds.braker2.gff3') downloaded from BIPAA portal (https://bipaa.genouest.org/sp/myzus_persicae/) (Mathers et al., 2021) with STAR (v2.7.6a) using default parameters and quality was again checked with MultiQC. Between 85.6% and 88.7% of reads were uniquely mapped to the aphid genome for Arabidopsis and between 81.8% and 87.6% of reads were uniquely mapped to the aphid genome for Camelina. Gene counts were obtained with featureCounts (v2.0.1). 87.4% to 88.3% of uniquely aligned reads were assigned to a gene for aphids on Arabidopsis and 83.4% to 86.8% aligned reads were assigned to a gene for aphids on Camelina. Differential gene expression was then analyzed with SARTools (v1.7.3) and the DESeq2 method (i.e. aphids on TuYV-infected plants vs. mock-inoculated plants, aphids on CaMV-infected plants vs. mock-inoculated plants, and aphids on TuYV-infected plants vs. CaMV-infected plants). GO enrichment analysis of the DEGs was performed with GOseq (v1.36.0) (Young et al., 2010).

Results and discussion

Quality control and validation of RNA-seq data

Ca. 32 to 44 million mRNA-seq reads per sample were obtained for aphids on Arabidopsis, of which >85 % could be aligned to the aphid reference genome (Supplementary Table S1a). For aphids on Camelina, ca. 30 to 41 million mRNA-seq reads per sample were obtained and >81 % of the reads could be aligned to the reference genome (Supplementary Table S1b).

Exemplarily, a similar trend of gene deregulation was confirmed by RT-qPCR for four Myzus genes with different degrees of deregulation (Supplementary Figure S1). Three genes showed the same trend of downregulation on CaMV- and TuYV-infected Arabidopsis, while g15329 was upregulated in all tests except RT-qPCR on TuYV-infected plants. The discrepancy in the results for g15329 expression was likely due its weak expression changes that in general difficult to detect by RT-qPCR because of the exponential amplification kinetics of this technique.

Principal component analysis of RNA-seq datasets (Figure 1a) indicated good clustering of the three biological replicates of aphids fed on mock-inoculated or virus-infected Arabidopsis. One of the three biological replicates of aphids fed on CaMV-infected Arabidopsis grouped less well with the other two but was still within an acceptable range. In the case of Camelina, the three replicates for each virus (TuYV and CaMV) clustered well together, indicating homogeneity of the replicates (Figure 1b). The Myzus data for Camelina infected with TuYV and CaMV were more similar to each other than those for Arabidopsis, indicating that the transcriptome changes in aphids fed on Camelina were less dependent on the virus species than those on Arabidopsis. In the case of mock-inoculated Camelina, two of the three replicates clustered together and were well separated from the data for virus-infected Camelina, while the third replicate clustered with the data from infected plants and was therefore excluded from further analysis. Taken together, all samples except one mock replicate of Camelina were of sufficient quality for transcriptome analysis.

Global analysis of differentially expressed aphid genes

Analysis of RNA-seq data revealed twice as many differentially expressed genes (DEGs) (false discovery rate <0.05) in aphids feeding on virus-infected Arabidopsis (4,060 for TuYV and 3,998 for CaMV) than in aphids feeding on virus-infected Camelina (1,771 for TuYV and 1,890 for CaMV), compared to aphids from mock-inoculated controls (Figure 1c, 1d and 1e). Remarkably, each virus modified the expression of about the same number of genes in aphids fed on the same host. Moreover, for each plant species, about 2/3 of aphid DEGs were common for the two viruses, indicating a profound common response of aphids to feeding on infected plants, independent of the virus species and of the transmission mode (Figure 1c and 1d). Like the number of aphids DEGs, also the proportion of up- and downregulated aphid genes was virus-independent, with ca. 45 % of the aphid DEGs being upregulated after feeding on TuYV- or CaMV-infected Arabidopsis, and ca. 75 % and ca. 81 % being upregulated after feeding on TuYV- and CaMV-infected Camelina, respectively (Figure 1e). The differences in the number and proportion of up- and downregulated aphid DEGs between Arabidopsis and Camelina indicate an important plant species effect on the aphid transcriptome, which is independent of the virus. On the other hand, for each plant species, ca. 1/3 of the aphid DEGs was specific for each virus, indicating that the transmission mode (or the virus species) has a substantial and characteristic impact on the aphid transcriptome.

Impact of CaMV and TuYV infection on aphid processes

Gene ontology analysis of Myzus infesting CaMV- or TuYV-infected plants

Using gene ontology (GO) analysis, we first looked at the effects of virus-infected Arabidopsis on the aphid transcriptome (Figure 2). In aphids fed on TuYV-infected Arabidopsis, 11 of the Top 25 enriched GO categories of DEGs classified as Biological Processes (BP) (Figure 2a). The most affected processes were ‘oxidation-reduction’ (BP), ‘integral component of membrane’ (belonging to the category Cellular Component [CC]), and the rather general process ‘ATP-binding’ (belonging to the category Molecular Function [MF]). Other prominent processes were related to protein synthesis and metabolism (translation initiation, protein synthesis, endopeptidase activity, protein folding, proteasome-mediated protein degradation and unfolded protein binding). Similarly, the most deregulated processes of aphids feeding on CaMV-infected Arabidopsis were ‘oxidation-reduction (BP)’, ‘integral component of membrane (CC)’ and ‘ATP binding (MF)’, followed by protein synthesis and metabolism-related processes (Figure 2b).

A different picture was found for Myzus on virus-infected Camelina (Figure 2c). In the case of TuYV infection, only 8 categories (2 BP, 3 CC and 3 MF) were identified by GO analysis as being significantly deregulated. The deregulated processes included chitin-related processes (chitin binding, MF; chitin

metabolic processes, BP; structural constituent of cuticle, MF), transcription (transcription factor complex, CC), oxidation reduction (oxidoreductase activity, MF) and plasma membrane-related processes (homophilic cell adhesion via plasma membrane, BP; plasma membrane, CC; extracellular region, CC), with only the oxidation/reduction and plasma membrane processes being related to those identified in aphids fed on Arabidopsis. In the case of CaMV infection of Camelina, only three processes were deregulated in aphids, 'homophilic cell adhesion via plasma membrane (BP)', 'chitin binding (BP)' and 'chitin metabolic process' (MF), which were also deregulated by TuYV.

Taken together, GO analysis revealed distinct, plant host-specific impacts on the aphid gene expression, which are rather independent of thse virus type, with virus-infected Arabidopsis having a much more profound impact on aphids than virus-infected Camelina (Figure 2a,b vs 2c,d).

Since the current annotation of the Myzus genome is not as advanced as for other model organisms such as *Drosophila melanogaster*, we complemented our above-described GO analysis by analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa, 1996). This analysis showed that similar percentages of aphid genes involved in 'genetic information processing', 'metabolism' and 'signaling and cellular processes' were modified in both plant hosts (Figure S2).

General heatmap analysis of DEGs

To better visualize the aphid transcriptome changes, heatmaps presenting all DEGs in aphids infesting Arabidopsis and Camelina were generated (Figure 3a and 3b). The profiles of all aphid replicates fed on mock [M]-inoculated Arabidopsis or Camelina clustered well together, while the profiles from aphids feeding on virus-infected plants (CaMV and TuYV) did not. This again indicates that, in our experiment, effects of infection on aphid genes are largely independent of the virus species. Like the global analysis (Figure 1e), the heat maps also show that Myzus on virus (TuYV or CaMV)-infected Camelina displayed proportionally more up- than downregulated genes, compared to Myzus on mock-inoculated Camelina, whereas proportions of up- and downregulated DEGs in aphids feeding on virus-infected vs mock-inoculated Arabidopsis were similar. The significance of these plant host-specific effects remains to be investigated. We speculate that Myzus might have more difficulties in establishing infestation on Arabidopsis than on Camelina, visible by the higher number of DEGs that are indicative of extensive transcriptome reprograming to adapt to the new plant host.

In summary, both GO and a general heatmap analyses indicated an important effect of plant infection on the aphid transcriptome, which is strongly shaped by the host plant identity (2/3 of the DEGs) and less so by the virus species (1/3 of the DEGs) and therefore the virus transmission mode.

Discussion of DEGs by classes

In the following, we will class the aphid DEGs in several categories using as criteria whether or not the genes are deregulated in specific conditions (plant host species and virus identity). The rationale is to identify genes that are general players in plant-virus-aphid interactions (i.e. deregulated by both viruses and in both host plants) and genes that are specifically deregulated either by one virus species or in one host species. Then, we extract aphid DEGs related to one virus and conserved regardless of the host plant to highlight virus species/transmission mode-specific genes that are not sensitive to the host plant identity. Finally, we compare TuYV vs CaMV effects on each host plant to reveal additional, host plant-specific, 'manipulation strategies' linked to the virus transmission mode.

1 Common deregulated genes in aphids feeding on CaMV- and TuYV-infected Arabidopsis and Camelina

304 We first focus on aphid DEGs that are common for both CaMV and TuYV infections and both host
305 plants to identify DEGs related to general infection/stress responses (Table 1). We first discuss
306 upregulated DEGs followed by downregulated DEGs.

307 ***1a Aphid genes UPREGULATED by both VIRUSES on both PLANTS***

308 Only five genes were upregulated in Myzus feeding on both CaMV- and TuYV-infected vs mock-
309 inoculated plants. Two of them (g22946 and g22969) code for titins, which are structural muscle
310 proteins (Lemke and Schnorrer, 2017). Their upregulation could potentially affect locomotion
311 behavior and facilitate intra or inter-plants vector movement, as was recently observed for TuYV-
312 viruliferous aphids (Chesnais et al., 2020). The third commonly upregulated gene (g6068) codes for a
313 vasodilator-stimulated phosphoprotein-like (VASP) protein, which is associated with actin filaments
314 and focal adhesions (Ahern-Djamali et al., 1998) and can participate in neural development and
315 function (as suggested for its Drosophila homolog Ena; Ahern-Djamali et al., 1998). Transferred to the
316 present work, VASP might be induced by viruses to modulate aphid development and behavior, a
317 feature that has been reported several times in the recent literature (Mauck et al., 2018). The fourth
318 gene upregulated in all modalities encodes an angiotensin-converting enzyme-like protein (g22588),
319 the orthologue of *Acyrthosiphon pisum* ACE1. g22588 contains a signal peptide and could therefore
320 be a saliva protein. *Acyrthosiphon pisum* ACE1 is expressed in salivary glands and modulates aphid-
321 plant interactions by affecting the feeding behavior and survival of aphids on host plants (Wang et al.,
322 2015). More precisely, silencing of ACE1 and ACE2 shortened the lifespan of *A. pisum* on plants but
323 not in membrane feeding assays. Thus, the ACE1 g22588 possibly counteracts plant defenses and its
324 overexpression could help Myzus to better cope with plant defenses and to increase its lifespan. ACE
325 and other metalloproteases have also been found in the saliva of other phytophagous and blood-
326 feeding arthropods (Decrem et al., 2008; Stafford-Banks et al., 2014) and are believed to be part of
327 their arsenal counteracting defense responses of their hosts (reviewed by Chen and Mao, 2020; Hopp
328 and Sinnis, 2015; Pham et al., 2021; Wang et al., 2017). Thus, the increased expression of ACE, as
329 observed here in the interaction of Myzus with two viruses and on two host plants, could be a
330 common 'manipulation strategy' shared among plant viruses to facilitate aphid feeding on virus-
331 infected host plants and accelerate virus acquisition. The fifth commonly upregulated gene is the
332 uncharacterized Myzus gene g27731 encoding a protein with MATH and LRR domains. Since the LRR
333 domain is evolutionary conserved in many proteins associated with innate immunity pathways (Ng
334 and Xavier, 2011), this protein might be a good candidate for further studies on virus-mediated
335 manipulation of insect vectors.

336 ***1b Aphid genes DOWNREGULATED by both VIRUSES on both PLANTS***

337 In the case of downregulated genes, besides the identical genes (with the same 'g' accession
338 number) downregulated under all conditions, we included in our analysis homologs of a given gene
339 when one homolog was significantly downregulated for a virus and the second homolog for the other
340 virus (Table 1). For example, we identified two potentially secreted homologous cathepsin B-like
341 proteases (g8486 for aphids infesting TuYV-infected plants and g24532 for aphids infesting CaMV-
342 infected plants, respectively). The rationale was that one specific host or infection condition might
343 deregulate a specific gene but that the overall effect on plant aphid interactions might be the same
344 or very similar for both genes (in this case the two cathepsin Bs).

345 We found 18 common downregulated genes (including some homologs) in aphids fed on virus-
346 infected plants, 13 of which are implicated in aphids' physiological responses to plant defenses, i.e.
347 stress-related genes (Table 1). Previously, we demonstrated that plant infection with CaMV and TuYV
348 alters primary and secondary metabolism in Arabidopsis and Camelina strongly (Chesnais et al.,

349 2022a). Consequently, aphids could also be stressed on the infected plants because of these
350 important alterations of the plant composition. However, we found that stress-related aphid genes
351 were downregulated in Myzus feeding on virus-infected plants. This suggests that plant infection
352 with TuYV and CaMV could facilitate aphid infestation. The downregulated stress genes included
353 those encoding the metabolic enzymes cytochrome P450s, glutathione-S-transferase and UDP-
354 glucuronosyl transferases which can play a key role in detoxifying plant secondary metabolites
355 (Brierley and Burchell, 1993; Li et al., 2007). We also noticed downregulation of genes encoding FE4-
356 like esterases that belong to another class of enzymes involved in detoxification that can confer
357 insecticide resistance in Myzus (Field and Devonshire, 1998). Likewise, Myzus genes coding for CHK
358 domain-containing proteins were downregulated (Table 1). CHKs (checkpoint kinases) are major
359 mediators of cell cycle checkpoints in response to genotoxic and other stresses (de Vries et al., 2005).

360 Another aphid gene downregulated under all conditions is the one coding for a facilitated
361 transmembrane trehalose transporter Tret1. Trehalose (α-D-glucopyranosyl-(1,1)-α-D-
362 glucopyranoside) is the main hemolymph sugar, and Tret1 is necessary for the transport of trehalose
363 produced in the fat body and its uptake into other tissues that require a carbon source (Kanamori et
364 al., 2010). Deregulation of this gene following virus acquisition has already been reported in other
365 insect vectors and is not linked to the virus transmission mode (e.g. Ding et al., 2019; Gamage et al.,
366 2018).

367 As mentioned above, we observed downregulation of distinct Cathepsin B3 (CathB)-encoding genes
368 in aphids feeding on TuYV- or CaMV-infected vs mock-inoculated plants. CathBs are detoxifying
369 proteases found in saliva and intestine and are subject to gene amplification, which is thought to be
370 an adaptation of saliva and intestine of aphids feeding on phloem sap from different plant species
371 (Mathers et al., 2017; Rispe et al., 2008). The CathB identified in Myzus feeding on TuYV-infected
372 plants (g24532) is the same as the one described by Guo et al. (2020), and the one found in Myzus
373 infesting CaMV-infected plants (g8486) is closely related to it (87% identity on the amino acid level).
374 The latter paralog (CathB3) is a saliva protease and an effector that induces plant defenses.
375 Therefore, its downregulation can be proviral by facilitating plant infestation. Up- or downregulation
376 of CathB-encoding genes during host-virus interactions has been observed in many arthropod vectors
377 (aphids, whitefly, thrips, leafhoppers, mites and mosquitos), suggesting importance of the cathepsin
378 Bs in virus-host-vector interactions and possibly transmission (Caicedo et al., 2019; Gamage et al.,
379 2018; Gupta et al., 2019; Hasegawa et al., 2018; Li et al., 2019; Li et al., 2020; Pinheiro et al., 2017; Xu
380 et al., 2021). Another feeding-related downregulated gene in aphids infesting virus-infected
381 Arabidopsis and Camelina encodes a sialin (g26345). A mammalian ortholog of the sialin gene
382 encodes a membrane protein of salivary gland cells controlling osmolarity and composition of saliva
383 (Li et al., 2018). Finally, among commonly downregulated Myzus genes we identified a gene encoding
384 a farnesol dehydrogenase-like protein (g24472), implicated in hormone metabolism. This gene could
385 be responsible for the oxidation of farnesol to farnesal, a precursor of the juvenile hormone as
386 shown for mosquitoes (Mayoral et al., 2009). Downregulation of juvenile hormone can favor wing
387 development (Zhang et al., 2019), which might facilitate viral spread.

388 Taken together, we observed that among the 'common' genes those involved in locomotion, neural
389 development and lifespan were rather upregulated in aphids feeding on virus-infected plants. This
390 might favor aphid mobility and survival and in turn virus dispersion. Genes involved in stress
391 responses and saliva functions were mostly downregulated (except the saliva protein ACE1
392 contributing to lifespan), indicating that viral infection facilitates aphid infestation of the host plants,
393 for example by dampening anti-herbivore plant defenses as observed in our previous study (Chesnais
394 et al., 2022a).

2 *Virus-specific aphid DEGs on both host-plants*

2a TuYV-specific DEGs in Myzus feeding on Arabidopsis and Camelina

To know whether plant viruses can impact aphid genes independently of the plant host, we first screened for common aphid DEGs deregulated after feeding on TuYV-infected Camelina and Arabidopsis. We found 19 upregulated genes (see a complete list in Table S3). Two of them might influence aphid feeding behavior (Table 2a). One (g26473) codes for a putative stylet sheath protein. Stylet sheaths are formed by gelling saliva that is secreted during stylet penetration in plant tissue. The sheaths insulate the stylets and potentially protect them from plant defenses and seal cell and phloem puncture sites (Will et al., 2012). Silencing of an *A. pisum* sheath protein gene disrupted sheath formation and disturbed phloem-feeding (Will and Vilcinskas, 2015), suggesting that upregulation, as observed here, might conversely facilitate and accelerate aphid feeding behavior on TuYV-infected plants (as observed by Chesnais et al., 2020), and hence TuYV acquisition. The second TuYV-specific feeding-related gene (g15241) codes for a receptor for the insect neuropeptide SIFamide that might control feeding indirectly by modulating behavior, as shown for SIFamide in the Chagas disease vector, the kissing bug *Rhodnius prolixus* (Ayub et al., 2020). The other upregulated genes are mostly related to development. Interestingly, two of them, forkhead box protein O (Foxo) and ATP-binding cassette sub-family G member 4-like (ABCG4) could be involved in aphid wing formation (Grantham et al., 2020; Shang et al., 2020). Induction of wings could considerably increase virus propagation by aphids, especially over long distances, as recently shown for CMV transmission (Jayasinghe et al., 2021). In this specific case, the wing formation was attributed to a virus satellite co-infecting the plant. The few downregulated genes (n = 14, see a complete list in Table S3) specific for TuYV are involved in detoxification and are closely related to the detoxification genes downregulated by both viruses in all conditions (see the previous section).

2b CaMV-specific DEGs in Myzus feeding on Arabidopsis and Camelina

We also analyzed the common and specific DEGs only found in aphids fed on CaMV-infected Arabidopsis and Camelina. We identified a total of 48 deregulated genes (31 upregulated and 17 downregulated DEGs, see the complete list in Table S4). One of the upregulated genes codes for a glucose dehydrogenase (Table 2b). Since glucose dehydrogenases are involved in multiple pathways, it is difficult to attribute a precise role for these enzymes in CaMV-aphid interactions. Several other upregulated genes might modulate aphid development and feeding behavior. For example, the gene g21498 codes for a structural RR-2 cuticle protein 3. Other RR-1 and RR-2 cuticle proteins are involved in virus–vector interactions (Deshoux et al., 2018), and it would be interesting to investigate a possible role of the RR-2 cuticle protein 3 in CaMV transmission. Another upregulated gene codes for an astacin (g7709), which belongs to a group of metalloproteases with various functions (Sterchi et al., 2008). Since the astacin identified here contains a signal peptide for secretion, it is tempting to speculate that it could be released during salivation or digestion and that upregulation might improve feeding.

As mentioned in the above section, both TuYV and CaMV infections deregulated some genes linked to salivary proteins (for example ACE1 and CathB). CaMV acquisition, but not TuYV acquisition, upregulated in Myzus another potential saliva gene coding for a mucin-2-like protein (g27683). In animals, saliva mucins protect by lubrication soft and hard tissues in the mouth (Turner, 2016). Their aphid homologs could have similar functions in protecting the stylet surface. Insect mucins have been studied thoroughly in the brown planthopper *Nilaparvata lugens*. One of them, NlMul, is a major component of watery and gelling saliva, required for proper feeding (Huang et al., 2017). Another one, NlMLP, is also involved in sheath formation, but in addition this mucin elicits plant defense responses (Shangguan et al., 2018). Transferred to aphids, genes encoding mucins could be involved

in the significant phagostimulation observed in aphids on CaMV-infected plants compared to healthy plants (Chesnais et al., 2021).

Among genes downregulated by CaMV (but not TuYV) in Myzus when feeding on both hosts were other genes coding for potential saliva proteins. One of them (g22531) codes for a 5'-nucleotidase with some similarities to a 5'-nucleotidase downregulated by various stresses in *A. glycines* (Enders et al., 2015) and to a saliva-contained 5-'nucleotidase of the mosquito *Aedes aegypti* (Champagne et al., 1995). A second gene codes for a pancreatic lipase-related 2-like protein (g16515). Similar enzymes have been identified in the salivary proteome of the potato aphid *Macrosiphum euphorbiae* (Chaudhary et al., 2015). Other pancreatic lipases are involved in vector interactions with circulative viruses. A pancreatic lipase from *Rhopalosiphum padi* binds to the CP and RT of barley yellow dwarf virus (family *Luteoviridae*) in yeast two-hybrid assay (Wang et al., 2015) and the gene expression of another pancreatic lipase is downregulated in *Bemisia tabaci* fed on TYLCV-infected tomato (Hasegawa et al., 2018). Thus, an impact of downregulation of this gene on non-circulative CaMV transmission could be indirect. Among other downregulated DEGs was the sugar transporter SWEET1-like gene (g20667), which codes for the midgut receptor of at least three planthopper-transmitted circulative, propagative viruses (Qin et al., 2018). A role, if any, for this gene in non-circulative transmission of CaMV by aphids could also be indirect, possibly by increasing feeding activity and concomitant virus acquisition, due to reduced sugar uptake.

3 Host plant-specific aphid DEGs for TuYV vs CaMV

To reveal an additional, host plant-specific, contribution to viral manipulation strategies linked to circulative vs non-circulative transmission modes, we analyzed DEGs in aphids feeding on TuYV- vs CaMV-infected Arabidopsis and in aphids feeding on TuYV- vs CaMV-infected Camelina (Figure 1e, TuYV vs CaMV). Since for Arabidopsis the total number of such aphid DEGs was 380, we applied a cut-off of logFC (fold changes) > 0.5 for upregulated genes and < -0.5 for downregulated genes to limit the number to 90 genes. This step was not necessary in the case of Camelina, where in total only 22 aphid DEGs were observed (see the complete lists in Tables S3, S4 and S5).

3a TuYV vs CaMV in Arabidopsis

A higher proportion of DEGs was upregulated in aphids feeding on TuYV-infected Arabidopsis, compared to aphids fed on CaMV-infected Arabidopsis (see Table 3a and Supplementary Table S5-6). Two of them (g5369 and g10419) encode chitinases that are essential for insect survival, molting and development (Arakane and Muthukrishnan, 2010). Four other genes encode the development-related proteins octopamine receptor Oamb (g15146), homeotic protein distal-less-like protein (g5303), zinc finger protein Elbow (g24564) and bombyxin C-2 like protein (g7214) (Campbell and Tomlinson, 1998; Ding et al., 2017; Wang et al., 2016; Weihe et al., 2004)). Thus, compared to CaMV, TuYV infection of Arabidopsis specifically induces higher expression of genes potentially involved in wing formation/development. This could promote, as discussed above, the formation of alate individuals with consequences on TuYV dispersal to new plants.

Interestingly, Myzus feeding on CaMV-infected Arabidopsis showed a different subset of developmental genes expressed at higher levels than Myzus feeding on TuYV-infected Arabidopsis. Four of these genes encode cuticle proteins (Table 3b and Supplementary Table S6). The fatty acyl-CoA reductase wat-like isoform X1 gene (g11235) that was also expressed at a higher level in the presence of CaMV, compared to TuYV, belongs to a gene family mediating the synthesis of insect cuticular hydrocarbons that are involved in the waterproofing of insect cuticles but also functions in signaling (Blomquist and Ginzel, 2021). Further experiments are needed to understand the impact of these gene on acquisition and transmission of non-circulative vs circulative viruses from Arabidopsis.

486 In addition to developmental genes, a high number of Myzus DEGs related to defense and
487 detoxification responses were differentially expressed in Myzus after acquisition of TuYV on
488 Arabidopsis, compared to acquisition of CaMV on Arabidopsis (Table 3a,b and Supplementary Table
489 S5). For example, variable deregulations in the different conditions were observed for four genes of
490 the UDP-glucuronosyl transferase gene family encoding detoxification enzymes (Brierley and
491 Burchell, 1993). However, other genes that could be related to defense and/or detoxification were
492 expressed at higher levels in CaMV-exposed aphids compared to aphids fed on TuYV-infected plants,
493 such as the gene encoding the Hayan serine protease (g21180), which activates the melanization
494 immune response to physical or septic wounding (Nam et al., 2012) and a gene encoding a histidine-
495 rich glycoprotein (g10551). A gene coding for an anti-microbial peptide, repetitive proline-rich cell
496 wall protein 2-like (g27577) (Li et al., 2012), was also expressed at higher levels in aphids fed on
497 CaMV-infected plants than in aphids fed on TuYV-infected plants. A similar trend was found for the
498 nuclear transcription factor Y subunit beta-like (g25790), which might interact with PLRV virions
499 (DeBlasio et al., 2021) and a homolog of which belongs to the upregulated genes associated with the
500 KEGG category "viral infectious disease" in whiteflies feeding on tomato infected with semi-
501 persistent cucurbit yellow stunting disorder virus (genus *Crinivirus*, family *Closteroviridae*) (Kaur et
502 al., 2019). Overall, we observed that different immune defense and detoxification pathways are
503 deregulated in Myzus feeding on CaMV-infected Arabidopsis, compared to Myzus feeding on TuYV-
504 infected Arabidopsis. This might be related to the different transmission modes of the two viruses.
505 TuYV being circulative is expected to interact delicately with the vector and maybe even evade
506 immune responses. On the other hand, CaMV interaction with the vector is confined to the stylet tip.
507 Therefore, CaMV might rather modulate feeding responses. This might be illustrated by the strong
508 activation of saliva genes (see below) following CaMV acquisition, whereas the impact of CaMV on
509 developmental genes was comparably lower. However, one needs to keep in mind that we discuss
510 here only a subset of the most strongly deregulated genes in CaMV-exposed aphids compared to
511 TuYV-exposed aphids.

512 Interestingly, in aphids feeding on CaMV-infected Arabidopsis, considerably more genes related to
513 salivary proteins were expressed at higher levels, compared to those feeding on TuYV-infected
514 Arabidopsis. Among them is the gene encoding a regucalcin (g15329) that was identified earlier in
515 the saliva of other aphid species (van Bel and Will, 2016). Regucalcin and other calcium-binding
516 proteins could reduce calcium availability, and subsequently inhibit aphid-induced calcium-mediated
517 sieve tube occlusion, which is observed in incompatible aphid-plant interactions (Will et al., 2009).
518 Another gene encodes the soluble calcium-activated nucleotidase 1-like isoform X2 (g12364), which
519 has previously been annotated in whitefly salivary glands (Su et al., 2012) and is predicted to be a
520 secretory ATP-hydrolyzing protein that could be involved in reducing the concentration of
521 extracellular ATP and suppressing plant defenses during whitefly feeding (Roux and Steinebrunner,
522 2007). Altogether, these aphid DEGs and the genes discussed above (see section 2b) indicate that
523 CaMV acquisition affects aphid saliva secretion on infected Arabidopsis. To explain this finding, we
524 propose two non-exclusive hypotheses. In the first one, the more severe phenotype of CaMV-
525 infected Arabidopsis, compared to TuYV-infected Arabidopsis, could induce adaptive changes of the
526 aphid secretome to allow successful settlement on the plants. In the second hypothesis, CaMV could
527 directly alter the saliva transcriptome. Whatever the mechanisms, these deregulations could be
528 responsible for the changes in the feeding behavior of aphids on CaMV-infected Arabidopsis plants
529 (Chesnais et al., 2021).

530 ***3b TuYV vs CaMV in Camelina***

Only 22 DEGs were found for aphids on TuYV- vs CaMV-infected Camelina, 17 expressed at higher levels in TuYV-exposed aphids and 5 expressed at higher levels in CaMV-exposed aphids (Fig. 1e). This small number of deregulations, in comparison to aphids fed on Arabidopsis, indicates strong host plant effects, which might be caused by differential host plant susceptibility to the viruses or different host-vector associations/suitability.
Among the genes expressed at higher levels in aphids on TuYV-infected vs aphids on CaMV-infected Camelina, we identified genes related to development, such as the gene encoding a glycine-rich cell wall structural protein-like (g7216) implicated in chitin-based cuticle development (Table 4, see the complete list in Table S7). This again suggests that TuYV may target aphid performance by inducing morphological changes, for example, the formation of wings that could enhance transmission.

Two immune-responsive aphid DEGs on Camelina were different from those observed in aphids feeding on Arabidopsis, again denoting some host specificity. One gene (g9870), expressed at higher levels in TuYV-exposed aphids than in CaMV-exposed aphids, encodes dual oxidase maturation factor 1 that is required for activation of dual oxidases and is involved in the control of reactive oxygen species (ROS) generation and signaling (De Deken et al., 2014). Its fruit fly ortholog is involved in antimicrobial defense mechanisms in the Drosophila intestine (Kim and Lee, 2014). Another gene (g18794), expressed at higher levels in TuYV-exposed compared to CaMV-exposed aphids, encodes the calcium release-activated calcium channel protein 1-like isoform X1 protein that regulates calcium entry into non-excitable cells and is required for proper immune function in Drosophila (Hou et al., 2020).

Finally, we observed that the gene coding for the protein THEM6-like (g24259) was expressed in TuYV-exposed aphids at a higher level than in CaMV-exposed aphids.

The five genes expressed at higher levels in CaMV aphids compared to TuYV aphids are already discussed in previous sections of this manuscript.

Taken together, our results show that TuYV vs CaMV DEGs on one host plant, Arabidopsis, are quite different from TuYV vs CaMV DEGs on another plant, Camelina, even if these two plants have strong phylogenetical proximity. This reinforces the idea that DEGs of insect vectors have a strong host-virus specificity.

Concluding remarks

We here compared the transcriptome profiles in Myzus aphids infesting two host-plant species from the family *Brassicaceae* (Arabidopsis and Camelina) infected with two viruses from different families with different transmission modes (circulative persistent TuYV and non-circulative semi-persistent CaMV). We found a strong plant-specific response of the aphid transcriptome, likely related to the differences in susceptibility of the plant hosts to these viruses (and/or the specific virus isolates studied here). This is evidenced by the higher number of aphid DEGs and stronger deregulations on virus-infected Arabidopsis compared to Camelina, regardless of the virus. It is worth noting that a plant transcriptome analysis revealed a lower number of plant DEGs in Arabidopsis and Camelina infected with TuYV compared to CaMV-infected plants, suggesting a strong virus-specific effect on the two plant hosts (Chesnais et al., 2022a). Thus, the global aphid transcriptome response to plant infection by the two viruses described here does not correlate with the global plant transcriptome response to the virus infection.

We found that stress-related aphid genes were downregulated in Myzus on both infected plants (regardless of the virus). This suggests that both CaMV and TuYV infections facilitate the establishment of Myzus on the plants, likely by downregulating in the plants expression of genes

implicated in anti-herbivore secondary metabolism such as the jasmonic acid pathway as shown in by us in the same experimental setup (Chesnais et al., 2022a). Apart from common transcriptomic changes induced by both viruses, our results indicate that there are also virus-specific gene expression changes, which might be related to the transmission mode. Overall, the circulative non-propagative TuYV tended to affect developmental genes, which could increase the proportion of alate (winged) aphids in TuYV viruliferous aphids, but also contribute to their locomotion, neuronal activity and lifespan, whereas the non-circulative semi-persistent (stylet-borne) CaMV had a stronger impact on feeding-related genes and in particular those related to salivary proteins. Overall, these transcriptome alterations target pathways that seem to be particularly adapted to the transmission mode of the corresponding virus. Long-term interactions of TuYV and its aphid vectors are expected and alterations of developmental genes, potentially promoting aphid dispersion at the population level (alate morphs with higher mobility and longer lifespan), could be a suitable strategy. In support of this, we have shown increased locomotory properties of wingless TuYV-carrying aphids (Chesnais et al., 2020), but whether Myzus aphids on TuYV-infected plants form also more alate morphs remains to be shown. On the other hand, the short-term association of CaMV with the tip of the aphid stylets, together with a relatively brief retention time, should favor manipulation of rather fast processes, such as initial probing and phloem feeding, encouraging fast aphid dispersion.

Next research steps should include functional validation of the candidate genes identified in our study for their role in viral manipulation, such as aphid behavior and performance, and consequently on viral transmission.

Tables and figures

Table 1. Selected deregulated aphid genes in common for aphids feeding on both CaMV and TuYV-infected Arabidopsis and Camelina.

						TuYV				CaMV			
						Arabidopsis thaliana		***Camelina sativa***		***Arabidopsis thaliana***		***Camelina sativa***	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**	**log2FC**	**padj**	**log2FC**	**padj**
Structural muscle proteins	Locomotion behavior	(Lemke and Schnorrer, 2017)	g22946	Titin isoform X1	*Acyrthosiphon pisum*	0,83	4,18E-24	0,79	1,44E-06	1,07	2,56E-05	2,86	1,98E-44
			g22969	Titin-like, partial	*Myzus persicae*	0,68	1,02E-02	2,49	3,39E-33	1,00	7,99E-35	0,80	8,59E-07
Cell function - Development (neurons)	Aphid development and behavior	(Ahern-Djamali et al., 1998)	g6068	Vasodilator-stimulated phosphoprotein-like	*Sipha flava*	0,58	1,94E-03	1,18	4,38E-04	0,75	3,85E-05	0,83	2,47E-02
Innate immunity	Unknown	(Ng & Xavier, 2011)	g27731	MATH and LRR domain-containing protein PFE0570w-like	*Sipha flava*	1,16	2,83E-05	1,11	1,44E-04	1,24	7,66E-06	1,05	3,71E-04
Salivary protein	Aphid feeding behavior and survival	(Wang et al., 2015)	g22588	Angiotensin-converting enzyme-like	*Myzus persicae*	0,66	2,78E-11	0,72	1,82E-02	0,71	2,81E-13	0,97	4,57E-04
Development (Hormones)	Aphid wing development	(Mayoral et al., 2009)	g24472	Farnesol dehydrogenase-like	*Myzus persicae*	-0,83	2,02E-05	-0,79	4,61E-05	-1,36	2,58E-13	-0,86	6,90E-06
Potential saliva effector	Aphids feeding behavior / Hydrolysis of toxic proteins	(Mathers et al., 2017; Rispe et al., 2008; Guo et al., 2020)	g24532	Cathepsin B-like cysteine proteinase 3	*Myzus persicae*	-0,71	5,45E-06	-0,51	2,19E-04	*padj > 0.05 or log2FC < |0.5|*			
			g8486	Cathepsin B-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,52	1,23E-11	-0,50	2,22E-02
Immune response and Detoxification (plant defense)	Aphid physiological response to plant defense	(Field and Devonshire, 1998) (Brierley and Burchell, 1993)	g22540	Esterase FE4-like	*Myzus persicae*	-0,93	6,09E-28	-0,50	9,43E-03	*padj > 0.05 or log2FC < |0.5|*			
			g19915	Esterase FE4-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,74	2,66E-07	-1,03	1,25E-06
			g26167	UDP-glucuronosyl transferase 344L3	*Myzus persicae*	-0,99	2,04E-04	-0,87	7,63E-05	-1,26	1,62E-06	-0,78	4,11E-04
			g18945	UDP-glucuronosyl transferase 344E7	*Myzus persicae*	-1,27	8,21E-03	-0,86	6,63E-03	-1,23	1,16E-02	-1,23	1,59E-05
			g26165	UDP-glucuronosyl transferase 344L3	*Myzus persicae*	-1,20	1,34E-05	-0,63	8,28E-03	*padj > 0.05 or log2FC < |0.5|*			
			g26170	UDP-glucuronosyltransferase 2B2-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-1,21	8,40E-11	-0,61	1,68E-02
			g12372	Glutathione S-transferase-like	*Myzus persicae*	-0,53	1,33E-08	-0,57	1,50E-02	-0,78	4,04E-18	-0,67	2,67E-03
			g24191	Glutathione S-transferase-like	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,81	2,48E-05	-0,84	6,97E-04
			g19821	Probable cytochrome P450 6a13 isoform X1	*Myzus persicae*	-2,56	2,55E-03	-1,34	1,15E-08	*padj > 0.05 or log2FC < |0.5|*			
			g18042	Probable cytochrome P450 6a13	*Myzus persicae*	*padj > 0.05 or log2FC < |0.5|*				-0,60	1,37E-02	-0,82	1,20E-03
Protein of salivary gland cells	Aphid feeding behavior	(Li et al., 2018)	g26345	Sialin-like	*Aphis craccivora*	-0,85	3,11E-04	-0,51	4,71E-02	-0,53	3,35E-02	-0,57	2,05E-02
Stress Response (DNA damages and genotoxic stresses)	Aphid physiological repsonse to plant defense	(de Vries et al., 2005)	g21951	CHK domain-containing protein	*Aphis craccivora*	-0,60	2,14E-16	-0,50	6,28E-04	*padj > 0.05 or log2FC < |0.5|*			
			g21958	CHK domain-containing protein	*Aphis craccivora*	-0,69	4,55E-12	-0,55	4,96E-03	-0,92	3,54E-21	-0,59	1,72E-03
			g21950	CHK domain-containing protein	*Aphis craccivora*	-1,46	1,83E-08	-0,57	2,70E-04	-1,60	6,26E-10	-0,71	1,80E-06
Transport of trehalose	Unknown - Aphid physiology	(Kanamori et al., 2010)	g14418	Facilitated trehalose transporter Tret1-like	*Myzus persicae*	-0,93	1,54E-04	-0,74	3,50E-04	-1,40	5,46E-09	-1,13	4,81E-09

595

596 **Table 2.** Selected genes commonly deregulated in aphids feeding on **a)** TuYV-infected and **b)** CaMV-infected host plants (Arabidopsis and Camelina).

597 **a) TuYV**

						Arabidopsis thaliana		*Camelina sativa*	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**
Saliva protein	Aphid feeding behavior	(Will et al., 2012)	g26473	Putative sheath protein, partial	*Sitobion avenae*	0,52	1,76E-11	0,69	9,93E-03
Insect neuropeptide	Aphid behavior (sleep, sexual and feeding)	(Ayub et al., 2020)	g15241	Neuropeptide SIFamide receptor-like	*Myzus persicae*	0,57	5,75E-03	1,22	4,50E-03
Membrane-associated transporter	Aphid development (wing)	(Shang et al., 2020; Jayasinghe et al., 2021)	g16568	ATP-binding cassette sub-family G member 4-like	*Myzus persicae*	0,52	2,15E-15	0,82	2,76E-07
Transcription factor	Aphid development (wing)	(Grantham et al., 2020)	g24925	Forkhead box protein O	*Myzus persicae*	0,50	9,81E-04	1,11	3,62E-06

598

599 **b) CaMV**

						Arabidopsis thaliana		*Camelina sativa*	
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**log2FC**	**padj**	**log2FC**	**padj**
Development (multiple pathways)	Aphid behavior and development		g19210	Glucose dehydrogenase [FAD, quinone]-like isoform X1	*Myzus persicae*	0,87	6,77E-10	1,20	7,11E-04
			g19209	Glucose dehydrogenase [FAD, quinone]-like	*Myzus persicae*	0,51	6,21E-03	0,98	8,07E-04
Structural protein	Aphid development - Virus interaction	(Deshoux et al., 2018)	g21498	RR-2 cuticle protein 3, partial	*Myzus persicae*	1,03	1,21E-10	0,85	9,89E-04
Saliva protein	Aphid feeding behavior	(Huang et al., 2017; Shangguan et al., 2018)	g27683	Mucin-2-like	*Myzus persicae*	1,01	9,40E-42	1,54	9,71E-06
Metalloproteases - Secreted protein	Aphid feeding behavior - Digestion	(Sterchi et al., 2008)	g7709	Astacin-like	*Myzus persicae*	1,13	1,26E-09	0,93	1,69E-02
Saliva protein - Lipase activity	Aphid feeding behavior	(Chaudhary et al., 2015)	g16515	Pancreatic lipase-related protein 2-like	*Myzus persicae*	-0,71	8,11E-14	-0,50	8,80E-03
Saliva protein	Aphid feeding behavior	(Enders et al., 2015; Champagne et al., 1995)	g22531	Protein 5NUC isoform X1	*Acyrthosiphon pisum*	-0,62	4,17E-02	-0,70	6,29E-04
Carbohydrate metabolism	Aphid metabolism	(Qin et al., 2018)	g20667	Sugar transporter SWEET1-like	*Myzus persicae*	-0,53	1,87E-08	-0,54	1,18E-02

600

Table 3. Selected genes deregulated in aphids feeding on TuYV-infected vs CaMV-infected Arabidopsis. **a)** up-regulated on TuYV-infected Arabidopsis and **b)** up-regulated on CaMV-infected Arabidopsis.

a)

Functional category	Potential effects on aphids	Reference(s)	Name	Gene description annotation	Top hit Taxon	Counts			log2FC	padj
						Mock	TuYV	CaMV		
Chitin degradation/reconstruction	Aphid survival, molting or development	(Arakane and Muthukrishnan, 2010)	g5369	Chitinase-like protein 4	*Myzus persicae*	56	77	37	1,06	3,78E-02
			g10419	Chitinase-like protein PB1E7.04c	*Rhopalosiphum maidis*	270	354	220	0,68	1,49E-02
Insulin-like insect hormone	Development (wing)	(Ding et al., 2017)	g7214	Bombyxin C-2-like	*Myzus persicae*	274	274	174	0,65	2,25E-02
Developmental protein (embryo, tracheal)		(Weihe et al., 2004)	g24564	Zinc finger protein Elbow-like	*Myzus persicae*	954	1305	880	0,57	4,52E-03
Hormone / Neurotransmitter		(Wang et al. 2016)	g15146	Octopamine receptor Oamb	*Myzus persicae*	64	117	50	1,21	8,65E-03
Transcription factor		(Campbell and Tomlinson, 1998)	g5303	Homeotic protein distal-less-like	*Myzus persicae*	279	416	267	0,63	2,79E-03
Immune response and Detoxification (plant defense)	Aphid physiological response to plant defense	(Field and Devonshire, 1998) (Brierley and Burchell, 1993)	g21619	UDP-glucuronosyl transferase 344D9	*Myzus persicae*	119	150	48	1,65	4,00E-03
			g26170	UDP-glucuronosyltransferase 2B2-like	*Myzus persicae*	6090	4348	2641	0,72	2,50E-03
			g23179	UDP-glucuronosyltransferase 2C1-like isoform X1	*Myzus persicae*	610	374	228	0,72	2,48E-02
			g21618	UDP-glucuronosyltransferase 2B9-like isoform X9	*Myzus persicae*	1109	495	335	0,56	3,10E-02

b)

Functional category	Potential effects on aphids	Reference(s)	Name	Gene description annotation	Top hit Taxon	Counts			log2FC	padj
						Mock	CaMV	TuYV		
Structural protein	Aphid development - Virus interaction	(Deshoux et al., 2018)	g21495	Cuticle protein 7-like	*Myzus persicae*	125	152	60	1,34	8,60E-05
			g27579	Cuticular protein-like precursor	*Acyrthosiphon pisum*	2106	4663	2153	1,12	2,34E-06
			g21493	Cuticle protein-like	*Myzus persicae*	2075	2794	1558	0,84	1,33E-10
Cuticle synthesis	Aphid development - Virus interaction	(Blomquist and Ginzel, 2021)	g21498	RR2 cuticle protein 3, partial	*Myzus persicae*	4063	8289	5229	0,67	7,25E-04
			g11235	Fatty acyl-CoA reductase wat-like isoform X1	*Myzus persicae*	89	240	130	0,89	2,94E-04
Membrane	Immune system	(Patton et al., 2021a)	g10551	Histidine-rich glycoprotein-like	*Myzus persicae*	2176	3132	2060	0,61	1,28E-13
Melanization immune response		(Nam et al., 2012)	g21180	Serine protease Hayan	*Acyrthosiphon pisum*	3376	6374	4356	0,55	1,60E-12
Antimicrobial peptide		(Li et al., 2012)	g27576	Repetitive proline-rich cell wall protein 2-like	*Myzus persicae*	3286	4109	1615	1,35	4,58E-09
			g27577	Repetitive proline-rich cell wall protein 2-like	*Myzus persicae*	7450	10736	6467	0,73	1,74E-11
Transcription factor		(DeBlasio et al., 2021)	g25790	Nuclear transcription factor Y subunit beta-like	*Myzus persicae*	10302	14330	9022	0,67	1,16E-03
Saliva protein	Aphid feeding behavior	(Will et al., 2009)	g15329	Regucalcin-like isoform X1	*Myzus persicae*	3804	10859	5495	0,98	9,82E-05
		(Roux and Steinebrunner, 2007)	g12364	Soluble calcium-activated nucleotidase 1-like isoform X2	*Myzus persicae*	9335	5354	2971	0,85	1,32E-02

605 **Table 4.** Selected genes upregulated in aphids feeding on TuYV-infected vs CaMV-infected Camelina.

						Counts				
Functional category	**Potential effects on aphids**	**Reference(s)**	**Name**	**Gene description annotation**	**Top hit Taxon**	**Mock**	**TuYV**	**CaMV**	**log2FC**	**padj**
Chitin-based cuticle development	Aphid development		g7216	Glycine-rich cell wall structural protein-like	*Myzus persicae*	3672	4861	3099	0,65	1,74E-02
Control of ROS and signaling	Immune system / Defense	(De Deken et al., 2014)	g9870	Dual oxidase maturation factor 1	*Myzus persicae*	1699	2215	1448	0,61	2,88E-03
Regulates calcium entry		(Hou et al., 2020)	g18794	Calcium release-activated calcium channel protein 1-like isoform X1	*Myzus persicae*	1714	2254	1695	0,41	3,13E-02
Hydrolase / Amino acid metabolism	Aphid metabolism		g24259	Protein THEM6-like (Thioesterase-like superfamily)	*Myzus persicae*	1060	1205	779	0,63	1,30E-02

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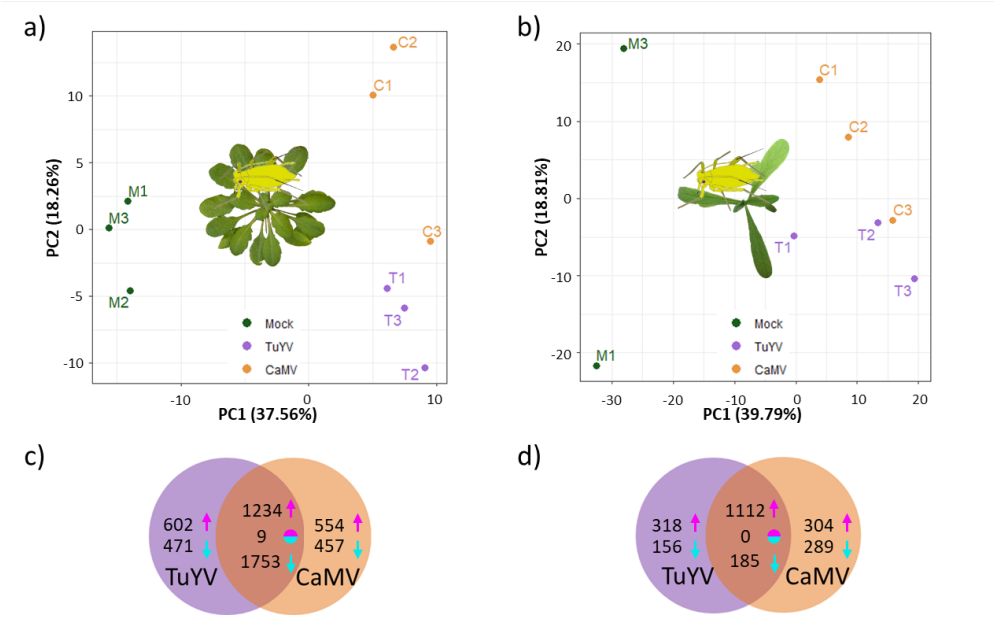

e)

	Arabidopsis			Camelina		
	TuYV vs. Mock	CaMV vs. Mock	TuYV vs. CaMV	TuYV vs. Mock	CaMV vs. Mock	TuYV vs. CaMV
n° DEGs (%DEGs/All genes)	4060 (14.24%)	3998 (14.02%)	380 (1.33%)	1771 (6.21%)	1890 (6.63%)	22 (<0.01%)
n° DEGs upregulated (%up/DEGs)	1836 (45.22%)	1797 (44.95%)	154 (40.53%)	1430 (80.75%)	1416 (74.92%)	17 (77.27%)
n° DEGs downregulated (%down/DEGs)	2224 (54.78%)	2201 (55.05%)	226 (59.47%)	341 (19.25%)	474 (25.08%)	5 (22,73%)
n° enriched GO terms	31	37	5	8	3	0

Figure 1. Analysis of the transcriptome profiles of aphids fed on mock-inoculated vs TuYV- and CaMV-infected plants. (a-b) Principal component analysis of three biological replicates for each condition of *Myzus persicae* feeding on (a) *Arabidopsis thaliana* and (b) *Camelina sativa*. The dots of the same color correspond to the biological replicates for each condition. The mock 2 (M2) Camelina sample was excluded from the analysis because it did not cluster with the other two replicates. (c-d) Venn diagrams presenting the number of differentially expressed genes (DEGs) in aphids fed on TuYV- and CaMV-infected Arabidopsis (c) and Camelina (d), compared to respective mock-inoculated controls. Magenta arrows: number of up-regulated genes, cyan arrows: number of down-regulated genes and two-color circles: inversely regulated genes (up-regulated genes in one virus-infected modality and down-regulated in the other virus-infected modality). e) The number of DEGs and enriched GO categories in aphids fed on TuYV and CaMV-infected plants vs mock controls as well as on TuYV- vs CaMV-infected plants.

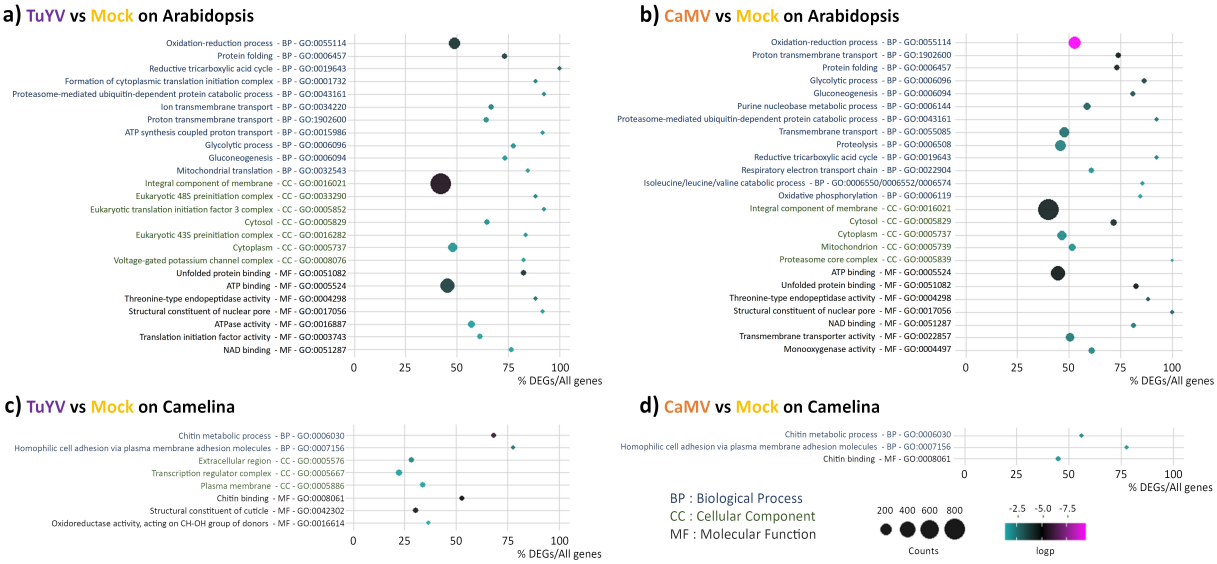

Figure 2. Gene ontology (GO) analysis of deregulated genes in *Myzus persicae* feeding on TuYV- and CaMV-infected Arabidopsis and Camelina. a) *Myzus persicae* on TuYV-infected vs mock-inoculated Arabidopsis, b) *Myzus persicae* on CaMV-infected vs mock-inoculated Arabidopsis, c) *Myzus persicae* on TuYV-infected vs mock-inoculated Camelina, and d) *Myzus persicae* on CaMV-infected vs mock-inoculated Camelina. The deregulated processes and the corresponding GO categories and IDs are specified in the vertical axis. For each GO category (BP: Biological Process, CC: Cellular Component, and MF: Molecular Function), the GO terms/processes are sorted according to decreasing log2 (1/p-value), also indicated by the color of each spot, to place the most significantly enriched GOs on top of the graph. The absolute number of DEGs that matched the GO term is indicated by the size of each spot, whereas the horizontal axis shows the percentage of DEGs belonging to the GO term.

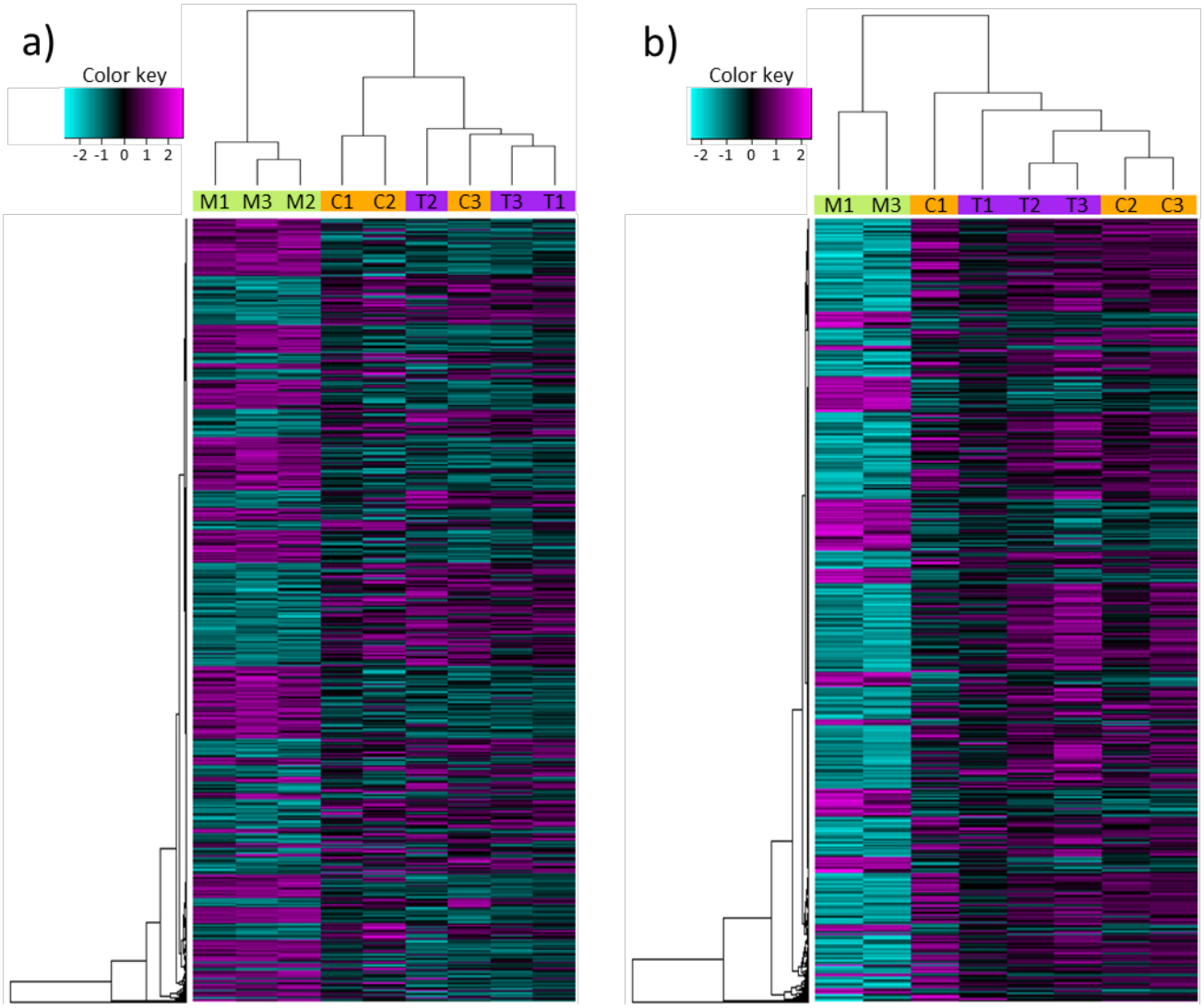

Figure 3. Hierarchical clustering of all differentially expressed genes (DEGs) in *Myzus persicae* feeding on CaMV- and TuYV-infected *Arabidopsis thaliana* (a) and *Camelina sativa* (b), compared to mock-inoculated control plants (Mock-inoculated [M], TuYV-infected [T] and CaMV-infected [C]) (Supplementary Dataset S1). The color key scale displays the row Z-score (normalized counts) from -2 to +2 as a gradient from cyan to magenta.

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Conflict of interest disclosure

The authors declare no conflict of interest.

Author contributions

Conceptualization, Q.C., V.B., M.P. and M.D.; methodology, Q.C., V.G. and M.D.; software, Q.C., A.V. and C.R.; validation, Q.C. and V.G.; formal analysis, Q.C., A.V., C.R., M.V. and M.D.; investigation, Q.C. and V.G.; Data curation, Q.C., A.V. and C.R.; Writing – Original Draft Preparation, Q.C., M.P. and M.D.; Writing – Review & Editing, Q.C., A.V., C.R., M.V., V.B., M.P. and M.D.; Visualization, Q.C.; supervision, M.P. and M.D.; project administration, M.D.; funding acquisition, M.P. and M.D.

Data, script and code availability

The raw RNA-seq data are available under project number PRJEB54781 at the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/view/PRJEB54781). The data used to create Figures 1 and 3 and Tables 1 through 4 are contained in the supplementary data set deposited on BioRXiv (https://doi.org/10.1101/2022.07.18.500449). The scripts used to process data are listed in the 'Materials and methods' section, subsection 'Raw data processing and quality control for transcriptome profiling'.

Supplementary information

The following supplementary data are available on doi: https://doi.org/10.1101/2022.07.18.500449:

Table S1. Aligned reads for transcriptome profiling

Table S2. Oligonucleotides used for RT-qPCR

Table S3. Complete list of deregulated aphid genes in common for aphids feeding on both CaMV and TuYV-infected Arabidopsis and Camelina.

Table S4. Complete list of genes commonly deregulated in aphids feeding on CaMV-infected host plants (Arabidopsis and Camelina) (padj < 0.05 and log2FC $> |0.5|$).

Table S5. Complete list of genes upregulated in aphids feeding on TuYV-infected vs. CaMV-infected Arabidopsis (padj < 0.05 and log2FC $> |0.5|$).

Table S6. Complete list of genes upregulated in aphids feeding on CaMV-infected vs. TuYV-infected Arabidopsis (padj < 0.05 and log2FC $> |0.5|$).

Table S7. Complete lists of genes upregulated in aphids feeding on CaMV-infected vs. TuYV-infected Camelina and of genes upregulated in aphids feeding on TuYV-infected vs. CaMV-infected Camelina (padj < 0.05 and log2FC > |0.5|).

Figure S1. Quantitative reverse transcription PCR (RT-qPCR) validation of differentially expressed genes (DEGs) determined by Illumina RNA-seq profiling of the aphid transcriptome.

Figure S2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of DEGs (log2FC > 1) in *Myzus persicae* in response to TuYV or CaMV infection in Arabidopsis or Camelina plants.

Dataset S1. RNA-seq data used to establish the heatmap.

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