

1 **Aphid transmission of a Potexvirus, *Foxtail mosaic virus*, in the presence of the Potyvirus**
2 **helper component proteinase**

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30 **Abstract:**

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32 To establish successful infections, plant viruses compete with the host plants for limited
33 resources and thus alter the physiological state of the plants. After successful infection, insect
34 vectors are required for the transmission of some plant viruses to the next host plant. One of the
35 largest groups of plant viruses, the *potyvirus*, can be transmitted by aphids. During transmission,
36 the potyvirus protein helper component proteinase (HC-Pro) binds to the yet-to-be-defined aphid
37 receptor on the stylet, as well as to the virus particles through the Asp-Ala-Gly (DAG) motif of
38 the viral coat protein. Previously it was determined that a naturally occurring DAG motif in the
39 non-aphid transmissible *potexvirus*, *Potato aucuba mosaic potexvirus* (PAMV), is functional
40 when the HC-Pro is provided through co-infection with a potyvirus. Further, the DAG motif of
41 PAMV can be successfully transferred to another non-aphid transmissible potexvirus, *Potato*
42 *virus X* (PVX), to convey aphid transmission capabilities. We expand on this previous work by
43 demonstrating, the DAG motif from two different potyviruses, *Sugarcane mosaic virus* and
44 *Turnip mosaic virus*, as well as the DAG motif from the previous potexvirus PAMV, can be
45 added to another non-aphid transmissible *potexvirus*, *Foxtail mosaic virus* (*FoMV*), to make it
46 aphid transmissible. Transmission efficiency varied from less than 10% to over 80% depending
47 on the DAG motif and host plant used in transmission, suggesting not all DAG motifs are equal
48 for engineering aphid transmission. The underlying mechanisms mediating this variation still
49 need to be explored.

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52 **Keywords:**

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54 Aphids, Coat protein, DAG motif, Potexvirus, Potyvirus, Virus transmission

55 **1. Introduction:**

56

57 Plant viruses must take over the metabolism of the host to obtain resources and establish a
58 successful viral infection. Interference with cellular homeostasis causes a variety of pathogenic
59 effects, including stunting, discoloration, and malformation (Gergerich and Dolja, 2006). Many
60 plant viruses move among plant hosts and plant populations through insect vectors. The rapid
61 reproduction of some vectors and their ability to travel over long distance have led to the fast
62 spread of plant viruses in ecosystems and significant amounts of economic loss. Fortunately, the
63 transmission of plant viruses by insect vectors requires specificity, with a particular plant virus
64 transmitted by a particular insect species in general (Casteel and Falk, 2016; Whitfield et al.,
65 2015).

66

67 Depending on the virus transmission mode, insect vector-mediated plant virus transmission can
68 be classified as persistent, semi-persistent, or non-persistent transmission. For persistent
69 transmission, the plant viruses enter the insect body and may or may not replicate within the
70 insect. For example, the *Rice dwarf virus* (RDV) enters the alimentary canal, further reaches the
71 midgut of its leafhopper vector, and can multiply in the insect host (Chen et al., 2011b). On the
72 contrary, the *Barley yellow dwarf virus* (BYDV) circulates in the aphid but does not replicate in
73 this insect vector (Li et al., 2001). In these cases, the insect vectors are viruliferous for their
74 entire life cycle. Other viruses, such as the *Lettuce infectious yellows virus* (LIYV), can be
75 transmitted by their insect vectors in a non-circulative, semi-persistent manner, where the virions
76 are retained for hours to days in the insect anterior foregut, or cibarium, and are lost after the
77 insect molts (Chen et al., 2011a; Tian et al., 1999). In contrast, some plant viruses can be
78 transmitted by their insect vectors in a non-persistent manner where they are only retained for
79 minutes to hours in the insect stylet and are lost after the insect molts. For example, *Cauliflower*
80 *mosaic virus* (CaMV), *Cucumber mosaic virus* (CMV), and *Potato virus Y* (PVY) are transmitted
81 in a non-persistent manner by aphids, the primary insect vector for this transmission mode
82 (Nanayakkara et al., 2012; Severin H, 1948; Swenson and Marsh, 1967).

83

84 The underlying molecular mechanism of non-persistent plant virus transmission by aphids has
85 been studied extensively from the perspective of viral components through the purification of

86 virus particles, the generation of mutated viruses, and the purification of transmission-related
87 viral proteins. For example, the viral coat protein (CP) is the major determinant of aphid-
88 mediated CMV transmission, especially the negatively charged loop structure on the surface of
89 the virus particle which is critical for aphid transmissibility (Liu et al., 2002). The transmission
90 of CaMV requires multiple viral proteins, of which the viral protein P2 binds to the aphid stylet
91 through its N-terminus, and the C-terminus of P2 interacts with the virion-associated viral
92 protein P3, for aphid-mediated transmission (Blanc et al., 2014; Hoh et al., 2010; Plisson et al.,
93 2005). The transmission of potyviruses, including *Turnip mosaic virus* (TuMV), *Sugarcane*
94 *mosaic virus* (SCMV), and PVY, is mediated by the Asp-Ala-Gly (DAG) motif (or the related
95 variants, such as DTG, DAE, and DAA motif) of the CP and the Lys-Ile-Thr-Cys (KITC) and
96 Pro-Thr-Lys (PTK) motif of the viral helper component proteinase (HC-Pro) (Blanc et al., 1998;
97 Gadhave et al., 2020; Peng et al., 1998). In this case (and the previous with CaMV), a bridge
98 model has been proposed: the viral protein HC-Pro simultaneously binds to the aphid stylet and
99 the virus particle for the transmission of the virus. Many other viral proteins are dispensable for
100 vector-mediated virus transmission, but they can facilitate virus transmission by modulating
101 plant physiology and insect vector behavior and biology. For instance, the potyvirus NIa-Pro can
102 disrupt the ethylene response of the host plant, thus attracting the insect vectors to the virus-
103 infected plant (Bak et al., 2019; Casteel et al., 2015). The 2b protein of CMV also can alter the
104 emission of volatile compounds, as well as enhance the production of reactive oxygen species, to
105 promote the virus transmission by the aphid vectors (Guo et al., 2019; Tungadi et al., 2017).
106 However, the aphid receptors involved in the virus transmission are still largely unknown, thus
107 far only a few potential candidates have been discovered (Deshoux et al., 2020; Yang et al.,
108 2008).

109
110 Potexviruses are not aphid transmissible, however, the CP from *Potato aucuba mosaic*
111 *potexvirus* (PAMV) contains a DAG motif. While PAMV cannot be transmitted by aphids in a
112 single infection (only PAMV), it can be transmitted by aphids when the potyviral HC-Pro is
113 provided in mixed infections with a potyvirus (PVY) (Baulcombe et al., 1993). It was further
114 demonstrated that aphid transmissibility can be successfully transferred to another potexvirus,
115 *Potato virus x* (PVX), with the addition of PAMV'S DAG motif to the N-terminus of PVX'S CP
116 (Baulcombe et al., 1993). To expand on this work, we evaluated if the DAG motifs from

117 different potyviruses can be added to another potexvirus, *Foxtail mosaic virus* (FoMV), to
118 convey aphid transmissibility. We compared the transmission efficiency of FoMV with different
119 DAG motif additions to its CP, including the addition of 15 or 35 amino acid residues from the
120 CP of two potyviruses (SCMV or TuMV), or the addition of 20 or 40 amino acid residues of
121 PAMV's CP. The addition of the 35 amino acid residues from the N-terminus of SCMV's CP
122 resulted in the highest aphid transmission efficiency (up to 75%) to maize plants (*Zea mays*),
123 while transmission success was less than 20% for all other recombinant viruses to maize.
124 Overall, aphid transmission was more successful to the model plant *Nicotiana benthamiana*
125 compared to maize, where transmission efficiency varied from 30% to over 80%, depending on
126 the N-terminus addition. These results demonstrate not all DAG motifs are equal for engineering
127 aphid transmissibility in potexviruses.
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129 **2. Materials and methods:**

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131 **2.1. *Potexvirus and potyvirus sequence comparison***

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133 The CP amino acid sequence of potexvirus, *Cymbidium mosaic potexvirus* (CymMV, accession
134 number AAL12628), *Narcissus mosaic virus* (NMV, accession number NP_040782), *Pepino*
135 *mosaic virus* (PepMV, accession number AIL23124), *Bamboo mosaic virus* (BaMV, accession
136 number AOS51152), PVX (accession number AAA47181), PAMV (accession number
137 AXL97636), FoMV (accession number AWT40560), and of the potyvirus, PVY (accession
138 number ADH52720), *Soybean mosaic virus* (SMV, accession number QBB78854), *Tobacco etch*
139 *virus* (TEV, accession number ABH10566), *Plum pox virus* (PPV, accession number
140 ABU97781), *Pepper mottle virus* (PepMoV, accession number BAB91329), *Bean yellow mosaic*
141 *virus* (BYMV, accession number ABM69144), *Potato virus A* (PVA, accession number
142 NP_734368), TuMV (accession number ANW35618) and SCMV (accession number P32652),
143 were aligned with ClustalW2 online server (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

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145 **2.2. *Molecular cloning***

146

147 The FoMV infectious clone pCambia1380/FoMV was a generous gift from Professor Steven A.
148 Whitham (Iowa State University, USA), and the TuMV infectious clone
149 pCambia0380/TuMV:6K2-GFP (Cotton et al., 2009) was kindly provided by Professor Jean-
150 François Laliberté (INRS-Institut Armand-Frappier, Canada). To make the mutants
151 pCambia1380/FoMV^{V8A-T9G}, pCambia1380/FoMV^{T12G}, pCambia1380/FoMV^{Y14A-K15G},
152 pCambia1380/FoMV^{V8A-T9G-T12G}, and pCambia1380/FoMV^{A6V-V8A-T9G}, three-step cloning
153 processes were performed. First, the HSSB fragment, of which the *SphI-SacII* fragment of wild
154 type (WT) FoMV that contains the N-terminal part of the FoMV CP coding sequence, with the
155 addition of *HindIII* restriction site at the 5' end and *BamHI* restriction site at the 3' end, was
156 cloned into the smaller vector pBluescript SK(+), and the resulting clone was pSK-HSSB. Site-
157 directed mutagenesis was then performed, and the corresponding mutations were then introduced
158 into pSK-HSSB using a complementary primer set. The *SphI-SacII* fragment of pSK-HSSB with
159 the desired mutations was then subcloned into pCambia1380/FoMV.

160

161 To construct the FoMV mutants (pCambia1380/FoMV^{SCMV N15}, pCambia1380/FoMV^{TuMV N15},
162 pCambia1380/FoMV^{PAMV N20}, pCambia1380/FoMV^{SCMV N35}, pCambia1380/FoMV^{TuMV N35} and
163 pCambia1380/FoMV^{PAMV N40}), three-fragment Gibson assembly reactions were performed. In
164 general, fragment 1 was PCR amplified with Primer F1 and R1, using the plasmid
165 pCambia1380/FoMV as the template. This fragment was approximate 200 bp and located right
166 upstream of the FoMV CP coding sequences. The coding sequences of SCMV N15, TuMV N15,
167 PAMV N20, SCMV N35, TuMV N35, and PAMV N40 were incorporated into the primer R1.
168 Fragment 2 was about 700 bp and was PCR amplified with primer F2 and R2 using the plasmid
169 pCambia1380/FoMV as the template. This fragment contained mostly the FoMV CP coding
170 sequences. Fragment 3 was the vector backbone of pCambia1380/FoMV that was digested with
171 *Bsu36I* and *XbaI*. The primer sequences are listed in Supplemental Table 1. All constructs were
172 confirmed by DNA sequencing.

173

174 **2.3. Virus infection**

175

176 All FoMV derived constructs were transformed into *Agrobacterium tumefaciens* (strain GV3101)
177 and the positive transformants were selected on LB Kanamycin-Rifampicin agar plates. The
178 positive transformants were cultured overnight, centrifuged, and suspended in a 10 mM MgCl₂
179 and 150 μM acetosyringone solution. For viral RNA accumulation assays, the OD₆₀₀ of *A.*
180 *tumefaciens* suspensions was adjusted to 0.03; And to purify the virus particles for transmission
181 electron microscopy (TEM) observations, the OD₆₀₀ of *A. tumefaciens* suspensions was adjusted
182 to 0.2. To co-infect *N. benthamiana* plants with FoMV recombinant virus and TuMV/6K2:GFP,
183 the OD₆₀₀ was 0.2 and 0.05 for the FoMV recombinant virus and TuMV/6K2:GFP *A.*
184 *tumefaciens* suspensions, respectively. Agroinfiltration was done with four-week-old *N.*
185 *benthamiana*, and the agro suspensions were infiltrated into the underside of the leaves with a
186 needleless syringe. To test the infectivity of the recombinant viruses in maize plant, the inoculum
187 was prepared by grinding the FoMV mutant infected *N. benthamiana* leaf tissues in 50 mM
188 potassium phosphate buffer, pH 7.0 (1 g tissue to 4 ml buffer), and rubbed onto 1-week-old
189 maize plants with carborundum. All plants were grown at 24 °C, 16-h-light/ 8-h-dark
190 photoperiod.

191

192 **2.4. Virus purification and preparation for TEM observation**

193

194 Systemic infected *N. benthamiana* leaf tissues were collected 2 weeks after agroinfiltration. Leaf
195 tissues were then homogenized with the same amount (1 g tissue to 1 ml buffer) of buffer
196 containing 0.1 M Tris-Citric acid (pH 8.0), 0.2% β -mercaptoethanol, and 0.01 M sodium
197 thioglycolate. Triton X-100 was added gradually to a final concentration of 1% in a period of 15
198 min with constant stirring at 4 °C. Chloroform was added to a final concentration of 25%, and
199 constant stirring at 4 °C for 30 min. The mixture was then centrifuged at 12,000x g for 15 min to
200 separate the water phase and organic phase. PEG6000 was then added to the water phase, to a
201 final concentration of 5% and constant stirring at 4 °C overnight. The mixture was then
202 centrifuged at 10,000x g for 15 min, and the resulting pellet was resuspended with 300 μ l of the
203 extraction buffer described above. To observe the virions under TEM, 5 μ l of purified virions
204 was loaded onto the pretreated grid. The solution was then absorbed with a filter paper after 5
205 sec. The same step was repeated 5 times but with 5 μ l 2% phosphotungstic acid solution. The
206 grid was then air-dried for 2 min and was ready for TEM observation. TEM observation was
207 done with a JEOL 2100F transmission electron microscope at 200 kV accelerating voltage.

208

209 **2.5. Aphid transmission**

210

211 All aphid colonies and plants were maintained in the growth chamber (24 °C, 16-h-light/ 8-h-
212 dark photoperiod). The green peach aphid (*Myzus persicae*) colony was reared on *N. tabacum*.
213 The aphid adults were collected and then starved for 4 h. The aphids were then allowed to
214 acquire the virus from the infected source tissues for 5 min and then were transferred to healthy
215 *N. benthamiana* plants (2-week-old) or sweet corn (*Zea mays* cv. Golden Bantam, 1-week-old).
216 Pesticide was sprayed to kill the aphids 24 h later. Two to three weeks after aphid transmission,
217 the newly emerged leaves were collected for RT-PCR.

218

219 **2.6. RT-PCR and RT-qPCR Analysis**

220

221 The agroinfiltrated leaf tissues were collected for RT-qPCR analysis, while either the systemic
222 infected or newly emerged leaf tissues were collected for RT-PCR analysis. The total RNAs
223 were isolated from the leaf tissues using SV Total RNA Isolation System (Promega, Madison,
224 WI, USA). A thousand ng of total RNAs were used for cDNA synthesis using the SMART
225 MMLV Reverse Transcriptase (Takara Bio, Mountain View, CA, USA). For RT-PCR, one
226 microliter of cDNA was used as the template, and a 348-bp FoMV CP fragment was amplified
227 using GoTaq DNA polymerase (Promega, Madison, WI, USA). The cDNA was diluted 40 times
228 and then 2 ul were used for the RT-qPCR. The RT-qPCR was done using SsoAdvanced
229 Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) as instructed. The *N.*
230 *benthamiana* Actin2 gene and *Z. mays* Actin gene were used as the internal control. The relative
231 gene expression was quantified using the delta-delta Ct method. A C1000 and a CFX384
232 thermocycler were used for RT-PCR and RT-qPCR (Bio-Rad, Hercules, CA, USA), respectively.
233 RT-PCR and RT-qPCR primer sequences are listed in Supplemental Table 1.

234

235

236 3. Results:

237

238 *3.1. The DAG motif and its variants are commonly present in the N-terminus of potyvirus coat* 239 *protein*

240

241 To gain insights into setting up the conditional transmission of FoMV by introducing the
242 potyvirus CP DAG motif, we first compared the CP amino acid sequence of different
243 potexviruses and potyviruses. The alignment showed that both the N-terminus of potexvirus and
244 potyvirus CP are highly variable (Fig. 1A & 1B). The C-terminus of the potexvirus CP is also
245 not conservative, while the C-terminus of the potyvirus CP is comparably conserved. For the
246 potexvirus, the DAG motif was only found in the N-terminus of PAMV CP at the position of 14-
247 16 (Fig. 1A). While a single or double DAG motif, or its variants, was found in the N-terminus
248 of all the aligned potyvirus CPs (Fig. 1B). The DAG motifs were usually located near the N-
249 terminus of the CPs, within the range of their N-terminus 15 amino acid residues. This further
250 confirms the DAG motif (or its variant) is indispensable for the aphid transmissibility of
251 potyviruses, thus we sought to set up the conditional transmission of FoMV by introducing the
252 DAG motif to the N-terminus of its CP.

253

254 *3.2. The recombinant FoMV viruses form infectious virus particles*

255

256 Based on the sequence alignment information, we first introduced the potyvirus DAG motif into
257 the FoMV CP by substituting amino acid residues within its N-terminus. In this case, we
258 maintained the authentic full-length sequence of the FoMV CP, changing only a few amino acid
259 residues, and expected these mutants would still be capable of forming infectious virus particles.
260 Five mutants, FoMV^{V8A-T9G}, FoMV^{T12G}, FoMV^{Y14A-K15G}, FoMV^{V8A-T9G-T12G}, and FoMV^{A6V-V8A-}
261 ^{T9G} were constructed (see supplemental Table 2). In the mutants FoMV^{V8A-T9G}, FoMV^{T12G}, and
262 FoMV^{Y14A-K15G}, a single DAG motif was introduced at positions 7-9, 10-12, and 13-15 within the
263 N-terminus of FoMV CP, respectively. For mutant FoMV^{V8A-T9G-T12G}, double DAG motifs were
264 introduced at positions 7-9 and 10-12. For mutant FoMV^{A6V-V8A-T9G}, a DAG motif was
265 introduced at position 7-9, and the alanine residue at position six was substituted with valine.
266 This mutant was made because we found an alanine residue that preceded the DAG motif quite

267 often in the N-terminus of potyvirus CPs. The systemic infection of the WT and all five mutated
268 FoMV was detected by RT-PCR at 5 days post agroinfiltration (data not shown). However, none
269 of the mutated viruses that carry the DAG motif were aphid transmissible in the presence of
270 TuMV HC-Pro protein (data not shown).

271
272 We then explored an alternative strategy where the N-terminal part of SCMV's, TuMV's, or
273 PAMV's CP, which contains the DAG motif, was added to the N-terminus of the full-length
274 FoMV CP (Fig. 1C). The addition of N-terminal 15 or 35 amino acid residues of SCMV and
275 TuMV CP, resulting in the recombinant virus termed FoMV^{SCMV N15}, FoMV^{TuMV N15}, FoMV^{SCMV}
276 ^{N35}, and FoMV^{TuMV N35}. The addition of the N-terminal 20 and 40 amino acid residues to the N-
277 terminus of FoMV CP are named FoMV^{PAMV N20} and FoMV^{PAMV N40} thereafter, respectively.
278 First, we wanted to check if these recombinant viruses still can replicate. As shown in Fig. 2A,
279 the WT FoMV viral RNA (vRNA) accumulation reached the highest level in inoculated *N.*
280 *benthamiana* leaves at 5 days post agroinfiltration. The vRNA accumulation for the recombinant
281 viruses was then compared in *N. benthamiana* leaves 5 days after agroinfiltration. We found all
282 the recombinant viruses were still replicable, although the replication level was about 20-40% of
283 the WT virus (Fig. 2B). The systemically infected leaves of the above agroinfiltrated *N.*
284 *benthamiana* were then checked by RT-PCR at 5 days post agroinfiltration. The recombinant
285 virus FoMV^{SCMV N15}, FoMV^{TuMV N15}, FoMV^{PAMV N20}, FoMV^{SCMV N35}, and FoMV^{PAMV N40} were
286 able to establish a systemic infection in *N. benthamiana* (Fig. 2C, upper panel). Systemically
287 infected *N. benthamiana* leaf tissues were used as the source tissues for rub inoculation into *Zea*
288 *mays* cv. Golden Bantam. All FoMV recombinants, except FoMV^{TuMV N35} could also infect *Z.*
289 *mays* systemically at 2 weeks after rub inoculation (Fig. 2C, lower panel). Next, virus particles
290 were purified from systemically infected *N. benthamiana* leaf tissues, and the purified virus
291 particles were observed under TEM. As expected, the above mutated virus FoMV^{SCMV N15},
292 FoMV^{TuMV N15}, FoMV^{PAMV N20}, FoMV^{SCMV N35}, and FoMV^{PAMV N40} were able to form virus
293 particle. This means the addition of exogenous N-terminal tails does not affect the assembly of
294 the virus particles, which is a prerequisite for aphid-mediated virus transmission. For aphid
295 transmission experiments, the recombinant virus FoMV^{TuMV N35} was not considered as it could
296 not establish virus systemic infection, probably because this recombinant virus could not form
297 infectious virus particles or lose the movement ability.

298

299 **3.3. The recombinant viruses, in particular the *FoMV*^{SCMV N35}, are aphid transmissible**

300

301 Next, we then explored if the recombinant FoMV viruses are aphid transmissible. *N.*
302 *benthamiana* leaf tissues co-infected with the above FoMV recombinant viruses and
303 TuMV/6K2:GFP. The co-infected plants were then used as the source tissue for virus
304 transmission (Fig. 3A). *N. benthamiana* co-infected with TuMV/6K2:GFP and WT FoMV or
305 with the recombinant FoMV's, were used as the negative control. As shown in Fig. 3B, the
306 recombinant virus FoMV^{SCMV N15}, FoMV^{PAMV N20}, FoMV^{SCMV N35}, and FoMV^{PAMV N40}, could be
307 transmitted to *N. benthamiana* efficiently by aphids. However, only the recombinant virus
308 FoMV^{SCMV N35} could be transmitted to *Z. mays* efficiently (Fig. 3C). On average, the transmission
309 efficiency ranged from 30-80% for *N. benthamiana* and 10-75% for *Z. mays* (Fig. 3D). The
310 negative controls did not show any virus transmission (data not shown). In conclusion, these data
311 suggest the recombinant viruses, in particular the FoMV^{SCMV N35}, are aphid transmissible in the
312 presence of TuMV HC-Pro protein.

313

314 **4. Discussion:**

315

316 The naturally occurring DAG motif of the potexvirus PAMV can make PAMV and other non-
317 aphid-transmissible potexviruses, such as PVX, aphid-transmissible in mixed infections with a
318 potyvirus (Baulcombe et al., 1993). We demonstrated that the N-terminal portion of different
319 potyvirus CPs, which contains the DAG motif, can also be used to engineer aphid transmission
320 for potexviruses. The N-terminal portion of the CP from the aphid-transmissible potyviruses,
321 TuMV or SCMV, or from the previously studied DAG containing potexvirus (PAMV), made
322 FoMV aphid transmissible when co-infected with a potyvirus (Fig. 3). The presence of the
323 potyvirus protein HC-Pro is required as the transmission can only happen during co-infection
324 with a potyvirus.

325

326 The importance of the context of the CP DAG motif in virus aphid transmission has been
327 demonstrated (López-Moya et al., 1999). The presence of the DAG motif does not guarantee
328 transmissibility, and the context in which the DAG or equivalent motif is found plays a role in
329 the process. This is consistent with what we have found, simply introducing the DAG motif in
330 the N-terminus of FoMV CP by substituting certain amino acids did not make FoMV aphid
331 transmissible. The virus transmission could happen only when part of the N-terminal tail of
332 potyvirus CPs were added to the N-terminus of FoMV CP (Fig. 3). We also noticed that the
333 DAG motifs from different viruses showed varied transmission efficiency in the presence of the
334 same HC-Pro protein (Fig. 3B & 3C). Our sequence alignment showed that the N-terminus of
335 potyvirus CP is highly variable, although the DAG motif is commonly present near the N-
336 terminus of the CP (Fig. 1B). These indicate the amino acid residues surrounding the DAG motif
337 are important for the virus aphid transmission, probably by affecting the HC-Pro accessibility to
338 the DAG motif.

339

340 Overall, our data shows the aphid transmission was more successful to the model plant *N.*
341 *benthamiana* compared to *Z. mays*, where transmission efficiency varied from 30% to over 80%,
342 depending on the N-terminus addition. We also noticed that one recombinant virus, FoMV^{PAMV}
343 ^{N20}, could only be transmitted by aphids to *N. benthamiana* plant but not to *Z. mays* (Fig. 3D).
344 Similarly, the recombinant virus, FoMV^{SCMV N15}, could be transmitted to *N. benthamiana* plant

345 efficiently, but this was not the case when it was transmitted to *Z. mays* (Fig. 3D). The highest
346 aphid transmission efficiency was observed for FoMV^{SCMV N35} for both host plants (Fig. 3). The
347 DAG motif may play dual functions by mediating aphid transmission and virus movement for
348 some viruses. In the case of *Zucchini yellow mosaic virus* (ZYMV), the N-terminal tail of its CP
349 doesn't seem important for the virus infection as the virus is still infectious when the N-terminal
350 tail 43 amino acid residues are deleted (Arazi et al., 2001). However, for the *Tobacco vein*
351 *mottling virus* (TVMV), the DAG motif is critical for the virus systemic infection (López-Moya
352 and Pirone, 1998). In this previous study mutation of the DAG motif abolished the virus cell-to-
353 cell movement, but not the vRNA replication in the cells. Thus, it will be interesting to
354 investigate whether the higher transmission efficiency of the recombinant virus, FoMV^{SCMV N35},
355 to *Z. mays*, is due to a better fitness of this virus in sweet corn, which is a susceptible host of
356 SCMV infection.

357

358 Although we have established engineering aphid transmission of FoMV can be successful, our
359 results demonstrate not all DAG motifs are equal and additional work is needed. In our study,
360 only one aphid species (*M. persicae*) and one HC-Pro (TuMV) were used. It is known that the
361 HC-Pro protein of Watermelon mosaic virus (WMV) can assist the aphid transmission of TuMV,
362 while the HC-Pro protein of TuMV is ineffective for the transmission of WMV (Sako and Ogata,
363 1981). Also, different aphid species can transmit the same virus at different efficiency, in the
364 case of ZYMV and TuMV (Dombrovsky et al., 2005). Efforts are still needed to figure out the
365 best combination of HC-Pro source and aphid species for a particular aphid-mediated plant virus
366 transmission. It is also not known whether this strategy can be applied to other non-aphid
367 transmissible plant viruses, such as viruses with icosahedral virions, of which the nucleic acid
368 encapsidation capacity is limited, so the additional amino acids may influence virus particle
369 assembly. The strategy that we have established here only applies to aphid-mediated virus
370 transmission. Thus, it is necessary to explore the other applicable strategies for the conditional
371 transmission of plant viruses.

372 **Declaration of Competing Interest**

373

374 The authors have no competing interests to declare.

375

376 **Author Contributions**

377 CLC conceived the original research plans; JJ and EY performed 565 the experiments; JJ
378 analyzed the data; CLC and JJ supervised the experiments; JJ and CLC wrote the article with
379 contributions of all the authors; CLC agrees to serve as the contact author responsible for
380 communication and distribution of samples

381

382 **Acknowledgments**

383

384 We thank Professor Steven A. Whitham for kindly providing the pCambia1380/FoMV infectious
385 clone. We thank Professor Jean-François Laliberté for the pCambia0380/TuMV:6K2-GFP
386 infectious clone. This research was supported by Defense Advanced Research Projects Agency
387 (DARPA) agreement HR0011-17-2-0053 t and by a US National Science Foundation award
388 award 1723926 to CLC. The views and conclusions contained in this document are those of the
389 authors and should not be interpreted as representing the official policies, either expressed or
390 implied, of DARPA or the U.S. Government.

391

392 **Figure legends:**

393

394 **Figure 1: Potexvirus and potyvirus coat protein amino acid sequence alignment and the**
395 **mutants constructed in this research.** The coat protein amino acid sequence of several
396 Potexviruses (A) and potyviruses (B) are aligned. CymMV, *Cymbidium mosaic potexvirus*;
397 NMV, *Narcissus mosaic virus*; PepMV, *Pepino mosaic virus*; BaMV, *Bamboo mosaic virus*;
398 PVX, *Potato virus x*; PAMV, *Potato aucuba mosaic potexvirus*; FoMV, *Foxtail mosaic virus*;
399 PVY, *Potato virus y*; SMV, *Soybean mosaic virus*; TEV, *Tobacco etch virus*; PPV, *Plum pox*
400 *virus*; PepMoV, *Pepper mottle virus*; BYMV, *Bean yellow mosaic virus*; PVA, *Potato virus A*;
401 TuMV, *Turnip mosaic virus*; SCMV, *Sugarcane mosaic virus*. The accession number of each
402 sequence is listed after the virus abbreviation. The dashed underlines highlight the unconserved
403 N- and C-terminus. The boxes indicate the DAG motif or its variants. Identical amino acid
404 residues that are highly conserved are highlighted by stars, and similar amino acid residues are
405 indicated by dots. FoMV mutants constructed in this research are shown in C, with the amino
406 acid residues of the introduced N-terminal tail are shown.

407

408 **Figure 2: FoMV mutant characterization.** *N. benthamiana* leaf tissues were agroinfiltrated
409 with WT FoMV, and the accumulation of vRNA was quantified by RT-qPCR at different days
410 up to 7 days (A). The FoMV mutant agroinfiltrated *N. benthamiana* tissues were collected at 5
411 days after agroinfiltration, and the tissues were processed for RT-qPCR (B). Mean values \pm
412 standard deviations (SD) from three independent experiments are shown (A & B). The infection
413 of FoMV mutant systemic infected *N. benthamiana* (C, upper panel) or *Z. mays* (C, lower panel)
414 leaf tissues were checked by RT-PCR. Virions were purified from the systemic infected *N.*
415 *benthamiana* leaf tissues and observed with transmission electron microscopy (D).

416

417 **Figure 3: FoMV aphid transmission.** Fig. 3A shows how the aphid transmission was
418 performed in the presence of TuMV/6K2:GFP co-infection. *N. benthamiana* leaf tissues co-
419 infected with TuMV/6K2:GFP and FoMV recombinant virus were used as the source tissues for
420 aphid transmission. The aphids were starved for 4 h, and fed on the source tissue for 5 min
421 before transferred to the healthy plants. One representative aphid transmission from infected *N.*
422 *benthamiana* to healthy *N. benthamiana* (B) or healthy *Z. mays* (C) is shown. (B and C) The

423 presence of virus infection was confirmed by RT-PCR. Upper panels: the amplification of a
424 348bp fragment of FoMV; Lower panels: the amplification of internal control; (+): the plasmid
425 pCambia1380/FoMV used as the PCR template. The data from two times experiments are shown
426 as (D). The value of how many plants were infected out of the tested plants are shown.

427

428 **Supplemental Table 1: Primers used in this study.**

429

430 **Supplemental Table 2: Additional mutants constructed in this research.**

431

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- 516

A

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CymMV_AAL12628 -----MG-EPT-----PTPAATYSAADPTSSPKLADLTAI
NMV_NP_040782 -----MATPSTQT-----DPKPNADLSDPNRAPSLDLKKI
PepMV_AIL23124 -----MEN-QPTASNPSDVPPTAAQ-----AGAQSADFSPNPTAPSLDLKKI
BaMV_AO551152 MSG-----TGTGTTGATGTGAGGTTGGTGGIGVAGRTQQASQWEAKFTKDDLAAI
FXV_AAA47181 MTT-----P--ANTTQAVGSKSTT-----T--TTAGATPANSGLFTTPDGDFFRTA
PAMV_AXL97636 MVDSSKTEIPQVVDAGKKTSSKTSHAGRVQF-----LTPAKQFSASDVRSSPSLTDLDEI
FoMV_AWT40560 -----MATQNAVDFDADTDYKPKPPEAKOAL
-----M-----

CymMV_AAL12628 KYSPTVSSSIATPEEIKAITQLWVNNLGLPADTVGTAAIDLARAYADVGSKSATLLGVCP
NMV_NP_040782 KYESTTTAVATPAEIQLLGLDF--KKLGLDANSVAPAMWDLARAYADVQASRSVLSGTT
PepMV_AIL23124 KYVSTVTSVATPAEIEALGKIF--TAMGLAANETGPAMWDLARAYADVQSSKSAQLIGATP
BaMV_AO551152 EPKASANVPNTKQWINIQAQLKAGATDAN--FMKVLGLSLEAFDRGSEATTWDGTF--
FXV_AAA47181 KAVVASDVAATKEELSEIQSIW--KNNKVPDMDTQAAWTLVRHCADGSSAQTEMTIGTP
PAMV_AXL97636 AYEVRTTISIAPAEIEAVCOLWIKNTEIPADKVALIADMARAYADVGSKRKAVLLDAPA
FoMV_AWT40560 TIQPRSNKAPSDLELVRIINAAGKRLTPAA--FVQAAIVFTMESMDKGTASTITPTGY-
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CymMV_AAL12628 TKPDVRAALAAQIFVANVTRQFCAYAYKVVNMLMANTDPPANWAKAGFQEDTRFAAF
NMV_NP_040782 SNPAITRQALARQFVINITRQFCMFAKVVNMLLDSNVPPAGWAKQLPDDCKFAFG
PepMV_AIL23124 SNPALSRRAAAQFDRNITRQFCMFAKTVNMLLDSNVPPANWAKAGFQEDTRFAAF
BaMV_AO551152 --EGVEHRAANAIKEANCPHVKVTYLAKPTFAIRQSKNLPANWAKKVPDQYKWCAP
FXV_AAA47181 YSNVSRARLAAAIKE--VCTLRQFCCKYAPVVNMLTNNSSPANWAOAGFKPEHKAFAF
PAMV_AXL97636 LAPTVARSRLAQMAGAGISPRQFCSYAKIIVNMLMHNKPEPANWAKIGKEDYKFAAF
FoMV_AWT40560 --NTPMKSALACKDAGVPHKLCYFYTKPAYANRRVANQPAPRWNTENVPKANKWAAF
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CymMV_AAL12628 DFFDAVDSAALEPAE--WQRPTDRERAGHSIGKYGALARORIQGNLITN--IAEVTKGH
NMV_NP_040782 DFFEGVLSPAALDADGLIRPSPQREIQAHSYAKYGALARORRYMETSEFPWLKSLTVGS
PepMV_AIL23124 DFFDGVNTPASLOPADGLIRPSPQREIQAHSYAKYGALAROKTSTGNYITN--LGEVTRGH
BaMV_AO551152 DAFDGLYDPTCLASEL--PYDAPSEIDRMAYATFKYIQIKTANDOKGFNLN--YNPVTOAR
FXV_AAA47181 DFFDGVNTPAAITPKEGLMRPSEAEINAAQTAAPVKITKARAGSNDPFS--LDAAVNRGH
PAMV_AXL97636 DFFDAVDSFAALEFSQ--WVRHPTDKERAAGVVKWASLGRERLQEGTSTIT--VAELNKGH
FoMV_AWT40560 DTFDALLDPEYVPSV--PYDEPTPEDRQVNEIFKDKDLQAASARNQL--LG--TQASITRGR
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CymMV_AAL12628 LGFPTNTLY--ALAA-----PPTF-----
NMV_NP_040782 AVSTPCTPKLHQNCRNRTSKLKLVCGL
PepMV_AIL23124 HGGAN--TMYAID--APPEL-----
BaMV_AO551152 LPHTPALPEPASP-----
FXV_AAA47181 ITGTTVREA--VV--SLEPP-----
PAMV_AXL97636 LGGVNNLP--ALMA--PDS-----
FoMV_AWT40560 LAGA--PALPNNQGFIEAPQ-----
-----M-----

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B

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PVY_ADH52720 -----GNDTIDAGSSTK-----
SMV_QBB78854 -----SCTV-----D-----
TEV_ABH10566 -----SCTV-----D-----
PPV_ABU97781 ADEKEDDEEVDAGRPVVVTFAPAAVATTOPAPVPIQAPOTTAMPFNIPT--PATTOPAV
PepMoV_BAB91329 --SSRSRDTIDAGKEK-----
BYMV_ABM69144 --SDQEKIDAGANKKKE-----
PVA_NP_734368 -----AETIDASA--LA-----
TuMV_ANW35618 -----AGETIDAGLDEQK-----QAEK
SCMV_P32652 -----SCTVIDAGQGGSGSQ-----GTPPATGSGGAKPATSAGSGSGTGAGTGVTGQGA
-----M-----

PVY_ADH52720 -----KDA-----KBOQSGIQPNLNEKEKDVNVCSTVTVPRIKAI
SMV_QBB78854 --SGKEK-----EG-----DIDAGKDPKKNITSSSKGAGTSSKDVNVCSTVTVPRIKAI
TEV_ABH10566 -----DIDAGKDPKKNITSSSKGAGTSSKDVNVCSTVTVPRINAM
PPV_ABU97781 RP--VPPISGAKPR--SFGVGDNEDASPTSNLTVNTRDRDSDAGSITGFVPRKTM
PepMoV_BAB91329 -----KKN-----EVATVSDGMKKVEVSTRSDVNVAGTCTVTVPRIKSI
BYMV_ABM69144 -----RKNENPDKNSEQSSQRIVPRDRDSDAGSITGFVPRKTKM
PVA_NP_734368 -----KQSEGRKERSMSKAVAVKRDVDLGTAGTSTVPRIKSM
TuMV_ANW35618 ER--KEKERAEKER-----ERQKQLAKKGAALAEGERDNEVAGTSTVPRKSL
SCMV_P32652 RTGSGTCTGSGATGCGSGSGSQFQVNSGATNATGQRDRDVAICTGKISVFLKAM
-----M-----
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PVY_ADH52720 TSKMRMPKSKGATVNLHLEHLEYAPQIDISNTRATQSQDFTWVEAVOAYDIGETEMPT
SMV_QBB78854 TRKMLNPMVEGKILLSLDHLLLEYKPNQVDLFTNTRAFRTQFEAWYNAVKDEYHLDDEQMGV
TEV_ABH10566 ATKLQYPRMKGVEVVMNLNHLGYKQPDLSNARATHEQFPAHWQAVNTAYGVNNEQMKI
PPV_ABU97781 TSKLSLKVKGKAIMLNLNHLNLAISPAQVDSLMTNRAEQSCFQIWEVGRDRTDDEEESI
PepMoV_BAB91329 TEKMRMPKQKKGAVMLNHLLEYKPSQVDSIMSTRSTQAQFDMWYSEVMKAYDLQEFAMGT
BYMV_ABM69144 AGKLNIPKIGKIVLMLDHLLEYPMQDGSIMVIAIQAQFEAVNGVIAQVETVEDSQMGI
PVA_NP_734368 TSKLTLPLMKKGSVNLHLLGYKQVDLSNARATHEQFQWYDGMASYLEESESSEMI
TuMV_ANW35618 TSKMRVFKYKRVALLDHLNLAISPAQVDSLMTNRAEQSCFQIWEVGRDRTDDEEESI
SCMV_P32652 SKMRLPKAKGRDVLHLDFLQYKQPDQISNTRATKEEFDRAWYDAIKKEYEIDDTQTV
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PVY_ADH52720 VMNGLMVCIENGTSPNINGVWMDGDEQVEYPLKPIVENAKPTRLQIMAHFSDVAEAY
SMV_QBB78854 VMNGFMVCIDNGTSPDANGVWMDGDEQVEYPLKPIVENAKPTRLQIMHHSDAEAY
TEV_ABH10566 LLNGFMVCIENGTSPNINGVWMDGDEQVEYPLKPIVENAKPTRLQIMHFSDLAEAY
PPV_ABU97781 LLNGFMVCIENGTSPNINGVWMDGDEQVEYPLKPIVENAKPTRLQIMAHFSDVAEAY
PepMoV_BAB91329 VMNGLMVCIENGTSPNINGVWMDGDEQVEYPLKPIVENAKPTRLQIMAHFSDVAEAY
BYMV_ABM69144 LLNGFMVCIENGTSGDQGEVWMDGDEQVEYPLKPIVENAKPTRLQIMHFSDAEAY
PVA_NP_734368 LLNGFMVCIENGTSPDINGVWMDMDEQVEYPLKPIVENAKPTRLQIMHFSALAEAY
TuMV_ANW35618 LLNGFMVCIENGTSPNINGVWMDGDEQVEYPLKPIVENAKPTRLQIMAHFSDVAEAY
SCMV_P32652 VMSGLMVCIENGTSPNINGVWMDMDEQVEYPLKPIVENAKPTRLQIMHHSDAEAY
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PVY_ADH52720 IEMRNKPEYMPRYGLVRLNLDGLARAYAFDFEYVTSRTFVRAREAHIQMAAALKSAQS
SMV_QBB78854 IEMRNSESPYMPRYGLLRLNLDRELARAYAFDFEYVTSKTFNRAEAIQMAAALSGVNN
TEV_ABH10566 IEMRNREYMPRYGLQRNITDMSLRYAFDFEYVTSKTFVRAREAHQMAAALVRSNGST
PPV_ABU97781 IEKRNKPEYMPRYGLQRNITDMSLRYAFDFEYVTSKTFVRAREAHQMAAALRVNQN
PepMoV_BAB91329 IEMRNKPEYMPRYGLVRLNLDGLARAYAFDFEYVTSRTFVRAREAHIQMAAALKSAQT
BYMV_ABM69144 IEKRNATERYMPRYGLQRNITDMSLRYAFDFEYVTSKTFVRAREAHQMAAALVRSNGST
PVA_NP_734368 IEMRSREKPYMPRYGLQRNITDMSLRYAFDFEYVTSKTFVRAREAHQMAAALKNSNT
TuMV_ANW35618 IEKRNQDRPYMPRYGLQRNITDMSLRYAFDFEYVTSRTFVRAREAHQMAAALRGANN
SCMV_P32652 IEYRNTERYMPRYGLQRNITDMSLRYAFDFEYVTSRTFVRAREAHQMAAALVRSNGST
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PVY_ADH52720 RLFGLDGGISTQENTEERHTTDEVSFMSHTLLGVKNM
SMV_QBB78854 KLFGLDGNISTNSENTEERHTARDVQNMHTLLGMGFGQ
TEV_ABH10566 RLFGLDGNVGTAEEDTERHTAHDVNRNMHTLLGVRQ--
PPV_ABU97781 RLFGLDGNVGTQEDTERHTAGDVNRNMHTLLGVRGV-
PepMoV_BAB91329 RLFGLDGGISTQENTEERHTTDEVSFMDHTLLGVRM-
BYMV_ABM69144 RLFGLDGNVGTAEEDTERHTAGDVNRNMHTLLGVRV--
PVA_NP_734368 NMFGLDGNVTTSEEDTERHTATDVNRNMHTLLGVRG-
TuMV_ANW35618 NLFGLDGNVGTVENTERHTTDEVSFMSHTLLGVKGL-
SCMV_P32652 RLFGLDGNVGTQENTEERHTAGDVSRNMHTLLGVQGH
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