Cauliflower Mosaic Virus Isolate, CM1841 Can Be Transmitted by Aphids from Infected Plants

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Cauliflower mosaic virus (CaMV) isolate, CM1841 was derived from a chlorotic local lesion of *Brassica campestris* after inoculation with a California isolate of CaMV¹⁰. Lung and Pirone¹¹ reported the CM1841 isolate as aphid nontransmissible (AT⁻). This result was confirmed in some papers thereafter^{3,12,15}. Complete nucleotide sequence of the CM1841 isolate was determined⁷, and its viral DNA was cloned at own *Sa*I site to pBR322¹⁰. Nucleotide sequence data revealed that the CM1841 isolate retained an intact ORF II which was the gene for aphid transmission factor (ATF) of CaMV^{1,2}. The product of ORF II, a protein with molecular weight of 18,000 dalton (p18) was synthesized in leaves infected with the CM1841 isolate but its concentration was lower than that in leaves infected with aphid transmissible (AT⁺) CaMV⁹. It was suggested that a single amino acid change in ORF II (Gly to Arg at position 94) was responsible for the lack of aphid transmissibility and the decrease of p18 accumulation in infected leaves with the CM1841 isolate^{12,15}. On the other hand, Blanc *et al.*³ showed that p18 of the CM1841 isolate, produced in a baculovirus system, possessed the ATF activity to a similar level of that of AT⁺ CaMV isolate when it was fed to aphids through a membrane. These results contrary to former observations were explained by the hypothesis that the lack of aphid transmissibility of the CM1841 isolate is due to the low accumulation level of p18 in infected leaves³. However, it has not been known whether p18 of the CM1841 isolate facilitates the aphid transmission of the isolate in infected plants. We previously showed that the low accumulation level of p18 in CM1841-infected leaves was due to its instability and that the accumulation level of p18 in CM1841-infected leaves was comparable to that in leaves infected with AT⁺ recombinant CaMV at the early stage in symptom development¹³. Now, we report that CM1841 isolate is transmitted by aphids from infected plants at early stage in symptom development when large numbers of aphids were used in a test.

Plasmid, pCa122 containing 1.2 mer (redundant end) copy of CM1841 DNA¹⁴, was mechanically inoculated to turnip plants at a concentration of 0.2 $\mu\text{g}/\mu\text{l}$. Symptom development was observed on inoculated plants at about 20 days post inoculation. Aphid transmission test was performed using a leaf with vein clearing at 3 to 5 days post symptom emergence (p.e.) or a young stunted leaf at 3 to 5 days post leaf emergence. Aphids, *Myzus persicae* (Sulz) were maintained on healthy turnip plants (*B. campestris* cv. Marubakomatsuna) in cages. Following starvation period (3 to 4 hr), normal numbers (10 to 20) or large number (200 to 300) of aphids were placed on infected leaves and allowed to feed for 15 min, and then, were transferred to a leaf of healthy test plants (third or fourth leaf stage). After being caged on test plants for overnight, the aphids were killed by spraying chemicals. Then test plants were transferred to a glasshouse at approximately 25°C. Symptom development was monitored up to 4 weeks

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Table 1. Aphid transmission of the CM1841 isolate assessed at early stage of symptom development

No. of aphid used	No. infected/ no. tested
10 to 20	0/120 ^{a)}
200 to 300	2/6

Aphid transmission test was performed using a leaf with vein clearing at 3 to 5 days post symptom emergence (p.e.) or a young stunted leaf at 3 to 5 days post leaf emergence.

a) Summation of results of several experiments.

Fig. 1. Transition of the amount of p18 in leaves infected with the progeny virus transmitted from CM1841-infected leaves by aphids. Total proteins were extracted from leaves at 5 to 25 days post symptom emergence (p.e.) and analyzed by Western blotting using anti-p18 serum. Lane 1 : 5 days p.e., lane 2 : 10 days p.e., lane 3 : 15 days p.e., lane 4 : 20 days p.e., lane 5 : 25 days p.e. Arrow indicates the position of p18.

after inoculation. Symptom of CaMV appeared in none of 120 plants tested with normal number of aphids, but in 2 of 6 plants tested with large numbers of aphids (Table 1). Progeny virus and their products from test plants which developed symptom were examined at following points: i) the property of p18 in infected leaves, ii) nucleotide sequence of ORF II region and iii) aphid transmissibility.

i) The property of p18 produced by the CM1841 isolate differed from that produced by AT⁺ CaMV with regard to the accumulation pattern in infected leaves and the electrophoretic mobility in SDS-PAGE¹³⁾. The amount of p18 accumulated in CM1841-infected leaves was higher at early stage of symptom emergence than at later stage while that in AT⁺ CaMV was continuously increased from early stage of symptom emergence, and p18 of the CM1841 isolate run slower than that of AT⁺ CaMV in SDS-PAGE. Leaf discs (8 mm in diameter) were sampled at 5, 10, 15, 20 and 25 days p.e. from a single leaf infected with the progeny virus. The amount of p18 and the electrophoretic mobility of p18 in leaf discs were examined using Western blotting¹²⁾. Results showed that p18 of the progeny virus retained the properties same as the CM1841 isolate in both accumulation pattern and electrophoretic mobility in SDS-PAGE (Figs. 1, 2).

ii) Viral DNA of progeny virus was purified from infected leaves as described by Gardner and Shepherd⁶⁾ with slight modification, and cloned to pUC118 at *Sa*I site. Complete sequence of the ORF II in two viral DNA clones (pPV-1, pPV-2) were determined using synthesized oligo-nucleotide primers. The ORF II region of both clones retained the point mutation, leading to amino acid change at position

Fig. 2. Electrophoretic migration difference among p18s in SDS-PAGE. Lane 1: progeny virus transmitted from CM1841-infected leaves, lane 2: CM1841, lane 3: CMBX (aphid transmissible CaMV refer to Nakayashiki *et al.*¹³⁾). Total proteins were extracted from leaves and analyzed by Western blotting using anti-p18 serum. Arrows indicate the position of p18s.

94 (data not shown). Compared to the ORF II sequence of the CM1841 isolate published by Gardner *et al.*⁷⁾, the sequence of ORF II of pPV-1 was perfectly identical to it, but one change was found in the nucleotide sequence of ORF II of pPV-2 at position 1667 (T to C) which results in amino acid substitution at position 107 (Ser to Pro).

iii) Aphid transmissibility of progeny virus was tested at 10 days p.e. using normal numbers of aphids. No transmission was observed in 20 test plants.

Thus, the progeny virus conserved the character of the CM1841 isolate, although one base change was found in the ORF II of one cloned progeny virus (pPV-2). However, the change did not seem to be involved in the aphid transmissibility of the virus, because progeny virus was not transmitted when normal numbers of aphids were used.

The results presented here showed that the CM1841 isolate could be transmitted by aphids at early stage of symptom development when numerous numbers of aphids were used in a single trial. However, it is not likely that the CM1841 isolate efficiently transmitted by aphids under the natural condition. Therefore, we would like to term the CM1841 isolate an aphids pseudo-nontransmissible isolate. Another CaMV isolate, Campbell¹⁵⁾ showing a same phenotype with the CM1841 isolate, may be classified into the same category with the CM1841 isolate because it was suggested that the same amino acids change (Gly to Arg at position 94) in both isolates was responsible for the phenotype of the isolates^{12,15)}.

Recently, it has been shown that paracrystalline form is one of the characteristic properties of active p18⁴⁾. The paracrystalline form was detected in leaves infected with CM1841 isolate (Blanc, personal communication).

The amino acid change occurred in p18 of the CM1841 isolate seemed to reduce the stability of p18 in infected leaves but not to directly affect the ATF activity^{3,13)}. It was reported that p18 influenced the structure of the viral inclusion body and that p18 of AT⁺ Cabb B-JI formed distinct inclusion body in infected plant cells which was not observed in infection with AT⁻ CaMV isolates^{5,8)}. The *in vivo* structures of viral products may be involved in stable accumulation of p18 rather than direct involvement in ATF activity, because it was shown that p18 possesses ATF activity in its soluble form⁴⁾.

There are two possible explanations for necessity of numerous numbers of aphids to transmit the CM1841 isolate on plants. (i) It increase the possibility for aphids to meet aphid transmissible complex in CM1841-infected leaves, which may compensate for low concentration of aphid transmissible complex in CM1841-infected leaves, (ii) It enlarge the inoculum potential of virus enough to cause successful infection on plants, which may compensate for low amount of CaMV acquired by each aphid from CM1841-infected leaves. The work on this problem will be addressed in future.

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和 文 摘 要

中屋敷均・小林括平・津下誠治・奥野哲郎・古澤 巖：カリフラワーモザイクウイルス、CM 1841 系統はアブラムシによって感染葉から媒介される

カリフラワーモザイクウイルス (CaMV)、CM 1841 系統は、昆虫媒介性に関与するウイル遺伝子 (ORF II) にアミノ酸置換がある非昆虫媒介性の CaMV 分離株として知られてきた。CM 1841 感染葉では、ORF II 産物 (p18) 蓄積量が昆虫媒介性の CaMV と比較して少ないが、病徴発現初期には昆虫媒介性の CaMV 系統の 25~50% 程度の p18 が蓄積している (Nakayashiki *et al.* *J. Gen. Virol.* 74 : 2469-2472, 1993)。そこで病徴発現初期における CM 1841 系統の

昆虫媒介性を検討した。通常のアブラムシ頭数(10~20)による検定では媒介は認められなかったが、多頭数(200~300)のアブラムシを用いて検討を行ったところ、これまで非昆虫媒介性と考えられていた CM 1841 系統でアブラムシによる媒介が初めて確認された。

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