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Short communication

RNA 2 of cucumber mosaic virus subgroup I strain NT-CMV is involved in the induction of severe symptoms in tomato

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Abstract

A selection of cucumber mosaic virus (CMV) subgroup I strains originating from Asia and Fny-CMV isolated in USA were studied for their interaction with tomato plants. All strains caused mosaic, fernleaf expression and stunting of tomato plants. Symptom expression was relatively mild after infection with Fny-CMV, T-CMV, Le-CMV and MB-CMV, whereas strains PRC-CMV, NT-CMV and K-CMV caused more severe symptoms. Biologically active clones of NT-CMV RNAs 2 and 3 were generated to construct pseudorecombinant viruses with Fny-CMV to map the symptom determining RNA. The pseudorecombinant FNF-CMV (RNAs 1 and 3 from Fny-CMV, RNA 2 from NT-CMV) showed a similar phenotype on tomatoes to those caused by NT-CMV, whereas FFN-CMV (RNAs 1 and 2 from Fny-CMV, RNA 3 from NT-CMV) induced symptoms comparable to Fny-CMV. The data indicate that CMV RNA 2 of NT-CMV is involved in the induction of severe symptoms in tomato plants.

Cucumber mosaic virus (CMV), one member of the *Bromoviridae*, is a tripartite (+) single stranded RNA virus. It is distributed worldwide and has a very large host range infecting more than 700 plant species. It is aphid transmissible in a nonpersistent manner by more than 60 aphid species, and causes severe diseases in various crops all over the world. The CMV genome has been extensively studied, and replicase functions have been attributed to RNAs 1 and 2 encoded proteins 1a and 2a, whereas RNA 3 encodes the viral movement protein 3a as well as the viral coat protein in a subgenomic manner (for review, see Palukaitis et al., 1992). In addition RNA 2 has a second overlapping open reading frame encoding protein 2b (Ding et al., 1994).

Cucumber mosaic virus strains are separated into two subgroups I and II, which differ in their nucleotide sequence and can be distinguished by polymerase chain reaction (PCR) (Rizos et al., 1994). The role of single genomic components of CMV in induction of disease symptoms has been studied in different host plants. In general all CMV genes can play a role in the phenotypic expression of a certain plant/virus interaction, but RNAs 2 and 3 seem to play a major role in the induction of symptoms as compared to RNA 1 (Rao and Francki, 1982). In tomato, to our knowledge only one report has been published characterizing the symptom determining RNA of CMV strain NT9 on tomato plants. In this study, Hsu et al. (1988) determined that RNA 3 was responsible for the induction of severe symptoms in tomato plants by NT9-CMV.

Here we have also used biologically-active clones from RNAs of strain NT-CMV that induce severe symptoms in tomato plants. Tomato plants (*Lycopersicon esculentum* Mill.) cvs. Vollendung and Hellfrucht were used for greenhouse experiments. Plants were grown in pots of 12 cm diameter containing the commercially available substrate Fruhstorfer Einheitserde Type P under greenhouse conditions at a temperature interval of 28–20 °C under a 15 h/9 h day/night

cycle with supplemental light. All plant experiments were repeated twice with 5 plants and 8 plants per variant. Strains T-CMV, P-CMV, K-CMV and PRC-CMV originated from China. Strains Le-CMV and MB-CMV were isolated in Japan and Sri Lanka, respectively. Fny-CMV originated from USA. Strain NT-CMV was kindly supplied by Sylvia K. Green from the Asian Vegetable Research and Development Center, Taiwan. It was observed to cause severe symptoms on tomato in the field. NT-CMV belongs to CMV subgroup I as determined by RT-PCR according to methods described in Anonymous (1998) (data not shown). CMV strains were propagated in *Nicotiana tabacum* cv. Xanthi-nc. Ten days after inoculation, infected leaves were harvested and CMV strains were purified essentially as described in Kaplan et al. (1995). A purified virus suspension in a concentration of 100 μg/ml was used to inoculate two leaves per plant (10 μl per leaf) including Celite as an abrasive. A selection of CMV subgroup I strains was inoculated on tomato plants to compare disease severity between these strains. The CMV strains tested induced different degrees of mosaic patterns, leaf deformation like leaf curling or fernleaf expression and stunting. Symptoms were evaluated 5 weeks after inoculation. Different classes of symptoms were distinguished with regard to mosaic symptoms, fernleaf expression and stunting including plants not showing the symptom as well as plants showing mild, moderate or severe symptoms, respectively (data not shown).

After symptom evaluation, plants were harvested for dry weight estimations of the plant parts above ground. Symptom classification was compared to dry weight estimations. It was evident that severe symptom expression after infection of CMV strains was accompanied by reduced biomass production. As a consequence of this observation dry weight reduction was used as a parameter for symptom severity in CMV infected tomato plants. The results of dry weight estimations of tomato plants after inoculation with different strains of CMV are shown in Figure 1. As variation of the data obtained within one group of plants infected by a certain strain of CMV was relatively high in some experiments (as indicated by standard deviation bars, Figure 1), statistically significant differences were not obtained in all experiments. In this case additional experiments were performed. From these experiments it was evident that NT-CMV caused more severe symptoms in tomato plants than Fny-CMV, which caused only moderate symptoms (Figure 1 and data not shown, see also Figure 2).

A biologically-active clone of NT-CMV RNA 2 was generated using the strategy described by Hellwald and Palukaitis (1994), and three full-length clones of NT-CMV RNA 3 were generated (Roossinck et al.,

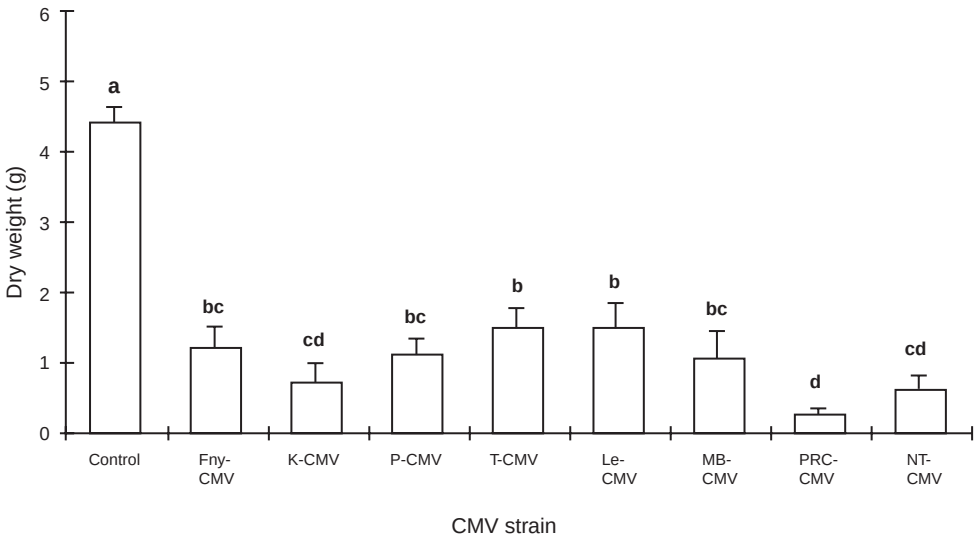

Figure 1. Dry weight analysis of tomato plants 5 weeks after inoculation with different strains of CMV compared to mock-inoculated control plants. Plants were inoculated with 20 μl of a purified virus solution in a concentration of 0.1 mg/ml. Bars with common letters indicate values that are not significantly different according to Tukeys Honest Significant Difference Test ($P = 0.05$).

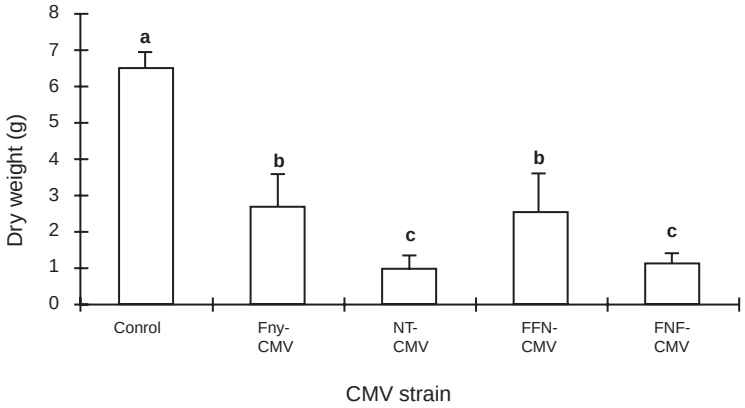

Figure 2. Dry weight analysis of tomato plants cv. Vollendung after inoculation with pseudorecombinant FNF-CMV and FFN-CMV in comparison to the corresponding wildtype viruses Fny-CMV and NT-CMV. Plants were harvested for dry weight analysis 6 weeks after inoculation. Bars with common letters indicate values that are not significantly different according to Tukeys Honest Significant Difference Test ($P = 0.05$).

1999). Clone pNT301 was used for the generation of RNA transcripts. Full-length cDNA clones of RNAs 1, 2 and 3 from Fny-CMV (Rizzo and Palukaitis, 1990) were kindly supplied by Prof. Dr. Peter Palukaitis from the Scottish Crop Research Institute, Dundee, U.K. *In vitro* transcription was performed as described in Zhang et al. (1994). Pseudorecombinant viruses were generated by replacing either RNA 2 or RNA 3 in Fny-CMV by the corresponding RNAs from the strain NT-CMV. Pseudorecombinant viruses were named in a three-letter code by using the first letter of the strain for origin of the RNA. FNF-CMV, for example, contains RNAs 1 and 3 derived from Fny-CMV, and RNA 2 derived from NT-CMV. These RNA transcript mixtures were first inoculated to tobacco plants. Symptom-bearing leaves were harvested 10 days after inoculation and CMV was purified from these leaves and inoculated to tomato plants.

Fny-CMV as well as NT-CMV caused fernleaf symptoms and stunting of the tomato plants. Figure 2 shows the dry weight reduction of the tomato plant parts above ground 6 weeks after inoculation. The data indicate that the phenotype observed after infection with NT-CMV maps to RNA 2, as the pseudorecombinant virus FNF-CMV and NT-CMV showed similar phenotypes. Accordingly, Fny-CMV and FFN-CMV showed similar phenotypes after systemic infection of tomato. Plants from these experiments are shown in Figure 3.

Hsu et al. (1988) characterized a CMV-strain NT9 causing a severe type of disease symptoms on tomato plants and compared this strain to a mild strain M48-CMV. The severe strain NT9-CMV was fully sequenced and RNA 3 of strain NT9-CMV was determined to be involved in the induction of severe symptoms in tomato plants. Hsu et al. (1988) used viral RNA extracted from polyacrylamide gels for the generation of pseudorecombinant viruses. The advantage of using RNA transcripts from biologically active clones is the generation of a genetically-defined virus. On the other hand, a biologically-active clone represents only one RNA molecule out of a population of the 'quasispecies' CMV (for review, see Roossick, 1997) that might not represent RNAs present in the RNA population potentially inducing severe phenotypes. As our data did not confirm the results obtained by Hsu et al. (1988), this question was addressed by generating RNA transcripts from three independent NT-CMV RNA 3 clones as well as directly from full-length PCR products of NT-CMV RNA 3. For these experiments, full-length PCR products were generated with a primer specific to the 3′ end of all CMV subgroup I strain RNAs (Hellwald and Palukaitis, 1994) and a 5′ terminal CMV RNA 3 primer described in Roossinck et al. (1999). PCR-products were separated on a 1% agarose gel in Tris–acetate buffer, isolated from the gel with a Sephaglas Band Prep Kit (Amersham Pharmacia Biotech, Freiburg) and purified with a PCR Purification Kit (Boehringer, Mannheim) prior to *in vitro* trancription. Again FFN-CMV derived from the corresponding RNA transcripts induced similar symptoms as Fny-CMV on tomato plants (data not shown).

This study confirmed that the severe symptoms in tomato plants observed after infection with NT-CMV from Asia were also expressed under greenhouse

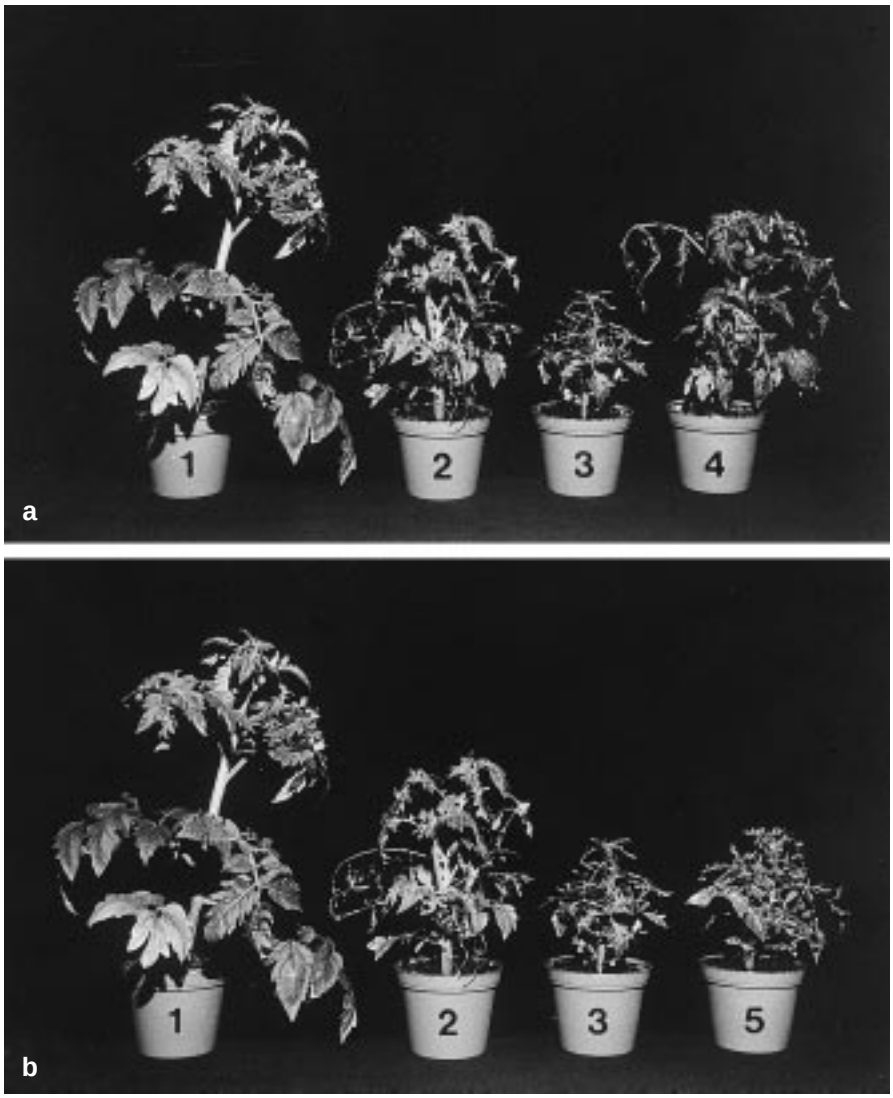

Figure 3. Tomato plants 6 weeks after mock-inoculation (1) or inoculation with Fny-CMV (2) or NT-CMV (3) in comparison to inoculations with FFN-CMV (4, panel a) or FNF-CMV (5, panel b).

conditions. Our results indicate that CMV RNA 2 of NT-CMV is involved in the induction of severe symptoms. With regard to the data obtained by Hsu et al. (1988), these results indicate that severe symptom expression of CMV on tomato can be induced by different viral genes.

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