Yellow Mosaic Symptoms Induced by Y Satellite RNA of Cucumber Mosaic Virus is Regulated by a Single Incompletely Dominant Gene in Wild *Nicotiana* Species

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ABSTRACT

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Y satellite RNA (Y-satRNA) of cucumber mosaic virus (CMV) together with the helper virus induces a bright yellow symptom when inoculated on tobacco plants (*Nictotiana tabacum*) and develops a green mosaic symptom in *N. clevelandii,* while *N. bigelovii* exhibits a yellow symptom when inoculated with CMV-O plus Y-satRNA, even though the two wild species are very closely related to each other. *N. bigelovii* and *N. clevelandii* were crossed to analyze the symptom expression of the progeny plants. When inoculated with CMV+Y-satRNA, all the F_1 plants initially showed a faint yellow mosaic, which eventually turned into a green mosaic with traces of yellow, indicating intermediate symptoms. The symptom responses of F_2, F_3 selfed plants, and backcross plants to inoculation with CMV+Y-satRNA were also analyzed. The segregation ratios were consistent with the presence of a single incompletely dominant gene that is involved in the production of bright yellow symptoms during infection by Y-satRNA.

Satellite RNAs (satRNAs) of cucumber mosaic virus (CMV) depend on CMV for replication and encapsidation and have no significant sequence similarity with CMV genomes (1,16). These satellites are single-stranded, linear RNA molecules 330–400 nucleotides long (3,6,7,10). The plants infected with CMV in the presence of satRNA often show attenuated disease symptoms, but in some cases, quite dramatic symptom expression is observed (4,11,19,20).

We have analyzed the molecular basis of the symptom alteration by Y-satRNA, which causes a bright yellow mosaic on tobacco plants (19). The sequence domain responsible for the symptom induction by chlorosis-inducing satRNAs has already been identified by two other laboratories and by this laboratory (2,9,13,15,17). However, little is understood about the molecular or genetic interactions between satRNAs, CMVs, and the host plant, which in concert lead to the yellow induction.

In this report, we have investigated a host factor that controls the host specificity of the disease induction. To understand the mode of inheritance on symptom development, we examined the genetics of crosses between two *Nicotiana* spp.: *N. clevelandii* and *N. bigelovii*, which show different symptoms upon infection by Y-satRNA.

MATERIALS AND METHODS

CMV-O (8) was used as a helper virus for inoculation. The origin of Y-satRNA has been previously described (19). The seeds of all the wild *Nicotiana* spp. were maintained in the Plant Breeding and Genetics Research Laboratory of Japan Tobacco Inc. The male sterile tobacco, G101, was a kind gift from T. Kumashiro (12).

Plants were maintained in a greenhouse with a natural photoperiod and temperatures of 24–26 C. Wild *Nicotiana* spp. and the progenies from crosses between *N. clevelandii* and *N. bigelovii* were analyzed for symptom development following infection by CMV+Y-satRNA. Y-satRNA (1 μg/ml) was inoculated together with CMV-O (10 μg/ml) onto about 1.5-mo-old seedlings. Leaves were dusted with Carborundum and rub-inoculated, followed by rinsing with water. Plants were scored for virus symptoms for more than 2 mo until anthesis. To confirm the accumulation of single-stranded and double-stranded Y-satRNAs, total RNA from the infected leaf tissue was analyzed on a 1.5% agarose gel under nondenaturing conditions (14) and by Northern blot hybridizations. After evaluation, several plants were selfed to obtain F_2 and F_3 plants.

RESULT AND DISCUSSION

Symptom expression on wild species and G101. In the presence of CMV-O, Y-satRNA caused a brilliant yellow symptom on *N. tabacum* and most of the other wild species tested. Only *N. debneyi* and *N. clevelandii* developed a green mosaic, which is actually an attenuated symptom of a whitish-green mosaic induced by CMV alone (Table 1). The accumulation of Y-satRNA in the wild species showing green mosaics was analyzed by agarose

TABLE 1. Symptom appearance on wild species and hybrids of *Nicotiana* infected with cucumber mosaic virus (CMV-O) + Y-satRNA

Species[a]	Symptoms[b]
N. rustica	Y
N. tabacum	Y
N. glutinosa	Y
N. alata	Y
N. sylvestris	Y
N. repanda	Y
N. miersii	Y
N. bigelovii	Y
N. clevelandii	G
N. debneyi	G
G101	Y
N. clevelandii × *N. bigelovii* (CB)	Y/G
N. bigelovii × *N. clevelandii* (BC)	Y/G
CB (F_2)	Y+Y/G+G
BC (F_2)	Y+Y/G+G
CB-Y (F_3)	Y
CB-G (F_3)	G
CB × *N. clevelandii*	Y/G+G
CB × *N. bigelovii*	Y/G+Y

[a] F_2 = self-fertilized CB, BC; CB-Y = self-fertilized F_2 plants that showed yellow mosaic; CB-G = self-fertilized F_2 plants that showed green mosaic.
[b] Y = yellow mosaic; Y/G = intermediate symptoms; G = green mosaic.

TABLE 2. Reactions of crossed hybrid tobacco plants to Y-sat and χ^2 analysis for goodness of fit

Crosses[a]	Total	Y[b]	Y/G[b]	G[b]	Segregation[c]	χ^2	P
Nicotiana bigelovii × *N. clevelandii* (BC)	20	0	20	0			
N. clevelandii × *N. bigelovii* (CB)	20	0	20	0			
BC(F_2)	50	10	26	14	(1:2:1)	0.72	0.5–0.75
CB(F_2)	50	9	24	17	(1:2:1)	2.64	0.1–0.5
CB-Y(F_3)	20	20	0	0			
CB-G(F_3)	20	0	0	20			
CB × *N. clevelandii*	32	0	15	17	(0:1:1)	0.13	0.7–0.8
CB × *N. bigelovii*	32	18	14	0	(1:1:0)	0.5	0.3–0.5

[a] F_2 = self-fertilized BC, CB; CB-Y = self-fertilized F_2 plants that showed yellow mosaic; CB-G = self-fertilized F_2 plants that showed green mosaic.
[b] Y = yellow mosaic; Y/G = intermediate symptoms; G = green mosaic.
[c] Expected ratio.

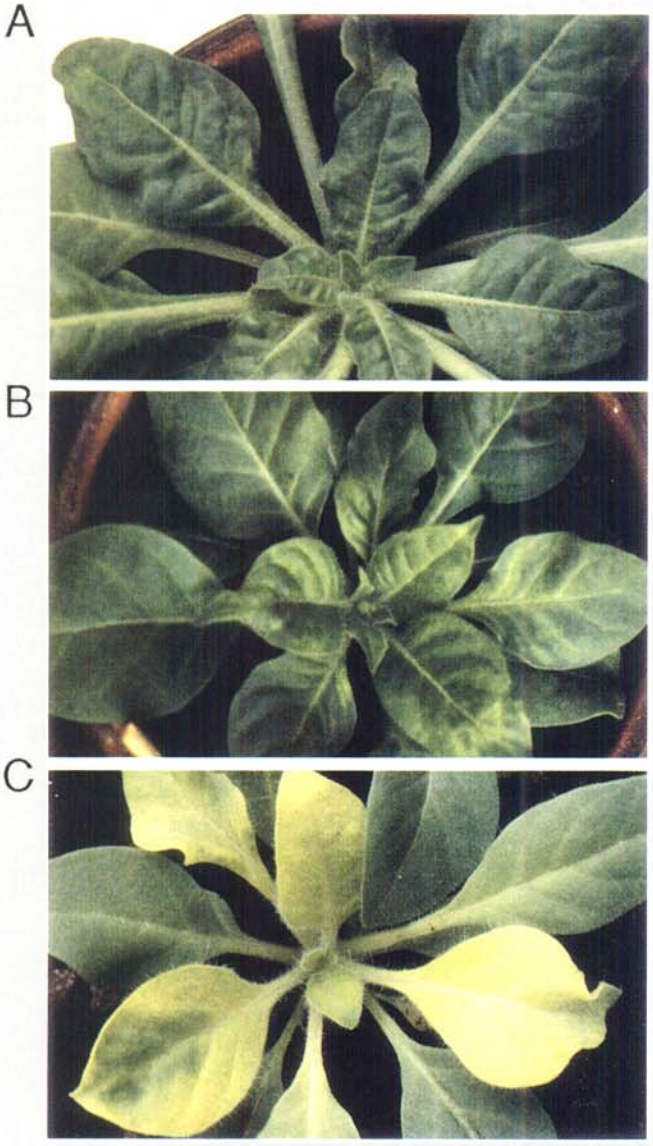

Fig. 1. Symptoms on the F_2 plants from the F_1 plants made between *Nicotiana clevelandii* and *N. bigelovii* upon infection of CMV-O+ Y-satRNA. The segregation classes developed **A,** green mosaic **B,** intermediate symptoms; and, **C,** yellow mosaic phenotypes.

gel electrophoresis and Northern blots and was found to be indistinguishable from that of the tobacco showing a yellow symptom. The yellowing resulted from a significant reduction of chlorophyll content in chloroplasts (one third to one fourth that of the healthy plant) (unpublished). Chlorophyll was extracted from newly developed leaf tissue showing the typical symptoms about 2 wk after inoculation, then its content was photometrically determined. To analyze the host factor(s) for the yellow induction by Y-satRNA, we first questioned whether the factor is organellar (chloroplasts) or nuclear encoded. G101, which is a male sterile tobacco produced by cell fusion following X-ray irradiation and contains the cytoplasm of *N. debneyi* and the nuclear genome of *N. tabacum* (cv. Consolation 402), was used for this purpose (12). This tobacco exhibited a typical yellow symptom upon infection with CMV-O and Y-satRNA, suggesting that nuclear factor(s) is involved in the symptom expression. This was clearly established by the results from the reciprocal crosses between *N. clevelandii* and *N. bigelovii* described below (Table 2).

In the experiment shown in Table 1, we observed that although *N. clevelandii* and *N. bigelovii* share very close genetic background, the former did not develop the typical yellow symptom, whereas the latter did. The two species are thought to have differentiated from their immediate progenitor in southernmost California, where they today overlap in distribution (5,18). The karyotype of *N. clevelandii* is essentially a replica on a reduced scale of that of *N. bigelovii* (5). In addition, their genome constitutions are very similar except for the existence of the two satellite chromosomes known to occur in *N. bigelovii*. In many cases, it may be impossible to perform genetic analyses by interspecies crossing because a simple Mendelian segregation is rarely observed, due mainly to the failure of pairing of homologous chromosomes in the different genetic background. However, based on the above similarities, the two wild species show very close chromosomal and genetic homology and so appear to be sufficiently related to be genetically analyzed at least for their major genes by crossing. Until the fifth generation tested, the fertility of our crosses remained unchanged when *N. clevelandii* was used as the maternal plant while the fertility of *N. bigelovii* × *N. clevelandii* decreased to some extent (<50%). This may be due to the incompatibility between the nuclei from *N. clevelandii* and the cytoplasm from *N. bigelovii*.

Symptom expression on the F_1, F_2, and F_3 populations from the crosses between *N. clevelandii* and *N. bigelovii*. All the F_1 plants from the reciprocal crosses showed neither yellow mosaics nor green mosaics but developed intermediate symptoms, which are distinguishable from the typical yellow mosaics and green mosaics observed on the parental plants (Fig. 1). We distinguished these three phenotypes as follows: 1) Green mosaic is the symptom without any yellow chlorosis during the observation period. 2) An initital expression of a very faint, dull yellow mosaic which faded was considered as the intermediate symptom type. 3) The yellowing phenotype consisted of a brilliant yellow chlorosis over the observation period (Fig. 1). All the plants with intermediate symptoms were checked for the presence of normal levels of Y-satRNA. A relatively high level of accumulation of Y-satRNA was observed in the plants with intermediate symptoms as well as in those showing the typical yellow mosaic or the green mosaic; one example of the agarose gel electrophoresis is shown in Figure 2. However, variability of yellow mosaics on the plants with yellowing phenotype was observed in some experiments. This variability could be attributed mainly to the growth stage of the plants used for inoculation and the light intensity in the greenhouse. All possible efforts were made to minimize such environmental effects on symptom expression. The F_2 plants from the F_1 plants segregated 1 yellow : 2 intermediate : 1 green mosaics. The chi-square and *P* values are given in Table 2. To verify the F_2 ratios, backcross populations were made with the F_1 CB plants, and

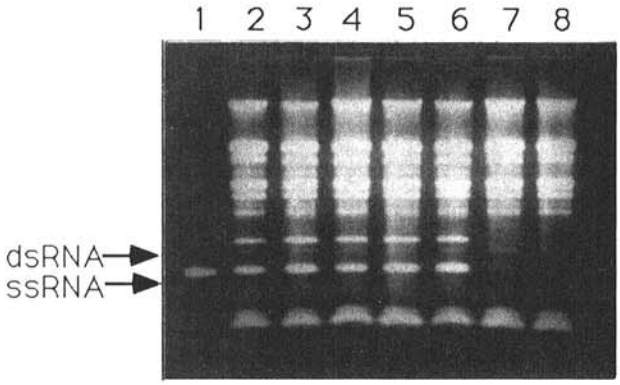

Fig. 2. Electrophoretic mobility of single- and double-stranded Y-satRNAs in the Y-satRNA-infected F_2 plants resulting from crossing between *Nicotiana clevelandii* and *N. bigelovii*. Lane 1 is authentic Y-satRNA (369 nucleotides). Lanes 2, 3, and 4 contain the total RNAs from the F_2 plants that developed yellow, intermediate, and green mosaics, respectively. Lanes 5 and 6 are the total RNAs from *N. clevelandii* and *N. bigelovii*, respectively, when inoculated with CMV-O+Y-satRNA. The total RNAs from *N. clevelandii* and *N. bigelovii*, when inoculated with only CMV-O, are shown in lane 7 and 8, respectively. The total RNAs from every healthy plant used for this experiment do not contain any distinct bands around the positions for Y-satRNAs (data not shown).

the symptoms were scored upon CMV-O plus Y-satRNA infection. The F_1 plants backcrossed to *N. clevelandii* segregated 1 intermediate : 1 green and those backcrossed to *N. bigelovii* segregated 1 yellow : 1 intermediate. From the cross *N. clevelandii* × *N. bigelovii,* the F_2 plants showing typical yellow symptoms and those showing green mosaics were allowed to self to obtain F_3 populations, CB-Y and CB-G, respectively. The symptoms of these populations did not segregate; all the CB-Y plants showed yellow symptoms while all the CB-G plants resulted in green mosaics. Again, due to the symptom attenuation by Y-satRNA, the green mosaic symptoms on the above progeny plants are visually distinguishable from those of the plants infected with CMV alone.

Our results suggest that Y-satRNA modulation of CMV symptom development in tobacco is controlled by a nuclearly encoded incompletely dominant gene. This symptom induction appears to be determined by a three-way interaction between the plant, Y-satRNA, and the helper CMV. In this paper, we demonstrated that the tobacco nuclear genome contains at least one gene involved in the regulation of symptom modulation by Y-satRNA. At this stage, we have no idea what this gene product is. To our knowledge, this is the first report of a single incompletely dominant gene involved in differential symptom induction by a satRNA supported by a helper virus. Though the mechanism by which Y-satRNA can induce yellow chlorosis on tobacco remains a matter for speculation at present, the identification of the host factor described in this paper will certainly facilitate understanding of the molecular basis of Y-satRNA pathogenicity.

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