Resistance-Breaking Variants of Cowpea Chlorotic Mottle Virus in Soybean

O. R. PAGUIO, C. W. KUHN, and H. R. BOERMA, Departments of Plant Pathology and Agronomy, University of Georgia, Athens 30602

ABSTRACT

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Disease reactions and virus concentration levels of seven strains of cowpea chlorotic mottle virus (CCMV) were studied in six soybean genotypes, each with a different disease reaction to the soybean strain (S). Although differences occurred among genotypes, both disease reactions and concentration levels were similar among virus strains within each genotype. In general, variable environmental conditions (time of year, constant 24 or 30 C) did affect disease reactions and levels of virus concentration, but any variations observed were similar among the virus strains. One notable exception was that the bean yellow stipple (BYS) strain produced more virus than other virus strains in some genotypes. Furthermore, at 30 C it caused systemic symptoms and frequently death in genotype Bragg, which developed no systemic symptoms when inoculated with other strains. In subsequent studies, two new CCMV isolates, designated D and N, were derived by passage of the BYS strain through Bragg. Both isolates D and N overcame the hypersensitive-type resistance in genotypes Bragg and Williams. Isolate D caused leaf distortion and severe stunting, while isolate N caused a mild mottle.

Cowpea chlorotic mottle virus (CCMV) is one of about 11 soybean (*Glycine max* (L.) Merr.) viruses that may have some economic importance (12). Cowpea chlorotic mottle virus can reduce soybean yield from 20 to 37% (5,10) and cause alterations in the quality and quantity of oil and protein content in seeds (4).

After screening over 500 soybean genotypes for their reaction to CCMV strain S, one susceptible and five resistant categories were established on the basis of disease reactions and levels of virus concentration (1). A field study confirmed the practicality of the six categories and established the value of different types of resistance to CCMV-S with regard to seed yield (10).

Since it has been established that soybean genotypes can vary in their reaction to CCMV, the primary objective of this study was to identify genetic diversity among strains of CCMV. Six soybean genotypes were evaluated for their reaction to seven known strains of the virus. Ultimately, we expected to find differences among the strains that would enable us to identify both viral and host genes related to resistance.

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MATERIALS AND METHODS

Virus. Seven strains of CCMV were used in the initial studies. Six have been identified previously: type (T) (6), soybean (S) (7), mild (M) (8), Arkansas (A) (3), bean yellow stipple (BYS) (3), and R (12). Four serological groups have been recognized (3,8,13): T, S, and M belong to one group, and A, BYS, and R are each distinct from all other strains. A seventh strain, designated PSM (S. D. Wyatt and C. W. Kuhn, *unpublished*), can overcome resistance in cowpea, similar to strain R, but PSM belongs to the T, S, and M serological group.

Soybean genotypes. Six soybean genotypes that have variable disease reactions to strain S were studied (1,10). Davis is susceptible, Jackson is moderately resistant, plant introduction (PI) 346304 is resistant, PI 96983 is resistant to virus accumulation but becomes stunted and sometimes has systemic necrosis, and Bragg and Williams are highly resistant. Necrotic local lesions develop on the latter two genotypes, similar to a hypersensitive reaction. However, virus can be isolated from uninoculated, new trifoliolate leaves (1,10).

Inoculation and experimental design. Inoculum of the original seven strains was prepared by partial purification (one cycle of ultracentrifugation) of the virus 7–12 days after inoculation of Davis soybean (1). New isolates D and N were cultured in Bragg soybean. Inoculum (170 μg/ml) in 0.01 M of potassium phosphate (pH 7.0) containing 1% Celite was rubbed onto unifoliate leaves 9–11 days after seeding. Virus strains and soybean genotypes were included as fixed effects in a factorial treatment design. For disease reaction and virus concentration studies, there were three or four replications per treatment (replication = one pot with three plants). Pots were located on greenhouse benches or in growth chambers in a randomized block or a completely randomized design.

Virus quantification. Virus concentration in infected plants was determined by a method described previously (1). It involved partial purification by one cycle

of ultracentrifugation followed by density gradient centrifugation and ultraviolet absorption analysis [E = 5.8 $(mg/ml)^{-1}$ cm^{-1} at 260 nm] (1). Each sample contained one leaflet from each of the second, third, and fourth uninoculated trifoliolate leaves on 40 day-old plants (nine leaflets/treatment/replication).

In the interest of efficiency of time and cost, all virus samples were clarified and concentrated by one cycle of ultracentrifugation. Samples with more than 170 $\mu g/g$ of tissue were adjusted by subtracting 30 $\mu g/g$, an average estimate of ultraviolet-absorbing, normal host constituents that was determined by subjecting healthy soybean tissue to the same procedure. Samples with less than 170 $\mu g/g$ were subjected to density gradient centrifugation and the area of virus peaks was measured with a planimeter.

RESULTS

Disease reactions. In general, both local and systemic symptoms caused by the seven CCMV strains were similar in each of the six soybean genotypes and also similar to the reactions caused by strain S in previous reports (1,10). Differences were observed among the genotypes. Symptoms on inoculated unifoliate leaves varied from chlorosis (Davis, Jackson, PI 346304) to diffuse necrosis (PI 96983) to discrete necrotic lesions (Bragg, Williams). On uninoculated trifoliolate leaves, symptoms varied from mosaic and stunt (Davis, Jackson, PI 96983) to none or mild mottle on individual plants (PI 346304) to none on any plants (Bragg, Williams). The relative degree of mosaic and stunt from strain to strain was not consistent when tests were repeated. One notable symptom variation was observed on genotype Bragg. Strain BYS caused systemic mosaic and stunt on about 10% of the plants, particularly in warm months, while all other strains caused no symptoms.

Virus concentration. In general, the virus concentration of six CCMV strains in uninoculated trifoliolates of various genotypes was similar to the pattern previously reported for strain S (1,10) (Table 1). Two data points, T concentration in Davis and BYS concentration in Jackson, were apparently anomalous. Neither of these high concentrations was confirmed in two subsequent experiments, while the relative virus concentrations of other strain-genotype combinations were substantiated. Strain BYS virion yields were higher in PI 96983, Bragg, and Williams than the other virus strains (Table 1). The higher yields were substantiated in two other tests in which concentration values were similar to those in Table 1.

Strain A was included in the experiment reported in Table 1, and virus concentration was very low in all replications for all genotypes. In three subsequent tests, however, strain A concentration was similar to that of strains S, T, M, PSM, and R. Concentration levels were similar to those in Table 1.

When the relative average concentration of all strains is considered, Davis produced the most virus and Bragg and Williams the least. Virus concentration in Jackson was different from that in the other genotypes. Although PI 346304 and PI 96983 had similar quantities of virus, the disease reaction was much stronger in PI 96983 than in PI 346304.

Temperature effect. When experiments were conducted from November to April, the types of symptoms for all strains and genotypes were similar but usually more severe than those conducted in June and July. Furthermore, higher virus concentration levels for all strains were noted during the cooler months.

A similar temperature effect was observed in growth chambers adjusted to constant temperatures of 24 and 30 C. The disease reactions were milder at 30 C, with two exceptions: with genotype PI 96983, all strains caused severe stunting and 33–83% of the plants died within 3 wk after inoculation; on genotype Bragg, strain BYS caused vein necrosis, distortion, and stunting of uninoculated trifoliolate leaves on about half of the plants, instead of no symptoms.

Table 1. Concentration of six strains of cowpea chlorotic mottle virus (CCMV) in uninoculated leaves of susceptible and resistant soybean genotypes in greenhouse tests[a]

Soybean genotype	Concentration of virus strains (μg of virus/g fresh wt)[b]						Genotype average ($\mu g/g$)
	S	T	M	PSM	BYS	R	
Davis (S)[c]	191	403	279	262	278	278	282
Jackson (MR)	104	149	60	22	402	72	135
PI 346304 (R)	72	37	12	155	96	63	73
PI 96983 (C)	24	25	79	9	158	17	52
Bragg (HR)	ND[d]	<1	<1	ND	1	ND	<1
Williams (HR)	ND	<1	ND	ND	6	ND	1
			LSD (0.05)[e] 125				51

[a] Greenhouse study conducted in June and July. Three replications per treatment.
[b] Fresh weight of uninoculated leaf tissue. Virus concentrations were determined spectrophotometrically from partially purified test samples either before or after density gradient centrifugation (1).
[c] Reaction to CCMV strain S based on virus concentration and symptoms (1): S = susceptible, MR = moderately resistant, R = resistant, C = complex reaction, HR = highly resistant.
[d] Virus not detectable by procedure that can measure slightly less than 1.0 $\mu g/g$ of tissue.
[e] Analysis of variance was calculated without data from Bragg and Williams.

Table 2. Disease reaction and virus concentration of two new variants (D and N) and the soybean strain (S) of cowpea chlorotic mottle virus in uninoculated leaves of susceptible and resistant soybean genotypes in greenhouse tests[a]

Genotype	Symptoms[b]						Plant height (cm)[c]			Virus concentration ($\mu g/g$)[d]		
	S		D		N							
	Local	Systemic	Local	Systemic	Local	Systemic	S	D	N	S	D	N
Davis	C	SM,St	C	SM	C	M	43	45	53	322	158	225
Jackson	C	M	C	M	C	M	61	65	72	188	173	175
PI 346304	C	M	C	M	C	M	58	60	64	<1	19	38
PI 96983	DN	M,St	C,VN	D,St	C	M	42	42	56	52	106	111
Bragg	NL	None	C,VN	D,St	C	M	64	30	59	<1	203	335
Williams	NL	None	C,VN	M,St	C	M	63	28	59	<1	86	204
								LSD (0.05) 7			LSD (0.05) 86	

[a] Greenhouse experiment replicated four times; replication = one pot with three plants.
[b] C = chlorosis, D = leaf distortion, DN = diffuse necrosis, M = mosaic, NL = necrotic lesions, SM = severe mosaic, St = stunt, VN = vein necrosis.
[c] Soil line to top of plant. Measurements were made at 30 days after inoculation of 10-day-old seedlings.
[d] Fresh weight of uninoculated trifoliolate leaf tissue. Virus concentrations were determined spectrophotometrically from partially purified test samples either before or after density gradient centrifugation (1).

New variants of CCMV. Sap from diseased uninoculated tissue of Bragg infected with BYS was used to inoculate other Bragg plants. After four to six passages through Bragg, two distinct isolates of CCMV became apparent. After 3 mo, their uniqueness from other strains and from each other was regarded as stable because they routinely caused the same disease reactions on the six soybean genotypes, and symptoms were not strongly affected by environment in the greenhouse or in growth chambers set at 24 or 30 C. The isolates were designated D (leaf distortion in Bragg soybean) (Table 2) and N (systemic necrosis in cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*)) (11). In immunodiffusion tests (0.8% Noble agar, pH 5.0), both isolates D and N reacted with precipitin lines to antisera raised to each of the seven original CCMV strains.

Disease reactions of isolates D and N in soybean genotypes were compared in the same experiments with those of strain S (Table 2). Symptoms were similar for S, D, and N in Davis, Jackson, and PI 346304. Davis and Jackson plants infected with N were taller than those infected with S and D. The symptoms produced by strains D and N were strikingly different from S in PI 96983, Bragg, and Williams, particularly the latter two. Strain S caused no systemic symptoms in Bragg and Williams, while N caused a mild mottle and D caused leaf distortion and severe stunting (Fig. 1, Table 2).

Virus concentration of D and N was the same or less than S in Davis and Jackson, the two most susceptible genotypes (Table 2). In highly resistant Bragg and Williams, N produced similar or higher levels of virus than that found in the susceptible genotype Davis. Isolate D produced less virus than N in the highly resistant genotypes, and similar quantities in both the susceptible and highly resistant genotypes. Although differences were not significant in test data in Table 2, isolates D and N consistently produced more virus in PI 346304 and PI 96983 than strain S in three subsequent experiments. In three experiments with PI 346304 the data were as follows: S = <1 (± 0.0) μg of virus/g of tissue, D = 27 (± 4.3), and N = 22 (± 9.5). In a fourth experiment, no differences were observed. In four experiments with PI 96983, the data were as follows: S = 42 (± 5.5) μg of virus/g of tissue, D = 89 (± 6.7), and N = 75 (± 12.5).

DISCUSSION

Two resistance-breaking variants of CCMV were recovered from soybean cultivar Bragg that has the hypersensitive-type resistance gene *Rcv* (2). Variants D and N overcame the *Rcv* resistance. No necrotic local lesions were produced, and uninoculated leaves had distinct symptoms and virus concentration levels similar to those in the susceptible soybean Davis. In Bragg, variant D caused a severe stunting disease, while N caused a very mild disease. Disease symptoms caused by D were more severe in some genotypes than those caused by N, even though the latter isolate produced similar or larger quantities of virus in the hosts. A similar disease severity-virus concentration phenomenon has been noted previously with CCMV in Davis and PI 96983 soybean and California Blackeye and Clay cowpea (1,9,10). Preliminary (11) and unpublished results (O. R. Paguio and C. W. Kuhn) show other biological differences between variants D and N, and we believe they should be considered distinct strains of CCMV.

None of the previously known seven strains of CCMV was able to overcome the various types of resistance in soybean. Both disease reactions and virus concentration levels were relatively similar for each soybean genotype representing different levels of resistance. Strain BYS was an exception, particularly under certain environmental conditions. It appears that our isolate of BYS contained a mixture of variants, and strains D and N were relatively easily selected from soybean genotypes having the resistance gene *Rcv*.

More virus was produced by strains D and N than by S in PI 96983, but the complex reaction (low virus concentration, strong disease reaction) (1,10) in the genotype cannot be interpreted as being susceptible or resistant at this time. In three experiments with direct comparisons, strains D and N appeared to partially overcome the resistance (not related to hypersensitivity) in PI 346304. Symptoms caused by S, D, and N in this genotype were similar, but the latter two strains caused more virus to be produced, although not as much as that produced in susceptible Davis. It should be noted that concentration of S can be variable in PI 346304 (10) (Tables 1 and 2).

In cowpea PI 186465, CCMV strains R and PSM can overcome resistance gene *Mv* that controls systemic movement of the virus (9). Neither of these strains could overcome any type of resistance to CCMV in soybean. Therefore, it appears that distinct resistance mechanisms against CCMV occur in soybean and cowpea.

These studies support the virus concentration-disease reaction categories for soybean genotypes that were reported by Bijaisoradat and Kuhn (1), particularly when the virus concentration for all strains is averaged for each genotype. Furthermore, a distinction between Bragg and Williams, both giving a hypersensitive reaction, is strengthened by the fact that virus concentrations of new strains D and N are different in the two hosts. In the original study (1), the two genotypes were categorized primarily by size of necrotic local lesions. In this study, strains D and N produce one-and-a-half to two times more virus in Bragg than in Williams.

Fig. 1. Leaf distortion and stunting in Bragg soybean caused by cowpea chlorotic mottle virus strain D.

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