Variation in Double-stranded Ribonucleic Acid among Pepper Cultivars

Rodrigo A. Valverde¹ and James F. Fontenot²

Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center Baton Rouge, LA 70803

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Abstract. Double-stranded ribonucleic acid (dsRNA) was found associated with 51 of 80 healthy pepper (*Capsicum annuum* L., *C. frutescent* L., *C. chinense* Jacq.) cultivars analyzed. In general, dsRNAs were consistent within particular cultivars. Twelve distinct dsRNA profiles that varied in the number and size of the dsRNA segments were obtained. All bell and pimento pepper cultivars analyzed had a similar dsRNA profile. Furthermore, all six cherry pepper cultivars tested were free of dsRNAs. However, an association between the dsRNA profile and the pepper group was not obtained with other cultivars. Selected dsRNAs were transmitted at a high rate through the seed of self-pollinated plants but were not transmitted through grafts to plants that lacked them.

DsRNA is not normally found in plants unless they are infected with an RNA virus (Morris and Dodds, 1979). However, there have been several reports of dsRNAs occurring in healthy plants, including pepper (Abou-El Nasr et al., 1985; Grill and Garger, 1981; Valverde et al., 1986; Wakarchuk and Hamilton, 1985). A dsRNA of ≈ 12 kb from 'California Wonder' pepper has been partially characterized (Valverde et al., 1990).

During a survey for viral diseases of pepper in Louisiana, we noticed that most commercial pepper cultivars contained dsRNAs. These dsRNAs varied in size and number depending on the cultivar analyzed. Furthermore, they appeared to be consistent within specific pepper cultivars. Therefore, the purpose of this investigation was to: a) screen pepper cultivars from various horticultural groups for dsRNAs, b) determine the consistency of the dsRNAs among individual plants from a particular cultivar, and c) explore the possibility of using the dsRNA analysis to identify pepper genotypes.

Materials and Methods

Cultivars and plant species. Seventy-three cultivars of *C. annuum* (bell, pimento, ancho, Anaheim chili, cayenne, Cuban, jalapeno, serrano, cherry, and yellow wax), seven cultivars of *C. frutescens* (tabasco), and one line of *C. chinense* were used in this study. Cultivars and seed sources (Table 1) were grouped according to the horticultural classification of Smith et al. (1987). Leaf tissue from at least 10 individual plants from each cultivar grown in the greenhouse was used for dsRNA extraction.

Extraction and analysis of dsRNA. DsRNA was extracted as described by Valverde et al. (1986) with some modifications. Leaf tissue (3.5 g) was squeezed through a leaf roller in 7.0 ml of $2 \times$ sodium chloride, tris, EDTA (STE) buffer [$1 \times$ STE = 0.1 M NaCl, 0.05 M Tris-HCl and 0.001 M ethylenediamine tetraacetic acid (EDTA), pH 6.8.]. The extract was collected in a 50-ml centrifuge tube, and 10 ml of $1 \times$ STE-saturated phenol, 1 ml of 10% sodium dodecyl sulphate, and 0.5 ml of bentonite

suspension (25 mg·ml⁻¹, were added. Tubes were shaken for 30 min and then centrifuged at $8000 \times g$ for 15 min in a RC-5B Sorvall (DuPont Co., Wilmington, Del.) centrifuge using a SS-34 rotor at 10C. The aqueous phase (upper) was collected, and ethanol was added to a final concentration of 16%. The solution was subjected to two cycles of cellulose (CF-11 Whatman) column chromatography. Columns were made by pouring a suspension of cellulose and 25 ml of $1 \times$ STE with 16% ethanol (final concentration) into an empty barrel of a 30-ml plastic syringe that had a disk of miracloth paper at the bottom. Samples (aqueous phase) were then added to the column. After washing columns with 100 ml of a solution of $1 \times$ STE and 16% ethanol, the dsRNA was eluted from the columns with 7 ml of $1 \times$ STE. DsRNA was precipitated at -20°C overnight in 50-ml tubes with 2.5 volumes of 95% ethanol and 0.5 ml of 3 M sodium acetate, pH 5.5. Tubes containing dsRNA were centrifuged for 30 min at $8000 \times g$. The dsRNA pellets were resuspended in 200 μ l of electrophoresis buffer (0.04 M Tris-HCl, 0.02 M sodium acetate, and 0.001 M EDTA, pH 7.8) that contained 20% glycerol and 0.01% bromophenol blue. Electrophoresis was performed for 3 h at 100 V by use of 6% polyacrylamide gels (8×10 cm $\times$ 1.5 mm) in a vertical gel apparatus. Gels were stained in ethidium bromide (20 ng·ml⁻¹) for 15 min, visualized with a UV light transilluminator (300 rim), and photographed with Polaroid film, type 57. Size standards used for comparison were dsRNAs extracted from 'California Wonder' pepper (12 kb) and tobacco plants infected with tobacco necrosis virus (3.3, 1.1, and 1.0 kb) and tobacco mosaic virus (6.2, 3.0, 1.4, and 0.6 kb) (Valverde et al., 1986).

Seed and graft transmission of dsRNA. To determine the level of seed transmission of selected dsRNAs through three generations of self-pollination, individual plants from five pepper cultivars ('Yolo Wonder', 'Cayenne Long Red', 'Hungarian Wax', 'Jalapeno M-84', and 'Mexican Chili'), each having distinct dsRNA banding patterns, were grown in the greenhouse for three generations. Ten plants from each generation were randomly chosen for dsRNA analysis. The same five cultivars were selected for graft transmission experiments. At least two scions from each of the five cultivars (2-month-old plants) were grafted on rootstock of these cultivars in various combinations. One month after grafting, 3.5 g of newly developed leaves from each scion were collected and analyzed for dsRNA.

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¹Assistant Professor.

²Professor, Dept. of Horticulture.

Abbreviation: dsRNA, double-stranded ribonucleic acid.

Table 1. DsRNA profiles obtained from pepper cultivars.² (Cultivars are grouped according to their horticultural type.)

Group and cultivar	dsRNA profile	Seed source ³
Bell		
Agronomico-8	b	UCR
Delray Bell	b	TAES
Domino Hybrid	b	Asgrow
Florida VR-2	b	LSU
Gedeon Hybrid	b	UCR
Grande Rio 66	b	TAES
Jupiter	b	TAES
Keystone Resistant Giant #3	b	Asgrow
Olympic Hybrid	b	Asgrow
Marengo	b	Asgrow
Shamrock Hybrid	b	Asgrow
Tambel-2	b	TAES
Tasty Hybrid	b	UCR
TB-2	b	TAES
Yolo Wonder	b	UCR
Pimento		
Pimento	b	UCR
Pimento L	b	UCR
Pimento Perfection	b	TAES
Tam F89	b	TAES
Ancho		
Ancho 101	b	TAES
Mexican Chili	a	UCR
Anaheim chili		
Anaheim Chili	g	UCR
Anaheim Mild Chili	a	UCR
New Mexico 6	a	TAES
Papri Mild	g	TAES
Super Chili	g	LSU
Tam Mild Chile-2	h	TAES
Tam Mild Chile-3	a	TAES
Tam Sweet Chile	b	TAES
Cayenne		
Cajun-1	f	LSU
Cajun-2	a	LSU
Cayenne 709	g	LSU
Cayenne Large Red Thick	b,c	LSU
Cayenne Long Red	g	UCR
Cayenne Long Slim	c	LSU
Durkee	m	TAES
Panola Cayenne	a	LSU
Sport	m	LSU
Super Cayenne	d	Peto
Tam Cayenne	b	TAES
Cuban		
Cubanelle	b	Peto
Pepperoncini	b	LSU
Jalapeno		
Jalapa	h	Peto
Jalapeño	a	LSU
Jalapeño M-84	f	UCR
Jalapeño M-88	f,h,j	Peto
Jalapeño M-89	h	TAES
Jalapeto	g,h	Peto
Jumbo Jalapeño	a	Peto
Tam Mild Jalapeño	a	TAES
Tam Sweet Jalapeño	a	TAES
Tam Veracruz Jalapeño	a	TAES
Tam Yellow Jalapeño	a	TAES
Serrano		
Serrano Chili	i,k	Peto
Tam Hidalgo	i	TAES
Tampiqueño	a	TAES

Table 1. Continued.

Group and cultivar	dsRNA profile	Seed source ³
Cherry		
Cherry	a	LSU
Large Cherry	a	UCR
Sweet Cherry	a	UCR
Tam Hot	a	TAES
Tam Sweet	a	TAES
Tiger	a	LSU
Yellow wax		
Gold Spike	g	LSU
Habanero	l	LSU
Habanero Orange	a	TAES
Habanero Red	g	TAES
Hungarian Wax	c	UCR
Hungarian Yellow Wax	a	TAES
Sante Fe Grande	c	TAES
Sweet Banana	a	UCR
Tam Cascabella	a	TAES
Tam Rio Grande	a	TAES
Tam Gold Spike	a	TAES
Tabasco		
Dat'l	a	TAES
Greenleaf Tabasco	e	LSU
Greenleaf Tabasco	a	TAES
LSU Tabasco	e	LSU
McLhenny Select	a	MC
McLhenny Tabasco	a	TAES
Tam Tabasco	b	TAES

²DsRNA profile designation was assigned according to the number and relative mobility of dsRNAs after polyacrylamide gel electrophoresis. Typical profiles are shown in Fig. 1.

³UCR = Univ. of California, Riverside; LSU = Louisiana State Univ.; Peto = Petoseed, Inc; TAES = Texas Agricultural Experiment Station; Asgrow = Asgrow Seed; MC = McLhenny Co.

Results and Discussion

Twelve distinct dsRNA banding patterns (profiles) were obtained after electrophoretic analysis of *C. annuum* and *C. frutescens* (Fig. 1). DsRNA was not detected in *C. chinense*. An arbitrary designation (a through m) was given to each dsRNA profile according to the number and relative mobility (size) of the dsRNAs detected by gel electrophoresis (Table 1).

Most of the dsRNAs found in pepper are similar to the genomic dsRNAs of cryptic viruses (Boccardo et al., 1987). Cryptic viruses are spherical, multisegmented dsRNA (1–3 kb) plant viruses that do not induce symptoms in their hosts, are transmitted at a high rate through seed, and are not transmitted mechanically or by grafting. Some dsRNAs found here could be the genomes of cryptic viruses. They were transmitted at a high rate through seed of self-pollinated plants and have sizes similar to the dsRNAs of cryptic viruses. It is unlikely that these dsRNAs constitute the genome of conventional plant viruses. Attempts to transmit several dsRNA types by grafting, which is a standard method for transmission of plant viruses and virus-like agents, failed. Furthermore, pepper plants that had these dsRNAs did not show visible virus symptoms. Regardless of the origin, these dsRNAs are consistently associated with some specific pepper cultivars and, therefore, may have great utility.

Smith et al. (1987) have grouped peppers grown in the United States according to fruit morphology, size, color, and usage. DsRNA profile b was associated with all 15 bell pepper cultivars examined. Furthermore, previous analysis of 10 other bell pepper cultivars yielded a similar dsRNA profile (Valverde et al.,

Fig. 1. Polyacrylamide gel electrophoresis (6%) profiles of dsRNA extracted from individual plants of 12 pepper cultivars. To each dsRNA profile, an arbitrary designation was given. Channels are labeled according to the dsRNA profile. Lane a, 'Jumbo Jalapeño'; lane b, 'Pimento L'; lane c, 'Cayenne Large Red Thick'; lane d, 'Super Cayenne'; lane e, 'Greenleaf Tabasco'; lane f, 'Jalapeño M-84'; lane g, 'Gold Spike'; lane h, 'Jalapa'; lane i, 'Serrano Chili'; lane j, 'Jalapeño M-88'; lane k, 'Serrano Chili'; lane l, 'Habanero'; and lane m, 'Sport'. Size standards are dsRNAs extracted from tobacco plants infected with tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV) and 'California Wonder' pepper (CW). Molecular size is given in kilobases (kb). Electrophoresis was conducted at 100 V for 3 h. Migration of dsRNAs is from top to bottom [cathode (-) to anode (+)].

1990). 'Cubanelle' and 'Pepperoncini', which belong to the Cuban group, and all four pimento cultivars also had this dsRNA profile. This commonality may indicate common ancestry. All six cultivars of the cherry group lacked dsRNAs. DsRNA profiles from cultivars of other groups were variable. Therefore, except for the above groups, dsRNA profiles did not coincide with the present horticultural classification.

Most pepper cultivars with dsRNAs yielded profiles that were consistent in all 10 plants examined; the exceptions were 'Serrano Chili', 'Cayenne Large Red Thick', 'Jalapeto', and 'Jalapeño M-88'. In these cases, individual plants within a cultivar had different dsRNA profiles (Table 1). Furthermore, 'Jalapeño M' plants from two seed lots had distinct dsRNA types. Plants from the 1984 seed lot had dsRNA type f, those from a 1989 seed lot had dsRNA type h, but those from the 1988 seed lot had either type f, h, or j (Table 1). Similarly, 'Greenleaf Tabasco' from two sources yielded different dsRNA profiles. These variations could indicate an actual mixture of seed from more than one cultivar, a partial infection of the seed lot by a cryptic virus, cross pollination, or segregation of the different dsRNAs encoded in parental genomes. The latter could be related to lack of homozygosity in these cultivars.

Preliminary studies on the inheritance of these dsRNAs indicate that they are transmitted through the seed, both maternally and paternally. Some dsRNA profiles shown in Fig. 1 (c, d, e, h, and j) appear to be mixtures of two profiles. Since many cultivars are derived from crosses, these mixtures were not surprising.

'Jalapeño M' plants of two seed lots were phenotypically indistinguishable. Therefore, dsRNA profiles could be used to differentiate seed lots. The genetic background of pepper cultivars may also be traced by looking at their dsRNA profiles. This could be done not only by electrophoretic analysis but also by molecular hybridization using a cloned cDNA probe specific for a selected dsRNA segment.

The development of a computer database of the dsRNA profiles of all commercial pepper cultivars could be useful not only for plant breeders, but also for commercial seed companies. Analysis of dsRNA can be complimentary to other techniques, such as enzyme analysis for studying the taxonomy and evolution of peppers (McLeod et al., 1983).

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