HortScience 45(11):1756–1758. 2010.

'TAM Ben Villalon', a New, Multiple-virus Resistant, Mild, Green-chile Pepper

Kevin M. Crosby[1,4], Justin Butcher[2], and Kil Sun Yoo[1]
Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, 1500 Research Parkway, Suite 120, College Station, TX 77845

Daniel I. Leskovar[3]
Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas AgriLife Research Center, 1619 Garner Field Road, Uvalde, TX 78801

Additional index words. Capsicum annuum, potyviruses, germplasm, open-pollinated

The Vegetable and Fruit Improvement Center at Texas A&M University announces the release of 'TAM Ben Villalon', a multiple-virus resistant, mildly hot Anaheim-type pepper (*Capsicum annuum* L.). Very few Anaheim or New Mexico pod cultivars possess resistance to potyviruses (Greenleaf, 1986; Villalon, 1981). The project to breed virus-resistant peppers at the Texas A&M Agricultural Experiment Station–Weslaco began in 1971. Several cultivars of virus-resistant peppers have been released over the last 20 years (Villalon, 1983; Villalon et al., 1992, 1994). The production of hot and sweet peppers in Texas has fluctuated from 1200 to 7000 ha over the last 30 years. Currently, close to 3000 ha of hot peppers are cultivated statewide (U.S. Department of Agriculture, National Agricultural Statistics Service, 2010). One of the most persistent problems of pepper production in south Texas has been virus infection. The two most serious virus pathogens are *Tobacco etch virus* (TEV) and *Pepper mottle virus* (PepMoV), both transmitted primarily by the cotton aphid (*Aphis gossipyi* Glover) and the green peach aphid (*Myzus persicae* Sulzer). Historically, most pepper cultivars grown in south Texas have been susceptible to local strains of both TEV and PepMoV. Yield reductions from infected plants have been as high as 45%, particularly when fruit is intended for the fresh market (Greenleaf, 1986). The best solution to the problem is the development of resistant cultivars with high-quality fruit. Multiple sources of single resistance genes against these potyviruses have been documented in various germplasm lines (Cook, 1960; Greenleaf, 1956; Kyle and Palloix, 1997; Zitter and Cook, 1973).

Origin

In 1971, two sources of potyvirus resistance were inoculated with south Texas isolates of TEV, *Tobacco mosaic virus* (TMV), and *Potato virus Y* (PVY). 'Avelar' and 'Agronomico 8' both demonstrated resistance to these three viruses, and 'Avelar' demonstrated resistance to PepMoV (Cook, 1960; Zitter and Cook, 1973). 'Agronomico 8' possesses the pvr2 gene and 'Avelar' possesses the pvr3 gene (Kyle and Palloix, 1997). These cultivars were crossed together and the F_2 progeny were mechanically inoculated to identify lines resistant to TEV, TMV, and PVY (Villalon, 1981). In addition, resistance to PepMoV was verified in several lines as well. These resistant lines were used as sources of potyvirus resistance genes to develop additional breeding lines. 'TAM Mild Chile-2' was developed by crossing one of these multivirus-resistant lines to 'Anaheim 23' followed by controlled virus inoculations, backcrosses, and field selection for desirable horticultural attributes (Villalon et al., 1986). This cultivar exhibits resistance to the four viruses but has smaller fruit size than current industry standards.

'TAM Ben Villalon' originated from a cross of 'TAM Mild Chile-2' and 'Arabel', a large, sweet, elongated (16 cm × 5 cm) Spanish lamuyo-type pepper. The pedigree method was used from the F_2 to F_9 generations with single plant selections for yield, fruit quality, and virus resistance (Fig. 1). At each selfed generation, progeny were tested for resistance to TEV and PepMoV by mechanical inoculation with highly virulent south Texas isolates. The F_{10} to F_{15} generations were bulked seed of selected plants from isolation blocks. Ten F_{15} plants were then inoculated with both TEV and PepMoV in a greenhouse experiment. All plants exhibited resistance compared with the control, 'New Mexico 6'. Breeder's seed was increased in a greenhouse to produce the F_{16} generation.

This seed was planted for field evaluation at Uvalde, TX, during the spring of 2006. Plants were grown with standard commercial practices of drip irrigation and chemical pest control. The popular green chile cultivars, Sonora and NM 6, were included as checks. 'TAM Ben Villalon' produced significantly higher yields of larger fruit than both 'Sonora' and 'NM 6' (Table 1). A separate yield trial was established at Pearce, AZ, during the spring of 2009. Five acres of 'TAM Ben Villalon' were planted adjacent to 20 acres of 'NM 6'. All plants were grown with standard production practices, including chemical fertilization, pest control, and furrow irrigation. Yields and fruit size of 'TAM Ben Villalon' were significantly greater (31%) than those of 'NM 6' (Table 2).

Total capsaicinoid concentrations of mature fruits from 'TAM Ben Villalon', 'NM 6', and 'Sonora' plants grown at Uvalde and Pearce were measured by high-performance liquid chromatography (HPLC) (Hoffman et al., 1983). Fruits from the hot jalapeño cultivar Grande, grown in College Station, TX, were also analyzed for comparison as a hot control. Total capsaicin, on a dry weight basis, was converted to Scoville Heat Units (SHU) following the method of Collins et al. (1995). 'TAM Ben Villalon', 'NM 6', and 'Sonora' fruit all had very low levels of capsaicin (Table 1). By comparison, the hot jalapeño 'Grande' had a capsaicin concentration of 1,332 $\mu g \cdot g^{-1}$ (19,980 SHU). Total flavonoids were extracted with 100% methanol and analyzed by HPLC with a Nova-pak C-18 column (Lee et al., 1995). Among the three chile cultivars, only 'TAM Ben Villalon' had detectable levels of flavonoids at 31 $\mu g \cdot g^{-1}$. In a separate experiment at Pearce, fruits from replicated plots of both 'TAM Ben Villalon' and 'NM 6' were harvested at the mature red stage from a commercial field and dried using a commercial fruit drier. Dried tissue was ground in a coffee grinder and capsaicin was extracted using methanol. Under these desert conditions, capsaicin was produced in fruit of both cultivars (Table 2). The average (four replications) concentration of total capsaicinoids for 'TAM Ben Villalon' was 19.1 $\mu g \cdot g^{-1}$, whereas 'NM 6' contained 60.3 $\mu g \cdot g^{-1}$. Although these values were statistically different ($P < 0.05$), neither cultivar would be considered very hot. In contrast to the results from Uvalde, at Pearce both cultivars produced flavonoids in the fruit with 'NM6' containing 90 compared with 37.6 $\mu g \cdot g^{-1}$ for 'TAM Ben Villalon'.

In separate experiments, seedlings of both 'TAM Ben Villalon' and a susceptible control, 'NM 6', inoculated with local isolates of either TEV, PVY, or PepMoV, were transplanted to 10-L pots in the greenhouse and into field plots to rate the expression of virus symptoms and observe the effects on plant growth and fruit quality. In both greenhouse

Received for publication 19 July 2010. Accepted for publication 16 Aug. 2010.
This research was funded in part by USDA grants 2003-34402-13647, 2004-34402-14768, and 2006-34402-17121: "Designing Foods for Health."
We thank the New Mexico Chile Commission for financial support of this research. We also thank Dr. Benigno Villalon, Mr. Rick Hernandez, Mr. Cory Dombrowski, and Mr. Alfredo Rodriguez for their valuable help in conducting greenhouse and field experiments.
Use of trade names does not imply endorsement of the products named nor criticism of similar ones not named.
[1]Associate Professor.
[2]Graduate Research Assistant.
[3]Professor.
[4]To whom reprint requests should be addressed; e-mail k-crosby@tamu.edu.

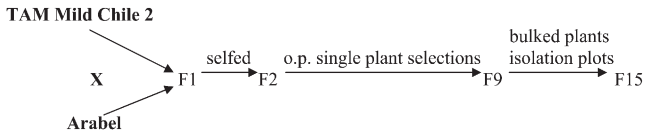

Fig. 1. Pedigree of 'TAM Ben Villalon' pepper.

Table 1. Performance of 'TAM Ben Villalon' at Uvalde, TX, in 2006.

Traits	Ben Villalon	Sonora	NM 6
Fruit length (cm)	18.1 a[w]	16.0 b	14.8 c
Wall thickness (mm)	2.6 a	1.8 b	2.0 b
Yield (kg·ha^{-1})[z]	16,632 a	14,228 b	12,243 c
Total capsaicin (μg·g^{-1})[y]	41 b	62 a	45 b
Scoville Units	615	930	675
Total flavonoids (μg·g^{-1})	31	ND	ND
Days to red maturity[x]	75	72	78

[z]Fresh, red pods; average of four replicated plots.
[y]Dry weight basis.
[x]Based on 6-week-old transplants.
[w]Mean separations within rows by least significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.
ND = none detected.

Table 2. Performance of 'TAM Ben Villalon' at Pearce, AZ, in 2009.

Traits	Ben Villalon	NM 6
Fruit length (cm)	18.2 a[w]	16.3 b
Wall thickness (mm)	1.8 a	1.8 a
Yield (kg·ha^{-1})[z]	22,866 a	17,435 b
Total capsaicin (μg·g^{-1})[y]	19 b	60 a
Scoville Units	285	900
Total flavonoids (μg·g^{-1})	37.6 b	90 a
Days to red maturity[x]	125	125

[z]Fresh, red pods; average of four replicated plots.
[y]Dry weight basis.
[x]Based on direct seeding in the field.
[w]Mean separations within rows by least significant difference, $P \leq 0.05$. Means followed by the same letter are not significantly different.

Table 3. Virus symptom expression based on enzyme-linked immunosorbent assay and plant visual ratings after mechanical inoculation.

			Leaf and fruit symptoms[y]		
Cultivar	Virus	ABS[z]	Clorosis	Distortion	Plant stunting
NM 6	TEV	4.00	Severe	Severe	Moderate
Ben Villalon	TEV	0.84	Mild	None	None
Capistrano	TEV	4.00	Severe	Severe	Severe
Positive control	TEV	1.36	—	—	—
NM 6	PepMoV	3.22	Severe	Severe	Severe
Ben Villalón	PepMoV	1.24	Mild	None	None
Capistrano	PepMoV	2.37	Severe	Severe	Severe
Positive control	PepMoV	1.80	—	—	—
NM 6	PVY	1.11	Mild	None	None
Ben Villalón	PVY	0.05	None	None	None
Capistrano	PVY	1.12	Moderate	None	None
Positive control	PVY	1.29	—	—	—

[z]Absorbance at 405 nm
[y]Ratings: severe = entire plant affected, complete loss of yield; moderate = more than 50% of leaves chlorotic and stunted and minor plant stunting; mild = slight chlorosis of young leaves, no distortion or stunting; none = completely unaffected.
TEV = *Tobacco etch virus*; PepMoV = *Pepper mottle virus*; PVY = *Potato virus Y*.

and field experiments, TEV-inoculated plants of 'NM 6' had small, misshapen fruit and chlorotic leaves, whereas inoculated plants of 'TAM Ben Villalon' had normal fruit and mild chlorosis. PepMoV-inoculated plants of 'NM 6' exhibited severe leaf distortion, plant stunting, and reduced yield with misshapen fruit. None of the fruit from inoculated 'NM 6' plants were of commercial quality. 'TAM Ben Villalon' plants inoculated with PepMoV exhibited mild chlorosis on older leaves but no distortion or misshapen fruit. No symptoms were observed on 'TAM Ben Villalon' and only mild symptoms were evident on 'NM 6' after inoculation with PVY in the greenhouse. Double antibody sandwich–enzyme-linked immunosorbent assay tests were conducted on leaf samples from inoculated greenhouse plants of both 'TAM Ben Villalon' and 'NM 6' to determine the presence of TEV, PepMoV, and PVY (Clark and Adams, 1977). Absorbance readings were taken with a microplate reader at 405 nm to determine virus infection. Leaves from 'NM 6' tested positive for TEV, PepMoV, and PVY (Table 3). Leaves from 'TAM Ben Villalon' tested weakly positive for TEV and PepMoV and negative for PVY. Leaves of a susceptible control, 'Capistrano' bell pepper, tested positive for TEV, PVY, and PepMoV.

Fig. 2. 'TAM Ben Villalon' plant and fruit.

The large, mildly pungent fruit of 'TAM Ben Villalon' makes it well suited for both fresh and processed green chile products (Fig. 2). These would include mild salsas and canned chiles for markets that prefer less pungent foods. Additionally, the high yield potential of this new cultivar makes it desirable for dry red chile production (Nick Carson, Rio Valley Chiles, Rincon, NM, personal communication), which is important in the spice industry. The multiple-virus resistance and adaptation to diverse climates will be valuable attributes to U.S. growers. 'TAM Ben Villalon' is unique among Anaheim-type peppers with its resistance to three potyviruses. It may be a useful parent to develop other virus-resistant chile cultivars with varying degrees of heat or other attributes. In addition, seeds of this open-pollinated cultivar will be more affordable for direct seeding into the field than comparable hybrid cultivars.

Availability. Breeder's seed will be maintained by the Texas A&M University. Application for plant variety protection is being filed for 'TAM Ben Villalon'. This variety may be licensed through Texas A&M University, Office of Technology Commercialization, for commercial seed production.

Literature Cited

Clark, M.F. and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475–483.

Collins, M.D., L.M. Wasmund, and P.W. Bosland. 1995. Improved method for quantifying capsaicinoids in Capsicum using high-performance liquid chromatography. HortScience 30:137–139.

Cook, A.A. 1960. Genetics of resistance in *Capsicum annuum* to two virus diseases. Phytopathology 50:364–367.

Greenleaf, W.H. 1956. Inheritance of resistance to tobacco etch virus in *Capsicum frutescens* and in *Capsicum annuum*. Phytopathology 46:371–375.

Greenleaf, W.H. 1986. Pepper breeding, p. 67–134. In: Basset, M.J. (ed.). Breeding vegetable crops. AVI Publishing Co., Westport, CT.

Hoffman, P.G., M.C. Lego, and W.G. Galetto. 1983. Separation and quantification of red pepper major heat principles by reverse-phase high-pressure liquid chromatography. J. Agr. Food Chem. 31:1326–1330.

Kyle, M.M. and A. Palloix. 1997. Proposed revision of nomenclature for potyvirus resistance genes in Capsicum. Euphytica 88:231–239.

Lee, Y., L.R. Howard, and B. Villalon. 1995. Flavonoids and anti-oxidant activity of fresh pepper (*Capsicum annuum*) cultivars. J. Food Sci. 60:473–476.

U.S. Department of Agriculture, National Agricultural Statistics Service. 2010. 22 Feb. 2010. <http://www.nass.usda.gov/Statistics_by_State/Texas/index.asp>.

Villalon, B. 1981. Breeding peppers to resist virus diseases. Plant Dis. 65:557–562.

Villalon, B. 1983. TAM mild jalapeño pepper-1 pepper. HortScience 18:492–493.

Villalon, B., F.J. Dainello, and D.A. Bender. 1992. 'TAM Veracruz' hot jalapeño pepper. HortScience 27:184–185.

Villalon, B., F.J. Dainello, and D.A. Bender. 1994. 'Jaloro' hot yellow jalapeño pepper. HortScience 29:1092–1093.

Villalon, B., F.J. Daniello, W.N. Lipe, and R.M. Taylor. 1986. 'TAM Mild Chile-2' chile pepper. HortScience 21:1468–1469.

Zitter, T.A. and A.A. Cook. 1973. Inheritance of tolerance to a pepper virus in Florida. Phytopathology 63:1211–1212.