Resistant Responses of Peach Somaclone 122-1 to *Xanthomonas campestris* pv. *pruni* and to *Pseudomonas syringae* pv. *syringae*

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Additional index words. bacterial leaf spot, bacterial canker, disease resistance, *Prunus persica*, somaclonal variation

Abstract. A detached-leaf bioassay was used to evaluate peach [*Prunus persica* (L.) Batsch] somaclone 122-1 (derived from callus produced on an immature embryo of peach cultivar Redhaven) for resistance to several virulent strains of *Xanthomonas campestris* pv. *pruni* [E.F. Sm.) Dows], causal agent of bacterial leaf spot, and to a virulent isolate of *Pseudomonas syringae* van Hall pv. *syringae*, causal agent of bacterial canker. The detached-leaf bioassay was also used to evaluate progeny of 122-1 for resistance to *X. campestris* pv. *pruni* virulent strain XP1. Somaclone 122-1 was significantly more resistant to most strains of *X. campestris* pv. *pruni* than was 'Redhaven', and all of its progeny exhibited high levels of resistance to *X. campestris* pv. *pruni* strain XP1. Somaclone 122-1 exhibited significantly higher levels of resistance to *Pseudomonas syringae* pv. *syringae* than did 'Redhaven' and this resistance was retained over time in the greenhouse and following a 2-year cycle of tissue culture propagation.

A narrow germplasm base and long generation time have impeded progress in generating peach trees with high levels of disease resistance through conventional breeding (Scorza and Hammerschlag, 1992; Scorza and Okie, 1991). One alternative to conventional breeding for obtaining useful genetic variation is to generate somaclonal variants from organs, tissues or cells (Larkin and Scowcroft, 1981). Several examples of increasing levels of disease resistance in crop plants by generating somaclonal variants include apple (*Malus domestica* Borkh.) with resistance to *Erwinia amylovora* (Burrill) Winslow et al. (Donovan et al., 1994), celery (*Apium graveolens* L.) with resistance to *Fusarium oxysporum* f.sp. *apii* (R.R. Nelson & Sherb.) W.C. Snyder & H.N. Hans. (Heath-Pagliuso et al., 1988), and tomato (*Lycopersicon esculentum* Mill.) with resistance to *Clavibacter michiganensis* subsp. *michiganensis* (Smith) (Davis et al.) (De Vries and Stephens, 1997). Tissue culture technologies developed to regenerate peach plants from cell cultures (Hammerschlag et al., 1985) have

led to the identification of somaclonal variants of peach with increased levels of resistance to bacterial leaf spot (Hammerschlag, 1988, 1990) and to the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood (Hashmi et al., 1995). Preliminary studies (Hammerschlag and Ognjanov, 1990) have indicated that variants with resistance to *Pseudomonas syringae* pv. *syringae* can be obtained by screening unselected peach regenerants for resistance to this bacterium. Stability of resistance to bacterial leaf spot over time in the greenhouse, through in vitro propagation and after several years in the field, has been exhibited by peach somaclone 122-1 (Hammerschlag et al., 1994).

The objective of this study was to examine the response of: 1) peach somaclone 122-1 (Hammerschlag, 1990) to several strains of *X. campestris* pv. *pruni*; 2) progeny of open-pollinated somaclone 122-1 to *X. campestris* pv. *pruni* virulent strain XP1; and 3) somaclone 122-1 to *P. syringae* pv. *syringae*.

Materials and Methods

Plant material. Four types of plant material were used in these experiments: 1) 5-year-old 'Redhaven' peach trees (moderately resistant to bacterial spot and susceptible to bacterial canker), 2) greenhouse-grown 'Redhaven' plants obtained from axillary shoots of field-grown trees by micropropagation ('Redhaven' TC) (Hammerschlag et al., 1987), 3) somaclonal variant 122-1 derived from callus generated on an immature 'Redhaven' embryo (Hammerschlag et al., 1985) and screened for resistance to *X. campestris* pv. *pruni* virulent strain XP1 (Hammerschlag, 1990), and 4)

seedlings obtained from seeds of open-pollinated (probably selfed) 122-1 trees as described previously for seedlings of 'Redhaven' (Hammerschlag, 1990). About 2 months after seedlings or in vitro plantlets were transferred to the greenhouse, actively growing plants were tested for response to *X. campestris* pv. *pruni* and *P. syringae* pv. *syringae* using a modified detached-leaf bioassay (see below)

For studies on the effects of plant aging on the response to *P. syringae* pv. *syringae*, somaclone 122-1 was maintained under greenhouse conditions from March through August, hardened off outside the greenhouse from September through October, and stored in the dark at 1.7 to 7.2 °C from November through February. This regime was repeated for 2 years, after which actively growing plants were evaluated for their response to *P. syringae* pv. *syringae* by a detached-leaf bioassay (see below).

For studies on the effects of in vitro propagation on the response to *P. syringae* pv. *syringae*, axillary shoots were removed from the original 122-1 regenerant and propagated in vitro (Hammerschlag, 1982a; Hammerschlag et al., 1987). After 9 months of propagation, shoots were placed on 1/2 strength MS medium (Murashige and Skoog, 1962) supplemented with 58.7 mM sucrose and 0.6% Phytagar (GIBCO/BRL, Grand Island, N.Y.) and incubated in the dark at 4 to 7 °C. After 6 to 8 weeks of cold, shoots were transferred to 1/2 strength MS medium supplemented with 0.57 to 2.9 μM of α-naphthaleneacetic acid, 58.7 mM sucrose and 0.6% Phytagar and incubated at 25 °C under a 16-h photoperiod provided by cool-white fluorescent lights at 40 μmol·m⁻²·s⁻¹. Rooted shoots were acclimated and plantlets transferred to the greenhouse as described previously (Hammerschlag, 1982b; Hammerschlag et al., 1987). Axillary shoots produced from these plants were collected, propagated, cold acclimated, and rooted similarly to the axillary shoots collected from the original regenerant (as described above). Actively growing greenhouse 122-1 plants from shoots following a 2-year cycle of in vitro propagation were evaluated for response to *P. syringae* pv. *syringae* by a detached-leaf bioassay (see below).

Culture of bacteria and standardization of inoculum. *Xanthomonas campestris* pv. *pruni*, highly virulent strains XP1 (from Beltsville, Md.), XP21 (from South Africa), XP27 (from Canada), and XP31 (from North Carolina) were obtained from Dr. E. Civerolo, U.S. Dept. of Agriculture, Agricultural Research Service, Beltsville, Md. (currently in Davis, Calif.), and were maintained at 4 °C on Difco nutrient agar supplemented with dextrose (20 g·L⁻¹). Cultures were transferred every week. To prepare standard inoculum (SI), a loopful of *X. campestris* pv. *pruni* from a nutrient agar plate culture was transferred to 0.8% Difco nutrient broth containing 5% NaCl and 2% dextrose. The culture was incubated for 24 h at 27 °C (120 rpm). Bacterial cells were collected by centrifugation at 5000 g₀ for 10 min. The cells were resuspended in 0.05 M phosphate buffer, pH 6.8, and adjusted with this buffer to

Received for publication 30 Mar. 1999. Accepted for publication 23 May 1999. Special thanks are due to L. Douglas for assistance with statistical analyses; to D. Ritchie for providing the F₁ seed; and to R. Griesbach, J. Maas, D. Ritchie, and D. Werner for critical review of this manuscript. Use of company or product name does not imply approval or recommendation of the product to the exclusion of others, which may also be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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an optical density of 0.165 at 620 nm [in a Milton Roy Spectronic 21 spectrophotometer (Thomas, Swedesboro, N.J.) zeroed with a buffer blank] to give the SI that contained $\approx 2 \times 10^8$ colony forming units (cfu)/mL. For inoculations, the SI was diluted with buffer to 2×10^6 cfu/mL.

Pseudomonas syringae pv. *syringae*, highly virulent strain B3, was obtained from M. Canfield, Oregon State Univ., Corvallis, and stored at -80°C in medium (pH 7.2) containing (g·L⁻¹): 1 Difco Bacto tryptone, 0.5 Difco Bacto yeast extract, 1 NaCl and 15% (v/v) glycerol. For inoculations, the bacteria were grown for 36 h on King's medium B (King et al., 1954), harvested, pelleted, and resuspended in 0.02 M sterile phosphate buffer (pH 7.0) to an OD₅₉₀ of 0.155 to give a density of 1×10^7 cfu/mL.

Detached-leaf bioassay. Leaves were removed from actively growing: 1) 'Redhaven' trees, 2) greenhouse-grown regenerant 122-1, 3) plants propagated from 122-1, and 4) plants derived from seedlings of open-pollinated 122-1, and were evaluated for their response to either *X. campestris* pv. *pruni* or *P. syringae* pv. *syringae* using a modified detached-leaf bioassay (Hammerschlag, 1988; Randhawa and Civerolo, 1985). Symptoms of infection with *X. campestris* pv. *pruni*, at each inoculation site, were evaluated 3 weeks after inoculation and were rated on a 0 to 3 scale: 0 = no symptoms; 1 = distinct chlorotic spot and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; and 3 = distinct, dark necrotic spot >2 mm in diameter, with or without a chlorotic halo. Symptoms of infection with *P. syringae* pv. *syringae*, at each inoculated site, were evaluated 7 d after inoculation and were rated on a 0–4 scale. Ratings of 0 to 2 were the same as those for *X. campestris* pv. *pruni*. A rating of 3 = distinct, dark necrotic spot >2 mm, and 4 = distinct necrotic spot with a distinct chlorotic halo. At least three leaves, replicated a minimum of three times, were inoculated per genotype per treatment.

Statistical analyses. Data were analyzed using the Linear Mixed Model procedure of SAS version 6.12 (SAS Institute, 1997). For the response to strains of *Xanthomonas campestris* pv. *pruni*, comparisons were between 'Redhaven' and somaclone 122-1 for all four strains, plus 13 progeny of somaclone 122-1 for strain XP1 only. These models were one way with genotype as the only source of variation and were run separately for each strain. In addition, a combined analysis across all strains was conducted including only the 'Redhaven' and 122-1 genotypes. The model for this analysis was a complete 4×2 factorial of strain by genotype. For *P. syringae* pv. *syringae*, three separate one-way analyses were run comparing genotype for three treatment groups: 1) original regenerant or cultivar, 2) plants derived from the original regenerant after 2 years of micropropagation, and 3) the original regenerant 2 years after transfer to soil in the greenhouse. In addition, a combined analysis was conducted using only the 'Redhaven' TC and 122-1 genotypes and the

three treatment groups. The model for this analysis was a complete 2×3 factorial of genotype by treatment. For several models, the residual variances were found to be heterogeneous. Goodness of fit criteria, provided in the Mixed procedure, were used to partition the residual variances into relatively homogeneous variance groups for the final model. Significant differences between treatment means were based on the LSD test, $P \geq 0.05$.

Results and Discussion

Response to different strains of *X. campestris* pv. *pruni*. Peach plants propagated vegetatively from somaclone 122-1 exhibited a high level of resistance to all strains of *X. campestris* pv. *pruni* and were significantly more resistant to most of these strains than was the parent 'Redhaven' TC (Table 1). Resistance of somaclone 122-1 to non-native strains of *X. campestris* pv. *pruni* suggests that this somaclone may be useful in countries other than the United States.

Response of progeny of somaclone 122-1 to *X. campestris* pv. *pruni*. All progeny of somaclone 122-1 exhibited high levels of resistance to the highly virulent XP1 strain of *X. campestris* pv. *pruni* and all were significantly more resistant to this bacterium than was 'Redhaven' (Table 1). Of the 20 open-pollinated 'Redhaven' progeny evaluated for resistance to *X. campestris* pv. *pruni* in a previous study (Hammerschlag, 1990), 16 were as susceptible, and six were significantly more susceptible to this bacterium than was the parental 'Redhaven'. Other studies (Evans and Sharp, 1983; Larkin et al., 1984) that have compared tissue culture-derived and seed-propagated plants have demonstrated that more variation occurs when plants are regenerated from cell cultures. Most peach cultivars are genetically heterozygous, and although peach is predominantly self-pollinating, considerable outcrossing can occur (Scorza and Okie, 1991). Thus, one would expect segregation for bacterial spot susceptibility among sexually derived

offspring of 'Redhaven' and somaclone 122-1. Although progeny populations were small, the absence of high levels of resistance in 100% of 'Redhaven' progeny (20 seedlings tested) (Hammerschlag, 1990) and the presence of high levels of resistance in all the progeny of 122-1 (13 seedlings tested) suggests that bacterial spot resistance may be controlled by only a few genes.

Response to *P. syringae* pv. *syringae*. Somaclone 122-1 exhibited high levels of resistance to *P. syringae* pv. *syringae* and was significantly more resistant to this pathogen than were 'Redhaven' trees from the field (Table 1, Fig. 1) or 'Redhaven' derived through in vitro propagation and maintained in the greenhouse (Table 2). Continued resistance of 122-1 to *P. syringae* pv. *syringae* after 2 year's growth in the greenhouse and following a 2-year cycle of micropropagation (Table 2) suggests some degree of stability of disease resistance. However, other peach somaclones exhibiting stability of resistance to *X. campestris* pv. *pruni* over time, and after in vitro propagation, have reverted to susceptible reaction types in the field (Hammerschlag et al., 1994). Epigenetic (change in gene expression) variation has also been reported for somaclones of lucerne (*Medicago sativa* L.) (Latunde-Dada and Lucas, 1988) where resistance to Verticillium wilt in the regenerant populations was greater than in the parental controls; however, plants recovered via a second tissue culture cycle reverted to mainly susceptible reaction types. Both studies on peach and lucerne point out the importance of field testing and progeny evaluation in determining the stability and usefulness of somaclonal variation. Preliminary evidence for its resistance to bacterial canker suggests that this somaclone should be evaluated further, in light of the fact that *P. syringae* pv. *syringae* plays a role in premature peach tree mortality or peach tree short life in the southeastern United States (Miller, 1994), and causes crop losses in all commercially grown *Prunus* species in California (Ogawa and English, 1991).

Table 1. Reactions of detached leaves from peach cultivar Redhaven TC, peach somaclonal variant 122-1 and progeny of 122-1 to *Xanthomonas campestris* pv. *pruni* strains XP1, XP21, XP27, and XP31.

Genotype ^a	Avg lesion rating ^z			
	XP1	XP21	XP27	XP31
Redhaven TC	3.0 a ^x	2.9 a	2.3 a	2.6 a
122-1	1.2 b	1.0 b	1.3 a	1.4 b
122-1-SA	0.9 b–d	---	---	---
122-1-SB	1.2 bc	---	---	---
122-1-SC	0.8 c–f	---	---	---
122-1-SD	0.6 ef	---	---	---
122-1-SE	0.4 f	---	---	---
122-1-SF	1.1 bc	---	---	---
122-1-SG	1.1 dc	---	---	---
122-1-SH	0.7 c–f	---	---	---
122-1-SI	0.8 c–f	---	---	---
122-1-SJ	0.5 cd	---	---	---
122-1-SK	1.0 b–f	---	---	---
122-1-SL	0.9 b–d	---	---	---
122-1-SM	1.0 b–d	---	---	---

^aAverage lesion rating 3 weeks after inoculation: 0 = no symptoms; 1 = distinct chlorotic spots/and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; and 3 = distinct, dark necrotic spot >2 mm in diameter, with or without a chlorotic halo.

^bRedhaven TC is greenhouse-grown 'Redhaven' derived from tissue-cultured 'Redhaven' shoots. 122-1-S A-M are the sexual progeny from open-pollinated somaclone 122-1.

^zMean separation within columns by LSD, $P \leq 0.05$.

Fig. 1. Reaction of detached leaves from peach somaclonal variant 122-1 (left) and from a 'Redhaven' tree (right), 7 d after inoculation with *Pseudomonas syringae* pv. *syringae*.

Table 2. Reactions of detached leaves from peach somaclonal variant 122-1 and 'Redhaven' grown in the field or in tissue culture (TC) to infection with *Pseudomonas syringae* pv. *syringae*.^z

Somaclonal variant or cultivar	Original somaclonal variant or cultivar	Greenhouse plants	
		After 2 years of micropropagation	2 years after transfer to soil
Redhaven field	3.2 a ^y	---	---
Redhaven TC ^x	2.8 a	2.8 a	3.0 a
122-1	1.1 b	1.4 b	1.0 b

^zAverage lesion rating 7 d after inoculation: 0 = no symptoms; 1 = distinct chlorotic spot and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; 3 = distinct, dark necrotic spot > 2 mm; and 4 = distinct necrotic spot with a distinct chlorotic halo.

^yMean separation within columns by LSD, $P \leq 0.05$.

^xRedhaven TC is greenhouse-grown 'Redhaven' derived from tissue-cultured 'Redhaven' shoots.

In conclusion, this study provides strong evidence that: 1) variation induced by tissue culture is a viable approach to generating useful genetic variation in peach; 2) somaclone 122-1 provides a source of bacterial spot resistant germplasm for breeding and a potential source of germplasm resistant to bacterial canker; and 3) this somaclone should be evaluated further for its potential as a new peach cultivar. Although tissue culture of peach may be neither faster nor easier than breeding for disease-resistant germplasm, it has provided a means of obtaining high levels of resistance to bacterial leaf spot, combined with high fruit quality (data not shown), which has been difficult to obtain thus far through conventional breeding (Werner et al., 1986).

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