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Screening Peaches in Vitro for Resistance to *Xanthomonas campestris* pv. *pruni*

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Abstract. **A wick bioassay was developed to screen peach [*Prunus persica* (L.) Batsch] shoot cultures for resistance to bacterial spot [*Xanthomonas campestris* pv. *pruni* (E. F. Sm.) Dows)]. Three weeks after inoculation, the number of colony forming units (CFU) from 1-cm stem sections excised from highly susceptible cultivars was significantly greater than CFU from several resistant cultivars. Neither growth regulators nor the length of time that shoots were maintained in vitro prior to inoculation significantly influenced the response of these cultivars to bacterial leaf spot. This technique should be useful in screening for somaclonal variants obtained from peach cell cultures.**

Bacterial leaf spot is a serious disease where peaches are grown in warm humid environments. Chemical control is costly and often ineffective, and the use of resistant cultivars is suggested in areas where the disease is prevalent. Werner et al. (14) recently reported that during a severe epiphytotic of this disease, several previously reported highly resistant cultivars (3) exhibited only moderate resistance. In addition, none of the 58 plant introductions evaluated exceeded the level of resistance currently in commercial cultivars. The scarcity of germplasm with high resistance suggests that approaches other than conventional breeding need to be undertaken to obtain highly resistant peach germplasm.

Tissue culture techniques have been used successfully to obtain disease-resistant plants (2, 5). Ideally, potential variant plants produced in vitro should be screened in vitro because large numbers can be evaluated in a limited amount of space and, once identified, a variant can be rapidly micropropagated. However, in vitro screening is not recommended if the response of a plant to the pathogen in vitro does not correlate with the response in vivo (1, 5).

Because it has been demonstrated that altering the plant growth regulator level in a tissue culture medium can alter the response of a plant to a pathogen (10) or pest (11) in vitro, and that duration in culture can alter such responses as rooting (8, 13), these factors also should be evaluated as a prerequisite to determining the feasibility of screening in vitro.

The objectives of this research were to: a) examine the response of in vitro-propagated shoots of leaf spot-resistant and -susceptible peach cultivars to bacterial leaf spot, b) compare the response of peaches to bacterial leaf spot in vitro with what occurs in the field c) determine if the response of peach shoots to bacterial leaf spot is influenced by either the presence or absence of plant growth regulators in the tissue culture medium or the length of time that shoots are maintained in vitro prior to inoculation, and d) determine the feasibility of screening peaches in vitro for leaf spot resistance.

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Materials and Methods

Plant material. In vitro-propagated shoots, maintained on shoot elongation medium (4), were used for all inoculation studies. For most studies, shoots grown in vitro for 6 months were used. For studies on the effects of duration in vitro on response of shoots to bacterial leaf spot, shoots grown in vitro for at least 12 months also were used. Shoots were transferred to fresh medium every 3 weeks during the culture period and were transferred to fresh tissue culture medium 1 week prior to inoculation.

For studies on the effects of growth regulators on the response of shoots to bacterial leaf spot, shoots were also placed on elongation medium without growth regulators [*N*-(phenylmethyl)-1*H*-purin-6-amine (BA) and 1*H*-indole-3-butyric acid (IBA)] 1 week prior to inoculation and kept on this medium for 3 weeks after inoculation.

Wick bioassay. Standard inoculum (SI) of peach pathogen *X. c.* pv. *pruni* strain XPI (highly virulent) and geranium pathogen *X. c.* pv. *pelargonii* strain L125 (nonpathogenic to peaches) containing $\approx 2 \times 10^8$ colony-forming units (CFU)/ml of 0.05 M phosphate buffer (pH 6.8) were prepared and maintained as previously described (6, 9). For inoculations, the SI was diluted with buffer to 2×10^4 CFU/ml. Sterile cotton thread, threaded through a sterile tapestry needle, was dipped into diluted SI or phosphate buffer (control) and then inserted and pulled through the stem of a peach shoot, leaving a 1-cm thread segment within the stem (Fig. 1). The thread was inserted halfway up the stem to prevent its touching the agar when the shoot was placed back into the tissue culture medium. At weekly intervals after inoculation, shoots were removed from the medium and 1-cm stem sections around the point of inoculation were excised. These sections were ground with a mortar and pestle in 10 ml of phosphate buffer (same as above) and 10-fold dilutions were made. From several dilutions, 0.1 ml was removed and plated onto 15 × 100 mm petri dishes containing 2.3% Difco nutrient agar supplemented with 0.5% NaCl and 0.2% dextrose. Petri dishes were incubated at room temperature for 3 days, after which colonies were counted and CFU per stem section determined. Inoculations were replicated a minimum of 3 times, with 3 shoots inoculated per cultivar per replicate.

Results

Cultivar differences. The response of in vitro-propagated peach shoots to bacterial spot can be seen in Table 1. At 1 and 2 weeks after inoculation, there were no significant differences

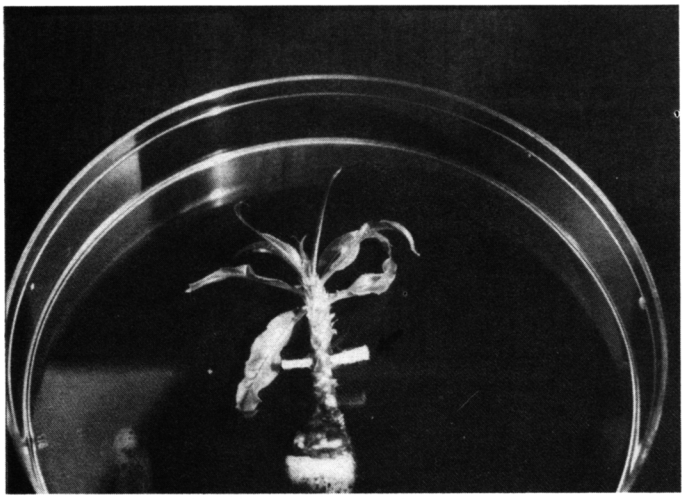

Fig. 1. Peach shoots containing wick (arrow) that was dipped in a suspension of *Xanthomonas campestris* pv. *pruni* containing 1 × 10^4 colony forming units/ml.

among the number of CFU isolated from resistant cultivars when compared with susceptible cultivars. At 3 weeks after inoculation, the number of CFU in stem segments from leaf spot-resistant cultivars was significantly lower than in segments from susceptible cultivars. The number of CFU of the nonpathogen, *X. c.* pv. *pelargonii*, in stem segments from leaf spot-susceptible 'Suncrest' was significantly lower than the number of CFU of *X. c.* pv. *pruni* in any of the leaf spot-resistant or -susceptible cultivars at 2 and 3 weeks after inoculation. No bacteria were isolated from leaf spot-susceptible 'Suncrest' inoculated with phosphate buffer.

Deleting growth regulators. The effect of eliminating BA and IBA from the tissue culture medium on the response of peach shoots to *X. c.* pv. *pruni* can be seen in Table 2. There were no significant differences among the number of CFU from shoots on medium with growth regulators compared with the number of CFU from shoots on media without growth regulators. Regardless of the presence or absence of growth regulators in the tissue culture medium, the number of CFU from leaf spot-susceptible 'Sunhigh' were significantly greater than from leaf spot-resistant 'Redskin'.

Length of time in vitro. The effect of length of time in vitro on response of peach shoots to bacterial leaf spot can be seen in Table 3. The number of CFU from a cultivar maintained in vitro for 6 months prior to inoculation was similar to that from the same cultivar maintained in vitro for at least 12 months. Regardless of length of time shoots were maintained in vitro prior to inoculation, the number of CFU from resistant cultivars was significantly less than that from susceptible cultivars.

Discussion

Since it has been reported that peach plants can be regenerated from cell cultures (7), it is essential that reliable screening procedures be developed to identify somaclonal variants. For tissue culture screening to be useful, the results must relate to what occurs at the whole plant level in nature (1, 5). Once a variant is identified, it can be propagated rapidly in vitro. Somaclonal variation can be evaluated in the greenhouse, but, in doing so, there is the risk of losing a clone during rooting or acclimatization, and much time can be lost if the plant enters dormancy once placed in the greenhouse. In addition, greenhouse screening requires space, and may also be subject to genetic × environmental interaction.

These results demonstrate that resistance and susceptibility of peach cultivars to bacterial leaf spot exhibited in the field (3, 14) can be demonstrated in vitro. The number of CFU from resistant cultivars was consistently 10 times lower than that from susceptible cultivars. Counting CFU did not help distinguish between highly resistant and resistant cultivars, or between highly susceptible and susceptible cultivars; however, field evaluations by different researchers (3, 14) suggest that degrees of resistance and susceptibility may not be clear-cut. The response to nonpathogen *X. c.* pv. *pelargonii* should be noted. The number of CFU of *X. c.* pv. *pelargonii* from leaf spot-susceptible 'Suncrest' was significantly less than the number of CFU of pathogen *X. c.* pv. *pruni* from each of the resistant cultivars tested. These data suggest that the level of resistance to *X. c.* pv. *pruni* may not be very high. These observations correlate well with a recent report by Werner et al. (14) for the field response of peaches

Table 1. Number of colony forming units (CFU) of *Xanthomonas campestris* pv. *pruni* in peach shoot cultures at 1, 2, and 3 weeks after inoculation.

Cultivar	Field resistance[z] to *X. c.* pv. *pruni*	CFU/stem section[y] Weeks after inoculation 1	2	3
Suncrest	HS (3, 14)	1.7 × 10^7 a[w]	8.0 × 10^4 b	8.6 × 10^4 c
Suncrest	HS (3, 14)	3.5 × 10^7 a	2.9 × 10^8 a	2.1 × 10^9 a
Sunhigh	HS (3, 14)	2.5 × 10^7 a	2.5 × 10^8 a	1.5 × 10^9 a
Jerseyqueen	S (3, 14)	---[v]	---	2.7 × 10^9 a
Rio Oso Gem	S (3)	---	---	1.9 × 10^9 a
Redskin	HR (3) MR (14)	2.5 × 10^7 a	1.5 × 10^8 a	1.2 × 10^8 b
Redhaven	R (3) MR (14)	1.5 × 10^7 a	1.5 × 10^8 a	1.9 × 10^8 b
Nemaguard	HR (3) MR (14)	---	---	1.7 × 10^8 b

[z]Field resistance previously recorded (3, 14). HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, and HS = highly susceptible.
[y]Inoculum concentration was 1 × 10^4 CFU/ml.
[x]Inoculated with *Xanthomonas campestris* pv. *pelargonii* strain L-126 (1 × 10^4 CFU/ml).
[w]Data were analyzed by analysis of variance. Statistics were performed on $\log_{10}$-transformed data. Mean separation in columns by Duncan's multiple range test, 5% level. Means were back-transformed for presentation.
[v]Not sampled.

Table 2. Effect of deleting growth regulators from tissue culture medium on the number of colony forming units (CFU) of *Xanthomonas campestris* pv. *pruni* in peach shoots 3 weeks after inoculation.

Cultivar	Field resistance[z]	Presence (+)/ absence (−) of BA and IBA	CFU/stem section[y]
Sunhigh	HS (1)	+	1.5×10^9 a[x]
		−	1.4×10^9 a
Redskin	HR (1), MR (11)	+	1.2×10^8 b
		−	1.4×10^8 b

[z]Field resistance previously recorded (3, 14). HR = highly resistant, MR = moderately resistant, and HS = highly susceptible.
[y]Inoculum concentration was 1×10^4 CFU/ml.
[x]Data were analyzed by analysis of variance. Statistics were performed on $\log_{10}$-transformed data. Mean separation by Duncan's multiple range test, 5% level. Means back-transformed for presentation.

Table 3. Effect of length of time in vitro prior to inoculation on the number of colony forming units (CFU) of *Xanthomonas campestris* pv. *pruni* in peach shoots 3 weeks after inoculation.

Cultivar	Field[z] resistance	CFU/stem section[y] Length of time in vitro (months) 6	12
Jerseyqueen	S (3)	2.7×10^9 a[x]	2.2×10^9 a
Suncrest	HS (3)	2.1×10^9 a	1.8×10^9 ab
Sunhigh	HS (3)	1.5×10^9 b	6.5×10^8 b
Redhaven	R (3), MR (14)	1.9×10^8 c	5.2×10^7 c
Redskin	HR (3), MR (14)	1.2×10^8 c	2.5×10^8 c

[z]Field resistance previously recorded (3, 14). HR = highly resistant, R = resistant, MR = moderately resistant, S = susceptible, and HS = highly susceptible.
[y]Inoculum concentration was 1×10^4 CFU/ml.
[x]Data were analyzed by analysis of variance. Statistics were performed on $\log_{10}$-transformed data. Mean separation of all 12 means by Duncan's multiple range test, 5% level. Means were back-transformed for presentation.

to *X. c.* pv. *pruni*. Such observations demonstrate the need for additional approaches, besides conventional breeding, for obtaining high levels of resistance to *X. c.* pv. *pruni*.

Shoot cultures used for propagation purposes are generally maintained on several culture media (12), each with different levels of growth regulators, and can remain in vitro for long periods of time while being transferred from one medium to another. In previous reports, growth regulator levels (10, 11) and length of time shoots were maintained in vitro (8) altered specific physiological responses. In this study, the number of CFU isolated from shoot cultures was not influenced by the presence or absence of growth regulators in the tissue culture medium or by the length of time shoots were maintained in vitro. The number of CFU from resistant cultivars was consistently less than the number from susceptible cultivars.

In conclusion, the wick bioassay in a reliable, quantitative assay that correlates well with field performance and should thus be a useful screening system for identifying new germplasm with leaf spot resistance.

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