Resistance Responses of Plants Regenerated from Peach Callus, Cultures to \textit{Xanthomonas campestris pv. pruni}

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\textbf{Abstract.} A detached-leaf bioassay was used to evaluate peach [\textit{Prunus persica} (L.) Batsch] regenerants derived from zygotic embryo callus cultures of cultivars Sunhigh (susceptible to leaf spot) and Redhaven (moderately resistant to leaf spot) for resistance to \textit{Xanthomonas campestris pv. pruni} [(E.F. Sm.) Dews], the causal agent of bacterial leaf spot. Regenerants obtained from calli produced on two ‘Sunhigh’ embryos, #61 and #156, and on three ‘Redhaven’ embryos were evaluated. Sixty-four percent of the regenerants derived from ‘Sunhigh’ embryo #61 demonstrated significantly greater spot resistance than ‘Sunhigh’. Regenerants with resistance greater than ‘Redhaven’ were also obtained from both ‘Sunhigh’ embryos. The frequency of variation in the ‘Sunhigh’ seedling population, with respect to the response to bacterial leaf spot, was not so great as that exhibited by the regenerants derived from ‘Sunhigh’ embryo #156. None of the ‘Redhaven’ seedlings or any of the regenerants derived from ‘Redhaven’ embryos were more resistant than ‘Redhaven’. These studies suggest that the frequency of somaclonal variation is genetically determined and that screening for somaclonal variation may be a feasible approach to obtaining leaf spot-resistant peach plants.

The recommended approach for controlling diseases of fruit trees is to breed for resistant cultivars (Dayton et al., 1983). However, the germplasm base is quite narrow for most commercial peach cultivars grown in the United States (Okie et al., 1985; Scorza et al., 1985) and resistance to many pathogens and pests is either nonexistent (Cochran, 1975; Okie and Reilly, 1983; Petersen, 1975) or, as in the case of bacterial leaf spot, only moderate (Werner et al., 1986). This information suggests that approaches other than conventional breeding are needed to obtain disease-resistant cultivars. Tissue culture techniques, i.e., selecting at the cellular level or screening at the whole plant level for somaclonal variation, have been used to obtain disease-resistant crop species (Daub, 1986; Hammerschlag, 1984). Somaclonal variants of peach with leaf spot resistance have been obtained by selecting cells for insensitivity to a toxic culture filtrate of \textit{X. campestris pv. pruni} and regenerating resistant plants from these cells (Hammerschlag, 1988). A brief report was also presented recently on obtaining leaf spot and bacterial canker-resistant peach variants by screening unselected regenerants (Hammerschlag and Ognjanov, 1990). The objective of this research was to determine if peach plants with moderate to high levels of leaf spot resistance could be obtained by screening regenerants from unselected cell cultures derived from susceptible and moderately resistant cultivars.

\textbf{Materials and Methods}

\textbf{Plant material.} Field-grown ‘Sunhigh’ (susceptible to leaf spot) and ‘Redhaven’ (moderately resistant to leaf spot) peach trees were used as a source of seedlings, immature embryos, and axillary shoots. All trees were evaluated for response to \textit{X. c. pv. pruni} by a modified detached-leaf bioassay (Hammerschlag, 1988; Randhawa and Civerolo, 1985) and the responses of all trees (unpublished data) correlated well with responses of these cultivars under field conditions (Werner et al., 1986). Greenhouse-grown ‘Sunhigh’ and ‘Redhaven’ plants produced by micropropagation (Hammerschlag et al., 1987) from axillary shoots, as well as plant regenerants and seedlings described below, were used to supply leaves for the detached-leaf bioassay.

\textbf{Culture and screening.} Highly regenerative callus and multiple plant regenerants were obtained from immature ‘Sunhigh’ embryos #61 and #156 and from immature ‘Redhaven’ embryos #30, #46, and #122 by a method described by Hammerschlag et al. (1985). Regenerants were removed from calli that were subculture (every 3 weeks) three to nine times. The same procedure was used to produce single regenerants from individual ‘Sunhigh’ embryos except that calli were subculture three to five times. Following regeneration, shoots were rooted, acclimatized (Hammerschlag et al., 1987), and grown in the greenhouse. Peach seedlings were obtained from open-polli nated ‘Sunhigh’ and ‘Redhaven’ trees. Seeds from mature fruit were surface-sterilized in 0.5% (w/v) sodium hypochlorite, washed three times in sterile distilled water and plated onto half-strength Murashige and Skoog (1962) salts medium supplemented with 0.6% Phytagar (GIBCO, Grand Island, N.Y.). Seeds were incubated in darkness at 4C for 2 to 3 months, and then placed at 25C under a 16-hr photoperiod provided by cool-white fluorescent lights at 100 \textmu mol-s\textsuperscript{-1}\textcdot m\textsuperscript{2}. When seedlings were 4 to 6 cm high, they were transferred to the greenhouse. About 2 months after transfer to the greenhouse, actively growing regenerants and seedlings were evaluated for their response to \textit{X. campestris pv. pruni} using a modified detached-leaf bioassay (Hammerschlag, 1988; Randhawa and Civerolo, 1985). Symptoms of infection with \textit{X. campestris pv. pruni} were evaluated at each inoculated site 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 greyish-white lesion < 2 mm in diameter, and 3 = distinct, dark necrotic spot > 2 mm in diameter, with or without...
plant level for somaclonal variants with increased levels of bac-

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chromatosis halo. Three leaves, replicated a minimum of three
times, were inoculated per clone or cultivar. Analysis of vari-
ance was performed on data and means were separated by Fish-
er’s least significant difference test.

**Results**

Sixty-four percent of the regenerants from ‘Sunhigh’ embryo
#156 were significantly more spot-resistant than ‘Sunhigh’; of
these, 9% were more resistant than ‘Redhaven’. Only 13% of
the regenerants from ‘Sunhigh’ embryo #61 were significantly
more resistant than ‘Sunhigh’ and none were more resistant than
‘Redhaven’ (Table 1). None of the regenerants derived from
‘Redhaven’ embryos were more resistant than ‘Redhaven’ (Ta-
ble 2). Of the ‘Sunhigh’ seedlings, 39% were significantly more
resistant than ‘Sunhigh’ and 3% were more resistant than ‘Red-
haven’ (Table 3). None of the ‘Redhaven’ seedlings were more
resistant than ‘Redhaven’. Variance components calculated for
the response of peach regenerants to *X. campestris* pv.
pruni.

Average lesion rating 3 weeks after inoculation: O = no symptoms;
1 = distinct chlorotic spots; 2 = distinct but pale necrotic spot
or greyish-white lesion < 2 mm in diameter; and 3 = distinct, dark
necrotic spot > 2 mm in diameter, with or without chlorotic halo.

Values followed by the same letter do not differ significantly (P =
0.05) according to Fisher’s *LSD* test.

| Regenerant No. of | Average Regenerant No. of | Average Regenerant lesion rating** or cultivar | Average Regenerant lesion rating** or cultivar | Average Regenerant lesion rating** or cultivar |
|-------------------|---------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| or cultivar subcultures | | | | |
| Sunhigh | --- | 2.4 bc | Sunhigh | --- | 2.4 abc |
| Redhaven | --- | 1.6 bc | Redhaven | --- | 1.6 de |
| 156-12 | 3 | 1.6 6 | 156-10 | 6 | 1.1 d |
| 156-11 | 8 | 1.6 9 | 156-9 | 6 | 1.5 de |
| 156-5 | 5 | 1.6 8 | 156-17 | 8 | 1.6 cd |
| 156-6 | 5 | 1.6 8 | 156-6 | 6 | 1.6 cd |
| 156-7 | 5 | 1.6 7 | 156-6 | 7 | 1.6 ce |
| 156-13 | 7 | 1.6 7 | 156-12 | 8 | 1.6 bc |
| 156-1 | 3 | 1.6 9 | 156-4 | 8 | 1.6 ab |
| 156-9 | 5 | 1.6 2 | 156-2 | 6 | 2.6 ab |
| 156-12 | 3 | 1.6 8 | 156-13 | 8 | 2.6 ab |
| 156-10 | 4 | 1.6 4 | 156-3 | 7 | 2.6 a |
| 156-5 | 7 | 1.6 5 | 156-1 | 7 | 2.6 a |

**Average lesion rating 3 weeks after inoculation:** O = no symptoms; 1 =
distinct chlorotic spots; 2 = distinct but pale necrotic spot or
greyish-white lesion < 2 mm in diameter; and 3 = distinct, dark necrotic
spot > 2 mm in diameter, with or without chlorotic halo.

Values followed by the same letter do not differ significantly (P =
0.05) according to Fisher’s *LSD* test.

**Discussion**

These results suggest that it is possible to screen at the whole
plant level for somaclonal variants with increased levels of bac-

terial spot resistance. In vitro selection methods to obtain bac-
terial spot resistance in peach will only be effective if a selective
agent that is involved in disease development and acts at the
cellular level is available. An advantage to this method is that
very large populations of cells ( > 10^6) can be screened at one
time. A much simpler approach, but one in which only limited
numbers can be evaluated, is to regenerate plants and screen
them at the whole plant level. This approach has been used to
take obtain disease resistance in alfalfa (Hartman et al., 1984; La-
tunde-Dada and Lucas, 1983), maize (Brettell and Thomas, 1980),
sugar cane (Krishnamurthi and Tlaskal, 1974), and tomato (Bar-
den et al., 1986), and, in some cases, the resistance was shown
to be heritable (Barden et al., 1986; Brettell and Thomas, 1980;
Hartman et al., 1984; Krishnamurthi and Tlaskal, 1974; Sac-
rayan, 1982). In all cases, populations of < 500 were screened.
Isolation of mutants from plant tissue cultures without applying
any selection pressure can be achieved when small populations are
used because growing cells in vitro yields a high frequency
of somaclonal variants that can express agriculturally useful traits
(Larkin and Scowcroft, 1981). The frequency of variation has
been estimated to be as high as 30% to 40% for the number of
plants showing some type of variation, and from 0.2% to almost
3% for variation in a single trait (Evans et al., 1984; Irvine,
1984; Lorz and Scowcroft, 1983; Zong-xiu et al., 1983). In the
present study, much greater than 3% variation was observed in
regenerants derived from embryos #156 and #61, but whether this
variation is heritable is unknown. Also unknown is whether
these variants are leaf spot-resistant or -susceptible because the
genotype of each embryo could not be determined. ‘Sunhigh’
seedlings have been reported to be consistently spot susceptible
(Civerolo, 1975), but the results reported here demonstrate that a
‘Sunhigh’ seedling population may contain individuals with
spot resistance. Therefore, it cannot be assumed that the spot-
susceptible plants are the variants in each set of regenerants. What
can be said from the present study is that considerable variation
in response to *X. campestris* pv. *pruni* was observed in regen-
erants.

Although some somaclonal variability has been shown to be
due to pre-existing mutations in the cells of the explant (Lorz
and Scowcroft, 1983; Orton, 1984), a large part of the variation is
induced during the cell culture cycle and is attributed to chro-

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Table 3. Reaction of detached leaves from two peach cultivars, regenerants from nine ‘Sunhigh’ embryos, and ‘Sunhigh’ and ‘Redhaven’ seedlings to Xanthomonas campestris pv. pruni.

| Regenerant or cultivar | Average lesion rating | Cultivar or ‘Sunhigh’ seedling (S) | Average lesion rating | ‘Sunhigh’ seedlings continued | Average lesion rating | Cultivar or ‘Redhaven’ seedling (S) | Average lesion rating |
|------------------------|-----------------------|-----------------------------------|-----------------------|-------------------------------|-----------------------|-----------------------------------|----------------------|
| Sunhigh                | 2.40                  | Sunhigh                           | 2.40                  | S-8                           | 2.08                  | Sunhigh                           | 2.40                 |
| Redhaven              | 1.60                  | Redhaven                          | 1.60                  | S-5                           | 2.10                  | Redhaven                          | 1.60                 |
| 19-3                  | 0.90                  | S-20                              | 1.10                  | S-6                           | 2.13                  | S-22                              | 1.40                 |
| 13-1                  | 1.57                  | S-3                               | 1.25                  | S-9                           | 2.20                  | S-19                              | 1.60                 |
| 31-1                  | 1.67                  | S-22                              | 1.38                  | S-19                          | 2.18                  | S-13                              | 1.63                 |
| 130-1                 | 1.70                  | S-23                              | 1.50                  | S-25                          | 2.20                  | S-1                               | 1.70                 |
| 107-2                 | 2.23                  | S-18                              | 1.58                  | S-24                          | 2.20                  | S-4                               | 1.73                 |
| 73-1                  | 2.27                  | S-7                               | 1.68                  | S-30                          | 2.23                  | S-10                              | 1.75                 |
| 12-1                  | 2.37                  | S-13                              | 1.70                  | S-31                          | 2.23                  | S-18                              | 1.80                 |
| 114-1                 | 2.40                  | S-17                              | 1.73                  | S-34                          | 2.40                  | S-9                               | 1.83                 |
| 74-2                  | 2.60                  | S-10                              | 1.78                  | S-35                          | 2.43                  | S-21                              | 1.90                 |
|                       |                       | S-11                              | 1.80                  | S-27                          | 2.48                  | S-17                              | 2.00                 |
|                       |                       | S-4                               | 1.80                  | S-26                          | 2.55                  | S-14                              | 2.00                 |
|                       |                       | S-15                              | 1.93                  | S-39                          | 2.55                  | S-7                               | 2.05                 |
|                       |                       | S-16                              | 1.95                  | S-37                          | 2.58                  | S-8                               | 2.10                 |
|                       |                       | S-1                               | 2.00                  | S-32                          | 2.60                  | S-3                               | 2.20                 |
|                       |                       | S-14                              | 2.05                  | S-36                          | 2.75                  | S-15                              | 2.20                 |
|                       |                       | S-21                              | 2.08                  | S-38                          | 2.78                  | S-24                              | 2.35                 |
|                       |                       |                                   |                       |                               |                       |                                   |                      |
|                       |                       |                                   |                       |                               |                       |                                   |                      |

Lesion rating 3 weeks after inoculation: 0 = no symptoms; 1 = distinct chlorotic spots; 2 = distinct but pale necrotic spot or greyish-white lesion <2 mm in diameter; and 3 = distinct, dark necrotic spot >2 mm in diameter, with-or-without chlorotic halo.

LSD value at 0.05 = 0.60.
LSD value at 0.05 = 0.42.
LSD value at 0.05 = 0.54.

Table 4. Components of variance for the response of peach regenerants derived from ‘Sunhigh’ embryos and ‘Sunhigh’ seedlings to Xanthomonas campestris pv. pruni.

| Variance component | Var. regenerants or seedlings | Var. error |
|-------------------|-------------------------------|------------|
| #156 regenerants  | 0.32                          | 0.04       |
| #61 regenerants   | 0.17                          | 0.12       |
| Single regenerants from Sunhigh embryos | 0.28 | 0.13 |
| Sunhigh seedlings | 0.15                          | 0.08       |

mosomal changes (Bayliss, 1980; Evans et al., 1984; Lapitan et al., 1984; Orton, 1984). It is clear from the studies reported in this paper that variation was generated during the cell cycle since each series of regenerants was pedigree-related, i.e., each set came from the same embryo. However, the genetic nature of the variation still needs to be determined. Because ploidy changes are common but generally undesirable (Larkin and Scowcroft, 1981), I used for these studies highly morphogenic calli whose cells were uniformly diploid, 2n = 2x = 16, through six subcultures (Hammerschlag and Bauchan, 1984), and determined that the ploidy level of peach regenerants from ‘Sunhigh’ embryos was diploid, 2n = 2x = 16 (Hammerschlag et al., 1985).

The frequency of variation observed within different sets of multiple regenerants derived from single embryos (#156 series vs. #61 series) was significantly different, which suggests that the frequency of variation is genotype-dependent. Other studies have also shown that the genotype of the plant can affect the amount of variability that occurs as a consequence of culturing tissues in vitro (Lorz, 1984; McCoy et al., 1982). The above suggests that in studies to obtain somaclonal variation, explants with different genetic backgrounds should be used.

The frequency of variation among regenerants derived from a single embryo (#156 series), as well as among single regenerants derived from different ‘Sunhigh’ embryos, was double that observed in a ‘Sunhigh’ seedling population. Previous studies that have compared tissue culture-derived and seed-propagated plants have demonstrated that more variation occurs when plants are regenerated from culture (Evans and Sharp, 1983; Larkin et al., 1984).

The frequency of variation did not correlate with duration of in vitro culture. Although numerous studies have reported that gross karyotype changes in cell cultures increase with length of in vitro culture (Scowcroft and Larkin, 1985), Ogihara (1981) reported that karyotype changes in regenerants are much reduced relative to those observed in cell culture and that these types of changes are selected against during regeneration.

In conclusion, this study, together with in vitro mutant selection studies (Hammerschlag, 1988), provides some evidence that regenerating plants from either selected or unselected cell cultures may be a feasible approach for obtaining much-needed variation in peach. Yet to be determined, and critical to establishing this as a definitive approach, is to determine the stability and heritability of spot resistance. Somatic cell hybridization and gene transfer techniques represent two other useful tissue-culture approaches to obtain variation, but both still need to be adapted for peach.

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