

Resistance Responses of Plants Regenerated from Peach Callus, Cultures to *Xanthomonas campestris* pv. *pruni*

F.A. Hammerschlag

U.S. Department of Agriculture, Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD 20705

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

Abstract. A detached-leaf bioassay was used to evaluate peach [*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 *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 #156 and 13% 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 *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.

Materials and Methods

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

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.

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-pollinated '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 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. 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 *X. campestris* pv. *pruni* using a modified detached-leaf bioassay (Hammerschlag, 1988; Randhawa and Civerolo, 1985). Symptoms of infection with *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

Received for publication 22 Nov. 1989. Mention of a trademark, proprietary product, or vendor by the U.S. Dept. of Agriculture does not imply its approval to the exclusion of other products or vendors that may also be suitable. I thank Edith Huang and Adrian Goldstein for their excellent technical assistance and Marla McIntosh for assistance with the statistical analyses. 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.

Table 1. Reaction of detached leaves from two peach cultivars and from regenerants derived from 'Sunhigh' embryos #156 and #61 to *Xanthomonas campestris* pv. *pruni*.

Regenerant No. of or cultivar subcultures	Average lesion rating ^{xy}	Regenerant or cultivar	No. of subcultures	Average lesion rating ^{xy}
Sunhigh	---	Sunhigh	---	2.4 abc
	2.4 bc	Redhaven	---	1.6 de
Redhaven	---	61-10	6	1.1 e
	1.6 efg	61-9	6	1.5 de
		61-14	8	1.8 cd
156-12	3	61-8	6	2.1 bcd
156-11	8	61-16	9	2.2 abc
156-5	5	61-17	5	2.2 abc
156-6	5	61-6	6	2.2 abc
156-7	5	61-7	4	2.3 abc
156-13	7	61-12	8	2.4 abc
156-1	3	61-4	5	2.6 ab
156-9	5	61-1	8	2.6 ab
156-4	5	61-2	3	2.6 ab
156-8	3	61-13	8	2.6 ab
156-10	4	61-3	4	2.7 ab
	2.9 a	61-5	7	2.7 a

^xAverage 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.

^yValues followed by the same letter do not differ significantly ($P = 0.05$) according to Fisher's LSD test.

chlorotic halo. Three leaves, replicated a minimum of three times, were inoculated per clone or cultivar. Analysis of variance was performed on data and means were separated by Fisher'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' (Table 2). Of the 'Sunhigh' seedlings, 39% were significantly more resistant than 'Sunhigh' and 3% were more resistant than 'Redhaven' (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* (Table 4) indicate that the frequency of variation among regenerants from embryo #156, as well as the frequency of variation among single regenerants from different 'Sunhigh' embryos, were double the frequency of variation among 'Sunhigh' seedlings. The frequency of variation observed within different sets of multiple regenerants derived from single embryos (#156 set vs. #61 set) was significantly different. Regenerants with greater leaf spot resistance than 'Sunhigh' were obtained regardless of the duration of in vitro culture. In addition, the number of regenerants with increased levels of leaf spot resistance did not correlate with the number of subcultures.

Discussion

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

Table 2. Reaction of detached leaves from two peach cultivars and from regenerants derived from 'Redhaven' embryos #30, #46, and #122 to *Xanthomonas campestris* pv. *pruni*.

Regenerant or cultivar	Average lesion rating ^{xy}	Regenerant or cultivar	Average lesion rating ^{xy}	Regenerant or cultivar	Average lesion rating ^{xy}
Sunhigh	2.4 a	Sunhigh	2.4 ab	Sunhigh	2.4 ab
Redhaven	1.6 bc	Redhaven	1.6 C	Redhaven	1.6 bc
30-1	1.6 c	46-3	1.9 bc	122-1	0.9 c
30-3	1.7 bc	46-2	2.3 ab	122-3	2.4 a
30-5	1.8 bc	46-4	2.3 ab	122-2	2.5 a
30-4	2.1 abc	46-1	2.3 ab		
30-8	2.2 ab	46-6	2.4 ab		
30-2	2.3 a	46-8	2.5 ab		
30-7	2.4 a	46-7	2.6 a		
30-6	2.5 a	46-5	2.7 a		

^xLesion 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.

^yValues followed by the same letter do not differ significantly ($P = 0.05$) according to Fisher's LSD test.

terial spot resistance. In vitro selection methods to obtain bacterial 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 obtain disease resistance in alfalfa (Hartman et al., 1984; Latunde-Dada and Lucas, 1983), maize (Brettell and Thomas, 1980), sugarcane (Krishnamurthi and Tlaskal, 1974), and tomato (Barden 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; Sacristan, 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-resistant 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 regenerants.

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-

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 ^{z,y}	Cultivar or 'Sunhigh' seedling (S)	Average lesion rating ^{z,x}	'Sunhigh' seedlings continued	Average lesion rating ^{z,x}	Cultivar or 'Redhaven' seedling (S)	Average lesion rating ^{z,w}
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-12	1.70	S-31	2.23	S-18	1.80
114-1	2.40	S-13	1.73	S-34	2.40	S-9	1.83
74-2	2.60	S-17	1.78	S-35	2.43	S-21	1.90
		S-10	1.78	S-2	2.45	S-6	1.95
		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
						S-2	2.38
						S-5	2.48
						S-23	2.48

^zLesion 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.

^yLSD value at 0.05 = 0.60.

^xLSD value at 0.05 = 0.42.

^wLSD 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*.

Class	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 Baughan, 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), Ogi-hara (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.

Literature Cited

- Barden, K. A., S.S. Smith, and H.H. Murakishi. 1986. Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. *Plant Sci.* 45:209-213.
- Bayliss, M.W. 1980. Chromosomal variation in plant tissues in culture. *Intl. Rev. Cytol. Suppl.* 11A:113-144.
- Brettell, R.I.S. and E. Thomas. 1980. Reversion of Texas male-sterile cytoplasm maize in culture to give fertile T-toxin resistant plants. *Theor. Applied Genet.* 58:55-58.
- Civerolo, E.L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. *Phytopathology* 65:258-264.
- Cochran, L.C. 1975. Viruses, p. 363-366. In: N.F. Childers (ed.). *The peach*. Horticultural Publications, New Brunswick, N.J.
- Daub, M.E. 1986. Tissue culture and the selection of resistance to pathogens. *Annu. Rev. Phytopathol.* 24:159-186.
- Dayton, D. F., R.L. Bell, and E.B. Williams. 1983. Disease resistance, p. 189-215. In: J.N. Moore and J. Janick (eds.). *Methods in fruit breeding*. Purdue University Press, West Lafayette, Ind.
- Evans, D.A. and W.R. Sharp. 1983. Single gene mutations in tomato plants regenerated from tissue culture. *Science* 221:949-951.
- Evans, D. A., W.R. Sharp, and H.P. Medina-Filho. 1984. Somaclonal and gametoclonal variation. *Amer. J. Bot.* 71:759-774.
- Hammerschlag, F.A. 1984. In vitro approaches to disease resistance, p. 453-490. In: G.B. Collins and J.G. Petolino (eds.). *Applications of genetic engineering to crop improvement*. Martinus Nijhoff/Junk, Dordrecht, Netherlands.
- Hammerschlag, F.A. 1988. Selection of peach cells for insensitivity to culture filtrates of *Xanthomonas campestris* pv. *pruni* and regeneration of resistant plants. *Theor. Applied Genet.* 76:865-869.
- Hammerschlag, F.A. and G. Bauchan. 1984. Genetic stability of callus cells derived from peach embryos. *HortScience* 19:554.
- Hammerschlag, F.A. and V. Ognjanov. 1990. Somaclonal variation in peach: screening for resistance to *Xanthomonas campestris* pv. *pruni* and *Pseudomonas syringae* pv. *syringae*. *Acts Hort.* (In press.)
- Hammerschlag, F. A., G. Bauchan, and R. Scorza. 1985. Regeneration of peach plants from callus derived from immature embryos. *Theor. Applied Genet.* 70:248-251.
- Hammerschlag, F. A., G.R. Bauchan, and R. Scorza. 1987. Factors influencing *in vitro* multiplication and rooting of peach cultivars. *Plant Cell Tissue Organ Culture* 8:235-242.
- Hartman, C. L., T.J. McCoy, and T.R. Knous. 1984. Selection of alfalfa (*Medicago sativa*) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f. sp. *medicaginis*. *Plant Sci. Let.* 34:183-194.
- Irvine, J.E. 1984. The frequency of marker changes in sugarcane plants regenerated from callus culture. *Plant Cell Tissue Organ Culture* 3:201-209.
- Krishnamurthi, M. and J. Tlaskal. 1974. Fiji disease resistant *Saccharum officinarum* var. Pindar subclones from tissue cultures. *Proc. Intl. Soc. Sugarcane Technol.* 15:130-137.
- Lapitan, N. L. V., R.G. Sears, and B.S. Gill. 1984. Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. *Theor. Applied Genet.* 68:547-554.
- Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theor. Applied Genet.* 60:197-214.
- Larkin, P. J., S. A. Ryan, R. I. S. Brettell, and W. R. Scowcroft. 1984. Heritable somaclonal variation in wheat. *Theor. Applied Genet.* 67:443-455.
- Latunde-Dada, A.O. and J.A. Lucas. 1983. Somaclonal variation and reaction to *Verticillium* wilt in *Medicago sativa* L. plants regenerated from protoplasts. *Plant Sci. Let.* 32:205-211.
- Lorz, H. 1984. Variability in tissue culture derived plants, p. 103-114. In: W. Arber (ed.). *Genetic manipulation; impact on man and society*. Cambridge Univ. Press, Cambridge.
- Lorz, H. and W.R. Scowcroft. 1983. Variability among plants and their progeny regenerated from protoplasts of Su/su heterozygotes of *Nicotiana tabacum*. *Theor. Applied Genet.* 66:67-75.
- McCoy, T. J., R.L. Phillips, and H.W. Rines. 1982. Cytogenetic analysis of plants regenerated from oat (*Avena sativa*) tissue cultures; high frequency of partial chromosome loss. *Can. J. Genet. Cytol.* 24:37-50.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
- Ogihara, Y. 1981. Tissue culture in *Haworthia*. Part 4: Genetic characterization of plants regenerated from callus. *Theor. Applied Genet.* 60:353-363.
- Okie, W.R. and C.C. Reilly. 1983. Reaction of peach and nectarine cultivars and selections to infection by *Botryosphaeria dothidea*. *J. Amer. Soc. Hort. Sci.* 108:176-179.
- Okie, W. R., D.W. Rammings, and R. Scorza. 1985. Peach, nectarine and other stone fruit breeding by the USDA in the last two decades. *HortScience* 20:633-641.
- Orton, T.J. 1984. Genetic variation in somatic tissues: method or madness? *Adv. Plant Pathol.* 2:153-189.
- Petersen, D.H. 1975. Bacterial canker of peach, p. 358-359. In: N.F. Childers (ed.). *The peach*. Horticultural Publications, New Brunswick, Canada.
- Randhawa, P.S. and E.L. Civerolo. 1985. A detached leaf bioassay for *Xanthomonas campestris* pv. *pruni*. *Phytopathology* 75:1060-1063.
- Sacristan, M.D. 1982. Resistant responses to *Phoma lingam* of plants regenerated from selected cell and embryogenic cultures of haploid *Brassica napus*. *Theor. Applied Genet.* 61:193-200.
- Scorza, R., S.A. Mehlenbacher, and G.W. Lightner. 1985. Inbreeding and coancestry of freestone peach cultivars of the Eastern United States and implications for peach germplasm improvement. *J. Amer. Soc. Hort. Sci.* 110:547-552.
- Scowcroft, W.R. and P.J. Larkin. 1985. Somaclonal variation, cell selection and genotype improvement, p. 153-168. In: M. Murray (ed.). *Comprehensive biotechnology: the principles, applications, and regulations of biotechnology in industry, agriculture, and medicine*. Pergamon, Oxford.
- Werner, D. J., D.F. Ritchie, D.W. Cain, and E.I. Zehr. 1986. Susceptibility of peaches, nectarines, plant introductions, and other *Prunus* species to bacterial spot. *HortScience* 21:127-130.
- Zong-xiu, S., Z. Cheng-zhang, S. Kangle, Q. Xiu-fang, and F. Ya-ping. 1983. Somaclonal genetics of rice, *Oryza sativa* L. *Theor. Applied Genet.* 67:67-73.