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Evaluation of diploid potato clones for resistance to tuber soft rot induced by strains of *Erwinia carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*

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Summary

Tubers of clones from a diploid hybrid population of *Solanum phureja* and *S. stenotomum* were screened for resistance to tuber soft rot caused by strains of *Erwinia carotovora* subsp. *atroseptica* (Eca), *E. carotovora* subsp. *carotovora* (Ecc) and *E. chrysanthemi* (Ech). Significant, positive correlations between resistance to the different strains and species were observed, indicating that screening for resistance to tuber soft rot could be accomplished by using just one of the three pathogens. Strains of Ecc and Ech were found to be much more virulent than strains of Eca. Inoculating with Ecc and Ech resulted in more distinct differences among susceptible, moderately resistant and resistant clones than inoculating with Eca.

Introduction

Blackleg, tuber soft rot and aerial stem rot, caused by species of *Erwinia*, are serious, worldwide diseases of potatoes (*Solanum tuberosum* L.) (Pérombelon & Kelman, 1980). *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye (Eca), *E. carotovora* subsp. *carotovora* (Jones) Bergey et al. (Ecc) and *E. chrysanthemi* Burkholder, McFadden and Dimock (Ech), can cause losses during growth of the potato crop, as well as post-harvest, through decay of tubers in storage and transit (Elphinstone, 1987; Lapwood et al., 1984; Pérombelon & Kelman, 1980).

Prevalent species, as well as strains of *Erwinia*, differ with geographical areas (Elphinstone, 1987; Maher et al., 1986). Eca and Ecc are common in potato growing areas in the northern hemisphere (Bång, 1989; DeBoer & Kelman, 1975; Powelson & Apple, 1986). Ech occurs only occasionally in these areas but is a constant threat to potato cultivation in the tropics (Hidalgo & Echandi, 1983; Pérombelon & Kelman, 1980). Lapwood et al. (1984) found that in general, potato cultivars ranked the same for susceptibility of tubers to rotting after inoculation with either a strain of Eca or a strain of Ecc. Austin et al. (1988) found similar results for Eca, Ecc and Ech in tubers from several *Solanum tuberosum* clones and from somatic hybrids between *S.*

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tuberosum and *S. brevidens*. Correlation of resistance of potato tubers to bacterial soft rot caused by different strains of Eca, Ecc and Ech has not been reported. If clones show a similar pattern in resistance when inoculated with different species and/or strains of *Erwinia*, screening for resistance could be done by inoculating with only a single strain.

The objective of this study was to determine if there is a correlation between resistance to different strains and species of *Erwinia*, in clones from a diploid hybrid population of *Solanum phureja* and *S. stenotomum*.

Material and methods

Bacterial strains. Strains used in 1989 were SR155 of Eca (Eca1), SR022 of Ecc (Ecc1) and SR298 of Ech (Ech1). In addition, strains EA64 (Eca2), 099 (Ecc2) and SR10 (Ech2) were used in 1990. All strains were obtained from Dr E. Echandi (North Carolina State University), and were stored at room temperature in test tubes containing sterile distilled water. Strains were grown on nutrient agar plates by incubating at 20 °C for 48 h (Eca) or at 25 °C for 48 h (Ecc and Ech).

Plant material. For inoculations, tubers were used from randomly chosen clones from a diploid hybrid population of *Solanum phureja* and *S. stenotomum*. In 1989 and 1990 eight and ten clones were selected, respectively.

Tuber inoculations. Bacterial suspensions were prepared by washing cells from 48 h nutrient agar cultures with sterile, distilled water. The suspension was adjusted to an optical density of 0.42 at 530 nm in a spectrophotometer (Perkin-Elmer Coleman 575, The Perkin Elmer Corporation, Oakbrook, IL 60521) which was equivalent to 5.5×10^8 colony forming units (cfu) per ml. Dilutions with sterile distilled water were made to obtain 5.5×10^6 cfu/ml. Tubers were inoculated using a modification of the procedure described by Hidalgo & Echandi (1982). In short, tubers were surface sterilized with 0.5% NaOCl for 20 min., rinsed with tap water and air dried. Ten µl of the bacterial suspension was injected at a depth of 1.5 cm using a Hamilton #1705 microliter syringe (The Hamilton Co., Reno, NV 89520) at two locations on one side of the tuber: one near the stolon end and the other near the bud end. Each place of inoculation was covered with petroleum jelly. Tubers were individually wrapped in a wet paper towel and sealed with two layers of polyvinylchloride. Preliminary experiments indicated that Ech caused rotting much more rapidly than either Eca or Ecc. Therefore, a shorter incubation period for tubers inoculated with Ech was necessary to prevent rotting of the entire tuber. Tubers inoculated with strains of Eca, Ecc and Ech were incubated for 96 h at 20 °C, 96 h at 25 °C and 72 h at 25 °C, respectively. After incubation, tubers were cut vertically through each place of injection, and the diameter of decay was measured at the widest point at right angles to the injection. The diameter of decay of the two places of injection were averaged, and the mean was used as measure of resistance of the tuber. As a control, 10 µl sterile distilled water was injected at two locations in one tuber of each clone.

Experimental design and data analyses. The experiment was arranged as a split plot design with bacterial strains as main-plot, and clones as sub-plot factors. The experiment consisted of three replications over time. For each replication, the resistance of five inoculated tubers per clone and per bacterial strain were averaged. Data were analysed using standard analyses of variance. Mean separation analyses were done using Duncan's multiple range test. To determine if there was a correlation between resistance to different strains and species of *Erwinia* in clones from a hybrid diploid population of *Solanum phureja* and *S. stenotomum*, Pearson's correlation coefficients were calculated.

Results

Significant differences in resistance to different strains and species of *Erwinia* were observed among clones in 1989 (Table 1) and 1990 (Table 2). Generally, inoculation with strains of Ecc and Ech resulted in a better separation between susceptible, moderately resistant and resistant clones, than inoculation with strains of Eca (Tables 1 and 2). Inoculation with Ech1 in 1989 resulted in three groups of clones that were significantly different from each other (Table 1). The most resistant group consisted of two clones (81DM54-6 and 85P73-2A), the intermediate group consisted of five clones (82DM51-5, 81DM14-5, 81DM29-1, 81DM68-1 and 81DM18-2) and the most susceptible group consisted of one clone (81DM80-1) (Table 1). After inoculation with Ecc1, three clones were found to be significantly more resistant than the others (81DM68-1, 81DM54-6 and 85P73-2A) (Table 1). Inoculation with Eca1 did not result in a distinct group of resistant clones, but mainly gave significant differences between the most susceptible clones and the rest. Similar observations were made in 1990, where inoculation with Eca1 and Eca2 could only distinguish the most susceptible clones from all the others, while inoculation with Ecc1, Ecc2, Ech1 and Ech2 resulted in more pronounced differences among susceptible, moderately resistant and resistant clones (Table 2).

Table 1. Diameter of soft rot lesions (mm) from tubers of eight clones of a diploid hybrid population of *Solanum phureja* and *S. stenotomum*, caused by inoculation with different species of *Erwinia* (1989).

Clone	Ech1	Ecc1	Eca1
81DM80-1	19.6 *a**	15.7 *a*	7.1 *a*
82DM51-5	16.4 *b*	13.4 *ab*	5.2 *bc*
81DM14-5	15.9 *b*	10.2 *c*	5.9 *ab*
81DM29-1	15.9 *b*	13.5 *ab*	4.0 *cd*
81DM68-1	15.6 *b*	7.0 *d*	2.6 *de*
81DM18-2	14.3 *b*	11.4 *bc*	2.6 *de*
81DM54-6	10.1 *c*	7.2 *d*	2.0 *e*
85P73-2A	9.7 *c*	7.5 *d*	3.0 *de*

* Means with the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 2. Diameter of soft rot lesions (mm) from tubers of ten clones of a diploid hybrid population of *Solanum phureja* and *S. stenotomum*, caused by inoculation with different strains and species of *Erwinia* (1990).

Clone	Ech1	Ecc1	Eca1	Ech2	Ecc2	Eca2
76DP1-1	19.4 *a**	23.1 *a*	9.4 *a*	32.9 *a*	15.2 *a*	7.7 *a*
H85P4-3	13.1 *b*	9.9 *bcd*	1.0 *c*	25.2 *b*	7.2 *bcd*	0.4 *c*
DEBP-1	13.0 *b*	10.8 *bcd*	0.5 *c*	22.2 *bc*	5.5 *d*	0.5 *bc*
DM14-5	12.4 *bc*	12.1 *bc*	3.6 *b*	23.2 *bc*	8.7 *b*	2.4 *b*
EB66P6-01	12.1 *bc*	12.8 *b*	1.2 *c*	26.3 *b*	6.9 *bcd*	0.9 *bc*
DMEBP-2	12.0 *bc*	11.9 *bc*	0.3 *c*	21.6 *bc*	7.3 *bcd*	0.5 *bc*
EB85P38-1	11.3 *bc*	8.7 *cd*	1.1 *c*	21.4 *bc*	8.2 *bc*	1.1 *bc*
D85P6-1	10.3 *bcd*	9.0 *bcd*	1.2 *c*	12.0 *d*	5.9 *cd*	0.9 *bc*
D85P55-1	9.8 *cd*	7.7 *de*	0.7 *c*	17.0 *cd*	5.9 *cd*	1.3 *bc*
D85P76-9	8.0 *d*	4.9 *e*	1.2 *c*	17.4 *cd*	7.2 *bcd*	1.0 *bc*

* Means with the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

For each bacterial strain, values for the diameter of soft rot lesions were averaged over all clones to determine which strains were the most virulent. In 1989 (Fig. 1a) as well as in 1990 (Fig. 1b) strains of Eca caused the smallest soft rot lesions and strains of Ech the largest.

To determine the coefficients of correlation between diameter of soft rot lesions caused by the different species, the 1989 and 1990 data for Eca1, Ecc1 and Ech1 were combined. Significant, positive coefficients of correlation were found for resistance to the different species (Fig. 2). Correlation coefficients between the diameter of lesions caused by Eca1 and Ecc1, Eca1 and Ech1, and Ecc1 and Ech1, were 0.74, 0.81 and 0.78, respectively (Fig. 2a, b and c). Similar results were found when an additional

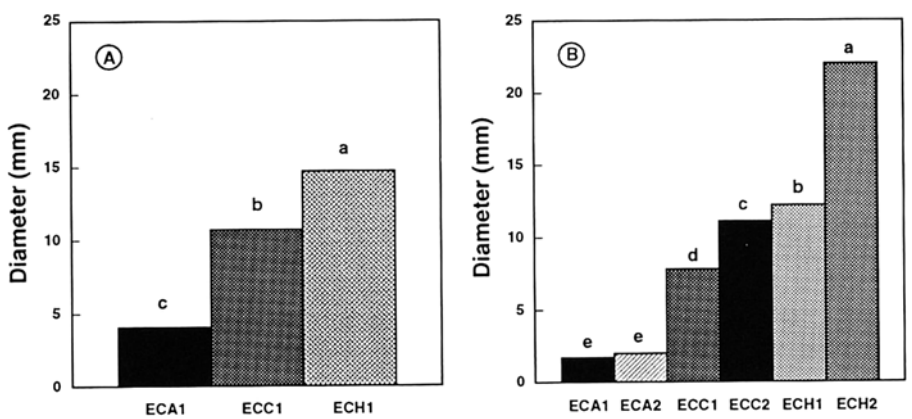

Fig. 1. Diameter of soft rot lesions, averaged over all clones for each bacterial strain in 1989 (A) and 1990 (B). Means with the same letter above the columns are not significantly different at $P<0.01$, as determined by Duncan's miltiple range test.

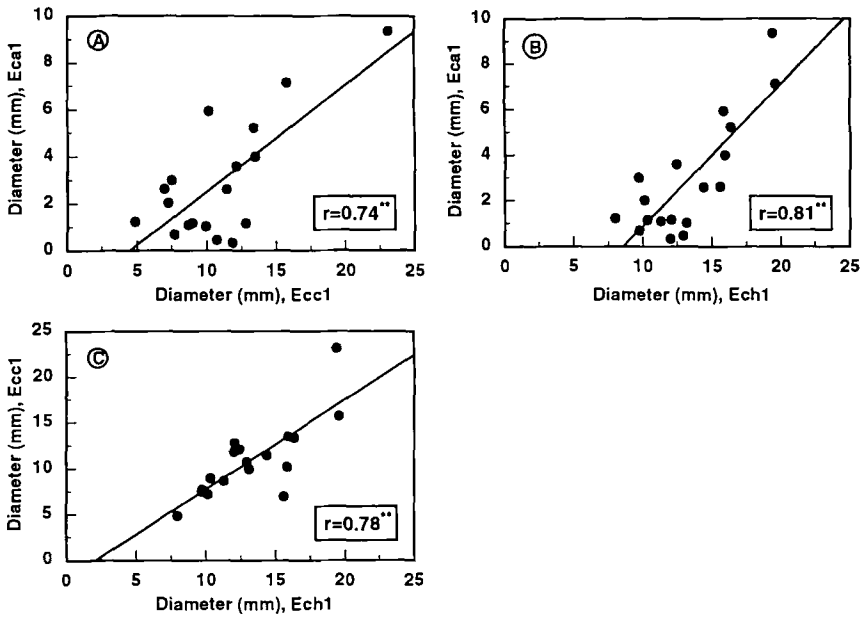

Fig. 2. Relationship between diameter of soft rot lesions in diploid potatoes caused by Eca1 and Ecc1 (A), Eca1 and Ech1 (B) and Ecc1 and Ech1 (C). Inserts represent coefficients of correlation. Data for 1989 and 1990 were combined. ** Significant at $P<0.01$.

Table 3. Correlation coefficients between resistance to different strains and species of *Erwinia* in clones from a diploid hybrid population of *Solanum phureja* and *S. stenotomum* (1990).

	Ech2	Ecc2	Eca2	Ech1	Ecc1	Eca1
Ech2	-	0.73*	0.63*	0.85**	0.82**	0.67*
Ecc2		-	0.95**	0.82**	0.84**	0.96**
Eca2			-	0.80*	0.85**	0.99**

* significant at $P<0.05$
** significant at $P<0.01$

strain was used for each of the three species (Eca2, Ecc2 and Ech2 respectively). Coefficients of correlations ranged from 0.63 to 0.99 (Table 3).

Discussion

Inoculation with Ecc and Ech had a greater possibility of selecting the most resistant clones as compared with inoculation with Eca, which mainly identified the most susceptible ones. The fact that selection for clones resistant to soft rot erwinias was more effective when Ecc or Ech was used could be explained by the strains of Eca being less virulent than those of Ecc and Ech (Fig. 1a and b). Disease development

after inoculation with Eca1 and Eca2 was not severe enough to give significant differences among moderately resistant and resistant clones. As shown in previous research (Hidalgo & Echandi, 1982), strains of Eca are not always less virulent than strains of Ecc and Ech, and can be used to test clones for resistance to tuber soft rot. Our results indicate that strains should be tested for an adequate level of virulence before using them to screen potato clones for resistance.

Significant, positive correlations between the diameter of the lesions caused by the different species indicate that clones resistant to one species were also resistant to the other two. Furthermore, clones resistant to one strain of an *Erwinia* species were also resistant to the second strain of that species as well as to strains of the two other species, suggesting that screening for resistance to tuber soft rot in diploid potato clones may be done using only one strain of either of the three species. These results correspond with reports from previous research. Tubers from somatic hybrids between *S. tuberosum* and *S. brevidens* showed resistance to strains of Eca, Ecc and Ech, and tubers from *S. tuberosum* clones were susceptible to all three strains (Austin et al., 1988). Lapwood et al. (1984) suggested that subsp. *carotovora* could be omitted from screening tests since cultivars showed a similar pattern of resistance to the two strains used (one of Eca and one of Ecc). Michalik et al. (1992) reported similar findings in carrots: two lines were screened for resistance using three strains of Ecc and one strain of Eca. One line was always more susceptible than the other, regardless of the strain used.

Resistance as measured in our experiments is the resistance of the tubers to tissue maceration. This measurement may have some disadvantages, since it bypasses the periderm, which may have a role in resistance to wounding and bruising. However, Tzeng et al. (1990) found that immersing mechanically harvested tubers in a suspension of Eca resulted in the same ranking of clones for susceptibility as did injecting them with a bacterial suspension. This suggests that the periderm does not play a major role in determining resistance to tuber soft rot, since tubers wounded during harvest no longer have the protection of an intact periderm. Thus, it appears that measuring resistance by injecting the tubers with a bacterial suspension, can give an indication of resistance to tuber soft rot.

Our data suggest that resistant potato clones, selected after testing with a specific species or strain of *Erwinia*, will also be resistant in areas where different strains and/or species are prevalent. This could facilitate the selection and utilization of clones resistant to tuber soft rot by eliminating extensive re-testing in different geographical areas.

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