Field Testing Bacterial Wilt-resistant Tomato Somaclones

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Abstract. ‘Healani’ tomato (*Lycopersicon esculentum* Mill.) somaclones were tested in a bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) infected field. Survival percentages of selected somaclonal lines were from 40% to 100%, while the original ‘Healani’ had a survival rate of 0% and resistant ‘Kewalo’ had 30%. Eighteen bacterial wilt resistant somaclonal lines were selected and tested for retention of horticultural characters in a noninfected field. ‘Healani’ significantly outyielded all tested somaclonal lines in total fruit weight and total number of fruit per plant. ‘Healani’ had greater fruit diameter than seven of the lines, greater width of the outer wall of the fruit pericarp than seventeen of the lines, and fewer locules in the fruit than seven of the lines.

Somaclonal variation, arising from tissue culture, was initially reported as useful for crop improvement (Larkin and Scowcroft, 1981; Orton, 1984; Scowcroft et al., 1983; Scowcroft and Larkin, 1988; van den Bulk et al., 1990). It has been reported that single gene mutations occur in tomato plants regenerated from callus culture (Evans and Sharp, 1983). The occurrence of classical point mutations has been concluded from genetic analysis of other crops such as rice, wheat, maize, lettuce, tobacco, celery and rapeseed (Scowcroft and Larkin, 1988). Thus, a proposed strategy has been to culture the best cultivars in vitro and select for improvements in the desired character in the regenerants while retaining favorable characters (Evans et al., 1984). Generation of disease resistance through somaclonal variation has been proposed (Skirvin, 1978; van den Bulk et al., 1991). Since there is a dearth of bacterial wilt resistant tomato cultivars, somaclonal variation is a possible method for generating resistance. Thus, it was the purpose of this study to obtain bacterial wilt (*Pseudomonas solanacearum*) resistance in ‘Healani’, a susceptible Hawaiian tomato cultivar, through somaclonal variation with the retention of favorable horticultural characters.

Materials and Methods

Tissue culture. ‘Healani’ tomato seeds were germinated on sterile, wet filter paper in flasks and placed under fluorescent lights at about 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in a 25C culture room at a 12-h photoperiod. Hypocotyl or cotyledon sections of these seedlings were cultured on modified Murashige and Skoog (MS) media (Murashige and Skoog, 1962) containing concentrations of 2.5/10, 5/10 and 10/10 μM indole-3-acetic acid (IAA)/ μM 6-benzyladenine (BA). These sections produced calli when grown under the same conditions as the flasks of germinated seedlings. When shoots began to appear from the calli, they were separated and removed from the flask. Rooted shoots were transferred directly into pots in the greenhouse, while the unrooted shoots were placed on MS media with 5 or 10 μM IAA to promote rooting. The remaining calli were transferred to medium with 0/2, 2.5/10 or 0/0 (μM IAA/ μM

BA) for further shoot growth. The number of transfers varied, and the period of culture ranged from 3 to 6 months.

Greenhouse and field. Seeds from the self-pollinating regenerants growing in the greenhouse were collected for progeny evaluation.

Table 1. Survival percentages of ‘Healani’, ‘Kewalo’, and the R_0 progeny of the R_0 somaclonal lines tested in the bacterial wilt (*Pseudomonas solanacearum*) field.

Cultivar or R_0 line	No. of plants	Survival percentage
H070101C	15	87 ^z
H070301	15	47 ^z
H070301A	15	93 ^z
H071002	15	73 ^z
H071002A	15	53 ^z
H071002C	14	43 ^z
H071003	15	60 ^z
H071004	15	40 ^z
H071005	15	40 ^z
H071006	15	20
H071009	15	53 ^z
H071009A	15	53 ^z
H071011	15	27
H071011A	15	27
H071012A	15	60 ^z
H071014	15	33
H082701	15	33
H082701A	15	67 ^z
H082703	15	40
H082703A	15	33
H082703B	15	47 ^z
H082710	15	80 ^z
H082712	15	40
H090801	11	100 ^z
H090803	15	53 ^z
H090803A	15	20
H090803B	15	33
H102713 OP	11	91 ^z
Kewalo	27	30
Healani	30	0

^zThese are the R_0 lines that were selected for subsequent testing in a noninfected field.

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Initially, R_1 seeds from 41 'Healani' somaclone R_0 plants were transplanted into a bacterial wilt infected field in a randomized complete-block design with two replications. (Each R_0 plant is an original regenerant; R_0 breeding lines descend from one plant. R_1 s, R_2 s, R_3 s, etc. indicate the progeny generation.) There were five plants per R_0 line per replication. Only those plants that survived to maturity were labeled resistant. While no cutting of the stems was done to inspect pith browning, resistance was assumed to be differing levels of tolerance (and by no means immunity). Seeds were collected from those R_1 plants surviving to maturity for the next trial.

'Kewalo', a cultivar resistant to bacterial wilt disease (Gilbert et al., 1974), was tested along with 'Healani' (susceptible to bacterial wilt) and the R_2 seeds from 28 of the surviving R_0 lines in the bacterial wilt infected field in the following year. The design was a randomized complete block with three replications of five plants in each replicate. R_3 seeds were collected from the surviving plants.

Eighteen R_0 lines were selected as bacterial wilt resistant based on a minimum survival rate of 40%. R_3 seedlings were planted in a noninfected field along with 'Healani' and 'Anahu' cultivars to evaluate phenotypic characters. The statistical design was a randomized complete block with three replicates and ten plants in each replicate.

Height of the first flower was measured. Plant vigor was rated on a scale of 1 to 5 about 2 months after the seedlings were transplanted into the field. Yield data were the average number and weight of fruit per plant. A sampling of Hawaii no. 1 grade of fruit was taken, usually five per replicate per R_0 line or cultivar. Data were taken on fruit diameter, width of the outer wall of the fruit pericarp, number of locules in the fruit, fruit firmness (rating scale of 1 to 5), and percentages of fruit with radial cracks, concentric cracks or hollowness.

Slide preparation for chromosome counts. Root tips from R_3 plants were collected from tomato cuttings held in water. Root tips were initially put in a vial of 0.002 M 8-hydroxyquinoline solution for 4 hours in cold water in a thermos, and then fixed with a 1:1:2 solution of 95% ethanol, chloroform and glacial acetic acid. Root tips were then hydrolyzed with 1 N hydrochloric acid for 2.5 min under running hot tap water, and then stored in 45% acetic acid. Root squashes were stained with aceto-orcein. Extensive chromosome counts of plants grown from seed of the same R_3 generation could not be pursued since difficulties arose in obtaining suitable roots and later, nonviable seeds precluded any further work.

Results

R_2 progeny of the lines planted in the bacterial wilt infected field showed survival rates ranging from 20% to 100% (Table 1). Degree of resistance for a cultivar may vary from location to location, according to field and environmental conditions. Cultivars 'Healani' (susceptible) and 'Kewalo' (resistant) had survival rates of 0% and 30%, respectively. Hence, somaclonal bacterial wilt resistance greater than that of 'Kewalo' (under our conditions) was obtained in some lines. These R_2 progeny having at least 40% to 100% surviving plants in the infected field were selected for further testing.

In the subsequent testing of the R_3 progeny of the R_0 lines in a noninfected field, mean vigor ratings ranged from 2.7 to 3.6 (scale of 1 = dead to 5 = excellent vigor) for all R_0 lines while the mean ratings of 'Healani' and 'Anahu' were significantly higher at 4.7 and 4.9, respectively (Table 3). 'Healani' differed statistically from only two other lines (H090801 and H090803) in height of the first flower (Table 2).

'Healani' significantly outyielded the R_0 lines in total fruit weight per plant and total number of fruit per plant (Table 2). Few

Table 2. Mean values for the characters of height of the first flower, fruit weight per plant, number of fruit per plant, fruit diameter, width of the outer wall of the pericarp of the fruit, and the number of locules per fruit of 'Healani', 'Anahu', and the R_3 progeny of the somaclonal lines.

Line or cultivar	Ht of first flower (cm)	Fruit wt per plant (kg)	Fruit per plant (no.)	Fruit diam (cm)	Width of outer wall of fruit pericarp (cm)	Locules in fruit (no.)
H102713	25.4 cd ²	1.83 d	23.2 d	6.05 cd	0.44 bc	8.7 cd
H070101C	28.2 bcd	3.27 cd	36.3 bcd	6.66 abc	0.47 bc	9.6 bcd
H070301	26.1 bcd	3.55 bcd	38.9 bcd	6.88 ab	0.47 bc	9.3 bcd
H070301A	26.7 bcd	4.03 bc	42.8 bc	6.51 abcd	0.44 bc	9.5 bcd
H071002	26.8 bcd	3.36 bcd	34.5 bcd	6.49 abcd	0.42 c	14.0 a
H071002A	28.3 bcd	3.27 cd	36.4 bcd	6.25 bcd	0.45 bc	10.3 bc
H071002C	27.2 bcd	2.76 cd	31.9 bcd	6.14 cd	0.43 c	8.8 bcd
H071003	29.4 abc	3.56 bcd	36.9 bcd	6.68 abc	0.51 ab	10.4 bc
H071004	28.1 bcd	4.31 bc	49.7 b	6.54 abcd	0.45 bc	8.4 cd
H071005	29.7 ab	3.90 bc	40.2 bcd	6.61 abc	0.46 bc	9.7 bcd
H071009	26.6 bcd	3.14 cd	36.6 bcd	6.46 abcd	0.42 c	9.6 bcd
H071009A	26.9 bcd	3.53 bcd	38.7 bcd	6.57 abcd	0.39 c	12.1 ab
H071012A	28.6 abcd	4.05 bc	46.9 b	6.11 cd	0.45 bc	10.4 bc
H082701A	27.2 bcd	2.90 cd	32.3 bcd	6.28 bcd	0.43 c	9.9 bcd
H082703B	29.5 abc	3.31 bcd	34.2 bcd	6.57 abcd	0.45 bc	11.5 abc
H082710	27.1 bcd	2.08 d	25.6 cd	5.90 d	0.43 c	8.5 cd
H090801	24.7 d	3.04 cd	35.9 bcd	6.17 cd	0.46 bc	8.5 cd
H090803	24.2 d	3.03 cd	33.9 bcd	6.39 abcd	0.40 c	10.8 bc
Healani	29.5 abc	6.48 a	69.1 a	7.04 a	0.54 a	6.9 d
Anahu	32.4 a	5.05 ab	43.5 bc	7.07 a	0.47 bc	9.4 bcd

²Mean separation in columns by Duncan's multiple range test, $P = 0.05$.

differences in yield occurred among the R_0 lines.

'Healani' and 'Anahu' were significantly greater in fruit diameter than seven of the 18 R_0 lines (Table 2). The width of the outer pericarp wall was significantly greater in 'Healani' than in 17 of the lines. 'Healani' had the least number of locules in the fruit and significantly fewer than seven of the lines. H071002 had the greatest number of locules. There was a lack of radial symmetry in fruit with many locules. In such fruit there were many small locules throughout the fruit with more pericarp tissue observed between the locules.

No significant differences among all R_0 lines and cultivars were found for percentages of fruit with concentric cracks or radial cracks (Table 3). Significant differences in percentages of fruit with hollowness (empty cavities within the fruit) were obtained ($P < 0.01$) for all lines and cultivars; however, Duncan's multiple range test revealed that 'Healani' yet did not differ significantly from all R_0 lines. Lines H070301 and H071004 had 0% hollowness and were significantly lower than nine of the other lines.

On a scale from 1 (firm) to 5 (soft), 'Healani' fruit had a mean rating of 2.3 for firmness, but did not differ from the R_0 lines which ranged in ratings from 1.7 to 2.5 (Table 3).

Table 4 indicates that the normal diploid number, $2n = 24$, was generally predominant in the tomato somaclones. Variants from this number were aneuploids and tetraploids. Hence, a mixoploid or mosaic nature in the root tips was apparent.

Discussion

This study produced plants of 'Healani' that were bacterial wilt resistant. Survival percentages were from 40% to 100%, which was higher than a 30% survival rate of resistant 'Kewalo'. Other characters of the somaclonal lines (yield, diameter of fruit, width

of the outer pericarp, and locule number) were statistically different from those of the parent cultivar 'Healani'. No significant differences from 'Healani' were found for the percentages of fruit with radial cracks, concentric cracks and hollowness. Yield reduction was a major constraint of this somaclonal variation. Reduced yield has also been observed in somaclones of wheat (Ahloowalia and Sherington, 1985; Qureshi et al., 1992). Inferior fruit were characteristic of many of the tomato somaclonal lines (smaller fruit and abnormalities in radial symmetry accompanied by a higher number of locules dispersed throughout the fruit).

As in wheat (Larkin et al., 1984), different morphological traits in 'Healani' somaclones varied from the original cultivar; however, not all characters changed. Regardless of being polygenically controlled or simply inherited, characters are not always altered in somaclonal variation (Karp and Bright, 1985). In contrast with our results, Daub and Jenns (1989) found progeny of tobacco somaclones to be of a normal phenotype, similar to parental types in yield and leaf chemistry, but resistant in varying degrees to black shank and bacterial wilt.

Limitations of the use of somaclonal variation for bacterial wilt resistance were apparent in 'Healani' tomato due to the accompanying changes. The limited use of somaclonal variation for crop improvement has been noted (Ahloowalia, 1986; van Harten, 1989; Baillie et al., 1992). Somaclonal variation is cultivar dependent (Karp, 1991). While single gene mutations through tissue culture have been reported in tomato (Evans et al., 1984), probabilities are such that several mutations are likely though not always recognized. While Nambisan et al. (1992) observed no variation at the phenotypic level for 950 somaclones of tomato, yet 2 of the 17 somaclones tested showed DNA polymorphism for the *Cab* locus. The question still arises whether too many characters are altered in somaclonal variation to be useful in crop improve-

Table 3. Mean values of the characters of plant vigor, fruit firmness, percent radial cracks, percent concentric cracks, and percent hollowness in 'Healani', 'Anahu', and the R_3 progeny of the somaclonal lines.

Line or cultivar	Plant vigor ^z	Fruit firmness ^{y,x}	Fruit with radial cracks (%) ^x	Fruit with concentric cracks (%) ^x	Fruit showing hollowness (%)
H102713	2.7 c ^w	2.4	21	57	7 cd
H070101C	3.4 bc	2.1	47	47	40 abc
H070301	3.6 b	2.2	27	33	0 d
H070301A	3.6 b	2.4	27	27	40 abc
H071002	3.3 bc	2.2	38	38	38 abc
H071002A	3.2 bc	2.3	20	40	40 abc
H071002C	3.1 bc	2.5	7	50	8 cd
H071003	3.5 bc	2.5	33	44	13 bcd
H071004	3.2 bc	2.2	13	33	0 d
H071005	3.5 bc	2.3	33	53	33 abc
H071009	3.6 b	2.4	29	36	14 bcd
H071009A	3.3 bc	2.1	13	20	21 bcd
H071012A	3.5 bc	1.7	33	33	31 abc
H082701A	3.2 bc	2.2	56	33	33 abc
H082703B	3.5 bc	2.1	33	40	47 ab
H082710	2.9 bc	2.4	31	33	27 abcd
H090801	3.4 bc	1.8	40	44	20 bcd
H090803	3.5 bc	2.2	21	29	50 ab
Healani	4.7 a	2.3	0	7	20 bcd
Anahu	4.9 a	2.7	6	7	60 a

^zRating is 1 = dead, 2 = weak, 3 = moderate growth, 4 = good vigor, 5 = excellent vigor.

^yRating is 1 = firm, 2 = moderately firm, 3 = average, 4 = moderately soft, 5 = soft.

^xANOVA shows no significant differences.

^wMean separation in column by Duncan's multiple range test, $P = 0.05$.

Table 4. Distribution of chromosome numbers found in root tips of R₃ plants of 'Healani' tomato.

Plant	Chromosome number distribution									
	21	22	23	24	25	42	43	44	47	48
H070301A #2				3	2				1	2
H070301A #3			2	3						
H070301A #4			2	3			1		1	
H071005 #1				1				1		
H071005 #2		1		2						1
H071005 #3			6							
H071005 #5			1	12	1					1
H082701A #1	1	2		1		1				
H090803 #1				3						

ment (Larkin and Scowcroft, 1983). Therefore, stringent evaluation procedures need to be used (Lorz and Brown, 1986; Larkin and Scowcroft, 1981).

In this study desirable as well as undesirable traits were mutated in the same R₀ plants. It is possible to incorporate disease resistance or other desirable characters into 'Healani' with the technique of callus culture. Under our experimental conditions, 'Healani' mutated in several characters along with the trait of bacterial wilt resistance. These mutations generally made the fruit commercially undesirable. Aberrations in chromosome number, mosaicism, have been detected in regenerants of other species or their hybrids (Fedak and Grainger, 1986; Alicchio et al., 1984; Kumar and Walton, 1992; D'Amato et al., 1980). This chromosome mosaicism, in part, may account for the undesirable traits. Crossing to incorporate the bacterial wilt resistance into the original 'Healani' is a possibility; however, the undesirable traits and the chromosome mosaicism may make it too cumbersome and costly to achieve benefits. Traditional breeding with other resistant germplasm may be more desirable for 'Healani'.

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