

# Increasing Resistance in Celery to *Fusarium oxysporum* f. sp. *apii* Race 2 with Somaclonal Variation

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## ABSTRACT

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Somaclones were regenerated from cell suspension cultures derived from callus of celery cultivar Tall Utah 52-70 HK (moderately resistant to *Fusarium oxysporum* f. sp. *apii* race 2). Eighty-one of 350 somaclones screened for resistance to *F. o. apii* race 2 in either the greenhouse or the field had increased resistance over parental plants grown from seed. Thirty-five highly resistant somaclones ( $R_0$  generation) were self-pollinated, and 10 of the resulting  $R_1$  progeny lines were rated as highly resistant when screened in the field. Most of the  $R_1$  lines resembled parental Tall Utah 52-70 HK plants in appearance, growth rate, and habit. Twenty-six highly resistant plants from 11  $R_1$  lines were self-pollinated, and nine of the resulting  $R_2$  lines were rated as highly resistant to *F. o. apii* race 2 in field trials. These lines were very similar horticulturally to the parent cultivar. The lines developed from this work could provide a source of increased resistance to *F. o. apii* race 2 for celery producers.

Additional keywords: *Fusarium* yellows, tissue culture

*Fusarium* yellows, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *apii* (R. R. Nelson & Sherb.) W. C. Snyder & H. N. Hans. race 2, is the limiting factor in celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.) production throughout most of the celery-growing regions of the United States (1,5,8,16). Symptoms include vascular discoloration and necrosis in roots and crowns, leading to chlorosis, stunting, and wilt (8). Host resistance is the only economically feasible means for controlling *F. o. apii* race 2. A few cultivars with moderate resistance are available, but none have the high level of resistance necessary for profitably growing celery in *Fusarium*-infested soils (6).

Conventional breeding techniques to increase resistance have involved interspecific crosses with celeriac (20). This procedure requires several years to develop acceptable cultivars because of the back-crossing and selection required to obtain both resistance and acceptable horticultural type. Tissue culture techniques offer a faster and easier way to induce heritable increases in resistance in existing cultivars while maintaining other desirable characteristics. Experimentally, tissue culture procedures have increased resistance to phytopathogenic

fungi, bacteria, and viruses in a number of crops, including sugarcane (14), tomato (2,4), soybean (19), oats (22), and alfalfa (13). Cultivars derived from tissue cultures include the sugarcane cultivar Ono (3), cultivars of tomato (7), a pepper cultivar (7), Scarlet sweet potato (17), and Velvet Rose geranium (23).

This study was undertaken to determine if somaclonal variation could generate a high level of heritable resistance to *F. o. apii* race 2 from a moderately resistant celery cultivar adapted to the northeastern United States.

## MATERIALS AND METHODS

**Somaclone regeneration.** Because cultivars with moderate levels of resistance yielded higher frequencies of somaclones with increased resistance than did susceptible cultivars (24), celery cultivar Tall Utah 52-70 HK, moderately resistant to *F. o. apii* race 2 (6), was used as the explant donor for the tissue culture procedure. Axillary buds from 11-wk-old greenhouse-grown plants were excised, surface-disinfested in 0.525% NaOCl for 10 min, and rinsed 3 $\times$  with sterile distilled water.

Murashige and Skoog medium (18) (MS), modified for celery by Heath-Pagliuso et al (9) and Wright and Lacy (24), was used for all aspects of the tissue culture procedure. Surface-disinfested buds were placed on MS agar containing 4.5  $\mu$ M of (2,4-dichlorophenoxy)acetic acid (2,4-D) and 8.9  $\mu$ M of 6-benzyladenine. The cultures were kept at 22 C under a 12-hr photoperiod. After 9 days, buds with callus formation were transferred to MS agar with 2.3  $\mu$ M of 2,4-D and 0.47  $\mu$ M of kinetin. Six weeks later, the callus was transferred to 250-ml

flasks containing 50 ml of MS broth with 2.3  $\mu$ M of 2,4-D and 0.47  $\mu$ M of kinetin. Broth cultures were incubated on a rotary shaker at 100 rpm in the dark at 21–23 C.

After 2 mo, cell suspension cultures were supplied monthly with fresh MS broth with the same hormone concentrations as listed earlier. Cell suspensions were diluted 1:1 (v/v) with fresh sterile broth, and 50 ml of the diluted cell suspension was poured into a sterile 250-ml flask and placed back on the shaker.

After 15–20 wk of incubation, 2-ml aliquots from cell suspensions containing cell aggregates were plated onto MS agar containing no hormones, 20 g of sucrose, and 0.5 g of activated charcoal per liter (24). Green plantlets typically appeared in 2 wk. The somaclones were kept at 26–27 C under 20–40  $\mu$ E $\cdot$ m<sup>-2</sup>·s<sup>-1</sup> of photosynthetically active radiation (PAR) for a 16-hr photoperiod. Somaclones were transferred every 2–4 wk to new MS agar with no hormones and 20 g of sucrose in either petri dishes (100  $\times$  15 mm) or culture boxes (7.5  $\times$  7.5  $\times$  10 cm) (GA7 vessels, Magenta Corporation, Chicago, IL). Somaclones that were 8–10 cm tall (4–8 mo after regeneration) were planted individually in polystyrene cups (8.5 cm high and 7.0 cm wide at top) containing autoclaved (30 min at 121 C) commercial peat-vermiculite potting soil, watered, and immediately placed inside plastic bags. Somaclones were kept at 7  $\mu$ E $\cdot$ m<sup>-2</sup>·s<sup>-1</sup> PAR during a 10-hr photoperiod for 2 days, then at 20–40  $\mu$ E $\cdot$ m<sup>-2</sup>·s<sup>-1</sup> PAR during a 16-hr photoperiod for 1–3 wk to minimize heat retention by the plastic bags. The plastic bags were removed and the plants were kept under intermittent mist (15 min on, 15 min off) in the greenhouse for 5 days. Somaclones could then be placed on a greenhouse bench under fluorescent lights (16-hr photoperiod) without wilting.

**Screening somaclones for resistance.** Somaclones ( $R_0$  generation) were grown for 1–2 mo (until approximately 15 cm tall) and then transplanted into muck soil naturally infested with *F. o. apii* race 2 either in the greenhouse or in commercial celery fields near Decatur or Hudsonville, MI, during 1987. The farm near Decatur was the site of a trial screening celery cultivars and breeding lines for resistance to *F. o. apii* race 2 in 1986, and similar trials were held at both Decatur and Hudsonville locations from

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1987 to 1990 (K. F. Toth and M. L. Lacy, unpublished). Somaclones and their progeny lines were planted adjacent to cultivar trials. In all cultivar trials from 1986 to 1990, plants of susceptible cultivars were dead or severely diseased by harvest (K. F. Toth and M. L. Lacy, unpublished), indicating a high infestation of *F. o. apii* race 2 in all fields used.

Somaclones screened in the greenhouse were kept under sodium vapor lamps (16-hr photoperiod) for 15–18 wk, and then crowns were cut in half and examined for vascular discoloration. Somaclones without vascular discoloration in the root or crown area were replanted and grown in the greenhouse for 3.5–4.0 mo before vernalization. Somaclones screened for resistance in the field were uprooted with a shovel 11–13 wk after transplanting and their crowns were cut in half and rated for vascular discoloration. Somaclones free of vascular discoloration (or with only a trace) in the taproot or lower crown area were returned to the greenhouse. These somaclones were trimmed to a few inner petioles and relatively few leaves, the roots and crowns were washed in 0.26% NaOCl to control secondary rotting organisms, and rooting hormone (Rootone, Pratt-Gabriel, Hanover, PA) was sprinkled on the cut area before transplanting in potting mix. The plants were then replanted into 25-cm-diameter pots and kept under a greenhouse bench for 5 days to reduce transplanting shock. Somaclones were fertilized once with 20-20-20 (NPK) and placed under sodium vapor or fluorescent lights in the greenhouse for 5–6 wk before vernalization.

**Vernalization and self-pollination.** Somaclones were vernalized at 4–6 C under a 12-hr photoperiod for 8 wk in a large temperature-controlled room, then placed back into the greenhouse under a 16-hr photoperiod to induce flowering (11). During and after vernalization, crowns of somaclones were sprayed three times per week with approximately 5 ml of a solution of CaCl<sub>2</sub> (14 g/L) to prevent black heart disease. Vernalized somaclones were fertilized weekly with soluble 20-20-20 (NPK) (11 g/3.8 L), and every 2 wk with modified Hoagland's solution (2.28 g of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 0.81 g of KNO<sub>3</sub>, 0.54 g of KH<sub>2</sub>PO<sub>4</sub>, 1.97 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.17 g of NaCl, 0.5 mg of FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.28 mg of H<sub>3</sub>BO<sub>3</sub>, 4 µg of CuCl<sub>2</sub>·2H<sub>2</sub>O, 3 µg of ZnCl<sub>2</sub>, and 39 µg of MnCl<sub>2</sub>·4H<sub>2</sub>O per liter) at 100 ml per pot.

When somaclones started to flower (2 mo after vernalization), the flower stalks were placed within a cheesecloth cage to prevent cross-pollination by insects. Cages were cylinders made of a single layer of cheesecloth 100–125 cm long, closed at the top and shaped by a 30- to 35-cm-diameter wire ring near the top. The cages were suspended in the greenhouse from overhead supports and the bottom

was tied around the flower stalks. Celery flowers are perfect but do not self-fertilize because anthers and stamens do not develop synchronously. Plants were shaken daily to spread pollen among the receptive flowers to encourage self-pollination. Seed was harvested when the pedicel had turned yellow and the seed was brown. Seed was cleaned of debris and stored at 4 C.

**Screening somaclone progeny for resistance.** Seeds collected from somaclones were soaked in constantly aerated water (18 C) for 7 days, germinated in potting mix, and 3- to 4-wk-old seedlings were transplanted into flats. Seedlings (R<sub>1</sub> generation) were grown in the greenhouse for four more weeks, then transplanted into the field. Because *Fusarium* yellows is more severe and disease escapes are less likely in the field than the greenhouse (6), the R<sub>1</sub> and R<sub>2</sub> progenies were screened in field trials.

Thirty 8-wk-old R<sub>1</sub> plants from individual parent plants were transplanted 15 cm apart into 4.5-m-long rows in a muck field naturally infested with *F. o. apii* race 2 near Decatur during 1988. Transplants of Tall Utah 52-70 HK grown from commercial seed served as controls. After approximately 12 wk of growth in the field, six to seven plants per R<sub>1</sub> line were uprooted, the crowns were cut open, and the plants were rated for disease severity. Ratings were on a 1–5 scale as follows: 1 = no vascular discoloration in root or crown area; 2 = trace (<1%) of vascular discoloration in root or crown area; 3 = <50% crown area discolored; 4 = >50% crown area discolored; and 5 = dead or nearly dead plant (severely stunted and chlorotic). Plants with no vascular discoloration were saved for seed production. The R<sub>1</sub> plants were trimmed, replanted in the greenhouse, vernalized, self-pollinated,

**Table 1.** Disease ratings for somaclone R<sub>1</sub> progenies screened for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in a nonreplicated field trial in Decatur, MI, in 1988 and from which plants were saved for seed production

R <sub>1</sub> progeny	No. of plants in disease rating classes <sup>x</sup>					Mean disease rating
	1	2	3	4	5	
K-328	4	2	0	0	0	1.3 <sup>*y</sup>
K-108	4	3	0	0	0	1.4 <sup>*</sup>
K-233	3	3	0	0	0	1.5
K-16	3	3	0	0	0	1.5
K-26	2	4	0	0	0	1.6
K-237	2	5	0	0	0	1.7
K-33	1	5	0	0	0	1.8
K-128	1	5	0	0	0	1.8
K-344	1	5	0	0	0	1.8
K-134	1	6	0	0	0	1.9
K-129	1	3	2	0	0	2.2
Tall Utah 52-70 HK <sup>z</sup>	1	3	2	0	0	2.2

<sup>x</sup> Disease rating was based on a 1–5 scale where: 1 = no vascular discoloration; 2 = trace of vascular discoloration in root or crown area; 3 = <50% crown area discolored; 4 = >50% crown area discolored; and 5 = dead or nearly dead plant. Data presented for six to seven plants per line.

<sup>\*y</sup> = Mean disease ratings significantly lower than that for Tall Utah 52-70 HK according to Student's *t* test (*P* < 0.05).

<sup>z</sup> Moderately resistant celery cultivar from which somaclones were regenerated. Seeds of tall Utah 52-70 HK were from a commercial source.

**Table 2.** Disease ratings of R<sub>2</sub> progeny from somaclones for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in a replicated field trial in Decatur, MI, in 1989

R <sub>2</sub> progeny <sup>w</sup>	No. of plants in disease rating classes <sup>x</sup>					Mean disease rating
	1	2	3	4	5	
K-328 [4]	11	7	2	0	0	1.6 <sup>*y</sup>
K-233 [4]	11	7	2	0	0	1.6 <sup>*</sup>
K-26 [1]	9	9	2	0	0	1.7 <sup>*</sup>
K-128	7	13	0	0	0	1.7 <sup>*</sup>
K-233 [2]	2	12	5	1	0	2.3
Tall Utah 52-70 HK <sup>z</sup>	2	10	6	2	0	2.4
K-233[3]	3	6	10	1	0	2.5

<sup>w</sup> Letter and number refers to parental somaclone. Number in brackets refers to the R<sub>1</sub> plant that was selfed to produce that particular R<sub>2</sub> line.

<sup>x</sup> Plants were rated on a 1–5 scale where: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = <50% crown area discolored; 4 = >50% crown area discolored; and 5 = dead or nearly dead plant. Data presented for 20 plants per line (10 from each of two replications).

<sup>\*y</sup> = Mean disease ratings significantly lower than that for Tall Utah 52-70 HK, LSD = 0.6 (*P* = 0.05).

<sup>z</sup> Moderately resistant celery cultivar from which somaclonal lines were derived. Seeds of Tall Utah 52-70 HK were from a commercial source.

and cared for as described earlier.

The R<sub>2</sub> progeny from six R<sub>1</sub> lines were screened for resistance in a field trial in 1989 in a randomized block with two replications at the Decatur site. At harvest, 10 plants per R<sub>2</sub> line for each replication were rated for disease severity. The remaining 20 R<sub>2</sub> lines produced seed too late for the 1989 season and were screened in 1990 in a nonreplicated trial near Hudsonville, MI. Ten plants per line were rated for disease severity. Seeds were pregerminated and plants grown as described for R<sub>1</sub> progeny.

## RESULTS

**Somaclonal regeneration and screening for resistance.** A total of 544 somaclones regenerated from Tall Utah 52-70 HK were selected and transplanted into potting mix. Somaclones not selected for transplanting were either extremely stunted or were morphologically abnormal. Of the 350 somaclones that survived transfer from culture to the greenhouse, 160 were screened for resistance to *F. o. apii* race 2 in the greenhouse, and 59 of these had no vascular discoloration after 15–18 wk (*data not shown*). Twenty-three of these somaclones were off-type with short, thin petioles and an open heart and were discarded before vernalization. Of 190 somaclones screened for resistance to *F. o. apii* race 2 in the field, seven developed severe disease. Twenty-two were free of

or had just a trace of discoloration and were replanted in the greenhouse (*data not shown*). Two of these somaclones developed soft rot before or during vernalization and were discarded.

Of the 56 somaclones surviving from greenhouse and field screens, 35 flowered and set enough viable seed for a progeny screen. Unique variants were observed in the R<sub>1</sub> progeny from two somaclones. Four of 17 seedlings from one line were dwarfs. In a second line, seven of 56 seedlings were albinos that did not grow past the cotyledon stage in the greenhouse.

**Screening somaclone progeny for resistance.** Ten R<sub>1</sub> lines (K-328, K-108, K-233, K-16, K-26, K-237, K-128, K-33, K-344, and K-134) were rated as highly resistant to *F. o. apii* race 2 (mean disease rating <2) in the field (Table 1). A total of 28 highly resistant plants were saved from these 10 R<sub>1</sub> lines and from line K-129 (Table 1), 26 of which flowered and were selfed. Plants from these lines (except K-129) all were within the disease rating classes of 1 or 2 (Table 1), whereas Tall Utah 52-70 HK control plants were in disease rating classes of 1, 2, or 3 (Table 1). Most of the R<sub>1</sub> lines were uniform in height and appearance in the field and resembled parental Tall Utah 52-70 HK plants in appearance and growth habit. Lines K-233 and K-26 were the two most vigorous lines and were uniform in appearance under field conditions. Lines K-108, K-328, and K-237

were also fairly uniform in appearance and disease reaction, but sizes of K-16 plants varied.

Twenty-two of the remaining 24 R<sub>1</sub> lines were rated as moderately resistant (disease ratings of 2–3) (*data not shown*). The last two R<sub>1</sub> lines had susceptible disease ratings of 3.3 and 4.0, respectively, which were significantly higher than the 2.2 rating for Tall Utah 52-70 HK plants from commercial seed ( $P = 0.05$ ) (*data not shown*).

The R<sub>2</sub> progeny from six lines were screened for resistance to *F. o. apii* race 2 in a replicated field trial near Decatur in 1989 (Table 2). Four lines, (K-328[4], K-233[4], K-26[1], and K-128[1],) had mean disease ratings that were significantly less than the disease ratings for Tall Utah 52-70 HK plants ( $P = 0.05$ ). (The number in brackets refers to the R<sub>1</sub> plant that was selfed to produce that particular R<sub>2</sub> line.) Eighteen to 20 of 20 (90–100%) plants from these four lines had disease ratings of 1 or 2, compared with only 60% of plants from Tall Utah 52-70 HK (Table 2). Plants with disease ratings of 1 were saved from these four somaclonal lines for seed production. Lines K-233[2] and K-233[3] had disease ratings that were not significantly different from Tall Utah 52-70 HK plants (Table 2).

An additional 20 R<sub>2</sub> lines were screened in a nonreplicated trial in Hudsonville in 1990 (Table 3). Tall Utah 52-70 HK had a higher mean disease rating in this field trial (Table 3) than in either 1988 or 1989 at Decatur (Tables 1 and 2). Most Tall Utah 52-70 HK plants were stunted in the Hudsonville field trial, and no plants from this cultivar had a disease rating of 1 or 2 (Table 3). All of the 20 R<sub>2</sub> lines screened for resistance in 1990 had significantly lower disease ratings than Tall Utah 52-70 HK control plants ( $P < 0.01$ ) (Table 3). Lines K-108[4], K-108[3], K-16[2], and K-328[3] were rated highly resistant, and eight or nine of 10 (80–90%) plants from these lines had disease ratings of 1 or 2 (Table 3). Most of the R<sub>2</sub> lines were uniform in height and appearance. K-108[3] was a tall, uniform line with full, compact hearts and wide petioles. Line K-108[4] was the shortest line and had small plants with thin petioles. K-16[2] plants were tall and of uniform height but had petioles of variable widths. Even R<sub>2</sub> lines with disease ratings  $\geq 2.5$  were tall with full, compact hearts.

## DISCUSSION

Celery somaclones that expressed a higher level of resistance to *F. o. apii* race 2 than the parental cultivar and that transmitted that resistance to their progeny through self-fertilization were successfully regenerated. Highly resistant somaclones were regenerated at a high frequency (23%) from the moderately resistant cultivar Tall Utah 52-70 HK.

**Table 3.** Disease ratings of R<sub>2</sub> progeny from somaclones for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in a replicated field trial in Hudsonville, MI, in 1990

R <sub>2</sub> progeny <sup>w</sup>	No. of plants in disease rating classes <sup>x</sup>					Mean disease rating
	1	2	3	4	5	
K-108 [4]	5	4	1	0	0	1.7 <sup>*y</sup>
K-108 [3]	2	7	1	0	0	1.9*
K-16 [2]	2	6	2	0	0	2.0*
K-328 [3]	3	5	1	1	0	2.0*
K-233 [1]	2	4	4	0	0	2.2*
K-134 [1]	2	4	4	0	0	2.2*
K-108 [2]	0	7	3	0	0	2.3*
K-237 [2]	1	4	5	0	0	2.4*
K-328 [1]	0	6	4	0	0	2.4*
K-344 [1]	2	3	4	1	0	2.4*
K-108 [1]	0	5	5	0	0	2.5*
K-16 [1]	0	5	4	1	0	2.6*
K-16 [4]	0	4	6	0	0	2.6*
K-26 [2]	0	4	6	0	0	2.6*
K-237 [1]	0	3	7	0	0	2.7*
K-16 [5]	0	3	7	0	0	2.7*
K-129 [2]	0	3	7	0	0	2.7*
K-16 [3]	0	2	8	0	0	2.8*
K-33 [2]	0	1	9	0	0	2.9*
K-26 [3]	0	1	6	3	0	3.2*
TU 52-70 HK <sup>z</sup>	0	0	2	7	1	3.9

<sup>w</sup>Letter and number refers to parental somaclone. Number in brackets refers to the R<sub>1</sub> plant that was selfed to produce that particular R<sub>2</sub> line.

<sup>x</sup>Plants were rated on a 1–5 scale where: 1 = no vascular discoloration in root or crown area; 2 = trace of vascular discoloration in root or crown area; 3 = <50% crown area discolored; 4 = >50% crown area discolored; and 5 = dead or nearly dead plant. Data presented for 10 plants per line.

<sup>y</sup>\* = Mean disease ratings significantly lower than that for Tall Utah 52-70 HK according to Student's *t* test ( $P < 0.01$ ).

<sup>z</sup>Moderately resistant celery cultivar from which somaclonal lines were derived. Seeds of Tall Utah 52-70 HK were from a commercial source.

Ten of the R<sub>1</sub> lines were rated highly resistant, although most were not statistically different from the moderately resistant cultivar Tall Utah 52-70 HK. Plants of the highly resistant R<sub>1</sub> lines had disease ratings of 1 or 2, whereas one-third of Tall Utah 52-70 HK plants had ratings of 3. Five R<sub>1</sub> lines gave rise to eight R<sub>2</sub> lines which were highly resistant under the high disease pressure during 1989 and 1990, and, again, a higher percentage of the plants from these lines had disease ratings of 1 or 2 than of the plants from Tall Utah 52-70 HK. Lines with disease ratings  $\leq 2$  would yield a greater number of marketable plants than existing cultivars. Plants with disease ratings  $\geq 3$  are usually unacceptable for fresh market at harvest, taste bitter, or rot quickly after harvest (1).

Wright and Lacy (24) and Heath-Pagliuso et al (9) also regenerated highly resistant celery somaclones from cell cultures. Wright and Lacy (24) reported that 4 and 28% of somaclones regenerated from celery cultivars susceptible and moderately resistant to *F. o. apii* race 2, respectively, had no disease symptoms when inoculated with *F. o. apii* race 2. Some of the highly resistant somaclones were also resistant to either Septoria late blight, Cercospora early blight, or bacterial leaf blight, but no somaclone was resistant to all of the pathogens, and all were overcome by the pathogens to which they were susceptible before flowering. Heath-Pagliuso et al (9) reported that only 0.5% of the somaclones regenerated from *F. o. apii* race 2-susceptible celery cultivar Tall Utah 52-70 R were highly resistant to the pathogen. They self-pollinated two highly resistant somaclones. The progeny from one somaclone were moderately resistant to *F. o. apii* race 2; the second, UC-T3 (which resulted from two cycles of cell culturing), had progeny that were highly resistant.

Resistance in Tall Utah 52-70 HK is coded for by at least one locus (21). Somaclonal variation could affect this resistance in several ways. The resistance could be increased by amplification of the existing gene or by a mutation resulting in the addition of another gene. The gene could also be rendered inoperative by the tissue culture procedure, resulting in somaclones susceptible to *F. o. apii* race 2. This was apparently the case in 4% of the somaclones screened for resistance in the field.

Not all disease-free somaclones gave rise to progeny that were highly resistant to *F. o. apii* race 2. Epigenetic changes induced by the stresses of culturing conditions (15) can give rise to an R<sub>0</sub> phenotype that is not heritable. Likewise, genetic changes that are not stable through meiosis (12) or a somaclone es-

caping infection attributable to a micro-environment unfavorable for pathogen survival or infection could lead to an apparent loss of resistance from the R<sub>0</sub> to R<sub>1</sub> generation. Segregation of genetic changes induced by in vitro culturing, combined with segregation of the resistance gene already present in the Tall Utah 52-70 HK parents during selfing of the R<sub>0</sub> and R<sub>1</sub>, could also account for the decrease in resistance in some of the R<sub>1</sub> and R<sub>2</sub> lines. Approximately 40% of the R<sub>1</sub> progeny from the highly resistant somaclone UC-T3 were susceptible to *F. o. apii* race 2 (10). The authors concluded that the variability of resistance in the R<sub>1</sub> lines was attributable to segregation of at least two resistance genes in UC-T3, which had resulted after two cell culture cycles.

The primary benefit of somaclonal variation is its use in the creation of useful genetic variation without hybridization. Celery somaclones produced in this study were also selected for morphology and growth habits resembling the parental cultivar, Tall Utah 52-70 HK. Most R<sub>1</sub> and R<sub>2</sub> plants from the somaclonal lines resembled Tall Utah 52-70 HK plants in the field.

We did not attempt to select for resistance to *F. o. apii* race 2 at the cellular level. No host-specific toxin has been implicated in the infection of celery by *F. o. apii* race 2. Heath-Pagliuso et al (9) reported that exposing cell cultures to fusaric acid did not produce somaclones resistant to *F. o. apii* race 2. After an extensive review of tissue culture research, Daub (3) concluded that screening at the whole plant level is as effective in providing pathogen-resistant variants as using a selective agent at the cellular level.

Somaclonal lines described here could provide a source of increased resistance to *F. o. apii* race 2 for celery breeding programs. We are examining further the disease resistance and horticultural characteristics of the R<sub>3</sub> and F<sub>1</sub> (crosses between selected R<sub>2</sub> plants) generations. Somaclonal lines that are horticulturally similar to or superior to Tall Utah 52-70 HK may be released as improved celery cultivars.

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