

## Potential of Somaclonal Celeries for Use in Integrated Pest Management

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**ABSTRACT** The somaclonal celery lines 'K-26[1]', 'K-108[3]2', 'K-128', 'F-128[3]1', and 'F-128[4]', developed for resistance to the *Fusarium* yellows caused by *Fusarium oxysporum* f. sp. *apii*, were investigated for susceptibility to *Spodoptera exigua* (Hübner), and for hazardous concentrations of linear furanocoumarins, a group of compounds toxic to humans and animals, and found in *Apium* spp. The somaclonal lines K-26[1], K-108[3]2, and K-128 were all significantly more resistant to *S. exigua* than the commercial standard celery 'Tall Utah 52-70R'. None of the somaclonal lines had total leaf contents of linear furanocoumarins significantly different from levels found in 52-70R. Petiole contents of furanocoumarins in the different celeries were variable, but below levels reported to cause acute dermatitis (18 µg/g fresh weight) or even chronic dermatitis (7 µg/g) in humans. Because the new somaclonal lines have resistance to a major celery disease, *Fusarium* yellows, the K-26[1], K-108[3]2, and K-128 lines represent good potential components for integration into breeding programs for integrated pest management for celery.

**KEY WORDS** *Spodoptera exigua*, *Apium graveolens*, somaclones, host plant resistance, linear furanocoumarins

THE DISEASE-CAUSING FUNGAL PATHOGEN *Fusarium oxysporum* f. sp. *apii* is a major limiting factor for celery, *Apium graveolens* L. *dulce*, production in the United States (Hart and Endo 1976, Elmer and Lacy 1984, Awuah et al. 1986, Martyn 1987, Toth and Lacy 1991). *Fusarium* yellows is characterized by vascular discoloration and necrosis of the roots and crowns, resulting in chlorotic, stunted, and wilted plants. A 2nd threatening factor to celery production in California is larval infestations of beet armyworm *Spodoptera exigua* (Hübner) (Van Steenwyk and Toscano 1981, Trumble et al. 1990). Beet armyworm feeding on leaves, and especially on the plant heart, prevents new petiole production, and damage to petioles results in reduced product marketability because of cosmetic injury. Use of *S. exigua*-resistant celery cultivars remains the best insect control tool because insecticidal sprays against these larvae usually result in increased secondary infestations by the leaf miner *Liriomyza trifolii* (Burgess) caused by the destruction of the natural enemies of this insect (Oatman and Kennedy 1976, Trumble 1990). Heavy *L. trifolii* infestations frequently stunt and sometimes kill plants by mining in the mesophyll tissues. Plant resistance also has been reported to be the only

economically viable means for controlling *F. o. f. sp. apii* in celery (Toth and Lacy 1991), and both conventional breeding efforts and tissue culture techniques have resulted in the development of *Fusarium*-resistant celery cultivars (Elmer and Lacy 1984, Orton et al. 1984, Elmer et al. 1986, Quiros et al. 1987, Toth and Lacy 1991).

Somaclonal variation, a mechanism used to regenerate large numbers of genetically variable plants through tissue culture (Larkin and Scowcroft 1981), has been used successfully to develop resistance against nematodes, fungi, bacteria, or viruses in several crop plants. These plants include alfalfa, *Medicago sativa* L. (Latunde-Dada and Lucas 1983); oat, *Avena sativa* L. (Rines and Luke 1985); tomato, *Lycopersicon esculentum* Mill (Barden et al. 1986); soybean, *Glycine max* L. (Olah and Schmitthenner 1988); cottonwood, *Populus deltoides* Bartr. (Prakash and Thielges 1989); pepper, *Mahus domestica* L. (Evans 1989); celery (Heath-Pagliuso and Rappaport 1990, Toth and Lacy 1991); sugar beet, *Beta vulgaris* L. (Yu and Pakish 1991); and apple, *Capsicum annum* L. (Donovan et al. 1994). Although much is known about the response of somaclonal celery lines to pathogens (Toth and Lacy 1991), their potential for use in insect pest management has not been reported. The primary goal of our study was to examine the new somaclonal lines 'K-26[1]', 'K-108[3]2', 'K-128', 'F-128[3]1', and 'F-128[4]' developed at Michigan State University, Department of Botany and Plant Pathology, for *Fusarium*

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resistance, for susceptibility to *S. exigua* with respect to the widely grown commercial celery cultivar 'Tall Utah 52-70R'. Information on the identification and characterization of insect resistance in *Fusarium*-resistant celery genotypes could be valuable for multiple pest-resistant cultivar development programs.

The development of new celery cultivars for pest resistance has often resulted in increased production of linear furanocoumarins in the plant (Trumble et al. 1990, McCloud et al. 1992, Afek et al. 1994). The furanocoumarins commonly isolated from *Apium* spp. (psoralen, 5-methoxypsoralen or bergapten, and 8-methoxypsoralen or xanthotoxin) have been used since ancient times to cure human skin disorders such as skin depigmentation (vitiligo) and psoriasis (Musajo and Rodighiero 1962, Scott et al. 1976, Van Scott 1976). However, the use of these furanocoumarins for medicinal purpose was associated with higher incidence of skin cancer in humans (Musajo and Rodighiero 1962, Stern et al. 1979, Grekin and Epstein 1981). For the past 30 yr (Scheel et al. 1963, Finkelstein et al. 1994), celery and the closely related species *Apium rapaceum* L. (celeriac) have been among the most extensively studied vegetables for linear furanocoumarin content because of their potential for high concentrations of these compounds in the plant (Beier et al. 1983; Chaudhary et al. 1985; Trumble et al. 1990; Diawara et al. 1992, 1994) and risks associated with handling (Finkelstein et al. 1994) or ingestion (Ljunggren 1990). The furanocoumarins have been reported to be carcinogenic, mutagenic (Roelandts 1984, Koch 1986, Young 1990) and photodermitic (Ljunggren 1990, Berkeley et al. 1986, Fleming 1990). Consequently, the 2nd major objective of our research was to investigate the new somaclonal lines for hazardous concentrations of linear furanocoumarins. Worker safety and consumer health concerns associated with celery handling and consumption require testing of new celery germplasm for composition and concentration of linear furanocoumarins before their release for large scale commercial production.

### Materials and Methods

The new somaclonal celery lines K-26[1], K-108[3]1, K-128, F-128[3]1, and F-128[4] were obtained from the germplasm collection held at the Michigan State University Department of Botany and Plant Pathology. The somaclonal lines beginning with K are  $R_2$  lines derived from the celery cultivar Tall Utah 52-70HK, which is moderately resistant to *F. oxysporum* f. sp. *apii* race 2 (Toth and Lacy 1991). The somaclonal lines beginning with F are  $R_2$  lines derived from the cultivar 'Florida 683', highly susceptible to *Fusarium* yellows (M. L. Lacy, unpublished data). Regeneration and testing were conducted as described by Toth and Lacy (1991). The commercial celery Tall Utah 52-70R was obtained from germplasm resources held

at the University of California, Davis, Department of Vegetable Crops. Beet armyworm larvae were from a laboratory colony maintained on artificial diet (Shorey and Hale 1965) at  $27 \pm 2^\circ\text{C}$  and a photoperiod of 16:8 (L:D) h. Field-collected larvae were added to the colony every year.

All 6 celery entries were seeded on 11 January 1993 and transplanted on 25 March 1993 in single rows of 8 m  $\times$  76.2 cm on sandy loam soil at the University of California's Agricultural Operations field in Riverside, CA. Test entries were transplanted in complete randomized blocks with 3 replicates. Plots were furrow irrigated to maintain adequate soil moisture; local standard cultural practices were followed.

**Beet Armyworm Resistance Bioassays.** Samples were collected for feeding 11 wk after the celery was transplanted. This period was chosen because all petioles produced 8 wk after transplanting would become part of the harvested product (Van Steenwyk and Toscano 1981) and celery is usually harvested  $\approx 16$  wk after transplanting. Because of the preference for leaves by 1st instars (Criswold and Trumble 1985), beet armyworm infestations initiated by egg deposition 15 wk after transplanting would not be a threat to commercial celery production. Tissue produced just before harvest was tested because the bioassay period usually lasts 3 wk.

The relative susceptibility of the 5 new somaclonal lines K-26[1], K-108[3]2, K-128, and F-128[3]1, F-128[4] to *S. exigua* with respect to 52-70R was determined by rearing the insects on fresh tissue. Samples were collected by cutting the petiole just above the 1st pair of leaflets located at the plant base. Samples were pooled within test entry in each replicate. Cups (30 ml) containing a solidified agar solution (Diawara et al. 1992) were filled with fresh plant parts (leaf + petiole) of each test entry. One neonate beet armyworm was placed in each cup, which then was capped with a plastic lid pierced with pinholes. Plant tissue was renewed every other day and the agar solution every week. For each bioassay, cups were arranged as a complete block with 3 replicates of 20 cups per replicate. All tests were maintained in environmental chambers at  $27 \pm 2^\circ\text{C}$ , 75% RH, and a photoperiod of 16:8 (L:D) h. Weight of larvae at 9 d, time to pupation, weight of pupae, time to adult emergence, and survivorship were recorded for each test entry.

**Linear Furanocoumarin Analysis.** All 6 celery entries were analyzed for the 3 major linear furanocoumarins isolated in *Apium* spp.: (1) psoralen, (2) 5-methoxypsoralen (bergapten), and (3) 8-methoxypsoralen (xanthotoxin) (Trumble et al. 1990, Diawara et al. 1992). Sample collections for determination of furanocoumarin composition and concentration were made at celery harvest on 19 July 1993. Three plants per entry per replicate were harvested at the marketable growth stage; these plants were not used during the feeding bioassay. For each plant, all senescing, unmarketable

**Table 1.** Development and survival of *S. exigua* on somaclonal celeries and the commercial celery variety Tall Utah 52-70R, *A. graveolens* L. *dulce* (mean  $\pm$  SEM)

Celery	Wt (mg)		Development time, d		% survival	
	9 d larva	Pupa	To pupa	To adult	To pupa	To adult
K-26[1]	14.2 $\pm$ 1.8ab	70.4 $\pm$ 4.1ab	17.6 $\pm$ 0.5a	25.8 $\pm$ 0.6ab	33.3 $\pm$ 9.2a	27.0 $\pm$ 5.0a
K-108[3]2	15.3 $\pm$ 1.8ab	63.3 $\pm$ 3.1a	17.5 $\pm$ 0.5a	25.6 $\pm$ 0.5ab	43.3 $\pm$ 7.2a	31.7 $\pm$ 5.1a
K-128	11.6 $\pm$ 1.2a	67.1 $\pm$ 2.7ab	18.9 $\pm$ 0.5a	27.6 $\pm$ 0.5b	50.0 $\pm$ 5.0ab	41.7 $\pm$ 1.7a
F-128[3]1	17.7 $\pm$ 2.0ab	66.3 $\pm$ 3.1ab	16.6 $\pm$ 0.5a	24.8 $\pm$ 0.5a	43.0 $\pm$ 9.4a	43.0 $\pm$ 8.0ab
F-128[4]	19.9 $\pm$ 2.1b	67.9 $\pm$ 2.6ab	17.4 $\pm$ 0.5a	26.0 $\pm$ 0.5ab	65.0 $\pm$ 7.6b	48.3 $\pm$ 1.7ab
52-70R	20.7 $\pm$ 2.6b	75.6 $\pm$ 4.1b	17.5 $\pm$ 0.4a	25.6 $\pm$ 0.4ab	78.7 $\pm$ 4.1b	61.3 $\pm$ 1.9b
<i>P</i>	0.002	0.004	0.130	0.064	0.028	0.050
<i>F</i>	3.971	3.337	1.732	2.147	4.097	3.685
df	3, 335	5, 171	5, 171	5, 137	5, 10	5, 10

Means within a column not followed by the same letter are statistically different at the 5% level using the Tukey-Kramer test (Abacus Concepts 1989).

petioles were removed. Then, the 2 outermost petioles and associated leaves were cut. Sample leaves and petioles were separated and stored at  $-65^{\circ}\text{C}$  until time of chemical analysis. Extraction of linear furanocoumarins for the different test entries was conducted as previously described (Diawara et al. 1992, Trumble et al. 1992). Briefly, sample tubes were spiked with 5  $\mu\text{g}$  of a synthetic internal standard, 7-benzoyloxycoumarin (synthesized from commercially available 7-hydroxycoumarin, Aldrich, Milwaukee, WI) (unpublished data). Plant samples were homogenized in distilled  $\text{H}_2\text{O}$ , extracted with toluene and the crude extract was partially purified by passage through an Extract Clean solid-phase extraction cartridge tube (Alltech, Deerfield IL) and eluted with acetone:chloroform (95:5). The purified extracts were concentrated to dryness, then dissolved in 250  $\mu\text{l}$  of hexane. High-performance liquid chromatography (HPLC) analyses were carried out with a Hewlett-Packard 1040 HPLC pump and an H.-P. 1050A diode array detector with a Chemstation data system (Hewlett-Packard, Avondale, PA). Peaks were monitored and quantified at 280 nm. An Alltech Econosil silica column (25 cm  $\times$  4.6 mm, 5  $\mu$  particle size) with a guard column (10  $\times$  4.6 mm) filled with the same packing material were used, and eluted isocratically with hexane:tetrahydrofuran (81:19). The tetrahydrofuran (HPLC grade) from Aldrich gave markedly better resolution than tetrahydrofuran from Fisher (St. Louis, MO).

**Statistical Analyses.** All data were analyzed as a randomized complete block design using analysis of variance (ANOVA) (Abacus Concepts 1989). Survival data were analyzed by comparing the percentage mortality for the 20 cups in each replicate. Statistically different means of each variable were separated at the 5% significance level using the Tukey-Kramer test (Abacus Concepts 1989).

## Results

**Beet Armyworm Feeding Bioassay.** *S. exigua* fed the somaclonal line K-128 weighed significantly less at 9 d ( $P = 0.002$ ;  $df = 5, 355$ ;  $F = 3.791$ )

or during the pupal stage ( $P = 0.004$ ;  $df = 5, 171$ ;  $F = 3.557$ ) compared with individuals fed the commercial celery 52-70R or the somaclonal line F-128[4] (Table 1). No significant differences were observed at the 5% level among the celery genotypes for insect developmental time from egg to pupa ( $P = 0.130$ ;  $df = 5, 71$ ;  $F = 1.732$ ) or to adult ( $P = 0.064$ ;  $df = 5, 137$ ;  $F = 2.147$ ). At the 10% level, cohorts reared on the somaclonal line K-128 took significantly longer to emerge as adults than those reared on F-128[3]1 ( $P = 0.064$ ;  $df = 5, 137$ ;  $F = 2.147$ ).

The somaclonal lines K-26[1], K-108[3]2, and K-128 were all significantly more resistant than the commercial celery 52-70R based on percentage of larvae surviving to adult ( $P = 0.050$ ;  $df = 5, 10$ ;  $F = 3.685$ ). Also, significantly less *S. exigua* survived to the pupal stage on K-26[1] and K-108[3]2 than on 52-70R ( $P = 0.028$ ;  $df = 5, 10$ ;  $F = 4.097$ ). Approximately 50% of the larvae fed K-128, if they survived to adults, developed into unfit moths that were either unable to unfold their wings or had deformed wings or body, or both. Overall, insect survival to adult varied from 27.0 to 48.3% on the somaclonal lines whereas over 60% of cohorts reared on the commercial celery emerged as adults. Previous research found that *S. exigua* survival on 52-70R varied from 34 to 82% (Diawara et al. 1992, 1994).

**Linear Furanocoumarin Composition and Concentration.** All 6 celeries were tested for the linear furanocoumarins psoralen, bergapten, and xanthotoxin. Only bergapten and xanthotoxin were detected in the petioles of the different celery genotypes; no psoralen was found in this plant part (detectable level 0.005  $\mu\text{g/g}$ ) (Table 2). The somaclonal lines K-108[3]2 and K-128[3]1 contained significantly higher levels of bergapten in the petioles than the commercial celery 52-70R ( $P = 0.004$ ;  $df = 5, 46$ ;  $F = 3.965$ ). None of the new somaclonal lines had concentrations of xanthotoxin in petioles significantly higher than the concentrations found in 52-70R ( $P = 0.055$ ;  $df = 5, 46$ ;  $F = 2.356$ ); this may be caused, in part, by the relatively high variations (SEM) in these results. The

**Table 2. Linear furanocoumarin contents ( $\mu\text{g/g}$  fresh petiole) of somaclonal celeries and Tall Utah 52-70R, *A. graveolens* L. dulce, grown under field conditions (mean  $\pm$  SEM)**

Celery	Psoralen <sup>a</sup>	Bergapten	Xanthotoxin	Total
K-26[1]	—	1.610 $\pm$ 0.242ab	0.905 $\pm$ 0.141a	2.515 $\pm$ 0.364ab
K-108[3]2	—	1.900 $\pm$ 0.172b	0.989 $\pm$ 0.105a	2.890 $\pm$ 0.248ab
K-128	—	1.195 $\pm$ 0.189ab	0.551 $\pm$ 0.092a	1.746 $\pm$ 0.264a
F-128[3]1	—	2.018 $\pm$ 0.153b	1.136 $\pm$ 0.133a	3.154 $\pm$ 0.263b
F-128[4]	—	1.267 $\pm$ 0.263ab	0.755 $\pm$ 0.199a	2.022 $\pm$ 0.446ab
52-70R	—	0.994 $\pm$ 0.176a	0.555 $\pm$ 0.185a	1.580 $\pm$ 0.334a
<i>P</i>	—	0.004	0.055	0.007
<i>F</i>	—	3.965	2.356	3.636

Means within a column not followed by the same letter are statistically different at the 5% level using the Tukey-Kramer test (Abacus Concepts 1989). Means represent actual data; analyses based on square-root transformations.

<sup>a</sup> Zero variance violates the assumptions of ANOVA; therefore ANOVA was not performed for psoralen.

total amount of bergapten plus xanthotoxin in the petioles was significantly higher in F-128[3]1 compared with the commercial celery 52-70R or somaclonal line K-128 ( $P = 0.007$ ;  $df = 5, 46$ ;  $F = 3.636$ ).

In the leaves, all 3 linear furanocoumarins were present at much higher concentrations than in the petioles of all 6 celeries (Table 3). None of the newly developed somaclonal lines had leaf contents of psoralen ( $P = 0.748$ ;  $df = 5, 46$ ;  $F = 0.536$ ), bergapten ( $P = 0.455$ ;  $df = 5, 46$ ;  $F = 0.955$ ), xanthotoxin ( $P = 0.060$ ;  $df = 5, 46$ ;  $F = 2.301$ ), or all 3 combined significantly higher compared with the commercial celery 52-70R ( $P = 0.376$ ;  $df = 5, 46$ ;  $F = 1.094$ ). Statistically significant differences were observed only at the 10% level in the concentration of leaf xanthotoxin between K-128 (2.6  $\mu\text{g/g}$ ) and F-128[3]1 (6.9  $\mu\text{g/g}$ ) ( $P = 0.060$ ;  $df = 5, 46$ ;  $F = 2.301$ ).

### Discussion

The somaclonal lines K-26[1], K-108[3]2, and K-128 were more resistant to *S. exigua* feeding than the commercial celery 52-70R based on insect survival to adult. Although the somaclonal line F-128[3]1 had significantly more combined bergapten plus xanthotoxin than the commercial line, the concentrations of linear furanocoumarins in petioles of all 6 celeries were far below the critical levels known to cause acute dermatitis (18  $\mu\text{g/g}$  fresh weight, Austad and Kavli 1983) or even

chronic dermatitis (7  $\mu\text{g/g}$ , Seligman et al. 1987). Total concentration of all 3 furanocoumarins in leaves of K-26[1], K-108[3]2, K-128, F-128[4], or 52-70R would not cause acute dermatitis; however, they are high enough to induce chronic dermatitis. Although the levels of linear furanocoumarins detected in F-128[3]1 were just below the 18  $\mu\text{g/g}$  reported to be hazardous to human and animal health, these levels are high enough to raise concerns given the fact that several environmental conditions, not tested here, can influence production of these compounds in crop plants (McCloud et al. 1992, Trumble et al. 1992, Diawara et al. 1994). The levels of linear furanocoumarins detected here represent no direct threat to health of consumers, who usually have contact only with the petioles. Thus, the new somaclonal lines have petioles that are safe for handling and consumption, unlike some of the previous breeding lines selected for disease resistance (Trumble et al. 1990). However, with concentrations exceeding even 7  $\mu\text{g/g}$  of fresh leaf, safety measures (for example, protective clothing) should be used by pest control advisors and workers handling celery leaves in the field.

Resistance to *S. exigua* in 52-70R and celery breeding lines varied as plants matured, with higher resistance in older plants (Diawara et al. 1993, 1994). Diawara et al. (1995) found that linear furanocoumarin concentrations varied significantly within leaves and within petioles of the same plant, with older, outer plant parts having higher concen-

**Table 3. Linear furanocoumarin contents ( $\mu\text{g/g}$  fresh leaf) of somaclonal celeries and Tall Utah 52-70R, *A. graveolens* L. dulce, grown under field conditions (mean  $\pm$  SEM)**

Celery	Psoralen	Bergapten	Xanthotoxin	Total
K-26[1]	0.406 $\pm$ 0.159a	10.471 $\pm$ 1.579a	4.981 $\pm$ 0.760ab	15.857 $\pm$ 2.157a
K-108[3]2	0.436 $\pm$ 0.102a	6.639 $\pm$ 0.975a	4.128 $\pm$ 0.598ab	11.203 $\pm$ 1.483a
K-128	0.567 $\pm$ 0.276a	8.469 $\pm$ 1.746a	2.617 $\pm$ 0.815a	11.653 $\pm$ 2.752a
F-128[3]1	0.756 $\pm$ 0.241a	9.635 $\pm$ 1.079a	6.877 $\pm$ 1.611b	17.269 $\pm$ 2.119a
F-128[4]	0.911 $\pm$ 0.498a	5.421 $\pm$ 1.807a	3.290 $\pm$ 0.802ab	13.746 $\pm$ 2.813a
52-70R	0.457 $\pm$ 0.171a	5.762 $\pm$ 1.921a	4.933 $\pm$ 1.010ab	15.975 $\pm$ 2.921a
<i>P</i>	0.748	0.455	0.060	0.376
<i>F</i>	0.536	0.955	2.301	1.094

Means within a column not followed by the same letter are statistically different at the 5% level using the Tukey-Kramer test (Abacus Concepts 1989). Means represent actual data; analyses based on square-root transformations.

trations than inner parts. This explains some of the variation in standard errors observed during the study (Tables 2 and 3). How both insect resistance and furanocoumarin content of the new somaclonal lines may change in plants tested at younger or older growth stages warrants further investigation.

The new somaclonal lines all had concentrations of linear furanocoumarins in petioles and leaves comparable to levels found in the commercial Tall Utah 52-70R. The somaclonal lines K-26[1], K-108[3]2, and K-128 also were less susceptible to *S. exigua* larval feeding than 52-70R. Because these celeries have been developed for resistance to a major celery disease, *F. oxysporum* f. sp. *apii*, K-26[1], K-108[3]2, and K-128 represent good potential breeding lines for future development of integrated celery pest management programs.

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