Increase of Disease Resistance in Celery Cultivars by Regeneration of Whole Plants from Cell Suspension Cultures

J. C. WRIGHT, Former Graduate Research Assistant, and M. L. LACY, Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824

ABSTRACT

Wright, J. C., and Lacy, M. L. 1988. Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Disease 72: 256-259.

Callus cultures started from axillary buds of celery cultivars Florida 683 and Tall Utah 52-70 HK were used to initiate shaken liquid cultures of single plant cells and cell clumps. Whole plants (somaclones) regenerated from embryoids formed in shaken cultures were screened in the greenhouse for reaction to three fungal and one bacterial pathogen of celery: *Septoria apiicola* (late blight), *Cercospora apii* (early blight), *Fusarium oxysporum* f. sp. *apii* (Fusarium yellows), and *Pseudomonas cichorii* (bacterial blight). Regenerated plants varied from highly susceptible to highly resistant to all four pathogens, whereas parent plants of Florida 683 were uniformly highly susceptible to all pathogens. Parent plants of Tall Utah 52-70 HK were moderately resistant to the Fusarium yellows pathogen and highly susceptible to the remaining pathogens. Resistance to a given pathogen appeared to arise independently from resistance to other pathogens, since plants were rarely found with high resistance to two or more pathogens. This technique may become an important way of introducing disease resistance into susceptible cultivars of celery.

Somaclonal variation, first noted in tobacco (19,23), has been reported in several plant species (7,10–14). Plants showed differences in phenotypic or genotypic traits after regeneration of whole plants from callus or single cells derived from axillary buds from a single parent plant. Cytogenetic variations have also been observed in regenerated plants (1,5,15,26).

These observations led to screening of somaclonal progeny for agronomically valuable traits. Plant cells have produced somaclones resistant to the herbicides paraquat (17,27) and asulam (16) and to the plant pathogens *Phytophthora infestans* (Mont.) de Bary (2,10,24,29), *Alternaria solani* Sorauer (10), *Sclerospora sacchari* Miyaki, *Helminthosporium sacchari* (B. de Haan) Butler (8,10–14), and *Fusarium oxysporum* f. sp. *apii* (R. Nelson & Sherb.) Snyd. & Hans. (22).

This research was undertaken to determine whether variation in disease responses to four celery (*Apium graveolens* L. var. *dulce* (Miller) Pers.) pathogens occurs in celery somaclones regenerated from cell suspension cultures.

Michigan Agricultural Experiment Station Journal Article 12298.

Accepted for publication 11 October 1987 (submitted for electronic processing).

MATERIALS AND METHODS

Celery cultivars Florida 683 and Tall Utah 52-70 HK were chosen for this work because Florida 683 is highly susceptible to Fusarium yellows, caused by *F. o.* f. sp. *apii* race 2, whereas Tall Utah 52-70 HK is considered to be moderately resistant (6); high levels of resistance to Fusarium yellows do not exist in commercial celery. These two cultivars are highly susceptible to leaf blights caused by *Cercospora apii* Fres., *Septoria apiicola* Speg., and *Pseudomonas cichorii* (Swing.) Stapp (28). No resistance exists to these leaf blights.

Our methods of inducing callus, growing cells, and regenerating plants were similar to those of Rappaport et al (22). Axillary buds from 10-wk-old plants of Florida 683 and Tall Utah 52-70 HK were excised, surface-sterilized with 0.525% sodium hypochlorite for 10 min,

rinsed twice with sterile double-distilled water, and placed on modified Murashige-Skoog (MS) medium (19) containing, per liter, 10 g of agar, 20 g of sucrose, 2 g of glycine, 100 mg of myoinositol, 0.5 mg of nicotinic acid, 0.5 mg of pyridoxine hydrochloride, 0.1 mg of thiamine, 1 mg of (2,4-dichlorophenoxy)acetic acid (2,4-D), and 2 mg of benzyladenine in 9-cm petri dishes after the method of Rappaport et al (22). Four to five weeks after buds were placed on agar and callus had formed from the buds, the callus was transferred to 100 ml of MS liquid medium in 250-ml Erlenmeyer flasks. This medium differed from the above by omission of agar, reduction of the 2,4-D to 0.5 mg/L, and substitution of 0.1 mg of kinetin for benzyladenine. The flasks of callus liquid medium were incubated on a rotary shaker at 100 rpm in the dark at 22–24 C. Suspensions of predominantly single cells and cell clumps formed in the liquid cultures. These were subcultured and examined for embryoid formation every 2 wk. When embryoids had formed, usually after 8–9 wk, 1.5-ml samples from liquid medium containing embryoids were transferred to petri dishes of solid modified MS medium containing 1% agar but without 2,4-D or kinetin. These embryoids were incubated in 12 hr light/12 hr dark at 22–24 C until plantlets were regenerated.

We assumed that most embryoids formed from single cells because the cell suspensions were predominantly of single cells, but we could not be certain whether embryoids arose from single cells or clumps of cells. Plantlets arising from embryoids were transferred to fresh MS agar every 4 wk until large enough to transplant into soil. When plantlets were 3–4 cm high from the base of the stem to the uppermost leaf, they were transplanted into a commercial potting mix (peat/perlite/vermiculite, 1:1:1, pH 5.2) and placed in 200-ml Styrofoam cups with drain holes punched in the bottom. Cups were placed in plastic bags, and the tops of the bags were sealed to keep the humidity high. After 1 wk, a small hole was made in each bag, and the hole was enlarged each week for 4 wk until the plants were completely exposed to the greenhouse environment.

When plantlets were approximately 20 cm tall, one of two compound leaves of each somaclone was sprayed with 3 ml of aqueous suspensions of conidia (5×10^5 conidia/ml) of either *S. apiicola* (incitant of late blight) or *C. apii* (incitant of early blight). The other leaves were shielded with a plastic bag while the spore suspension was applied. A third leaf was inoculated with *P. cichorii* by placing a drop of aqueous bacterial cell suspension (10^5 cells/ml) onto individual leaflets of the petiole, then immediately pricking leaflets with a device containing six needles spaced within a 4-cm^2 area. Spore suspensions of *S. apiicola* were obtained by soaking infected celery leaves containing pycnidia in distilled water for 15 min and diluting the suspension to 5×10^5 conidia/ml. Spore suspensions of *C. apii* were obtained from cultures grown on carrot leaf agar for 2 wk, washed with distilled water to obtain conidia, and diluted to 5×10^5 conidia/ml (18). The bacterial cell suspension was obtained by growing *P. cichorii* on nutrient agar for 1 wk, washing the plates with distilled water, and diluting the cell suspension to 10^5 cells/ml.

After inoculations, somaclones were placed into a mist chamber in the greenhouse with intermittent mist (on 15 min, off 15 min) to keep the leaves wet for 4 days. Somaclones were transplanted into 4-in. pots containing muck soil naturally infested with *F. o.* f. sp. *apii* race 2, incitant of Fusarium yellows, and placed on benches under a 14-hr photoperiod at temperatures of 20–25 C. Plants grown from seeds of Florida 683 and Tall Utah 52-70 HK were used as controls in somaclone evaluations.

Plants were rated visually for disease responses. Ratings for early, late, and bacterial blights were made 3 wk after inoculation on a scale of 1–5, with 1 = highly resistant (<1% diseased tissue), 2 = moderately resistant (1–5%), 3 = moderately susceptible (6–25%), 4 = susceptible (26–50%), and 5 = highly susceptible (>50%). Ratings for Fusarium yellows were made 10 wk after transplantation on a scale of 1–5, with 1 = <1% vascular discoloration, 2 = 1–5%, 3 = 6–10%, 4 = 11–25%, and 5 = >25% of crown tissue discolored.

RESULTS

Somaclones of Florida 683 celery obtained from cell suspension cultures (254 plants) gave varying disease responses to early blight, late blight, bacterial blight, and Fusarium yellows (Table 1). Twelve plants (4.7%) appeared to be moderately resistant to early blight, whereas the rest were susceptible. Two plants (0.8%) were highly resistant to late blight, one (0.4%) was moderately resistant, and the rest were susceptible. Four somaclones (1.6%) were highly resistant to bacterial blight, 23 (9.1%) were moderately resistant, and the rest were susceptible. Nine somaclones (3.5%) were highly resistant to Fusarium yellows, 17 (6.7%) were moderately resistant, and the rest were susceptible. Control plants grown from seed were susceptible or highly susceptible to all four pathogens.

Somaclones of Tall Utah 52-70 HK produced from cell suspension cultures (148 plants) also gave varying disease responses to early blight, late blight, bacterial blight, and Fusarium yellows (Table 2). One plant (0.7%) was highly resistant to early blight, two (1.4%) were moderately resistant, and the rest were susceptible; control plants grown from seed were susceptible to highly susceptible. One plant (0.7%) was highly resistant to late blight, three (2.0%) were moderately resistant, and the rest were susceptible; control plants were all highly susceptible.

Table 1. Disease response of celery cv. Florida 683 somaclones regenerated from cell suspension cultures and inoculated with four pathogens in the greenhouse

	Number of plants in each class							
	Cercospora apii		*Septoria apiicola*		*Pseudomonas cichorii*		*Fusarium oxysporum* f. sp. *apii* race 2	
Disease rating[a]	Controls[b]	Somaclones	Controls	Somaclones	Controls	Somaclones	Controls	Somaclones
1	0	0	0	2	0	4	0	9
2	0	12	0	1	0	23	0	17
3	0	76	0	29	0	64	0	57
4	5	64	0	48	6	57	2	89
5	20	102	25	174	19	106	23	82

[a] Disease rating: 1 = <1% diseased tissue, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, and 5 = >50%.
[b] Plants grown from seed.

Table 2. Disease response of celery cv. Tall Utah 52-70 HK somaclones regenerated from cell suspension cultures and inoculated with four pathogens in the greenhouse

	Number of plants in each class							
	Cercospora apii		*Septoria apiicola*		*Pseudomonas cichorii*		*Fusarium oxysporum* f. sp. *apii* race 2	
Disease rating[a]	Controls[b]	Somaclones	Controls	Somaclones	Controls	Somaclones	Controls	Somaclones
1	0	1	0	1	0	4	0	41
2	0	2	0	3	0	14	22	50
3	0	41	0	18	0	27	3	29
4	8	40	0	20	4	37	0	22
5	17	64	25	106	21	66	0	6

[a] Disease rating: 1 = <1% diseased tissue, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, and 5 = >50%.
[b] Plants grown from seed.

Four somaclones (2.7%) were highly resistant to bacterial blight, 14 (9.5%) were moderately resistant, and the rest were susceptible; control plants were susceptible to highly susceptible. Forty-one somaclones (27.7%) were highly resistant to Fusarium yellows, 50 (33.8%) were moderately resistant, and the rest were susceptible; almost all control plants were moderately resistant.

In order to determine the influence of increased resistance to one disease on reaction in the same plant to other diseases, the disease reactions of 16 somaclones whose resistance to *C. apii* had increased from highly susceptible to moderately or highly resistant were used as controls (Table 3). Correlation coefficients were determined between disease reactions to *C. apii* and to the other three pathogens, using individual somaclones as observations. Correlations between resistance to *C. apii* and resistance to *S. apiicola* ($r = 0.19$), *P. cichorii* ($r = 0.31$), and *F. o.* f. sp. *apii* race 2 ($r = 0.20$) were low, indicating there was not a strong relationship between resistance to *C. apii* and resistance to other diseases.

DISCUSSION

Even though highly resistant responses to *C. apii, S. apiicola, P. cichorii*, and *F. o.* f. sp. *apii* race 2 were found in relatively few somaclones produced from cell suspension cultures, such responses may be significant. These cell cultures may have greater potential than callus to produce recoverable genetic mutations because mutated cells are not influenced by surrounding cells as in callus cultures.

Highly resistant plants appeared at a higher frequency in this study when the parent material was moderately resistant. Although both celery cultivars were highly susceptible to early, late, and bacterial blights, Tall Utah 52-70 HK had moderate resistance to Fusarium yellows. Parental material that was typically susceptible to Fusarium yellows produced highly resistant plants less frequently than more resistant material. Plants showing increased resistance to Fusarium yellows have been vernalized and selfed, and seed progeny from these plants are being screened in the field for resistance to Fusarium yellows to determine if the resistance is stable and heritable.

In this study, no chemical or other mutagens were employed, although this is a common process for producing mutant cell lines that give rise to desired plant traits (10). None of the pathogens used produced a known characterized toxin or growth inhibitor that would allow for direct selections of resistant cells.

Recent work has shown that the source of somaclonal variation in celery is probably chromosome loss, chromosome deletion, chromosome inversion, or chromosome translocation (21). In liquid culture, single plant cells are genetically unstable (23). Cells from celery suspension cultures have produced cells that are polyploid, aneuploid in the hypodiploid range ($2n < 22$), or in excess of $2n = 22$ (3) and also cells that have lost chromosome segments (20). These cytogenetic variations are usually seen at anaphase in which chromatin bridges, lagging chromosomes, and multipolar spindles play a role in chromosome number variation (1,26).

Regenerated plants do not usually reflect the full range of cytogenetic abnormalities reported in cultured cells, indicating that some genotypes do not survive long enough for regeneration to take place. Production of numbers of somaclones not showing disease responses different from those of parent material was probably because cells that have not been subjected to drastic changes in genetic material are able to regenerate into whole plants, whereas many other cells may have lost the capacity to differentiate into whole plantlets because of lethal genetic changes. This hypothesis was supported by the work of Browers and Orton (4), who found that callus containing 70% nondiploid cells failed to regenerate into plants.

Plant improvement via somaclonal variation can exploit genetic changes that occur in cultured cells. This is a new and useful source of genetic variation that has become available since somaclonal variation traits have been shown to be heritable (8–14,25). Somaclonal variation may help in the production of more vigorous disease-resistant celery lines faster than would be possible with conventional breeding and selection methods.

Table 3. Interactions of disease responses to four pathogens in celery somaclones

Somaclone number	Source	Disease rating[a]			
		Cercospora apii	*Septoria apiicola*	*Pseudomonas cichorii*	*Fusarium oxysporum* f. sp. *apii* race 2
34	HK[b]	2	5	4	1
355	HK	2	5	5	2
381	HK	1	5	3	2
38	683[c]	2	5	5	1
62	683	2	4	3	2
73	683	2	5	4	3
83	683	2	4	2	5
101	683	2	5	5	4
121	683	2	2	5	5
156	683	2	5	5	5
187	683	2	4	5	3
237	683	2	1	4	4
256	683	2	4	5	4
302	683	2	5	4	3
392	683	2	4	4	1
454	683	2	4	3	5
r values[d]		...	0.19	0.31	0.20

[a] Disease rating: 1 = <1% diseased tissue, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, and 5 = >50%.
[b] Cultivar Tall Utah 52-70 HK.
[c] Cultivar Florida 683.
[d] Correlation coefficients between *C. apii* disease ratings and other disease ratings.

LITERATURE CITED

1. Bayliss, M. W. 1980. Chromosomal variation in plant tissue culture. Pages 113-144 in: International Review of Cytology, Suppl. 11A: Perspectives in Plant Tissue Culture. T. K. Vasil, ed. Academic Press, New York.
2. Behnke, M. 1979. Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. Theor. Appl. Genet. 55:69-71.
3. Browers, M. A. 1981. Chromosomal variability in tissue cell cultures in celery (*Apium graveolens*). M.S. dissertation. University of California, Davis.
4. Browers, M. A., and Orton, T. J. 1982. A factorial study of chromosomal variability in callus cultures of celery (*Apium graveolens*). Plant Sci. Lett. 26:65-73.
5. D'Amato, F. 1977. Cytogenetics of dedifferentiation in tissue and cell cultures. Pages 343-357 in: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. J. Reinert and Y. P. S. Bajaj, eds. Springer-Verlag, Berlin.
6. Elmer, W. H., Lacy, M. L., and Honma, S. 1986. Evaluations of celery germ plasm for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 in Michigan. Plant Dis. 70:416-419.
7. Engler, D. E., and Grogan, R. G. 1984. Variation in lettuce plants regenerated from protoplasts. J. Hered. 75:426-430.
8. Heinz, D. J., Krishnamurthi, M., Nickell, L. G., and Maretzki, A. 1977. Cell, tissue and organ culture in sugar cane improvement. Pages 3-17 in: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. J. Reinert and Y. P. S. Bajaj, eds. Springer-Verlag, Berlin.
9. Larkin, P. J., Ryan, S. A., Brettel, R. I. S., and Scowcroft, W. R. 1984. Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67:443-455.
10. Larkin, P. J., and Scowcroft, W. R. 1981. Somaclonal variation—A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.
11. Liu, M. C. 1981. In vitro methods applied to sugarcane improvement. Pages 299-333 in: Plant Tissue Culture. Methods and Applications in Agriculture. T. A. Thorpe, ed. Academic Press, New York.
12. Liu, M. C., and Chen, W. H. 1976. Tissue and cell culture as aids to sugar cane breeding. I. Creation of genetic variation through callus culture. Euphytica 25:393-403.
13. Liu, M. C., and Chen, W. H. 1978. Tissue and

cell culture as aids to sugar cane breeding. 2. Performance and yield potential of callus derived lines. Euphytica 27:273-282.
14. Liu, M. C., and Chen, W. H. 1978. Improvement in sugar cane by using tissue culture methods. (Abstr.) Page 163 in: Int. Congr. Plant Tissue Cell Culture 4th.
15. Meins, J. 1983. Heritable variation in plant cell culture. Annu. Rev. Plant Physiol. 34:327-346.
16. Merrick, M. M. A., and Collin, H. A. 1981. Selection for asulam resistance in tissue cultures of celery. Plant Sci. Lett. 20:291-296.
17. Miller, O. K., and Hughes, K. W. 1980. Isolation of paraquat-tolerant mutants from tomato cell cultures. In Vitro 16:1085-1091.
18. Murakishi, H. H., Honma, S., and Knutson, R. 1960. Inoculum production and seedling evaluation of celery for resistance to *Cercospora apii*. Phytopathology 50:605-607.
19. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:478-497.
20. Murata, M., and Orton, T. J. 1983. Chromosome structural changes in cultured celery cells. In Vitro 19:83-89.
21. Orton, T. J. 1983. Spontaneous electrophoretic and chromosomal variability in callus cultures and regenerated plants of celery. Theor. Appl. Genet. 67:17-24.
22. Rappaport, L. 1980. Cloning celery for plant propagation, inducing variation, and screening for disease resistance. Pages 19-31 in: California Celery Research Program. 1979–1980 Annual Report. F. Pusateri, ed. California Celery Research Advisory Board, Bakersfield.
23. Reisch, B. 1983. Genetic variability in regenerated plants. Pages 748-769 in: Handbook of Plant Cell Culture. Vol. 1. D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada, eds. Macmillan Publishing Co., New York. 970 pp.
24. Shephard, J. F., Bidney, D., and Shanin, E. 1980. Potato protoplasts in crop improvement. Science 208:17-24.
25. Skirvin, R. M., and Janick, J. 1976. 'Velvet Rose' Pelargonium. A scented geranium. HortScience 11:61-62.
26. Sunderland, M. 1977. Nuclear cytology. Pages 177-205 in: Plant Tissue and Cell Culture. H. E. Street, ed. University of California Press, Berkeley.
27. Thomas, B. R., and Pratt, D. 1982. Isolation of paraquat tolerant mutants from tomato cell cultures. Theor. Appl. Genet. 63:169-176.
28. Walker, J. C. 1952. Diseases of celery and parsley. Pages 104-122 in: Diseases of Vegetable Crops. McGraw-Hill Book Co., Inc., New York.
29. Wenzel, G., Schieder, O., Przewozny, T., Sopory, S. K., and Melchers, G. 1979. A comparison of single cell derived *Solanum tuberosum* plants and a model for their application in breeding programs. Theor. Appl. Genet. 55:49-55.