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SOMACLONAL VARIATION OCCURRING IN DISEASE RESPONSE OF REGENERATED CELERY PLANTS FROM CELL SUSPENSION AND CALLUS CULTURES

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ABSTRACT

SOMACLONAL VARIATION OCCURRING IN DISEASE RESPONSE OF REGENERATED CELERY PLANTS FROM CELL SUSPENSION AND CALLUS CULTURES

BY

Jane Christie Wright

Axillary buds from celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' placed on Murashige-Skoog (MS) agar medium containing 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 mg/l benzyladenine (BA) produced callus. These callus tissues were transferred to MS agar medium and to MS liquid medium containing 0.5 mg/l 2,4-D and 0.1 mg/l kinetin. Regeneration of whole plants (somaclones) from callus and from shaken single cell suspension cultures occurred after transfer to MS agar medium containing a variety of hormone levels and on MS agar medium containing activated charcoal and no hormones. There was a significant amount of mortality when somaclones were transferred from agar media to potting soil and placed in the greenhouse. Highest mortality occurred during the first 2 weeks after transfer. Those somaclones allowed to develop longer in culture before transfer to the greenhouse showed a higher survival rate.

Leaves of surviving somaclones were inoculated with spore suspensions of *Cercospora apii* and *Septoria apiicola* and with a cell suspension of *Pseudomonas cichorii*. Somaclones were then placed in muck soil containing *Fusarium oxysporum* f.sp. *apii* Race 2. Variable disease responses to all pathogens were observed, ranging from highly resistant to highly susceptible. Somaclones that appeared to be highly resistant to each pathogen were obtained primarily from plants regenerated from cell suspension cultures.

To my parents,

without their love, moral support and encouragement,

this work would never have been completed.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Culturing of plant tissues on artificial media was initiated in 1934 when White (58) succeeded in growing tomato roots indefinitely in aseptic culture. Later, Went and Thimann (56) discovered plant hormones (called auxins) that stimulate callus (undifferentiated masses of cells) and inhibit organized cell development in tissue culture. This enabled researchers to see the potential of callus cultures. They showed that carrot tissues (16,38) and tobacco tissues (59) grown on tissue culture medium supplemented with auxin supported undifferentiated cell proliferation. Not until the use of coconut milk (a liquid endosperm) became widely used as an additional nutrient in tissue culture medium were callus cultures maintained for extended periods of time. With the discovery of the phytohormone kinetin (31) in coconut milk , a completely defined medium for tissue culture was possible. Cytokinins promote cell division in tissue culture and *in vivo*. This complete tissue culture medium contains inorganic salts, vitamins (eg. thiamin, inositol, nicotinic acid, pyridoxine), phytohormones and carbohydrates.

Experiments testing the relative effects of auxins and cytokinins at different concentrations led to regeneration of entire plants from callus cultures (52). A tissue culture cycle consisting of 3 steps was established: (1) the establishment and growth of undifferentiated cell masses under defined cultural conditions; (2) continued growth of undifferentiated cell masses over several generations; and (3) regeneration of whole plants from these cell masses (22).

Cell suspension cultures were discovered by Muir et al. (34).

Fragments of callus agitated in liquid culture medium and on a reciprocal shaker produced suspensions containing single cells and cell aggregates which reproduced continuously when subcultured. Steward et al. (53) found that carrot callus produced free viable cells when placed in a shake culture. Although these cells did not resemble the original cells of the carrot explant, they survived, divided and gave rise to small cell clusters. These clusters grew either in an undifferentiated way, or in some cases in an organized manner which produced embryoids that were identical with carrot embryos produced *in vivo*. Thus cell suspension culture had potential for studying plant morphognensis and cell totipotency, as well as for micropropagation (36).

Plantlet regeneration from tissue culture occurs either by organogenesis or embryogenesis. Organogenesis is defined as the biochemical and anatomical changes in callus cultures that occur when unorganized growth becomes differentiated growth of either root or shoot (49). Somatic embryogenesis occurs when cultured cells undergo division and organization in a manner similar to seed embryo formation. Embryogenesis can be broken into 3 phases: the globular, heart and torpedo phases. The globular stage is represented by a compact circular cell mass. The heart phase occurs as the shoot meristmatic pole begins to form cotyledons, thus giving the embryoid a heart shape. The last stage is the elongation of the root tip, giving the embryoid the appearance of a torpedo. Embryoids formed in tissue culture have been used as developmental models for embryo formation *in vivo* (1,2,53).

Several terms have been coined for plants regenerated from tissue

culture. Somaclone is the general term given to those plants derived from any cell culture form, whereas calliclones has been used for plantlets regenerated from stem calluses, and protoclones for those derived from leaf protoplasts (22).

Historically, tissue culture cycles have been employed to clone a particular plant genotype (22). Phenotypic variants in somaclones from tissue culture were recorded more than a decade ago (34,47). These variations were first considered to be "artifacts of tissue culture" due to exposure to exogenous phytohormones, and were termed epigenetic traits (22).

Variation in plants regenerated from callus or cell cultures can cause unwanted changes in cloned material. Yet spontaneous and induced genetic changes arising in culture may provide novel opportunities for germplasm variation which may be used by plant breeders for improving horticultural traits and plant disease resistance. Skirvin and Janick (51) developed a somaclone of *Pelargonium sp.* with an improved scent. It was named 'Velvet Rose' and is believed to be the first named cultivar derived from tissue culture. Several sugarcane somaclone lines exhibiting high yield and smut resistance are being field tested (23) along with other tissue culture-derived lines showing resistance to downy mildew and Fiji disease (18). These studies indicated the potential usefulness of somaclonal variation in improving disease resistance levels in sugar cane and other plants.

Callus Initiation

Dicotyledonous plants are mainly used for tissue culture due to the culturability of a wide range of species, and their rapid response to

callus inducers. Several types of plant tissue have been utilized for callus formation. These include stem and petiole segments, leaves, roots and dormant shoots. Celery, a member of the family Umbelliferae, has been the subject of several tissue culture studies.

Callus initiation in celery (*Apium graveolens*) was first reported by Reinert et al. (44) using Murashige and Skoog's (37) basal medium (MS) (16.5mg/l NH_4NO_2, 19.0mg/l KNO_3, 4.4 mg/l $CaCl_2 \cdot 2H_2O$, 3.7 mg/l $MgSO_4 \cdot 7H_2O$, 1.7 mg/l KH_2PO_4, 0.062 mg/l H_3BO_3, 0.169 mg/l $MnSO_4 \cdot H_2O$, 0.106 mg/l $ZnSO_4 \cdot 7H_2O$, 0.0083 mg/l KI), White's vitamins (0.5mg/l nicotinic acid, 0.1 mg/l pyridoxine, 3 mg/l glycine, 0.1 mg/l thiamine) (58,49), 0.06 uM sucrose, and 0.23 uM 2,4-dichlorophenoxyacetic acid (2,4-D). This medium was not effective in promoting callus in celery when used by others (60). Young petiole sections of celery cv 'Florimart' produced callus after 3 weeks on MS basal medium using vitamin concentrations of 0.5mg/l nicotinic acid, 0.5 mg/l pyridoxine, 2.0 mg/l glycine, 0.1 mg/l thiamine, and 10 mg/l myo-inositol, plus 5.4 uM naphthalene acetic acid (NAA), 0.47uM kinetin, and 2% sucrose (9). Williams and Collin (60) used a callus initiation medium similar to Chen's (9), but employed 2.3 uM 2,4-D and 2.8 uM kinetin. Using celery cultivar 'Lathom Blanching', these researchers found that callusing occurred within 2 weeks, with tissue from the inner 3 petioles being most active in callus formation compared to the outer petioles. Also the upper half of the petiole formed callus better than the lower half. Al-Abta and Collin (1) utilized Williams and Collin (60) medium and found that the cultivar 'New Dwarf' formed callus within 10 days.

Explants from the axillary buds at the base of older petioles were

determined to be the best for forming callus using the cultivar 'Tall Utah 5270 R'(43). These workers used MS basal medium, Chen's vitamins, 9.1 uM 2,4-D and 4.4uM benzyladenine (BA) for callus initiation. Experiments performed by Fujii (15) on the same cultivar showed that BA was more effective than kinetin or zeatin for callus initiation, and that 9.1 uM 2,4-D was more active than 2.3 uM 2,4-D used in Williams and Collin's (60) modified medium (15). Browers (5), using the celery cultivar 'Tendercrisp' and celeriac (*Apium graveolens* var. rapaceum) lines 'PI 169001', 'PI 171500' and 'PI 177266', determined that a broad range of auxin (2,4-D) / cytokinin (kinetin and BA) ratios in MS basal medium promoted callus from both leaf and petiole explants. The optimal levels appeared to be 6.8 uM 2,4-D and 2.8 uM kinetin. In his study, petiole explants were consistently better than leaves in callus initiation, but no apparent differences were noted among plant lines.

Experiments to determine effects of physical environment on callus initiation are few. Chen (9) conducted his experiments at 25^{o} C in darkness, while others (60,2) used 26^{o} C and a 12 hour light-dark photoperiod. Rappaport et al. (43) and Fujii (15) utilized continous light at 25^{o} C.

Callus and Suspension Culture Maintenance

The maintenance of continued cell division requires that cells be supplied with fresh nutrients periodically. This can be performed either by transferring callus serially onto fresh media or by frequent subculturing of cell suspension cultures.

Celery callus can be maintained by employing a serial transfer technique utilizing the same medium and physical conditions as in callus

initiation (60). Fujii (15) found that the growth rate of serially transferred callus was greater when auxin concentrations were lowered to 0.45 uM of 2,4-D from 9.1 uM. He also found that a BA concentration of 4.4 uM in callus initiation medium was toxic to continued growth in MS basal medium containing 2.3 uM 2,4-D. If the original callus was continually subcultured, the cell mass consisted of undifferentiated parenchyma cells and embryoids (60).

Propagation of celery from cell suspensions was first recorded by Williams and Collin (60). By introducing established callus into liquid medium similar to the initiation medium and agitating on a rotary shaker, undifferentiated cell clusters and single cells appeared within 3 weeks. Subculturing of these shake cultures produced a continual source of embryoids (60).

Rappaport et al. (43) performed a similar procedure, but modified the medium by using 1.1 uM BA in place of kinetin. Cultures were shaken continuously under continuous light at 25 C. This allowed the cell volume to double every 5 days with cultures consisting of undifferentiated cells either in aggregates or singly (41).

Plant Regeneration

The process of somatic embryogenesis in celery is similar to that found in carrot (28). However, celery responds differently than carrot to hormones and retains embryonic potential longer (60). Embryogenesis was first noted in celery by Williams and Collin (60) in the process of callus initiation and maintenance. Root formations were observed from callused petioles in a few instances, suggesting that plant regeneration can occur via organogenesis (60).

Auxin:cytokinin ratios are crucial in initiation of somatic embryoid initiation. Early progression from cell to globular stage in embryogenesis was dependent on the presence of both 2,4-D and kinetin. Increased globular embryoid formations could be induced by adding kinetin and removing auxin (62). High concentrations of kinetin promoted embryoid formation, but formation decreased with the addition of 2,4-D (2). After 10 weeks on medium containing high 2,4-D concentrations, ability of celery callus to regenerate plantlets was irreversibly lost (2).

Zee and Yue (63) found 3 distinct morphological events prior to globular formation: (1) a lag period usually consisting of 1-4 days; (2) proliferation of cortical cells surrounding vascular bundles and the formation of meristematic layers after 4-10 days; and (3) meristematic layers giving rise to proembryoids.

Absence or low concentrations of sucrose as well as excessively high levels (10%) in culture medium resulted in low numbers of embryoids being formed. Of those formed, most were in the globular stage. A sucrose concentration of 3% was optimal for producing high numbers of the advanced torpedo-shaped embryoids (2).

After placing differentiated callus into liquid culture, cell aggregates consisting of vacuolated cells were formed (1). Embryoids developed on the surface of these cell aggregates within 2 weeks, suggesting that surface cells were the origin of embryoids in cell suspension (2).

Spontaneous embryogenesis persisted for over 2 years in several cultures of Al-Abta and Collin (2). Spontaneous embryogenesis, along

with undifferentiated growth, occurred when callus tissue was dispersed in agitated liquid medium with the majority being younger embryoids (globular stage). Presence of 2,4-D usually arrested embryoid development at the globular stage (9,60). Continued growth and development of embryoids could be supported if hormones were deleted (2,9,60,62,43,15) and by reducing salts and vitamin concentrations by 50% (43).

Chen (9) showed that celery callus tissue cultured on 5.4 uM NAA and 0.47 uM kinetin exhibited limited regeneration powers, while callus cultured on 4.5 uM 2,4-D and 4.7 uM kinetin remained undifferentiated. Embryogenesis could be induced by transferring both differentiated and undifferentiated callus to MS medium containing 0.54 uM NAA and 13.9 uM kinetin, but embryogenesis in differentiated and undifferentiated callus diminished within 8 months after serial propagation on medium containing 2,4-D (9).

Zee and Wu (62) found that globular embryoids were obtained from the cut surface of petiole explants from Chinese celery (*Apium graveolens*) on MS medium containing 2.3 uM 2,4-D. Further development did not occur on this medium, but when 2,4-D was replaced with 2.8 uM kinetin, torpedo-shaped embryoids appeared. Somatic embryogenesis in celery callus was shown to occur in cortical cells proximal to vascular bundles at the cut surface of the explants (63).

Rappaport et al. (43) and Fujii (15) reported that plant regeneration occurred in callus and in suspension cultures of celery cultivar 'Tall Utah 5250 R'. MS agar medium containing 2,4-D and kinetin (43) promoted embryogenesis from callus cultures, and rapid plantlet

formation from undifferentiated callus tissue occurred after removal of 2,4-D from the culture medium. Cells and tissues in liquid suspension culture could be regenerated either directly in liquid culture by deleting hormones or by plating cell suspension aliquots onto solid medium devoid of hormones (43). These suspension cultures contained single cells and aggregates with up to 10^5 cells (41). Suspension samples from the bottom layer of shaken liquid cultures gave the highest number of regenerated plants when placed on MS agar medium without hormones compared to samples taken from the middle and top regions (43).

Hardening and Adaptation to the Greenhouse

Somaclones are accustomed to sterile, highly humid conditions when developing *in vitro*. Hardening processes acclimate these plants to the more rigorous environments of the greenhouse and the field.

Williams and Collin (60,61) hardened their plants by transferring them to Vitax soil in seed trays covered by perspex lids and storing them under diffuse light for 2 days. When the somaclones were placed in the greenhouse, plantlets were exposed to the atmosphere gradually by removing the perspex lids for a short time daily. These somaclones were later transferred to individual pots and placed on greenhouse benches. It was discovered that 4-cm-high somaclonal shoots with cotyledons that were obtained from tissue culture within 7 weeks were more difficult to root and were more susceptible to wilting damage in the first few days while being exposed to the greenhouse atmosphere than somaclones of larger stature obtained after 7 weeks (60). Rappaport et al. (43) transferred celery somaclones directly to Speedling trays

containing vermiculite. These were then placed in the greenhouse without any reported coverings with MS medium supplied to the plantlets for the first month. No percent fatality was reported by either group.

Somaclonal Variability

Variability has been observed not only in plant regenerants, but in callus cultures and in cell cultures as well. The variations in callus and cell cultures included tissue morphology, growth rate of cells, friability of callus, sliminess, pigmentation, allinase activity, auxin and cytokinin habituation, ability to produce alkaloids and other secondary metabolites, and resistance to different antimetabolites, usually in the form of toxic amino acids (22).

Plants regenerated from callus were reported to vary in morphological characteristics, yield production, physiological activities (CO_2 absorption, photoperiod requirements) and cytogenetic traits , such as polyploidy, aneuploidy, and chromosomal rearrangements. Orton (42) stated that somaclonal variation is influenced by genetic factors (chromosome number) or by developmental state (age) of the explant tissue, type of tissue culture cycle (callus, cell suspension, protoplast), and by physical and environmental factors employed in the tissue culture cycle. Plants regenerated by use of a tissue culture cycle may be encouraged to display increased variation by using several techniques: (1) a long-term culture cycle; (2) a protoplast culture cycle; (3) a callus culture cycle; (4) the use of explants from specified tissues; (5) the generation of random variation concomitant with the selection of a specific nutrient medium or hormone formulation; and (6) the use of certain genotypes that tend to produce

increased amounts of variation (45).

Somaclonal variation can be associated with and may be an underlying cause of the loss of capacity for redifferention into whole plants, and those abnormal cells that successfully regenerate and survive as whole plants often exhibit decreased viability and abnormal morphology (42). Liu and Chen (24,25,26) found significant variation in sugarcane somaclones which included cane yield, sugar yield, stalk number, length, diameter, volume, density and weight, percent fibre, auricle length, dewlap shape, hair group and top leaf attitude. Several somaclones had better field performances than the parents.

Larkin and Scowcroft (22) have reviewed the types of plants that have shown somaclonal variation. Regenerated potato plants exhibited variability in compactness of growth habit, maturity date, tuber uniformity, tuber skin color, photoperiod requirements, and fruit production. Tobacco somaclones varied in CO_2 absorption and chlorophyll content, in days to flowering, plant height, stem diameter, total leaf number, leaf length, leaf width, total alkaloids and yield. Plants regenerated from oats had altered plant height, heading dates, awn morphology, fertility, and some had twin culms or yellow leaf stripes. Somaclones of corn displayed variability in abphyl syndrome, number of stalks from a node, reduced pollen fertility and height. *Brassica* somaclones exhibited variations in leaf wax, multiple branching of stems, precocious flowering from apices, stems or leaves, abnormally cupped leaves, reduced laminae in leaves, spontaneously aborting vegetative buds, slow growth, flowering failure and pollen grain size. *Pelargonium* somaclones derived from callus demonstrated variability in

leaf shape, size and form, flower morphology, plant height, fasciation, pubescence, anthocyanin pigmentation and oil composition. A more recent study showed several lettuce protoclones to have reduced fertility (13).

Cytogenetic Variation

Plant cells cultured from a wide range of species show a high rate of polyploidy, aneuploidy and chromosomal rearrangements (29,3,10,50,54). Somatic tissues cultured *in vitro* exhibited chromosomal/genetic variability more frequently and to a greater degree than those which were uncultured, and all hereditary units studied so far (genes, chromosomes, cytoplasmic genomes) have shown variability in culture (42). Cytogenetic alterations have been shown to increase with increasing age of cells or tissue culture (27,12). Barley callus and suspension cultures have exhibited several cytogenetic variations (39), while the majority of corn callus was dipolid, but some nondiploid cells were found that were tetraploids or triploids (12). Of the corn plants regenerated, all cytological traits observed in the callus cultures were present in regenerated plants (12). Oat cultures exhibited a high frequency of cytogenetically abnormal plants with the most common alteration being chromosome breakage, followed by a loss of chromosome segments which resulted in heteromorphic pairs at diakinesis (27). Trisomy, monosomy and interchanges were also observed in this study (27).

The nature of cytogenetic changes may be different between cultivars of the same species. Karp et al. (21) found that somaclones derived from one variety displayed a wide range (chromosome numbers of 46-92) and high percent of aneuploidy, while somaclones from the other variety

produced regenerants with a smaller aneuploid range (chromosomes numbers of 46-49) and a lower rate of aneuploid occurance. Both cultivars did show chromosomal variation within individual calluses, between calluses, and within shoot cultures.

Evans and Sharp (14) recovered tomato regenerants which had single gene mutations and were able to locate one mutation site. Lettuce somaclones showed reduced fertility in 20 plants with 12 being tetraploids (13).

Considerable work has been done on the nature of cytological variations in celery (6,7,35,40). Browers and Orton (6) found that 30 to 80% of cells in callus tissue were diploid (2n=22), while the rest were hypodiploid or polyploid. However, they did not detect karyological differences among cultured cells originating from the same plant. Another study examined 2 suspension cultures, one of which produced 70% nondiploid cells which failed to regenerate plants, and the other contained 80% diploid cells which readily regenerated plants (7). The plants regenerated in this experiment did not contain polyploids or aneuploids in excess of 2n=22, but aneuploids in the hypodiploid range were present in both the suspension cultures and the regenerants. Murata and Orton (35) found losses of long acrocentrics and gains of short acrocentrics in cultured celery cells when compared to native types. Orton (40) made celery crosses which produced phosphoglucomutase (PMG) and shikimic acetic acid (SDH) isozymes in the hybrids. These hybrids were taken through a tissue culture cycle, and the isozyme was produced in regenerated plants which were karyologically and developmentally abnormal. There appeared to be no consistent

differences between normal and variant clones with respect to chromosome number, structure and anomalous disjunction.

Plant Tissue Culture and Pathogen Studies

Host/pathogen interactions in tissue culture were first examined when Morel (33) performed experiments using grape callus cultures and the obligate parasite *Plasmopara viticola*. From this study stemmed other experiments utilizing tissue culture and pathogens to examine resistant and susceptible responses.

Helgeson et al. (19) used callus tissue of resistant and susceptible varieties of tobacco to study the phenomenon of resistance to *Phytophthora parasitica*. Follow-up studies showed that plants derived from resistant calluses expressed resistance, and those from susceptible tissue were susceptible to the pathogen (20,11). Haberlach et al. (17) found that differing concentrations of cytokinin and auxin had effects on resistance in *P. parasitica* in tobacco. Low levels of kinetin with high levels of auxin produced a hypersensitive response in callus inoculated with the pathogen, whereas high levels of cytokinin allowed for high infection of both susceptible and resistant callus. Callus of resistant and susceptible cotton lines screened against the phytopathogenic bacterium *Xanthomonas malvacearum* showed resistant and susceptible reactions respectively (46).

Plant Selection Processes

With measurable variations occurring, selection processes for crop improvement became possible. These selection processes using herbicides, growth-inhibiting chemicals, pathogen culture filtrates and

toxins have been performed on cells, on callus tissue or on the somaclones. Paraquat was used to select for paraquat-resistant lines in tobacco and tomato (32,55). Miller and Hughes (32) found that recovery of phenotypically stable resistant tobacco cell lines was higher from cell suspensions than from callus cultures. Fifteen of 43 somaclones regenerated from the paraquat-resistant cell lines of suspension cultures retained their resistance, while those from calluses of both resistant and suspectible plants exhibited either partial or complete resistance. Merrick and Collin (30) screened celery suspension cultures against the herbicide asulam and recovered one somaclone showing resistance. Carlson (8) used methionine sulfoximine (a structural analog of a toxin produced by *Pseudomonas tabaci* for selecting cells resistant to this compound in cell suspension cultures. Several haploid tobacco cells showed resistance, but the trait was less evident in regenerated mutant plants.

Dihaploid potato cultures were screened against culture filtrates of *Phytophthora infestans*, and regenerated plantlets retained the resistant phenotype (4). Shepard et al. (48) screened 500 somaclones against *Alternaria solani* toxin resulting in 5 resistant plants, of which 4 displayed field resistance to the pathogen. In the same study, 800 somaclones were screened against *P. infestans*, with 20 showing resistance to late blight and a few exhibiting multiple race resistance. Wenzel et al. (57) performed experiments similar to Shepard's using protoplasts from dihaploid plants derived from seed. Little variation was seen among the regenerated population, but results did show the same types of variation observed in Shepard's work. These observations

included disease resistance when tissues were cultured over long time periods prior to shoot regeneration.

Sugarcane somaclones were screened for resistance to Fiji disease (Reovirus group) and Downy Mildew (*Sclerospora sacchari*). These somaclones showed an increase in resistance to both diseases over the parent plants (22). Somaclones were also tested for resistance to eyespot disease (*Helminthosporium sacchari*). These plants varied greatly in their disease response (22). Rappaport et al. (43) screened celery somaclones against *Fusarium oxysporium* f.sp. *apii*, and found several plants exhibiting the desired resistant trait.

The objectives of this research were: (1) to determine the best medium for growing celery callus and suspension cultures, (2) to discover the best medium for regenration of celery plants (cultivars Florida 683 and Tall Utah 5270 HK) from callus or from single cells, and (3) to determine disease response variation in celery somaclones inoculated with the early blight (*Cercospora apii*), late blight (*Septoria apiicola*), bacterial blight (*Pseudomonas cichorii*), and Fusarium yellows (*Fusarium oxsporum* f. sp. *apii*) pathogens.

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CHAPTER 2

NUTRIONAL REQUIREMENTS FOR TISSUE CULTURE OF CELERY CULTIVARS 'FLORIDA 683' AND 'TALL UTAH 5270 HK'

INTRODUCTION

The propagation of celery (*Apium graveolens*) clonal material by tissue culture has been investigated by several workers (1,2,3,6,7,9,10,14,16,17,18). Callus cultures have been initiated from a broad range of celery genotypes, plant ages, and tissue types, including petiole segments (6,7,8,9,10,13,16,17,18,19), leaf pieces (6,7) and dormant shoot tissues (10,16). The optimal medium for callus initiation appeared to be Murashige-Skoog (MS) basal medium supplemented with a range of 2.3 - 9.1 uM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.3 uM kinetin (14). With some cultivars and some types of tissue, benzlyadenine (BA) appeared to be more effective than kinetin in callus initiation (10). There appeared to be different hormone requirements for each cultivar and tissue type used in tissue culture (1,9,10,16,17,18).

Maintaining callus cultures of celery on agar media over time may be performed by serial transfers of callus onto fresh solid medium, or by placing callus into liquid shake culture with frequent subculturing. By decreasing the concentration or omitting the 2,4-D, embryogenesis can be initiated (2,3,9,10,16,17,18,19) with plantlet formation the final product. These somaclones are then transferred to potting mix in the greenhouse where they undergo hardening, and then, if they survive the environmental change, begin to grow normally (16,17).

The present research was carried out to discover the optimal tissue

culture, regeneration, and hardening conditions for regeneratian of whole plants of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' from somatic cells and from callus tissue.

MATERIALS AND METHODS

Callus initiation. Axillary buds from 10-week-old plants of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' were excised and surface-sterilized by washing the buds in 0.525% sodium hypochlorite for ten minutes, followed by rinsing twice in sterile double-distilled water. These buds were then placed on one of several modified MS media (11) (pH 5.8) all of which contained 1% agar, 2% sucrose, 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, and 0.5 mg/l pyridoxin. In addition to the above ingredients, other ingredients were added as follows: medium #1 (16) contained 0.1 mg/l thiamin, 2 mg/l glycine, 1 mg/l 2,4-D, and 2 mg/l of the cytokinin benzyladenine (BA); medium #2 (17) contained 0.1 mg/l thiamin, 1 mg/l glycine, 0.5 mg/l 2,4-D, and 0.6 mg/l kinetin; medium #3 (12) contained 0.5 mg/l thiamin, 2 mg/l glycine, 0.5 mg/l 2,4-D, and 0.1 mg/l kinetin; and medium #4 contained 0.5 mg/l thiamin, 2 mg/l glycine, 0.5 mg/l 2,4-D, and 0.6 mg/l kinetin.

Callus and cell suspension maintenance. When callus was formed, the tissue was transferred to MS agar basal maintenance medium containing a decreased level of 2,4-D (0.5 mg/l), and a reduced amount of cytokinin (0.1 mg/l) with kinetin substituted for BA. All MS basal maintenance media contained 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 0.1 mg/l kinetin, and 2 mg/l glycine. The callus was serially transferred onto fresh MS agar medium every 4 weeks. Callus of 'Florida 683' and 'Tall Utah 5270 HK' was also placed into liquid culture of two types: MS liquid basal maintenance medium (above) used for serial transfers, and MS liquid medium containing 2,4-D (0.05 mg/l) and kinetin (0.6 mg/l). Callus clumps of 2 to 17 weeks age were

transferred into liquid shake culture of MS medium containing 5 mg/l 2,4-D and 1 mg/l kinetin. All liquid cultures were incubated at 22° C + or - 1° C with a 12 hour diurnal photoperiod on a rotary shaker (100 rpm). Cultures were transferred to fresh liquid media every 4 weeks for the first 2 to 3 transfers, and then were transferred every 2 weeks thereafter, with samples of the suspension cultures taken every 10 days to determine which stages of embryoids were present, if any.

Plant regeneration. When embryoids appeared in the suspension cultures of 'Florida 683' and 'Tall Utah 5270 HK', 1.5 ml samples were removed and pipetted onto several modified MS agar media to promote whole plant regeneration. All media contained nicotinic acid, pyridoxine, myo-inositol, and thiamin, and glycine unless stated otherwise. The media used were: (1) MS agar medium minus 2,4-D and kinetin; (2) MS agar medium plus 0.6 mg/l kinetin; (3) MS agar medium without vitamins and glycine, but with 0.6 mg/l kinetin; (4) MS agar medium containing 0.05 mg/l 2,4-D and 0.6 mg/l kinetin; and (5) MS agar medium minus 2,4-D and kinetin plus 0.1% activated charcoal. In an additional experiment to determine the number of plants produced per petri plate, 1.5 ml samples from the top, middle and bottom thirds of the liquid suspension cultures were removed after 5 minutes settling at and were pipetted onto MS agar medium without hormones. For whole plant regeneration from callus, callus tissues of 'Florida 683' and 'Tall Utah 5270 HK' were placed onto MS agar medium containing no hormones.

All cultures were incubated at 23° + or - 1° C on a 12 hour light-dark regime (17).

Growth in the greenhouse. After whole plant regeneration occurred, the small plantlets were subcultured on fresh MS agar medium without

plant hormones every 4 weeks until they appeared large enough to plant into soil (approximately 6 cm in height). Plantlets were placed into potting soil in styrofoam cups and enclosed in sealed polyethylene bags. These were then placed under benches in the greenhouse. After a week, small holes were made in the tops of the bags to begin acclimating the plantlets to lower humidities. Holes were enlarged daily for approximately 3-4 weeks until bags completely opened. These plantlets were fertilized weekly with Hoagland's solution. Approximately 4 to 5 weeks after the start of the hardening process, surviving plantlets were repotted into muck soil and placed onto greenhouse benches.

RESULTS

Callus initiation. Several modifications of MS medium were used to promote growth of callus from axillary buds. MS modified agar medium #1 gave the highest percent age of buds yielding callus in both 'Florida 683' and 'Tall Utah 5270 HK' (Table 1). There was considerable variation in sample size due to fungal and bacterial contamination of bud transplants occuring during the callus initiation period. Both 'Florida 683'and 'Tall Utah 5270 HK' required 11-13 days for callus initiation(Table 2).

Plant regeneration. The time required for embryoid formation in liquid suspension culture of both 'Florida 683' and 'Tall Utah 5270 HK' appeared to decrease as age of callus used in initiating the suspension culture increased (Fig. 1). Callus 13-17 weeks old required only 11 days to form embryoids, while callus 2-5 weeks of age required up to 120 days to form embryoids.

Celery plantlets regenerated from embryoids went through the typical embryogenesis stages of cell clumping, followed by the globular, heart-shaped, and torpedo embryo stages (Fig.2). Complete plantlet regeneration from 'Florida 683' occurred on several MS modified agar media (Table 3). All media contained nicotinic acid, pyridoxine, thiamin, myo-inositol, and glycine unless stated otherwise. No regeneration occurred in cells from suspension cultures transferred to MS agar medium with kinetin but without the above vitamins and glycine. Cells from MS liquid culture medium containing 0.05mg/l 2,4-D and 0.6 mg/l kinetin regenerated into plantlets after transfer to MS agar medium without these hormones, or to MS agar medium containing 0.6 mg/l

Table 1. Effect of modified Murashige-Skoog (MS) media on initiation of callus from axillary buds of two celery cultivars after 12 days incubation at 20° C.

	Cultivar					
	Tall Utah 5270 HK			Florida 683		
Medium[a]	Number of Buds Transplanted	Number Forming Callus	Percent Forming Callus	Number of Buds Transplanted	Number Forming Callus	Percent Forming Callus
Medium #1[b]	25	22	88	9	8	88
Medium #2[c]	26	20	76	24	18	75
Medium #3[d]	12	7	58	8	5	62
Medium #4[e]	28	11	39	16	11	68

[a]All media are modifications of Murashige and Skoog's medium (9)
[b]MS medium also containing 0.1 mg/l thiamin, 2 mg/l glycine, 1 mg/l 2,4-D, and 2 mg/l BA (14).
[c]MS medium also containing 0.1 mg/l thiamin, 1 mg/l glycine, 0.5 mg/l 2,4-D, and 0.1 mg/l kinetin (15).
[d]MS medium also containing 0.5 mg/l thiamin, 2 mg/l glycine, 0.5 mg/l 2,4-D, and 0.1 mg/l kinetin (10).
[e]MS medium also containing 0.5 mg/l thiamin, 2 mg/l glycine, 0.5 mg/l 2,4-D, and 0.6 mg/l kinetin

Table 2. Number of days required for callus formation from axillary buds of celery cultivars 'Florida 683' and 'Tall Utah 5270HK' on Murashige-Skoog modified medium[a].

	Callus formation						
	Days after excision						
Cultivar	1	3	5	7	11	13	15
Florida 683							
Rep I	-[b]	-	-	-	-	+	+
Rep II	-	-	-	-	+	+	+
Tall Utah 5270 HK							
Rep I	-	-	-	-	-	+	+
Rep II	-	-	-	-	+	+	+

[a]MS medium also containing 0.1 mg/l thiamin, 2 mg/l glycine, 1 mg/l 2,4-D, and 2 mg/l BA (14).
[b] - = no callus formation; + = callus formation.

Figure 1. Effect of age of callus prior to transfer to liquid culture initiation on time required for initial embryoid formation from axillary buds of celery cv 'Florida 683' and 'Tall Utah 5270 HK' in liquid culture.

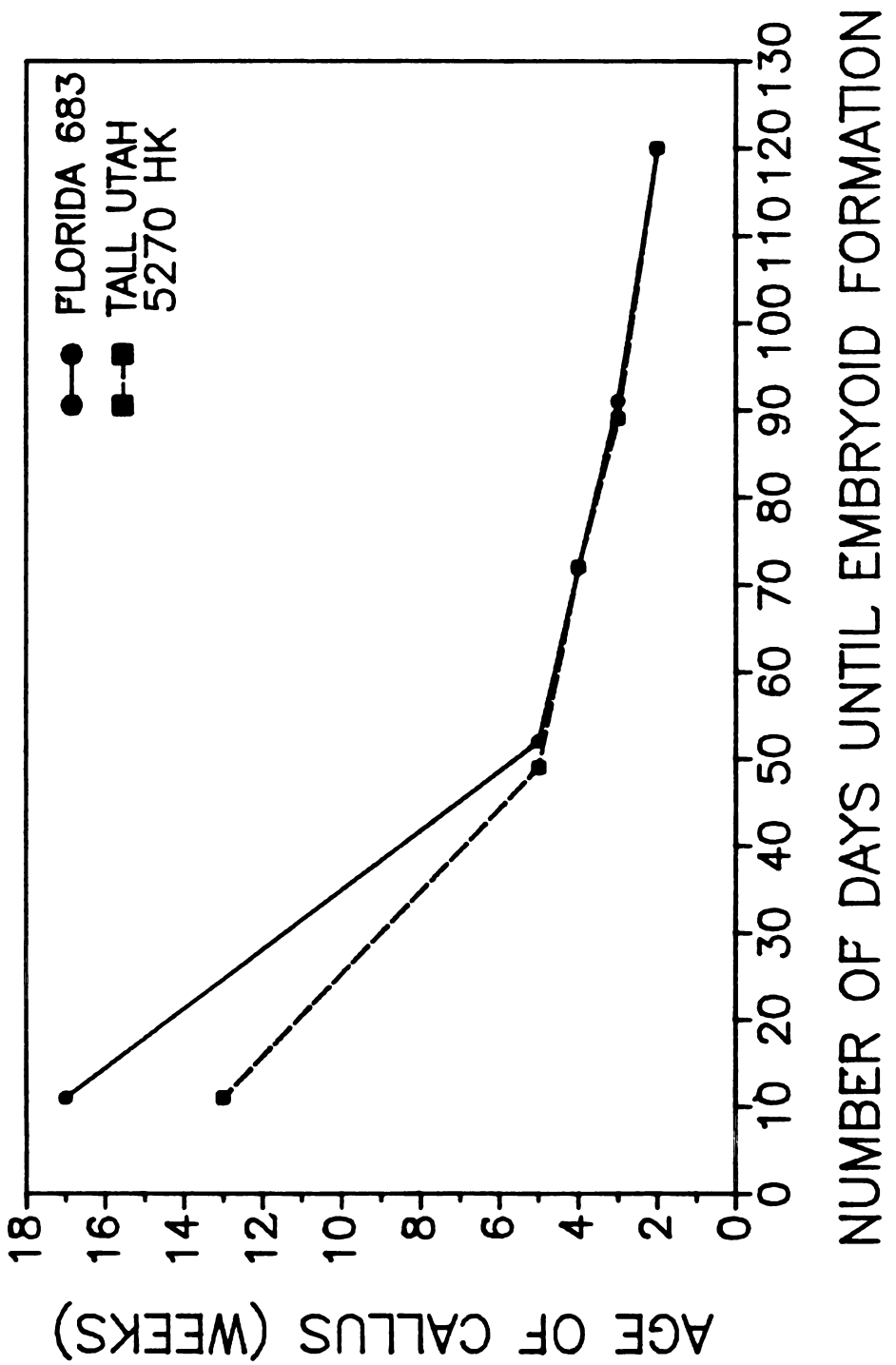

FLORIDA 683
TALL UTAH
5270 HK
AGE OF CALLUS (WEEKS)
NUMBER OF DAYS UNTIL EMBRYOID FORMATION
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50
60
70
80
90
100
110
120
130

Figure 2. A. Cell suspension consisting of single cells and cell clumps. B. Globular stage of embryogenesis. C. Heart-shape stage of embryogenesis. D. Torpedo stage with cotyledons beginning to develop.

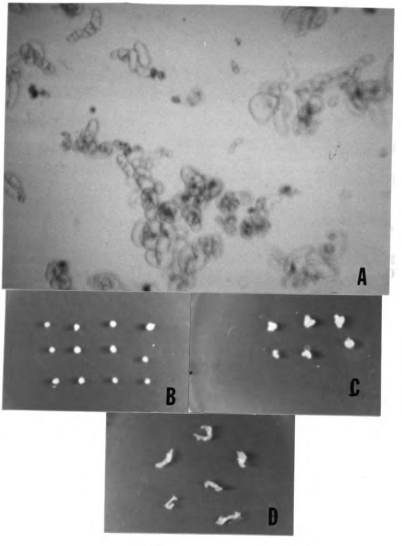

A
B
C
D

Table 3. Effectiveness of several modified Murashige-Skoog media on regeneration of celery cultivar 'Florida 683'plantlets from cell suspension culture.

	Regeneration Rating[a]				
Cell Suspension Media	Regeneration media				
	Medium #1[b]	Medium #2[c]	Medium #3[d]	Medium #4[e]	Medium #5[f]
Medium #1[g]	3	3	0	3	1
Medium #2[h]	2,3	3	0	0	1

[a]Rating System 0=no growth
1=root formation only
2=shoot formation only
3=both root and shoot formation

[b]MS medium containing vitamins, glycine, and no 2,4-D or kinetin

[c]MS medium containing vitamins, glycine and 0.6mg/l kinetin

[d]MS medium containing no vitamins or glycine, but 0.6mg/l kinetin

[e]MS medium containing vitamins, glycine 0.05mg/l 2,4-D, and 0.6mg/l kinetin

[f]MS medium containing vitamins, glycine, 1% activated charcoal and no 2,4-D or kinetin

[g]MS medium containing vitamins, glycine, 5mg/l 2,4-D and 1mg/l kinetin

[h]MS medium containing vitamins, glycine, 0.05mg/l 2,4-D and 0.6 mg/l kinetin

kinetin. Cell suspensions from media with higher hormone concentrations (0.5 mg/l 2,4-D and 0.1 mg/l kinetin) were regenerated into plantlets on MS agar medium without hormones, MS agar medium with 0.6 mg/l kinetin, and MS agar medium with 0.05 mg/l 2,4-D, and 0.6 mg/l kinetin. MS agar medium containing 1% activated charcoal, but no hormones, supported only root formation.

'Tall Utah 5270 HK' cell suspensions in MS liquid culture containing 5 mg/l 2,4-D and 1 mg/l kinetin could not be regenerated into plantlets on MS agar medium without vitamins and glycine, but with 0.6 mg/l kinetin (Table 4). Cell suspensions in MS liquid medium of lower hormonal concentrations could only be regenerated on MS agar medium containing 1% activated charcoal. Root formation occurred on MS agar medium containing no hormones, and on MS agar medium containing kinetin. Also, MS agar medium with 0.05 mg/l 2,4-D and 0.6 mg/l kinetin did not support plant regeneration from cell suspensions incubated in media with the same hormone concentration.

To determine the maximum number of plantlets regenerated from cell suspensions, 1.5 ml aliquots were removed from the upper, middle, and lower regions of suspension cultures onto MS agar medium containing no hormones after allowing the cell suspension to settle at for 5 minutes. The largest number of plantlets regenerated per plate came from the 1.5 ml samples removed from the lower third of the suspension cultures (Table 5). Large cell aggregates (1-3 mm) and embryoids were observed in this region.

Somaclones were ready for transplanting into potting soil within 6 months after bud collection, having passed through callus initiation, placement in cell suspension cultures, and embryoid and plantlet

Table 4. Effectiveness of several modified Murashige-Skoog media on regeneration of celery cultivar 'Tall Utah 5270 HK' plantlets from cell suspension culture.

	Regeneration Rating[a]				
Cell Suspension Media	Regeneration media				
	Medium #1[b]	Medium #2[c]	Medium #3[d]	Medium #4[e]	Medium #5[f]
Medium #1[g]	3	3	0	3	3
Medium #2[h]	1	1	0	0	3

[a]Rating System 0=no growth
1=root formation only
2=shoot formation only
3=both root and shoot formation

[b]MS medium containing vitamins, glycine and no 2,4-D or kinetin

[c]MS medium containing vitamins, glycinie and 0.6mg/l kinetin

[d]MS medium containing no vitamins or glycine but with 0.6mg/l kinetin

[e]MS medium containing vitamins, glycine, 0.05mg/l 2,4-D and 0.6mg/l kinetin

[f]MS medium containing vitamins, glycine, 1% activated charcoal, but no 2,4-D or kinetin

[g]MS medium containing vitamins, glycine, 5mg/l 2,4-D and 1mg/l kinetin

[h]MS medium containing vitamins, glycine, 0.05mg/l 2,4-D and 0.6mg/l kinetin

Table 5. Number of plantlets regenerated on Murashige-Skoog medium containing nicotinic acid, pyridoxine, thiamin, myo-inositol, glycine, but no hormones from 1.5 ml cell culture samples taken from the upper, middle, and lower third of liquid cultures after allowing cells to settle for 5 minutes.

Liquid Culture Region	Number of Plates	Total number of Plantlets	Average Number Plantlets per Plate
Upper Third	16	69	4.3
Middle Third	18	778	43.2
Bottom Third	20	3068	153.4

formation. Plant regenerants from callus culture were ready to be transplanted into potting soil within 5 months.

Growth in the greenhouse. 'Florida 683' had a 46.8% overall survival rate (668 of 1426) of somaclones during greenhouse hardening, while 'Tall Utah 5270 HK' had a 29.6% overall survival rate (324 of 1,093) (Fig. 3). The largest numbers of somaclonal mortalities of 'Florida 683' and 'Tall Utah 5270 HK' occurred within the first 2 weeks after the beginning of the greenhouse hardening process (Table 6). The second week showed the highest plant fatality rate with the rate decreasing during the third to fifth weeks. Percent survival of somaclones increased with increasing numbers of transfers to fresh media prior to transferring to potting soil (Fig. 4). There was a sharp drop after 2 transfers, then a steady increase to about 70% survival after 8 transfers with 'Tall Utah 5270 HK'. 'Florida 683' showed a drop in survival rate after 2 transfers with an increase at 4 transfers and a steady decrease until 6 transfers. A sharp jump in survival was seen at 7 transfers with an increase to over 70% at 8 transfers.

Figure 3. Total percent survival in the greenhouse of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' somaclones.

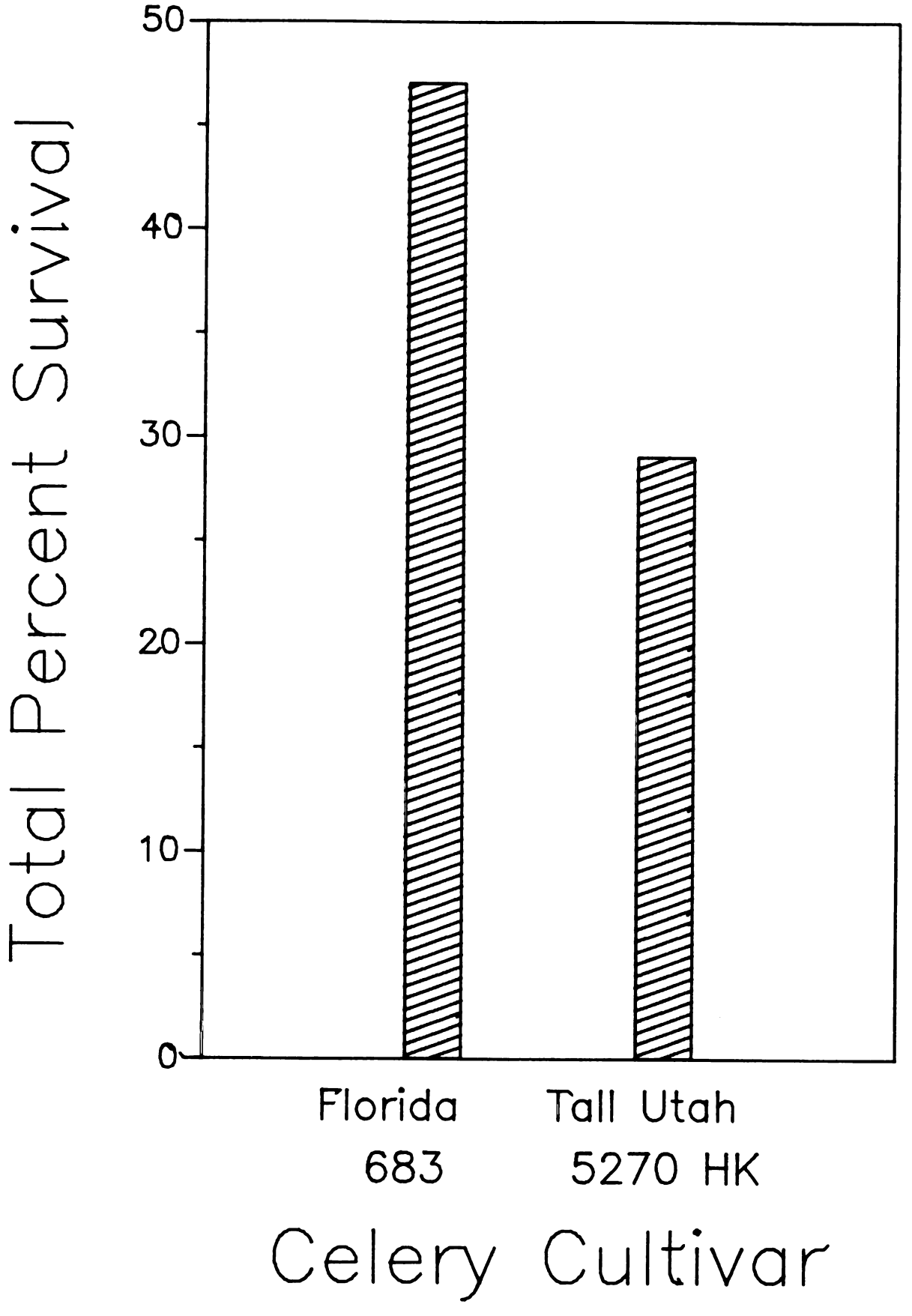
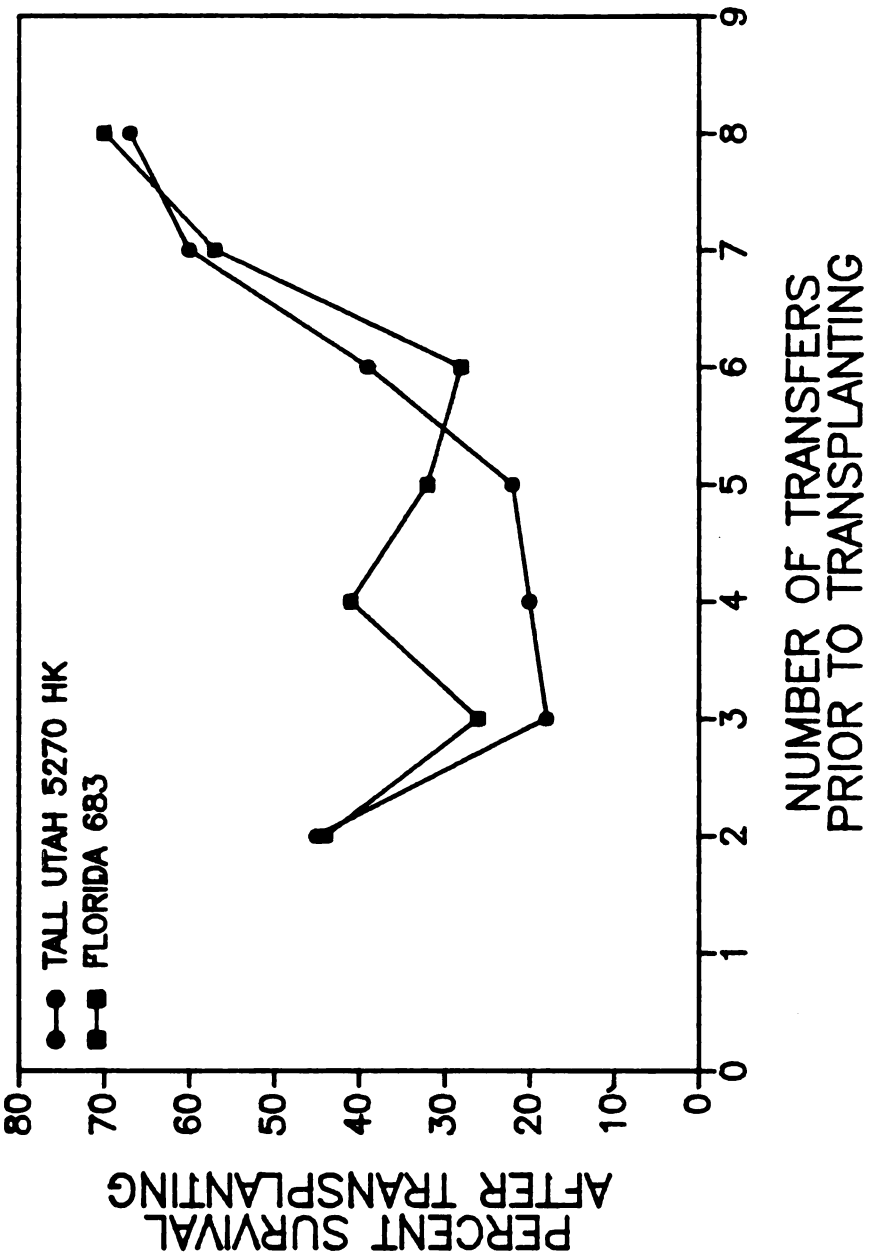

Total Percent Survival
50
40
30
20
10
0
Florida 683
Tall Utah 5270 HK
Celery Cultivar

Table 6. Percent mortality in surviving celery plantlets regenerated from callus and cell suspension cultures after transplanting into potting medium in the greenhouse.

	Percent Mortality				
	Weeks after Transplanting				
Cultivar	1	2	3	4	5
Tall Utah 5270 HK	25.7	32.4	20.0	15.3	12.9
Florida 683	18.6	26.9	13.2	7.1	2.3

Figure 4. The effect of number of transfers in vitro prior to soil transfers on survival rate of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' somaclones in the greenhouse.


TALL UTAH 5270 HK
FLORIDA 683
PERCENT SURVIVAL
AFTER TRANSPLANTING
NUMBER OF TRANSFERS
PRIOR TO TRANSPLANTING
0
10
20
30
40
50
60
70
80
0
1
2
3
4
5
6
7
8
9

DISCUSSION

Comparing different agar media for callus initiation enabled me to determine the best modified MS medium to use for tissue culture of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK'. MS agar medium containing 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 mg/l benzyladenine (BA) was developed specifically for callus initiation from axillary buds (10,16). The cytokinin BA appears to be better adapted for promoting cell division in callus initiation from axillary buds than the cytokinin kinetin used in other media (10).

Age of callus prior to transferring to cell suspension culture influenced the time when the first embryoids were observed. The older the callus when placed in liquid culture, the sooner embryoids appeared. Callus tissue that was serially transferred several times gave rise to embryoids quickly (17); thus embryoids were probably already present on older callus prior to cell suspension initiation. To obtain embryoids from single cells, it would be preferable to start cell suspension cultures with callus tissue 2 - 5 weeks of age.

Plantlet regeneration occured on several modified MS agar media. Amount of regeneration appeared to depend on the cultivar and liquid culture medium used. Although a decrease in nutrient concentration in culture allowed plant regeneration (16), the presence of myo-inositol, nicotinic acid, pyridoxine, and glycine appeared to be crucial at this stage of regeneration. 'Florida 683' and 'Tall Utah 5270 HK' did not regenerate whole plants on MS agar medium without vitamins and glycine.

Cell suspensions of 'Florida 683' exhibited only root formation on MS medium containing 1% activated charcoal, while 'Tall Utah 5270 HK' regenerated whole plants on MS agar medium containing 1% activated

charcoal. Activated charcoal is used in tissue culture for its ability to absorb growth inhibitors and inactivate hormones that cause continued cell proliferation. Media with charcoal have lower levels of phenylacetic and para-hydroxy benzoic acids that are inhibitory to embryogenesis (4). It also absorbs the growth inhibitor 5-hydroxymethylfurfural, a sucrose degradation product that occurs during autoclaving. Charcoal has been shown to enhance carrot embryogenesis when auxin is present in the medium and to promote initiation of root growth in *Allium cepa* (5). The differences in 'Florida 683' and 'Tall Utah 5270 HK' responses to activated charcoal could be attributed to its absorption ability. Charcoal could have bound the needed ingredients required for further embryoid development in 'Florida 683', making this cultivar highly sensitive to slight variations in hormone levels and growth inhibitors. However, regeneration took place on other media not containing charcoal. There is probably an interaction between 'Florida 683' and activated charcoal that promotes only root formation.

Cell suspensions with lower hormone levels (0.05 mg/l 2,4-D and 0.6 mg/l of kinetin) placed on MS agar medium containing the same hormone concentration did not support regeneration with either 'Florida 683' or 'Tall Utah 5270 HK'. This was probably due to hormone concentrations which were too low to develop the embryoids past the globular stage (2,3,9,10,17).

'Tall Utah 5270 HK' in liquid culture of a low hormonal concentration placed on MS agar medium containing no hormones and on MS agar medium containing kinetin promoted root formation. This was probably due to concentrations of auxin and cytokinin too low in cell suspension culture to allow embryoid development beyond the globular

stage (17).

The greenhouse hardening process was one of the greatest obstacles to overcome in somaclone production. Several explanations can be offered for the high fatality rates. First, decreased viability has been reported in several somaclones of different species (15), and this could have occurred in this study. Second, the plantlets were very tender and were possibly affected adversely by the greenhouse conditions (18). Third, secondary metabolites produced in tissue culture could weaken plantlets prior to transplanting to soil. Fourth, those plants lost could have become infected with Fusarium yellows by water splashing and draining from benches above the somaclones containing pots of Fusarium-infested soil.

The sharp decline in survival rate of somaclones after 2 subculturings in both 'Florida 683' and 'Tall Utah 5270 HK' might be due to secondary metabolites produced *in vitro*. Those that do survive can be further subcultured until transferred to soiil.

It can be concluded that MS medium modified by Rappaport et al (14) is the best medium for callus initiation from axillary buds for celery cultivars 'Florida 683' and 'Tall Utah 5270 HK.' Regeneration MS medium to be used would contain 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pryidoxine, and 2 mg/l glycine but no hormones, while the MS medium used for liquid shake culture would contain the same vitamin concentrations and 5 mg/l 2,4-D and 1 mg/l kinetin. Samples should be taken from the lower region of suspension cultures where large cell agrregates and embryos accumulate. To increase survival rate of somaclones, transfers to potting soil should take place after 8 subculturings in culture, and great care should be taken in gradual

exposure to harsh environmental conditions during the first 2 weeks of the hardening process.

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CHAPTER 3

VARIATION IN DISEASE SEVERITY AMONG SOMACLONES OF CELERY CULTIVARS 'FLORIDA 683' AND 'TALL UTAH 5270 HK'

INTRODUCTION

Somaclonal variation was first noted in tobacco (19,23). Since then, somaclones of several plant species have been reported to exhibit variation in phenotypic or genotypic traits. Somaclones often had characteristics qualitatively different from the parent plants. Furthermore, variation among somaclones obtained from the same parent occurred (7,10,11,12,13,14). Cytogenetic variations (polyploidy, aneuploidy, and chromosomal rearrangement) were observed in cell cultures and regenerated plants (1,5,15,27).

These observed variations led to plant cell selections and somaclonal screenings for agronomically valuable traits such as resistance to herbicides or plant diseases. Plant cell selections have produced somaclones of tomato and tobacco resistant to paraquat herbicide (17,28), celery somaclones resistant to asulam herbicide (16), and potato somaclones resistant to the pathogens causing late blight (*Phytophthora infestans*) (2,24,25,30) and early blight (*Alternaria solani*) (24). Sugarcane somaclones screened directly against Fiji disease (Reovirus group), downy mildew (*Sclerospora sacchari*), and eye spot (*Helminthosporium sacchari*) have produced several resistant clones (8,10,11,12,13,14). Celery somaclones have displayed resistance to *Fusarium oxysporum* f. sp. *apii* (22).

The present research was undertaken to determine disease responses

in celery somaclones regenerated from single cells or callus tissue to two fungal foliar pathogens (*Cercospora apii*, and *Septoria apiicola*), causing early blight and late blight, a bacterial pathogen (*Pseudomonas chicorii*) causing bacterial blight, and a soil-borne fungal pathogen (*Fusarium oxysporum* f.sp.*apii*) causing Fusarium yellows, and to determine if there were any relationships among disease reactions across the somaclone population.

Materials and Methods

Somaclones of celery cultivars Florida 683 and Tall Utah 5270 HK were produced as described in Chapter 2, and were hardened in the greenhouse for approximately 1 month prior to inoculations with pathogens. When plantlets were approximately 20 cm in height, one compound leaf each of 'Florida 683' and 'Tall Utah 5270 HK' somaclones were inoculated with aqueous spore suspensions of *Septoria apiicola* (late blight), *Cercospora apii* (early blight), and with an aqueous cell suspension of *Pseudomonas cichorii* (bacterial blight). Spore suspensions of *Cercospora apii* were obtained from cultures grown on carrot leaf agar for 2 weeks, washed with distilled water to obtain conidia, and diluted to 5 X 10^5 conidia/ml (18). Spore suspensions of *Septoria apiicola* were obtained by soaking infected celery leaves with pycnidia present in distilled water for 15 minutes, and diluting the suspension to 5 X 10^5 conidia/ml. The bacterial cell suspension was obtained by growing *Pseudomonas cichorii* on nutrient agar for 1 week, washing the plates with distilled water, and diluting the cell suspension to 10^5 cells/ml.

Two leaves were sprayed with 3 ml of aqueous spore suspensions *S. apiicola* and *C. apii* (5 X 10^5 conidia/ml). 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 and then immediately pricking leaflets with a device containing 6 needles within an approximate 4 cm^2 area. After inoculations, somaclones were placed into a mist chamber in the greenhouse with intermittent mist to keep the leaves wet for 4 days. Somaclones were then transplanted into 4 inch

pots containing muck soil naturally infested with *F. oxysporum* f. ap. *apii* Race 2, and placed on benches under a 14 hour photoperiod and temperatures of 20-25^{o}C.

Ratings of the foliar blights were taken 3 weeks after inoculations and Fusarium yellows symptoms were rated 10 weeks following inoculation. Plants were rated visually for early, late, and bacterial blights on a 1-5 scale, with 1 representing highly resistant (0% diseased tissue) and 5 highly susceptible (> 50% diseased tissue) disease reactions, and with 2, 3, and 4 representing moderately resistant, moderately susceptible, and susceptible reactions (1-5%, 6-25%, and 26-50% diseased tissue) respectively. Plants were also rated visually on a 1-5 scale for Fusarium yellows reaction, where 1 represented no vascular discoloration, 5 represented > 25% of crown tissue discolored, and 2,3, and 4 represented 1-5%, 6-10% and 11-25% of crown tissue discolored respectively.

'Florida 683' and 'Tall Utah 5270 HK' are cultivars considered to be highly suspecptible to all three leaf blights (29). 'Florida 683' is highly susceptible to Fusarium yellows, while 'Tall Utah 5270 HK' is considered to be moderately resistant (6).

RESULTS

Celery somaclones gave varying disease responses to early blight (*Cercospora apii*) (Figure 5.1-5.5). Twelve plants (4.7%) from cultivar 'Florida 683' somaclones obtained from cell suspension culture appeared to be moderately resistant, while 3 plants from callus culture (4.2%) had the same rating (Table 1). All other plants were rated as susceptible.

Among 'Tall Utah 5270 HK' somaclones produced by cell suspension culture, one plant (0.7%) was highly resistant to early blight, and two (1.4%) were moderately resistant (Table 2). Callus cultures produced one somaclone (4.3%) that was moderately resistant. The rest of the somaclones regenerated from both cell suspension and callus culture exhibited susceptible disease responses (ratings of 3-5).

Comparisons of somaclones obtained from both cell suspension and callus cultures of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' inoculated with *Cercospora apii* revealed that disease ratings were predominantly susceptible (3-5), but a few plants were rated as resistant (1 or 2 rating) (Table 3). 'Florida 683' somaclones produced no plantlets highly resistant to early blight (*Cercospora apii*), but there were 15 which were rated moderately resistant. All remaining plants were susceptible. 'Tall Utah 5270 HK' produced 1 somaclone highly resistant to early blight, and 3 that were moderately resistant. The remainder varied from moderately to highly susceptible. Parents of both cultivars were rated as susceptible to highly susceptible to this pathogen.

Celery somaclones gave varying disease responses to late blight

Figure 5. Visual disease rating of early blight (*Cercospora apii*) on celery somaclones. Fig. 5.1-5.3 = disease ratings of 1,2, and 3 respectivley.


5.1


5.2


5.3

Figure 5. Visual disease rating of early blight (*Cercospora apii*) on celery somaclones. Fig. 5.4,5.5 = disease ratings of 4 and 5 respectively.


5.4
5.5

Table 1. Disease response of celery cultivar 'Florida 683' somaclones from cell suspension and callus cultures to *Cercospora apii* (Early blight).

		Frequency distribution of:			
		somaclones from cellsuspension		somaclones from callus cultures	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	0	0.0	0	0.0
2	0	12	4.7	3	4.2
3	0	76	29.9	13	18.3
4	5	64	25.2	15	21.1
5	20	102	40.2	40	56.4

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly susceptible (>50% of tissue diseased)

[b]Controls were not somaclones,but plants from germinated seed.

Table 2. Disease response of celery cultivar 'Tall Utah 5270 HK' somaclones from cell suspension and callus cultures to *Cercospora apii* (Early blight).

		Frequency distribution of:			
		somaclones from cell suspension cultures		somaclones from callus cultures	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	1	0.7	0	0.0
2	0	2	1.4	1	4.3
3	0	41	27.7	6	26.1
4	8	40	27.0	8	34.8
5	17	64	43.2	8	34.8

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Suscseptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

Table 3. Comparison of variable disease response of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' from both cell suspension and callus to *Cercospora apii* (Early blight).

	Frequency distribution of:			
	'Florida 683'		'Tall Utah 5270 HK	
Disease Rating[a]	Number of plants	% of Total	Number of plants	% of Total
1	0	0.0	1	0.5
2	15	4.6	3	1.8
3	89	27.4	47	27.5
4	79	24.3	48	28.1
5	142	43.7	72	42.1

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly susceptible (>50% of tissue diseased)

(*Septoria apiicola*) (Figures 6.1-6.5). Two plants (0.8%) of 'Florida 683' somaclones from cell suspension culture were highly resistant to late blight, and one (0.4%) was moderately resistant (Table 4). One callus-derived somaclone (1.4%) showed a moderately resistant response. The rest of the somaclones displayed susceptible reactions. Parents were all rated 5.

Only one highly resistant (0.7%) and two moderately resistant (2.0%) 'Tall Utah 5270 HK' plants resulted from a total of 148 plants regenerated from cell suspension culture and inoculated with *Septoria apiicola* (Table 5). The rest of the somaclones displayed susceptible reactions (ratings of 3-5).

Comparing somcalones of cultivars 'Florida 683' and 'Tall Utah 5270 HK' inoculated with *Septoria apiicola*, only a few plants of each cultivar fell in the resistant range (1-2), with most somaclones falling in the susceptible range (3-5) (Table 6). 'Florida 683' produced 2 somaclones (0.6%) that were highly resistant to *S. apiicola* while 'Tall Utah 5270 HK' produced one somaclone (0.6%) with the same rating. 'Florida 683' also produced 2 (0.6%) moderately resistant plants, while 'Tall Utah 5270 HK' produced 3 (1.8%) moderately resistant plants. The rest of the somaclones of both cell suspension and callus culture displayed susceptible reactions. Parent material grown from seed was uniformly highly susceptible (Tables 4,5,).

Somaclones exhibited varying disease responses to bacterial blight (*Pseudomonas cichorii*) (Figures 7.1-7.5). 'Florida 683' cell suspension cultures produced 4 somaclones that were highly resistant (1.6%) and 23 plants (9.1%) that were moderately resistant to bacterial blight, with

Figure 6. Visual disease rating of late blight (*Septoria apiicola*) on celery somaclones. Fig. 6.1-6.3 = disease ratings of 1,2, and 3 respectivley.


6.1


6.2


6.3

Figure 6. Visual disease rating of late blight (*Septoria apiicola*) on celery somaclones. Fig. 6.4, 6.5 = disease ratings of 4 and 5 respectivley.

6.4

6.5

Table 4. Disease response of celery cultivar 'Florida 683' somaclones from cell suspension and callus cultures to *Septoria apiicola* (Late Blight).

		Frequency distribution of somaclones from cell suspension cultures		Frequency distribution of somaclones from callus cultures	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	2	0.8	0	0.0
2	0	1	0.4	1	1.4
3	0	29	11.4	13	18.3
4	0	48	18.9	10	14.1
5	25	174	68.5	47	66.2

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

Table 5. Disease response of celery cultivar 'Tall Utah 5270 HK' somaclones from cell suspension and callus cultures to *Septoria apiicola* (Late blight).

		Frequency distribution of somaclones from cell suspension culture		somaclones from callus culture	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	1	0.7	0	0.0
2	0	3	2.0	0	0.0
3	0	18	12.2	5	21.7
4	0	20	13.5	5	21.7
5	25	106	71.6	13	56.6

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but from germinated seeds.

Table 6. Comparison of variable disease reactions of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' somaclones from cell suspension and callus to *Septoria apiicola* (Late blight).

	Frequency distribution of:			
	'Florida 683'		'Tall Utah 5270 HK'	
Disease Rating[a]	Number of plants	% of Total	Number of plants	% of Total
1	2	0.6	1	0.6
2	2	0.6	3	1.8
3	42	12.9	23	13.4
4	58	17.9	25	14.6
5	221	68.0	119	69.6

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Suscetible (26-50% of tissue diseased)
5=Highly susceptible (>50% of tissue diseased)

Figure 7. Visual disease rating of bacterial blight (*Pseudomonas cichorii*) on celery somaclones. Fig. 7.1-7.3 = disease ratings of 1,2, and 3 respectively.


7.1


7.2


7.3

Figure 7. Visual disease rating of bacterial blight (*Pseudomonas cichorii*) on celery somaclones. Fig. 7.4, 7.5 = disease ratings of 4 and 5 respectively.


7.4


7.5

the rest having susceptible reactions (Table 7). Somaclones produced from callus cultures had 4 plants (5.6%) that were moderately resistant, with the rest rated in the susceptible range.

'Tall Utah 5270 HK' somaclones from cell suspension culture produced 4 plants that were highly resistant (2.7%) and 14 plants moderately resistant (9.5%) to *P. cichorii*, with the rest displaying susceptible responses (Table 8). Callus cultures produced two somaclones (8.7%) that were moderately resistant to *P. cichorii*, with the others exhibiting susceptible reactions.

Comaparing somaclones of each cultivar ('Florida 683' and 'Tall Utah 5270 HK) inoculated with *Pseudomonas cichorii*, 4 plants were identified as highly resistant (1.2% and 2.3% respectively) (Table 9). 'Florida 683' produced 27 plants (8.3%) that were moderately resistant, while 'Tall Utah 5270 HK'produced 16 somaclones (9.4%) that were moderately resistant The remaining somaclones displayed susceptible reactions (3-5). Control plants grown from seed were susceptible to highly susceptible.

Celery somaclones exhibited varying responses to *Fusarium oxysporum* f.sp. *apii* (FOA2) (Figures 8.1-8.5). 'Florida 683' produced 9 somaclones from cell suspension culture that were highly resistant (3.6%) to FOA2, and only one highly resistant somaclone (1.4%) came from callus culture (Table 10). Cell suspension culture also produced 17 moderately resistant plants (6.7%), while callus cultures produced 7 moderately resistant plants (9.9%). Plants grown from seed were predominantly highly susceptible.

Somaclones from cell suspension cultures of 'Tall Utah 5270 HK'

Table 7. Disease response of celery cultivar 'Florida 683' somaclones from cell suspension and callus cultures to *Pseudomonas cichorii* (Bacterial blight).

		Frequency distribution of:			
		somaclones from cell suspension culture		somaclones from callus culture	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	4	1.6	0	0.0
2	0	23	9.1	4	5.6
3	0	64	25.2	16	22.6
4	6	57	22.4	14	19.7
5	19	106	41.7	37	52.1

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

Table 8. Disease response of celery cultivar 'Tall Utah 5270 HK' somaclones from cell suspension and callus cultures to *Pseudomonas cichorii* (Bacterial blight).

		Frequency distribution of:			
		somaclones from cell suspension culture		somaclones from callus culture	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	4	2.7	0	0.0
2	0	14	9.5	2	8.7
3	0	27	18.2	3	13.0
4	4	37	25.0	6	26.1
5	21	66	44.6	12	52.2

[a]Disease Rating 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

Table 9. Comparison of disease response of somaclones of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' somaclones from both cell suspension and callus to *Pseudomonas cichorii* (Bacterial blight).

	Frequency distribution of:			
	'Florida 683'		'Tall Utah 5270 HK'	
Disease Rating[a]	Number of plants	% of Total	Number of plants	% of Total
1	4	1.2	4	2.3
2	27	8.3	16	9.4
3	80	24.6	30	17.5
4	71	21.9	43	25.2
5	143	44.0	78	45.6

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (50% of tissue diseased)

Figure 8. Visual disease rating of Fusarium yellows (*Fusarium oxysporum* f.sp. *apii* Race 2) on celery somaclones. Fig. 8.1-8.5 = disease ratings of 1,2,3,4 and 5 respectivley.

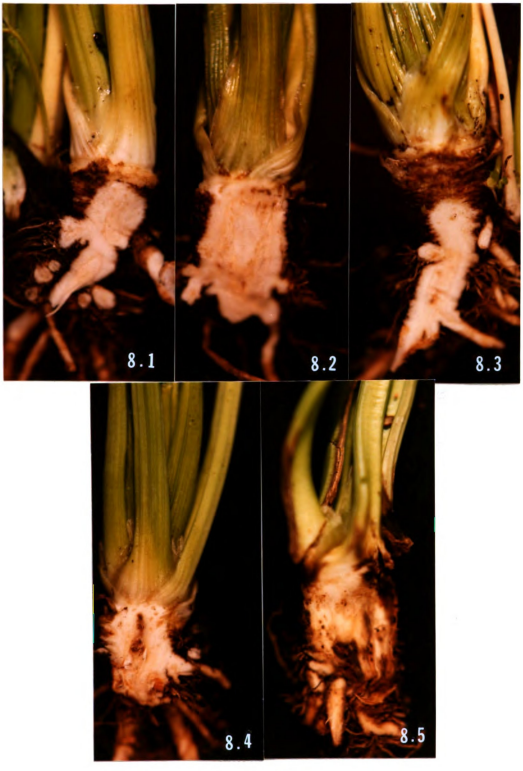
8.1
8.2
8.3
8.4
8.5

Table 10. Disease response of celery cultivar 'Florida 683' somaclones from cell suspension and callus cultures to *Fusarium oxysporum* f.sp. *apii* Race 2 (Fusarium yellows).

		Frequency distribution of:			
		somaclones from cell suspension culture		somaclones from callus culture	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	9	3.6	1	1.4
2	0	17	6.7	7	9.9
3	0	57	22.4	32	45.1
4	2	89	35.0	18	25.3
5	23	82	32.3	13	18.3

[a]Disease Rating 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

produced 41 highly resistant plants (27.7%) (Table 11). The remainder ranged from moderately resistant to highly susceptible to Fusarium yellows. Callus cultures produced no plants either highly resistant or highly susceptible to Parent FOA2. Parent plants were predominantley moderately resistant to FOA2.

Comparing the two cultivars in their response to Fusarium yellows 'Florida 683' yielded 10 somaclones that were highly resistant (3.1%) to FOA2, and 24 that were moderately resistant (7.4%) (Table 12). Celery cultivar 'Tall Utah 5270 HK' produced 41 highly resistant and 53 moderately resistant somaclones, and the remainder were moderately to highly susceptible. 'Florida 683' plants grown from seed were mostly highly susceptible and 'Tall Utah 5270 HK' parents were moderately resistant (6).

Disease response interactions were compared using *Cercospora apii*-resistant somaclones as the control. Correlation coefficients were low (r=.19) between early blight and late blight, between early blight and bacterial blight (r=.31) and between early blight and Fusarium yellows (r=2.0) (Table 13), indicating that there was not a strong relationship between resistance to *C. apii* and resistance to other pathogens.

Table 11. Disease response of celery cultivar 'Tall Utah 5270 HK' somaclones from cell suspension and callus cultures to *Fusarium oxysporum* f.sp. *apii* Race 2 (Fusarium yellows).

		Frequency distribution of:			
		somaclones from cell suspension culture		somaclones from callus culture	
Disease Rating[a]	Control[b]	Number of plants	% of Total	Number of plants	% of Total
1	0	41	27.7	0	0.0
2	22	50	33.8	3	13.0
3	3	29	19.6	10	43.5
4	0	22	14.9	10	43.5
5	0	6	4.0	0	0.0

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Controls were not somaclones, but plants from germinated seeds.

Table 12. Comparison of variable disease response of somaclones of celery cultivars 'Florida 683' and 'Tall Utah 5270 HK' from both cell suspension and callus to *Fusarium oxysporum* f.sp. *apii* Race 2 (Fusarium yellows).

	Frequency distribution of:			
	'Florida 683'		'Tall Utah 5270 HK'	
Disease Rating[a]	Number of plants	% of Total	Number of plants	% of Total
1	10	3.1	41	24.0
2	24	7.4	53	31.0
3	91	28.0	39	22.8
4	106	32.6	32	18.7
5	94	28.9	6	3.5

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3=Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

Table 13. Interactions of disease responses in celery somaclones.

Somaclone Number	Variety	Disease Rating[a] *Cercospora apii*	*Septoria apiicola*	*Pseudomonas cichorii*	*Fusarium oxysporum f.sp. apii* Race 2
34	'HK'[b]	2	5	4	1
355	"	2	5	5	2
381	"	1	5	3	2
38	'683'[c]	2	5	5	1
62	"	2	4	3	2
73	"	2	5	4	3
83	"	2	4	2	5
101	"	2	5	5	4
121	"	2	2	5	5
156	"	2	5	5	5
187	"	2	4	5	3
237	"	2	1	4	4
256	"	2	4	5	4
302	"	2	5	4	3
392	"	2	4	4	1
454	"	2	4	3	5

[a]Disease Rating: 1=Highly Resistant (0% of tissue diseased)
2=Moderately Resistant (1-5% of tissue diseased)
3= Moderately Susceptible (6-25% of tissue diseased)
4=Susceptible (26-50% of tissue diseased)
5=Highly Susceptible (>50% of tissue diseased)

[b]Cultivar 'Tall Utan 5270 HK'.

[c]Cultivar 'Florida 683'.

DISCUSSION

Recent work has shown that in celery, the sources of somaclonal variation are probably chromosome loss, chromosome deletion, chromosome inversion, or chromosome translocation (21). In tissue culture, cells of either cell suspensions or of callus cultures are genetically unstable (23). Cells of celery from suspension or callus cultures have produced cells that are polyploid, aneuploid in the hypodiploid range (2n <22) or in excess of 2n=22 (3), and have also produced cells that have lost chromosome segments (19). These cytogenetic variations usually seen at anaphase in which chromatin bridges, lagging chromosomes, and multipolar spindles play a role in chromosome number variation (1,27).

Regenerated plants do not usually reflect the full range of abnormalities reported in cultured cells, indicating that elimination of some genotypes prior to regeneration has taken place. Large numbers of somaclones that exhibit disease responses close to that of the parent plant are probably due to the ability of cells that have not been subject to drastic changes in genetic material to regenerate into whole plants, while many other cells may have lost the capacity to differentiate into whole plantlets because of genetic changes that are lethal. This is supported by work of Browers and Orton (4) who found that callus containing 70% nondiploid cells failed to regenerate plants.

There appeared to be slight differences in degree of variation in disease response between cell suspension and callus cultures. Highly resistant disease responses to *Cercospora apii*, *Septoria apiicola*, *Psuedomonas cichorii*, and *Fusarium oxysporum* f. sp. *apii* were displayed

by somaclones produced from cell suspension cultures in all cases but one (Tables 1,2,4,5,7,8,11,12). Cell suspension cultures probably have a greater potential to produce genetic mutations since they are not influenced by surrounding cells as in callus culture.

Highly resistant plants appeared at a higher frequency in this study if the parent material was moderately resistant. Although both celery cultivars were highly susceptible to early, late, and bacterial blights, 'Tall Utah 5270 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. Viability selection processes probably eliminated some cells with lethal mutations prior to regeneration.

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 cell selection. It has been reported that *S. apiicola* and *F. oxysporum* f. sp. *apii* may produce toxic substances (21), but this report has not been confirmed. All varying disease responses observed were due to mutations in the absence of chemical or other mutagens in cell suspension and callus cultures.

There appeared to be no correlation between disease response to the four pathogens among 16 somaclones compared (Table 13). This is probably due to different locations of genes coding for resistance or susceptiblity to the four pathogens. If on the same chromosome, they are probably not in close proximity.

Large chromosome number changes appear to be of little or no value in describing genetic variability at the chromosomal and whole plant level (21). Extensive genetic chracterization of celery and karyotypic cell culture analysis would facilitate celery breeding efforts by locating genes of desired traits, and increase the knowledge of genetic variation occuring in celery tissue culture by localizing the areas of mutations.

Mutation breeding and plant improvement via somaclonal variation may exploit genetic changes that preexist in the whole celery plant or changes that occur in cultured cells. This is a new and useful source of genetic varition available to plant breeders since somaclonal variation traits have been shown to be heritable (8,9,10,11,12,13,14,26). Somaclonal variation may help breeders in producing disease-resistant celery lines, and may also produce celery lines of higher vigor better able to co-exist with the pathogen.

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