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In Vitro Induction of Tetraploidy in *Vaccinium darrowi, V. elliottii,* and *V. darrowi* x *V. elliottii* with Colchicine Treatment

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Abstract. **Three diploid taxons (*Vaccinium darrowi* Camp, *V. elliottii* Chapm., and interspecific *V. darrowi* x *V. elliottii*) were treated with various colchicine concentrations and treatment durations to determine the best method for inducing autopolyploidy in *in vitro* blueberry cultures. Shoot-tip cuttings were the best *in vitro* planting material for induction of shoots with increased diameter, an indicator of polyploidy. Tetraploids were produced at colchicine concentrations from 0% to 0.20%. The best treatment combinations were genotype-dependent.**

Florida-native blueberry species include diploids ($2n = 2x = 24$) *Vaccinium darrowi, V. elliottii, V. atrococcum* Heller, *V. arboreum* Marsh, and *V. staminium* L.; tetraploids ($2n = 4x = 48$) *V. fuscatum* Ait. and *V. myrsinites* Lam.; and the hexaploid ($2n = 6x = 72$) *V. ashei* Reade (7). In the section *Cyanococcus,* which includes all of the above species except *V. arboreum* and *V. staminium,* there are only weak sterility barriers between species with the same chromosome number (2, 3). Heteroploid crosses give variable results ranging from partial success to almost complete failure. Hybrids derived from heteroploid crosses may exhibit ovule or pollen sterility (5). Because of a triploid block, few hybrids are obtained from diploid by tetraploid crosses and their reciprocals. Producing autotetraploids from diploids by colchicine treatment is one method of overcoming the crossing barrier between diploid and tetraploid *Vaccinium* species.

Chromosome doubling of woody perennial species by colchicine treatment has had limited success (4, 6). Treatment *in vitro* has several advantages over traditional methods, including a salutary environment that allows survival of the weaker autotetraploids. Shoots produced in tissue culture are very vigorous and have short internodes and numerous buds, which make them well-suited for chromosome doubling with colchicine. Tissue culture is also quite space conservative, allowing large numbers of shoots to be treated in a small area (8, 11). Polyploid shoots, characterized *in vitro* by increased shoot diameter, can be screened quickly and easily by visual examination (10).

The purpose of this study was to identify the best type of explant material for blueberry chromosome doubling by *in vitro* colchicine treatment and to determine optimal colchicine concentration and treatment duration.

Materials and Methods

Expt. 1. Plant material were mature *in vitro* colonies of *V. elliottii* growing on modified Knops medium (9) containing 5 mg/liter 6, γ, γ, dimethyl-allyl amino purine, (2iP). These colonies consisted of numerous shoots that filled completely the 50-ml culture vials. A callus-like mass was present at the base of each shoot cluster.

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Two kinds of explants were obtained from these cultures: 2-node cuttings obtained from rapidly growing shoots and explants obtained from the shoot bases, which contained numerous buds with high shoot-forming potential. Both explant sources were treated with 0.10% colchicine dissolved in liquid modified Knops medium. The treatment solution was aerated by rotating the treatment vials on a rotary wheel drum at about 3 rpm (1). After 24, 48, 72, or 120 hr of colchicine exposure, explants were rinsed and planted on modified Knops agar medium for regeneration. Nine vials were planted for each treatment duration-explant source combination. Five treated, 2-node cuttings or one treated shoot base were planted in each regeneration vial. After 8 weeks, vials were scored for colony vigor and for presence of shoots with increased diameter, previously found to be indicative of induced polyploidy in blueberry tissue culture (10).

Expt. 2. A factorial experiment was designed to find the best colchicine concentration and treatment duration for obtaining the maximum number of surviving plants with increased stem diameter. Two-node cuttings were tumbled in various colchicine concentrations on a rotary wheel as described in expt. 1. Colchicine concentrations tested were 0.00%, 0.01%, 0.05%, 0.10%, and 0.20% for treatment durations of 6, 12, 24, 48, and 72 hr. Three diploid populations of *Vaccinium* were used: *V. elliottii, V. darrowi,* and the interspecific hybrid *V. darrowi* x *V. elliottii*. Five 2-node cuttings were rinsed and planted in each of 10 vials of solid modified Knops medium for regeneration after treatment. Colonies were scored for vigor and frequency of increased-diameter shoots after 8 weeks.

Expt. 3. This experiment was conducted to determine the optimal colchicine concentration and treatment duration for explants treated on solid modified Knops medium rather than in a liquid medium on a rotating drum as in expt. 2. The experimental design was a factorial in which 2-node cuttings of *V. elliottii* were placed in vials of solid modified Knops medium with 5 levels of colchicine (0.00%, 0.01%, 0.05%, 0.10%, and 0.20%) for durations of 0, 2, 4, 6, or 8 weeks. Each vial was planted with 5 cuttings, and 10 vials were planted for each treatment combination. Cuttings were transferred to modified Knops medium for regeneration after treatment and rates of survival and increases in shoot diameter were determined after 8 weeks.

Results and Discussion

Expt. 1. Comparison of survival rate and doubling frequency of 2 explant types indicated a linear decrease in regrowth vigor with increasing colchicine treatment duration in both treated

Table 1. Ploidy level and stomate length of 10 large-diameter shoot colonies of *V. elliottii* screened from expt. 1.

Subclone	Ploidy level[z]	Stomate length (μm) Mean[y]	Range	SD
A	24	104	92–115	11
F	24	115	92–115	15
K	24	118	92–138	12
G	48	172	161–184	12
E	48	173	161–207	12
J	48	181	161–207	14
L	48	184	161–207	15
H	48	187	161–207	12
D	48	189	161–230	15
C	48	240	207–276	14

[z]Ploidy level determined by shoot tip squashes.
[y]Average derived from measurement of 15 stomates per subclone.

cuttings and bases. There was a significant difference between explant sources in frequency of shoots with increased diameter ($P = 5\%$, χ^2) although neither explant type differed significantly in survival after treatment. Clones regenerated from cuttings gave rise to 12 vials containing shoots of increased diameter whereas growth from bases contained none. Nine of the 12 vials containing increased-diameter shoots were regenerated from the 48-hr treatment duration with the remainder regenerated from the 72-hr treatment. For cuttings, the 48-hr treatment duration gave significantly more increased-diameter shoots than treatments of 24, 72, or 120 hr ($P = 5\%$, χ^2). Ten subclones were grown from the 12 vials (Table 1); of these, 7 proved to be tetraploid and 3 proved to be diploid by chromosome count.

Stomate measurements of leaves and chromosome counts of shoot tips from screened, thick shoots supported the correlation found earlier between increased stem diameter and induced polyploidy. Of colonies regenerating large-diameter shoots, 70% had increased stomatal guard cell lengths and higher ploidy levels (Table 1). Some of the shoots with increased diameter also showed other morphological anomalies, such as increased and coarse appearing pubescence, darker green, thicker leaves, or altered leaf shape. *In vitro* growth rate was decreased also for large-diameter shoots (Fig. 1).

Fig. 1. Diploid (center) and *in-vitro*-induced autotetraploid *V. elliottii*.

Table 2. Effects of colchicine concentration and treatment duration on regrowth vigor of treated *Vaccinium* explants.

Colchicine concn (%)	Regrowth vigor rating[z] Duration 6 hr	12 hr	24 hr	48 hr	72 hr	Mean
			V. darrowi			
0.00	2.6	1.4	2.1	1.8	2.9	2.2
0.01	2.3	1.2	1.2	1.7	0.9	1.5
0.05	1.7	0.7	1.0	1.6	1.0	1.2
0.10	1.8	0.6	1.0	1.4	0.2	1.0
0.20	1.6	0.1	0.9	1.5	0.1	0.8
Mean	2.0	0.8	1.2	1.6	1.0	
Vigor score = 1.8 − 5.1 concn; $r = 0.56$**						
			V. elliottii			
0.00	3.0	2.2	1.7	2.6	2.1	2.3
0.01	2.4	2.1	2.6	3.1	2.8	2.6
0.05	3.1	1.3	1.3	1.8	0.4	1.6
0.10	2.8	1.7	1.2	1.2	0.2	1.4
0.20	1.4	0.8	0.2	1.7	0.6	0.9
Mean	2.5	1.6	1.4	2.1	1.2	
Vigor score = 2.7 − 7.6 concn − 0.01 hr; $r = 0.68$*						
			V. darrowi x *V. elliottii*			
0.00	2.4	1.7	2.7	1.7	1.6	2.0
0.01	1.8	1.4	2.6	0.8	0.7	1.5
0.05	1.6	2.1	0.7	0.1	0.0	0.9
0.10	1.8	0.8	0.7	0.0	0.0	0.7
0.20	0.7	0.9	0.6	0.0	0.0	0.4
Mean	1.7	1.4	1.5	0.5	0.5	
Vigor score = 2.2 − 6.8 concn − 0.02 hr; $r = 0.81$**						

[z]Mean vigor score for 10 vials, each planted with five 2-node explants receiving this treatment (4 = very vigorous, 0 = dead).
*,**Significantly different from zero at 5% (*) or 1% (**) level.

Since the treated plant material has yet to flower, assessment of the L-II or gamete-producing layer has yet to be made. Chimeras are common problems in colchicine-treated plant material. Therefore, total assessment of increased-diameter shoots is, as yet, incomplete. Two of the 3 increased-diameter shoot colonies found to be diploid did flower, and pollen measurements fall within the range normally found within diploid *V. elliottii* pollen populations. The 3rd has not produced flowers yet.

Expt. 2. Survival rate of treated explants decreased with increasing colchicine concentrations and treatment durations in all 3 *Vaccinium* taxons tested (Table 2). *V. elliottii* had the highest overall survival rating and also the highest doubling frequency (Table 3). Optimal colchicine concentrations × treatment durations for each of the 3 taxons for polyploid induction were: *V. elliottii:* 0.01% for 72 hr, *V. darrowi:* 0.01% for 48 hr, *V. darrowi* x *V. elliottii:* 0.00% for 6 hr. Spontaneous chromosome doubling was responsible for the appearance of large-diameter shoots in the 0.00% colchicine treatments in *V. darrowi* and the *V. darrowi* x *V. elliottii* hybrid.

Expt. 3. Higher colchicine concentration and longer treatment duration both were correlated with decreased survival rates of treated 2-node cuttings (Table 4). Several regenerated vials

Table 3. Number of colonies containing one or more shoots of increased diameter following treatment with various colchicine concentrations for various lengths of time.

Colchicine concn (%)	No. colonies with large-diameter shoots[z] Treatment duration 6 hr	12 hr	24 hr	48 hr	72 hr	Total
			V. darrowi			
0.00	0	0	0	2	1	3
0.01	0	0	0	3	0	3
0.05	1	0	0	0	0	1
0.10	0	0	0	1	0	1
0.20	0	0	0	0	0	0
Total	1	0	0	6	1	
			V. elliottii			
0.00	0	0	0	0	0	0
0.01	0	0	0	1	7	8
0.05	5	0	0	2	1	8
0.10	1	5	1	2	1	10
0.20	0	0	1	1	1	3
Total	6	5	2	6	10	
			V. darrowi x *V. elliottii*			
0.00	3	1	1	0	0	5
0.01	0	0	2	0	0	2
0.05	0	2	0	0	0	2
0.10	0	1	0	0	0	1
0.20	1	0	0	0	0	1
Total	4	4	3	0	0	

[z]For each treatment, 10 vials were used, each planted with five 2-node cuttings.

Table 4. Effects of colchicine concentration and treatment duration on regeneration vigor of *V. elliottii* explants treated on solid modified Knops medium.

Colchicine concn (%)	Regeneration vigor rating[z] Duration 2 wk	4 wk	6 wk	8 wk	Mean
0.00	3.5[z]	3.7	3.9	4.0	3.8
0.01	3.3	1.5	2.5	1.2	2.1
0.05	1.7	0.9	0.7	0.1	0.9
0.10	1.3	0.1	0.4	0.0	0.5
0.20	0.2	0.1	0.0	0.1	0.1
Mean	2.0	1.3	1.5	1.1	

[z]Vigor score = 3.16 − 14.87 concn − 0.13 wk, r = 0.78*. Mean vigor score for 10 vials planted with five 2-node explants receiving this treatment (4 = very vigorous; 0 = dead).
*Significantly different from 0 at 5% level.

Table 5. Number of colonies of *V. elliottii* producing one or more shoots of increased diameter 8 weeks after planting with colchicine-treated explants.

Colchicine concn (%)	No. colonies with large-diameter shoots[z] Treatment duration 2 wk	4 wk	6 wk	8 wk	Mean
0.01	6	2	2	0	2.5
0.10	2	0	0	0	0.5
Mean	4	1	1	0	

[z]Five 2-node treated explants were planted per vial; 10 vials were used.

contained shoots of increased diameter (Table 5). Colchicine concentration of 0.01% for 2–6 weeks were the most successful treatments for production of increased-diameter shoots in *V. elliottii* explants.

Tissue culture appeared to be quite useful to induce tetraploidy along with colchicine, depending on taxa. Shoots with increased diameter could be screened easily by visual examination. The space conservative feature of *in vitro* culture allowed treatment of a great number of rapidly growing shoot-tip cuttings, which enhanced the probability for success. Doubled shoots could be cloned rapidly *in vitro* allowing large numbers of autotetraploid ramets to be grown. This is quite important due to the reduced vigor of autotetraploids and the fact that many die before they flower.

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