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BLUEBERRY CALLUS AND SHOOT-TIP CULTURE[1]

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Abstract. **Callus cultures were established from stem sections and anthers of rabbiteye and highbush blueberry** (Vaccinium) **on Murashige-Skoog medium with 2, 4-dichlorophenoxy acetic acid (2,4-D). Concentrations of 0.25 to 0.50 mg/liter 2,4-D produced abundant callus growth for several clones tested, but higher concentrations produced poor callus growth. Tetraploid highbush clones produced more callus than rabbiteye clones, but there were variations among clones in each group. Callus grew well when subcultured, but attempts to induce differentiation failed. Shoot-tip cultures from rabbiteye blueberry seedlings produced up to 40 secondary shoots per initial explant within 1 month on modified Murashige-Skoog medium with 6 gamma-gamma-Dimethylallyl Amino Purine (2iP) and indole acetic acid (IAA). The proliferated shoots could be rooted in a peat-perlite medium under mist in a greenhouse.**

The ability to grow blueberry plants from callus tissue would have several immediate and practical uses. Over 5 million blueberry plants are propagated annually by stem cuttings in the United States and wholesaled at prices ranging upward from 80¢ each. The eventual price of blueberry plants propagated by shoot-tip culture is difficult to estimate, and the technique may or may not prove feasible in normal commercial practice. In the first years after release of a new cultivar, however, when propagation material is in limited supply and plants are selling at high prices, tissue culture propagation would clearly be advantageous. The price of propagating broccoli plants by tissue culture was recently estimated at about 16¢ each (1), but the price of propagating plants of other species could differ considerably. Another use of blueberry tissue culture would be in production of disease-free plants that could be transferred from one blueberry growing area to another without having to be quarantined. Tissue culture could also be used to double and halve chromosome numbers and thus facilitate hybridizations among blueberry species differing in ploidy. The experiments reported in this paper were conducted to study the effects of medium composition and explant genotype on performance of callus and shoot-tip cultures in blueberry.

Materials and Methods

The effects of medium pH and 2,4-D concentration on callus growth from cut stems of 'Aliceblue' cultivar of rabbiteye blueberry (*V. ashei* Reade) were studied in the first experiment. A medium was prepared containing Murashige-Skoog salts (2), thiamine · HCL (0.4 mg/liter), myo-inositol (100 mg/liter), sucrose (30 g/liter), agar (9 g/liter), and 2,4-D at 0.5, 1.0, or 3.0 mg/liter. pH was adjusted to 5.8 for half the medium and to 4.8 for the other half.

Medium was placed in 35 ml screw-top vials and autoclaved. Stem tissue from suckers of field-grown plants was surface-sterlized in a 33% clorox solution for 15 minutes, rinsed in autoclaved water, stripped of bark, and cut into small discs which were placed on the cooled medium.

The second experiment compared callus growth for 5 blueberry clones, including 4 rabbiteyes and one tetraploid highbush. The medium was the same as in Experiment 1, except that pH was 5.7 and 2, 4-D level 0.2 mg/liter. The explant source again was stem tissue from field-grown plants.

The explant source in the third experiment was anthers removed from flowers from 2 to 7 days after meiosis. Four clones were compared, three of which were tetraploid breeding lines and the other a native Florida diploid blueberry of the *V. fuscatum* complex (3), and liquid medium was compared with agar medium. The media consisted of Murashige-Skoog salts, thiamine · HCL (0.4 mg/liter), myoinositol (100 mg/liter), sucrose (30 g/liter), glutamine (0.8 g/liter), alpha-naphthaleneacetic acid (NAA, 1 mg/liter), and IAA (1 mg/liter), and in the non-liquid medium, 9 g/liter agar.

Explants for the fourth experiment were shoot tips taken from blueberry seedlings grown in sterile culture from open-pollinated seed of rabbiteye clone Fla. 6-104 and from the cross of the tetraploid clone 2-9 x the highbush cultivar 'Avonblue'. Three levels of the cytokinin N6-Benzyladenine (BA, 1, 5 and 10 mg/liter) were tested in a medium containing Murashige-Skoog salts, thiamine · HCL (0.4 mg/liter), myo-inositol (100 mg/liter), sucrose (30 g/liter), (9 g/liter), and IAA (0.5 mg/liter).

For the 5th experiment, shoot tips from asceptically-grown open-pollinated seedlings of rabbiteye clone 6-104 were planted into medium containing 5 or 15 mg/liter of the cytokinin 2iP. Other components of the medium were the Murashige-Skoog salts modified to contain 800 mg/liter KNO_3, 2000 mg/liter NH_4 No_3, 30 mg/liter adenine sulfate, IAA at 4 mg/liter, thiamine · HCL (0.4 mg/liter), sucrose (30 g/liter), and agar (9 g/liter).

For all experiments, vials were incubated at approximately 27 C with 16 hours per day illumination of approximately 4200 Lux from white fluorescent tubes.

Results and Discussion

Best callus production from stem tissue of 'Aliceblue' blueberry was obtained with 2, 4-D at 0.5 mg/liter. Both 1.0 and 3.0 mg/liter gave poor callus growth (Table 1). In a subsequent experiment (data not shown), 0.25 mg/liter

Table 1. Effect of 2, 4-D level and pH on callus growth from Aliceblue blueberry.

Medium		Vials with callus[a] (no.)			
pH	2, 4-D	Good	Fair	Poor or none	Total
	(mg/liter)				
4.8	0.5	5	1	0	6
4.8	1.0	1	2	3	6
4.8	3.0	0	0	7	7
Total 4.8		6	3	10	
5.8	0.5	6	0	0	6
5.8	1.0	1	3	2	6
5.8	3.0	0	1	5	6
Total 5.8		7	4	7	

[a]Visual ratings based on amount and color of callus.

[1]Florida Agricultural Experiment Stations Journal Series No. 1499.

2,4-D gave callus growth equal to that obtained with 0.50 mg/liter. Even though blueberries grow best in the field at pH below 5.5, the callus in Experiment 1 grew as well at pH 5.8 as at 4.8.

Experiment 2 showed large differences among cultivars in callus growth from stem pith cultured on a medium containing 0.2 mg/liter 2,4-D (Table 2). The tetraploid highbush clone 7-9 produced much more callus than any of the 4 hexaploid rabbiteye clones tested. Among the rabbiteyes, cultivar 'Southland' produced much more callus than 'Briteblue', 'Delite', or 'Tifblue'.

Table 2. Effect of genotype on growth of blueberry callus.

Clone	Vials with callus growth[z]: (no.) Good	Fair	Poor	None	Total
Briteblue	0	0	2	6	8
Southland	0	7	0	5	12
Delite	0	0	1	8	9
Tifblue	0	0	0	15	15
7-9	10	0	0	0	10

[z]Visual ratings based on amount and color of callus.

There were also large differences (Table 3) among highbush clones in ability to produce callus from anthers cultured shortly after meiosis. Clones 4-75 and 'Sharpblue' produced abundant callus and clone 1-2 comparatively little. The diploid clone SG46 also produced little callus. Stationary liquid medium upon which anthers were floated gave slightly better callus growth than agar medium of similar composition.

Table 3. Effect of genotype on callus formation from blueberry anthers on agar and liquid media.

Anther source	Vials with callus growth: Agar medium Abundant	Intermediate	Slight or none	Liquid medium Abundant	Intermediate	Slight or none
			(no.)			
SG46	0	4	8	0	7	4
Sharpblue	6	3	4	5	2	4
1-2	0	1	12	0	8	7
4-75	7	1	3	8	2	1

BA at 1, 5 and 10 mg/liter gave only low levels of shoot proliferation from shoot-tip cultures of 6-104 and 2-9 x Avonblue seedlings (Table 4). On the other hand, 15 mg/liter 2iP in a modified Murashige-Skoog medium gave large numbers of shoots from approximately half of the vials planted with shoot tips of 6-104 seedlings (Table 5). A subsequent experiment (data not shown) suggested that 15 mg/liter 2iP was superior to either 10 mg/liter or 20 mg/liter. At the higher concentration, lateral proliferation was so great that the plantlets seemed to be reverting almost to a callus form. When transferred to a medium with 5 mg/liter 2iP, some of these shoot tips elongated, with individual tips resembling normal seedling apices. These tips were successfully used as explant sources from which proliferations again were obtained.

Table 4. Effect of BA concentration on proliferation of blueberry shoot tips.

BA(mg/l)	6-104 % proliferating reps.	6-104 Total no. of reps.	2-9 x Avonblue % proliferating reps.	2-9 x Avonblue Total no. of reps.
1	18	11	0	9
5	0	12	0	10
10	0	9	0	10

Table 5. Effect of 2iP concentration and medium pH on proliferation of blueberry shoot tips.

2iP (mg/l)	pH	% proliferating reps.	Total no. of reps.
5	5.7	24	17
15	5.7	53	17
15	4.5	33	18

Unsuccessful attempts were made to root shoot tips using media devoid of auxins and cytokinins, media diluted to 10% and 50% strength, and media in which the sucrose but not the salt concentrations were reduced. Chilling cultures to 50° C for 2 weeks and reducing light intensity also did not induce rooting. Shoots rooted readily if cut from the proliferating tissue when approximately 2 cm long and placed under conditions used commercially to root blueberry softwood cuttings. The cut ends were dipped in a commercial rooting powder (0.3% indole-3-butyric acid) and inserted to a depth of approximately 0.8 cm in a mixture of 50% peat moss and 50% perlite in a shallow tray. The tray was placed in a greenhouse where the shoots were kept moist by intermittent mist. Shoots also rooted in tissue culture vials when cut and inserted into autoclaved sand moistened with liquid Murashige-Skoog medium.

The success obtained with shoot-tip propagation in these experiments was encouraging, but further experiments will be needed to determine whether the same techniques that work with explants cut from germinating seeds will also work with explants from nonjuvenile clonal material.

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