Induced Tetraploidy in a *Vaccinium elliotii* Facilitates Crossing with Cultivated Highbush Blueberry

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abstract. A synthetic autotetraploid derived by colchicine treatment of a *Vaccinium elliotii* Chapm. plant ($2n = 2x = 24$) was used to study the effect of chromosome doubling on the ability of this noncultivated species to cross with the cultivated tetraploid highbush blueberry (*V. corymbosum* L.). Mean pollen germination was 28% for the autotetraploid plant, compared to 53% for the diploid *V. elliotii* plant. However, the number of seedlings obtained per flower pollinated on the tetraploid highbush cultivar O'Neal rose from 0.01 when diploid *V. elliotii* was the pollen source to 3.86 when pollen from the autotetraploid *V. elliotii* plant was used. Reciprocal crosses between diploid *V. elliotii* and its autotetraploid and selfs of the autotetraploid produced no seedlings. Meiotic irregularities, such as multivalent during metaphase, laggards, and unequal chromosome disjunction, were observed in the autotetraploid, but most chromosomes were associated as bivalents.

Vaccinium section *Cyanococcus* has been divided into nine diploid ($2n = 2x = 24$), 12 tetraploid ($2n = 4x = 48$), and three hexaploid ($2n = 6x = 72$) species (Camp, 1945; Darrow et al., 1944). Genes for important horticultural traits exist at all three ploidy levels; each ploidy level has useful genes not found at the others (Moore, 1965). Cultivated forms exist at the tetraploid and hexaploid levels, but diploid *Cyanococcus* species are not cultivated.

Vaccinium elliotii is a diploid found from Florida to Virginia and west to Texas. This species generally is low-chilling, early ripening, tolerant of dry upland soils, and disease resistant. Its fruit are very small but otherwise of medium to high quality (Lyrene and Sherman, 1980).

Several large-scale efforts have been made to cross tetraploid *V. corymbosum* cultivars with the diploid wild species *V. elliotii*. These crosses have produced few seedlings, but some vigorous triploid and tetraploid hybrids have been obtained (Lyrene and Sherman, 1983; Munoz and Lyrene, 1985). Two alternative explanations for the low success rate in this interspecific cross are the chromosome number difference and genetic differences not related to chromosome number.

Chromosome doubling has been induced in many diploid plant species (Dewey, 1979; Sanford, 1983). The resulting autotetraploids showed changes in fertility, chromosome behavior at meiosis, and plant morphology. In *V. atrococcum* larger pollen, flowers, and leaves resulted from autotetraploidy, as well as a slight breakdown in self-incompatibility (Draper et al., 1972).

Cytological studies in *Vaccinium* have revealed generally regular pairing in the diploid species (Longely, 1927), but a few pairing irregularities have been reported in tetraploids (Jelenkovic and Hough, 1970). Our study was carried out to determine the effect of chromosome doubling on overcoming the crossing barriers that exist between *V. elliotii* and the tetraploid highbush *V. corymbosum*.

Materials and Methods

The tetraploid *V. elliotii* clone used in this study, Fla. 519, was derived by in vitro colchicine treatment of two-node cut-

tings from a seedling diploid ($2n = 2x = 24$) *V. elliotii* (Perry and Lyrene, 1984). The tetraploid highbush (*V. corymbosum*) 'O'Neal' and 'Sharpblue', a diploid *V. elliotii* clone, and one clone of *V. darrowi* ($2n = 24$) were also used in the crosses.

In preparation for crossing, plants that had been growing in the field for ≈ 4 years were dug in December, potted, chilled at 7°C for 6 weeks, and then transferred to a greenhouse. The autotetraploid *V. elliotii* clone was self-pollinated and reciprocally crossed to both a cultivated highbush and a diploid *V. elliotii* clone.

Pollen viability was evaluated by in vitro pollen germination of ≈ 2000 pollen grains on an agar medium supplemented with sugar and nutrients (Goldy and Lyrene, 1983). Pollen stainability was measured on ≈ 1000 pollen grains using 1% acetocarmine for 1 h. Scanning electron microscopy was also used to determine the diameter and pollen morphology of ≈ 1000 pollen grains.

Flower buds from the autotetraploid and the diploid *V. elliotii* clones were used to study meiosis with a phase-contrast microscope (Dweikat and Lyrene, 1989). Leaf thickness was determined from 50 fully expanded leaves from the autotetraploid and diploid *V. elliotii*, and leaf area was measured with a LI-3000 portable leaf area meter (LI-COR, Lincoln, Neb.). Impressions were made by painting the abaxial surface of five mature leaves from the autotetraploid and diploid *V. elliotii* with clear fingernail polish. After the impressions had dried, they were stripped off, mounted in drops of water on microscope slides, and used to determine guard cell length under a light microscope.

As the berries ripened in the greenhouse, 20 random berries and 200 seeds were weighed from each clone. The seeds were extracted by hand from ripe berries, and the full-sized seeds were counted. The F_1 seeds from all greenhouse crosses were air-dried, stored in paper bags at 5°C for 6 months, and then sown on peatmoss under intermittent mist in the greenhouse. Germination percentage was determined before seedlings were transplanted to the field.

Results

Pollen germination on an artificial medium and pollen viability, estimated as percent stainable pollen, were lower in the autotetraploid than in the diploid *V. elliotii* (Table 1). The au-

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Table 1. Average phenotypes for autotetraploid and diploid plants of *V. elliotii* used in the greenhouse crosses.

Characteristic	Diploid		Autotetraploid		t value
	Range	Mean	Range	Mean	
Pollen germination (%)	46–60	53.3 ± 1.51	20–33	27.7 ± 1.33	12.26**
Pollen stainability (%)	82–94	87.3 ± 1.34	51–67	59.6 ± 1.40	14.25**
Fruit weight (g)	0.23–0.44	0.30 ± 0.01	0.60–0.87	0.66 ± 0.02	16.4**
Seed weight (mg)	0.57–0.65	0.62 ± 0.03	0.84–0.90	0.87 ± 0.06	10.8**
Leaf area (cm ²)	1.61–2.78	1.95 ± 0.13	2.75–5.82	3.89 ± 0.79	6.85**
Leaf thickness (μm)	160–200	182 ± 0.33	180–230	206 ± 0.52	3.91**
Guard cell length (μm)	12.6–15.7	13.7 ± 0.30	17.2–21.1	19.2 ± 0.41	10.79**
Pollen diameter (μm)	28–35	31.4 ± 1.77	37–47	42.5 ± 1.96	9.11**

*,** Significant at P = 0.05 and 0.01, respectively.

totetraploid had thicker stems, larger flowers (data not shown), and increased fruit and seed weight (Table 1, Fig. 1B). Leaf area of the autotetraploid averaged nearly twice that of the diploid *V. elliotii* (Table 1, Fig. 1A), but leaves of the former were only slightly thicker than those of the diploid. Stomatal guard cells of the autotetraploid averaged 40% longer than those of the diploid (Table 1, Fig. 1C and D). Scanning electron microscopy revealed that pollen from the autotetraploid had a larger diameter than that from the diploid (Table 1, Fig. 1E and F). The pollen walls of both the diploid and autotetraploid

appeared to be more concave than in previous microphotograph (Maas, 1977). These differences are probably due to technique.

Vaccinium elliotii clone Fla. 519 had medium to high fertility in crosses with the tetraploid *V. corymbosum* cultivar O'Neal - (Table 2). In this cross, Fla. 519 was more fertile as a seed parent than as a pollen parent. When 'O'Neal' was crossed in either direction with the diploid *V. elliotii* clone, fertility was extremely low. This result is an indication of the strength of the triploid block, which has been observed repeatedly in 4x × 2x crosses in *Vaccinium* section *Cyanococcus*. The tetraploid Fla. 519 set no seed when crossed with diploid *V. elliotii* was no-direction, suggesting that the triploid block is very strong within *V. elliotii*. Neither the diploid nor the tetraploid *V. elliotii* clone had significant self-fertility (Table 2).

Meiotic chromosomal behavior in diploid *V. elliotii* was normal in all cells examined (Fig. 2A and C). In the autotetraploid, univalent, bivalents, trivalent, and quadrivalents were found (Table 3, Fig. 2B and D). The mean frequency of chromosome associations other than bivalents was higher at diakinesis than at metaphase I (data not shown). Pairing associations involving more than four chromosomes were absent or rare, but abnormalities, such as clumping of chromosomes at metaphase I, were often observed. At anaphase I, ≈ 43% of the cells examined from the autotetraploid had an equal chromosome disjunction (24-24), while the rest of the cells contained one to two laggards (Fig. 2E). By anaphase II, most of the cells examined showed normal chromosome distribution (Fig. 2F).

Discussion

The reduced pollen viability of the autotetraploid *V. elliotii* clone compared to the diploid *V. elliotii* may be due, in part, to abnormal cytological behavior of the pollen mother cells, as observed in this study, or to genetic and physiological factors (Stebbins, 1947). Since meiosis was normal in the diploid studied, the cytological abnormalities at meiosis in the autotetraploid were regarded as a consequence of chromosome doubling. Univalent, trivalent, and quadrivalents at diakinesis and metaphase I are observed in doubled diploids of many species (Jackson and Casey, 1979). The decrease in trivalent and quadrivalent frequencies from diakinesis to metaphase I was also observed in autotetraploid *Fragaria* (Sebastiampillai and Jones, 1977).

In most gametophytic incompatibility systems, chromosome doubling has been found to weaken or eliminate the incompatibility (Lewis, 1954; Marks, 1966; Nettancourt, 1969; Pandey, 1956; Sebastiampillai and Jones, 1977). This was not the case in the autotetraploid of *V. elliotii*, supporting earlier reports (El-Agamy, 1979) that the incompatibility in *Vaccinium* is not gametophytic.

Fig. 1. Morphological characters of diploid and autotetraploid *V. elliotii*. (A) Branches of autotetraploid (left) and diploid (right). (B) Fruits of autotetraploid (left) and diploid (right). (C) Stomatal guard cells of autotetraploid. (D) Stomatal guard cells of diploid. (E) Autotetraploid pollen. (F) Diploid pollen. Scale = 20μm.

Table 2. Crossability and fertility data for the autotetraploid *V. elliotii* clone FL 519.

Cross		Flowers pollinated (no.)	Fruit set (%)	Seeds/fruit avg (no.)	Seedlings (no.)	Seedlings/ pollinated flower (no.)
Female	Male					
Fla. 519 (4x)	O'Neal (4x) ^z	118	62.0	15.5	850	7.21
O'Neal (4x)	Fla. 519 (4x)	185	53.0	7.7	528	3.86
O'Neal (4x)	Sharpblue (4x) ^z	50	86.0	18.3	613	12.27
Fla. 519 (4x)	<i>V. elliotii</i> (2x)	1120	0.0	0.0	0	0.00
<i>V. elliotii</i> (2x)	Fla. 519 (4x)	585	0.0	0.0	0	0.00
Fla. 519 (4x)	Self-pollinated	892	0.0	0.0	0	0.00
<i>V. elliotii</i> (2x)	Self-pollinated	650	4.3	1.7	35	0.05
<i>V. elliotii</i> (2x)	<i>V. darrowi</i> (2x) ^y	50	89.0	17.8	594	11.88
<i>V. elliotii</i> (2x)	O'Neal (4x)	520	2.7	1.3	6	0.011
O'Neal (4x)	<i>V. elliotii</i> (2x)	479	3.1	1.3	5	0.010

^zTetraploid *V. corymbosum* cultivar.^yA diploid species highly cross-compatible with *V. elliotii*.

Table 3. Range and mean of chromosome association at diakinesis (DK) and metaphase I (MI) in the autotetraploid.

Total no. of cells	Stage	Range and mean of chromosome association			
		I	II	III	IV
27	DK	0-3 (0.96)	9-14 (11.62)	0-3 2.36	2-5 4.18
29	MI	0-2 (0.71)	10-15 (14.25)	0-3 (1.75)	2-5 (3.82)

reported previously (Draper et al., 1972; Moore et al., 1964) and in many other species (Dewey, 1979; Sanford, 1983). The most important morphological feature resulting from chromosome doubling of *V. elliotii* was the increase in fruit size, since small fruit is a major factor preventing cultivation of diploid *Vaccinium* spp. (Sanford, 1983).

Induced tetraploids in *Vaccinium* may prove valuable in breeding because they facilitate gene exchange between diploid and tetraploid species. Important traits exist in both diploid and tetraploid species, and direct tetraploid × diploid crosses give a low frequency of tetraploids, along with triploids, pentaploids, and aneuploids (Lyrene and Sherman, 1983). Doubling the chromosome number of the diploids eliminates or greatly reduces the crossing barrier between diploid and tetraploid *Cyanococcus* spp. The ease of crossing the species at the tetraploid level suggests a close relationship between *V. elliotii* and *V. corymbosum* and indicates that the crossing barrier between these species is due largely to the difference in ploidy level and not to genic incompatibility.

After obtaining the data reported here, we crossed two unrelated *V. elliotii* autotetraploids produced by in vitro colchicine treatment and obtained ≈100 seedlings, which then were grown for 3 years in a high-density field nursery. These grew slower than diploid *V. elliotii* seedlings, but survived well in the field,

Fig. 2. Chromosome associations at diakinesis and metaphase I in *V. elliotii* and in Fla. 519. (A)

readily by their fertile pollen and used in backcrosses to the tetraploid gene pool. The two advantages of this method over induced polyploidy are avoidance of the task of inducing polyploidy and elimination of the 2 to 4 years required for induced polyploids to reach flowering age before they can be used in breeding.

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