HortScience 60(1):100–110. 2025. https://doi.org/10.21273/HORTSCI18263-24

# Artificial Induction of Polyploidy in Blueberry Breeding: A Review

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*Keywords.* blueberry, polyploidization, interspecific hybridization

***Abstract.*** **Blueberry planted acreage has increased rapidly during the past four decades, and blueberry consumption has kept pace. The environments across blueberry growing regions are highly heterogeneous. Variable factors include weather, soils, cultivation practices, biotic stress, and abiotic stress. Broadening the genetic diversity of the blueberry breeding gene pool will enable the development of blueberry cultivars adapted to specific growing regions. The primary gene pool for blueberry breeders includes cultivated tetraploids and hexaploids. The tetraploids include cultivars and advanced selections of northern highbush, southern highbush, lowbush, and half highbush; the hexaploids are the rabbiteye cultivars. The secondary gene pool encompasses diploid, tetraploid, and hexaploid wild blueberries in *Vaccinium* section *Cyanococcus*. The tertiary gene pool consists of 300 to 400 *Vaccinium* species in sections other than *Cyanococcus*. These are native to many parts of the world. Blueberry breeding began with interspecific hybrids in section *Cyanococcus*. Subsequent breeding has used diploid, tetraploid, and hexaploid *Cyanococcus* species with limited use of species from other *Vaccinium* sections. A strong triploid block and partial to total sterility in progeny from heteroploid crosses have limited the use of some species in breeding. Unreduced gametes allow the production of tetraploid hybrids from diploid × tetraploid crosses and hexaploid hybrids from triploid × hexaploid and triploid × triploid crosses. Production of polyploids by in vitro and in vivo treatments with antimitotic agents can expedite interspecific and intersectional hybridization. This review addresses the contribution of artificial induction of polyploidy to blueberry genetic improvement and discusses additional possible applications of artificial induction of polyploidy for blueberry breeding.**

Blueberry (*Vaccinium* sect. *Cyanococcus*) is a high-value fruit crop grown in many parts of the world (Fig. 1). For many years, the United States and Canada, where highbush blueberries are native, produced nearly all of the world's cultivated highbush blueberries. More recently, Europe, Chile, Peru, Mexico, China, and many other countries have begun blueberry production. Blueberries contributed approximately $4.7 billion to the economic impact in the United States in 2021 (https://ushbc.blueberry.org/all-resources/impact-report). Consumer appreciation of blueberry flavor, versatility, and health benefits has led to a 97% increase in per capita blueberry consumption in the United States during the past 10 years (https://www.thepacker.com/news/produce-crops/capita-availability-blueberries-raspberries-surging). The broad range of growing environments for blueberry production in various regions of the world requires the development of diverse cultivars adapted to specific growing conditions. Growers, packers, distributers, nursery operators, and consumers have varying needs for fruit quality, disease resistance, insect resistance, plant stress tolerance, and mechanical harvestability (Gallardo et al. 2018a). A survey conducted in the United States and Canada between 2016 and 2017 showed that fruit quality characteristics, including flavor, sweetness, texture, shelf life, small dry stem scar, size, shape, and color, are important to both producers and consumers. Resistance to biotic and abiotic stresses such as mummy berry disease, spotted wing drosophila, and tolerance to early and late freezes as well as high temperatures are high priorities for producers. Increasing labor costs (Gallardo et al. 2018b) have increased the demand for new cultivars suitable for machine harvest. Fruit and plant characteristics conducive to machine harvest include fruit firmness, resistance to mechanical bruising, concentrated ripening, upright growth habit, flexible canes, loose clusters, monopodial growth habit, and berry detachment such that mature berries detach readily, whereas immature berries do not. To meet these demands, a broad gene pool is needed.

One barrier to interspecific hybridization in *Vaccinium* is ploidy variation. Species include diploids, tetraploids, and hexaploids. There is a strong triploid block, and tetraploid × hexaploid crosses produce pentaploid hybrids with reduced fertility. Induced ploidy changes have been used since the 1960s to facilitate interspecific crosses. It is expected that artificial induction of polyploidy will continue to play an important role in broadening the gene pool and improving the resilience and sustainability of blueberry cultivation.

Received for publication 11 Oct 2024. Accepted for publication 16 Oct 2024.
Published online 17 Dec 2024.
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## *Vaccinium* Species that Have Been Used in Blueberry Breeding

Blueberry domestication and breeding have existed for more than 100 years. The first planned blueberry hybrids were created in 1909 by Frederick Coville, who crossed two tetraploid wild blueberry species in section *Cyanococcus* (*V. corymbosum* and *V. angustifolium*) (Coville 1927; Longley 1927; Moore 1965). Since then, intraspecific, interspecific, and intersectional hybridization have been important to blueberry improvement. The main gene sources used in blueberry breeding have been northern highbush ($2n = 4x = 48$; *V. corymbosum*), lowbush ($2n = 4x = 48$; *V. angustifolium*), and rabbiteye blueberries ($2n = 6x = 72$; *V. virgatum*) (Ballington 2001). Northern highbush and lowbush blueberries, which are endemic in north America, have a high chilling requirement and are adapted to areas with cold winters. Half highbush ($2n = 4x = 48$) blueberries were developed in Minnesota from crosses between highbush and lowbush cultivars. These can tolerate temperatures as low as −42 °C, partly because they are buried in snow during the coldest days of winter (Finn et al. 1990). Rabbiteye blueberries were domesticated from the hexaploid species, *V. virgatum* (*V. ashei* Reade), which is native in the southeastern United States (Lyrene 1987; Moore 1966). Southern highbush was initially developed by the introduction of low chill requirement, heat tolerance, soil adaptation, resistance to hot-weather diseases, and adaptive photoperiod responses, primarily from diploid *V. darrowii*, into northern highbush cultivars (Sharpe and Sherman 1971). Low-chill cultivars have allowed the spread of blueberry production to warmer regions, including the southeastern United States, Mexico, Peru, Morocco, southern Europe, South Africa, and southeastern China. Concerns regarding the narrow genetic base of cultivated blueberry were raised for both northern highbush and rabbiteye blueberries in the late 1980s. The genetic base for the original northern highbush cultivars, including Bluecrop, Blueray, Duke, Elliott, Jersey, and Weymouth, consists mainly of three wild tetraploid selections, Brooks, Sooy, and Rubel (Ehlenfeldt 1994; Moore 1993). Rabbiteye blueberry cultivars were largely developed from four wild hexaploid selections from the southeastern United States (Ethel, Clara, Myers, and Black Giant) (Lyrene 1987). Continued efforts to expand the genetic base of blueberry breeding are needed.

Worldwide, there are more than 400 *Vaccinium* species in more than 30 sections

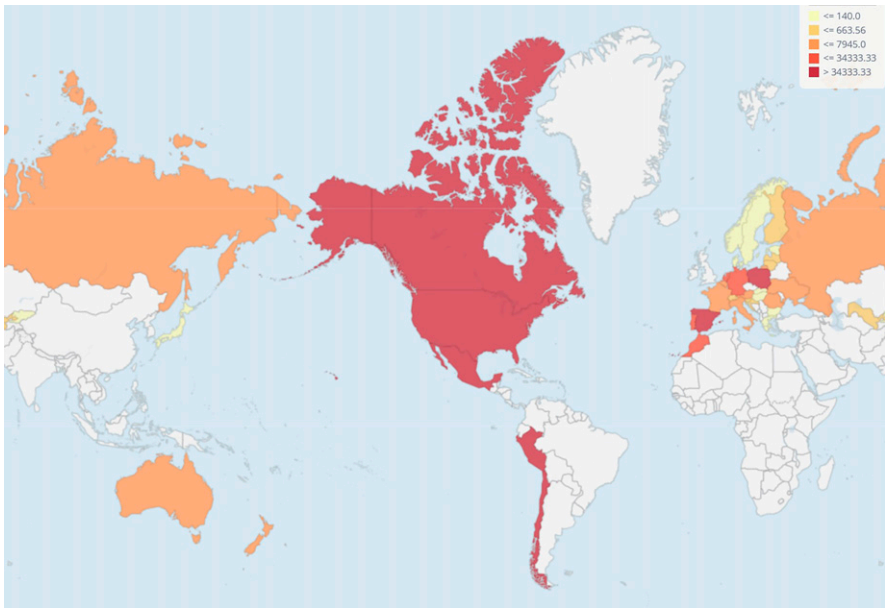


Fig. 1. World production of blueberries expressed as annual average production in tons from 2019 to 2021 (data source: https://www.fao.org/).

(Ballington 2001). Camp (1945) divided *V.* sect. *Cyanococcus*, which includes the principal cultivated species, into nine diploid, 12 tetraploid, and three hexaploid species (Camp 1945). Vander Kloet combined the diploid, tetraploid, and hexaploid highbush *Cyanococcus* species under the name *V. corymbosum*. The highbush group (Vander Kloet 1983, 1988) included the diploids *V. fuscatum* (also known as *V. atrococcum*) and *V. elliottii*, the tetraploids included *V. australe* and *V. simulatum*, and the hexaploids included *V. ashei* and *V. constablaei*. However, Vander Kloet's treatment was rejected by many blueberry researchers (Fritsch et al. 2024; Lyrene 2016a; Uttal 1986, 1987; Weakley 2024). One objection was that Vander Kloet's treatment did not consider the ploidy differences of *Vaccinium* species, which are important determinants of gene flow in nature. However, the treatment suggested by Camp (1945) needs further validation because of the insufficient evidence for ploidy determination based on a few chromosomal counts per species or prediction based on morphology (Fritsch et al. 2024). A phylogenetic analysis of five blueberry species through genotyping by sequencing suggested that there is a lack of clear delimitation between diploid *darrowii* and tetraploid *V. myrsinites* (Manzanero et al. 2023), but the chromosome number difference limits gene flow between these taxa. Camp postulated that *V. myrsinites* originated as a result of hybridization between *V. darrowii* and *V. tenellum* (Camp 1945). An admixture analysis indicated that *V. myrsinites* originated from *V. darrowii* by auto-tetraploidization (Fritsch et al. 2024; Manzanero et al. 2023). Further analyses using phenotypic, ecological, gene-flow, and molecular evidence will shed light on the species classification of *V.* sect. *Cyanococcus* (Fritsch et al. 2024). This current review adopts Camp's treatment because it was used most widely in the relevant literature.

Other than the primary gene pool consisting of cultivated blueberries, most of the species used in blueberry breeding have been from *V.* sect. *Cyanococcus*. Species from several other *Vaccinium* sections (the tertiary gene pool), such as sections *Batodendron*, *Polycodium*, *Bracteata*, *Myrtillus, Pyxothamnus*, *Hemimyrtillus*, and *Vaccinium*, have been hybridized with highbush cultivars (Table 1). Beneficial traits from these rich gene pools could be used in blueberry breeding (Ballington 2008). Adaptations to abiotic stresses (heat, cold, drought, higher soil pH, and low requirement of organic matters) were identified in wild blueberry relatives (Ballington 1990; Ballington 1996; Ballington 2008; Chandler et al. 1985; Contreras 2024; Ehlenfeldt and Polashock 2014; Ehlenfeldt et al. 2012; Li et al. 2022; Lyrene and Olmstead 2012; Lyrene et al. 2003, Lyrene 2016a; Miyashita et al. 2018; Moore 1965; Neill and Contreras 2022; Rousi 1966b; Tsuda et al. 2013). Resistance to stem canker (*Botryosphaeria corticis*), which was lethal to northern highbush cultivars when they were planted in North Carolina, came from North Carolina selections of wild tetraploid *V. corymbosum* (Demaree and Morrow 1951; Moore 1966). In *V.* sect *Cyanococcus*, high-level resistance to mummy berry caused by the fungal species *Monilinia vaccinii-corymbosi* (Reade) was identified in diploid species *V. boreale*, *V. darrowii*, *V. myrtilloides*, *V. pallidum*, and *V. tenellum* (Stretch et al. 2001). Resistance to blueberry stem blight (*Botryosphaeria dothidea*) and leafhopper was identified in *V. elliottii* and *V. stamineum* (Rooks et al. 1996).

Wild blueberries have architectural and fruit characteristics that could improve the mechanical harvestability and fruit quality of cultivated blueberries (Ballington et al. 1984, 1986; Lyrene and Sherman 1980). The upright and narrow crown formation of *V. arboreum* (Lyrene 2016a) and concentrated flowering time from *V. meridionale* (Ehlenfeldt and Ballington 2017; Ehlenfeldt and Luteyn 2021) could be used to improve the mechanical harvestability of blueberries. Fruit fragrance, flavor, upland adaptation, and short bloom-to-ripe interval from *V. elliottii* could be integrated into cultivars (Cabezas et al. 2021; Norden et al. 2020). The aromatic fruit flavors in the Florida cultivars Snowchaser and Kestrel are believed to have come from *V. elliottii* ancestors in their pedigrees. Introgression of soil and weather adaptability, disease resistance, fruit quality, and plant architecture from wild blueberries have enabled the cultivation of blueberry in a wide range of soils and climates worldwide. Hybridization of cultivars with wild blueberries results in reduced berry size and sometimes undesirable fruit taste, dark berry color, lack of firmness, and disease susceptibilities. Backcrosses are almost always needed to recover the commercial value in these introgressed breeding lines.

## Artificial Induction of Polyploidy Can Facilitate Interspecific and Intersectional Hybridization

Homoploid intraspecific crosses within species in *V.* sect. *Cyanococcus* usually produce numerous seeds and large numbers of vigorous, fertile hybrids (Moore 1966; Galletta 1975). Heteroploid interspecific crosses within *V.* sect. *Cyanococcus* are often difficult. Tetraploid × diploid and hexaploid × diploid crosses usually produce very few hybrids after thousands of pollinations. A strong triploid block, possibly related to endosperm underdevelopment in crosses between diploid and tetraploid parents, results in low hybrid progeny numbers (Ballington 2008; Darrow et al. 1944; Köhler et al. 2010; Lyrene et al. 2003). For instance, diploid species *V. caesariense* and *V. fuscatum* were reported to have a success rate of 0 to 0.008 hybrid seedlings per pollination when crossed with tetraploid highbush blueberries (Megalos and Ballington 1988). Rare interspecific triploids have very low fertility with abnormal meiotic chromosomal pairing (Dweikat and Lyrene 1988; Vorsa 1990; Vorsa and Ballington 1991). When the triploid progenies were crossed with highbush (4*x*), an average crossing success rate of 0.028 hybrid seedlings per pollination was reported (Vorsa and Ballington 1991). When triploids were crossed with hexaploids, a few hexaploid hybrids were obtained, and a few hexaploid seedlings have been obtained by crossing two triploids (Vorsa and Ballington 1991).

Crosses between tetraploid highbush cultivars and hexaploid rabbiteye cultivars are not difficult to make, but they give fewer seeds than homoploid crosses. Pentaploid seedlings from highbush × rabbiteye crosses are usually quite vigorous and partially fertile. The 2n gamete formation has made it possible to use triploids in blueberry breeding, and 2n gametes have enabled the production of fertile tetraploid hybrids from various diploid × tetraploid crosses (Lyrene et al. 2003; Ortiz et al. 1992a). However, frequencies of 2*n* pollen gametes in blueberries are normally low and

Table 1. Characteristics of *Vaccinium* species that could contribute to blueberry cultivar development through interspecific and intersectional hybridizations.

| Species | Ploidy | Desirable traits | Undesirable traits | References |
|---|---|---|---|---|
| *V.* sect. *Cyanococcus* | | | | |
| *V. darrowii* | 2*x* | Small fruit scar, firm berry, no chilling requirement, resistant to mummy berry disease, fire-adapted, drought-tolerant | Small fruits, twiggy plant structure, long fruit development period | Lyrene 2016a; Lyrene and Sherman, 1980; Ortiz et al. 1992; Stretch et al. 2001 |
| *V. elliottii* | 2*x* | High fruit aroma, tolerant to a wide range of soil types, short fruiting period, resistant to blueberry stem canker, stem blight, and leaf hopper | Small black fruits, berry softness | Lyrene 2016a; Norden et al. 2020; Ortiz et al. 1992; Rooks et al. 1996 |
| *V. boreale* | 2*x* | Resistant to mummy berry disease | Lowbush architecture | Lyrene et al. 2003; Stretch et al. 2001 |
| *V. myrtilloides* | 2*x* | Cold hardiness, resistant to mummy berry and stem blight diseases | Lowbush architecture | Darrow et al. 1944; Ortiz et al. 1992; Rooks et al. 1996; Stretch et al. 2001 |
| *V. tenellum* | 2*x* | Low chilling requirement, drought- and heat-tolerant, resistant to mummy berry disease | Dull to shiny black fruit color, susceptible to powdery mildew, small berry | Ballington 2008; Darrow et al. 1944; Ortiz et al. 1992; Stretch et al. 2001 |
| *V. pallidum* | 2*x* | Early ripening, small and dry stem scar, firm fruit, easy fruit detachment, resistant to mummy berry disease | N/A | Ballington et al. 1984; Stretch et al. 2001 |
| *V. vacillans* | 2*x* | Drought-resistant | N/A | Moore 1965 |
| *V. fuscatum* | 2*x* | Early ripening, resistant to blueberry stem canker | Small black fruits | Lyrene 2016a; Lyrene and Sherman 1980 |
| *V. fuscatum* | 4*x* | Shade-tolerant, cross-compatible with *V. corymbosum* | black berries | Lyrene 2016a |
| *V. corymbosum* | 4*x* | Early ripening, cold hardiness | N/A | Moore 1965 |
| *V. myrsinites* | 4*x* | Firm berry, small scar, no chilling requirement, drought- and heat-tolerant, tolerant to upland soil | Small fruits, lowbush architecture | Chandler et al. 1985; Lyrene 2016a |
| *V. australe* | 4*x* | Cold hardiness, resistant to fungal diseases | N/A | Moore 1965 |
| *V. lamarckii* | 4*x* | Early ripening, cold hardiness | N/A | Moore 1965 |
| *V. angustifolium* | 4*x* | Early ripening, cold hardiness, resistant to stem blight disease, tolerant to upland soil | Small berry, lowbush architecture | Ballington 2008; Chandler et al. 1985; Lyrene et al. 2003; Moore 1965 |
| *V. hirsutum* | 4*x* | Cross-compatible with highbush blueberries | Small, pubescent berries | Lyrene 1997 |
| *V. brittonii* | 4*x* | Cold hardiness | Lowbush architecture | Moore 1965 |
| *V. simulatum* | 4*x* | Late bloom, adapted to upland soil, deep root system, cold hardy | N/A | Ballington 1990 |
| *V. virgatum* | 6*x* | Small and dry stem scar, late-ripening, drought-tolerant, tolerant to upland soil, high plant vigor, tolerant to root rot phytophthora | Dark fruits, larger seeds, skins tend to get tough in storage, frozen berries | Chandler et al. 1985; Lyrene 2016a |
| *V. amoenum* | 6*x* | Small shallow scar, easy fruit detachment, short statue | Small black berries | Ballington et al. 1984; Lyrene 2016a |
| *V. constablaei* | 6*x* | Early ripening, late bloom, cold hardiness | Fruit soft | Ballington et al. 1986 |
| *V.* sect. *Batodendron* | | | | |
| *V. arboreum* | 2*x* | Late bloom, loose flower cluster and long pedicels, purple-colored berry flesh, adaptive to dry sandy soil, tolerant to slightly high soil pH | Dry, gritty, and barely edible berries | Lyrene 2016a |
| *V.* sect. *Polycodium* | | | | |
| *V. stamineum* | 2*x* | Large, juicy berries with high soluble solids content, berry flesh varies from green to red to purple, drought-tolerant, leaf hopper-resistant | Tough skin, bitter taste, berry falls when ripe, difficult to clonally propagate | Ballington 1996; Lyrene 2016a; Rooks et al. 1996 |
| *V.* sect. *Bracteata* | | | | |
| *V. bracteatum* | 2*x* | Wide and deep root system, drought-tolerant, tolerant to high pH soil | Small dark berries | Tsuda et al. 2013 |
| *V. boninense* | 2*x* | Heat- and drought-tolerant | N/A | Miyashita et al. 2018 |
| *V. wrightii* | 2*x* | Heat- and drought-tolerant | N/A | Miyashita et al. 2018 |
| *V.* sect. *Myrtillus* | | | | |
| *V. myrtillus* | 2*x* | Extreme cold hardiness, dark purple flesh | Lowbush architecture | Lyrene et al. 2003 |
| *V.* sect. *Pyxothamnus* | | | | |
| *V. meridionale* | 4*x* | Concentrated flowering time, monopodial architecture, suitable for machine harvest | Dark reddish to black berries, thick skin | Ehlenfeldt and Ballington 2017; Ehlenfeldt and Luteyn 2021 |
| *V. ovatum* | 2*x* | High land adaptation, evergreen | Small and tart berries | Contreras 2024; Neill and Contreras 2022 |
| *V.* sect. *Hemimyrtillus* | | | | |
| *V. padifolium* | 4*x* | Abundant flowering and fruiting, firm fruit, low fruit removal force, high sugar content, small dry scar, high self-fertility, upright structure, vigorous growth, drought-tolerant, adapted to neutral soil pH | N/A | Ballington et al. 1984; Ehlenfeldt and Polashock 2014; Ehlenfeldt et al. 2012; Ortiz et al. 1992 |

*(Continued on next page)*

Table 1. (*Continued*)

| Species | Ploidy | Desirable traits | Undesirable traits | References |
|---|---|---|---|---|
| *V. cylindraceum* | | Drought-tolerant, adaptive to neutral soil | N/A | Ehlenfeldt and Polashock 2014 |
| *V. arctostaphylos* | | Cold hardiness, tolerant to upland soil | N/A | Ehlenfeldt and Polashock 2014 |
| *V.* sect. *Vaccinium* | | | | |
| *V. uliginosum* | 4*x* | Cold hardiness | Lowbush habit | Li et al. 2022; Lyrene and Olmstead 2012; Rousi 1966 |

N/A = information not available.

variable (0% to 10%) (Ortiz et al. 1992a, 1992b). The frequencies reported for blueberries were like those for *Brassica napus* and *Descurainia sophia* (Kreiner et al. 2017). It was reported that 2*n* gamete production in blueberries resulted from first division restitution during meiosis (Qu and Hancock, 1995; Vorsa and Rowland 1997; Vorsa et al. 1986). Functional 2*n* pollen formation and 2*n* egg formation were not correlated in the same blueberry plant (Chavez and Lyrene 2009a). Production of large dyads instead of normal size tetrads, flow cytometry, and levels of success from diploid × tetraploid crosses have been used to detect 2*n* gamete production in blueberries (Kreiner et al. 2017; Lyrene et al. 2003; Megalos and Ballington 1988; Ortiz et al. 1992a). Progenies from diploid × tetraploid crosses in section *Cyanococcus* were mostly tetraploid, and the $F_1$ hybrids were highly fertile when backcrossed to highbush. Highly fertile interspecific tetraploid hybrids were produced by Darrow and Sharpe from crosses between tetraploid highbush and a diploid clone Florida-4B (Draper and Hancock 2003) because of the production of 2n gametes of Florida-4B. Florida-4B was confirmed as a hybrid between *V. darrowii* and *V. fuscatum* (Bassil et al. 2018). Crosses involving Florida-4B have made the most prominent contribution to the development of southern highbush blueberries, which have reduced chilling requirements and adaptation to the soils and climate of the southeastern United States (Draper and Hancock 2003). Most of the released southern highbush cultivars have Florida-4B in their pedigree (Ballington 2001). Florida-4B can also serve as a bridge to introgress alleles from diploid species that rarely produce 2*n* gametes (Ballington et al. 1996; Brooks and Lyrene 1998). Hybrids between hexaploid rabbiteye cultivars and diploid *V. darrowii*, which Sharpe and Darrow believed to be tetraploid, were later found to be pentaploid, apparently because only 2n gametes from *V. darrowii* functioned in this cross. The pentaploids were sufficiently fertile to produce seedlings when crossed with tetraploid highbush. The success of interspecific crosses indicates genome compatibility among *Vaccinium* species (Darrow and Camp 1945; Dweikat and Lyrene, 1989; Lyrene 2014; Moore 1966). This notion was further supported by the recent comparative genome analyses that suggested high collinearity of genome structures across tetraploid and diploid *Cyanococcus* species (Brevis et al. 2008; Edger et al. 2022; Mengist et al. 2023). Although blueberry breeders have been using 2*n* gametes to introgress desirable genetic traits, some diploid species make very few 2n gametes. Producing tetraploid plants of diploid species is an alternative way to obtain tetraploid hybrids using diploid species (Table 2).

*V. elliottii* ($2n = 2x = 24$) is native to the southeastern United States and is valued for its earliness, upright growth habit, and fruit flavor, but it has small black fruit (Lyrene 1997, 2014) and a low frequency of 2*n* gametes formation (Ortiz et al. 1992b). The crossing success rates between highbush (4*x*) and *V. elliottii* ranged from 0.002 to 0.008 hybrid seedlings per pollination (Lyrene and Sherman 1983; Megalos and Ballington 1988; Norden et al. 2020). The small number of $F_1$ hybrids recovered from crosses between tetraploid highbush blueberry cultivars and diploid *V. elliottii* included pentaploids ($2n = 5x = 60$), triploids ($2n = 3x = 36$), and tetraploids ($2n = 4x = 48$) (Lyrene and Sherman 1983; Norden et al. 2020). Somatic mixoploids suggested genome instability in these new hybrids. Triploid hybrids from highbush (4*x*) × *V. elliottii* (2*x*) produced little pollen. Most pollen was aborted except for a few well-formed dyads and monads (Norden et al. 2020). Backcrosses using triploid hybrids as male and highbush (4*x*) or *V. elliottii* (2*x*) as females were not successful (Norden et al. 2020; Vorsa and Ballington 1991). However, when the triploid hybrids were crossed with *V. virgatum* (6*x*), a success rate of 0.01 to 0.30 hybrid seedlings per pollination was achieved because of the formation of 2*n* gametes in the triploid hybrids (Dweikat and Lyrene 1988; Vorsa and Ballington 1991). The few tetraploid progenies from highbush × *V. elliottii* crosses were fertile and cross-compatible with southern highbush cultivars; such crosses produced an average of 0.89 hybrid seedlings per pollination (Norden et al. 2020). Despite the low success rates of interspecific crosses with *V. elliottii*, the early ripening and fruit aroma from *V. elliottii* were introgressed in highbush cultivars Carteret (Ballington 2009), Snowchaser, and Kestrel (Norden et al. 2020). It was reported that recovery of fruit size and commercial yield can be achieved through one backcross to southern highbush parents (Cabezas et al. 2021).

In contrast to the very low success rates of heteroploid crosses involving *V. elliottii* (2*x*), highbush (4*x*), and *V. virgatum* (6*x*), synthetic hexaploids produced by colchicine treatment of highbush × *V. elliotti* triploids crossed readily with hexaploid rabbiteye (Dweikat and Lyrene 1989; Perry and Lyrene 1984). The crossing success rates between colchi-*V. elliottii* (4*x*) and *V. corymbosum* (4*x*) were reported to be 3.8 to 7.2 hybrid seedlings per pollination (Dweikat and Lyrene 1991), which are comparable to those of homoploid intraspecific crosses. Although triploid hybrids from crosses between highbush (4*x*) and *V. elliottii* (2*x*) were highly sterile, hexaploid hybrids were fertile. Reciprocal crosses between hexaploids derived from 4*x V. corymbosum* × 2*x V. elliottii* crosses reached a success rate of five to six hybrid seedlings per pollination when crossed with hexaploid *V. virgatum*. This was at least 20-times higher than the success rates of crosses between triploid hybrids and *V. virgatum* (6*x*) (Dweikat and Lyrene 1988; Vorsa and Ballington 1991).

Compared with the low success rate of interspecific crosses between tetraploid *V. corymbosum* and diploid *V. elliottii*, a higher success rate of 0.01 to 0.11 hybrid seedlings per pollination was achieved with crosses between highbush (4*x*) and *V. darrowii* (2*x*) (Chavez and Lyrene 2009a; Sharpe and Darrow 1959) because *V. darrowii* naturally produced more 2*n* gametes than *V. elliottii* (Ortiz et al. 1992a, 1992b). *V. darrowii* introgression into highbush cultivars was made more efficient by using tetraploid *V. darrowii* produced using colchicine. Crosses of tetraploid *V. darrowii* with tetraploid blueberries reached a success rate of 4.54 ± 3.12 seeds per pollination from eight different cross combinations (Chavez and Lyrene 2009b).

Intersectional crosses between diploid *Vaccinium* species usually give few hybrids. These are less vigorous than both parents and are usually sterile, probably because of abnormal meiotic chromosomal pairing (Brooks and Lyrene 1998; Chavez and Lyrene 2010; Lyrene and Olmstead 2012). However, fertile tetraploid intersectional hybrids have been recovered. For instance, fertile hybrids with regular meiotic behavior from crosses between tetraploid *V. uliginosum* L. and tetraploid highbush cultivars were recovered (Rousi 1963, 1966a, 1966b). It was suggested that these fertile hybrids were potentially amphidiploids whose fertility was attributable to autosyndetic chromosomal pairing during meiosis (Lyrene and Olmstead 2012).

*V. arboreum* in *V.* sect. *Batodendron* (commonly known as sparkleberry) has deep and widespread roots that confer drought tolerance. Sparkleberry is also better adapted than highbush cultivars to soils with somewhat higher pH levels. Sparkleberry plants flower late, normally avoiding damage from spring freezes, and the berries ripen from late September through October in north Florida (Lyrene 2016a). It also has an upright growth

Table 2. Success rates of interspecific and intersectional crosses within genus *Vaccinium* are improved by artificial induction of polyploidy.

| Crossing combinations | Total number of pollinations | Number of crosses | Hybrid seedlings per pollination | References |
|---|---|---|---|---|
| Intraspecific crosses section *V. Cyanococcus* | | | | |
| Highbush (4*x*) (*Vaccicium*) | 1,828 | 12 | 3.19 ± 1.72 | El-Agamy et al. 1981 |
| *V. virgatum* (6*x*) | 3,838 | 18 | 1.55 ± 1.51 | El-Agamy et al. 1981 |
| Interspecific crosses within section *V. Cyanococcus* | | | | |
| Highbush (4*x*) × *V. elliottii* (2*x*) | 4,301 | 19 | 0.005 | Norden et al. 2020 |
| Highbush (4*x*) × *V. elliottii* (2*x*) | 7,000 | | 0.004 | Lyrene and Sherman 1983 |
| Highbush (4*x*) × *V. elliottii* (2*x*) | 932 | 4 | 0.002 ± 0.003 | Megalos and Ballington 1988 |
| *V. elliottii* (2*x*) × Highbush (4*x*) | 750 | 3 | 0.008 ± 0.01 | Megalos and Ballington 1988 |
| Colchi-*V. elliottii* (4*x*) × Highbush (4*x*) | 118 | 1 | 7.2 | Dweikat and Lyrene 1991 |
| Highbush (4*x*) × colchi-*V. elliottii* (4*x*) | 185 | 1 | 3.8 | Dweikat and Lyrene 1991 |
| [Highbush (4*x*) × *V. elliottii* (2*x*)] (3*x*) × *V. virgatum* (6*x*) | 10,853 | | 0.03 ± 0.04 | Dweikat and Lyrene 1988 |
| *V. virgatum* (6*x*) × [Highbush (4*x*) × *V. elliottii* (2*x*)] (3*x*) | 6,142 | | 0.01 ± 0.006 | Dweikat and Lyrene 1988 |
| Colchi-[Highbush (4*x*) × *V. elliottii* (2*x*)] (6*x*) × *V. virgatum* (6*x*) | 220 | 1 | 5.9 | Dweikat and Lyrene 1989 |
| *V. virgatum* (6*x*) × colchi-[Highbush (4*x*) × *V. elliottii* (2*x*)] (6*x*) | 237 | 1 | 5 | Dweikat and Lyrene 1989 |
| [Highbush (4*x*) × *V. elliottii* (2*x*)] (4*x*) × [Highbush (4*x*) × *V. elliottii* (2*x*)] (4*x*) | 1,211 | 6 | 0.89 ± 0.7 | Norden et al. 2020 |
| [Highbush (4*x*) × *V. elliottii* (2*x*)] (3*x*) × *V. elliottii* (2*x*) | 1,673 | 18 | 0 | Norden et al. 2020 |
| Highbush (4*x*) × *V. darrowii* (2*x*) | 1,027 | | 0.02 | Sharp and Darrow 1960 |
| *V. darrowii* (2*x*) × Highbush (4*x*) | 651 | | 0.01 | Sharp and Darrow 1960 |
| Highbush (4*x*) × *V. darrowii* (2*x*) | 5,145 | 10 | 0.11 ± 0.18 | Chavez and Lyrene 2009 |
| *V. darrowii* (2*x*) × Highbush (4*x*) | 5,866 | 10 | 0.08 ± 0.13 | Chavez and Lyrene 2009 |
| Highbush (4*x*) × colchi-*V. darrowii* (4*x*) | 1,575 | 8 | 4.54 ± 3.12 | Chavez and Lyrene 2009 |
| Intersectional crosses | | | | |
| *V. padifolium* (4*x*) × *V. corymbosum* (4*x*) | 398 | | 0.005 | Ehlenfeldt and Polashock 2014 |
| Highbush (4*x*) × *V. meridionale* (4*x*) | 134 | 4 | 1.1 ± 0.8 | Ehlenfeldt and Luteyn 2021 |
| Highbush (4*x*) × *V. aboreum* (2*x*) | 500 | 3 | 0 | Lyrene 2011 |
| Highbush (4*x*) × colchi-*V. aboreum* (4*x*) | 17,968 | 83 | 0.07 ± 0.08 | Lyrene 2011 |
| Highbush (4*x*) × *V. stamineum* (2*x*) | 490 | 2 | 0.02 | Lyrene 2016 |
| Highbush (4*x*) × colchi-*V. stamineum* (4*x*) | 2,702 | 13 | 0.68 | Lyrene 2016 |
| Highbush (4*x*) × [Highbush (4*x*) × colchi-*V. stamineum* (4*x*)] (4*x*) | 3,250 | 22 | 1.47 | Lyrene 2016 |

habit and loose fruit clusters. These characteristics are favorable for mechanical harvesting. Intersectional crosses between diploid *V. darrowii* and diploid *V. arboreum* gave more than 100 seedlings from 500 crosses (Lyrene 1991). The most vigorous of these were transplanted to a field nursery, where they were surrounded by tetraploid highbush cultivars. Some of the more vigorous intersectional hybrid plants heavily flowered each year and produced a few berries from open pollination. Most berries contained one viable seed. When these were planted, most of the seedlings were tetraploid as a result of 2n gametes from the diploid intersectional hybrids and normal gametes from the tetraploid cultivars. The tetraploid hybrids were fertile when backcrossed to tetraploid highbush cultivars, and such crosses eventually resulted in the cultivar Meadowlark (Brooks and Lyrene 1998). The narrow crown, upright bush architecture, and loose fruit cluster of 'Meadowlark' were thought to be caused by genes from *V. arboreum* (Olmstead et al. 2013). Although this strategy was successful, it was thought that the production of tetraploid forms of *V. arboreum* would make it easier to introduce a more diverse range of *V. arboreum* germplasm into the highbush genetic pool. A success rate of 0.08 hybrid plant per pollination was achieved from crosses between highbush and colchi-*V. arboreum* (4*x*) (Haring and Lyrene 2008; Lyrene 2011; Lyrene and Olmstead 2012). Encouragingly, the backcross progenies of these intersectional hybrids were somewhat fertile and highly variable (Lyrene 2013).

*V. stamineum* in *V.* sect. *Polycodium* (commonly known as deerberry or gooseberry) is another diploid species native in the eastern United States that has contributed to highbush blueberry breeding. *V. stamineum* is drought-tolerant and produces large, juicy berries with high soluble solids, high firmness, and small stem scars. The species is polymorphic for berry flesh color, and some plants have a red to dark purple berry flesh color. The flowers of *V. stamineum* shed copious amounts of pollen and have short and open corollas with exserted stigma, which can improve the effectiveness of pollination by honeybees (Lyrene 2016b). Approximately 500 emasculated flowers of diploid *V. fuscatum* (section *Cyanocossus*) pollinated with pollen from diploid *V. stamineum* resulted in only a few weak intersectional hybrids (Table 2). Like *V. arboreum*, colchicine-induced tetraploid *V. stamineum* plants were cross-compatible with tetraploid highbush cultivars (Lyrene 2016b). Backcrosses of highbush × *V. stamineum* tetraploid hybrids to highbush cultivars produced vigorous $BC_1$ seedlings, and some were highly fertile (Lyrene 2018). The successful production of intersectional hybrids between diploid evergreen shrub shashanbo (*V. bracteatum* section *Bracteata*) and northern highbush cultivar Spartan was also attributed to the use of tetraploid plants produced from diploid shashanbo (Tsuda et al. 2013). The strong root development in high pH medium in the intersectional hybrids suggested that shashanbo might be valuable for improving blueberry soil adaptability (Tsuda et al. 2014).

## Artificial Induction of Polyploidy in Blueberries

The production of 2*n* gametes is considered meiotic polyploidization because chromosome doubling occurred during gamete formation (Cui et al. 2023). Because of the low frequency of naturally occurring 2*n* gametes, polyploid induction with antimitotic chemicals has been used in blueberries to circumvent ploidy barriers in interspecific and intersectional hybridization. In mitotic polyploidization, the chromosome number of somatic tissues is doubled by treating actively dividing somatic cells with antimitotic agents. Commonly used antimitotic agents include colchicine, oryzalin, and trifluralin. These arrest the cell cycle at the end of S-phase (DNA synthesis phase) and before cytokinesis (Dhooghe et al. 2011; Eng and Ho 2019). The most frequently used antimitotic agent for

artificial induction of polyploidy in blueberry has been colchicine, with concentrations ranging from 250 to 12,518 μM (Table 3). Other methods, such as physical induction using γ-rays, X-rays, and ultraviolet rays, have been used for other species such as *Lemna minor* (Van Hoeck et al. 2015), but not yet in blueberry. The ploidy increase in *Vaccinium* has been achieved using in vivo and in vitro systems (Table 3).

In vivo, various methods have been used to apply the antimitotic agent to blueberry tissues including seeds and axillary buds. Blueberry seeds were soaked in antimitotic solution for various lengths of time (Aalders and Hall 1963; Chavez and Lyrene 2009b; Haring and Lyrene 2008; Lyrene 2011, 2016b; Rousi 1966a). Alternatively, blueberry seeds were sterilized and plated on culture medium containing the antimitotic agent before germination (Miyashita et al. 2009; Tsuda et al. 2013). Potted plants have been treated by dropping antimitotic agent solutions on the axillary buds or by spraying leaves and buds with the solution (Draper et al. 1972; Moore et al. 1964; Neill and Contreras 2022). Moore et al. (1964) produced a decaploid blueberry by treating axillary buds of a highbush × rabbiteye pentaploid hybrid with a drop of 0.5% colchicine solution five times with a 2-d interval between treatments. For colchicine treatment, young seedlings produced by sprinkling blueberry seeds on pots of Canadian peat have been used. After the seedlings were approximately 1 cm tall, they were removed from the pots, their roots were washed free of peat, and the plants were completely submerged for 40 to 60 h in a 0.2% colchicine solution contained in flasks. During treatment, the flasks were maintained in a shaded part of the greenhouse and manually swirled several times per day to increase aeration. Then, the seedlings were washed in water and transplanted into trays of peat. The seedlings were surprisingly tolerant of this treatment. Approximately half of them normally survived, and up to 10% of the surviving seedlings had one or more polyploid branches.

In the in vitro system, micropropagation through tissue culture was first performed to obtain stems or leaf explants for treatment with antimitotic agents (Dweikat and Lyrene 1989, 1991; Goldy and Lyrene 1984; Lei et al. 2023; Lyrene and Perry 1982; Marangelli et al. 2022; Perry and Lyrene 1984; Podwyszynska et al. 2021). Two- to three-node stem segments or leaf explants were either immersed in the antimitotic solution or cultured on shoot induction medium containing an antimitotic agent for 24 h to 10 d. The treated explants were subsequently placed on fresh medium free of the antimitotic chemical to allow shoot proliferation and elongation. Suppression of shoot growth was reported in most of the treated explants, but surviving explants usually gave vigorous colonies of shoots.

Polyploidization is frequently accompanied by phenotypic changes. These have included increased stem diameter, leaf size, and leaf thickness of azalea (Paden et al. 1990), flower size of lavandin (Urwin 2014), fruit size of gooseberry (Kumar et al. 2020), and chilling tolerance of caladium (Zhang et al. 2020). Compared with the source plants, synthetic polyploid blueberries had increased shoot diameter, leaf area, leaf thickness, leaf chlorophyll content, stomatal guard length, pollen tetrad diameter, and flower size and decreased stomata density (Chavez and Lyrene 2009b; Dweikat and Lyrene 1991; Lei et al. 2023; Lyrene 2016b; Marangelli et al. 2022; Moore et al. 1964; Perry and Lyrene 1984). These phenotypic changes were used to select blueberry explants with induced ploidy. To confirm ploidy increase in treated explants, chromosomes in blueberry have been counted in mitotic cells of shoot tips and in premeiotic and meiotic cells in flower buds (Goldy and Lyrene 1984; Longley 1927; Lyrene and Perry 1982; Perry and Lyrene 1984; Rousi 1966a; Tsuda et al. 2013; Vorsa et al. 1986). Alternatively, the increased DNA content in the cells of treated tissues can be detected by flow cytometry, which has the advantage of high throughput (Eng and Ho 2019). With this method, nuclei from leaf samples are extracted and labeled with a DNA-specific fluorochrome such as propidium iodide and DAPI (4′,6-diamidino-2-phenylindole). The fluorophore-labeled nuclei are sorted through the focus of intense light, and the fluorescence emission from the nuclei is captured. The high DNA content in the nuclei corresponds to increased fluorescence emission, which allows differentiation of nuclei clusters with varied chromosome numbers. The location of the peaks of the nuclei distribution compared with known control samples indicates the ploidy of the tissue sampled.

The success rates of chromosomal doubling were highly variable depending on many factors. These included genotype, application methods, dosage and exposure time of the antimitotic agent, and light and temperature conditions during treatment (Table 3). The recovery rate of chromosome-doubled seedlings from colchicine-treated seeds was three to five per 1000 germinated seedlings (Chavez and Lyrene 2009b; Lyrene and Olmstead 2012; Lyrene 2016b). Because colchicine only acts on dividing cells, treatment of dried seeds would be effective only if some of the colchicine remained in the seeds until they began to germinate. However, 2% to 11% of the stem segments that were treated with colchicine in vitro produced axillary buds with doubled chromosomal levels (Lei et al. 2023; Marangelli et al. 2022; Podwyszynska et al. 2021). The higher efficiency of in vitro chromosomal doubling compared with that of seed treatment could be attributable to the ease of penetration of antimitotic agents to actively dividing cells in the axillary buds (Goldy and Lyrene 1984). Blueberry genotypes that grow well in vitro can produce vigorous shoots with many internode buds that are exposed to the antimitotic treatment. In addition, the in vitro system offers an invigorating environment to sustain and propagate newly induced polyploid explants, which are often weaker than the noninduced explants (Lyrene 2021).

Chimeras are common after colchicine treatment (Dhooghe et al. 2011; Marangelli et al. 2022; Nukaya et al. 2019). Mixoploidy was reported in colchicine-treated pentaploid blueberry hybrids (Chavez and Lyrene 2009b; Miyashita et al. 2009). Shoot apical meristems have three histological layers: L1, which forms epidermal tissue; L2, which includes subepidermal tissue such as mesophyll cells and gametes; and L3, which produces the vascular bundle with cambium and pith (Burge et al. 2002; Dermen and Bain 1944; Frost and Krug 1942). Sectorial, mericlinal, and periclinal chimeras can be produced during artificial induction of polyploidy depending on the cell layers modified by the antimitotic treatment. Sectorial chimeras have a sector of the plant such as a stem, a branch, or a leaf with doubled chromosomes in all histogenic layers, and other sectors of the plant remain normal. Mericlinal chimeras have a portion of the histogenic layers polyploidized in certain sectors of the plant. Periclinal chimeras have polyploidization of one or more entire histogenic layers. Plants that are periclinal chimeras often maintain their chimeral status when propagated by stem cuttings or grafting. However, loss of polyploid cell layers by replacement with more vigorous cytotypes can occur. In chimeral blueberries, an increased stomate size, which involves cells in the L-1 histogenic layer, may not be associated with increased pollen tetrad diameter, which involves the L-2 histogenic layer. Sectorial chimeras were reported among colchicine-induced tetraploid *V. stamineum* plants (Lyrene 2018). Genetic instability and ploidy reversal of mericlinal and sectorial chimeras mean that special care is required when using them for further breeding (Eng and Ho 2019). Solid polyploids can be recovered from sectorial chimeras by propagating from the polyploid sectors. Although periclinal chimeras are relatively stable, if the chromosomal level of the gamete-producing L2 layer is not doubled, then the chimera is not useful as a parent. To overcome the issue of chimeric polyploid formation, adventitious shoot regeneration from periclinal chimeric explants has been shown to produce a high percentage of solid polyploid regenerants (Regalado et al. 2017; Zhou et al. 2017).

Polyploidization not only produces additional sets of chromosomes but also induces random genome changes such as loss of genes, gene duplication, changes in expression profile, and epigenetic alterations (Eng and Ho 2019). Meiotic abnormalities among induced polyploids have been reported (Dweikat and Lyrene 1989, 1991), with close to 80% of pollen mother cells demonstrating abnormal chromosome association during both anaphase I and anaphase II. Consequently, pollen viability was lower in the colchi-induced lines. Reduced fertility has characterized induced polyploids in other crops (Batiru and Lübberstedt 2024; Oates et al. 2012). However, the well-known "gigas effect" of artificial chromosome doubling in ornamental

Table 3. Optimized conditions for artificial induction of polyploidy in *Vaccinium* species.

| Species | Section | Ploidy of source plants | Explant for treatment | Antimitotic agent | Antimitotic agent concn (μM) | Treatment conditions | Polyploidy induction rate | Identification ploidy increase | References |
|---|---|---|---|---|---|---|---|---|---|
| In vivo | | | | | | | | | |
| *V. boreale* | *Cyanococcus* | 2*x* | Radical extending seeds | Colchicine | 12,518 | Immersion of seeds in the solution with aeration for 7 d | 5% of treated seeds | Stomatal guard cell size | Aalder and Hall 1963 |
| *V. myrtillus* | *Myrtillus* | 2*x* | Seeds | Colchicine | 12,518 | Immerse seeds in antimitotic agent solution | 1% | Stomata cell size and chromosome count | Rousi 1966 |
| *V. darrowii* | *Cyanococcus* | 2*x* | Seeds | Colchicine | 5,007 | Imbibe seeds in the solution for 24 h | 5 out of 4000 seedlings | Stomatal guard cell size, pollen size | Chavez and Lyrene 2009b |
| *V. corymbosum* (4*x*) × *V. virgatum* (6*x*) hybrids | *Cyanococcus* | 5*x* | Seeds | Colchicine | 1,252 | Lay seeds on filter papers soaked with antimitotic agent solution | 33% to 50% of surviving seedlings | Flow cytometry | Miyashita et al. 2009 |
| *V. arboreum* | *Batodendron* | 2*x* | Seeds | Colchicine | 5,007 | Imbibe seeds in colchicine solution for 96 h and 5-h per week of postplant treatment for up to 3 weeks | 12 out of >30,000 treated seeds | Thickened hypocotyls, asymmetrical leaf shape, flow cytometry, pollen size | Haring and Lyrene 2008; Lyrene 2011 |
| *V. bracteatum* | *Bracteata* | 2*x* | Seeds | Colchicine | 1,252 | Culture sterilized seeds on solid MS/WMP medium with colchicine | 2 out of an unknown number of treated seeds | Flow cytometry, chromosome counting | Tsuda et al. 2013 |
| *V. Stamineum* | *Polycodium* | 2*x* | Seeds | Colchicine | 5,007 | Immerse seeds in antimitotic agent solution | 13 out of thousands of seedlings from treated seeds | Hypocotyl thickness, pollen size | Lyrene 2016b |
| *V. corymbosum* (4*x*) × *V. virgatum* (6*x*) hybrids | *Cyanococcus* | 5*x* | Axillary buds | Colchicine | 12,518 | Apply a drop of solution to the upper axillary buds at 2-d intervals five times | N/A | Leaf size, venation, and shape, chromosome count | Moore 1964 |
| *V. fuscatum* | *Cyanococcus* | 2*x* | Axillary buds | Colchicine | 12,518 | Apply a drop of solution to the upper axillary buds at 2-d intervals five times | N/A | N/A | Draper et al. 1972 |
| *V. ovatum* | *Pyxothamnus* | 2*x* | Seedlings | Oryzalin | 150 | Spray leaves with the solution daily for 20 d | N/A | Flow cytometry | Neill and Contreras 2022 |
| In vitro | | | | | | | | | |
| *V. virgatum; V. elliottii* | *Cyanococcus* | 6*x*; 2*x* | Shoot tip from tissue culture | Colchicine | 5,007 | Immerse stem segments in sterile antimitotic agent solution | | Large stem diameter, chromosome count | Lyrene and Perry 1982 |

*(Continued on next page)*

Table 3. (*Continued*)

| Species | Section | Ploidy of source plants | Explant for treatment | Antimitotic agent | Antimitotic agent concn (μM) | Treatment conditions | Polyploidy induction rate | Identification ploidy increase | References |
|---|---|---|---|---|---|---|---|---|---|
| Highbush | *Cyanococcus* | 4*x* | Stems from tissue culture | Colchicine | 625 | Immerse stem segments in sterile antimitotic agent solution | 5% | Large stem diameter, chromosome count | Goldy and Lyrene 1984 |
| *V. darrowii*; *V. elliottii*; *V. darrowii* × *V. elliottii* hybrids | *Cyanococcus* | 2*x* | Stems from tissue culture | Colchicine | 250 | Culture stem segment on shoot induction medium with antimitotic agent | 16% | Large stem diameter, chromosome count | Perry and Lyrene 1984 |
| *V. corymbosum* × *V. elliottii* hybrids | *Cyanococcus* | 3*x* | Stems from tissue culture | Colchicine | 500 | Culture stem segment on shoot induction medium with antimitotic agent | 6 out of 350 explants | Large stem diameter | Dweikat and Lyrene 1989 |
| *V. elliottii* | *Cyanococcus* | 2*x* | Stems from tissue culture | Colchicine | | Culture stem segment on shoot induction medium with antimitotic agent | 23 out of 50 explants | Large stem diameter | Dweikat and Lyrene 1991 |
| *V. myrtillus* | *Myrtillus* | 2*x* | Stems from tissue culture | Amiprophos methyl | 33 | Culture stem segment on shoot induction medium with antimitotic agent | 8.60% | Flow cytometry | Podwyszynska et al. 2021 |
| *V. myrtillus* | *Myrtillus* | 2*x* | Stems from tissue culture | Colchicine | 625 | Culture stem segment on shoot induction medium with antimitotic agent | 10.40% | Flow cytometry | Podwyszynska et al. 2021 |
| Highbush | *Cyanococcus* | 4*x* | Leaf from tissue culture | Colchicine | 250 | Culture stem segment on shoot induction medium with antimitotic agent | 7.8% to 11% | Flow cytometry | Marangelli et al. 2022 |
| *V. duclouxii* | *Eococcus* *sleumer* | 2*x* | Stems from tissue culture | Trifluralin | 60 | Immerse stem segments in sterile antimitotic agent solution | 8.33% | Flow cytometry | Lei et al. 2023 |

and crop species results in larger flowers, fruits, seeds, leaves, stems roots (Niazian and Nalousi 2020), higher accumulation of secondary metabolites (Madani et al. 2021; Salma et al. 2017), and improved adaptation to biotic and abiotic stresses (Omere et al. 2023). Large populations of treated plants are needed to uncover mutants with beneficial phenotypes. Although artificial induction of polyploidy has been performed in multiple *Vaccinium* species, the main application has focused on eliminating ploidy differences to allow allele introgression. The full value of ploidy-induced blueberries has not been determined. In addition to cultivars for commercial fruit production, market demand for ornamental blueberries for home gardening and landscaping is increasing. Ploidy induction has created many new variations in the ornamental plant industry (Niazian and Nalousi 2020). It is expected that further work with ploidy induction in blueberries will create novel characteristics useful for both commercial and ornamental blueberries.

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