Variation in Ploidy and Nuclear DNA Content in Blueberry

Hamidou F. Sakhanokho[1], M. Nurul Islam-Faridi[2], Timothy A. Rinehart[1], Stephen J. Stringer[1]
[1]USDA-ARS, Southern Horticultural Laboratory, Poplarville, MS 39470
[2]US Forest Service, Tree Molecular Cytogenetics Laboratory, Texas A&M University, College Station ,TX 77843

Abstract

Commercial blueberry production in the U.S. relies on cultivars derived from combinations of different blueberry species. Interspecific hybridization is used to improve overall fruit quality as well as abiotic and biotic tolerance to expand the range of blueberry production. Knowing both ploidy and nuclear genome size can help in determining the evolutionary relationships among plant species and also lead to better breeding strategies for the development and selection of new cultivars. Blueberry species have many different genome sizes, presumably due to differing ploidy levels and/or DNA content. In some species, such as *V. pallidum* Aiton, there are different ploidy series available for breeding, further complicating the observed DNA content in elite selections. In this study we determined DNA content in 79 blueberry taxa including 11 species and 68 hybrid cultivars and selections. Chromosome spread was performed in selected taxa to verify presumptive ploidy levels or determine for the first time the chromosome numbers of some species or selections. Diploid, tetraploid, pentaploid, and hexaploid blueberry species and accessions were included in this study. Holoploid genome size (2C DNA) was significantly correlated with ploidy, or presumed chromosome number (2n), but only weakly correlated with monoploid genome size (Cx).

Introduction

Commercial blueberry cultivar development relied mostly on blueberry species in the section Cyanococcus including *V. angustifolium* Aiton (lowbush blueberry, 2*n* =4 *x*= 48) *V. corymbosum* L. (highbush blueberry, 2*n* = 4*x* = 48), *V. darrowii* (2*n* =2*x* = 24, and *V. virgatum* Aiton (syn. *V. ashei* Reade) (rabbiteye blueberry, 2*n* = 6*x* =72). Additional section Cyanococcus species such as *V. constablaei* A. Gray (2*n* = 6*x* = 72), *V. elliottii* Chapm.(2*n* = 2*x* = 24), *V. myrtilloides* Michx. (2*n* = 2*x* = 24), *V. pallidum* Aiton (2*n* = 2*x* = 24; 2*n* = 4*x* = 48), *V. tenellum* Aiton (2*n* = 2*x* = 24), and *V. boreale* V. Hall & Aalders (2*n* = 2*x* = 24) have also contributed to the development of blueberry cultivars. Knowing both ploidy and nuclear genome size can help in determining the evolutionary relationships among plant species and also lead to better breeding strategies for the development and selection of new cultivars. The objective of this study was to determine the nuclear DNA content of a large and diverse set of diploid, tetraploid, pentaploid, and hexaploid blueberry species and accessions and discuss the results in relation to available chromosomal data.

Table 1. Holoploid (2C DNA) and monoploid (1Cx-value) genome size of diploid, tetraploid, and hexaploid blueberries.

Ploidy	2C DNA (pg)			1Cx-DNA (pg)		
	Mean ± SD*	Median	Min-Max	Mean ± SD*	Median	Min-Max
Diploid	1.14 ± 0.09 c	1.13	1.04 - 1.31	0.57 ± 0.04 a	0.57	0.52 - 0.66
Tetraploid	2.25 + 0.15 b	2.24	2.01 - 2.58	0.56 + 0.04 a	0.56	0.50 - 0.65
Hexaploid	3.61 + 0.43 a	3.70	2.46 - 4.10	0.60 + 0.07 a	0.62	0.41 -0.68

*Means within the same column followed by the same letter are not significantly different according to Tukey's Studentized Range test (P = 0.05).

Table 2. Pearson correlation coefficient between chromosome number (2n) and nuclear DNA content of 74 blueberry (*Vaccinium* spp.) taxa.

	Chromosome number (2n)	2C DNA	1Cx-DNA
Chromosome number (2n)		0.95***	0.23*
2C DNA			0.50***

*Significant at P = 0.05; ***Significant at P = 0.0001.

Material and Methods

Seventy nine blueberry taxa including 11 species and 68 hybrid cultivars or selections. Among these taxa are southern highbush, northern highbush, low bush, rabitteye, and half high blueberries. Plants were collected from various sources including the USDA Germplasm Resources Information Network (GRIN), commercial nurseries, and USDA-ARS research station in Poplarville, Mississippi. To determine nuclear DNA content, propidium iodide (PI)-stained nuclei were analyzed using a BD Accuri C6 flow cytometer and a BD Accuri C6 software version 1.0.264.21 (BD BioSciences, Ann Arbor, MI). Standards used: *Glycine max* (cv 'Polonka',2.50 pg 2C) used for diploid taxa and *Zea mays* (CE-777, 5.43 pg 2C) for tetraploid, pentaploid, and hexaploid. Chromosome spread was accomplished following the method of Sakhanokho and Islam-Faridi (2013) with minor modifications. Actively growing root tips about 1.5 cm long were harvested from plants growing in potting soil in a greenhouse and mmediately transferred in an aqueous solution of 0.04% hydroxyquinoline for 3 h at room temperature in the dark. Roots were fixed in 4:1 ethanol:glacial: acetic acid for 10 min to arrest cell division at metaphase. After, roots were transferred to fresh 4:1 ethanol:glacial: acetic acid solution before storage or use. To prepare slides, fixative was removed by washing roots with ddH_2O, and enzyme treatment, chromosome spread, slide scanning, and imaging were performed.

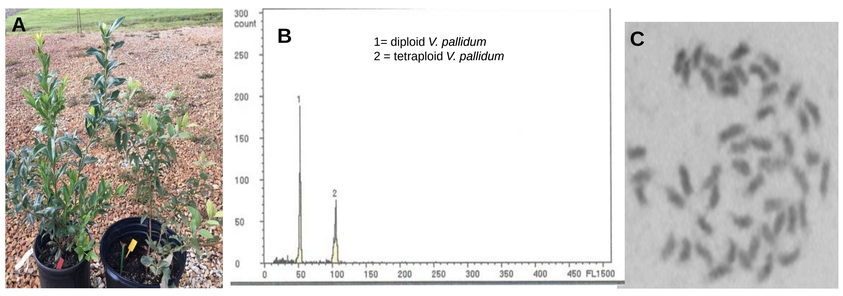

Fig. 1. (A): Two cytotypes of *Vaccinium pallidum*. The diploid (2*n* = 2*x* =24) on the right and the tetraploid (2*n* = 4*x* = 48) on the left are difficult to distinguish morphologically. (B): Histogram representing the two *V. pallidum* cytotypes. (C): Chromosome number of tetraploid (2*n* = 4*x* = 48) *V. pallidum*.

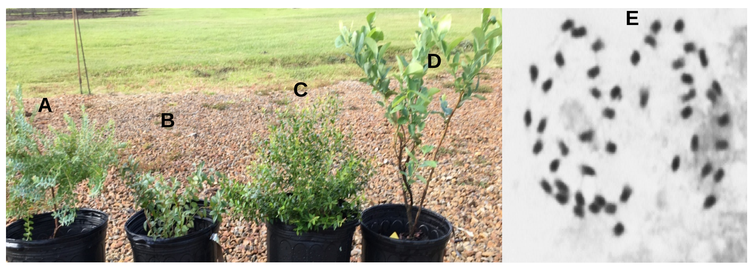

Fig.2. In general, diploid blueberries such as *V. darrowii* (A) tend to have smaller leaf size than tetraploids and hexaploids (D), but morphology is not always a reliable indicator of ploidy. Both *Vaccinium pensylvanicum* (B) and MS 775 (C) have small leaf sizes, but they are both tetraploid (2*n* = 4*x* = 48). The chromosome numbers of these two taxa were determined for the first time in this study. (E): Chromosome spread for *V. pensylvanicum* (E).

Results and Discussion

The absolute nuclear DNA content (2C-value) of 79 blueberry taxa was determined, and this is the first report on nuclear DNA content (2C DNA) and their derived monoploid (1Cx-DNA) genome sizes. Absolute genome sizes varied significantly (P = 0.05) among ploidy levels, with 2C-value means of 1.14 pg for diploid, 2.25 pg for tetraploid, and 3.61 pg for hexaploid taxa (Table 1). Clearly, there was a strong relationship between ploidy level and nuclear DNA content as increased ploidy resulted in increased nuclear DNA content (Table 2). This is particularly evident in *V. pallidum*, the only species in this study with two cytotypes and whose teraploid form has a nuclear DNA content ~2-fold larger than that of its diploid counterpart (Fig. 1).

Conclusion

Holoploid genome sizes and their derived monoploid genome sizes were determined for 79 diploid, tetraploid, pentaploid, and hexaploid blueberry taxa. Nuclear DNA content has been correlated with various plant characteristics such as growth indices, climatic factors, and latitudinal and elevational distributions (Ohri and Khoshoo, 1986; Wakamiya et al., 1993). Therefore, variability for nuclear DNA content could be helpful in the selection of plant materials for blueberry expansion and production beyond its traditional North American areas of cultivation. Also, chromosome numbers were determined for the first time for one species, *V. pensylvanicum* and one selection, MS 775. Both taxa were tetraploid (2*n* = 4*x* = 48) despite their diploid-like morphology (Fig. 2B, 2C, 2E).

References

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