HortScience 55(1):4–7. 2020. https://doi.org/10.21273/HORTSCI14274-19

Ploidy Level in American Persimmon (*Diospyros virginiana*) Cultivars

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Additional index words. fruit-set, germplasm, polyploidy, tree-fruit

***Abstract*. The American persimmon (*Diospyros virginiana*) is a slow-growing, moderately sized tree fruit native to the forests of Kentucky. This tree fruit is in the early stages of commercial production with many cultivars selected from the wild. Small orchards of commercially available cultivars are planted in Kentucky. Persimmons are normally dioecious, and female trees require cross-pollination to produce fruit. There are two races of persimmon: the tetraploid (60-chromosome) race is centered in the southern Appalachian region, while the hexaploid (90-chromosome) race generally occupies a range north and west of the tetraploid range. These ranges overlap in Kentucky. Because the ranges overlap, cross-pollination may cause sexual incompatibility, resulting in pollination without fertilization, and therefore seedless fruits of poor quality. The objective of this study was to assess the ploidy level of commercially available American persimmon cultivars and native Kentucky persimmon populations. Leaf samples were collected from 45 cultivars and advanced selections, as well as 45 trees from native populations in Bullitt, Barren, and Franklin Counties. Flow cytometer analysis showed that only four of the selected cultivars were from the tetraploid race: Ennis Seedless, Weeping, Sugar Bear, and SFES; the remaining cultivars were from the hexaploid race. Both hexaploid and tetraploid American persimmon trees were identified in the populations sampled in the Bullitt County locations, but only tetraploid races were found in Franklin and Barren Counties. Because pollen from native trees could result in seedless fruit formation of poor quality when native seedlings are used as pollinizers in commercial production of American persimmon, ploidy level of seedlings needs to be considered.**

The American persimmon (*Diospyros virginiana*) is a slow-growing, moderately sized tree-fruit native to the forests of the southeastern and midwestern United States (Nesom, 2018) and is in the early stages of commercial orchard production (Skallerup, 1953). The American persimmon is tolerant to a range of soil conditions and trees have a long productive life of more than 20 years (Goodell, 1982; McDaniel, 1973a, 1973b; Troop, 1895). Overall, American persimmon is well adapted to low input culture by small growers with few pests and diseases (Kaiser and Ernst, 2017; Nesom, 2018; Crandall and Baker, 1950; Kaiser and Ernst, 2017).

The American persimmon matures over a long ripening season, and trees have a long, productive life (McDaniel, 1973a, 1973b). The golden-orange fruit are sweet when fully ripe and astringency is reduced. A common misconception is that the persimmon fruit is not edible and less astringent until it has frozen, but this is not the case with most cultivars (Troop, 1895). The fruit is well suited for processing and preservation by freezing. Its pulp can be used in pudding, cookies, cake, custard, and ice cream (Briand, 2005; Goodell, 1982). Ground persimmon seeds have been used as a substitute for coffee (Briand, 2005) and tea made from the leaves of persimmon has antioxidant properties (Kobayashi et al., 2017). Flowers are a significant nectar source for bees (Troop and Hadley, 1895). The most widely cultivated persimmon species across the world is *Diospyros kaki,* the Oriental or Japanese persimmon, with its large, light yellow-orange to dark red-orange nonastringent fruit; however, this species is not well adapted to the Kentucky climate, and cultivars do not share a similar genetic background (Ames, 2010; Raddová et al., 2012).

American persimmon improvement began in the late 19th century with the work of Dr. James Troop at Purdue University (Plumb, 1896; Troop, 1895; Troop and Hadley, 1895). The first named American persimmon cultivar was selected out of the wild in Illinois in 1880 (Miller, 1894). This original cultivar, Early Golden, has served as the female parent of many of the cultivars developed throughout the 20th century. Professor J.C. McDaniel (1973a) from the University of Illinois had a strong interest in persimmon and was responsible for selecting the cultivars John Rick and Florence. James Claypool continued breeding American persimmon in the 1970s (Goodell, 1982). Over the course of 20 years, Claypool evaluated more than 2000 trees and kept extensive orchard records describing the characteristics of each tree in his breeding project (Jerry Lehman, personal communication). Some of the Claypool selections are commercially available from nurseries.

Troop and Hadley (1895) reported that American persimmons are normally dioecious and require cross-pollination with another cultivar to produce fruit. At least three American persimmon cultivars, Early Golden, Gatterson, and Killen, are usually pistillate (female-flower) trees; however, some branchlets of staminate (male) flowers occur and can furnish pollen for fruit set (McDaniel, 1973a, 1973b).

There are two races of American persimmon: a tetraploid (60-chromosome) race is centered in the southern Appalachian Mountains and adjacent areas and a hexaploid (90-chromosome) race occupies the range north and west of the tetraploid range (Fig. 1). Using light microscopy observation of root tips stained with crystal violet, hexaploid genotypes were identified for plants generated from seeds collected from trees in Delaware, Georgia, Iowa, Kansas, Louisiana, Mississippi, Missouri, Ohio, Pennsylvania, Texas, Virginia, and West Virginia, whereas tetraploid genotypes were identified from sites in Florida, Georgia, Kentucky, North Carolina, South Carolina, and Tennessee (Baldwin and Culp, 1941). This study included only single tree samples of seeds for most states, although several additional trees were sampled in Georgia, Tennessee, Texas, and Virginia. The ranges for persimmons with these two ploidy levels overlap in Kentucky (Baldwin and Culp, 1941).

Most cultivated American persimmon cultivars are considered likely to be the

Received for publication 6 June 2019. Accepted for publication 16 Oct. 2019.
Published online 16 December 2019.
Kentucky State University Agricultural Experiment Station Publication # KYSU-000061.
This research was supported by U.S. Department of Agriculture, National Institute of Food and Agriculture, Grant 2010-38821-21522 with Kentucky State University.
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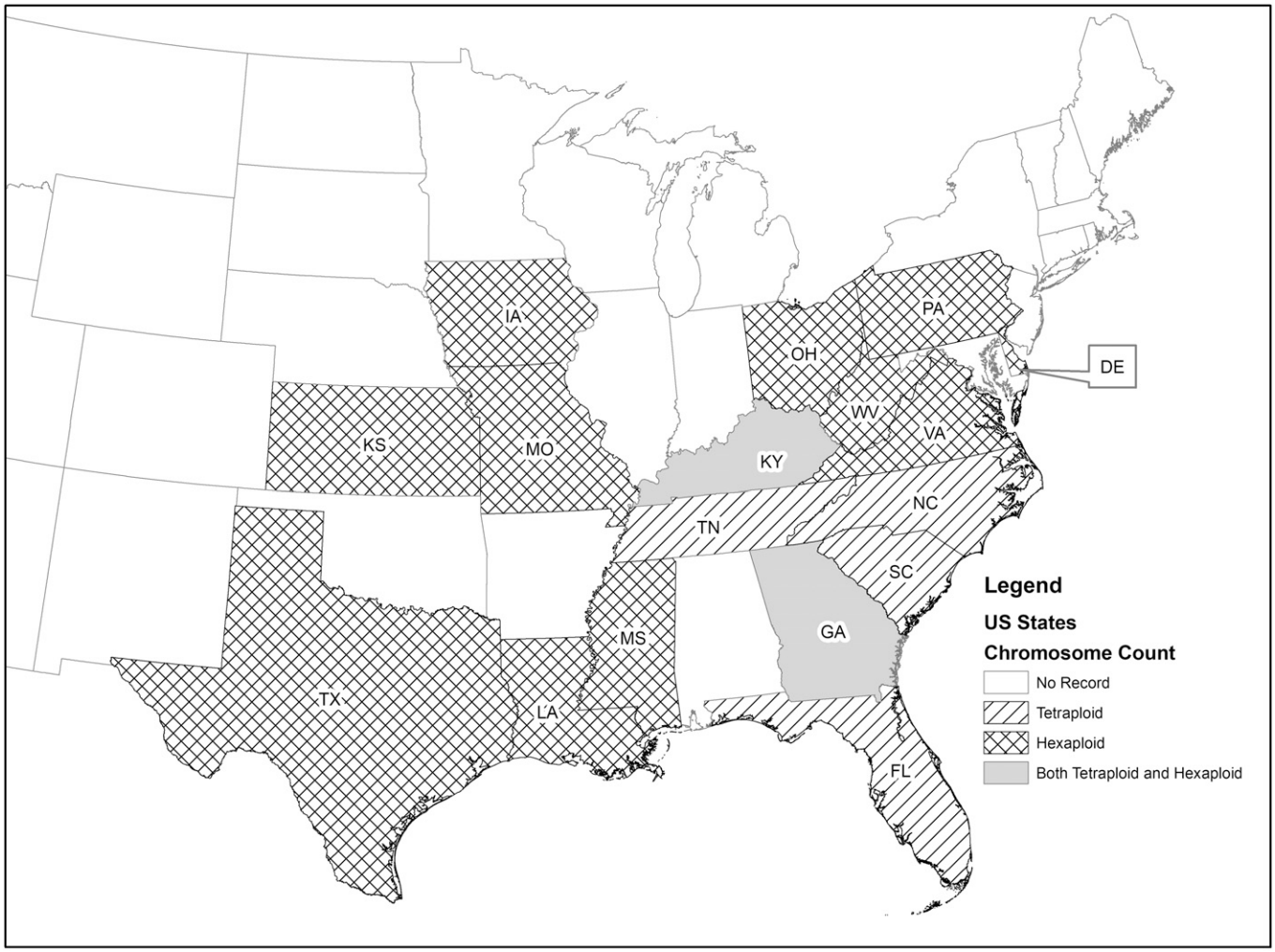

Fig. 1. Reported tetraploid and hexaploid American persimmon populations based on the data of Baldwin and Culp (1941) and data in the current study.

northern hexaploid race; however, there has not been a thorough examination of ploidy level for commercially available cultivars (Choi et al., 2003; McDaniel, 1973a; Nissen and Roberts, 2015). This study is the first to examine the contribution of the tetraploid race toward the commercially available cultivar base. Because both ranges overlap, pollen from a different race with a different pollen ploidy number would result in sexual incompatibility, resulting in pollination without fertilization and the production of seedless fruits. This could allow for the development of orchard production with staminate tetraploid race trees with hexaploid pistillate cultivars to produce seedless fruit. McDaniel (1973a) noted that trees of 'Wabash', a hexaploid, ripen seedless fruit when pollinated by tetraploid males. McDaniel (1973b) noted that seedless fruits of American persimmon do not have as edible a quality because seeded fruits on the same clone and seedless clones are consistently inferior in sweetness to the best seeded cultivars. The objective of this study was to assess the ploidy level of commercially available American persimmon cultivars and native Kentucky persimmon populations to compare tetraploid and hexaploid contributions to this germplasm for grower knowledge.

Materials and Methods

Plant material. Leaf samples, the youngest three terminal leaves, were collected from 46 cultivars and advanced selections, as well as 45 trees from native populations in Bullitt, Barren, and Franklin Counties. Leaf samples were collected in July 2017 from the American persimmon cultivars Prairie Star, Prairie Sun, Mohler, Early Golden, Garretson, and Meader trees located at the Harold R. Benson Research and Demonstration Farm in Frankfort, Kentucky (Table 1). Leaf samples from additional cultivars or numbered selections were obtained from the budwood mother blocks at England's Orchard and Nursery in McKee, Kentucky. Leaf samples from at least 10 trees at each site were also collected in June and July 2017 from wild American persimmon populations from Bullitt, Barren, and Franklin Counties in Kentucky at sites within one mile of the Global Positioning System coordinates listed in Table 2. Additionally, in October 2018, the average fruit weight and number of seeds per fruit were evaluated for at least 10 fruit from six trees in the area where leaves were previously sampled in Bullitt County, Kentucky (Table 3).

Ploidy level determination. Ploidy analysis was conducted by Plant Cytometry Services (Berkenhof 37, 6941 ZR, Didam, The Netherlands; https://plantcytometry.com/) based on the method of Arumuganathan and Earle (1991). Briefly, leaf material (1–2 cm^2, 50–100 mg) was chopped with a sharp razor blade in an ice-cold buffer in a plastic petri dish. The DNA buffer consisted of 0.1% 4′,6-diamidino-2-phenylindole (DAPI) with 0.1% dithiothreitol and 1% polyvinyl pyrrolidone 10. After chopping, the buffer (≈2 mL), containing cell constituents and large tissue remnants, was passed through a nylon filter (50-µm mesh size). Nuclei were isolated from the leaf samples and stained with the DNA specific dye DAPI. The solution with stained nuclei was sent through a flow cytometer (CyFlow Space; Sysmex Partec GmbH, Görlitz, Germany) with a high-power ultraviolet light–emitting diode (365 nm). Ploidy levels were determined by comparing them with standards *Pachysandra terminalis* Sieb. & Zucc. and *Vinca minor* L.

Table 1. Ploidy level for American persimmon cultivars.

Selection	Notes	Ploidy
100-29 Deer Magnet	Jerry Lehman cross	Hexaploid
100-46	Jerry Lehman cross	Hexaploid
Brace #1 Seedless	Selected by Lucky Pittman of Western Kentucky	Hexaploid
Dollywood	D-128 James Claypool cross	Hexaploid
Early Golden	Propagated since the 1890s, from Illinois.	Hexaploid
Elmo (A-118)	James Claypool cross	Hexaploid
Ennis Seedless	Indiana seedling	Tetraploid
F-100	Male selection	Hexaploid
F-34	James Claypool cross	Hexaploid
F-62	James Claypool cross	Hexaploid
Garretson	Seedling of Early Golden	Hexaploid
Golden Supreme	Very old cultivar, unknown background	Hexaploid
H-118 Early Jewel	James Claypool cross	Hexaploid
H-120	James Claypool cross	Hexaploid
H-55A	James Claypool cross	Hexaploid
H-63A Claypool	James Claypool cross	Hexaploid
H-63A Seedling Osage	Seedling from Wes Rice	Hexaploid
I-115	James Claypool cross	Hexaploid
J-127	James Claypool cross	Hexaploid
J-59 Claypool	James Claypool cross	Hexaploid
K1	James Claypool cross	Hexaploid
K2	James Claypool cross	Hexaploid
K6 Claypool	James Claypool cross	Hexaploid
Keener	Luther Burbank cross	Hexaploid
Keener X	Seedling was thought to be 120 chromosomes	Hexaploid
Korp	Bred by George Slate	Hexaploid
Meader	Bred by Corwin Meader of New York	Hexaploid
Mohler	Unknown background	Hexaploid
NC-10	Bred by Douglas Campbell of Southern Ontario	Hexaploid
Prairie Star	James Claypool Cross	Hexaploid
Prairie Sun	James Claypool Cross	Hexaploid
Prok	Bred by George Slate and propagated by John Gordon Jr. of upper New York	Hexaploid
R12T1	Seedling from Jerry Lehman from Terre Haute, IN	Hexaploid
SFES	Selected by Lucky Pittman of Western Kentucky	Tetraploid
Souvenir	Imported in 2006 from Crimea, Ukraine	Hexaploid
Sugar Bear	Seedling from Madison County, Kentucky	Tetraploid
Super Sweet	Bred by George Slate	Hexaploid
Szukus	Bred by George Slate	Hexaploid
U-20A	Jerry Lehman cross	Hexaploid
Union Star	Seedling from Rhodelia, Kentucky from Fred Blankenship	Hexaploid
Weeping	Seedling from Kentucky	Tetraploid
Wonderful	Cross of Yates ×F-100 by Clifford England	Hexaploid
WS 19-10	Selected by Jerry Lehman	Hexaploid
WSA 8-10	Selected by Jerry Lehman	Hexaploid
Yates	Found by Ed Yates, but was mislabeled AKA Juhl (cv)	Hexaploid

Table 2. Ploidy level in wild American persimmon trees.

County	GPS coordinates	No. of trees sampled	No. of tetraploid trees	No. of hexaploid trees
Bullitt	37.903884, –85.628716	19	6	13
Barren	37.101909, –86.058753	21	21	0
Franklin	38.175547, –84.915906	5	5	0

GPS = Global Positioning System.

Table 3. Average fruit weights and seeds per fruit for 10 fruit each from six wild American persimmon trees, sampled in Bullitt County, Kentucky.

Tree no.	Avg fruit wt (g)	Avg no. of seeds per fruit	No. of seedless fruit
1	11.5 b[z]	3.0 cd	0
2	19.3 a	5.5 a	0
3	8.4 bc	4.5 abc	1
4	16.3 a	3.7 cd	1
5	6.3 c	1.7 d	0
6	11.5 b	5.0 ab	0

[z]Lowercase letters indicate means are significantly different by Fisher's least significant difference at $P = 0.05$.

Results and Discussion

Ploidy levels were determined for 45 American persimmon cultivars commercially available from nurseries. Only four selected cultivars were from the tetraploid race: Ennis Seedless, Weeping, Sugar Bear, and SFES (Table 1). Both hexaploid and tetraploid American persimmon trees were found in the populations sampled in the Bullitt County locations, but only tetraploid races were found in Franklin and Barren Counties (Table 2).

Most cultivated American persimmon cultivars were assumed to be the northern hexaploid race; however, a thorough examination of ploidy level for commercially available cultivars had not been conducted (Choi et al., 2003; McDaniel, 1973a; Nissen and Roberts, 2015). On the basis of the results of this study, it would appear that the hexaploid race has led to the selection of most commercial cultivars although a few cultivars were tetraploid selections. Kentucky is in the unique position to have both races represented in forests around the Commonwealth. There were both hexaploid and tetraploid American persimmon trees in the populations sampled in the Bullitt County locations, whereas only tetraploid races were found in Franklin and Barren Counties. Because the ranges overlap, pollen from a different race may cause sexual incompatibility, resulting in pollination without fertilization and the production of seedless fruits. This could allow for the development of orchard production with male tetraploid race

trees with hexaploid pistillate cultivars to produce seedless fruit. Many native trees in the forests of Kentucky are of the tetraploid race and grow near commercial plantings of persimmons, and thus pollen from a different race may cause sexual incompatibility and result in pollination without fertilization—and therefore production of seedless fruits of poor quality for consumption. Planting hexaploid female cultivars with male seedling pollinizer trees of the tetraploid race could produce seedless fruit of reduced quality, because some Kentucky forests could have hexaploid native male trees nearby, which could result in seed set of fruit in the commercial hexaploid cultivars. However, in 2018, the number of seeds per fruit was evaluated from at least 10 fruit from six trees in the previously sampled area in Bullitt County Kentucky. Average fruit weight ranged between 6.3 to 19.3 g on individual trees with a 12.2-g average fruit weight across all trees sampled (Table 3). Average number of seeds per fruit ranged from 1.7 to 5.5 seeds per fruit on individual trees sampled, with an average number of seed per fruit of 3.9 across all trees sampled. Only two fruit of 60 sampled were seedless. Apparently, although pollen from native trees may result in seedless fruit formation in Kentucky and native seedlings, pollen from nearby trees with the same ploidy level is usually sufficient for seed formation in fruit.

Over the course of 20+ years, James Claypool has evaluated more than 2000 trees and made a number of cultivar releases (Whitson, 2007). Analysis of leaf samples collected from 18 Claypool selections and 10 commercially available showed that all Claypool selections, including Dollywood, Elmo (A-118), F-34, F-62, H-118 Early Jewel, H-120, H-55a, H-63a Claypool, H-63a Osage Seedling, I-115, J-127, J-59 Claypool, K1, K2, K6, Prairie Star, and Prairie Sun were all from the hexaploid race; the non–Claypool-selected cultivars Early Golden, Garretson, Golden Supreme, Meader, Mohler, NC-10, and Yates were also from this race. Four non–Claypool-selected commercial cultivars were from the tetraploid race: Ennis Seedless, Weeping, SFES, and Sugar Bear. These data support the idea that the hexaploid race is also strongly represented in the high-quality material of the Claypool germplasm that was selected (Goodell, 1982). It remains to be seen whether high-quality tetraploid germplasm can be located to contribute toward American persimmon improvement.

In conclusion, this was the first comprehensive study to assess the ploidy level of commercially available American persimmon cultivars and native Kentucky persimmon populations. Flow cytometer analysis of the leaf samples showed that only four selected cultivars were from the tetraploid race: Ennis Seedless, Weeping, Sugar Bear, and SFES. These results support the concept that most cultivated American persimmon cultivars have been selected from the northern hexaploid race. Both hexaploid and tetraploid American persimmon trees were found in the populations sampled in the Bullitt County locations, but only tetraploid races were found in Franklin and Barren Counties. Because pollen from native trees could result in seedless fruit formation in Kentucky, native seedlings could serve as pollinizers in commercial production of American persimmon and have seedless fruit of reduced quality.

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