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Preservation and DNA Fingerprinting of the Historic Tidal Basin Cherries[1]

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Abstract

The historic Japanese flowering cherry trees planted around the Tidal Basin in Washington, DC, were given to the United States in 1912 as a gift from Japan, yet only a small portion of the original trees remain. In cooperation with the National Park Service, the U.S. National Arboretum clonally propagated a portion of these trees. DNA from these and other *P*. x *yedoensis* plants obtained from domestic commercial nurseries were compared using RAPD markers. Twenty-one 10-nucleotide primers yielded 80 repeatable bands that were used to assess genetic distances among the accessions. The genetic distances ranged from 0.65 to 1.0, with thirteen accessions identical at all loci tested. The most genetically dissimilar trees were *P*. x *yedoensis* accessions that were collected as seed in Japan. Accessions obtained from commercial nurseries including 'Afterglow', 'Akebono', and Yoshino were also dissimilar to the Tidal Basin trees. This study indicated that most of the older trees planted around the Tidal Basin are genetically very similar, but that variability in *P*. x *yedoensis* exists, especially in accessions collected as seed from Japan.

Index words: *Prunus* x *yedoensis*, ornamental flowering cherry, Yoshino cherry, genetic diversity, RAPD, germplasm, clonal propagation, IBA.

Significance to the Nursery Industry

Each spring, hundreds of thousands of visitors to the Tidal Basin in Washington, DC, are awestruck by the display of Yoshino cherry trees in flower. In addition to supporting the local tourist industry, these cherry trees have helped to increase the public's appreciation of and demand for flowering cherry cultivars, which has a direct impact on the nursery and landscape industry. A unique marketing opportunity could exist in the propagation and promotion of this historic germplasm by commercial nurseries.

Introduction

The historic Japanese flowering cherry trees planted around the Tidal Basin in Washington, DC, were given to the United States in 1912 as a gift from Japan in response to President and First Lady Taft's expressed interest in and fondness for the tree. Originally planted as a means to beautify the newly reclaimed mud flat later known as Potomac Park, the cherry trees have become a popular tourist attraction that draws hundreds of thousands of visitors each spring and inspired the popular annual Cherry Blossom Festival in Washington, DC.

The original gift from Japan consisted of 3,020 trees, comprised of 11 cultivars of *P. serrulata* and 1800 trees of the now famous Yoshino cherry. Yoshino cherry is the common name for *P.* x *yedoensis* Matsum., which is a hybrid of unknown origin, discovered cultivated in Japan in 1868 (3). The trees were planted in 1912 around the Tidal Basin, in Potomac Park, and elsewhere in Washington, DC. Over the next 10–15 years, as the plants grew and required thinning, some of the original trees were moved to other locations in DC, including the grounds of the Library of Congress, the Naval Observatory, and the Dalecarlia Water Treatment Plant. Unfortunately, Potomac Park has been subject to frequent floods since 1912, and most of the *P. serrulata* cultivars did not survive. Replacement trees have consisted primarily of

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P. x *yedoensis* (including 'Akebono') purchased or donated from commercial nurseries in the United States. It is estimated that fewer than 125 of the original trees remain (2).

A timely news article, inspired partly by retired National Arboretum botanist R.M. Jefferson and his campaign to preserve the historic *Prunus* germplasm, appeared in the Washington Post during the week of the peak bloom in 1997 (5). In response to this article, representatives from the National Park Service (part of the Department of the Interior) and the U.S. National Arboretum agreed to attempt to identify and preserve at least some of the remaining original germplasm.

The purpose of the research described here was to identify and clonally propagate some of the *P.* x *yedoensis* trees that were part of the original 1912 gift from Japan, and to assess the genetic variability of this germplasm using a DNA marker technique called Randomly Amplified Polymorphic DNA (RAPD).

Materials and Methods

Plant materials. Nine original *P.* x *yedoensis* trees were identified based on historic records from the National Park Service (Table 1). These trees were accessions that had originally been planted around the Tidal Basin, but subsequently transplanted to the grounds of the Library of Congress or the Dalecarlia Water Treatment Plant. The accessions labeled 'Chinda' and 'Taft' were clonally propagated plants made by R.M. Jefferson, obtained by the National Arboretum in 1980, of two trees at the Tidal Basin that were allegedly planted in 1912 by First Lady Taft and Viscountess Chinda of Japan. Nine additional trees were selected from the Tidal Basin based on apparent age of the trees compared to other specimens. Definitive identification of these trees as original is not possible because regular records of the plantings were not kept until 1933 when Potomac Park came under the auspices of the National Park Service. Thus, trees that were replaced in the first 20 years of the original planting would be difficult to distinguish from original trees based on visual observation of age alone, and could be mistaken for original trees.

Two relatively old trees that consistently bloom 7–10 days prior to the Tidal Basin trees and planted at the Inlet Bridge near the Tidal Basin were also selected for propagation and genetic analysis. Seven additional *P.* x *yedoensis* trees from a commercial nursery and from the collection at the U.S. National Arboretum were also used in the genetic analysis (Table 1).

Propagation. Cuttings from 13 of the Tidal Basin trees were taken in June 1997, and cuttings from the seven trees at the Dalecarlia site were taken in May 1998. Cuttings were stored in plastic bags and transported to the National Arboretum in a cooler. Rooting was accomplished using standard procedures. Briefly, 13–15 cm (5–6 in) long, semi-hardwood cuttings were wounded and dipped in Wood's Rooting Compound, 10,000 ppm indole-3-butyric acid (IBA) and 5,000 ppm α-naphthaleneacetic acid (NAA), diluted 15:1 (Earth Science Products, Corp, Wilsonville, OR) or Hormoroot B, 3000 ppm IBA (Rockland Chemical Co. Inc., West Caldwell, NJ). For the few very soft cuttings, Hormodin 1, 1000 ppm IBA (Merck and Co., Rahway, NJ) was used. Cuttings were then placed in a peat-perlite medium under an automatic misting system in the greenhouse.

Table 1. Origin of *P.* x *yedoensis* accessions used in this project.

Accession[z]	NA Number	Origin
L276	69513	Tidal Basin, Wash., DC
Q138	69514	Tidal Basin, Wash., DC
Q148	69518	Tidal Basin, Wash., DC
Q155	69517	Tidal Basin, Wash., DC
Q266	69511	Tidal Basin, Wash., DC
Q269	69512	Tidal Basin, Wash., DC
Q270	69507	Tidal Basin, Wash., DC
Q330	69516	Tidal Basin, Wash., DC
Q331	69515	Tidal Basin, Wash., DC
Q248	(destroyed)	Inlet Bridge near Tidal Basin, Wash., DC
AE242	69510	Inlet Bridge near Tidal Basin, Wash., DC
WT1*	69523	Dalecarlia Water Treatment Plant, Wash., DC
WT2*	69524	Dalecarlia Water Treatment Plant, Wash., DC
WT3*	69525	Dalecarlia Water Treatment Plant, Wash., DC
WT4*	69526	Dalecarlia Water Treatment Plant, Wash., DC
WT5*	69527	Dalecarlia Water Treatment Plant, Wash., DC
WT6*	69528	Dalecarlia Water Treatment Plant, Wash., DC
WT7*	69529	Dalecarlia Water Treatment Plant, Wash., DC
2nd and EC*	69509	Library of Congress, Wash., DC
2nd and Ind.*	69508	Library of Congress, Wash., DC
Taft	42007	US National Arboretum, Wash., DC (originally from Tidal Basin)
Chinda	42006	US National Arboretum, Wash., DC (originally from Tidal Basin)
Yoshino-JFS		J. Frank Schmidt and Son Co., Boring, OR
'Afterglow'		seedling cultivar of 'Akebono'. J. F. Schmidt Co., Boring, OR
'Akebono'-JFS		seedling cultivar of Yoshino. J. F. Schmidt Co., Boring, OR
'Akebono'-NA	55534	seedling cultivar of Yoshino. US Nat. Arboretum, Wash., DC
Honshu	58834	seed collected from Takamatsu Park, Morioka, Honshu, Japan
Hokkaido1	58839	seed collected from Onjushi Park, Kayabe-Gun, Hokkaido, Japan
Hokkaido2	58851	seed collected from Tokiwa Park, Asahikawa, Hokkaido, Japan

[z]An asterisk (*) indicates that this accession is documented as a tree from the original 1912 planting.

DNA extraction. Fresh, newly expanding leaves were collected, freeze-dried, and stored at –70C until used. For each sample, three to four leaves were placed in a lysing matrix (Bio101, Vista, CA) with 500 µl CTAB buffer and processed in a FastPrep FP120 machine (Bio101) on speed 4 for 12 seconds. The resulting homogenate was incubated at 65C for 15 minutes then extracted with 500 µl of chloroform:isoamyl alcohol 24:1. The DNA from this crude first extraction was then isolated using the QIAamp Tissue Kit (Qiagen, Inc., Valencia, CA). DNA purity and quantity was estimated by visual comparison with known standards on a 1% agarose gel. All samples were diluted to 10 ng/µl.

RAPD-PCR. PCR was performed in 25 µl volumes containing PCR buffer (20 mM NaCl, 50 mM Tris pH 9.0, 1% Triton X-100 [1]), 3 mM $MgCl_2$, 200 µM dNTP, 0.2 µM primer (primers with 70–90% G+C content were selected from UBC set 100/4; UBC Nucleic Acid-Protein Service Unit, Vancouver, British Columbia, Canada), 0.25 U of Taq DNA polymerase, and 10 ng DNA template. DNA amplification was carried out in a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT) programmed for 45 cycles of 30 sec at 95C; 30 sec at 48C and 45 sec at 72C. RAPD reactions were analyzed on 1.4% agarose TBE gels stained with ethidium bromide. Gels were visualized and documented using an AlphaImager 2000 (Alpha Innotech Corp., Alameda, CA). Reactions were repeated at least once to insure reproducibility of scored amplification products.

Data analysis: RAPD PCR amplification products were listed as discrete character states per accession (presence or absence of band). Similarity coefficients between each accession were calculated using the SIMQUAL program in NTSYS-pc, version 1.70 (4). These data were subjected to UPGMA cluster analysis using the SAHN program of NTSYS to generate a phenogram.

Results and Discussion

Propagation. Cuttings from all trees sampled rooted well, with an overall rooting percentage of 85%. Cuttings had an average of 5–6 well-formed thick roots when they were planted to soil mix. Losses occurred with the very soft cuttings due to a period of extremely warm temperatures early in the rooting period. Clonal propagules from 11 of the first set of 12 trees that were propagated in 1997 were currently 6–7 ft (2–2.5 m) tall and were planted to a nursery in early May 1999. They will be planted back to the Tidal Basin in 2000–2001. All trees propagated from one of the earlier blooming Inlet Bridge trees, AE242, were destroyed in 1998 due to virus infection. Propagules from the seven original trees at the Dalecarlia Water Treatment Plant that were propagated in 1998 will be planted to the nursery in spring 2000.

Genetic variability. Of the 29 primers that produced bands, eight were not used in this analysis because the products were not reproducible. The remaining 21 primers amplified a total of 80 discrete bands, 20 of which were monomorphic among all *P.* x *yedoensis* tested, and thus were not informative. The number of bands scored for each primer ranged from 1–8, with an average of 2.3 scorable bands per accession (Fig. 1). The relatively small number of bands per accession is likely due to the high annealing temperature that is used for PCR (48C vs 36C for most RAPD-PCR (6)), which leads to more stringent and probably more reproducible results.

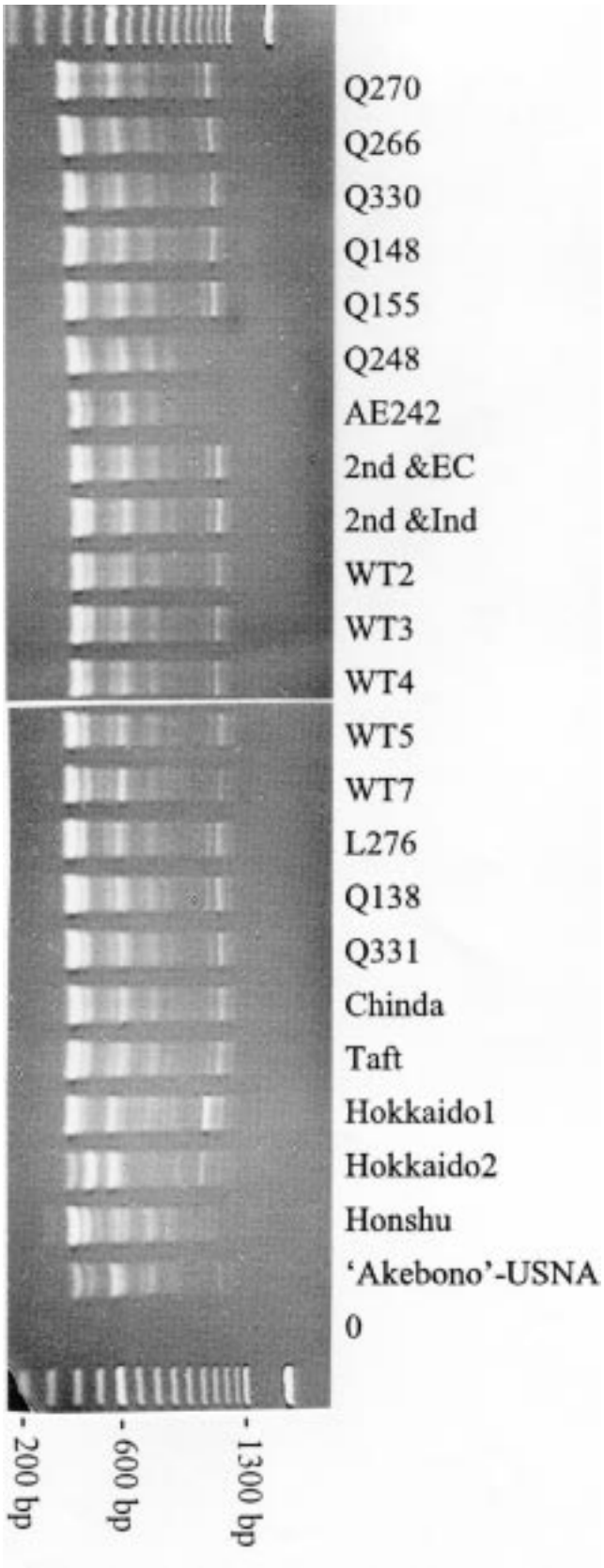

Fig. 1. Amplification of genomic DNA of *Prunus* x *yedoensis* from some of the trees used in this study by use of UBC primer #379. 100 bp size markers were run in the first and last lanes. Lane marked '0' contains the no-template control amplification. Slight differences in electrophoretic parameters account for the increased separation of products in the gel on the right.

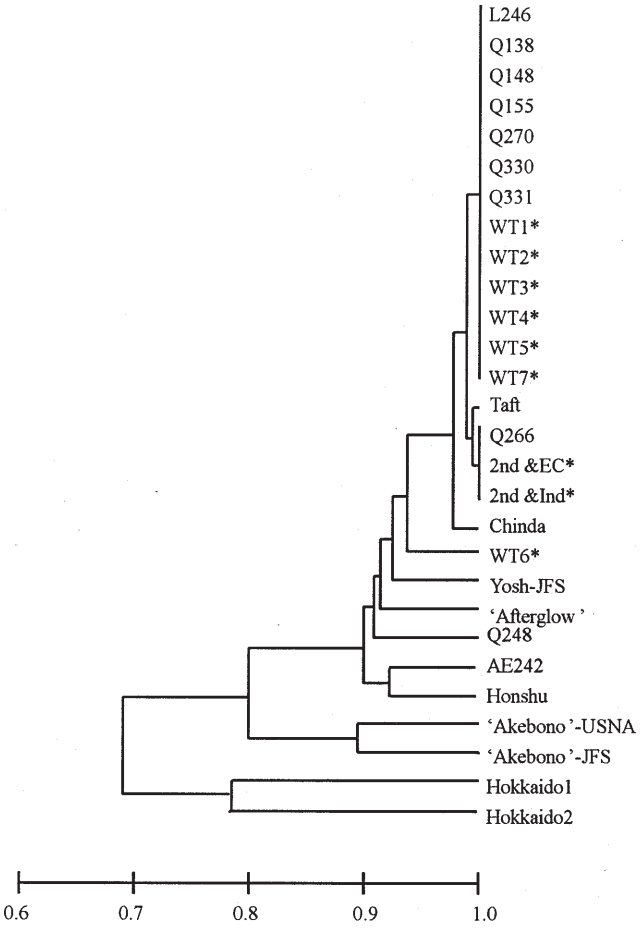

Fig. 2. Phenogram based on computed similarity data from RAPD analysis of various individual *Prunus* x *yedoensis* trees. Individuals clustered by a verticle line at the 1.0 tick mark are identical. Accession Q269 (Table 1) was not analyzed due to poor quality DNA.

The genetic distances between accessions ranged from 0.65 to 1.0. Thirteen of the accessions, including six of the nine that are known to be original germplasm, were identical at all loci tested (Fig. 2). Other accessions that are thought to be original trees, including Taft, Q266, 2nd&EC, and 2nd&Ind., had similarity values >0.99. The most dissimilar original tree was WT6, which had a similarity value of 0.93 compared to the other Tidal Basin trees. The two early-blooming trees planted at the Inlet Bridge were clearly different from the Tidal Basin trees, although are likely still *P.* x *yedoensis*, assuming correct classification of the other accessions.

The most genetically dissimilar trees were those trees that originated as seedlings of *P.* x *yedoensis*, including 'Afterglow', 'Akebono', and the three accessions grown from seed collected in Japan, most significantly those collected on the island of Hokkaido. The Yoshino tree obtained from a commercial nursery (Yoshino-JFS) was 92% similar to the Tidal Basin trees.

Although unrelated directly to the purpose of this study, an interesting discrepancy arose with the testing of 'Akebono' trees. Trees of 'Akebono' were originally planted in the 1920s as replacement trees for Tidal Basin trees. Thus, many of the older trees along the Tidal Basin are actually 'Akebono' and are distinguished from the other Yoshino trees by their light pink-colored bloom. Cultivar 'Akebono' is thought to be derived from seed of *P.* x *yedoensis* that was selected at the W.B. Clarke Nursery in California (2). Thus, the two 'Akebono' trees tested should, in theory, be identical at all loci, because this accession represents a clonally propagated cultivar. However, the two accessions have a similarity value of only 0.89 (Fig. 2). One cause for this discrepancy could be mislabelling or misidentification. The accession ''Akebono'-USNA' was obtained as a cutting in 1985 from a tree at the Supreme Court Building in Washington, DC. Perhaps this tree was misidentified or 'Akebono' was first released as a group of phenotypically similar seedlings rather than as a single clonally propagated selection. Further comparisons of these two accessions with other 'Akebono' trees obtained from different commercial sources and from the DC Tidal Basin would be a simple experiment that would help to clarify this interesting side story.

This study indicates that the older (putative original) trees planted around the Tidal Basin are genetically very similar to each other and to the documented original trees. Although preservation of historic germplasm is important, there are well-known risks associated with planting large numbers of accessions of limited genetic diversity. Based on this study, it is clear that variability in *P.* x *yedoensis* exists in accessions obtained commercially and abroad. Because quarantine restrictions make it difficult to import ornamental cherry seed or budwood, maximizing diversity in future Tidal Basin plantings could be realized by using commercially available *P.* x *yedoensis* and other ornamental cherry species such as *P. serrulata*, *P. takesimensis*, *P. subhirtella*, or *P. sargentii*.

Although most of the trees propagated for this project will be used as replacements for original trees around the Tidal Basin, one tree of each accession will be retained by the U.S. National Arboretum as part of the ornamental *Prunus* germplasm collection. These plants may be used as germplasm for ornamental cherry cultivar development. More significantly, the National Arboretum will serve as a long-term repository for this historic germplasm, from which propagations for replacement trees may be taken as necessary to ensure that at least part of the future plantings around the Tidal Basin reflect this genetic heritage and the goodwill it symbolizes.

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