Genetic Diversity of Japanese Strawberry Species Based on Microsatellite Markers

W. Njuguna
Oregon State University
Department of Horticulture
ALS 4017, Corvallis, Oregon, 97331
USA

C. Richards
United States Department of Agriculture
(AUSDA), Agricultural Research Service
(ARS), National Center for Genetic Resources Preservation (NCGRP)
1111 South Mason Street, Fort Collins
Colorado, 80521-4500
USA

T.M. Davis
Department of Biological Sciences
Rudman Hall, University of New Hampshire
Durham, New Hampshire, 03824
USA

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Abstract

The United States Department of Agriculture (USDA) - Agricultural Research Service (ARS) - National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, is a genebank that preserves strawberry genetic resources. The *Fragaria* L. collection consists of more than 1700 accessions of 17 species from 37 countries. In 2004, Asian diploid species, *F. iinumae* Makino and *F. nipponica* Makino, were collected during an expedition to Hokkaido, Japan. An ancestor of *F. iinumae* may be a progenitor of the “B” genome for octoploid strawberry species. The objective of this study was to evaluate the diversity of these two species using microsatellite markers. We report preliminary results obtained from SSR analysis of 139 accessions based on 20 SSRs. A higher genetic diversity was observed in *F. nipponica*, which tends to be cross pollinated, than in *F. iinumae*, which tends to be self-pollinated. This study identified putative hybrids between *F. iinumae* and *F. nipponica* as well as an unexpected octoploid accession from Hokkaido, Japan. The hybrid nature of these accessions and the possible source of this octoploid accession will be further evaluated using chloroplast and nuclear markers as well as morphological traits.

INTRODUCTION

The genus *Fragaria* contains approximately twenty species with variable ploidy levels and a base chromosome number of seven. This genus contains naturally occurring diploids (2n = 2× = 14), tetraploids (2n = 4× = 28), sterile pentaploids (2n = 5× = 35), one hexaploid (2n = 6× = 42), octoploids (2n = 8× = 56) and one decaploid (2n = 10× = 70) (Hummer et al., 2008). The cultivated strawberry, *F. ×ananassa* Duchesne ex Rozier, is an octoploid species and a commercially important perennial fruit crop (Hancock, 1999). Most *Fragaria* diploids, tetraploids and the decaploid are confined to Asia which is a center of diversity for *Fragaria* – not the center of origin. *Fragaria* is an ancient species that is a part of the Arcto-Tertiary Flora (Staudt, 1999). Several species from around the Sea of Japan include *F. iturupensis* Staudt (decaploid) found on Iturup Island, *F. mandshurica* (diploid) found on the continental Russian Far East to north Korea, *F. orientalis* (tetraploid) found along the Amur Valley and into China, *F. iinumae* Makino (diploid) in Honshu and Hokkaido, Japan and *F. nipponica* Makino (diploid) found in Honshu and Hokkaido Japan, Sakhalin, Russia and Kurils (Staudt, 2005; Staudt and

Kim.Hummer@ars.usda.gov
Olbricht, 2008). Wild ancestors of domesticated species, such as strawberries, provide reservoirs of genetic variation for crop improvement. The US strawberry genebank is located at the NCGR in Corvallis, Oregon. It preserves diverse strawberry genetic resources of more than 1700 accessions from 17 species and 37 countries. The study of the relationships among and within the wild strawberry species could provide insight into the origin of the cultivated species. Asian diploids are of special interest due to the rich distribution of species on the islands around the Sea of Japan, and the potential that their ancestors could be directly linked to the development of North American octoploid strawberry species, as Staudt (1999) suggests.

Microsatellite or simple sequence repeat (SSR) markers are stretches of tandemly repeated di-, tri-, or tetra-nucleotide DNA motifs that are highly polymorphic, reproducible, codominant, and multiallelic (Powell et al., 1996). These characteristics make them a preferred tool for molecular analysis (Hokanson et al., 2001; Tian-Ming et al., 2007). The utility of microsatellites in studying genetic diversity at the interspecific level has been facilitated by the high transferability of SSR primer sequences among species in various crops. A particularly high level of cross species transferability within the *Fragaria* genus has been reported (Bassil et al., 2006b; Monfort et al., 2006).

The objective of this study was to assess the genetic diversity of two wild Asian diploid species, *F. iinumae* and *F. nipponica* using microsatellite markers.

**MATERIALS AND METHODS**

Seeds and runner plants from *F. iinumae* and *F. nipponica* plants were collected from 12 and 10 locations, respectively, across Hokkaido, Japan in July 2004 (Hummer et al., 2006). Seeds were germinated and grown in greenhouses at the NCGR-Corvallis. 144 seedlings are being evaluated. Two *F. iinumae* clones, (PI 551751 and PI 616505) and one *F. nipponica* (PI 616506) from Honshu were used as outgroups.

DNA was extracted from actively-growing leaves using the Puregene kit (Genta Systems Inc. Minneapolis, Minn). Amplification and polymorphism of 82 SSRs from *Fragaria* were evaluated in 8 *F. iinumae* and 8 *F. nipponica* by 3% agarose gel electrophoresis. Polymorphism was verified in the correctly sized fragments and polymorphic SSRs were selected after preliminary fragment analysis on a Beckman CEQ 8000 genetic analyzer (Beckman Coulter Inc., Fullerton, CA).

In this preliminary study we report analysis of 139 accessions using 20 SSRs. 16 of these have been previously published (UAFv7648, UAFv8204, UAFv8936, UFFa 01H05, UFFa 02A03, UFFa 02G01, UFFa 03D11, UFFa 09B11, UFFa 14F08, UFFa 16H07, UFFa 19B10, FAC-001a, FAC-008, FAC-011, FAC-012 and ARSFL-19) (Bassil et al., 2006a, b; Lewers et al., 2005) while 4 are new (UFFf-1B07 UFFf 2-H12 UFFf 4-B12 UFFf 5-G02). PCR was performed in a 15 µl total reaction volume containing: 1 x PCR buffer, 2 mM MgCl₂, 0.2 mM each dNTP, 10 µM each primer, 0.05 U of Biolase enzyme (Bioline USA Inc., Randolph, Mass.) and 4.5 ng of DNA template. Allele sizing and visualization was performed using the fragment analysis module of the CEQ 8000 software. PowerMarker version 3.25 (Liu and Muse, 2004) was used to calculate genetic similarity based on the proportion of shared alleles, *Dsa*. Neighbor Joining (NJ) algorithm was used for cluster analysis and MEGA version 3.1 (Kumar et al., 2004) was used to generate the dendrograms.

**RESULTS AND DISCUSSION**

50 of the tested 82 SSRs amplified expected size fragments. 22 primers were polymorphic between and within the two species: 5 were genomic and the remaining were expressed sequence tagged (EST) SSRs. 2 primers and 4 accessions were omitted from this preliminary analysis due to missing values.

A high genetic diversity was observed in the outcrossing *F. nipponica* compared to self-pollinating *F. iinumae* as seen from the heterozygosity values (H) (0.4071 vs. 0.1336, respectively) and the number of alleles/locus (10.6 vs. 7.3, respectively). Outcrossing species tend to be more genetically variable than self-pollinating species.
NJ analysis separated the two species into two distinct clusters (Fig. 1). Putative hybrids were identified as accessions that did not group with either of the two major clusters. Possible hybrids will be further evaluated using chloroplast and nuclear genome markers and morphological traits. CFRA 1860.001 an accession that contained more than two alleles in the SSR loci examined was found to be an octoploid from flow cytometry. This octoploid accession will be further analyzed to determine its relationship to other *Fragaria* species.

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**Literature Cited**


Fig. 1. NJ cluster analysis of 139 accessions based on the proportion of shared allele distance using 20 SSRs. The putative hybrids 1861.003, 1861.001 J24, 1865.004 and 1868.004 did not group with the two major species groups: *F. iinumae* and *F. nipponica*. Two *F. iinumae* (PI 551751 and PI 616505) and one *F. nipponica* accession (PI 616506) each from Honshu were used as outgroups.