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Interspecific hybridization of diploids and octoploids in strawberry

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ABSTRACT

Interspecific crosses were performed among accessions of the wild strawberry species *F. vesca* and *F. nilgerrensis* and the cultivated strawberry, *F.* × *ananassa*, following reciprocal crossing. Crosses between both *F. vesca* species 'Hawaii-30' and 'UC-01' were successful regardless of the presence of reciprocal crossing, while crosses with 'Whiteberry' of the *F. nilgerrensis* species were not successful. In particular, intraspecific hybridization of 'Hawaii-30' and 'UC-01' in the *F. vesca* species produced a tetraploid plant. Although octoploid 'Akihime' did not cross with different species, it performed well with 'Hawaii-30' only through the parent seed. Progenies of interspecific hybridization between species of different ploidy, namely 'Hawaii-30' and 'Akihime', produced 12 pentaploid plants with an intermediate phenotype between the crossing parents and characterized by sterility. In addition, despite its poor hybridization, a cross-combination between 'Akihime' and 'Whiteberry' produced one pentaploid plant, which did not bloom.

1. Introduction

The genus *Fragaria* has a basic chromosome number of seven ($x = 7$) and a diverse ploidy level ranging from 2*x*, 4*x*, 5*x*, 6*x*, 8*x*–10*x*. There are 24 species of strawberry including 14 species of diploid strawberry that include *Fragaria vesca*, five tetraploid species including *F. orientalis* ($2n = 4x = 28$), the one hexaploid of *F. moschata* ($2n = 5x = 48$), three octoploid species, *F. chiloensis* ($2n = 8x = 56$), *F. virginiana*, and their hybrid *F.* × *ananassa*, and the one decaploid of *F. iturupensis* (Sargent et al., 2009). The total of 23 species includes the recently discovered *F.* × *bringhurstii staudt*, which is a pentaploid derived from interspecific hybridization between *F. vesca* and *F. chiloensis* (Hummer and Hancock, 2009).

Diploid species among the genus *Fragaria* show different characteristics, such as a white color and peach aroma (*F. nilgerrensis*), or no aroma (*F. daltoniana*), or an excellent aroma and sour taste (*F. iinumae*), a long calyx (*F. nipponica*), or a long neck (*F. nubicola*), or large raised achenes (*F. vesca*). Diploid species are used most frequently in genetic studies due to their various morphological characteristics (Staudt, 1989), the close interrelationship between species (Potter et al., 2000), their higher relative fertility compared to other species (Bors and Sullivan, 2005), a genome (164 Mb) sufficiently small to be compared with *Arabidopsis thaliana* (Akiyama et al., 2001), and a size small enough to grow in a lab. *F. orientalis*, a tetraploid species that is an autopolyploid of *F. mandschurica*, and *F. tibetica* is similar in characteristics to *F. pentaphylla* descendents. These tetraploid species are therefore believed to have been born from a natural chromosome polyploidization of diploid species. *F. moschata*, a hexaploid species found in Europe only, is dioecious. It is tall and vigorous, and produces few runners with a red fruit color and irregular fruit shape accompanied by a strong aroma (Hummer and Hancock, 2009). *F.* × *bringhurstii staudt*, a pentaploid species, was found in California, US for the first time, and was also recently found in Jilin, China. Despite its tall vigor and few runners, it is known to have no fertility (Lei et al., 2005).

F. × *ananassa*, an octoploid species, is the most important species commercially, with superior characteristics in terms of fruit shape, size, and aroma. *F. virginiana* is superior to *F. chiloensis* in crown number, fruit weight and sugar content, while *F. chiloensis* is superior to *F. virginiana* in runner production, peduncle length, and number of fruits, fruit color and hardness (Hancock et al., 2003). *F. iturupensis*, a decaploid species, is similar to *F. vesca*, a diploid species, in terms of fruit size and aroma (Hummer et al., 2009), but has upward sepals and large raised achenes.

Polyploids of the genus *Fragaria* are related to unreduced gametes or 2*n* gametes, which play an important role in increasing polyploidy. Bringhurst and Senanayake (1966) found the frequencies of giant pollen grain to be about 1% of the total, and more than 10% of the natural hybrids generated from two species resulted from unreduced gametes.

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Many studies on polyploidization from diploid to tetraploid, or the origins of triploid species, have been performed in potato (Wilkinson et al., 1995; Heleen et al., 1998) and banana (Ramanna and Jacobsen, 2003), but in the strawberry, the relations between diploids and different polyploids is not clear, despite extensive phylogenetic analyses (Sargent et al., 2004).

Intraspecific crosses of *F.* × *ananassa* have been extensively used to develop new cultivars with improved agronomic traits. However, this species is susceptible to fungal diseases and bacterial pathogens. On the other hand, *F. vesca* species are valuable sources of genetic determinants for disease resistance and stress tolerance (Marta et al., 2004). For this reason, hybridization between these two species can have a high economic value.

However, interspecific hybridization between *Fragaria* species results in a variety of gametophytic incompatibility and compatibility according to the cross-combination. In particular, interspecific hybridization between individuals with different ploidy level can hinder or prevent gene flow due to various internal and external barriers, and is often not successful (Marta et al., 2004). In addition, it is quite rare in nature and it is difficult to obtain normal plants through artificial hybridization(Nosrati et al., 2010). Although achenes are produced from normal interspecific hybridization, they often show poor vigor or die before reaching the adult stage, and do not germinate by apomixis (Li et al., 2000).

However, because the genome of *F. vesca* shows high genetic variability in comparison with other *Fragaria* species, *F. vesca* is more compatible than other *Fragaria* species (Hancock and Luby, 1993), and is more amenable to chromosome doubling by colchicine than other *Fragaria* species (Bors and Sullivan, 2005). This species has been widely used in interspecific hybridization, but the mechanisms behind the interference with interspecific hybridization are still unclear.

Despite these difficulties, attempts have been made to transfer valuable genes from wild species to cultivated octoploid species through colchicine treatment or using gametes with unreduced chromosome number. Crosses using *F. vesca* as bridges have also been attempted, but these methods showed little efficiency.

The purpose of the present study was to analyze the phenotypes of *F. vesca*, *F. nilgerrensis*, and *F.* × *ananassa* species, and to identify interspecific hybridization compatibility and changes in ploidy level, as well as phenotypic variation resulting from interspecific hybridization.

2. Materials and methods

2.1. Plant material

F. vesca 'UC-01' and 'Hawaii-30', which are diploid species, were introduced from the US, while the *F. nilgerrensis* 'Whiteberry', diploid species and *F.* × *ananassa* 'Akihime', an octoploid species, were from Japan. All plants were grown in the greenhouse under controlled conditions of 26 °C days/6 °C nights from September 2009 to July 2010 at the protected Horticulture Experiment Station, National Institute of Horticultural & Herbal Science in Korea.

2.2. Crossing and F_1 hybrids

'UC-01' and 'Hawaii-30', 'Whiteberry' and 'Akihime' were crossed as shown in Table 2. For hybridization, each accession or cultivar was clonally propagated from runner and planted in 2 L plastic pots with compost soil. Pollen was collected from the male parent and kept at 4 °C with a desiccator until used. Seed parents were emasculated at the 'white bud' stage, and the emasculated receptacle was covered with oiled paper to protect it from other pollen. The emasculated receptacles were pollinated and the oiled paper removed after 2 weeks of pollination. The fruits were allowed to develop normally. Seeds from crosses were collected and stored at 4 °C until they were sown. All seeds were sown on a 3 cm deep layer of peat moss in 70% humidity at 20 °C for 4 weeks.

For F_1 hybrid progenies, 4-week-old seedlings were planted in 2 L plastic pots with compost soil. The plants were planted in September 2010 in the greenhouse, and grown in the greenhouse under controlled conditions of 26 °C day/6 °C night. All plants, including F_1 hybrids and cross parents, were cultivated until June 2011 for assessment of characteristics.

2.3. Transmission electron microscopy

Leaves were cut with a razor to a length of 5 mm and a width of 1–2 mm, and fixed with 2.5% glutaraldehyde for 2–3 h. Leaves were then washed two to three times with buffer solution (potassium phosphate buffer, pH 7.4) and fixed with buffer solution at 4 °C for 12 h. Samples were then treated with 1 mL 2% osmium tetroxide (OsO_4) and 1 mL Dalton fixative solution ($K_2Cr_2O_7$ 0.5 mL + 3.4% NaCl 0.5 mL) for 1 h and 30 min, and dehydrated by incubation in an ethanol gradient (30%, 50%, 70% and 90%, 10 min each). Samples were then dehydrated two times for 5 min each using absolute ethanol. Samples were washed with propylene oxide for 15 min, and infiltrated with a solution of epoxy resin and absolute acetone (1:1) for 3 h at room temperature. Samples were then polymerized by embedding in a pure epoxy resin mixture for 8 h at 40 °C, 50 °C, and 60 °C.

Ultrathin sections (50–60 nm) of the embedded pellets were cut on a Reichert-Jung Ultracut, and mounted on grids. The sections were stained with 2% aqueous uranyl acetate and lead citrate for 20 min and 10 min at room temperature, respectively. Finally, the sections were dried and examined under a transmission electron microscope (DS1300, Hitachi, Japan).

2.4. Test of pollen viability

Pollen viability was estimated indirectly by staining a sample on a glass slide with Alexander's solution, and about 1000 pollens were randomly selected to repeatedly observe pollen viability three times. Pollens were selected from the largest inflorescence due to some difference in the size of pollen depending on inflorescence, and viable ones that stained red were counted. Pollen measurements were performed by placing a small amount of pollen onto a glass slide with Alexander's solution and covering with a coverslip. Pollen size was measured in approximately 100 pollens, three times each with a microscope at 40× magnification, and pollen grains that were at least two-times larger than the average size were scored as 2*n* pollens (Jackson and Hanneman, 1999). Pollen grains were germinated by placing on pollen germination media consisting of sucrose (100 g L^{-1}), agar (5 g L^{-1}), and H_3BO_3 (0.02 g L^{-1}), and then incubated at room temperature for 5 h at 20 °C.

2.5. RAPD

DNA was extracted from 200 mg of young leaf tissue and purified using a Dneasy plant mini kit (Qiagen GmbH, Hilden, Germany) Extracted DNA was quantified using a micro-volume spectrophotometer (Nanodrop 2000, Thermo Scientific Co. Ltd., USA). RAPD was performed through a modification of the method of Williams et al. (1990), and PCR amplification was conducted in a volume of 25 μL containing 10 ng of template DNA, 200 nM of primer (5′-GATGACCGCC-3′), 100 μM of dNTP, 1× *Tag* polymerase buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin) and 0.8 units of *Tag* DNA polymerase. Amplification was performed under the following conditions: 45 cycles of 94 °C for 3 min, 94 °C for 1 min, 37 °C for 1 min and extension for 2 min at 72 °C. PCR products