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Original article

Assessing the genetic similarity of F_1 strawberry plants (*Fragaria × ananassa* Duch.) using RAPD markers

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Summary

To determine the degree of genetic similarity between F_1 hybrids and the parents with Randomly Amplified Polymorphic DNA (RAPD) markers, 19 primers were selected from a total of 91 primers based on polymorphism. The polymorphism information content values of the 19 selected primers were substantially low ranging from 0.19 to 0.38, confirming that their genetic diversity was relatively low. The index of genetic similarity between the F_1 hybrid and the parents ranged from 0.55 to 0.84. The F_1 hybrid was genetically more similar to the pollen parent. Testing the degree of similarity among F_1 hybrid and parent individuals identified four distinct groups: F_1 hybrid, ovary parents, pollen parents, and control cultivar. All F_1 hybrid individuals ($n = 20$) were observed to be genetically identical, and so were the parents. Moreover, because the degree of genetic similarity among F_1 hybrid and parent individuals were significantly high, the F_1 hybrid and the parent plants appeared to be all genetically identical, similar to the results observed in the RAPD marker test.

Keywords
seed propagation, F_1 hybrid, inbred line, inbreeding depression, polymorphism, selfing generation

Significance of this study

What is already known on this subject?

- The generation of self-pollinated strawberry leads to the phenomenon of inbreeding depression. But a few studies have been performed on F_1 cultivar breeding wherein seed propagation in strawberry has been shown to be possible using inbred lines. No research has been conducted on genetic uniformity of F_1 hybrid strawberry and inbred lines.

What are the new findings?

- Each parent plant of the S9 generation used in the present study is a pure line to a certain extent. All F_1 hybrid 'Seeberry' progenies developed by crossbreeding would appear to be genetically uniform.

What is the expected impact on horticulture?

- Crossbreeding using inbred lines could be used in the breeding of F_1 seed-propagated strawberry cultivars instead of vegetative propagation via runners in the current.

Introduction

The octoploid strawberry (*Fragaria × ananassa* Duch.) currently being cultivated is a perennial herbaceous plant. It is vegetatively propagated via runners or crown division, which requires a substantial amount of labor and time to be successful (Jun et al., 2005). Moreover, if the mother plant is infected with a virus or pathogenic bacterium, there is a risk that the daughter plant would be infected (Ito et al., 2011a). Therefore, a substantial amount of labor and time are required for the clonal propagation and management of nursery plants as well as for the management of the mother plant and damage caused by infection. Thus, there has long been a need for strawberry cultivars that can be propagated by seed instead of vegetative propagation via runners.

Strawberry seed propagation presents certain advantages, including the reduction of labor, decrease in infections caused by viruses and pathogenic bacterium, and the organized cropping system. However, seed-propagated strawberry requires the inbred lines breeding; unfortunately, in strawberries, the generation of self-pollinated strawberry leads to the phenomenon of inbreeding depression (Kaczmarska et al., 2014). According to a report by Galletta and Mass (1990), this inbreeding depression in strawberries leads to challenges in F_1 cultivar breeding because of a decreased germination percentage, plant viability, and fertility as well as owing to an increase in homozygous plants and a decrease in heterozygous plants, thereby leading to weaker environmental adaptability. On the other hand, according to a study by Rho et al. (2008), octoploid strawberries tend to demonstrate a lower germination rate in the initial selfing generation. However, selecting vigorous individuals with a high germination rate resulted in successful inbred lines breeding. Although the inbred lines exhibited less vigor and smaller yields than the original cultivar, these traits were completely recovered in the F_1 hybrid. Simultaneously, the genomic constitution of the octoploid strawberry would presumably be AAA'A'BBB'B' (2A2A'2B2B') or AAA'A'BBBB (2A2A'4B), similar to those of the diploid plant. Similar to seed-propagated crops, it has been demonstrated that F_1 cultivar breeding would be possible by making inbred lines closer to the pure line (Bringhurst, 1990).

Recently, a few studies have been performed on F_1 cultivar breeding wherein seed propagation in strawberry has been shown to be possible using inbred lines (Bentvelsen et al., 1997; Bentvelsen and Bouw, 2000; Maruo et al., 2007; Ito et al., 2010, 2011a, b; Usui, 2003). Bentvelsen and Bouw (2000) developed 'Elan', an everbearing F_1 cultivar, by crossing inbred lines. In addition, a seed-propagated strawberry with no runners, called 'Bush Alpine' or 'Gaillon', has been

developed for ornamental purposes (Slovin et al., 2009). In addition, ABZ Seeds, a company in The Netherlands, presently grows and commercially sells cultivation cultivars including 'Elan', 'Milan', 'Durban' and 'Golan' as well as ornamental cultivars such as 'Merlan' and 'Tristan'. Moreover, in Japan, 'Yotsuboshi' ($2n=8x=56$), an everbearing F_1 cultivar, has been developed by crossing the S4 generations of octoploid strawberry (Mochizuki et al., 2017). In addition, the Chiba Prefectural Agriculture and Forestry Research Center in Japan has developed a cultivar of June-bearing 'Chiba F-1 gou' for the first time (Fukao et al., 2012).

In Korea, the selfing lines of S9 generation of octoploid strawberry was bred via the line-breeding method, which involves the elimination of individuals with severe inbreeding depression and the selection of vigorous individuals (Jeong et al., 2015a). The F_1 hybrid 'Seeberry' cultivar, a seed-propagated strawberry, was bred using this method (Jeong et al., 2015b). Although the yield of 'Seeberry' is lower than that of vegetatively propagated cultivars, the cultivar showed a morphological stability with a higher germination rate and uniform growth among individuals by seed propagation (Jeong et al., 2015b). However, unlike the diploid strawberry, the octoploid strawberry could not be considered genetically fixed via selfing over six or seven generations. Nevertheless, practically no research has been conducted on such genetic uniformity of F_1 hybrid strawberry and inbred lines derived from octoploid strawberry.

Therefore, the present study was conducted to assess the genetic similarity of F_1 hybrid strawberry cultivar and inbred lines of S9 generation using parent plants derived from octoploid strawberry using Randomly Amplified Polymorphic DNA (RAPD) markers.

Materials and methods

Plant materials

The F_1 hybrid 'Seeberry' was developed by crossbreeding between inbred lines: 'Wongyo 3115' × 'Wongyo 3116'. 'Wongyo 3115', used as the ovary parent, was of the S9 selfing generation ('Benihoppe 3-5-1-8-307-8-138-5-5'); its original cultivar was 'Benihoppe'. 'Wongyo 3116', used as the pollen parent, was of the S9 selfing generation ('Toyonoka 1-3-6-40-7-10-1-8-8'); its original cultivar was 'Toyonoka' (Figure 1).

Inbred lines of octoploid strawberry were selected from two original cultivars ('Toyonoka' and 'Benihoppe'), and individuals in each generation were selected or eliminated based on the vigor. The selected individuals were self-pollinated to produce seeds for the next generation; the generations were advanced to S9 generation by selfing. Among S9 selfing generations, the 'Benihoppe 3-5-1-8-307-8-138-5-5' line was named 'Wongyo 3115' and 'Toyonoka 1-3-6-40-7-10-1-8-8' line was named 'Wongyo 3116' (Rho et al., 2015). The F_1 hybrid 'Seeberry' was developed by crossbreeding between inbred lines of 'Wongyo 3115 × Wongyo 3116' (Figure 1).

For RAPD marker analysis, 20 individuals of F_1 hybrid 'Seeberry' and 5 individuals from each of the three groups – the ovary parent 'Wongyo 3115', the pollen parent 'Wongyo 3116', and the control cultivar 'Seolhyang' (vegetative propagation cultivar), – were used.

DNA extraction

For genomic DNA extraction, a clean young strawberry leaf was collected and ground using liquid nitrogen in a Mixer Mill (MM400, Retsch, Germany). Genomic DNA was extracted from the tissue using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The genomic DNA was confirmed using BioPhotometer (Eppendorf, Hamburg, Germany). The final concentration was adjusted to 30 ng μL^{-1} for polymerase chain reaction (PCR).

RAPD marker analysis

91 RAPD markers were selected based on previous studies on genetic analyses of strawberries (Landry et al., 1997; Kuras et al., 2004; Sugimoto et al., 2005; Degani et al., 1998; Thongthieng and Smitamana, 2003; Morales et al., 2011; Celik et al., 2017; Garcia et al., 2002; Hancock and Callow, 1994; Congiu et al., 2000; Gidoni et al., 1994). PCR amplification

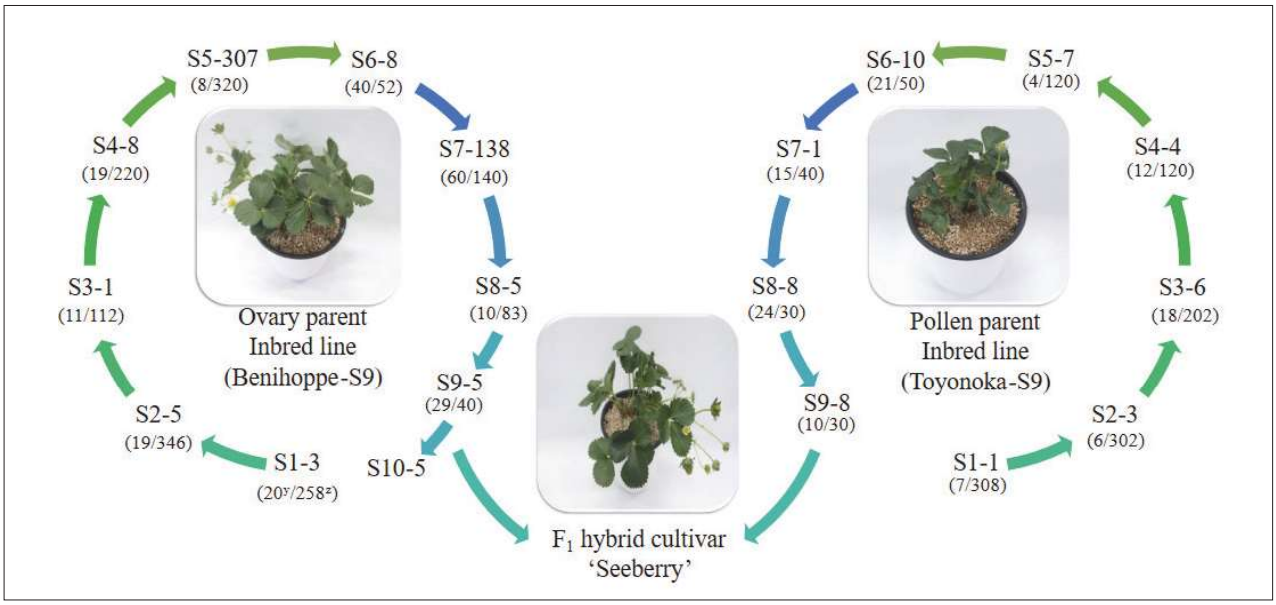


Figure 1. Pedigree diagram of parents for 'Seeberry' F_1 hybrid breeding in octoploid strawberry. Sn-number means that Sn shows selfing generation and number shows number of selected individuals among ^{y} in bracket, and ^{z} in bracket shows the number of surviving seedling after sowing in each generation; y in bracket shows the numbers of selected individuals among surviving seedlings for advancing selfing generation.

was performed in a 50 µL volume containing 60 ng gDNA, 4 µL dNTP, 5 µL 10× buffer (Tris pH 9.0, 15 mM $MgCl_2$), 1 µL DNA polymerase, 2 µL primer, and distilled water. The PCR conditions were as follows: 45 cycles of elongation at 72°C for 30 s, annealing at 35°C for 30 s, and denaturation at 94°C for 30 s. The PCR products were confirmed by electrophoresis on 1.5% agarose gel for 90 min.

Statistical analysis

To determine the degree of genetic similarity and that of genetic polymorphism using each marker, a score of 1 (present) or 0 (absent) was recorded depending on the presence of a band detected by electrophoresis. The polymorphic information content (PIC) value for each marker was calculated using the following formula:

$$PIC = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2 P_i^2 P_j^2$$

where *Pi* and *Pj* are the population frequencies of the i^{th} and j^{th} alleles (Nagy et al., 2012). Genetic similarity was calculated using Jaccard coefficient in POPGENE32 (v. 1.32) followed by analysis using the unweighted pair group method with arithmetic mean. Genetic similarity values were represented in a dendrogram constructed using PAleontological STatistics (PAST) v. 3.19.

Results and discussion

Analysis of RAPD polymorphism in parent and F_1 hybrid plants

The RAPD marker technique is frequently used in the selection of markers related to specific traits (Sugimoto et al., 2005) and in genetic similarity analyses among strawberry cultivars (Morales et al., 2011; Landry et al., 1997; Degani et al., 1998; Garcia et al., 2002; Kang et al., 1998; Park and Lee, 1996).

In the present study, 19 primers showing polymorphism were selected based on the genetic similarity among the F_1 hybrid 'Seeberry' plants and parent plants determined using 91 RAPD markers. The allele size of primers selected from all RAPD markers on the basis of polymorphism ranged from 150 to 2,000 bp (Table 1). The number of alleles per locus varied among the markers, ranging from 4 to 13 alleles (mean 8.9 alleles). Overall, 170 alleles were identified using the 19 primers. There were 97 alleles (57%) exhibiting polymorphism in 35 individuals examined, including the F_1 hybrid 'Seeberry' plant, parent plant, and control cultivar groups. Besides these, there were 73 alleles (43%) exhibiting monomorphism. According to Chavan et al. (2013), an increase in polymorphism results in an increase in genetic diversity, and vice versa. Morales et al. (2011) have reported that the mean rates of polymorphism and monomorphism in 11 strawberry cultivars obtained using markers (selected from 40 RAPD markers based on polymorphism) were 55.8% and 44.2%, respectively. Moreover, the selection of markers in this study was reportedly appropriate because the RAPD markers were sufficient to identify cultivars and analyze genetic similarity. Because the present study also showed greater polymorphism than monomorphism, the selected primers were considered appropriate in cultivar identification and similarity analysis.

Comparison of genetic diversity in parent and F_1 hybrid plants

The range of PIC values indicating the degree of diversity was 0.19–0.38 (mean, 0.30) in the present study. According to Yoon et al. (2012), the average PIC value of 25 strawberry cultivars originating in Japan was 0.519, that of 20 cultivars

Table 1. Characteristics of RAPD markers with polymorphism in the 35 strawberry accessions used breeding of F_1 hybrid cultivar.

Primer	Annealing temperature (°C)	Size (bp)	PIC	Number of bands		
				Polymorphic	Monomorphic	Total
OPA-08	35	300-1,200	0.32	5	0	5
OPA-14		600-1,000	0.32	2	2	4
OPA-19		300-2,000	0.33	5	3	8
OPB-01		500-2,000	0.36	7	3	10
OPB-11		450-1,600	0.36	6	3	9
OPC-19		300-1,200	0.33	5	6	11
OPD-01		400-2,000	0.19	2	5	7
OPD-18		200-1,200	0.31	8	1	9
OPE-07		200-1,100	0.11	2	7	9
OPG-14		300-1,600	0.38	6	2	8
OPJ-01		400-1,200	0.12	1	7	8
OPJ-06		500-1,200	0.30	4	2	6
OPJ-07		400-1,500	0.37	9	2	11
OPJ-09		400-1,600	0.25	3	5	8
OPV-07		150-2,000	0.36	10	3	13
OPV-15		350-1,800	0.32	7	5	12
OPV-20		200-1,600	0.33	5	5	10
OPX-11		300-1,600	0.24	3	7	10
OPX-18		250-2,000	0.37	7	5	12
Total		150-2,000	0.19-0.38	97	73	170

Table 2. Genetic diversity within four strawberry cultivars.

Locus	Observed number of alleles	Nei's gene diversity	Shannon's Information index
OPA-08	2.000	0.204	0.344
OPA-14	1.500	0.185	0.278
OPA-19	1.625	0.243	0.361
OPB-01	1.700	0.275	0.407
OPB-11	1.667	0.234	0.357
OPC-19	1.455	0.115	0.187
OPD-01	1.333	0.157	0.221
OPD-18	1.800	0.398	0.553
OPE-07	1.222	0.102	0.145
OPG-14	1.625	0.175	0.280
OPJ-01	1.125	0.046	0.069
OPJ-06	1.667	0.263	0.380
OPJ-07	1.800	0.190	0.309
OPJ-09	1.375	0.142	0.206
OPV-07	1.786	0.183	0.301
OPV-15	1.500	0.153	0.233
OPV-20	1.500	0.170	0.258
OPX-11	1.300	0.111	0.167
OPX-18	1.583	0.151	0.248
Mean	1.556	0.184	0.279

originating in Korea was 0.450, and that of 10 cultivars originating in the United States was 0.402. Therefore, the average PIC value in the present study was slightly lower than that in another research (Yoon et al., 2012). Because the present study used an F_1 hybrid cultivar and its parent plants as its main experimental targets, the gene pool was limited, which appears to lower the genetic diversity.

Other indicators frequently used to demonstrate the genetic diversity of various plants are Nei's gene distance and Shannon's information index (Hennink and Zeven, 1991; Kosman, 2003; Onate et al., 2018; Gyorgy et al., 2012). Typically, Nei's gene distance is used to analyze genetic distances among individuals within a certain population (Kosman and Leonard, 2005), whereas Shannon's information index is primarily used to measure diversity in population genetics (Glasenapp et al., 2015; Lewontin et al., 1972).

On analyzing the genetic diversity among the F_1 hybrid 'Seeberry' plants and parent plants according to Nei's gene distance and Shannon's information index, the range of allele by selected primer was from 1.222 to 2.000 (mean, 1.556; Table 2). The overall mean of the Nei's gene distance index was 0.184, with the highest value being 0.398 in OPD-18 and the lowest value being 0.046 in OPJ-01. The overall mean of Shannon's information index was 0.279, with the highest value being 0.553 in OPD-18 and the lowest value being 0.069 in OPJ-01 (Table 2). Although there were some numeric differences between Nei's gene distance and Shannon's information index, they both indicated the same tendency in each primer.

A report has shown that Nei's gene distance and Shannon's information index in 70 octoploid wild strawberries (*F. nubicola* L.) collected from the Tibetan highlands of China were 0.212~0.310 and 0.315~0.289, respectively (Peng et al., 2015; Meng et al., 2015). Another report has indicated that Nei's gene distance and Shannon's information index of *F. chiloensis* and *F. paragonica*, wild strawberries collected from Chile, were 0.24 and 0.37, respectively (Carrasco et al., 2007). In comparison with such reports, the present study showed a closer genetic distance and a lower genetic diversity; this confirms that the genetic similarity of the F_1 cultivar and parent plants used in this study was higher than the degree of genetic similarity within the cultivar or among cultivars in other previous studies.

Analysis of genetic similarity between parent plants and F_1 hybrid cultivar

Analysis using 19 selected RAPD markers among the F_1 cultivar, parent plant, and control cultivar showed that the genetic similarity between the F_1 cultivar and the ovary parent was 0.56, that between the F_1 cultivar and the pollen parent was 0.57, and that between the F_1 cultivar and the control cultivar was 0.55 (Table 3). Furthermore, the result indicated that although the degree of genetic similarity between the F_1 cultivar and parent plants tended to be similar, it tended

Table 3. Matrix of genetic similarity using (above diagonal) and genetic distance(below diagonal) among 35 strawberry individuals using F_1 hybrid cultivar breeding based on RAPD markers.

Cultivars and parents	F_1 hybrid	Ovary	Pollen	Cont.
F_1 hybrid	–	0.56	0.57	0.55
Ovary	0.44	–	0.67	0.64
Pollen	0.43	0.33	–	0.84
Cont.	0.45	0.36	0.16	–

to be slightly closer to the pollen parent. Jeong et al. (2015b) indicated that the F_1 hybrid 'Seeberry' cultivar traits, such as plant shape, leaf shape, fruit shape, and fruit color, were intermediates of its parent plant traits. Moreover, Rho et al. (2012) have indicated that the F_1 hybrid strawberry traits appeared to be intermediate, although heterosis occurred in the F_1 hybrid cultivar. Accordingly, the genetic similarity between the F_1 hybrid cultivar and ovary and pollen plants was extremely intermediate in the present study. Similar to the phenotype, the genotype of the F_1 hybrid cultivar were considered include the parent plant's genes. Additionally, the pollen parent had an extremely high degree of genetic similarity to 'Seolhyong', which was used as the control cultivar. This was caused by the use of the breeding material ('Toyonoka' cultivar) of the 'Seolhyong' ['Akihime' × 'Red pearl' ('Aiberry' × 'Toyonoka')] cultivar and the use of this 'Toyonoka' cultivar as the pollen parent in inbred lines breeding ('Toyonoka S9') of the F_1 hybrid cultivar.

Analysis of genetic similarity among parent and F_1 hybrid individuals

Based on the analysis of genetic similarity between individual strawberries of the F_1 hybrid cultivar and parent plants, the plants were categorized into four groups: F_1 cultivar, ovary parent, pollen parent, and control cultivar (Figure 2). The present study confirmed that 20 individual strawberries of the F_1 hybrid cultivar were perfectly genetically matched and that 5 individuals from each of three groups (ovary parent, pollen parent, and control cultivar) were genetically identical. Therefore, using RAPD marker, this study confirmed that each individual strawberry of the pollen parent 'Toyonoka S9' and the ovary parent 'Benihoppe S9', which were considered as inbred lines that were bred by selfing of the octoploid strawberry, as well as that of the F_1 hybrid 'Seeberry' cultivar developed by their crossbreeding were genetically identical.

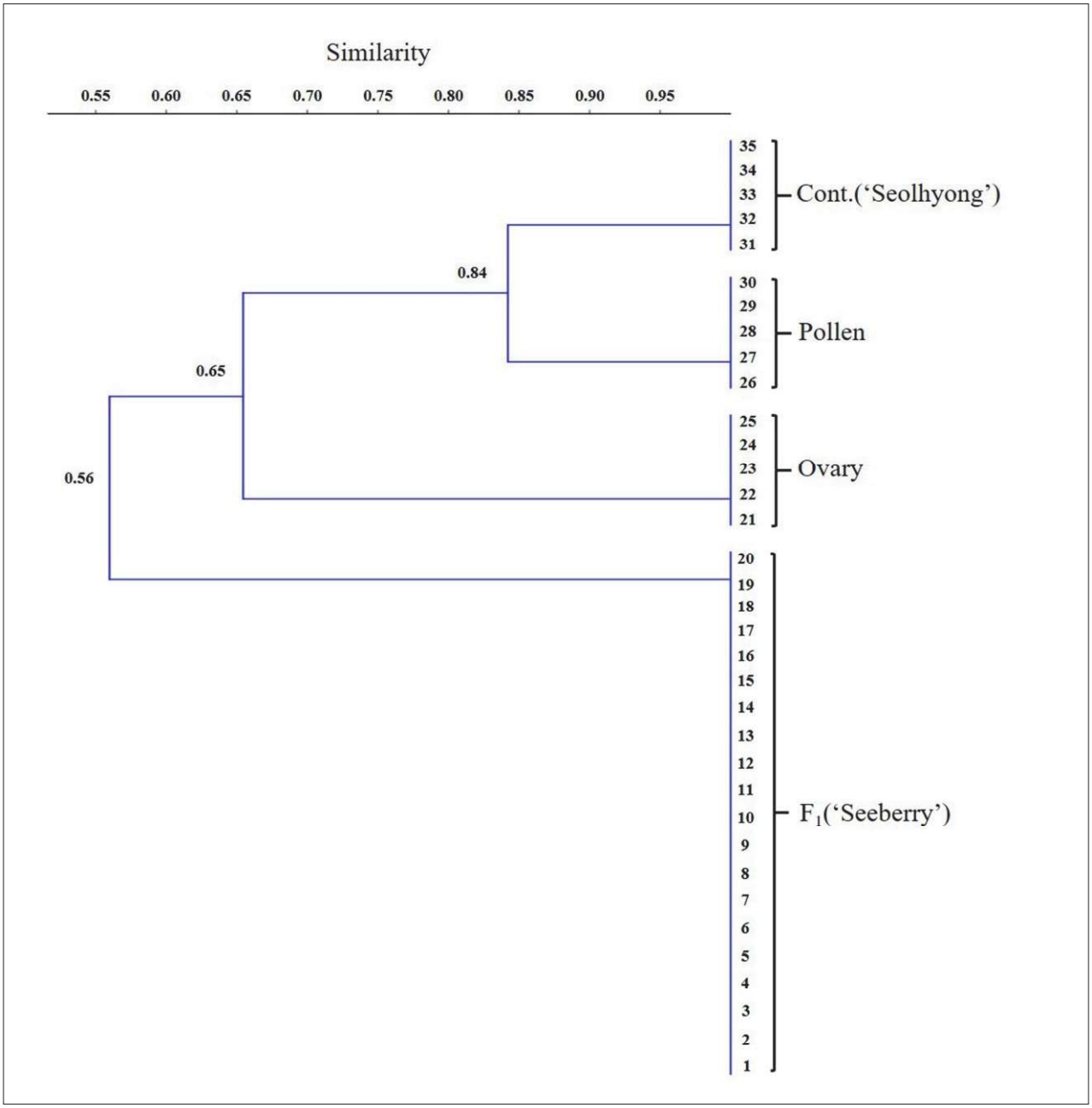


Figure 2. Dendrogram showing the genetic similarity among individuals of F_1 hybrid progeny plants and parent plants.

Development of F_1 hybrid cultivar using inbred lines in octoploid strawberry

To date, four genotype models of octoploid strawberry have been proposed: AABBBCC (Federova, 1946), AAA'A'BBBB (Senanayake and Bringhurst, 1967), AAA'A'BBB'B' (Bringhurst, 1990), and AABBCCDD (Bassil et al., 2015). Among these, AAA'A'BBB'B' (2A2A'2B2B') is considered the strongest model (Hirakawa et al., 2014). Therefore, one could predict that the homozygote rate based on the advancement of the selfing generation would demonstrate an inheritance aspect similar to that of the diploid strawberry. Typically, considering independent inheritance in the diploid strawberry, the homozygote rate in the S7 and S8 generations is ≥ 98%. Based on this, it could be presumed that each parent plant of the S9 generation used in the present study is a pure line to a certain extent. All F_1 hybrid 'Seeberry' progenies developed by crossbreeding would appear to be genetically uniform. However, considering the inheritance of associations between the genome A' or B' and the genome A or B among the strong strawberry genome model, it would be difficult to consider S9 generation as 100% pure lines. Moreover, considering that the RAPD markers randomly amplified DNA and that it was the dominant marker made it slightly difficult to consider the parent plants as 100% pure lines. Therefore, additional studies should be conducted using more precise analytical methods, including SSR and HRM markers, with regard to the pure line of the octoploid strawberry. However, although the parent plants are not genetically 100% pure lines, inbred lines can be used for the F_1 hybrid cultivar breeding because the phenotype uniformity in each individual of F_1 hybrid cultivar is substantially high (Jeong et al., 2015b). During the F_1 hybrid cultivar breeding, the advancement of excessive selfing generations from their parent plants would cause inbreeding depression. Even in instances of heterosis occurrence in F_1, because F_1 hybrid cultivars would have the disadvantages of lower plant vigor and yield compared with that of vegetatively propagated plants, such areas should be strengthened in the breeding process of F_1 seed-propagated strawberry cultivars.

Conclusions

The analysis of the genetic similarity between the F_1 hybrid 'Seeberry' cultivar and S9 generations as parent plants using RAPD markers in the present study evidently identified the following four groups: F_1 cultivar, ovary parent, pollen parent, and control cultivar. The genetic similarity between the F_1 cultivar and the ovary parent and that between F_1 cultivar and the pollen parent was substantially intermediate. Therefore, the rate of genes inherited from the parent plants in the F_1 hybrid cultivar would be similar. Moreover, five individual strawberries of each of two groups – the ovary parent and pollen parent – analyzed from the selfing S9 generation of the octoploid strawberries were genetically identical, indicating that they were of pure line to a certain extent. Furthermore, all 20 individuals of F_1 hybrid cultivar developed by crossbreeding were genetically identical.

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