

The genetic diversity among strawberry breeding resources based on SSRs

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ABSTRACT: Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is a high value horticultural crop. In this study, the genetic diversity of 160 strawberry accessions was determined using five highly polymorphic simple sequence repeat (SSR) markers. Sixty different alleles were identified, with allele frequencies in the range of 0.006 to 1. Similarity scores were in the range of 0.034 to 0.963 (average: 0.507). The accessions were categorized into five groups. Group 1 contained two diploid *Fragaria vesca* species and one unknown accession. Group 2 contained one accession (*F.* × *ananassa*). Group 3 contained 20 *F.* × *ananassa* accessions and six unknown accessions. Group 4 contained 48 *F.* × *ananassa* accessions, one octaploid *Fragaria chiloensis* species, and six unknown accessions while Group 5 contained 69 *F.* × *ananassa* accessions and six unknown accessions. Accessions within a pedigree were frequently grouped together. A total of 30 novel accessions were categorized alongside existing accessions. These results will allow breeders to develop strategies which incorporate more genetic diversity into new cultivars. **Keywords:** *Fragaria* × *ananassa*, simple sequence repeat, cultivar identification

Introduction

Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is an octoploid ($2n = 8x = 56$) generated by natural hybridization between two octoploid strawberry species, *F. chiloensis* and *F. virginiana* (Hancock et al., 2010). To date, 23 strawberry species have been reported, with various ploidy levels from diploid to decaploid (Nathewet et al., 2010; Rousseau-Gueutin et al., 2009).

Numerous strawberry breeding programs have been developed to generate new cultivars with improved taste and flavor or extended harvest period and shelf life (Capocasa et al., 2008; Faedi et al., 2000). Breeding success is associated with accessibility to breeding resources and genetic variations. Consequently, information regarding the genetic diversity and population structure of breeding resources is important for efficient development of new cultivars. Traditionally, the identification of strawberry cultivars was determined by the examination of differences in morphological or physiological characteristics such as leaf, flower, fruit parameters, and flowering habit (Dale, 1996; Nielsen and Lovell, 2000). However, such methods are unsuitable for cultivated strawberries as genetic and phenotypic variations between cultivars are minimal. As a consequence, DNA markers were developed to facilitate analysis of genetic diversity, linkage mapping, and identification of cultivars (Chambers et al., 2013; Congiu et al., 2000; Degani et al., 2001; Garcia et al., 2002; Govan et al., 2008; Isobe et al., 2013; Kuniyama et al., 2003; Tyrka et al., 2002). SSR markers for strawberry were first developed using primer pairs that amplified characterized regions such as expressed sequence tags (ESTs) or genomic libraries, in contrast to other primer pairs that amplified anonymous DNA fragments (Ashley et al., 2003; Bassil et al.,

2006; Gil-Ariza et al., 2006; James et al., 2003; Lewers et al., 2005; Monfort et al., 2006; Sargent et al., 2003). The completion of the genome sequence of diploid *F. vesca* (Shulaev et al., 2011) allowed robust SSRs to be developed and mapped on the *Fragaria* reference map (Rousseau-Gueutin et al., 2011; Sargent et al., 2011; Zorrilla-Fontanesi et al., 2011). In this study, we assessed the genetic diversity of 160 strawberry accessions used for breeding in Korea, including 34 newly collected accessions. The genotyping results will provide a molecular basis for future breeding programs and will facilitate the development of novel strawberry cultivars with increased genetic diversity.

Materials and Methods

Plant materials and DNA extraction

A total of 160 strawberry accessions (130 accessions from the Korea Rural Development Administration (RDA) Genebank Information Center, and 30 newly generated or collected accessions) were provided by the Nonsan Strawberry Experimental Station (Nonsan, Korea) (Table 1). Accessions were collected from Korea (26 accessions), Japan (46 accessions), the United States (45 accessions), Europe (13 accessions: four from the United Kingdom, three from the Netherlands, two each from Germany and Russia, and one each France and Spain), and other countries (nine accessions: three from each of Canada and Israel, two from China, and one from New Zealand). The countries of origin of 21 accessions were unknown. Genomic DNA was extracted from young leaves using a WizPrep Plant DNA Mini Kit (Wizbiosolutions) according to the manufacturer's protocol. The final DNA concentration was adjusted to 10 ng per μ l for use in a polymerase chain reaction (PCR).

Table 1 – List of 160 strawberry accessions used in this study including accession name, origin, IT number, parentage, scientific name, and status.

No.	Name	Origin	IT number ^a	Parentage	Scientific name	Status
1	Busan No.1101*	Korea, South	IT 232440	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
2	Chodong*	Korea, South	IT 232442	Harunoka × Yachio	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
3	Daehak1*	Korea, South	IT 232439	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
4	Daeun*	Korea, South	IT 232453	Akihime × Redpearl	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
5	Daewang ^b *	Korea, South	IT 245989	Maehyang × Wonkyo3111	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
6	Daewang ^b *	Korea, South	IT 245989	Maehyang × Wonkyo3111	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
7	Dahong*	Korea, South	IT 232450	Sachinoka × Maehyang	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
8	Danmi*	Korea, South	IT 245379	Maehyang × Amaou	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
9	Geumhyang*	Korea, South	IT 232448	Akihime × Tochiotome	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
10	Johong*	Korea, South	IT 232446	Nyoho × Akihime	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
11	Maehyang*	Korea, South	IT 232445	Tochinomine × Akihime	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
12	Manhyang*	Korea, South	IT 245381	Nyoho × Akanekko	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
13	Mihong*	Korea, South	IT 232444	Toyonoka × Reiko	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
14	NS970524*	Korea, South	IT 245343	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
15	Okmae*	Korea, South	IT 245987	Toyonoka × Maehyang	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
16	Seolhyang*	Korea, South	IT 232447	Akihime × Redpearl	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
17	Suhong*	Korea, South	IT 232441	Hokowase × Harunoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
18	Sulhong*	Korea, South	IT 232443	Suhong × Toyonoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
19	Wildstrawberry1*	Korea, South	IT 245312	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Wild
20	Wildstrawberry2*	Korea, South	IT 245335	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Wild
21	Wonkyo3111*	Korea, South	IT 245867	Open pollinated of Benihoppe	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
22	Aiberry*	Japan	IT 232465	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
23	Aihime*	Japan	IT 232463	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
24	Akanekko*	Japan	IT 232473	Aiberry × Hokowase	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
25	Akasyanomitsuko	Japan	IT 245334	Nyoho × Kunowase	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
26	Akihime	Japan	IT 245292	Kunowase × Nyoho	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
27	Alps	Japan	IT 245363	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
28	Amaou	Japan	IT 232483	Fukuoka S6	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
29	Aska	Japan	IT 232470	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
30	Asuka Wave	Japan	IT 232480	[Uzushio × ((Kurume Sokusei-3 × Hokowase) × (Donner × Hokowase))] × ((Kurume Sokusei-3 × Hokowase) × (Donner × (Kobe-1 × Hokowase))]	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
31	Bbiaberry	Japan	IT 245362	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
32	Benihoppe	Japan	IT 232482	Akihime × Sachinoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
33	Chiduru	Japan	IT 232457	[(Donner-s)-s × (Harunoka-s)-s]-s × Harunoka-s	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
34	Terunoka	Japan	IT 232461	Hokowase × Donner	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
35	Everberry	Japan	IT 245337	Oishi-Sikinary × Haruyoi	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
36	Goryeong	Japan	IT 245328	Takane Haikara × Donner	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
37	Guardian	Japan	IT 245289	NC-1768 [Fairpeake × (Aberdeen × Redheart)] × Tennessee Beauty × Surecrop	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
38	Harunoka	Japan	IT 232456	Kurume103 × Donner	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
39	Haruyoi	Japan	IT 232460	Hokowase × Harunoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
40	Hongbok	Japan	IT 245311	Kohuku	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
41	Houkouwase ^c	Japan	IT 232455	Kogyoku (Fairfax self seedling) × Tahoe	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
42	Houkouwase ^c	Japan	IT 232455	Kogyoku (Fairfax self seedling) × Tahoe	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
43	Jumbo	Japan	IT 232484	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
44	Jumbo Pure Berry	Japan	IT 232486	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
45	Kunowase	Japan	IT 245308	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
46	Kurume39	Japan	IT 245327	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
47	Kurume52	Japan	IT 245332	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
48	Myongbo	Japan	IT 245324	Meiho derived	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
49	Nyoho	Japan	IT 232469	Kei210 × Reiko	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
50	Pechika ^d	Japan	IT 245346	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
51	Pechika ^d	Japan	IT 245346	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
52	Red Pearl	Japan	IT 232475	Aiberry × Toyonoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar

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Table 1 - Continuation.

53	Regina	Japan	IT 245313	Amerikanischer Samling II (Geneva) × Deutsch Evern	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
54	Reiko	Japan	IT 232468	Fukuba × Harunoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
55	Reiyu	Japan	IT 232464	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
56	Rockyhara	Japan	IT 245329	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
57	Sachinoka	Japan	IT 232476	Toyonoka × Aiberry	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
58	Sagahonoka	Japan	IT 232458	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
59	Shinyurbong	Japan	IT 245336	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
61	Sungkang17	Japan	IT 245321	Morioka17	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
62	Sungkang19	Japan	IT 245322	Morioka19	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
63	Syuko	Japan	IT 232477	Shizutakara × Haruyoi	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
64	Tochinomine	Japan	IT 232479	(Florida69-266 × Reiko) × Nyoho	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
65	Tochiotome	Japan	IT 232472	Kurume49 × Tochinomine	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
66	Toyonoka	Japan	IT 232466	Himiko × Harunoka	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
67	Wonyuk	Japan	IT 245325	Himiko derived	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
68	Aiberry(ARS)	United States	IT 245365	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
69	Armora	United States	IT 232488	Blakemore × Aroma	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
70	Berry Star ^e	United States	IT 232501	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
71	Berry Star ^e	United States	IT 232501	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
72	Blackmore	United States	IT 245298	Missionary × Howard 17	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
73	Camarosa	United States	IT 232510	Douglas × Cal 85.218-605	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
74	Cardinal	United States	IT 232487	Earlibelle × ARK 5063	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
75	Cascade	United States	IT 232503	Shasta × Northwest	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
76	Catskill	United States	IT 232491	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
77	Columbia	United States	IT 232500	WA 157 × WA 175	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
78	Comet	United States	IT 232499	Earlibelle × ARK 5063	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
79	Cyclone	United States	IT 232492	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
80	Dabreak	United States	IT 245299	Headliner × {Klonmore × [L-117-1-45-3 × (Suwannee × Konvoy)]}	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
81	Diamante	United States	IT 245796	Cal. 87112-6 × Cal. 88270-1	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
82	Donner	United States	IT 245291	CAL 222 × CAL 145.52	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
83	Earlibrite ^f	United States	IT 245382	Rosalinda × FL 90-38	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
84	Earlibrite ^f	United States	IT 245382	Rosalinda × FL 90-38	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
85	Erie Star	United States	IT 245294	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
86	Flamenco	United States	IT 245349	Evita × EMRO77	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
87	Floridabelle	United States	IT 232502	Sequoia × Earlibelle	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
88	Huxley	United States	IT 232504	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
89	Juspa	United States	IT 245300	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
90	Klondike	United States	IT 245301	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
91	Lassen	United States	IT 232506	Blakemore × (Marshall × Fendalcinno) × Nich Ohmer × (Royal Sovereign × Howard 17) × (Marshall × Fendalcinno)	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
92	Linn	United States	IT 232505	MDUS 3184 × ORUS 2414	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
93	Mingoi	United States	IT 245353	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
94	Missionary	United States	IT 245302	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
95	NewYork884	United States	IT 245297	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
96	North West	United States	IT 245303	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
97	NY1406	United States	IT 245320	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
98	Pink Panda	United States	IT 232511	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Wild relatives
99	Premier	United States	IT 232509	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
100	Redgauntlet	United States	IT 245304	New Jersey 1051 × Auchincruive Climax	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
101	Red Glow	United States	IT 232489	Fairland × Tennessee Shipper	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
102	Red Rich	United States	IT 232497	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
103	Sari	United States	IT 245354	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
104	Shasta	United States	IT 232495	Marshall × Howard 17	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
105	Sweet Charlie	United States	IT 245345	FL 80-456 × Pajaro	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
106	Dahoe	United States	IT 244919	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar

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Table 1 - Continuation.

107	Victoria	United States	IT 232508	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
108	Bolero	United Kingdom	IT 245348	LA0988 × Selva	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
109	Dyd Babi	United Kingdom	IT 245318	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
110	Jonson's Early	United Kingdom	IT 245319	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
111	Jursey Belle	United Kingdom	IT 245296	(Lupton × Aberdeen) × Fairfax × (Pathfinder × Fairfax)	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
112	Cavalier	Canada	IT 232515	Valentine (Howard 17 × Vanguard) × Sparkle	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
113	Guards Man	Canada	IT 232496	Claribel × Sparkle	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
114	Micmac	Canada	IT 232516	Tioga × Guardsman S1	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
115	Nowang ^a	Israel	IT 245351	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
116	Nowang ^a	Israel	IT 245351	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
117	Tamar	Israel	IT 232517	Osogrande × Dorit	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
118	Chunseong	China	IT 245356	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
119	Gilgyung53	China	IT 245307	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Line
120	Senga Gigana	Germany	IT 232513	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
121	Senga Sengana	Germany	IT 232512	Markee × Sieger	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
122	Elsanta	Netherlands	IT 245875	Gorella × Holiday	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
123	Sarian	Netherlands	IT 245364	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
124	Bukaj	Russia	IT 245180	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
125	Kama	Russia	IT 245357	Sengasengana × Cavlier	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
126	Favette	France	IT 245373	(Souvenir des Halles × Regina) × (Pocahontas × Aliso)	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
127	Toteuklip	Spain	IT 245355	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
128	Chiloensis		IT 245331	Unknown	<i>Fragaria chiloensis</i>	Cultivar
129	<i>Fragaria</i> Wild		IT 245316	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Wild
130	Jasan		IT 232493	Unknown	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	Cultivar
131	Crop Station	Korea, South		Unknown		
132	Jirisan Wild	Korea, South		Unknown		
133	Sakyejul	Korea, South		Shikisetzu Unpolished	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
134	Ssanta	Korea, South		Maehyang × Seolhyang	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
135	Sukhyang	Korea, South		Seolhyang × Maehyang	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
136	Elan	Netherlands		Fern × Rapella	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
137	New Zealand	New Zealand		Unknown		
138	Caminoreal	United States		Cal 89.2307 × Cal 90.2533	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
139	Festival	United States		Rosalinda × Osogrande	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
140	Florida	United States		Unknown		
141	<i>Fragaria</i> Minnesota	United States		Unknown		
142	Ventana	United States		Cal 93.170-606 × Cal 92.35-601	<i>Fragaria</i> × <i>ananassa</i> subsp. <i>ananassa</i>	
143	Bious			Unknown		
144	EMC			Unknown	<i>Fragaria vesca</i>	
145	Island Wild			Unknown		
146	Jepter			Unknown		
147	Naoami			Unknown		
148	Niigata			Unknown		
149	No0241			Unknown		
150	No04113			Unknown		
151	No06155			Unknown		
152	No06172			Unknown		
153	No06182			Unknown		
154	No06183 ^b			Unknown		
155	No06183 ^b			Unknown		
156	No0636			Unknown		
157	No0641			Unknown		
158	No0643			Unknown		
159	UC1			Unknown	<i>Fragaria vesca</i>	
160	Wild Strawberry3			Unknown		

^aIT numbers were provided by the RDA-Genebank Information Center (<http://www.genebank.go.kr>); ^bSame accessions collected from distant two pots; *Accessions 1-24 were used to select the SSR markers.

SSR analysis

A subset of 24 strawberry accessions (accessions 1–24, Table 1) was used to select SSR markers that could discriminate effectively between the different accessions. Genotyping was performed using 16 SSR markers that were developed by three independent research groups (Chambers et al., 2013; Govan et al., 2008; Isobe et al., 2013). PCR products were analyzed using a MultiNA microchip electrophoresis system (MCE-202 MultiNA, Dongil Shimadzu). Peak sizes on electropherograms were automatically detected by MultiNA software (MultiNA Control Software and Data Analysis Software MultiNA Viewer, Dongil Shimadzu). Five SSR markers were selected from all the 160 strawberry accessions for analysis. Modified primer sequences for the markers are listed in Table 2. PCR reaction mixtures contained 1 ng of template DNA, 1 × PCR buffer, 0.25 mM of dNTP mixture, 2.5 μM of each primer, and 1 unit of i-Max II DNA polymerase (iNtRON) in a total volume of 20 μl. PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 36 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s, and then a final extension at 72 °C for 5 min. PCR reactions were performed using a C1000 Touch Thermal Cycler (Bio Rad Laboratories).

Genetic diversity analysis

The genetic diversity of 160 strawberry accessions was analyzed using five selected SSR markers. First, SSR marker peaks were scored as 1 (presence of peak) or 0 (absence of peak) for each accession. Rare alleles and accession-specific alleles were applied in this study to distinguish strawberry accessions (Cho et al., 2007; Govan et al., 2008; Yoon et al., 2012). The binary data set for all strawberry accessions was then assembled in a single matrix and analyzed using the unweighted pair group method with an arithmetic mean (UPGMA, <http://genomes.urv.cat/UPGMA/>) algorithm (using the Jaccard coefficient) to calculate genetic similarities and distances between the accessions. Output data in the Newick format were used with MEGA version 6 to produce a dendrogram (Tamura et al., 2013).

Results and Discussion

Evaluation of diversity markers and genotyping of 160 strawberry accessions

In this study, we strengthened strawberry breeding resources in Korea by analyzing 160 previously collected or newly generated strawberry germplasms. To determine the optimal markers for genotyping, 16 previously developed SSRs (nine from Govan et al. (2008), six from Chambers et al. (2013), and one from Isobe et al. (2013)) were used to test 24 of the 160 strawberry accessions (accession 1–24) (Table 1). Of the sixteen markers, eight yielded dim or difficult to interpret fingerprints, and three were insufficiently polymorphic (Figure 1). The remaining five markers, which were developed by Chambers et al. (2013), revealed clear fragments that were highly polymorphic between accessions (Figure 1). These five markers were chosen for genotyping from all 160 accessions. A total of 60 alleles (19 from FG7c/d, 13 from FG7a/b, 12 from FG1a/b, and 8 from each of FG2a/b and FG7e/f) were obtained from the five markers across all accessions (Table 2). All the markers were highly polymorphic: individual markers separated the 160 accessions into 17 (FG7e/f) to 54 (FG2a/b) allelic patterns. Allele sizes among the 160 accessions were in the range of 123 bp (FG1a/b) to 599 bp (FG7e/f) (Table 2), which differed slightly from those observed previously (Chambers et al., 2013). These differences may be attributable to the different strawberry accessions tested, slight modifications to primers (see Table 2), different PCR conditions, and different genotyping equipment.

Allele frequencies were in the 0.006–1 range (Figure 2). Of the 60 alleles identified in the 160 accessions, 21 alleles (35 %) occurred with low frequency (< 0.1). These 'rare alleles' were detected by all five markers: one rare allele was identified using FG2a/b, three alleles from FG7e/f, five alleles from FG1a/b and FG7a/b, and seven alleles from FG7c/d (Table 2). One allele detected with FG7c/d (291 bp) was detected only in a single accession, accession 80 (from United States), which was suggestive of an accession-specific allele. Of the 160 strawberry accessions, 53 % (84 accessions) possessed

Table 2 – List of five SSR primer pairs used for genotyping 160 strawberry accessions.

SSR ^a	Primer sequences ^b	No. of alleles scored	No. of rare alleles ^c	Peak ranges observed (bp)
FG1a/b	Forward : TGGTTTGCCGGTAGCAATAGCAGCA Reverse : TGACACACTCTCTGTCTGATCCCT	12	5	123-167
FG2a/b	Forward : TGAAGTGGTCCATCGGTGCTGAAA Reverse : TGATCACACAATACGCATTACCAAGCCCT	8	1	298-362
FG7a/b	Forward : GCAGTGCTACATCGACTCAGGTCCAA Reverse : ACCAAGGAAGTGCCGAAGTGGGTTT	13	5	153-240
FG7c/d	Forward : AGGTGTCCAAGAGGGTTGCTGTAGA Reverse : TCCCTCTCCAATAACCCCTTGCTTC	19	7	236-396
FG7e/f	Forward : ACGGTGCCGAGATGCCTGATTACT Reverse : GCTGATCTCCACTTCTCTCTATACCA	8	3	472-599

^aChambers et al., 2013, Mol Breeding; ^bReverse primers were shorter than those of reference due to deletion of M13 sequence; ^cAlleles with frequency lower than 0.1.

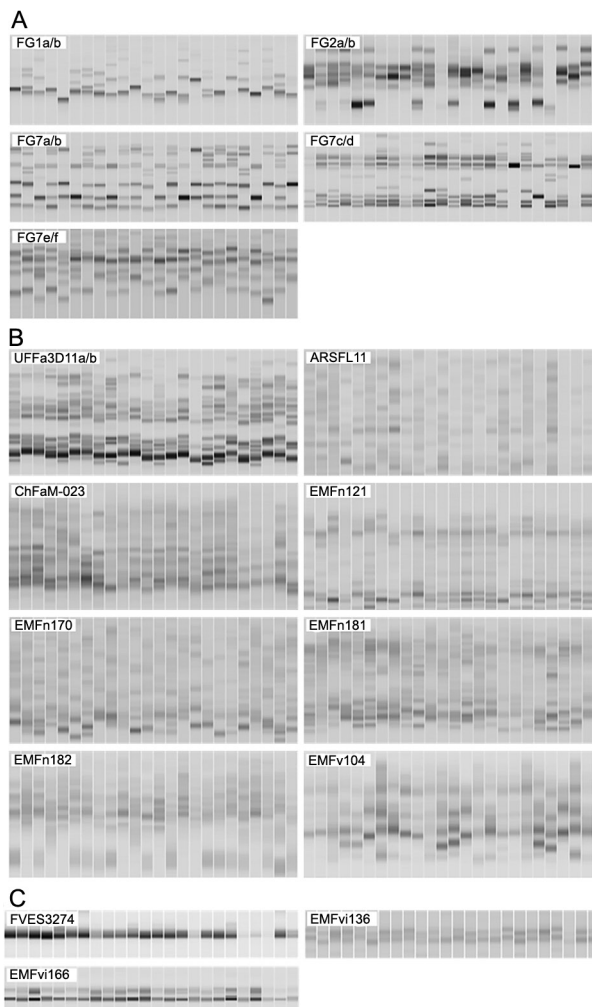


Figure 1 – Gel images of 24 strawberry accessions examined by 16 SSR markers. (A) Clear and highly polymorphic markers. (B) Dim or difficult to interpret markers. (C) Few polymorphic markers.

1–8 rare alleles, indicating the value of the rare alleles in discerning the different accessions. Of these 84 accessions, the largest number (32) was collected from the United States, followed by Japan (19), unknown origin (12), Korea (10), Europe (8), Canada (1), China (1), and New Zealand (1). One of the two Chinese accessions (accession 119) possessed five rare alleles, whereas the other Chinese accession (accession 118) showed no rare allele. This result indicated that the origin of the germplasm and the genetic relationship did not always correlated in strawberry resources.

Genetic diversity and classification of strawberry accessions

The genetic relationships between 160 strawberry accessions were analyzed using the 60 polymorphic alleles generated from the five SSR markers (Figure 3). Together, the five SSR markers distinguished 155 of the

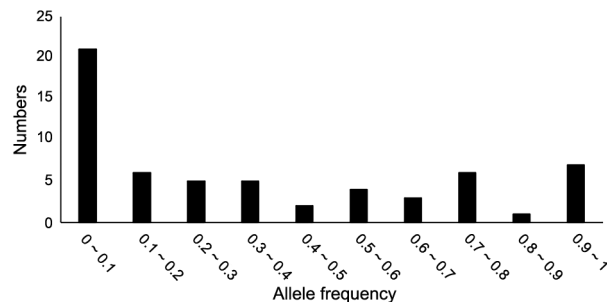


Figure 2 – A histogram of allele frequencies for 60 alleles in 160 strawberry accessions.

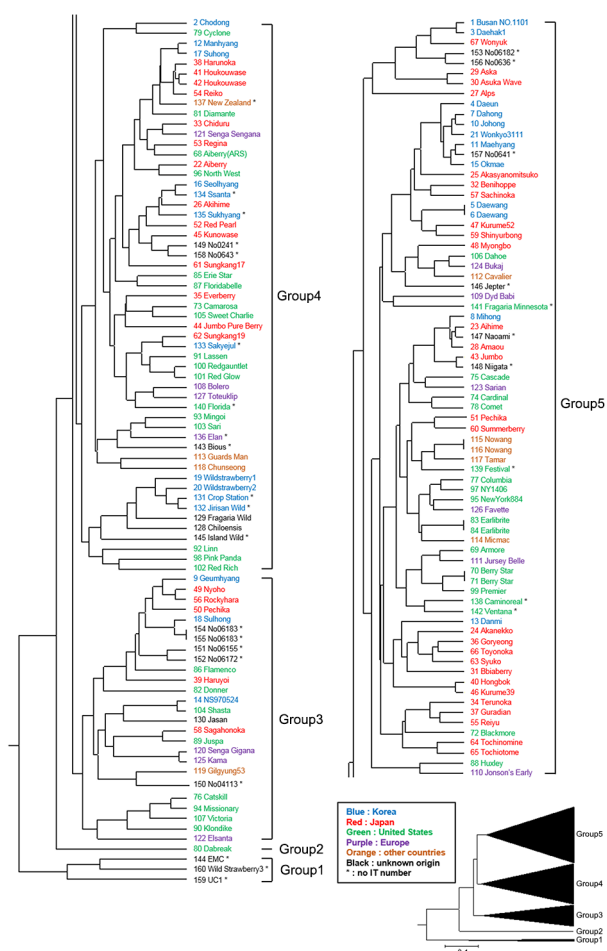


Figure 3 – Classification of 160 strawberry accessions based on their molecular diversity. Genetic similarities were calculated by UPGMA and the dendrogram was constructed using MEGA V.6.

accessions. The remaining accessions consisted of five pairs of duplicates (accession 83 (including its duplicate, accession 84), accession 115 (including its duplicate, accession 116), accession 70 (including its duplicate, accession 71), accession 5 (including its duplicate, accession 6), and accession 154 (including its duplicate, accession

155) that appeared to have the same allelic patterns for the five SSR markers (Figure 3). These results supported the effectiveness of our genotyping approach. However, two additional duplicates (accession 41 (including its duplicate, accession 42) and accession 50 (including its duplicate, accession 51)), which were collected separately, appeared to be of different genotypes. The two accessions 41 and 42 were similar but not identical (Figure 3), and both belonged to group 4. One allele (302 bp from FG7c/d) was present in accession 41 but was absent in accession 42. The two accessions 50 and 51 were genetically distant and belonged to groups 3 and 5, respectively (Figure 3). These two accessions differed by 10 alleles, suggesting that they were from different strawberry lines.

Excluding the five duplicate pairs, the genetic similarities of the strawberry accessions were in the range 0.034-0.963, with an average value of 0.507. The highest similarity value (0.963) was between accession 148 (unknown origin) and accession 43 (from Japan), and between accession 125 (from Russia) and accession 120 (from Germany). The lowest similarity value (0.034) was between accession 159 (unknown origin) and accession 119 (from China).

The 160 strawberry accessions were divided into five groups according to genetic distance (Figure 3). Group 1 contained two diploid *F. vesca* species (accession 159 and accession 144) and one new accession, accession 160. These accessions were dissimilar to other accessions and were genetically distant showing the lower genetic similarities with other accessions (0.1, 0.19, 0.16 for accession 159, accession 144, and accession 160, respectively) compared to the average genetic similarities among the 160 accessions (0.509). These three accessions possessed relatively few SSR alleles (5 in accession 159, 11 in accession 144, and 9 in accession 160), compared to the average from all accessions (21.9). This was consistent with the simpler genetic structure found in diploid species such as *F. vesca* compared to octoploid species (*F. chiloensis* and *F. × ananassa*). These data suggested that accession 160 belonged to *F. vesca* or another diploid species. Group 2 contained only one accession, accession 80, which was collected from the United States (Figure 3). One accession-specific allele (291 bp at FG7c/d) was detected in accession 80, alongside a further 16 SSR alleles.

Group 3 contained 20 *F. × ananassa* accessions (8 from the United States, 5 from Japan, 3 each from Korea and Europe, and 1 from China) and six unknown origin accessions (Figure 3). In this group, several accessions were closely grouped in line with their geographical origins. For example, three accessions from the United States (accession 94, accession 107, and accession 90) and three from Japan (accession 49, accession 56, and accession 50 (including its duplicate, accession 51)) were closely grouped. Four of the six unknown origin accessions (accession 154 (including its duplicate, accession 155), accession 152, and accession 151) were also closely

grouped, allowing for speculation that these may have originated in the same country. Of the 26 accessions in group 3, 16 (62 %) possessed at least one rare allele, which distinguishes the *F. × ananassa* accessions in this group from those in other groups.

The remaining 130 accessions were clustered into Groups 4 and 5, indicating minimal variability among these accessions within groups. Group 4 contained 48 *F. × ananassa* accessions (17 from United States, 13 from Japan, 11 from Korea, 4 from Europe, and 1 each from Canada, China, and New Zealand), one octoploid species (*F. chiloensis*), and six unknown origin accessions (Figure 3). *F. chiloensis*, which is one of the parents of *F. × ananassa* cultivars, was located in a subcluster with three wild strawberry accessions (accession 19, accession 20, and accession 129) and three new accessions (accession 131, accession 132, and accession 145).

Group 5 contained 69 *F. × ananassa* accessions (27 from Japan, 19 from the United States, 12 from Korea, 6 from Europe, 3 from Israel, and 2 from Canada) and six unknown origin accessions (Figure 3 and Table 1). Our five SSR markers confirmed the pedigree of some accessions such as accession 17 (the offspring of accession 41 and accession 42) and accession 38, and accession 54 (the grandparent of accession 12) (Figure 3 and Table 1). In addition, as shown in Figure 3 and Table 1, accession 134 was the offspring of accession 16, accession 26, and accession 52 were parents of accession 135, and accession 45 was one of the parents of accession 26. Certain accessions that shared at least one parent were also closely clustered. For example, accession 74 and accession 78 were both offspring of 'Earlibelle' and 'ARK5063'. Accession 139 and accessions 83 (including its duplicate, accession 84) shared the parent 'Rosalinda', and accession 139 and accession 117 shared the parent 'Osogrande' (Figure 3 and Table 1). By contrast, some accessions were in different groups despite sharing parents. For example, accession 39 (group 3) and accession 17 (group 4) were both offspring of accession 38 and 'Hokowase' (Figure 3 and Table 1). This result confirmed the previous observations of these accessions that clustered into the different group (Cho et al., 2007). Similarly, accession 16 (group 4) and accession 4 (group 5) were in different groups despite sharing parents (accession 26 and accession 52). This latter result was unexpected, as previous research had placed accession 16 and accession 4 in the same group (Hong et al., 2014) which might be due to the use of different batches of strawberry accessions or the number of accessions compared in two studies.

To date, most reports classifying Korean strawberry resources have examined only small numbers of accessions, such as those registered in the RDA-Genebank Information Center (<http://www.genebank.go.kr>). In this study, we presented an expanded dendrogram classifying 160 Korea strawberry breeding accessions, including 30 new, unregistered accessions. These new accessions were distributed throughout the dendrogram: three accessions in group 1, five in group 3, twelve in group 4,

and ten in group 5. Further phenotypic classification of the new accessions, coupled with our marker-associated analysis, will enhance their value in breeding programs. Many of the accessions characterized in this study were generated by preexisting breeding programs, and consequently exhibited minimal genetic variation. The characterization of 160 strawberry accessions in the study will allow breeders to design new breeding strategies to incorporate more genetic diversity into new cultivars.

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