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Geneva

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES  
AND DNA-PROFILING IN PARTICULAR**

**Fourteenth Session  
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CONSTRUCTION OF DNA PROFILE DATABASE OF STRAWBERRY VARIETIES USING SSR MARKERS

*Document prepared by an expert from Republic of Korea*

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The Annex to this document contains a copy of a presentation "Construction of DNA Profile Database of Strawberry Varieties using SSR Markers" made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Jee-Hwa Hong, Seed Testing & Research Center, Korea Seed & Variety Service (KSVS), Ministry of Agriculture, Food and Rural Affairs, Republic of Korea

[Annex follows]

[2014 BMT]

## Construction of DNA Profile Database of Strawberry Varieties using SSR Markers

Nov. 2014

Jee-Hwa Hong



Seed Testing & Research Center  
Korea Seed & Variety Service  
Ministry of Agriculture, Food and Rural Affairs



### Introduction

#### ◎ Strawberry

- The strawberry genus, *Fragaria*, belongs to the Rosaceae family, subfamily Rosoideae (Davis et al. 2007)
- The *Fragaria* is comprised of 23 species.
  - Cultivated strawberry is *Fragaria x ananassa*.
  - *F. vesca*, *F. nubiola*, *F. viridis*, *F. virginiana* and others

#### ◎ Karyotype of strawberry

- The basic chromosome number in *Fragaria* is  $x=7$  (Ichijima 1926).
- The *Fragaria* species comprise a polyploidy series.
  - Diploid( $2n=2x=14$ ), Tetraploids( $2n=4x=28$ ),  
Hexaploids( $2n=6x=42$ ), Octoploids( $2n=8x=56$ )



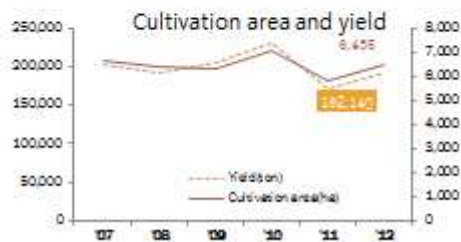


## Introduction

### ◎ Cultivation area of strawberry

- Cultivated area is located in Europe(63.3% of the total area), followed by Asia(14.8%) and North and Central America(13.8%)(Davis et al. 2007)

### ◎ Cultivation area and yield of strawberry in Korea



(Ministry of Agriculture, Food and Rural Affairs, 2013)

- Major cultivars
    - Seolhyang, Maehyang, Keumhyang
    - 78% of cultivation area(2013)
- (<http://www.krei.re.kr>)



## Introduction

### ◎ Status of plant variety protection(PVP) of strawberry in Korea

- **PVP** : A variety protection right have an exclusive right to exploit the protected variety commercially and industrially.
- From 7. Jan. 2012, strawberry was designated as crop for PVP

PVP Applications		Application by Origin	
2012	21	Korea	29
2013	18	Japan	12
2014	9	USA	5
<b>Total</b>	<b>48</b>	UK	1
		Netherlands	1

(KSVS, Oct. 2014)



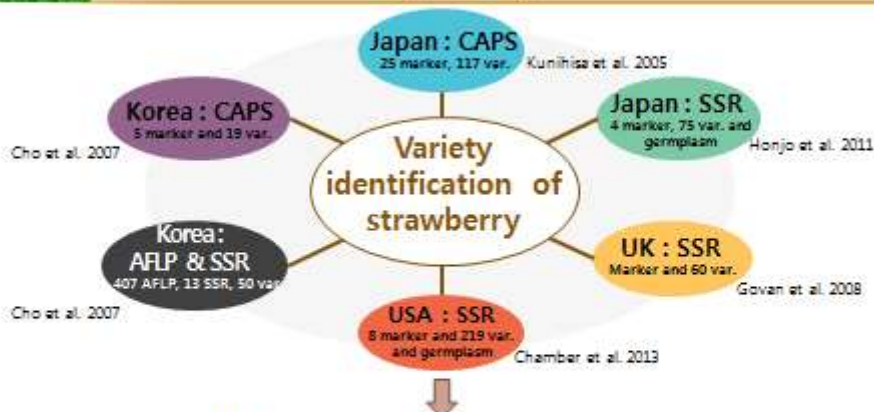
## Introduction

### ◎ Necessity of DNA marker development of strawberry in Korea

- Strawberry are capable of vegetative propagation via the production of runners.
- The unregulated propagation and distribution of patented varieties has become a serious problem (Kunihisa, 2010).
- Morphological characteristics are influenced by environment factors and difficult to impartial observer.
- Development of DNA marker and construction DNA profile database is needed to protect breeder's rights.



## Literature Reviews : Variety Identification of Strawberry using DNA Markers



- ◎ CAPS may also fulfill the BMT guideline but their use in DNA profiling database of plant varieties has not yet been explored.
- ◎ Limited SSR marker information and varieties
- ◎ New candidate varieties have strongly increased in Korea



## Objectives

- To select SSR markers for strawberry variety identification
- To construct of DNA profile database for strawberry varieties using SSR markers



## Materials

### ◎ 100 varieties (Origin : Korea 27)

No.	Variety name	Parentage	Origin
1	Nonsan 1	Tochinomina x Toyonoka	Korea
2	Daehak 1	Unpublished	Korea
3	Danmi	Maehyang x Amaou	Korea
4	Seonhong	Johong x Maehyang	Korea
5	Suhong	Hokowasa x Hatundea	Korea
6	Shinyeobong	Tissue culture	Korea
7	Sancheonwang	Hikangobuaka x Maehyang	Korea
8	Sinmyeong	Maehyang x Geumhyang	Korea
9	Sindaebong	Kaumhyang x Seolhyang	Korea
10	Sinseolmae	Seolhyang x Maehyang	Korea
11	Okhyang	Johong x Akihime	Korea
12	Santa	Maehyang x Seolhyang	Korea
13	Hanun	Seolhyang x Maehyang	Korea
14	Daesun	Akihime x Redpearl	Korea
15	Manhyang	Nyoho x Akanekko	Korea
16	Kaumhyang	Akihime x Tochtotome	Korea
17	Seolhyang	Akihime x Redpearl	Korea
18	Maehyang	Tochinomina x Akihime	Korea
19	Jukhyang	Redpearl x Maehyang	Korea
20	Damhyang	Akihime x Maehyang	Korea
21	Bukyong	Johong x Maehyang	Korea
22	Chodong	Hatunoka x Yachtlyo	Korea
23	Johong	Nyoho x Akihime	Korea
24	Daewang	Maehyang x Wongyo 3111	Korea
25	Josaenghongsim	Benizuru x Usosio	Korea
26	Eyebary Busan	Unpublished	Korea
27	Bagyedaeol	Unpublished	Korea



## Materials

### 100 varieties (Origin : Japan 33)

No.	Variety name	Parentage	Origin
20	Kurume	Selection of F. x ananassa	Japan
21	Kurume 49	Touropole x Nyohe	Japan
22	Reuchole	Unpublished	Japan
23	Tachinoma	Km 411 x Nyohe	Japan
24	Reuena	Unpublished	Japan
25	Nan-hoona	Akoma x Sechnole	Japan
26	Mitsuba 38	Fairfax x Akoma/Km 411 x Chusda	Japan
27	Mitsuba 47	Selection of F. x ananassa	Japan
28	Sakurita	(Akomy x Holokassa) x Touropole x (Akomy x Holokassa)	Japan
29	Imaru	Sakurita 24	Japan
30	Sauka Ruby	Sauka Wave x Nyohe	Japan
31	Sauka Wave	(Utsuho x (Kurume Sokusa'd x Holokassa) x (Donner x Holokassa)) x (Kurume Sokusa'd x Holokassa) x (Donner x (Kobari x Holokassa))	Japan
40	Raiko	Honolulu x Sukubara	Japan
41	Imurodashi	Unpublished	Japan
42	Chouji	(Honolulu x Honolulu/Km 411 x Honolulu)	Japan
43	Reuena	Mitsuba x The Sun x Sakuba	Japan
44	Raiko	Unpublished	Japan
45	Kurume 100	Mitsuba x The Sun x Sakuba	Japan
46	Sauka	Honolulu x Honolulu	Japan
47	Ko'oku	Unpublished	Japan
48	Suabaru	Chababikomi 2 x Honolulu	Japan
49	Honolulu	Kurume100 x Donner	Japan
50	Nagayatsuumi	Selection of F. x ananassa	Japan
51	Rikita	Unpublished	Japan
52	Akoma	Kurume x Nyohe	Japan
53	Nyohe	Km 210 x Raiko	Japan
54	Redheart	Akomy x Touropole	Japan
55	Reuchole	Touropole x Akomy	Japan
56	Fujaki 084	Donner x Rensburg	Japan
57	Touropole	Selection of F. x ananassa	Japan
58	Touropole/Kikomi	Selection of F. x ananassa	Japan
59	Touropole 17	Selection of F. x ananassa	Japan
60	Junho	Unpublished	Japan



## Materials

### 100 varieties (Origin : China 2, USA 27, other 1)

No.	Variety name	Parentage	Origin
61	Chuneeang	Unpublished	China
62	Chuncho	Unpublished	China
63		Unpublished	other
64	Albion	Diamanta x Cal94.16-1	USA
65	Blakemore	Missionary x Howard 17	USA
66	Ca31 5x1	Lesson x delf	USA
67	Jaspan	Unpublished	USA
68	Sweet Charlie	R80-456 x Palajo	USA
69	Summer	Titan x MDUS 2856	USA
70	Columbia	WA157 x WA175	USA
71	Donner	CA1222 x CA1145 52	USA
72	Marshall	Unpublished	USA
73	Camline	Joey Linda x R93-53	USA
74	Oriz	Cal. 37.20-45 x Sequoia	USA
75	Danow	MDUS2713 x MDUS2787	USA
76	Douglas	Tufts x 64.57-108	USA
77	Earldawn	Midland x Tennessee Shipper	USA
78	Earlflow	MDUS 2859 (Fairland x Midland) x MDUS 2713	USA
79	EarlMiss	Alborton x Tennessee Shipper	USA
80	MDUS 3839	Earldawn x MDUS 2713	USA
81	Midland	Howard 17 x Redheart	USA
82	Pelican	82-1556 P/Louisiana	USA
83	Prelude	Titan x NC 2987	USA
84	Red Cross	Ettersburg No. 216 x Teble	USA
85	Solana	CAL 177-12 x CAL 108-22	USA
86	Sunrise	USMD 4152 x Stelemaster	USA
87	Titan	NC 1787 x Alborton	USA
88	Toro	Unpublished	USA
89	Tai-Wan	Unpublished	USA
90	Vesper	Sparkle x Valentine	USA



## Materials

### © 100 varieties (Origin : UK 6, Canada 2, Netherlands 2)

No.	Variety name	Parentage	Origin
91	Eastern Pine	Cultivar grown since the late 1700s, original species from United States but grown in Europe since then.	UK
92	Amelia	Includes Pandora, Marmolada, Kent, and Providence	UK
93	Flamenco	Evita x EMR77	UK
94	Jucunda	Selection of cultivated strawberry from England	UK
95	Bolero	Selva x LA 988	UK
96	Pink Panda(Frel)	Fragaria x Comarum hybrid involving Fragaria chiloensis	UK
97	Blomidon	K72-4 x [Micmac x (Guardsman x Tioga)]	Canada
98	Cornwallis	Earlglow x Kent	Canada
99	Rabunda	Redgauntlet x Repita	Netherlands
100	Tango	Rapella x Selva	Netherlands



## Methods

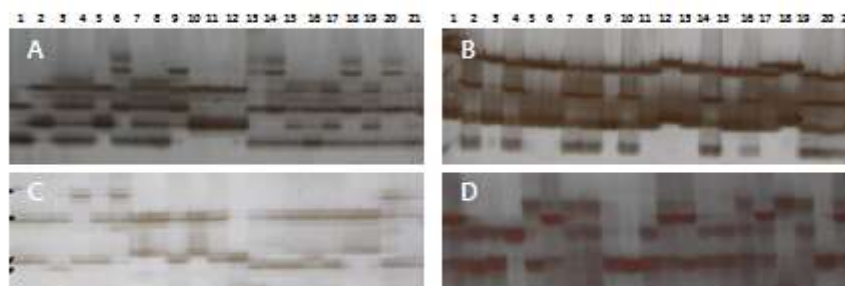
### © Simple Sequence Repeat(SSR) analysis

Classification	Selection of SSR markers	Construction of SSR profile database
Varieties	21 varieties	100 varieties
Primers	274 SSR markers	25 SSR markers
Genotyping	6% polyacrylamide gels, silver staining	ABI3130xl Genetic analyzer, GeneMapper software(Applied Biosystems, USA)
Data analysis	<ul style="list-style-type: none"> <li>• Polymorphism level : Polymorphism information content(PIC)(Anderson et al., 1993)</li> <li>• Genetic relationship : NTSYS-PC version 2.01(Rohlf, 1998), Jaccard's coefficient</li> </ul>	



## Results

### ◎ The selection of polymorphic SSR markers in 21 varieties



EMFvi179 (A), EMFvi109 (B), FAC-013 (C) and EMFv021 (D). The PCR products were separated in 6% polyacrylamide gel. Lane 1, 'Kurme 49'; 2, 'Seolhyang'; 3, 'Raiko'; 4, 'Radpean'; 5, 'Akihima'; 6, 'Jumbo'; 7, 'Chodong'; 8, 'Donner'; 9, 'Sukyong'; 10, 'Santa'; 11, 'Daeun'; 12, 'Albion'; 13, 'Blakemore'; 14, 'Fletcher'; 15, 'Akihima'; 16, 'Solar'; 17, 'Ca51Sr-1'; 18, 'Jaspan'; 19, 'Lihn'; 20, 'Marshall'; 21, 'Everberry'.



## Results

### ◎ Polymorphism of SSR markers between 21 varieties

No.	Type of SSR	Number of screened markers	Number of polymorphic markers	Polymorphism(%) of SSR markers	SSR marker source
1	gSSR	40	2	2/40 ( 5.0%)	Sargent et al. 2006
2	gSSR	19	1	1/19 ( 5.3%)	Monfort et al. 2006
3	gSSR	36	7	7/36 (19.4%)	Sargent et al. 2004
4	gSSR	13	5	5/13 (38.5%)	Sargent et al. 2003
5	gSSR	3	1	1/3 (33.3%)	Ashley et al. 2003
6	gSSR	70	11	11/70 (15.7%)	Lewers et al. 2005
7	gSSR and EST-SSR	37	7	7/37 (20.6%)	Bassil et al. 2006
8	gSSR	21	4	4/21 (19.0%)	Hadonou et al. 2004
9	EST-SSR	14	4	4/14 (28.6%)	Bassil et al. 2006
10	EST-SSR	21	3	3/21 (14.3%)	Rosaceae genome D/B
		<b>274</b>	<b>45</b>	<b>45/274 (16.4%)</b>	

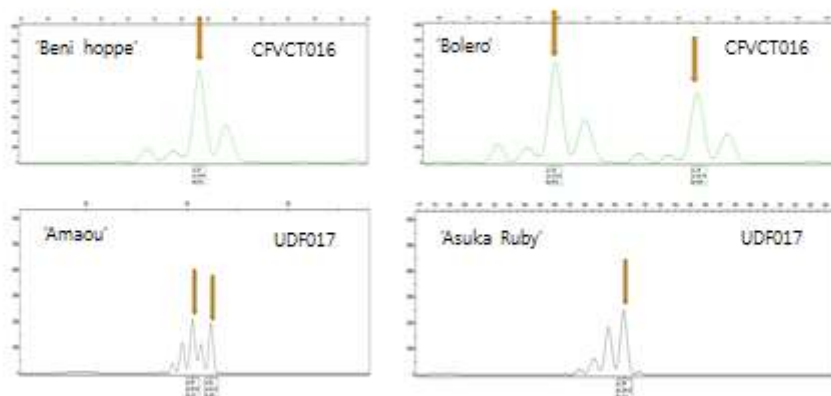
\*gSSR : genomic SSR, EST : Expressed sequence tag





## Results

### ① Detection of allele number and size at the CFVCT016 and UDF017 locus



The PCR products were separated using a Genetic Analyzer 3130XL and detected using GeneMapper software.



## Results

### ① SSR markers used to strawberry DNA profile database

No.	Type of SSR	Number of screened markers	Number of polymorphic Markers (First selection by polyacrylamide gel)	Number of database SSR markers (Second selection by sequencer)
1	gSSR	40	2	2
2	gSSR	19	1	1
3	gSSR	36	7	2
4	gSSR	13	5	5
5	gSSR	3	1	0
6	gSSR	70	11	6
7	gSSR and EST-SSR	37	7	6
8	gSSR	21	4	3
9	EST-SSR	14	4	0
10	EST-SSR	21	3	0
		<b>274</b>	<b>45</b>	<b>25</b>

- Ratio of SSR marker selection → **25/274 (9.1%)**



## Results

### Origin of 25 SSR markers of strawberry DNA profile database

Number of database SSR markers	Origin of SSR markers
12	<i>Fragaria x ananassa</i>
6	<i>F. vesca</i>
5	<i>F. viridis</i>
2	<i>F. nubicola</i>
Total 25	

- SSR markers of *F. vesca*, *F. viridis*, *F. nubicola* were also amplified in *Fragaria x ananassa*.



## Results

### Database of 25 SSR profiles for 100 strawberry varieties

Marker	Variety																								
SSR1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR7	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR9	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR11	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR12	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR13	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR14	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR16	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR17	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR18	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR19	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR21	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR22	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR23	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR24	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
SSR25	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25



## Results

### ⊙ Characteristics of 25 SSR markers in strawberry DNA database

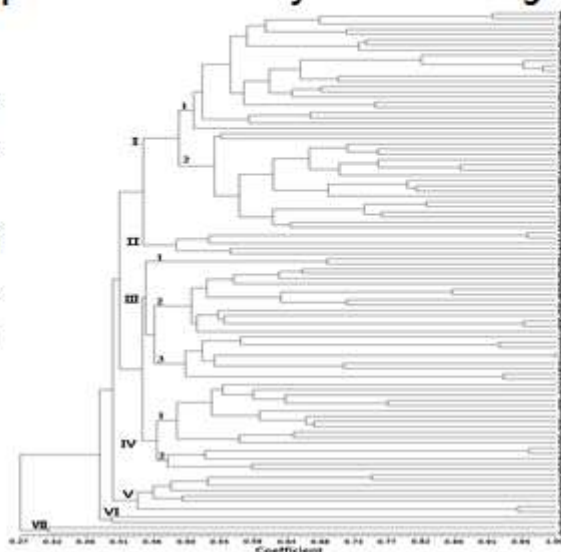
SSR marker	Repeat motif	Annealing temp	Product size (bp)	No. of alleles	PIC value
ARSFL_092	(CT)8(GA)13-1	55	170-183	8	0.759
ARSFL_099	(AC)13	55	163-183	7	0.668
ARSFL_100	(AT)7	55	242-264	5	0.553
ARSFL_101	(AG)6	55	184-219	7	0.740
CFVCT016	-	55	120-134	7	0.622
EMFnl160	(CT)24	55	135-195	13	0.764
EMFnl185	(GA)11	55	196-215	6	0.752
EMFV013	Perfect trinucleotide	55	206-218	6	0.711
EMFV016	Imperfect dinucleotide	55	211-256	9	0.790
EMFV021	Perfect dinucleotide	55	191-221	9	0.759
EMFVl025	(TG)8	55	244-256	8	0.631
EMFVl108	(AG)29	55	172-198	8	0.741
EMFVl109	(TC)4/(TC)7	55	251-275	7	0.801
EMFVl146	(AC)8	55	191-198	4	0.651
EMFVl179	(AG)36	55	133-155	7	0.721
FAC-003a	(TA)7	55	167-210	3	0.383
FAC-003b	(GA)3	55	301-327	10	0.779
FAC-013	(AAG)10	55	178-219	6	0.775
UDF017	-	55	176-202	11	0.789
UFFa02H04	(TC)6	55	197-213	6	0.750
UFFa11G07	(AT)8	55	164-178	7	0.698
UFFa11A11	(TC)11	55	220-278	11	0.841
UFFa13C07	(CA)6	55	163-184	5	0.621
UFFa18H04	(CT)14	55	133-168	6	0.726
UFFa20H10	(AT)7	55	211-236	9	0.709
<b>Total</b>				<b>188</b>	<b>18.347</b>
<b>Mean</b>				<b>7.5</b>	<b>0.706</b>



## Results

### ⊙ Genetic relationship of 100 strawberry varieties using 25 SSR markers

- 25 SSR markers identified 98 varieties except for 2 varieties
- Cluster analysis of 100 varieties was categorized into 7 major groups
- Genetic distance - 0.27~1.00





## Results

◎ **Group I : 42 varieties** → divided into 2 groups at 0.40 genetic similarity

Group I	No.	Variety name	Origin
Group I-1 (23 var.)	1	Nonsan 1	Korea
	40	Reiko	Japan
	28	Kunowase	Japan
	54	Redpearl	Japan
	55	Sachinoka	Japan
	33	Beni hoppe	Japan
	60	Jumbo	Japan
	41	Himesodachi	Japan
	5	Suhong	Korea
	15	Manhyang	Korea
	22	Chodong	Korea
	71	Donner	USA
	42	Chizuru	Japan
	49	Harunoka	Japan
	37	Amaou	Japan
	44	Takane	Japan
	62	Chuncho	China
	34	Morioka 16	Japan
	35	Morioka 17	Japan
	30	Derunoka	Japan
	46	Syuko	Japan
	56	Fujisaki 068	Japan
	36	Akanekko	Japan

- Varieties showed high genetic similarity
- 'Chodong' and 'Donner': 95% genetic similarity

<Pedigree>



## Results

◎ **Group I : 42 varieties**

Group I	No.	Variety name	Origin
Group I-2 (19 var.)	3	Danmi	Korea
	19	Jukhyang	Korea
	4	Seonhong	Korea
	23	Johong	Korea
	52	Akihime	Japan
	7	Sancheonwang	Korea
	18	Maehyang	Korea
	24	Daewang	Korea
	21	Sukyong	Korea
	8	Sinmyeong	Korea
	10	Sinseolmae	Korea
	16	Keumhyang	Korea
	9	Sindaebong	Korea
	12	Santa	Korea
	20	Damhyang	Korea
	14	Daemun	Korea
	17	Seolhyang	Korea
	31	Tochinomine	Japan
	53	Nyoho	Japan

- There was no high genetic similarity varieties in group I-2.
- The most strawberry varieties developed in Korea were categorized into group I-2.
- Because most korea varieties are crossed from 'Maehyang' and 'Tochinomine', 'Akihime', 'Nyoho'.



## Results

### ○ Group II : 5 varieties

Group II	No.	Variety name	Origin
Group II (5 var.)	2	Daehak 1	Korea
	25	Josaenghongsim	Korea
	26	Eyeberry Busan	Korea
	43	Benizuru	Japan
	45	Kurume 103	Japan

- Varieties showed high genetic similarity
- 'Daehak1' and 'Josaenghongsim' : 95% genetic similarity



## Results

### ○ Group III : 24 varieties → divided into 3 groups at 0.46 genetic similarity

Group III	No.	Variety name	Origin
Group III-1 (2 var.)	6	Shinyeobong	Korea
	69	Summer	USA
Group III-2 (13 var.)	11	Okhyang	Korea
	68	Sweet Charlie	USA
	13	Hanun	Korea
	66	Ca51 Sr-1	USA
	63		Other
	88	Toro	USA
	74	Cruz	USA
	89	To-Wan	USA
	64	Albion	USA
	73	Carmine	USA
	93	Flamenco	UK
	95	Bolero	UK
	76	Douglas	USA
Group III-3 (9 var.)	27	Sagyeojeol	Korea
	32	Rowang	Japan
	48	Everberry	Japan
	38	Asuka Ruby	Japan
	39	Asuka Wave	Japan
	85	Solana	USA
	86	Sunrise	USA
29	Kurume 49	Japan	
47	Kofuku	Japan	

- Varieties showed high genetic similarity
- 'Asuka Ruby' and 'Asuka Wave' : 100% genetic similarity

<Pedigree>

'Asuka Wave' X 'Nyoho'



'Asuka Ruby'



## Results

⊙ **Group IV : 17 varieties** → divided into 2 groups at 0.47 genetic similarity

Group IV	No.	Variety name	Origin
IV-1 (12 var.)	51	Okitsu	Japan
	57	Tonami	Japan
	79	EarliMiss	USA
	83	Prelude	USA
	87	Titan	USA
	70	Columbia	USA
	77	Earlidawn	USA
	80	MDUS 3839	USA
	81	Midland	USA
	78	Earliglow	USA
	98	Cornwallis	Canada
	97	Blomidon	Canada
IV-2 (5 var.)	59	Tohoku 13	Japan
	90	Vesper	USA
	82	Pelican	USA
	65	Blakemore	USA
	67	Jaspan	USA

- Varieties showed high genetic similarity
- 'Tohoku 13' and 'Vesper' : 95% genetic similarity



## Results

⊙ **Group V : 8 varieties**

Group V	No.	Variety name	Origin
V (8 var.)	58	Tonami-zairai-shik inari	Japan
	100	Tango	Netherlands
	99	Rabunda	Netherlands
	96	Pink Panda(Frel)	UK
	84	Red Cross	USA
	94	Jucunda	UK
	72	Marshall	USA
	92	Amelia	UK

- 'Marshall' and 'Amelia' : 94% genetic similarity

⊙ **Group VI : 2 varieties**

Group VI	No.	Variety name	Origin
VI (2 var.)	50	Nagoya-oomi	Japan
	61	Chunseong	China

⊙ **Group VII : 2 varieties**

Group VII	No.	Variety name	Origin
VII (2 var.)	75	Darrow	USA
	91	Eastern Pine	UK



## Conclusions

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- ◎ Out of 274 SSR primer pairs, **25 primer pairs** were produced reliable, reproducible and easy to interpret fingerprint.
- ◎ **DNA profile database for 100 cultivated strawberries were constructed by 25 SSR markers.**
- ◎ Cluster analysis was categorized into **7 major groups** reflecting breeding site and pedigree information.
- ◎ **98 varieties** except for two varieties could be discriminated by marker genotypes.
- ◎ These markers will be useful as a tool for **protection of plant breeders' intellectual property rights** in addition to means to resolve seed disputes relating to **variety authentication**.



## Future Plans

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- ◎ **Application of SSR markers to DUS test**
  - Selection of similar varieties through genetic relationship analysis between existing varieties and candidate varieties
- ◎ **Correlation relationship between marker genotypes and morphological characteristics** (UPOV option 2 approach)



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*Thank you*

Jee-Hwa Hong : hongjh19@korea.kr

[End of Annex and of document]