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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS**  
Geneva

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

**Fourteenth Session  
Seoul, Republic of Korea, November 10 to 13, 2014**

**DEVELOPMENT OF EST-SSR MARKERS OF LETTUCE AND VARIETY IDENTIFICATION USING  
EST-SSR MARKERS**

*Document prepared by an expert from Republic of Korea*

*Disclaimer: this document does not represent UPOV policies or guidance*

The Annex to this document contains a copy of a presentation “Development of EST-SSR Markers of Lettuce and Variety Identification using EST-SSR Markers” made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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[Annex follows]



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### ◎ Introduction

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## Lettuce (*Lactuca sativa* L.)

### ◎ Lettuce

- Lettuce is a member of the family Asteraceae(Compositae), a diverse group of plants with a global distribution
- Cultivated lettuce (*Lactuca sativa* L.)
- Wild species (*Lactuca serriola* L. *Lactuca saligna* L. *Lactuca virosa* L. )

### ◎ Morphological type of lettuce (Kristkova et al, 2008)



Number of PVP in vegetable crops	
1	Pepper (163)
2	Chinese cabbage (126)
3	Radish (74)
4	Lettuce (63)
5	Watermelon (47)



## Variety Identification (1.Morphological characteristics)

**Variety:** expression of the characteristics resulting from a given genotype or combination of genotypes (UPOV 1991 Act, Article 1, vi)

### ◎ Requirement of PVP

- **Distinctness**: Clearly distinct compared to the similar variety
- **Uniformity**: uniform per every individual
- **Stability**: uniform per year or growing cycle

### ◎ DUS test : Long time and labor intensive, influenced by environment

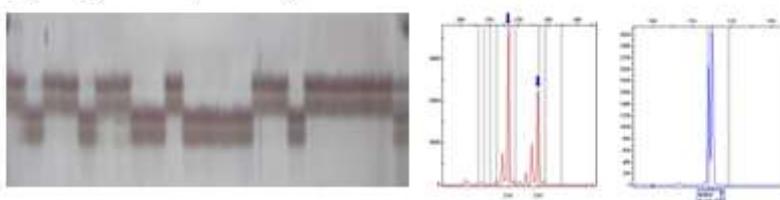


(Candidate variety : Similar variety)



## Variety Identification (2. DNA analysis)

- ◎ **DNA marker** : A short specific DNA sequence with a known location on a chromosome ↗ **Tool for testing difference of DNA sequence**
- ◎ Variety identification using difference of DNA sequence
- ◎ Independent from the environment, year, growth stage, easy to impartial observer
- ◎ Difficult to practical use due to the low correlation between genotype and phenotype



## Possible Application Models of DNA Markers in UPOV

### ◎ Application models of DNA markers in DUS test

- Option 1 : Use of DNA markers directly linked to characteristics (gene-specific markers) e.g. disease resistance characteristics
- Option 2 : Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics
- Option 3 : Use of DNA markers as threshold levels for judging distinctness ↗ **No consensus**
- Option 4 : Combining phenotypic and molecular distance in the management of variety collections



## BMT Guideline

### ◎ Molecular marker selection

- Reproducibility of data production between laboratories and detection platforms (different types of equipment)
- Repeatability over time
- Discrimination power
- Possibilities for databasing
- Accessibility of methodology



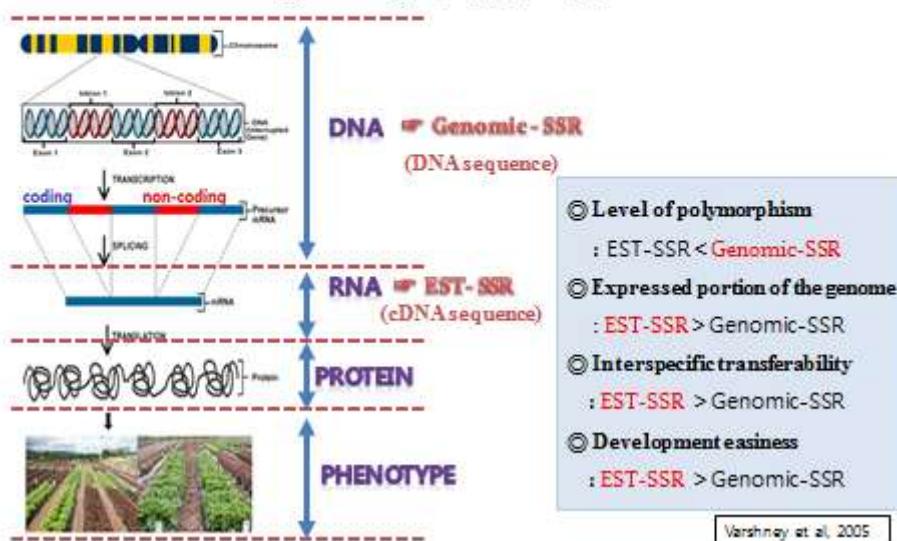
Simple Sequence Repeat (SSR)  
Single Nucleotide Polymorphism (SNP)

UPOV, 2010

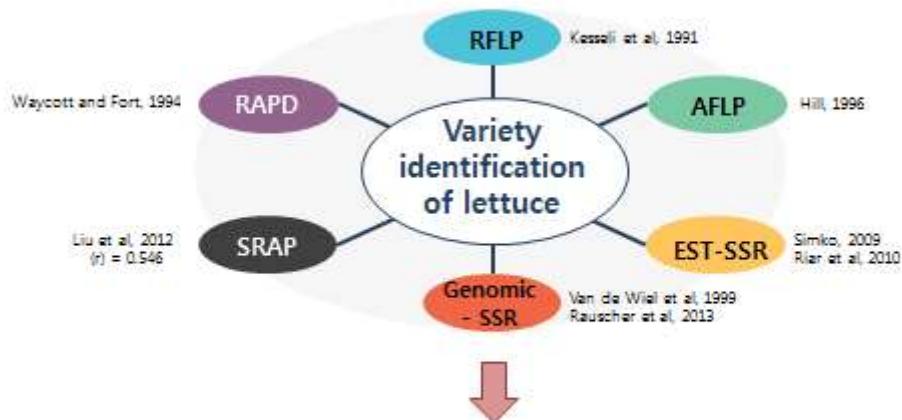


## Two Types of SSR Markers

- ◎ SSR markers : Different number of repeats in microsatellite regions e.g. (AT)<sub>18</sub> (TCT)<sub>12</sub>



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



- ◎ Limited SSR marker information
- ◎ Difficult to exact allele size due to the poly acrylamide gel analysis
- ◎ Low correlation between DNA markers and morphological characteristics

## Objectives and Experiments

### Objectives

- To develop EST-SSR markers of lettuce(*Lactuca sativa*)
- To investigate applicability of EST-SSR markers to DUS test

### Experiments

- Experiment 1 : Development of EST-SSR markers
- Experiment 2 : Variety identification and DNA profile database construction using EST-SSR markers
- Experiment 3 : Distinctness test of lettuce and correlation between EST-SSR markers and morphological characteristics



## Materials and Methods

### Experiment 1. Development of EST-SSR markers

① Download of 81,330 *L. sativa* ESTs from NCBI

② Sequence assembly

- Software : CAP3 (Huang and Madan, 1999)

③ SSR detection

- Di(6 times repeat), Tri(5), Tetra(5), Penta(4), Hexa(4)
- Software : MISA (<http://pgrc.ipk-gatersleben.de/misa/>)

④ EST-SSR primer design

- Parameters : 18-27 nucleotides with product size 100-300bp, annealing temperature of 50°C-60°C, GC content 20°C-80°C
- Software : Primer3 (Rozen et al, 2000)



### Experiment 2. Variety identification and DNA profile database construction using EST-SSR markers

Classification	SSR marker selection	SSR profile database
Varieties	8~23 varieties	156 varieties + 16 germplasm
Primer	352 primers Searched markers(81) + This study markers(271)	High reproducibility and peak clearance
Genotyping	6% polyacrylamide gels, silver staining	Genetic analyzer(ABI3130xl)
Data analysis	<ul style="list-style-type: none"><li>• Level of polymorphism : Polymorphism information content(PIC) (Anderson et al, 1993)</li><li>• Genetic relationship : NTSYS-PC version 2.01(Rohlf, 1998), Jaccard's coefficient</li><li>• Blast analysis between markers and proteins : BLASTX (Altschul et al, 1990)</li></ul>	



### Experiment 3 : Distinctness test and correlation between EST-SSR markers and morphological characteristics

- ① **Varieties** : 7 group containing 16 varieties(genetic similarity 93~100%)
- ② **Field and year** : Suwon, 2011 & 2012(2 years)
- ③ **DUS test** : 29 traits among 33 leaf traits
- ④ **Breeder's DUS test (Blind test)**
  - Kwonnong (1), Nongwoo (1), Asia (2); breeder 4 peoples



- ⑤ **Correlation between EST-SSR and morphological characteristics**
  - Use of 30 EST-SSR markers result
  - NTSYS-PC version 2.02 (Rohlf, 1998), Mantel (1767) matrix



### Results

<Experiment 1>  
**Development of EST-SSR markers in Lettuce**



## Assembly of EST Sequence

- ◎ Sequence alignment after download EST data of lettuce



## SSR Detection of EST Singletons

- ◎ Summary of the *in silico* search for EST singletons sequence for SSR in lettuce (*L. sativa*)

Parameters used in screening	Data generated by MISA	
Total number of sequences examined	41,609	
10.2% ↗ Total number of identified SSRs	4,229	(100%)
Dinucleotide(2)	1,256	(29.70%)
Trinucleotide(3)	2,500	(59.12%)
Tetranucleotide(4)	87	(2.06%)
Pentanucleotide(5)	106	(2.51%)
Hexanucleotide(6)	280	(6.62%)



## SSR Detection of EST Contigs

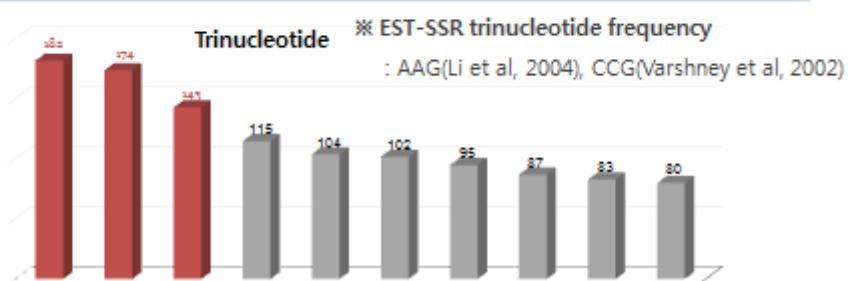
◎ Summary of the *in silico* search of EST contigs sequence for SSRs in Lettuce(*L. sativa*)

Parameters used in screening	Data generated by MISA	
Total number of sequences examined	8,452	
9.5% ↗ Total number of identified SSRs	807	(100%)
Dinucleotide(2)	266	(32.96%)
Trinucleotide(3)	455	(56.38%)
Tetranucleotide(4)	13	(1.61%)
Pentanucleotide(5)	31	(3.84%)
Hexanucleotide(6)	42	(5.20%)



## Nucleotide Frequency of SSR Repeat Motifs

◎ Number and frequency of major repeat motifs of EST-SSR in lettuce





## EST-SSR Primer Design

- Number of primers and SSR motifs designed from SSR sequences using primer3 program

SSR sequences source	Primer design	SSR motif	
Singleton 4,229 SSR	471	Dinucleotide	175
		Trinucleotide	68
		Tetranuclotide	1
		Pentanucleotide	1
		Compound formation	226
Contig 807 SSR	710	Dinucleotide	202
		Trinucleotide	374
		Tetranuclotide	13
		Pentanucleotide	28
		Hexanucleotide	39
		Compound formation	54
<b>Total</b>		<b>1,181</b>	



## SSR Markers from EST Singleton SSR

- Description of 15 EST-SSR markers among 471 EST-SSR markers developed from EST singletons of *L. sativa*.

No.	Primer name	EST ID	Repeat motif	Forward primer	Reverse primer	Product size
1	KSL-1	CLSS10549	(CAA)10	CACCACTCCATTTCATCCCA	GCTCATTCGCCAACCCAGAT	170
2	KSL-2	CLSS10515	(AAG)5aa(GAT)5	GCGAAGAACGAGAAAGATGA	AACTCCCGACGGTGTGTCAC	172
3	KSL-4	CLSS10504	(TC)2	CGGGGGTACTGTGTATATATCTA	ACGAGGAGTAGGATCGGGTT	145
			TCTG			
4	KSL-5	CLSS10587	(TC)15	TGGTCTTCATACTCAGGGGCT	CTTCTTGGGAGAGAGACCG	151
5	KSL-6	CLSS10530	(TC)21	GGGATCCTAACCAAAACACAAC	AGGGGGAACTGGTAAACACAG	144
6	KSL-7	CLSS10499	(TCT)12	TGCTCAATCTCGAGCTTATCT	ATGTCGCCAACAGGAAGACAA	279
7	KSL-8	CLSS10465	(AG)15	CTCTCGCTGAGGTTTTCT	CTTGGGGAGGGAAATTGGAA	277
8	KSL-9	CLSS10449	(AG)7(AG)8	ATCATCGCTAAGGCGGAACCT	TACAACTGCCTTGCTTCCTC	107
			(AAG)(delgggggggggggggggg)			
9	KSL-10	CLSS10273	GAAGATH	ACGAAAGGAGGAGGGAGAG	GCATCCGGCAACCCACTAATA	275
10	KSL-11	CLSS10145	(GAA)22	GGAAACCAAAAGGAGGAAACCA	CTACTAAACCGCTCCGGCGAG	255
11	KSL-12	CLSM9993	(TC)7tg(AC)8	GGGGGGGATCCATCTACAA	TTTGGTTGGGTGTTGTTGTTG	102
			(ATG)(delgggggggggggggggg)(A)			
12	KSL-13	CLSM9990	TG15	ACGAGGATGAGGATGAAGCA	AACAAAGCCCTTCATTTGCG	262
13	KSL-17	CLSM9357	(GT)14	CTGGCATGATGCGAGGTT	TGACAAAAATGCCCTCATGT	282
14	KSL-18	CLSM9350	(TC)8gggttg(GA)7	TCTGTATCCATAAGGGCCATC	AACAAAGGGTGGGGTTGAGG	203
			(TAC)(delaaaaaccggccggccccc)			
15	KSL-19	CLSM9307	gggggggggggggggggggggggt	GCTTTGGCAAAATGATCAA	TGAGAGGATTCAAGGGTGGAA	277
			ACGT			

A small green sprout with two leaves growing out of a patch of green grass against a blurred green background.

SSR Markers from EST Contigs SSR

- Description of 15 EST-SSR markers among 710 EST-SSR markers developed from EST contigs of *L. sativa*.

## Results

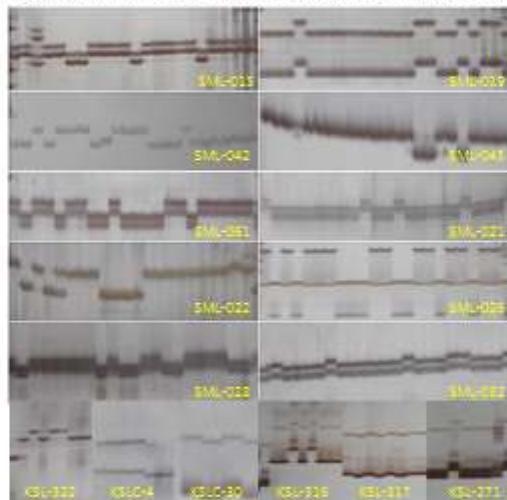
## <Experiment 2>

## Variety identification and DNA profile database construction using EST-SSR markers



## Selection of EST-SSR Markers

- Selection of polymorphic and band clearance markers from 352 SSRs



Silver-stained denaturing polyacrylamide gel electrophoresis(PAGE) of PCR amplification in 8~23 lettuce varieties.



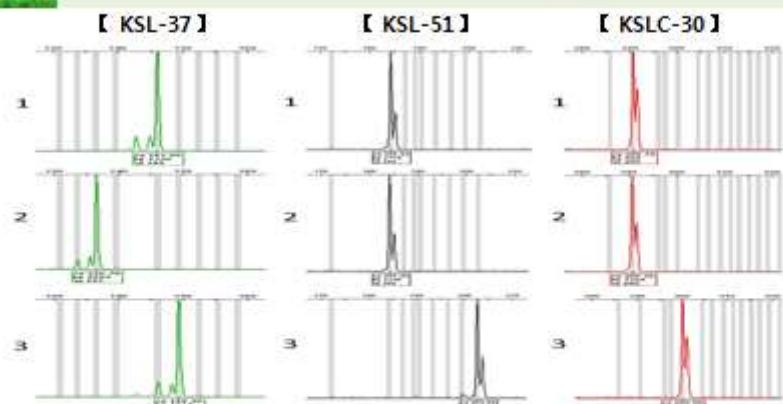
## Polymorphism of Screened SSR Markers

- SSR markers screened for identifying lettuce varieties and polymorphism of amplified SSR markers using polyacrylamide gel electrophoresis.

Type of SSR markers	Number of screened markers (A)	Polymorphism of amplified SSR markers (B)	Polymorphism % (B ÷ A)	SSR marker source
EST-SSR of lettuce	20	1	5%	Jeuken et al. (2008)
	61	30	49.2%	Simko (2009)
	271	47	17.3%	In this study
	352	78	22.2%	

- Polymorphism ratio showed 5% ~ 49.2%.
- Out of 78 SSR markers, selection of 60 SSR markers showing high polymorphism, repeatability and reproducibility.
- Attachment of fluorescence dye(VIC, NED, FAM, PET) to forward primers of 60 SSR.

Detection of Allele Size using Genetic Analyzer



Amplified fragments for 3 EST-SSR markers from 3 following samples.  
(1)Joara; (2)Evergreen; (3)Hotred.

- EST-SSR markers showed peak clearance more better than genomic-SSR (Leigh et al, 2003; Simko, 2009)
  - Easiness of allele size detection

 Construction of SSR Profile Database

© Construction of SSR profile database containing 156 lettuce varieties

- Allele present 1, absent 0

## Characteristics of 60 EST-SSR Markers

◎ 60 EST-SSR markers selected for identification of lettuce varieties

No.	Primer name	EST/Config ID	Repeat motif	Annealing (%)	Product size (bp)	No. of alleles	TIC	Primer labeling
1	SVL-001	OGC15N13	(CATGAT)6	55	176-198	5	0.526	VIC
2	SVL-002	OGH4c05	(TTC)17	55	171-235	6	0.294	NSQ
3	SVL-003	OGA14A20	(GTTTT)5	55	114-119	2	0.435	FAM
4	SVL-007	OGG10L02	(TCACCA)19	55	184-202	3	0.497	PET
5	SVL-013	Chrg-4735	(GAA)14...(CTG)5, (GAA)7...-(CTG)2	55	285-276	4	0.460	VIC
6	SVL-015	Chrg-1438	(TGTTA)16	55	254-269	3	0.484	NSQ
7	SVL-019	Chrg-1235	(ATATG)5, (ATATG)4	55	183-188	2	0.309	FAM
8	SVL-020	Chrg-419	(AATG)6, (AATG)5	55	223-237	2	0.397	PET
9	SVL-025	Chrg-4546	(CCA)4, (CCA)3	55	159-192	2	0.372	VIC
10	SVL-029	Chrg-5032	(CCCGTT)3, (CCCGCTT)1	55	229-235	2	0.397	NSQ
11	SVL-042	Chrg-6454	(GGAA)11, (AGGA)3(AAG(A)11)(GAAAGA)11	55	189-193	3	0.476	FAM
12	SVL-043	Chrg-649	(T)14, (T)10	55	192-198	4	0.419	PET
13	SVL-045	Chrg-T475	(AAG)9, (AAG)12	55	220-238	3	0.345	VIC
14	SVL-046	Chrg-7235	(T)12, (T)19	55	114-119	2	0.403	NSQ
15	SVL-051	OGC13L24	(TAA)6	55	237-240	2	0.380	FAM
16	SVL-052	Chrg-2202	(CAT)5, (CAT)5(GAGGGAAAT	55	219-297	4	0.306	PET
17	SVL-054	Chrg-3849	(TA)3, (TA)4	55	269-271	2	0.300	VIC
18	SVL-055	Chrg-2688	(TGA)15...(ATG)9	55	234-242	3	0.435	NSQ
19	SVL-056	OGG4h11	(TTA)14	55	183-189	2	0.339	FAM
20	SVL-057	OGG14L15	(GAA)14...-(CTG)5	55	266-276	4	0.462	PET

## Characteristics of 60 EST-SSR Markers

◎ 60 EST-SSR markers selected for identification of lettuce varieties

No.	Primer name	EST/Config ID	Repeat motif	Annealing (%)	Product size (bp)	No. of alleles	TIC	Primer labeling
21	SVL-059	Chrg-4484	(TCT)12, (TCT)13	55	177-212	4	0.535	VIC
22	SVL-060	OGG20P12	(TTC)12	55	174-188	2	0.025	NSQ
23	SVL-061	Chrg-2414	(AAC)6	55	203-207	2	0.412	FAM
24	SVL-021	Chrg-1077	(TA)5, (TA)4	55	172-180	4	0.418	PET
25	SVL-022	Chrg-2111	(ATC)13, (ATC)6	55	214-235	4	0.514	VIC
26	SVL-026	Chrg-2451	(GAA)11, (GAA)6	55	172-195	3	0.479	NSQ
27	SVL-028	Chrg-2759	(A)15, (A)9	55	170-181	2	0.311	FAM
28	SVL-032	Chrg-2480	(T)14, (T)6	55	214-215	2	0.484	VIC
29	SVL-036	Chrg-4484	(TCT)12...-(CCAAA)4, (TCT)13...-(CCAAA)4	55	214-230	3	0.149	NSQ
30	SVL-037	Chrg-4499	(AAC)3, (AAC)2	55	211-214	2	0.382	FAM
31	KSL-1	CLSS10549	(CAA)10	55	182-171	3	0.164	PET
32	KSL-27	CLSM1424	(AGA)15	55	123-175	7	0.673	VIC
33	KSL-31	CLSZ1624	(ATG)10	55	184-202	3	0.487	NSQ
34	KSL-371	CLSS1212	(ATG)12	55	218-230	3	0.448	PET
35	KSL-316	OGC5F02	(AAT)11	55	272-281	2	0.484	VIC
36	KSL-317	OGC5E09	(ATG)11	55	242-251	3	0.505	NSQ
37	KSL-357	CLSS10922	(TGA)14	55	255-283	2	0.431	VIC
38	KSL-37	CLSY3704	(CT)17	55	256-272	7	0.625	NSQ
39	KSL-172	CLSW1444	(CT)14	55	151-169	7	0.665	FAM
40	KSL-245	CLSM813	(AG)16	55	269-275	2	0.164	PET

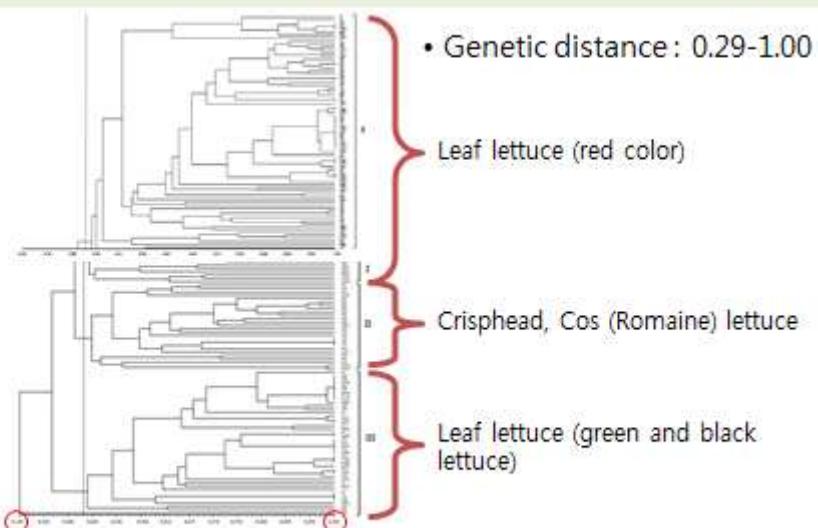
 Characteristics of 60 EST-SSR Markers

- 60 EST-SSR markers selected for identification of lettuce varieties

- Number of alleles : 3.5 per locus (van de Wiel et al, 1999)
  - PIC : 0.32 EST-SSR(Simko, 2009), 0.56 Genomic-SSR(Rauscher and Simko, 2013)



## Genetic Relationships between Varieties



Dendrogram constructed based on Jaccard's similarity coefficient from 60 EST-SSR data on 156 lettuce varieties.

## Groups for Varieties of genetic similarity 100%

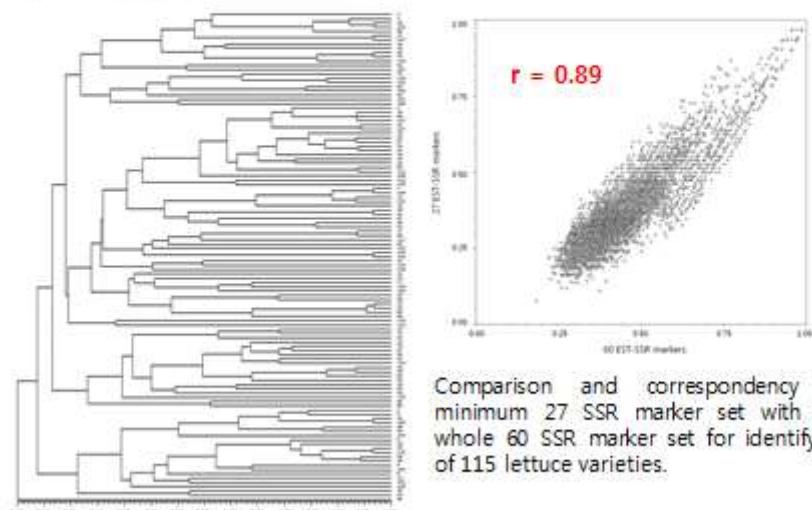
### ◎ Varieties depicting 100% of genetic similarity by SSR markers

No. of group	Varieties of genetic similarity 100%	Number of variety
1	Yeoljungeokchima(Kwon-nong), Mujeokchima(Mise), Todamjeokchima(ks)	3
2	Sunmangjeokchima(yeon)ion gwo (Bo.) Redpanjeokchima(yeo)(Daenong)	2
3	Ongungeokchima(Kwon-nong), Jinppajochachima(Ara)	2
4	Manchuricata(Takki), Manchussonita(Gyeongnong)	2
5	Sungungbo(Kwon-nong), Nuberone(Kwon-nong)	2
6	Mulgungjeokchima(yeon)Tangmangjeokchima(Kwon-nong), Mungjeokchima(Yeo), Gengjeokchima(yeon), Mai, Tukmepichima(Yeo), Tukmepichima(Sungyo), Ardeokchima(Yeo), Gammjeokchima(Yeo), Sungmepichima(Gwah), Bo, Tukmepichima(yeong), Tukmepichima(yeond), Yeonbiam(Gyeongnong)	12
7	Rubia(Seedless), Powanjeokchima(yeon)(Semi), Saebokchima(Yeo)	3
8	Hanjekchima(Greenheart Bo.), Jeokchima(Aram), Jeokchima(Sungyo)	3
9	Yeonunghyangdameo(Bo.), Cheongyongyo(Bo.), Saeokchima(Dongyimongnang)	3
10	Chonggeumchima(Kwon-nong), Ssakmacheongchima(Bo), Gengchima(Bo), Hwachachima(Bo), Hwangchima(Gwah), Cheongchima(Bo), Yeonchima(Dongyimongnang)	8
11	Yeonjeokchima(Bo.), Gangjapek(Aram)	2
12	Cheongeungchima(Kwon-nong), Blackaujeokchima(Semi Bo.)	2
13	Cheongeungchima(Kwon-nong), Iipmyeoreumcheongchima(Bo), Cheongchima(Aram), Greencheongchima(Greenheart Bo.), Cheongchima(Sungyo)	5
14	Meokchima(Kwon-nong), Meokchimmasangch(yeond), Sakatameokchima(Sakata)	3
15	Sunheukchima(Yeo), Sunheukchima(Aram)	2
16	Sembokmeokchima(Kwon-nong), Heukassemekchima(Ara), Meddo(Syngenta)	3
Total		57

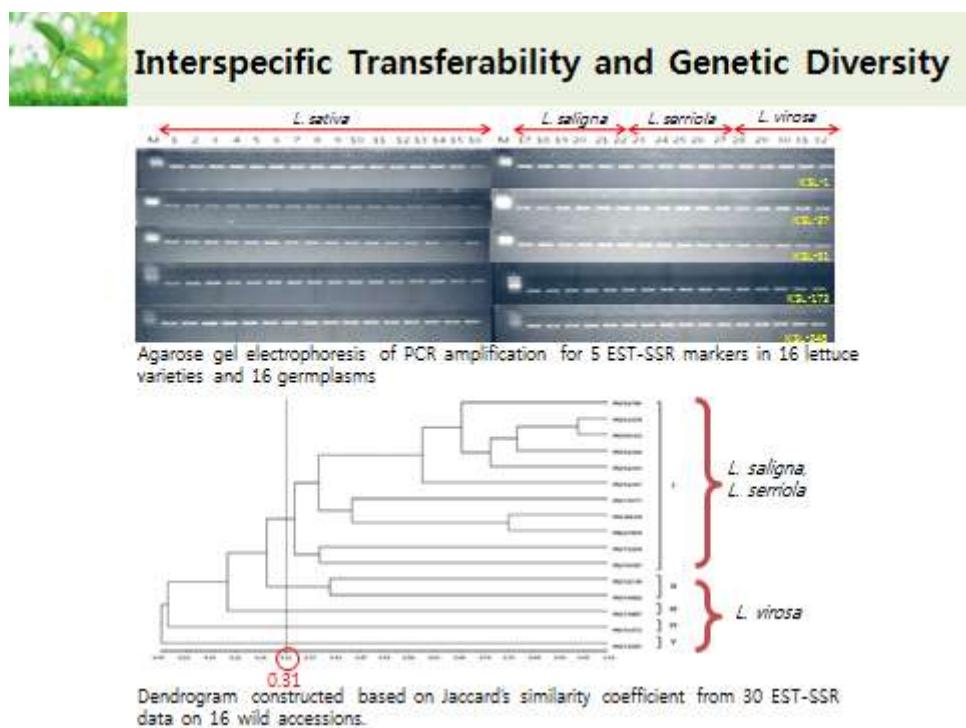
- From 156 varieties, 57 varieties showed 100% genetic similarity.
- 115 varieties were identified by 60 SSR markers.

## Selection of minimum SSR markers

### ◎ 27 minimum SSR markers



Comparison and correspondency of minimum 27 SSR marker set with the whole 60 SSR marker set for identifying of 115 lettuce varieties.



### Putative Function of 18 EST-SSR markers

◎ Blast analysis between 18 EST-SSR markers and proteins

No.	Primer name	EST ID	Putative function	Species	E-value
1	KSL-1	CLBS10849	TOP transcription factor 11	Solanum lycopersicum	4.00E-40
2	KSL-37	CLBM1424	Predicted protein	Populus trichocarpa	4.00E-72
3	KSL-271	CLBS8197	Flap endonuclease GBN-like 1	Vitis vinifera	1.00E-70
4	KSL-357	CLBS10992	Hypothetical protein MTR_1g089750	Medicago truncatula	3.00E-49
5	KSL-97	CLBY5704	Remorin, putative	Ricinus communis	5.00E-29
6	KSL-173	CLBM444	SEC14 cytosolic factor-like	Vitis vinifera	7.00E-114
7	KSL-245	CLBM513	Uncharacterized protein LOC100262380	Vitis vinifera	4.00E-58
8	KSL-7	CLBS10499	RING finger and WD Repeat domain-containing protein	Medicago truncatula	2.00E-27
9	KSL-26	CLBM14994	Hypothetical protein VITBV_044433	Vitis vinifera	2.00E-52
10	KSL-32	CLBM14764	Predicted protein	Populus trichocarpa	1.00E-64
11	KSL-43	CLBM1373	Unnamed protein product	Vitis vinifera	4.00E-37
12	KSL-75	CLBY7906	Le-related protein 6-like	Vitis vinifera	4.00E-67
13	KSL-83	CLBY6646	Os01g0886200	Oryza sativa Japonica group	2.00E-08
14	KSL-92	CLBY517	14-3-3 protein, putative	Ricinus communis	4.00E-126
15	KSL-97	CLBY4815	Hypothetical protein ROOM_0927570	Ricinus communis	2.00E-24
16	KSL-115	CLBM11208	DNA binding protein, putative	Ricinus communis	5.00E-27
17	KSL-119	CLBM10279	Alcohol oxidase domain-containing protein FAM110B-like	Glycine max	4.00E-93
18	KSL-123	CLBL2393	60S ribosomal protein L7a, putative	Ricinus communis	2.00E-48



## Results

### <Experiment 3>

#### Distinctness test of lettuce and correlation between EST-SSR markers and morphological characteristics



## Distinctness test

### ◎ Morphological variety descriptions of groups

Classification	Group 1	Group 2
Phenotype		
Genetic similarity	96%	93%
Distinctness test	No Distinctness	Distinctness (Intensity of color of outer leaves, bolting day)
Breeder's test	No Distinctness	Distinctness (3) No Distinctness (1)
Distinctness traits	-	Intensity of color of outer leaves, Intensity of anthocyanin coloration, glossiness

## Distinctness test

### ◎ Morphological variety descriptions of groups

Classification	Group 3	Group 4
Phenotype		
Genetic similarity	100%	100%
Distinctness test	<b>Distinctness(bolting day)</b> ※ distinctness using KSL-87 new marker → applicability associated with bolting trait	No Distinctness
Breeder's test	Distinctness (2) No Distinctness (2)	Distinctness (2) No Distinctness (2)
Distinctness traits	Leaf thickness, Intensity of anthocyanin coloration	Intensity of anthocyanin coloration, Leaf blistering

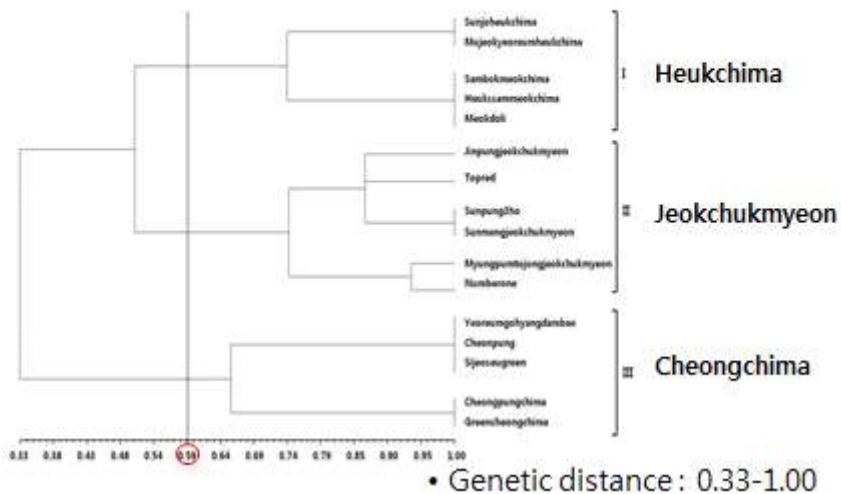
## Distinctness test

### ◎ Morphological variety descriptions of groups

Classification	Group 5	Group 6	Group 7
Phenotype			
Genetic similarity	100%	100%	100%
Distinctness test	No Distinctness	No Distinctness	No Distinctness
Breeder's test	No Distinctness	No Distinctness	No Distinctness

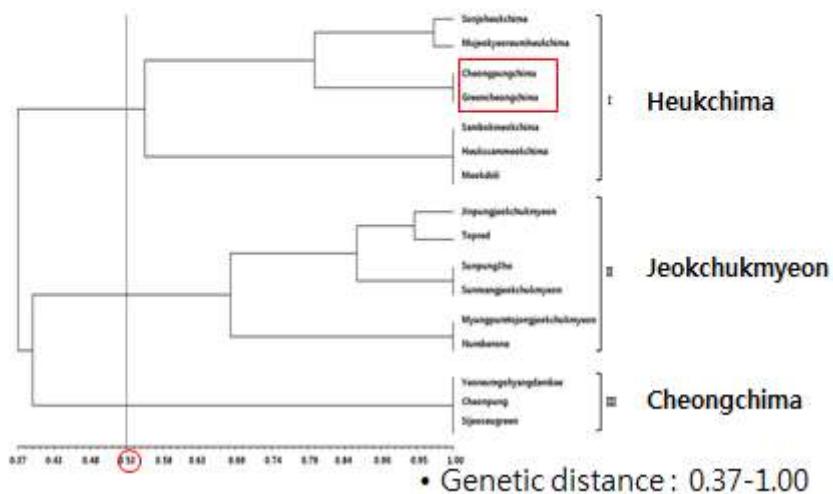
## Genetic Relationship of Morphological Characteristics

- ◎ Dendrogram constructed based on morphological data of 18 lettuce varieties



## Genetic Relationship of EST-SSR Markers

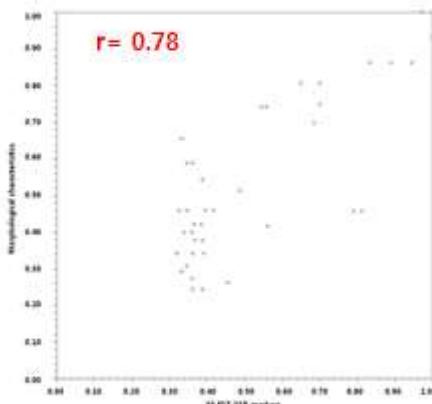
- ◎ Dendrogram constructed based on Jaccard's similarity coefficient from 30 EST-SSR markers on 18 lettuce varieties.





## Correlation between EST-SSR Markers and Morphological Characteristics

- 30 EST-SSR markers and morphological test (Mantel test)



- Correlation coefficient : 0.78
- Higher value than ever reported (0.05-0.68)



Applicability of option 2

Comparison between SSR markers and morphological traits using the mantel test.



## Conclusions

### Experiment 1. Development of EST-SSR markers in Lettuce

- Development of 1,181 EST-SSR primer
- Important tools for lettuce variety identification



Utility of these EST-SSR markers in molecular mapping  
and molecular breeding of lettuce

### Experiment 2. Variety identification and DNA profile database construction using EST-SSR markers

- Detection of exact allele size
- Construction of EST-SSR database of lettuce varieties
- Identification of wild germplasm using EST-SSR markers



Applicability of EST-SSR markers in variety and  
germplasm identification and diversity study



## Conclusions

### Experiment 3. Distinctness test of lettuce and correlation between EST-SSR markers and morphological characteristics

- ◎ Varieties showing high genetic similarity were very similar in morphological characteristics
- ◎ Correlation coefficient( $r$ ) = 0.78
- ◎ The reasons high  $r$  value that ever reported
  - ✓ Use of EST-SSR marker
  - ✓ A well-constructed DNA profile database (marker combination)
  - ✓ Similar result of genotype and phenotype data

 We used practically these EST-SSR markers for the selection of similar varieties and distinctness test of candidate varieties

