

**Working Group on Biochemical and Molecular Techniques
and DNA-Profiling in Particular**

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**USE OF MOLECULAR MARKER TECHNIQUES IN DUS TESTING AND ENFORCEMENT OF
BREEDER'S RIGHT IN THE REPUBLIC OF KOREA**

Document prepared by an expert from the Republic of Korea

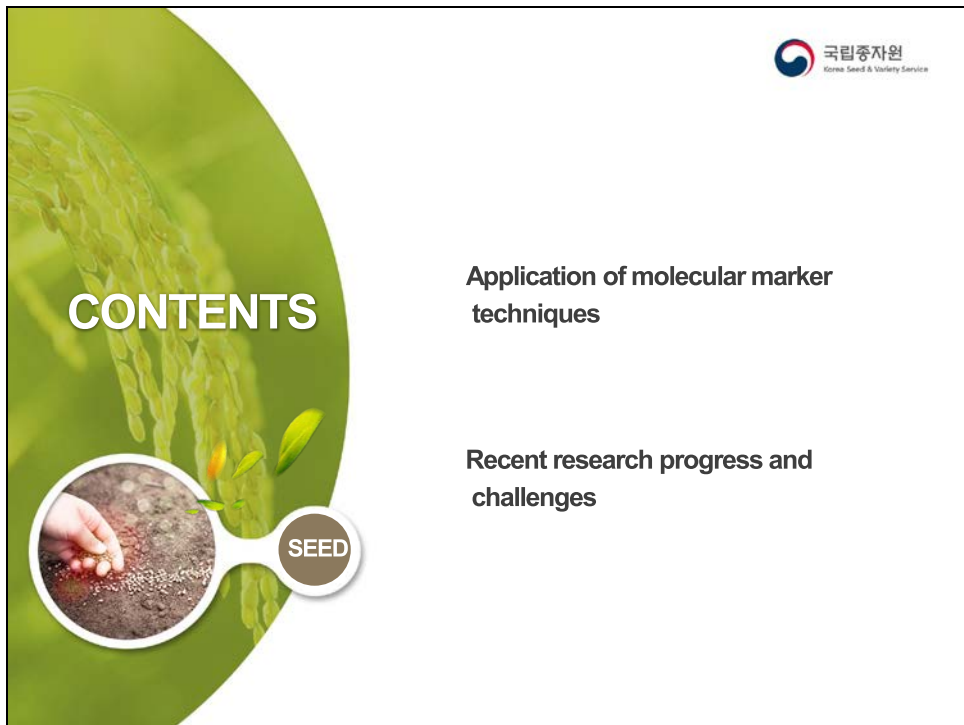
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Molecular Marker Techniques have been used in the Korea Seed and Variety Service (KSVS) as an effective tool not only for improving the reliability of DUS testing but also for enforcement of Breeder's right. KSVS has constructed the molecular marker database for more than 5,000 varieties of 30 species, including most staple food crops, vegetables, some fruits and ornamentals. In this presentation, applications of the techniques and some results in 2017 to 2018 are highlighted. Also some challenges and the prospects are discussed here.

[Annex follows]

USE OF MOLECULAR MARKER TECHNIQUES IN DUS TESTING AND ENFORCEMENT OF BREEDER'S RIGHT IN THE REPUBLIC OF KOREA

Presentation prepared by an expert from the Republic of Korea





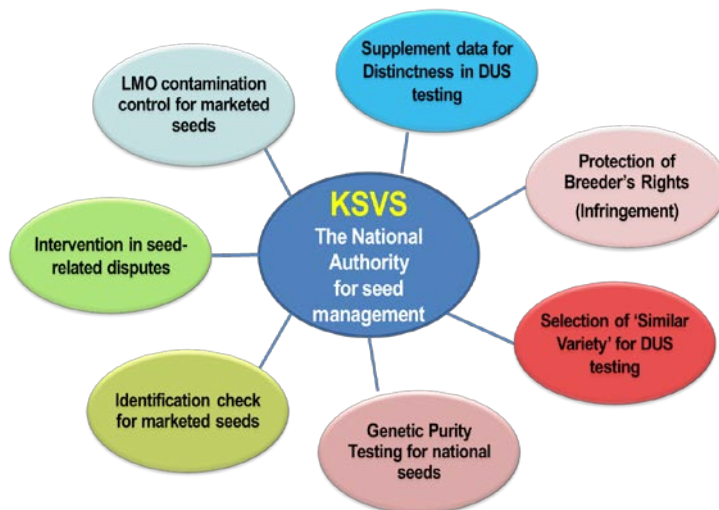
● **Status of DB construction in KSVS**

- 30 crops, 5,272 varieties

< Construct database for variety identification >

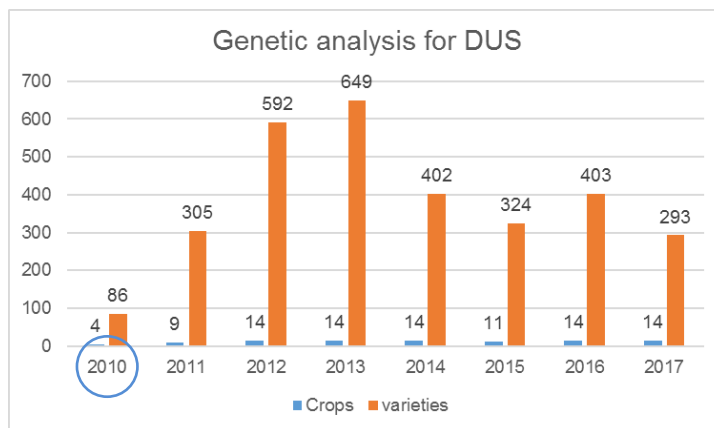
Category	Crop	Techniques	No. of Varieties	Category	Crop	Techniques	No. of Varieties
Vegetables	Pepper	SSR	672	Fruit tree	Citrus	SSR	113
	Watermelon	SSR	300		Plum	SSR	160
	Melon	SSR	180		Pear	SSR	87
	Tomato	SSR	122		Peach	SSR	174
					Apple	SSR	67
	Chinese cabbage	SSR	435		Blueberry	SSR	40
					Persimmon	SNP*	84
	Oriental melon	SSR	108	Persimmon	SNP*	48	
	Carrot	SSR	115	Flower	Rose	SSR	70
	Pumpkin	SSR	167		Chrysanthemum	SSR	128
	Radish	SSR	288		Gerbera	SSR	30
	lettuce	SSR	435	Cereals	Rice	SSR	373
	Cucumber	SSR	175		Rice	SNP*	161
	Onion	SSR	77		Barley	SSR	71
Strawberry	SSR	110	Soybean		SSR	148	
Cabbage	SNP*	108	Mushroom	Maize	SSR	90	
				Industrials	Sesame	SNP*	67
Total : 30 crops / 5,272 varieties							

● Use of Molecular Markers in KSVS



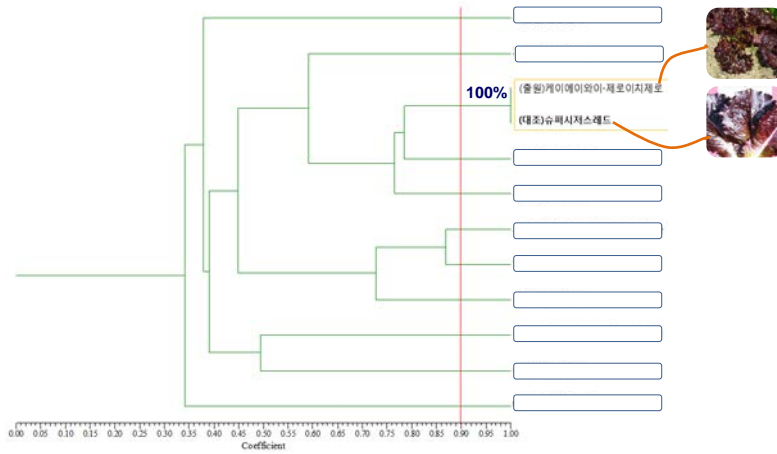
● The Selection of 'Similar Varieties' in DUS testing

- Use the Molecular marker for selection of 'Similar varieties' in DUS test since 2010



● The Selection of 'Similar Varieties' for DUS testing

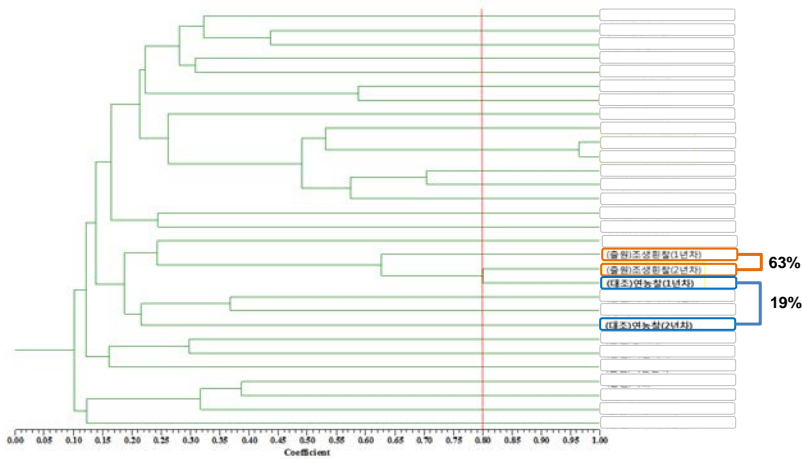
- 2018, Lettuce have no distinctness in marker test → Select as a similar variety



● The Test of 'Uniformity' in DUS testing in Korea

- 2018, A candidate variety showed distinctness in field test by year

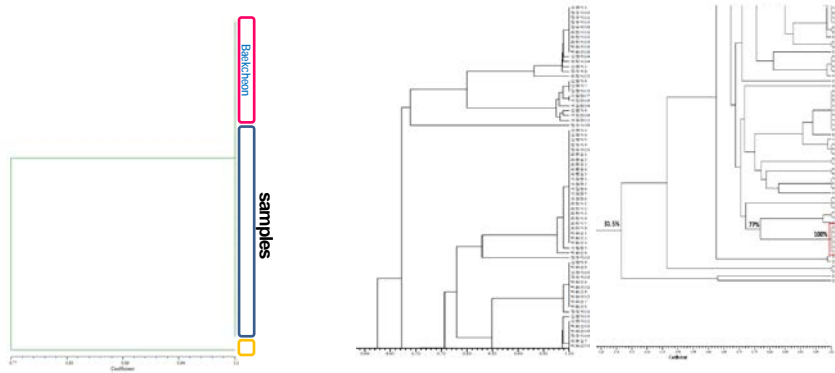
→ To be rejected



● **Infringement of PVP**

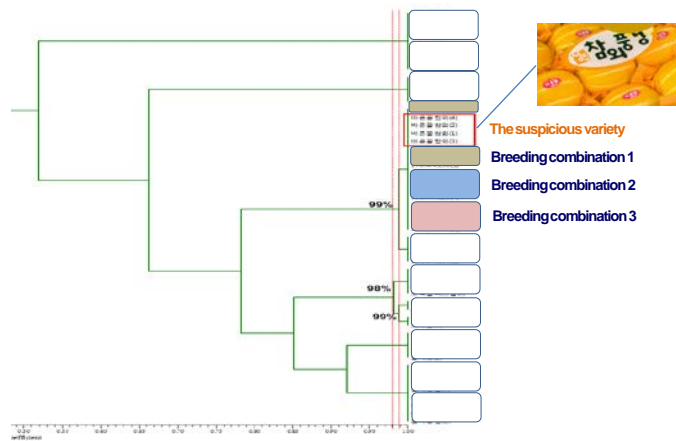
- Case: 2017 Infringement for PVP (Baekcheon)
- Variety / Species: Baekcheon / Peach
- Result: Violation of Plant Variety Protection Right

- Case: 2018 Infringement for PVP (Danwhangdo)
- Variety / Species: Danwhangdo / Peach
- Content: No Violation of Plant Variety Protection Right



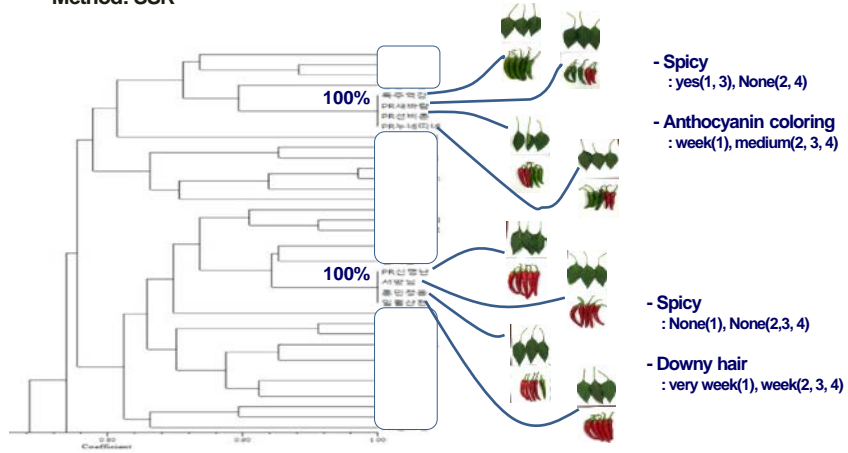
● **Infringement of PVP**

- 2018. 8. Case of technology leakages in breeding of an oriental melon



● Genetic analysis for seeds in market : pepper

- Sample: 336 varieties
- Time for analysis: 5 months
- Method: SSR



● Purpose of recent research

1. GWAS

- Increasing relationships between phenotype and genotype
- To develop traits-related marker
- Effective in both discrimination and similarity of varieties

2. SNP marker

- Reliable marker
- More distributed in chromosome
- Highly associated with function

3. High-throughput method

- Cost effective and fast

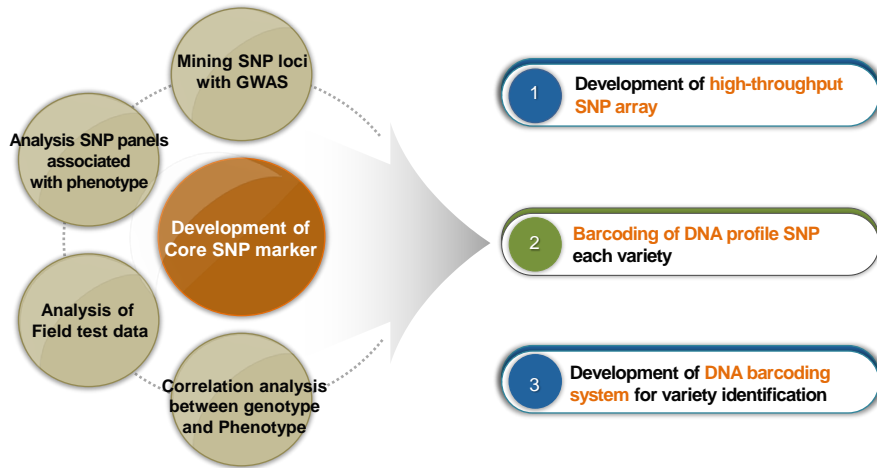
● SSR vs SNP

Work flow and items	Time required	
	SNP	SSR
Preparation of sample	20 min.	
DNA prep.	1.5 hr.	
PCR mixture	10 min. (multiplex)	60 min. (single rxn.)
PCR cycling	2 hr.	2 hr. ~ ※ dependent on No. of instruments
Electrophoresis	-	2 hr. ~ ※ dependent on No. of instruments
Data input & analysis	10 min.	2 hr.
Total time	4 hr.	9 hr. ~
Total cost*	~ 200\$	~ 5,000\$

* cost for beginning of initial operation

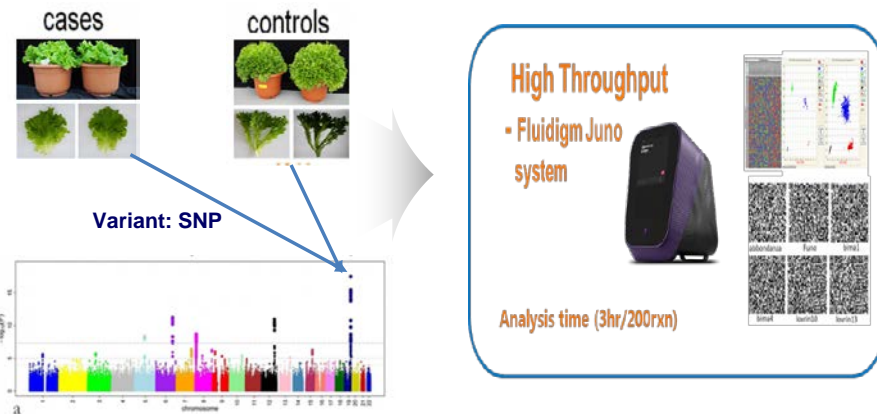
● Recent research for PVP in KSVS

- Flowchart for research



● Recent research for PVP

- Goal for research

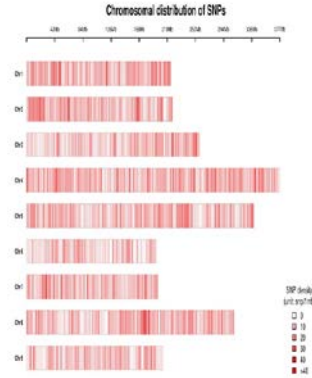


● Recent research for PVP

■ Lettuce: GBS analysis of 96 varieties (1st)

Chromosome	Gene	Gene	Gene
1	170101	170101	170101
1	170102	170102	170102
1	170103	170103	170103
1	170104	170104	170104
1	170105	170105	170105
1	170106	170106	170106
1	170107	170107	170107
1	170108	170108	170108
1	170109	170109	170109
1	170110	170110	170110
1	170111	170111	170111
1	170112	170112	170112
1	170113	170113	170113
1	170114	170114	170114
1	170115	170115	170115
1	170116	170116	170116
1	170117	170117	170117
1	170118	170118	170118
1	170119	170119	170119
1	170120	170120	170120
1	170121	170121	170121
1	170122	170122	170122
1	170123	170123	170123
1	170124	170124	170124
1	170125	170125	170125
1	170126	170126	170126
1	170127	170127	170127
1	170128	170128	170128
1	170129	170129	170129
1	170130	170130	170130
1	170131	170131	170131
1	170132	170132	170132
1	170133	170133	170133
1	170134	170134	170134
1	170135	170135	170135
1	170136	170136	170136
1	170137	170137	170137
1	170138	170138	170138
1	170139	170139	170139
1	170140	170140	170140
1	170141	170141	170141
1	170142	170142	170142
1	170143	170143	170143
1	170144	170144	170144
1	170145	170145	170145
1	170146	170146	170146
1	170147	170147	170147
1	170148	170148	170148
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1	170150	170150	170150
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1	170165	170165	170165
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1	170167	170167	170167
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1	170170	170170	170170
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1	170172	170172	170172
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1	170174	170174	170174
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1	170189	170189	170189
1	170190	170190	170190
1	170191	170191	170191
1	170192	170192	170192
1	170193	170193	170193
1	170194	170194	170194
1	170195	170195	170195
1	170196	170196	170196
1	170197	170197	170197
1	170198	170198	170198
1	170199	170199	170199
1	170200	170200	170200

< Chromosomal distribution of 17,877 SNP >



< SNP mining for Lettuce (*Lactuca sativa* L.) using GBS >

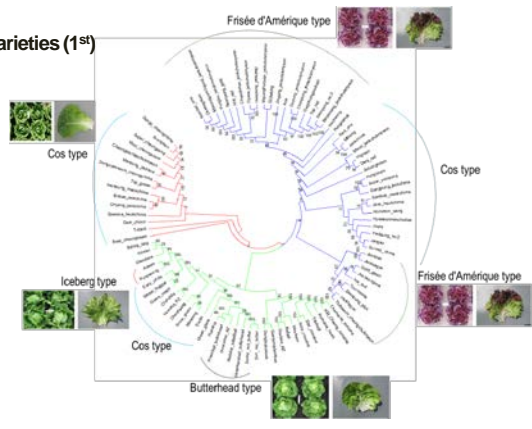
SNP mining	List of filtering	SNP matrix loci
1	Total SNP matrix	276,462
2	MAF (minor allele frequency)	100,611
3	> 5% missing rate < 30%	17,877

● Recent research for PVP

■ Lettuce: GBS analysis of 96 varieties (1st)

< Total SNPs, intergenic, genic, CDS, intron, UTR and gene number >

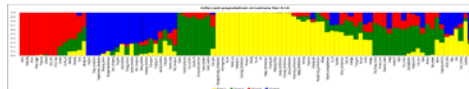
Category	Count
Total SNPs	276,462
Intergenic	150,000
Genic	126,462
CDS	100,000
Intron	26,462
UTR	0
Gene number	10,000



< Genetic relationships >

< Statistic results of transition and transversion of SNP >

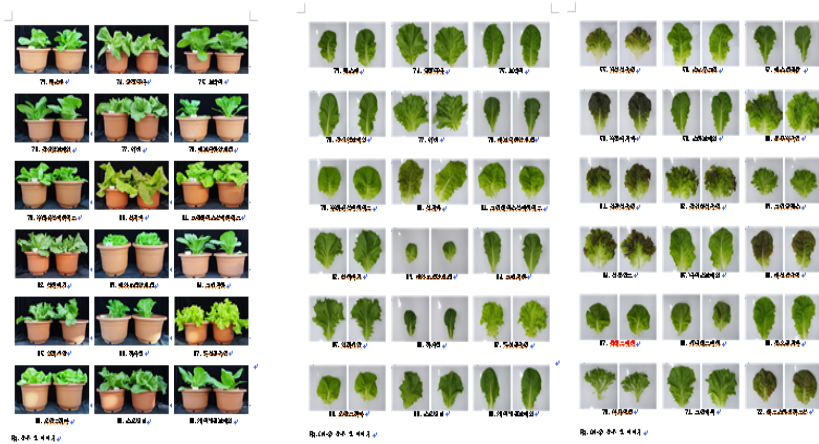
Substitution	SNPs
Transitions (Ti)	171,969
C-T	87,112
A-G	84,857
Transversions (Tv)	102,444
C-G	19,367
A-T	39,764
A-C	25,430
G-T	25,183
Tv/Ti ratio	1.99



< Population structure >

● Recent research for PVP

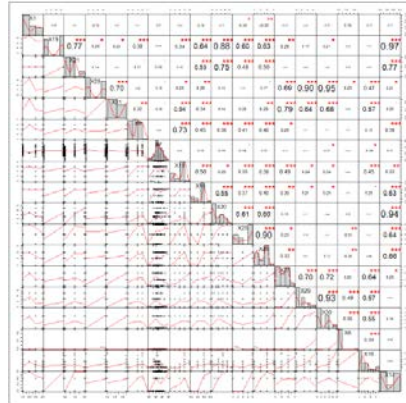
- Lettuce: 1st Field test of 96 varieties with 37 item in TG



● Recent research for PVP

- Lettuce: GWAS of lettuce (1st)

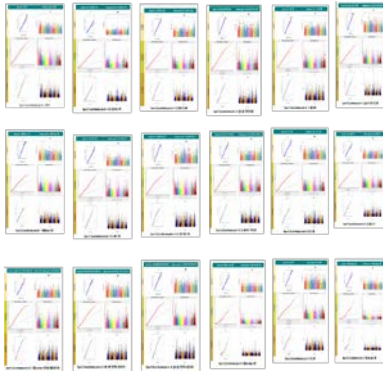
Classification	TG #	Character of plant
Qualitative	1	Seed: color
	19	Leaf: color of anthocyanin
	21	Leaf: distribution of anthocyanin
	28	Blade: incision of leaf edge
	31	Blade: vein
Quantitative	5	Leaf: attitude
	7	plant: diameter
	15	Leaf: attitude in harvests
	18	Leaf: color's intensity of outer leaf
	20	Leaf: color's intensity of anthocyanin
	25	Leaf: concavo-convex
	26	Leaf: size of concavo-convex
	27	Blade: wave shape of edge
	29	Blade: depth of incision of leaf edge
	30	Blade: degree of incision of leaf edge
Pseudo-qualitative	6	Leaf: number of divisions
	16	Leaf: shape
	17	Leaf: color of outer leaf



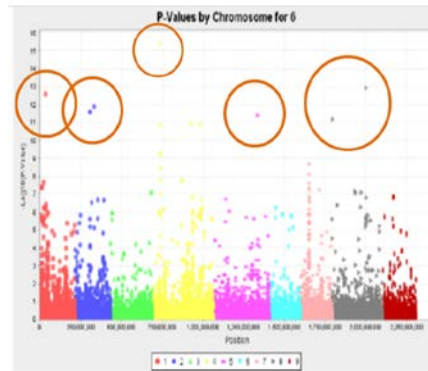
● Recent research for PVP in KSVS

- **Lettuce:** GWAS of lettuce (1st)

< Manhattan plots for each phenotypes >



< Leaf: number of divisions >



● Relocation of SSR DB

- Crop # : 25
- Method : SSR
- Marker # : 40 (in average)
- Sample # : 300 varieties (in average)



Total cost for DB relocation: - \$10,000 / crop



[End of Annex and of document]