UPOV
International Union for the Protection of New Varieties of Plants

E

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

BMT/17/14 Rev.

Seventeenth Session
Montevideo, Uruguay, September 10 to 13, 2018

Original: English
Date: September 5, 2018

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

Disclaimer: this document does not represent UPOV policies or guidance

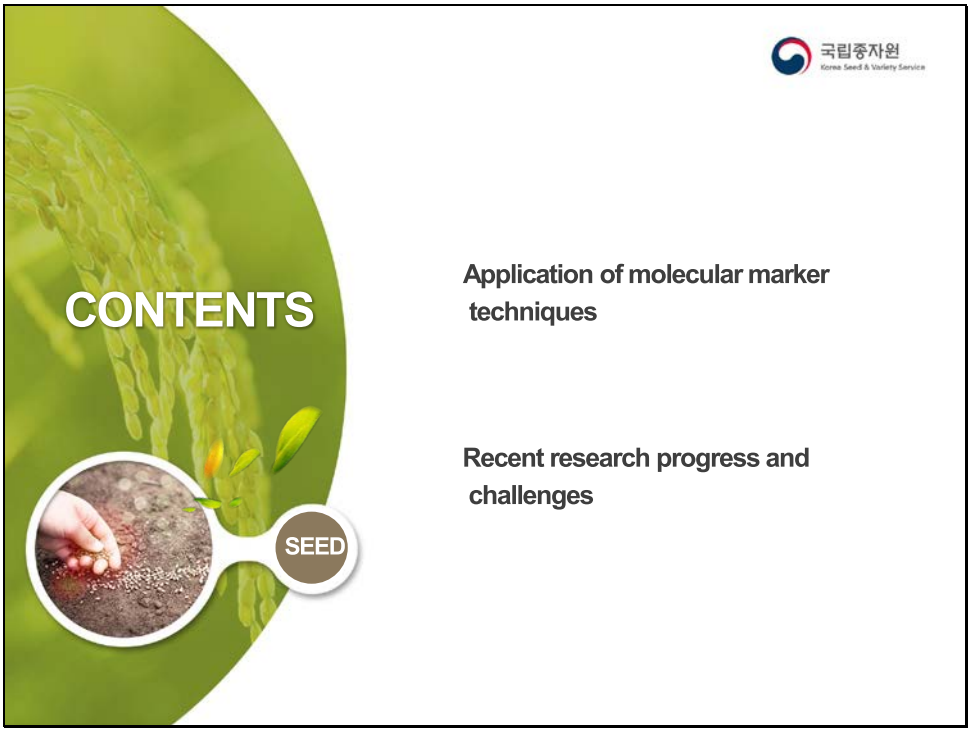
Molecular Marker Techniques have been used in the Korea Seed and Variety Service (KSVS) as an effective tool not only for improving the reliability of DUS testing but also for enforcement of Breeder's right. KSVS has constructed the molecular marker database for more than 5,000 varieties of 30 species, 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.

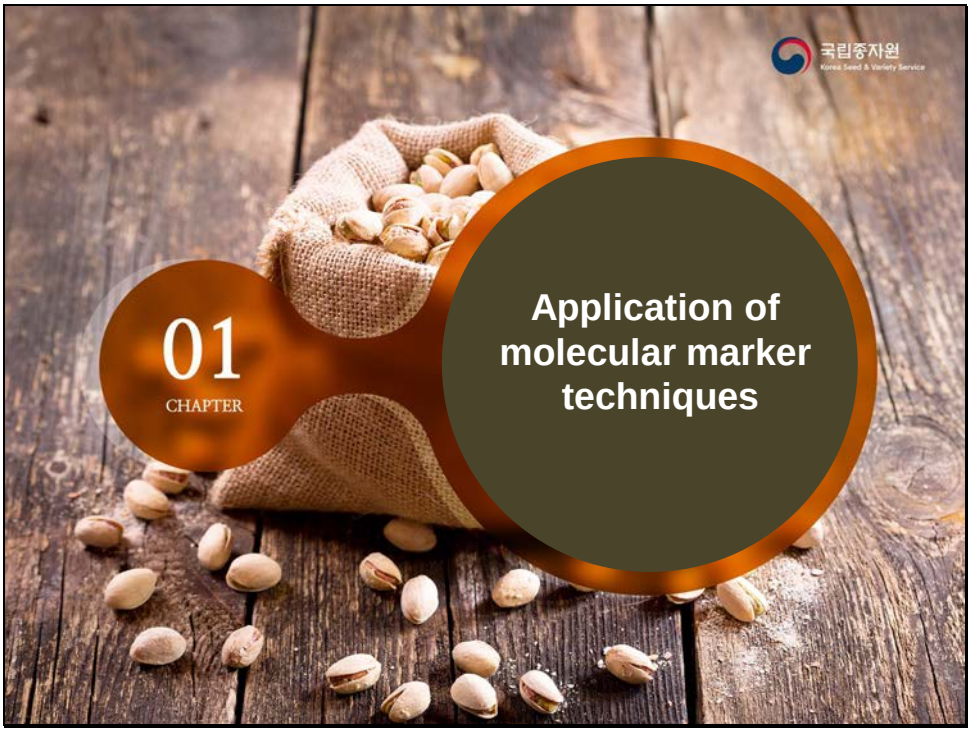
[Annex follows]

ANNEX

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





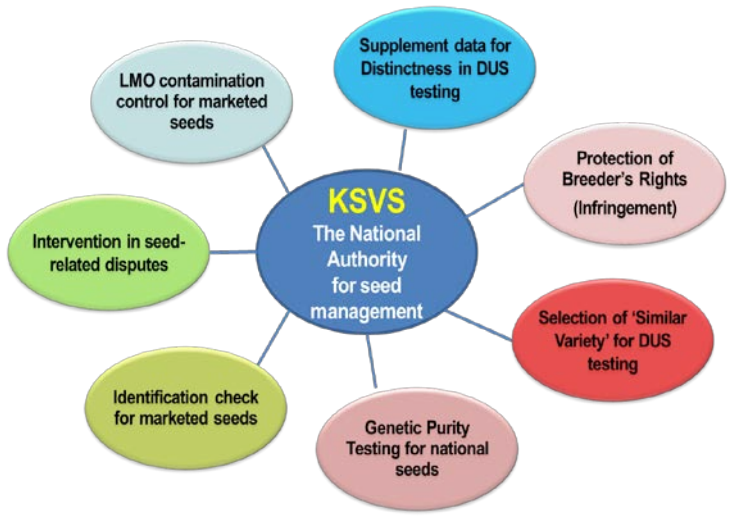
- **Status of DB construction in KSVS**
  - 30 crops, 5,272 varieties

< 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
					Plum	SSR	160
	Watermelon	SSR	300		Pear	SSR	87
	Melon	SSR	180		Peach	SSR	174
	Tomato	SSR	122		Apple	SSR	67
					Blueberry	SSR	40
						SNP*	84
					Persimmon	SNP*	48
	Chinese cabbage	SSR	435	Flower	Rose	SSR	70
	Oriental melon	SSR	108		Chrysanthemum	SSR	128
					Gerbera	SSR	30
	Carrot	SSR	115	Cereals	Rice	SSR	373
	Pumpkin	SSR	167			SNP*	161
	Radish	SSR	288		Barley	SSR	71
	lettuce	SSR	435		Soybean	SSR	148
	Cucumber	SSR	175		Maize	SSR	90
	Onion	SSR	77	Mushroom	*Pleurotus*	SSR	69
	Strawberry	SSR	110	Industrials	Sesame	SNP*	67
	Cabbage	SNP*	108				
Total : 30 crops / 5,272 varieties							

Korea Seed & Variety Service
Republic of Korea

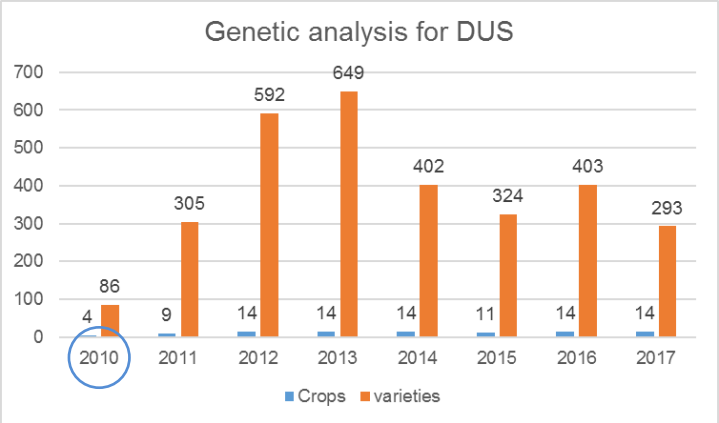
- **Use of Molecular Markers in KSVS**



Korea Seed & Variety Service

- **The Selection of 'Similar Varieties' in DUS testing**

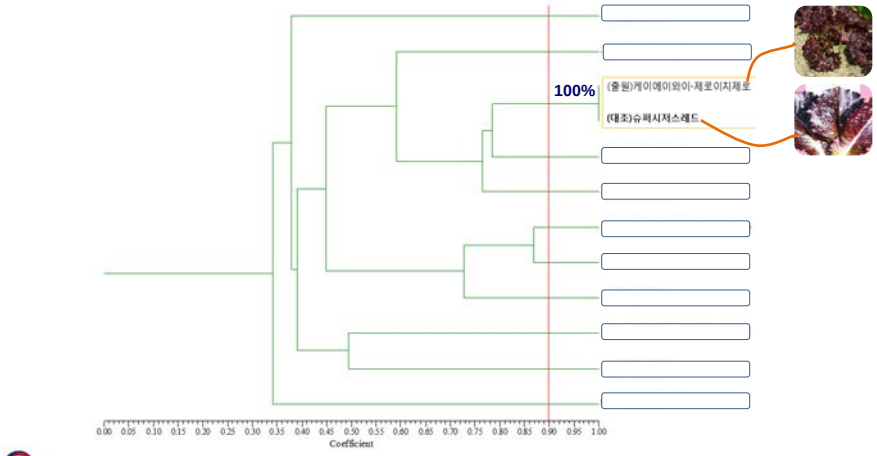
- Use the Molecular marker for selection of 'Similar varieties' in DUS test since 2010



Korea Seed & Variety Service

- **The Selection of '<u>Similar Varieties</u>' for DUS testing**

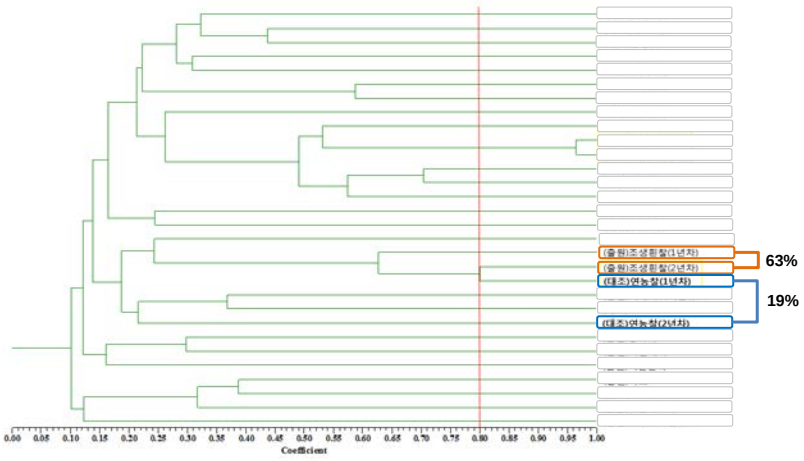
  - 2018, Lettuce have no distinctness in marker test ➡ Select as a similar variety



Korea Seed & Variety Service
Republic of Korea

- **The Test of '<u>Uniformity</u>' in DUS testing in Korea**

  - 2018, A candidate variety showed distinctness in field test by year
    ➡ To be rejected

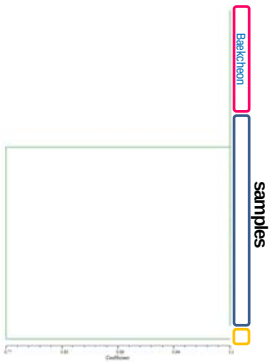


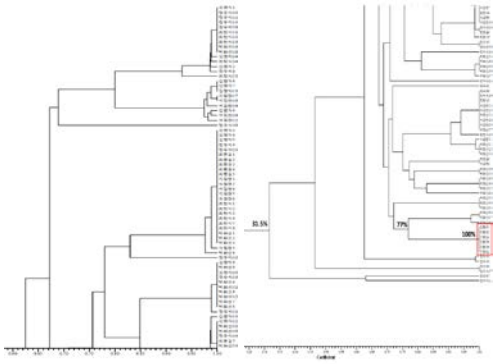
Korea Seed & Variety Service
Republic of Korea

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

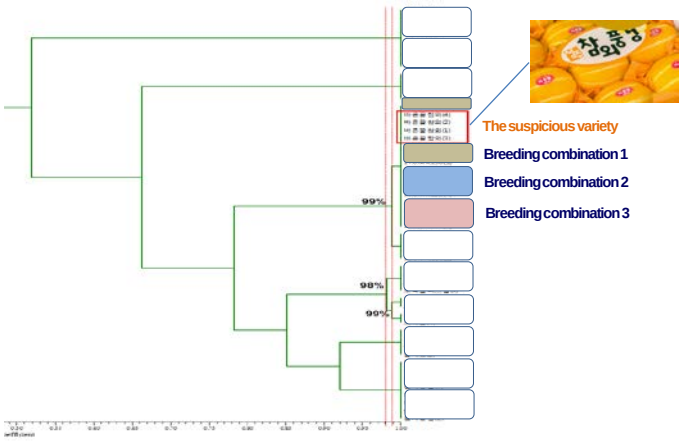




Korea Seed & Variety Service
Republic of Korea

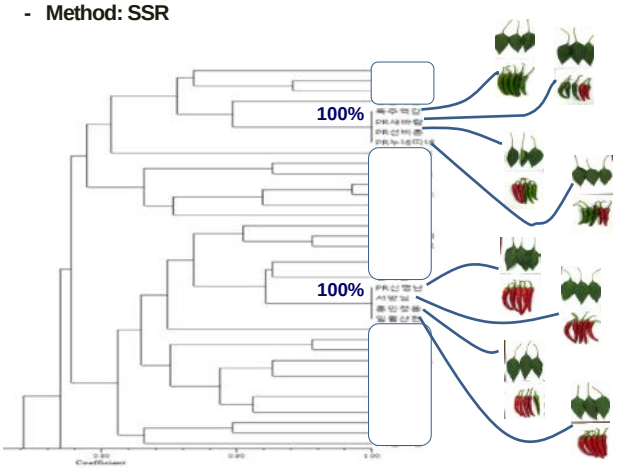
Infringement of PVP

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



Korea Seed & Variety Service
Republic of Korea

- **Genetic analysis for seeds in market : pepper**
  - Sample: 336 varieties
  - Time for analysis: 5 months
  - Method: SSR



- Spicy
  : yes(1, 3), None(2, 4)
- Anthocyanin coloring
  : week(1), medium(2, 3, 4)
- Spicy
  : None(1), None(2,3, 4)
- Downy hair
  : very week(1), week(2, 3, 4)

Korea Seed & Variety Service
Republic of Korea

- **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

Korea Seed & Variety Service
Republic of Korea

- **SSR vs SNP**

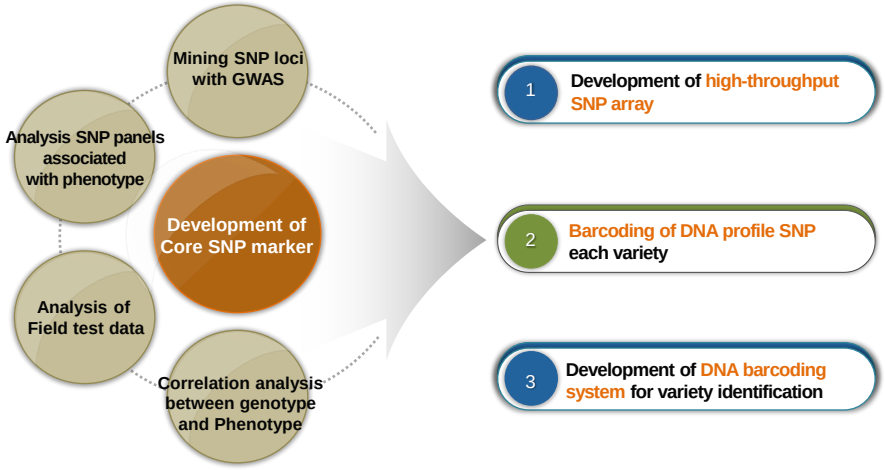
Work flow and items	Time required	
	SNP	SSR
Preparation of sample	20 min.	
DNA prep.	1.5 hr.	
PCR mixture	10 min. (multiplex)	60 min. (single rxn.)
PCR cycling	2 hr.	2 hr. ~ ※ dependent on No. of instruments
Electrophoresis	-	2 hr. ~ ※ dependent on No. of instruments
Data input & analysis	10 min.	2 hr.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

* cost for beginning of initial operation

Korea Seed & Variety Service
Republic of Korea

Recent research for PVP in KSVS

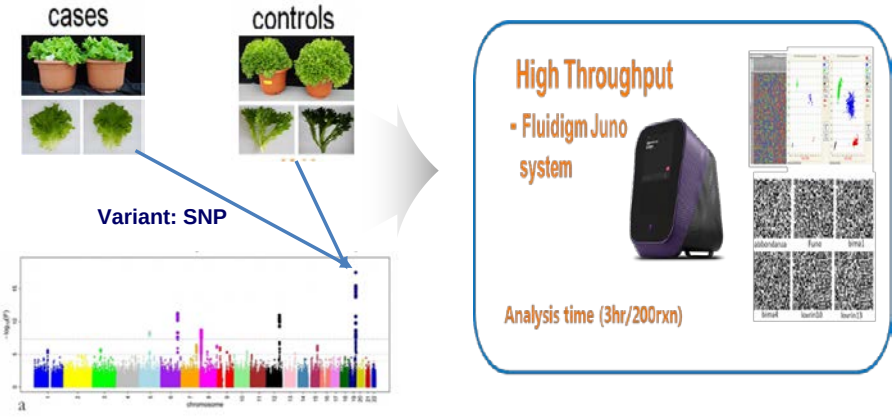
- Flowchart for research



Korea Seed & Variety Service

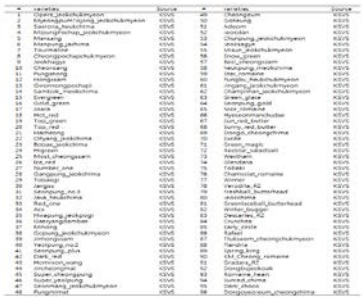
Recent research for PVP

- Goal for research

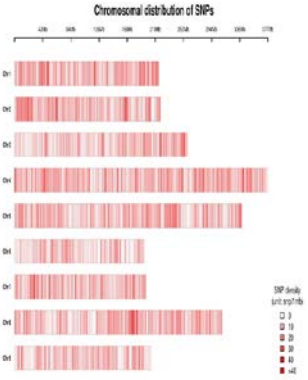


Korea Seed & Variety Service

- **Recent research for PVP**
  - **Lettuce: GBS analysis of 96 varieties (1st)**



< Chromosomal distribution of 17,877 SNP >



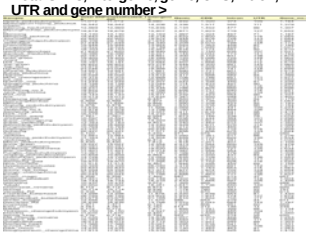
< SNP mining for Lettuce(*Lactuca sativa* L.) using GBS >

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

Korea Seed & Variety Service
Republic of Korea

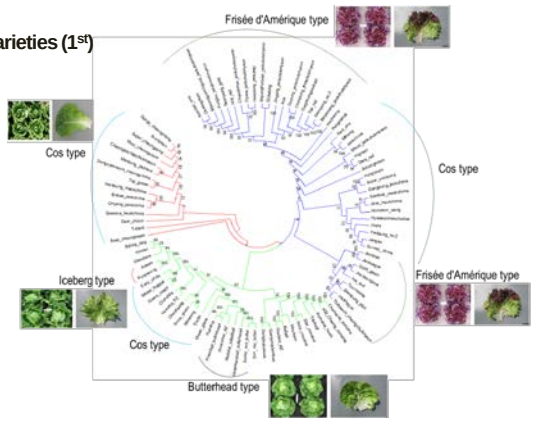
- **Recent research for PVP**
  - **Lettuce: GBS analysis of 96 varieties (1st)**

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

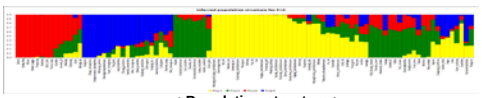


< Statistic results of transition and transverson of SNP >

Substitutions	SNPs
Transitions (Ts)	173,599
C>T	87,112
A>G	86,487
Transversions (Tv)	102,744
C>G	18,067
A>T	35,764
A>C	25,430
G>T	25,183
Ts/Tv ratio	1.69



< Genetic relationships >



< Population structure >

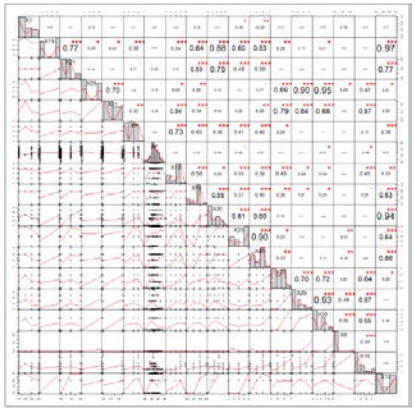
Korea Seed & Variety Service
Republic of Korea

- **Recent research for PVP**
  - **Lettuce: 1st Field test of 96 varieties with 37 item in TG**

Korea Seed & Variety Service
Republic of Korea

- **Recent research for PVP**
  - **Lettuce: GWAS of lettuce (1st)**

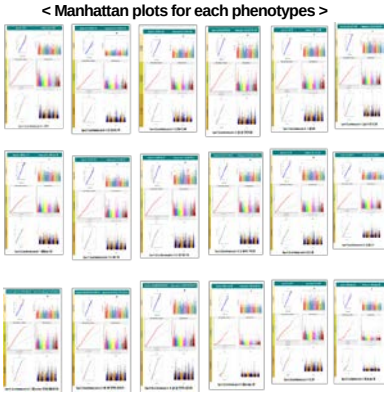
Classification	TG #	Character of plant
Qualitative	1	Seed: color
	19	Leaf: color of anthocyanin
	21	Leaf: distribution of anthocyanin
	28	Blade: incision of leaf edge
	31	Blade: vein
Quantitative	5	Leaf: attitude
	7	plant: diameter
	15	Leaf: attitude in harvests
	18	Leaf: color's intensity of outer leaf
	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



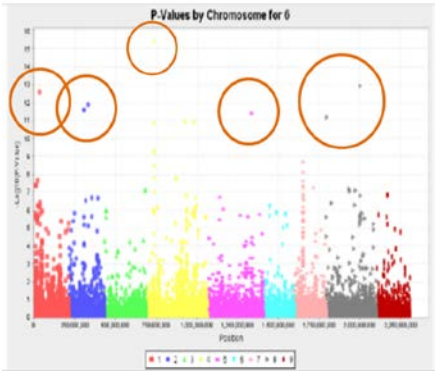
Korea Seed & Variety Service
Republic of Korea

- **Recent research for PVP in KSVS**
  - **Lettuce: GWAS of lettuce (1st)**

< Manhattan plots for each phenotypes >



< Leaf: number of divisions >



Korea Seed & Variety Service
Republic of Korea

- **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

Korea Seed & Variety Service
Republic of Korea

[End of Annex and of document]