UPOV

International Union for the Protection of New Varieties of Plants

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ADDENDUM TO DEVELOPMENTS AND USE OF MOLECULAR TECHNIQUES FOR PLANT VARIETY PROTECTION IN THE REPUBLIC OF KOREA

Document prepared by an expert from the Republic of Korea

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The Annex to this document contains a copy of a presentation on “Developments and Use of Molecular Techniques for plant variety protection in the Republic of Korea”, prepared by an expert from the Republic of Korea, to be made at the sixteenth session of the Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT).

[Annex follows]

ANNEX

DEVELOPMENTS AND USE OF MOLECULAR TECHNIQUES FOR PLANT VARIETY PROTECTION IN THE REPUBLIC OF KOREA

Presentation prepared by prepared by an expert from the Republic of Korea


국립종자원
01
CHAPTER
Developments


20th Anniversary of PVP in Korea
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Variety Identification Based on Molecular Techniques

Characteristics (locus)	Traits (allele)	Phenotypes
Stem: length	short(3)-medium(5)-long(7)	Length(cm)
Glutenins	Sub-unit compositions	MW(bands)
AFLP	Nucleotide compositions	Band composition
SSR	No. of repeat unit	Band size(in bp)
SNP_{AS-PCR}	Nucleotide compositions	Amplicon(+/-)
SNP_{HRM}	Nucleotide compositions	T_m curve
SNP_{NGS}	Nucleotide compositions	Fluorescence…

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Category	Crop	Techniques	No. of Varieties	Category	Crop	Techniques	No. of Varieties
Vegetables	Pepper	SSR	672	Fruit tree	Citrus	SSR	113
					Plum	SSR	180
					Pear	SSR	87
	Watermelon	SSR	300		Peach	SSR	174
	Melon	SSR	180		Apple	SSR	67
	Tomato	SSR	122		Blueberry	SSR	40
						SNP*	84
					Persimmon	SNP*	48
	Chinese cabbage	SSR	435	Ornamentals	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

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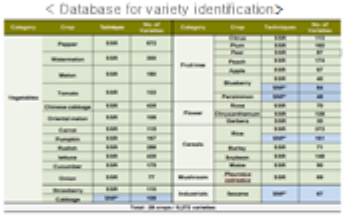
Status of DB construction in KSVS

- **Seed storage: 917 crop, 34,474 varieties**
  - Production and selling: 28,393, Plant variety protection: 2,099, National List:508, etc.: 3,474

< Database for variety identification>



- **Construction of National Standard Database for Varietal Characteristics using Morphological Descriptor and DNA Genotypes**(IPET R&D project: $180,000)
  - Crop : Food crop(rice, soybean, barley), vegetabes(pepper, tomato, radish, cabbage, watermelon, lettuce), Flowers(rose, Chrysanthemum), Fruit trees(blueberry, pear)
  - Penod : 2013. 12. ~ 2016. 12. (3 Year)

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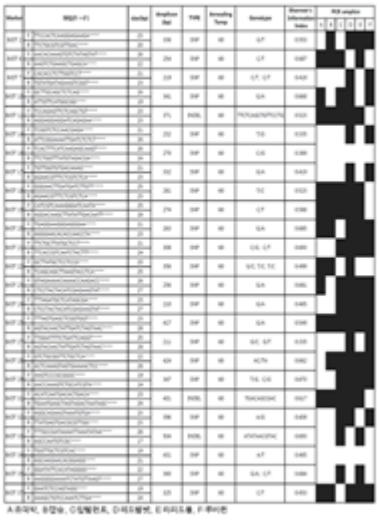
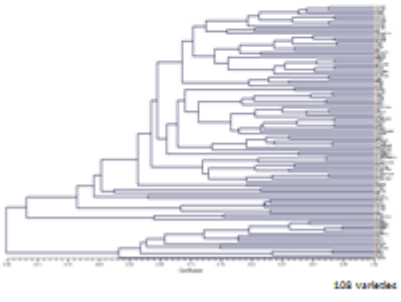
< Highly informative SNP marker set for Cabbage variety identification>



Steps	Filtering	SNP matrix loci
1	Total SNP matrix	488,882
2	MAF(minor allele frequency) > 5%	179,704
3	Missing rate < 30%	56,210
4	Core set	25



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Principles for use of molecular techniques for PVP

- "Minimum Distances in Variety;
  it is determined only in clear phenotypic differences or meaningful genetic differences."

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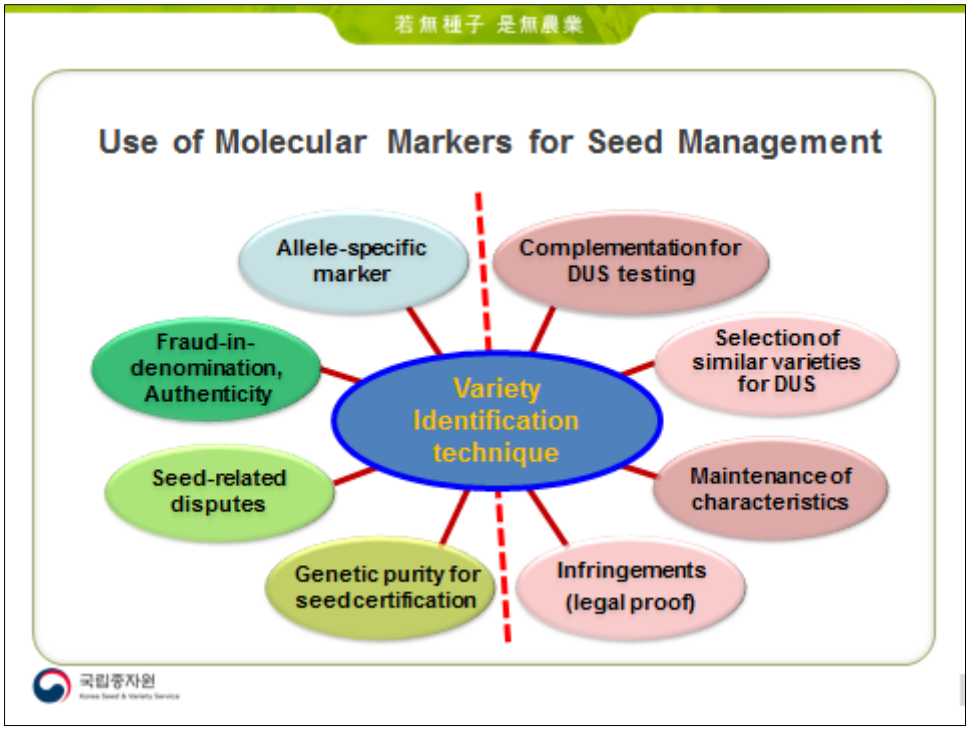
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Use of Molecular Markers for Seed Management
Allele-specific marker
Complementation for DUS testing
Fraud-in-denomination, Authenticity
Selection of similar varieties for DUS
Variety Identification technique
Seed-related disputes
Maintenance of characteristics
Genetic purity for seed certification
Infringements (legal proof)
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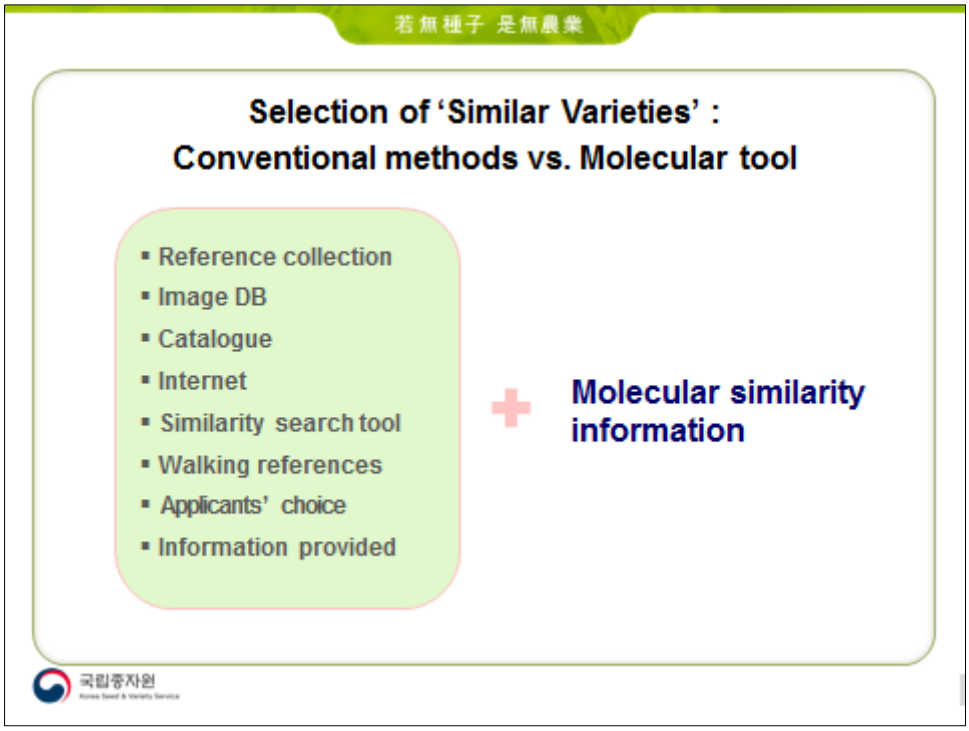
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Selection of 'Similar Varieties' :
Conventional methods vs. Molecular tool
▪ Reference collection
▪ Image DB
▪ Catalogue
▪ Internet
▪ Similarity search tool
▪ Walking references
▪ Applicants' choice
▪ Information provided
Molecular similarity information
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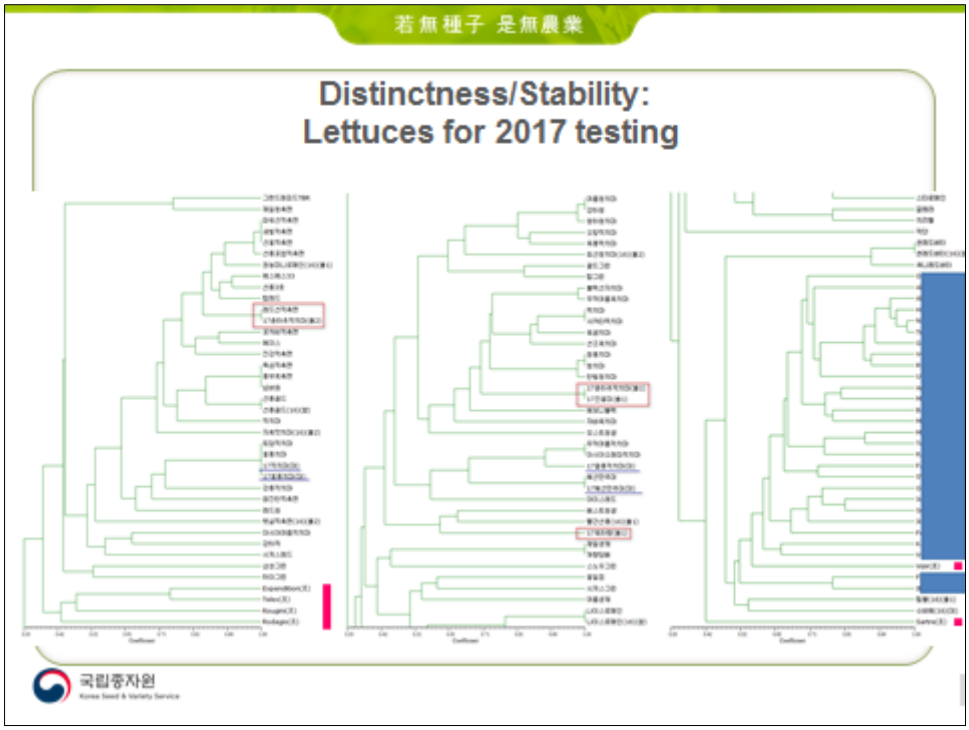
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Distinctness/Stability:
Lettuces for 2017 testing
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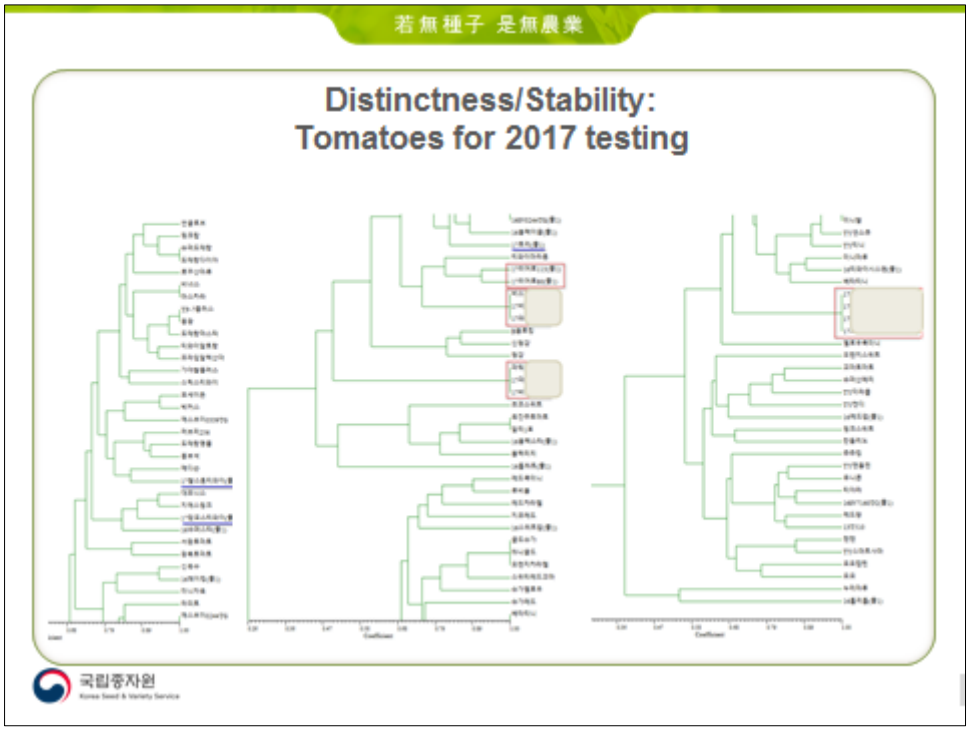
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Distinctness/Stability:
Tomatoes for 2017 testing
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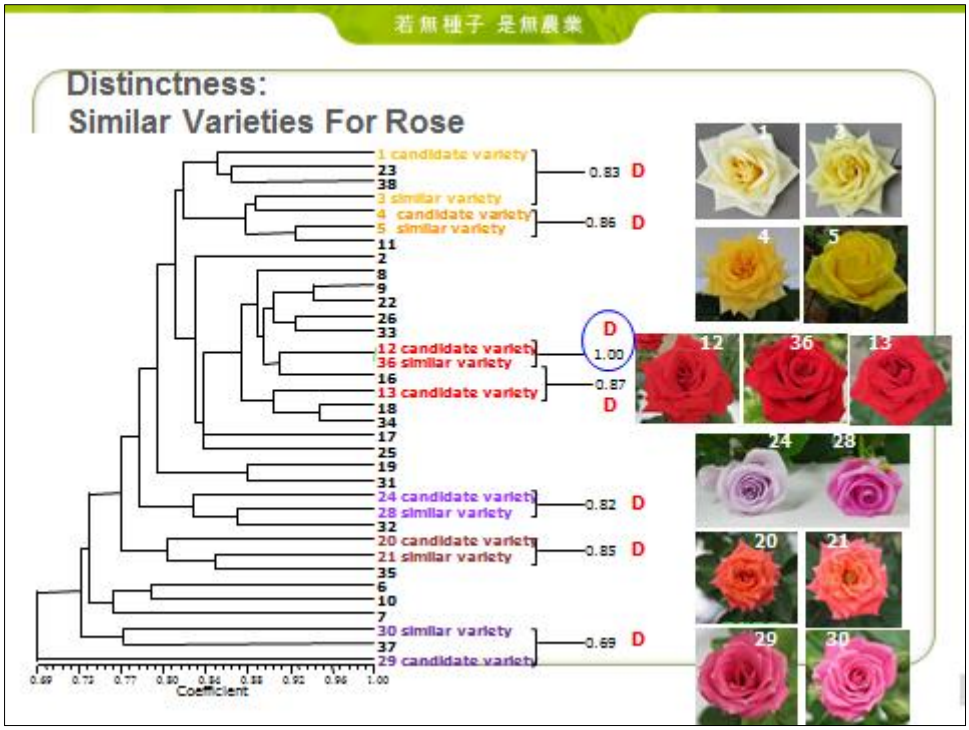
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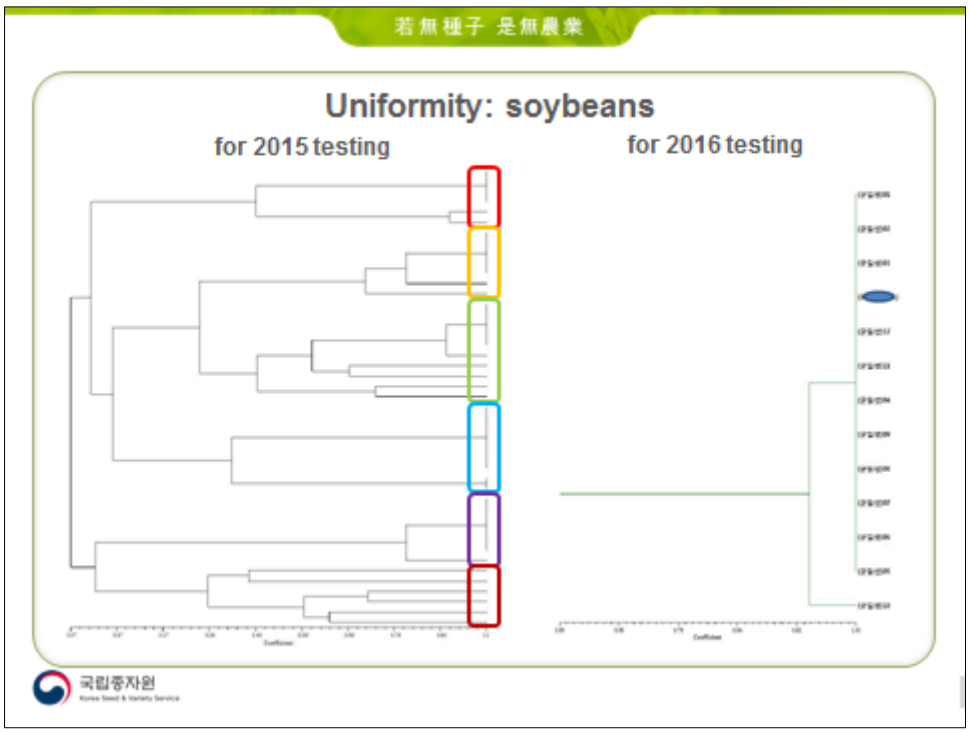
Distinctness:
Similar Varieties For Rose
1 candidate variety
23
38
3 similar variety
4 candidate variety
5 similar variety
11
2
8
9
22
26
33
12 candidate variety
36 similar variety
16
13 candidate variety
18
34
17
25
19
31
24 candidate variety
28 similar variety
32
20 candidate variety
21 similar variety
35
6
10
7
30 similar variety
37
29 candidate variety
0.83
0.86
D
1.00
0.87
0.82
0.85
0.69
Coefficient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Uniformity: soybeans
for 2015 testing
for 2016 testing
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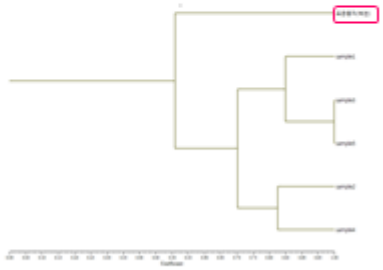
Peach (Baekcheon)

- Case: 2017 Infringement for PVP
- Variety / Species: Baekcheon / Peach
- Content: Violation of Plant Variety Protection Right and Exclusive License

<Case 1>



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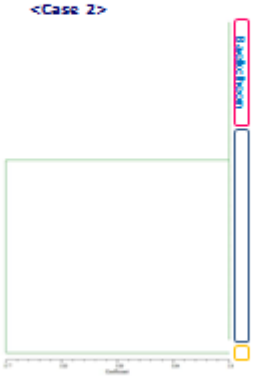

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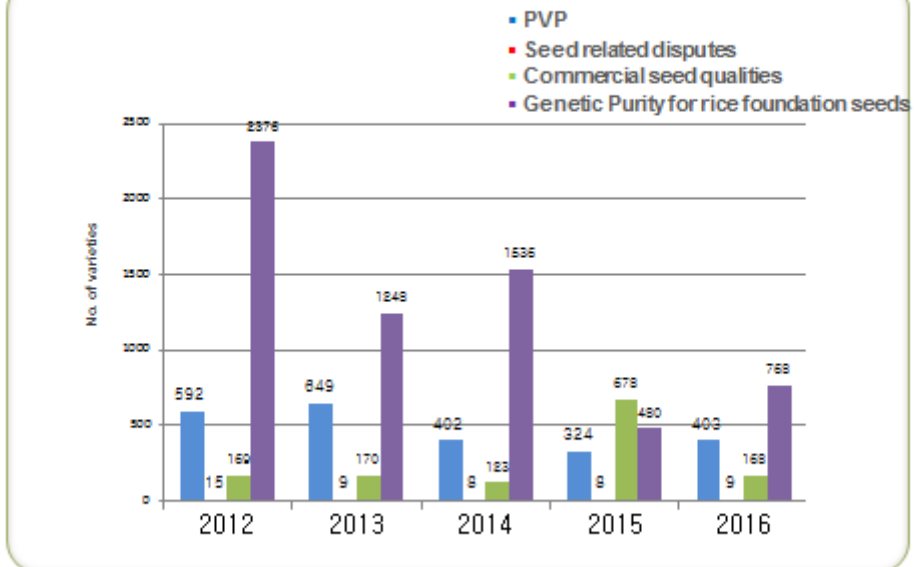
No. of applications for molecular techniques



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Next generation genotyping techniques

NG genotyping

1. NG sequencing techniques
   - allele mining in large number: SNPs
   - high-throughput method
   - instrument-dependent
2. GWAS
   - increasing relationships between phenotype and genotype
   - to develop traits-related marker
   - effective in both identification and similarity analysis of varieties

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Next generation genotyping techniques

Economic feasibility analysis

➢ **Rice purity analysis for 1 sample: 24 grains, 15markers**

work flow and items	time and cost	
	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. ~ ※ dependant on No. of instruments
Electrophoresis	-	2 hr. ~ ※ dependant on No. of instruments
Data input & analysis	10 min.	1 hr.
Total time	**4 hr.**	**8 hr. ~**
Cost*	**~ 200$**	**~ 500$**

* Including consumables, labor cost, depreciation cost and etc.

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Next generation genotyping technology for variety identification

Project Development of NG genotyping technology for variety identification and it's application for fostering seed industry

Study period 2017. 4. ~2020. 12. (45 months)

Participants KSVS(a managing institute), Donga Univ., Sejong Univ., Busan Univ., Jungang Univ., Jingchun Seeds, Misogene Co.

Target crops Chinse cabbage, lettuce, melon, watermelon, tomato, cucumber, squash

R&D fund $ 2200000

Particular development objects

- SNP discovery using NGS technology and selection of core set of SNPs for variety identification
- High throughput genotyping methodology using SNP markers and DNA barcording systems
- Development of cost effective high throughput technology and construction of business model
- Portable rapid analysis system for variety identification
- Utilization of SNP markers associated with morphological characteristics

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[End of Annex and of document]