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Geneva

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

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

ADDENDUM TO DOCUMENT BMT/14/12

POTENTIAL USES OF MOLECULAR MARKERS IN MANAGEMENT OF ROSE VARIETIES FOR THE PVP SYSTEM

Document prepared by experts from China

The Annex to this document contains a copy of a presentation “Potential uses of Molecular Markers in Management of Rose Varieties for the PVP System” made at its fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNS-Profiling in particular (BMT).

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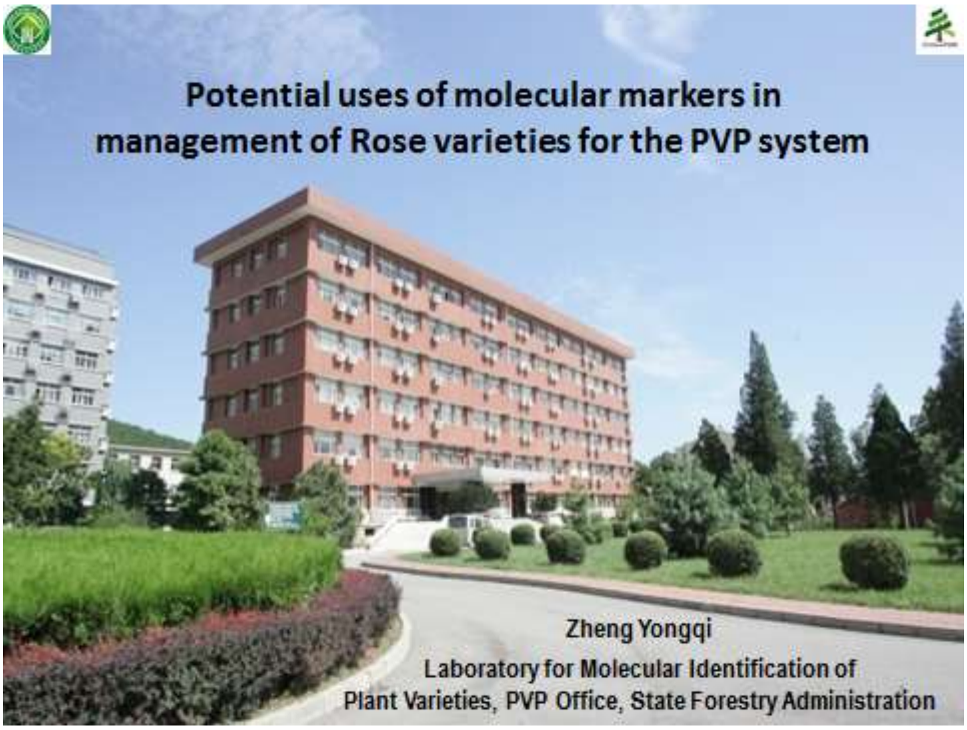
[Annex follows]

ANNEX



Potential uses of molecular markers in management of Rose varieties for the PVP system

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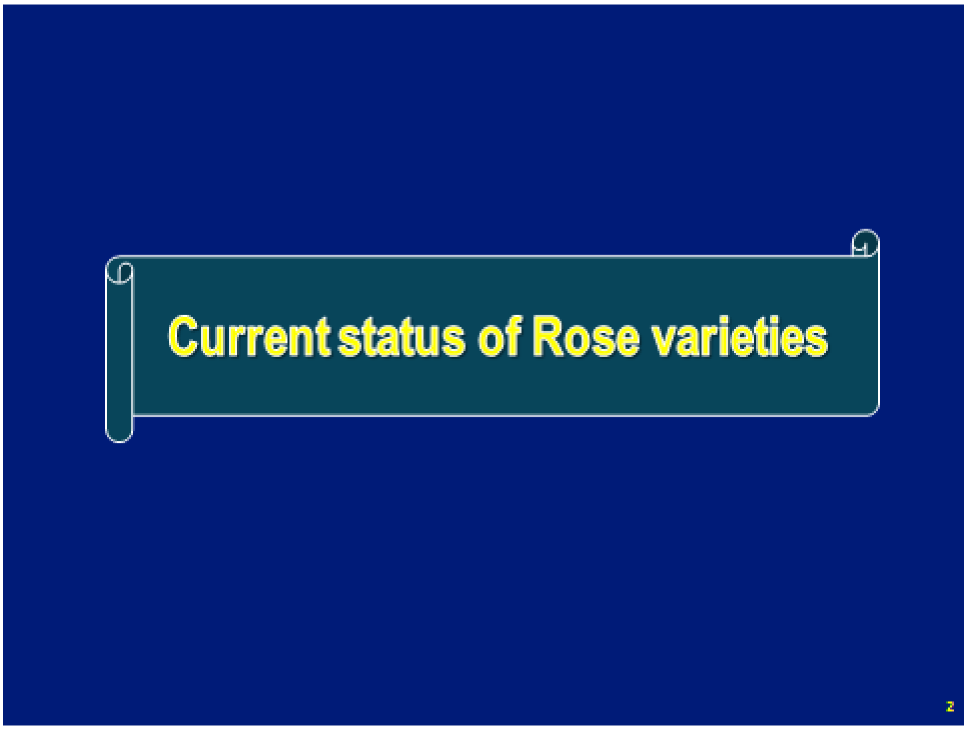
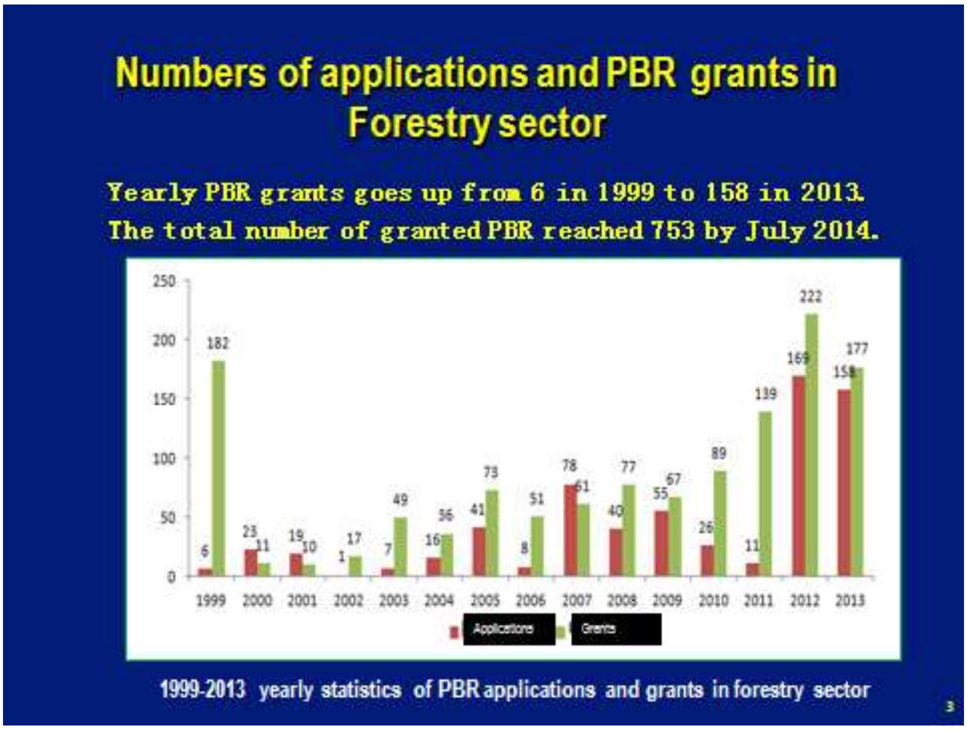


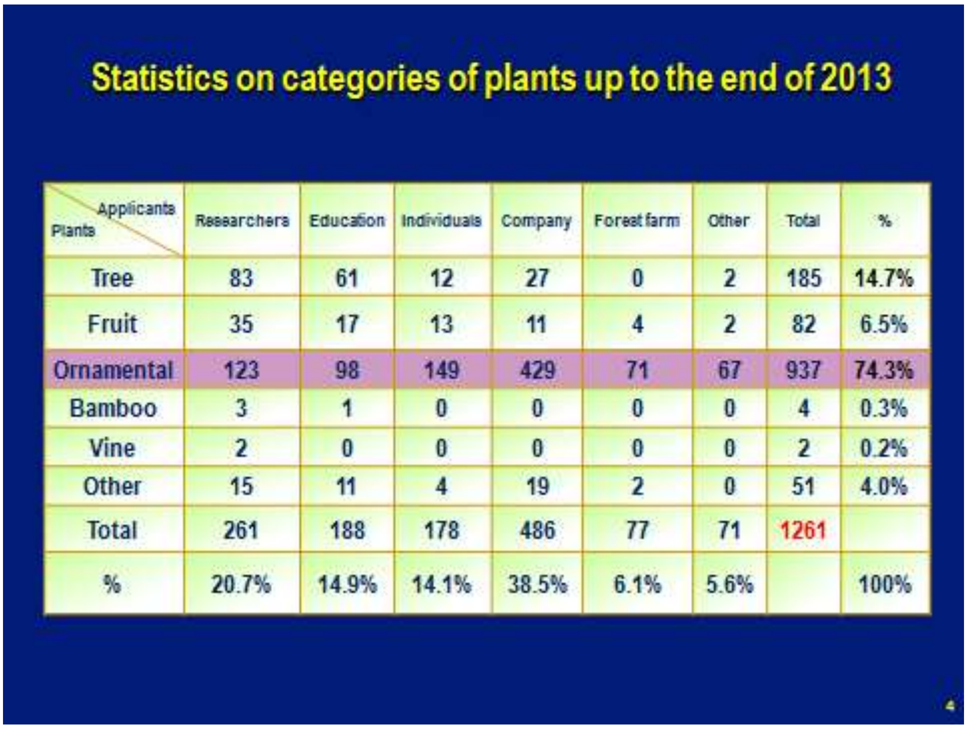
Current status of Rose varieties



Numbers of applications and PBR grants in Forestry sector

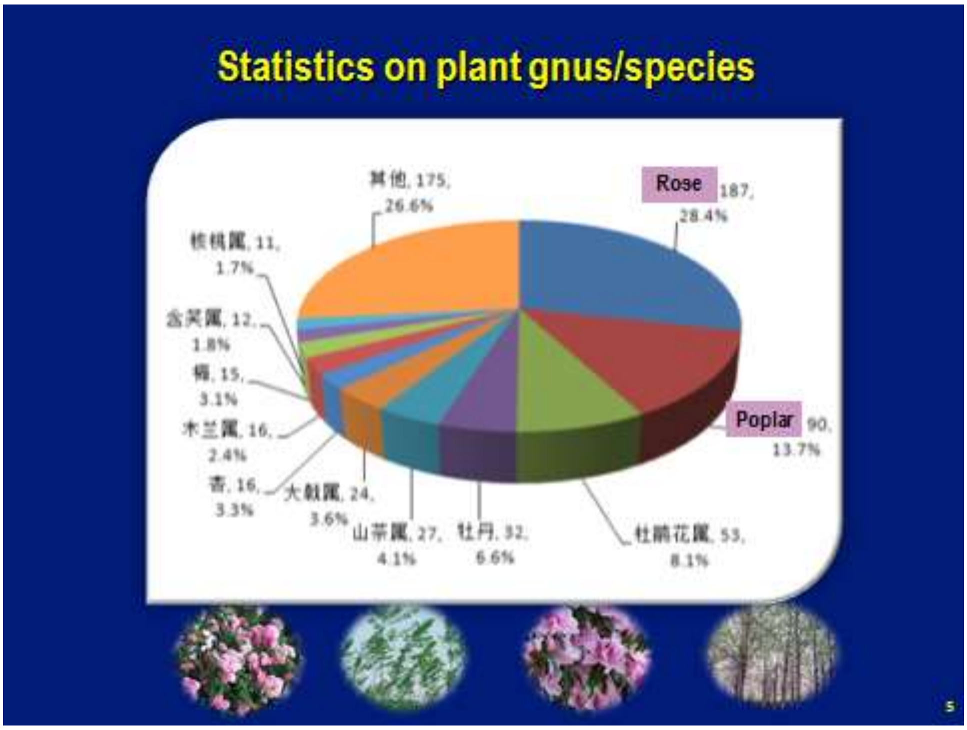
Yearly PBR grants goes up from 6 in 1999 to 158 in 2013.
The total number of granted PBR reached 753 by July 2014.

1999-2013 yearly statistics of PBR applications and grants in forestry sector

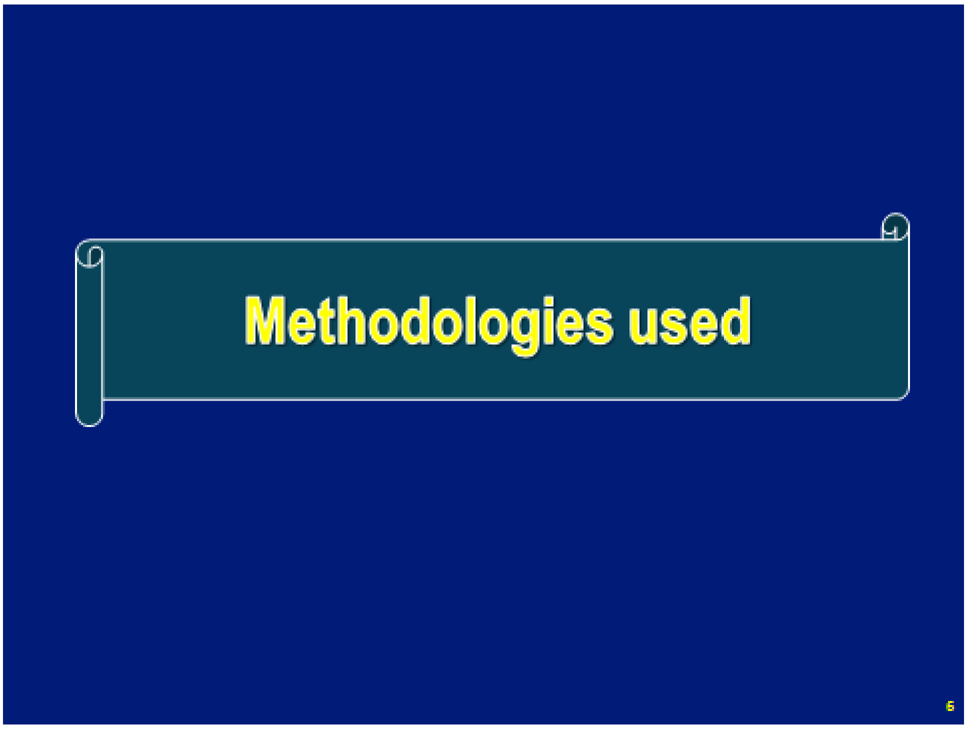


Statistics on categories of plants up to the end of 2013

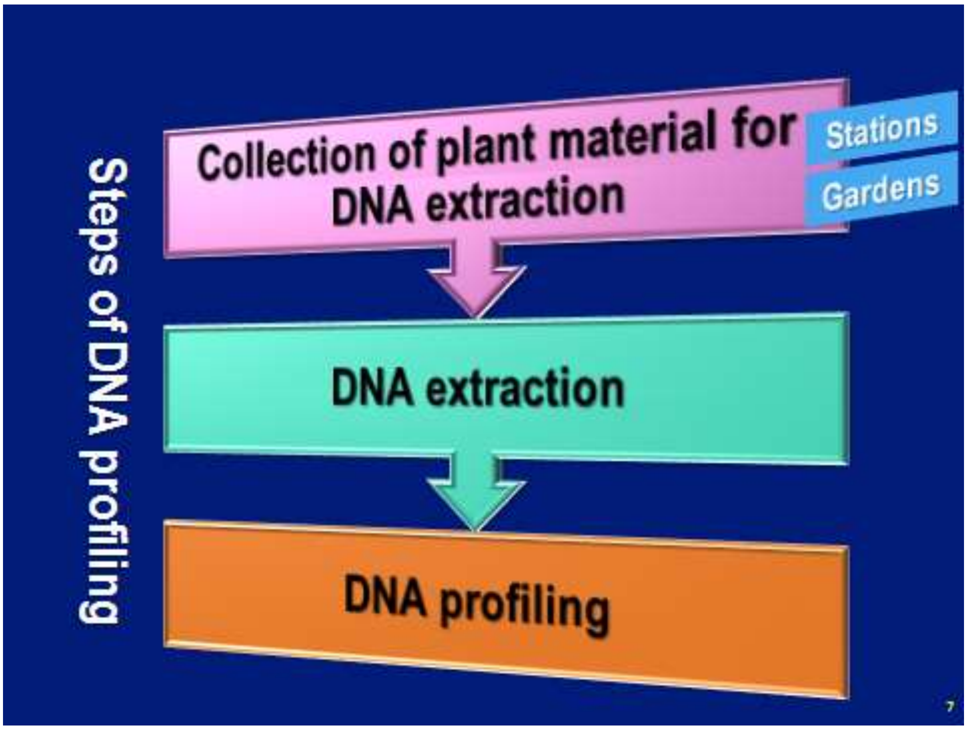
Applicants Plants	Researchers	Education	Individuals	Company	Forest farm	Other	Total	%
Tree	83	61	12	27	0	2	185	14.7%
Fruit	35	17	13	11	4	2	82	6.5%
Ornamental	123	98	149	429	71	67	937	74.3%
Bamboo	3	1	0	0	0	0	4	0.3%
Vine	2	0	0	0	0	0	2	0.2%
Other	15	11	4	19	2	0	51	4.0%
Total	261	188	178	486	77	71	1261	
%	20.7%	14.9%	14.1%	38.5%	6.1%	5.6%		100%



Statistics on plant gnus/species



Methodologies used



Steps of DNA profiling

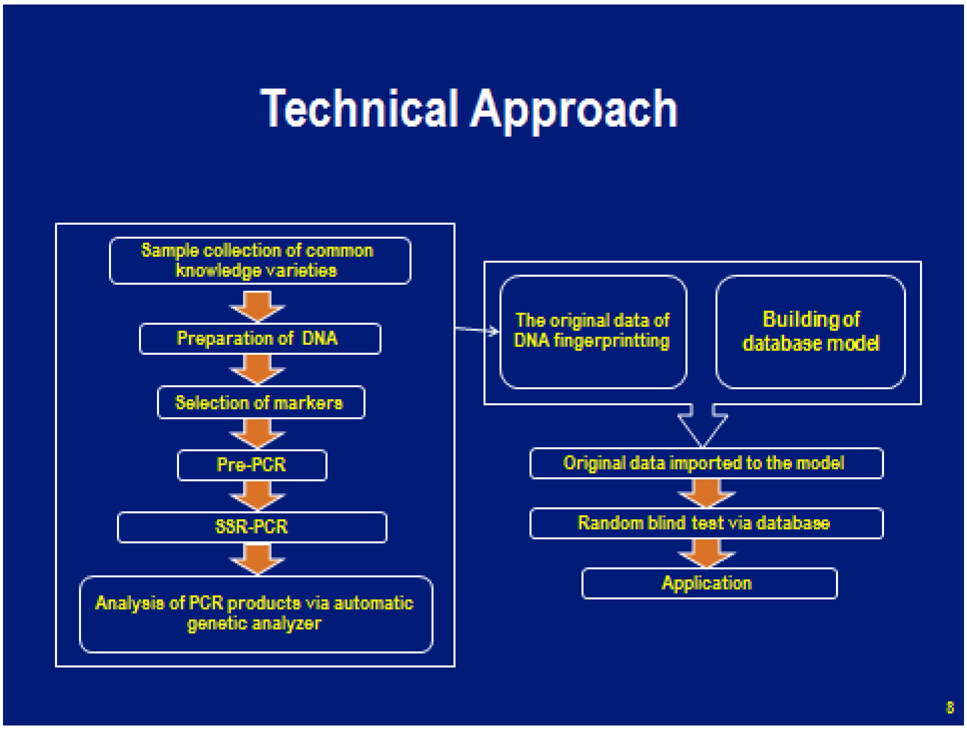
Collection of plant material for DNA extraction

Stations

Gardens

DNA extraction

DNA profiling



Technical Approach

Sample collection of common knowledge varieties

Preparation of DNA

Selection of markers

Pre-PCR

SSR-PCR

Analysis of PCR products via automatic genetic analyzer

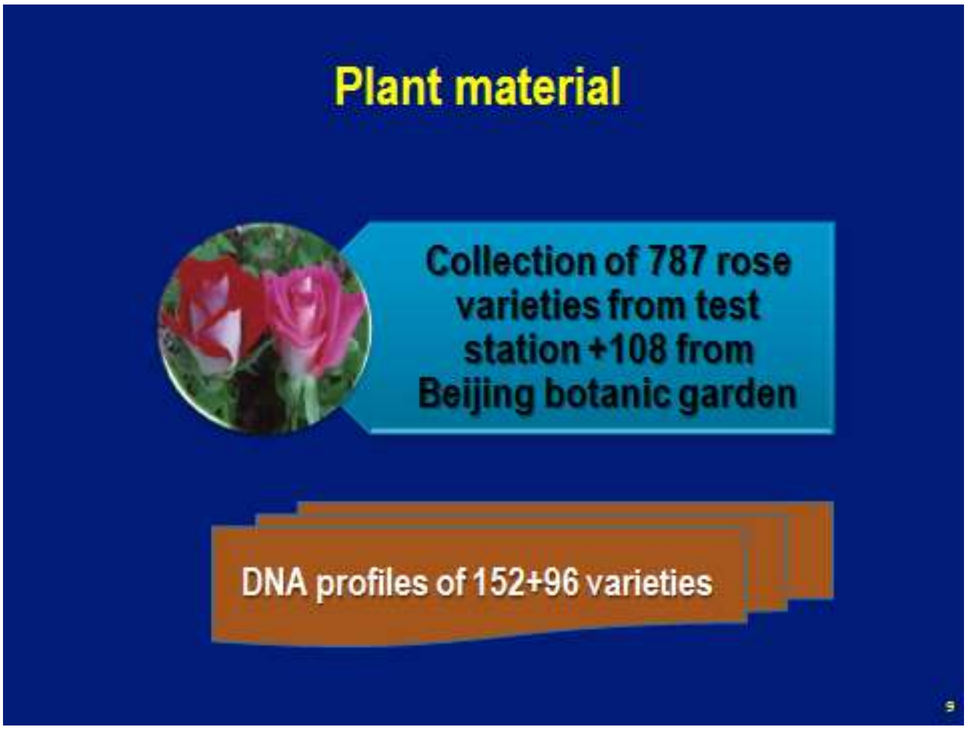
The original data of DNA fingerprintting

Building of database model

Original data imported to the model

Random blind test via database

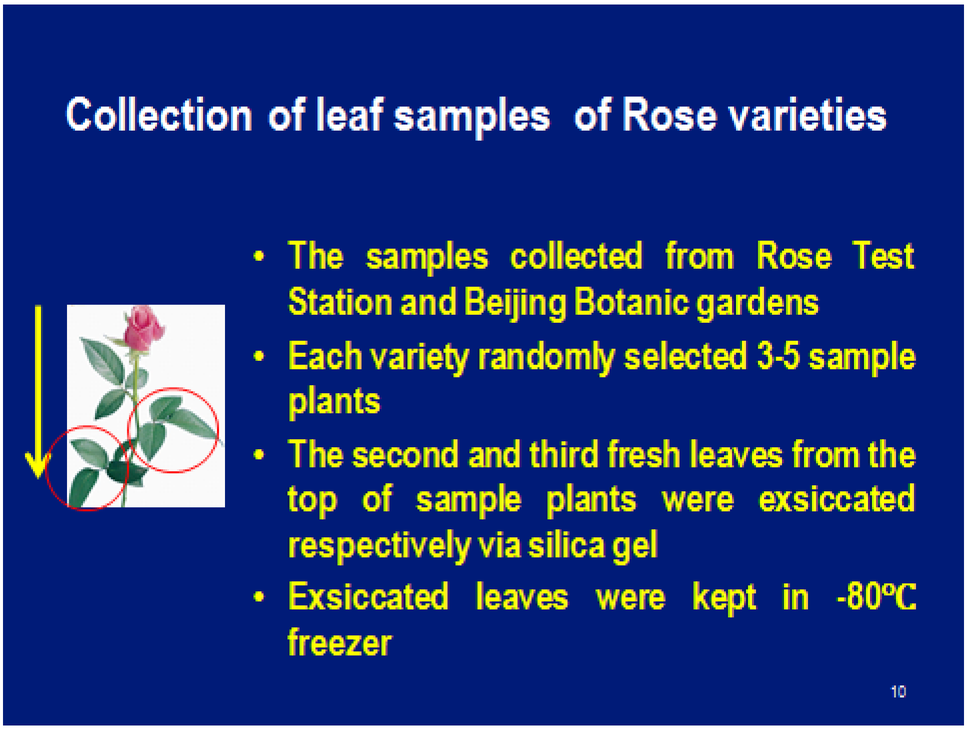
Application



Plant material

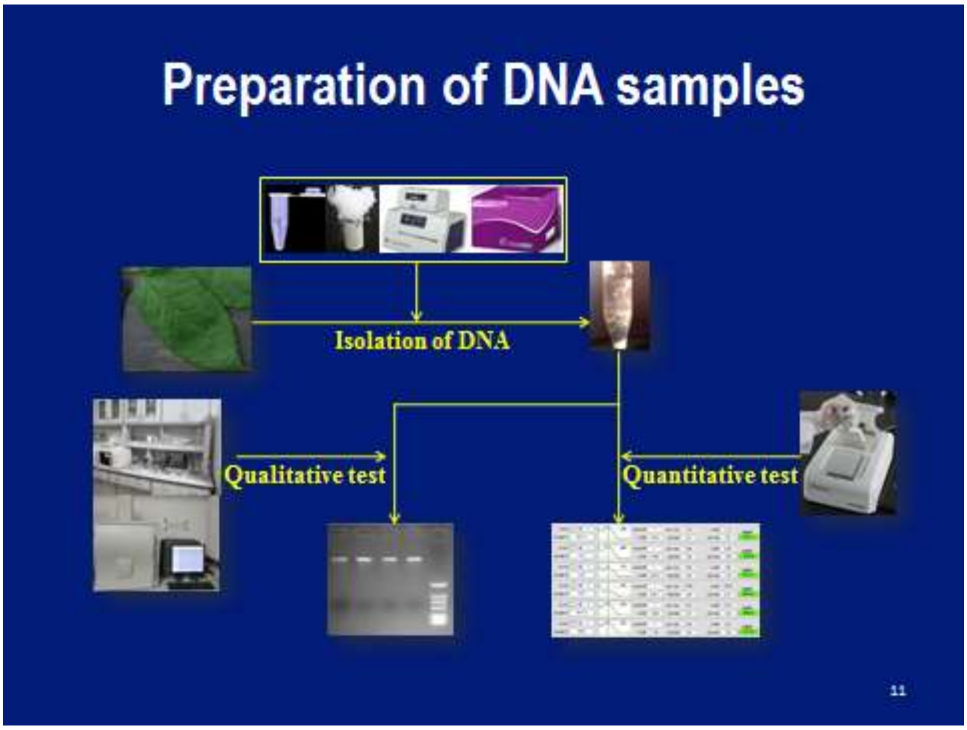
Collection of 787 rose varieties from test station +108 from Beijing botanic garden

DNA profiles of 152+96 varieties



Collection of leaf samples of Rose varieties

- The samples collected from Rose Test Station and Beijing Botanic gardens
- Each variety randomly selected 3-5 sample plants
- The second and third fresh leaves from the top of sample plants were exsiccated respectively via silica gel
- Exsiccated leaves were kept in -80℃ freezer

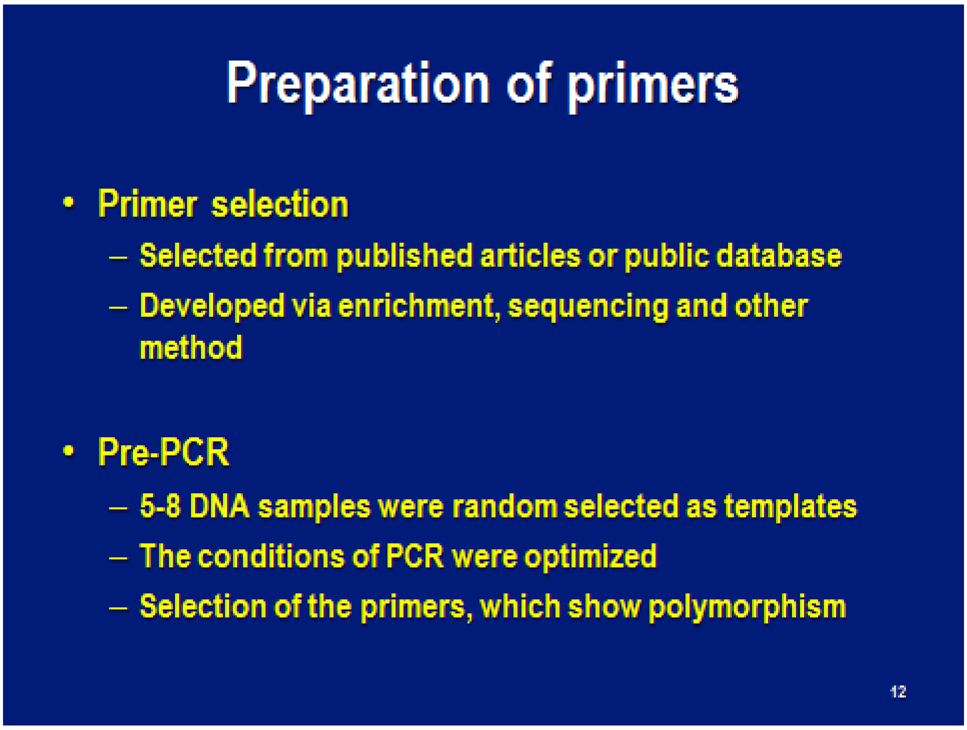


Preparation of DNA samples

Isolation of DNA

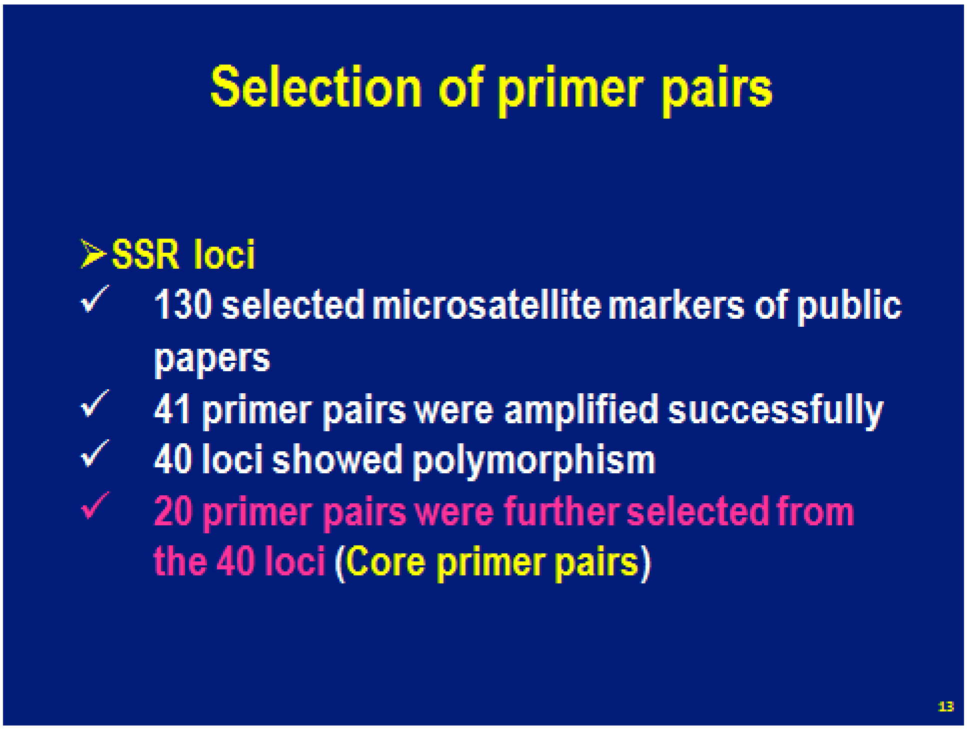
Qualitative test

Quantitative test



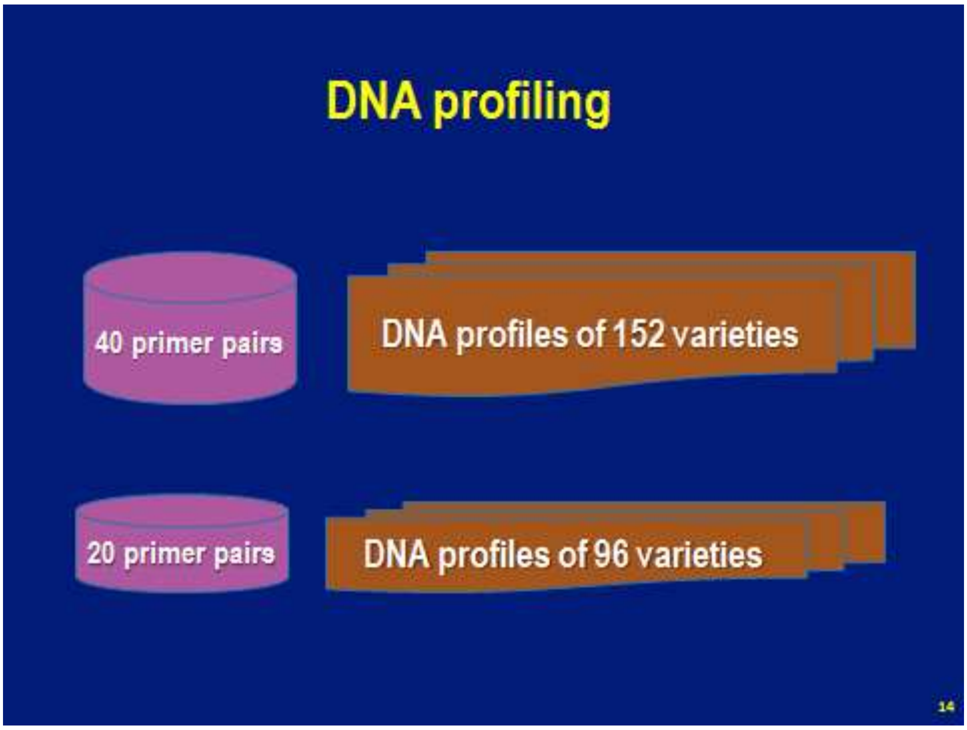
Preparation of primers

- Primer selection
  - Selected from published articles or public database
  - Developed via enrichment, sequencing and other method
- Pre-PCR
  - 5-8 DNA samples were random selected as templates
  - The conditions of PCR were optimized
  - Selection of the primers, which show polymorphism



Selection of primer pairs

➢ SSR loci
- ✓ 130 selected microsatellite markers of public papers
- ✓ 41 primer pairs were amplified successfully
- ✓ 40 loci showed polymorphism
- ✓ 20 primer pairs were further selected from the 40 loci (Core primer pairs)



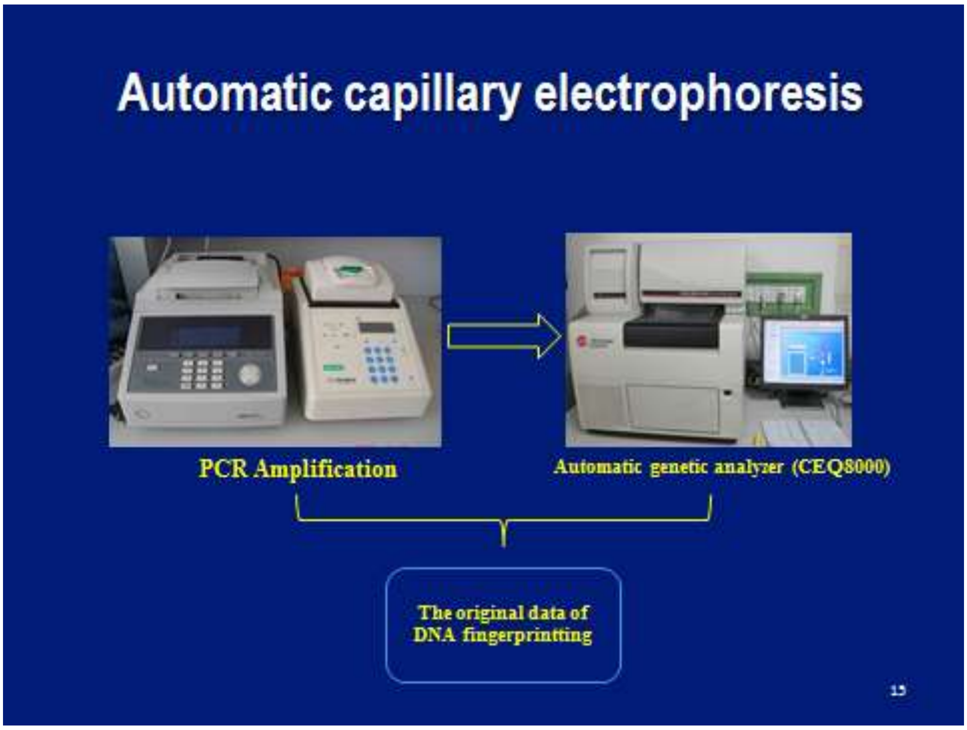
DNA profiling

40 primer pairs

DNA profiles of 152 varieties

20 primer pairs

DNA profiles of 96 varieties



Automatic capillary electrophoresis

PCR Amplification

Automatic genetic analyzer (CEQ8000)

The original data of DNA fingerprintting

Calculation of genetic parameters

Effective number of alleles:

$$N_e = 1 / \sum p_i^2$$

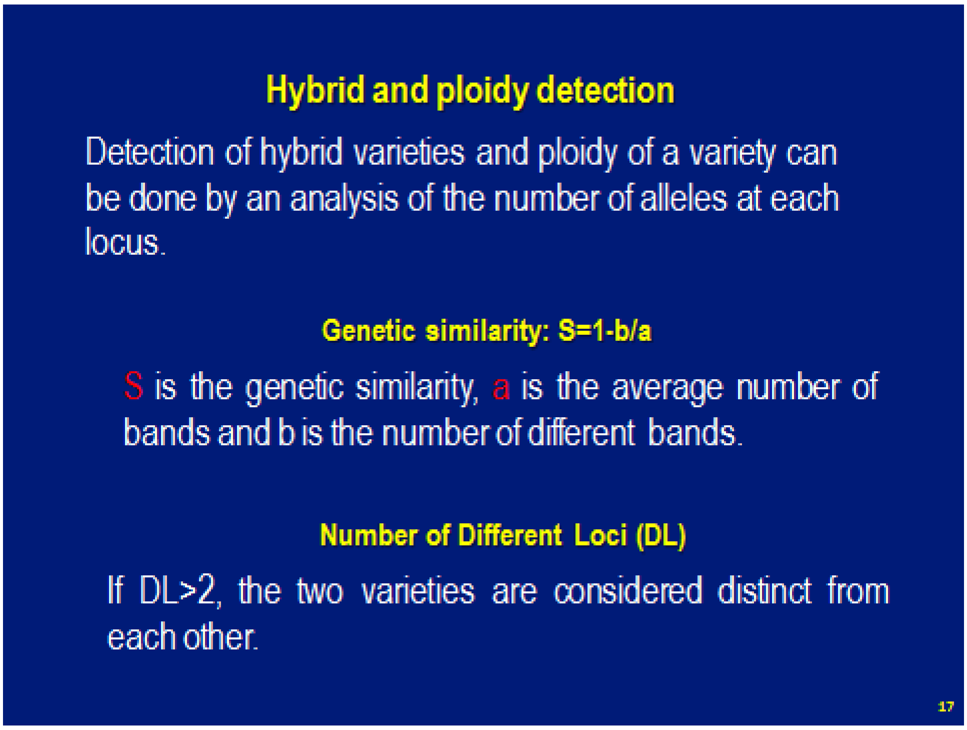
Polymorphic information content :

$$\mathrm{PIC} = 1 - \sum p_i^2$$

Ability of detecting a variety

$$\mathrm{D}_i = 1 - C_i = 1 - \sum_{i=1}^{I} c_i = 1 - \sum_{i=1}^{I} p_i \frac{(N\mathrm{P}_i - 1)}{N - 1}$$

Where D_i is the ability of detecting a variety at locus i, C_i is the extent of confusion of genotypes at locus i. P_i is the frequency of allelic genotypes at locus i, and N is the size of total samples.



Hybrid and ploidy detection

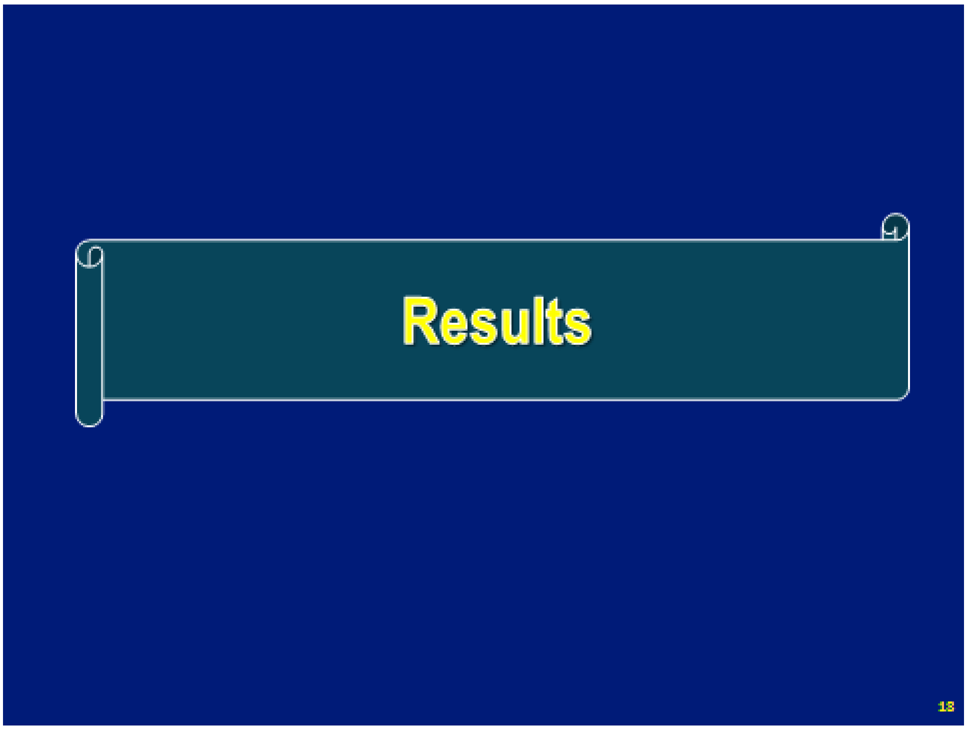
Detection of hybrid varieties and ploidy of a variety can be done by an analysis of the number of alleles at each locus.

Genetic similarity: S=1-b/a

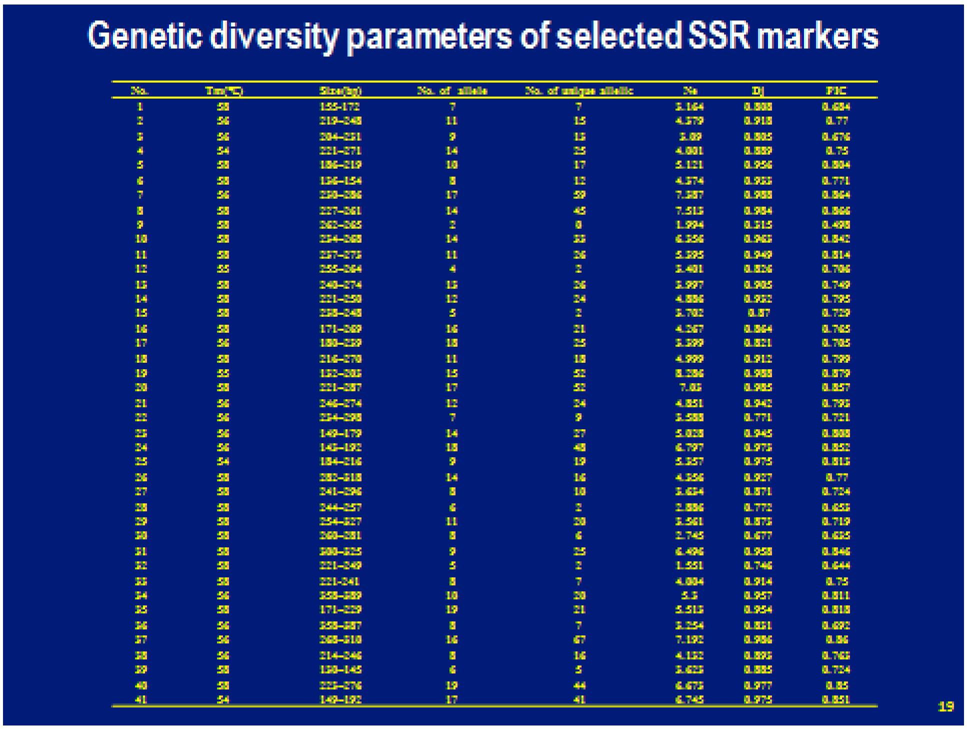
S is the genetic similarity, a is the average number of bands and b is the number of different bands.

Number of Different Loci (DL)

If DL>2, the two varieties are considered distinct from each other.

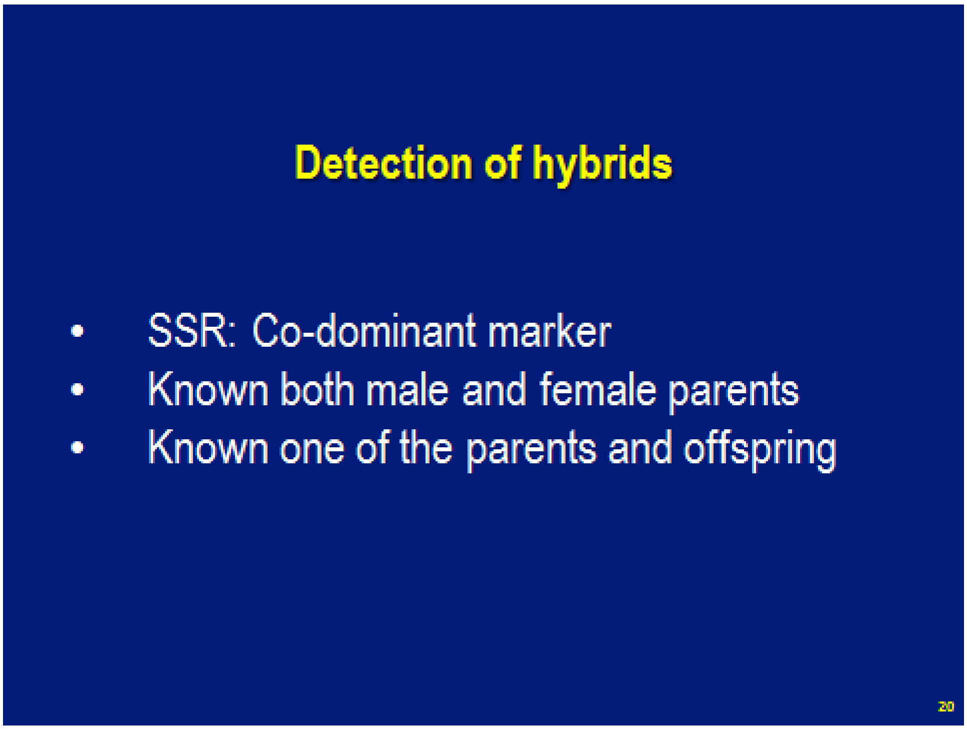


Results



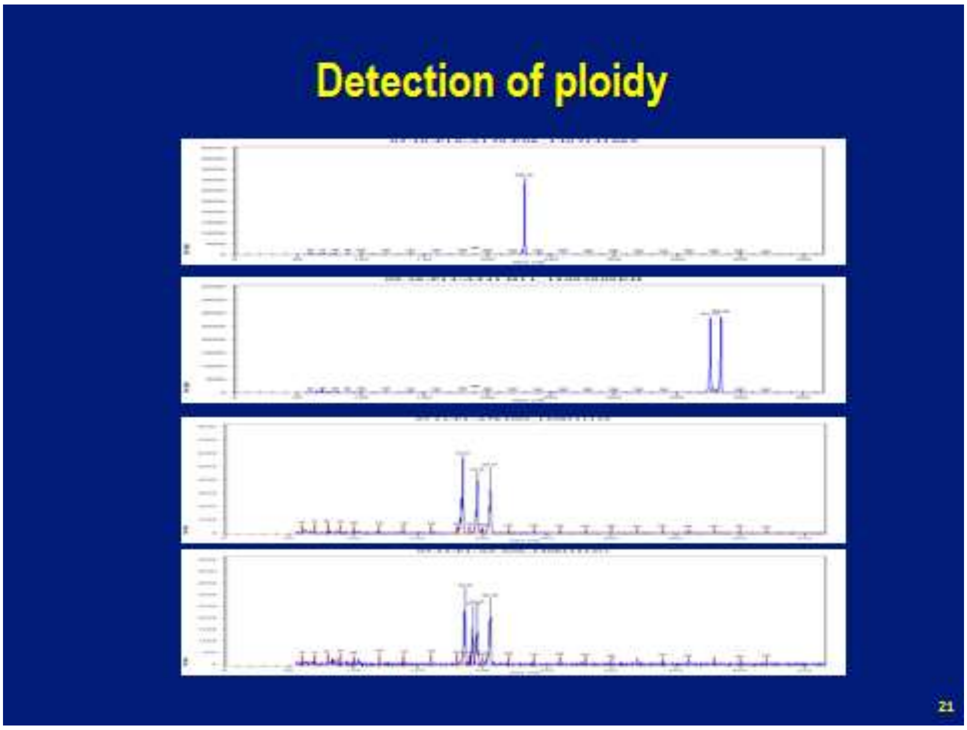
Genetic diversity parameters of selected SSR markers

No.	Tm(℃)	Size(bp)	No. of allele	No. of unique allelic	Ne	Dj	PIC
1	58	155-172	7	7	3.164	0.808	0.684
2	56	219–248	11	15	4.379	0.918	0.77
3	56	204–231	9	13	3.89	0.885	0.676
4	54	221–271	14	25	4.001	0.889	0.75
5	58	186–219	10	17	5.121	0.956	0.804
6	58	136–154	8	12	4.374	0.933	0.771
7	56	230–286	17	59	7.387	0.988	0.864
8	58	227–261	14	45	7.513	0.984	0.866
9	58	262–265	2	0	1.994	0.315	0.498
10	58	234–268	14	33	6.336	0.963	0.842
11	58	237–273	11	26	5.395	0.949	0.814
12	55	255–264	4	2	3.401	0.826	0.706
13	58	240–274	13	26	3.997	0.905	0.749
14	58	221–250	12	24	4.886	0.952	0.795
15	58	238–248	5	2	3.702	0.87	0.729
16	58	171–269	16	21	4.267	0.864	0.765
17	56	180–239	18	25	3.399	0.821	0.705
18	58	216–270	11	18	4.999	0.912	0.799
19	55	132–203	15	52	8.286	0.988	0.879
20	58	221–287	17	52	7.03	0.985	0.857
21	56	246–274	12	24	4.851	0.942	0.793
22	56	234–298	7	9	3.588	0.771	0.721
23	56	149–179	14	27	5.028	0.945	0.808
24	56	143–192	18	48	6.797	0.973	0.852
25	54	184–216	9	19	5.357	0.975	0.813
26	58	282–318	14	16	4.356	0.927	0.77
27	58	241–296	8	10	3.634	0.871	0.724
28	58	244–257	6	2	2.886	0.772	0.655
29	58	254–327	11	20	3.561	0.873	0.719
30	58	260–281	8	6	2.745	0.677	0.635
31	58	300–325	9	25	6.496	0.958	0.846
32	58	221–249	5	2	1.551	0.746	0.644
33	58	221-241	8	7	4.004	0.914	0.75
34	56	358–389	10	20	5.5	0.957	0.811
35	58	171–229	19	21	5.513	0.954	0.818
36	56	358–387	8	7	3.254	0.831	0.692
37	56	268–310	16	67	7.192	0.986	0.86
38	56	214–246	8	16	4.132	0.895	0.763
39	58	130–145	6	5	3.625	0.885	0.724
40	58	225–276	19	44	6.673	0.977	0.85
41	54	149–192	17	41	6.745	0.975	0.851

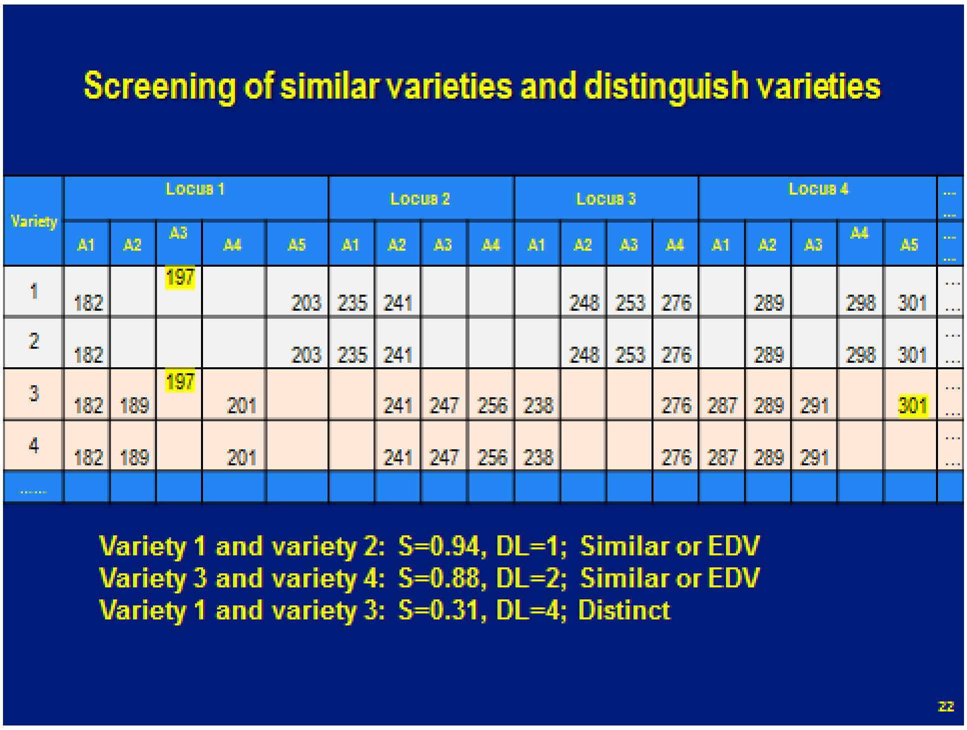


Detection of hybrids

- SSR: Co-dominant marker
- Known both male and female parents
- Known one of the parents and offspring



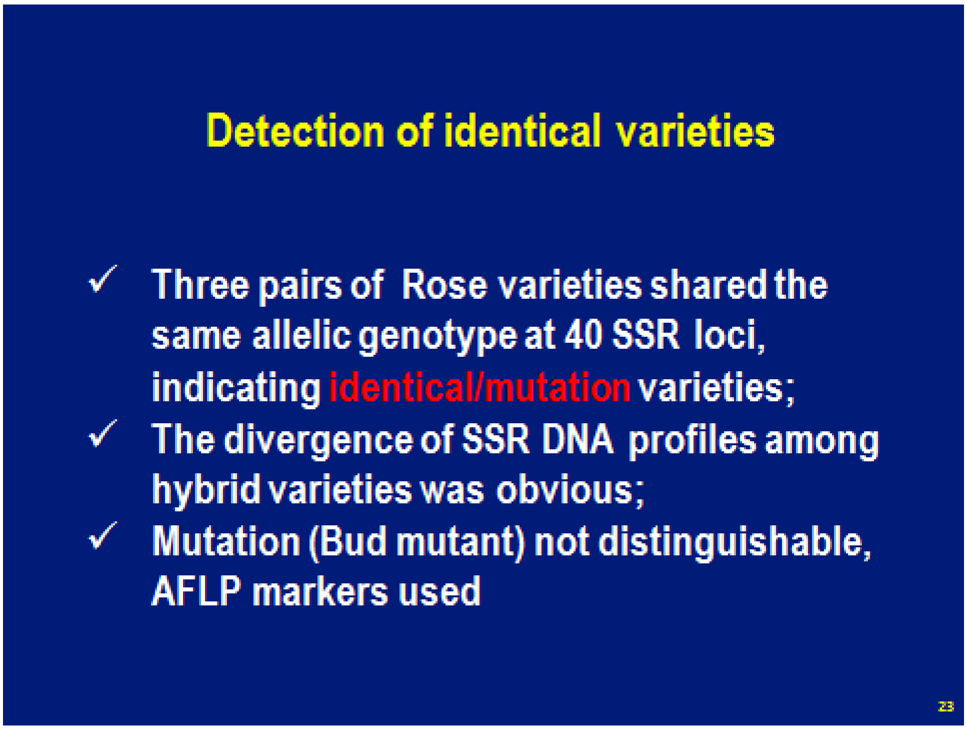
Detection of ploidy



Screening of similar varieties and distinguish varieties

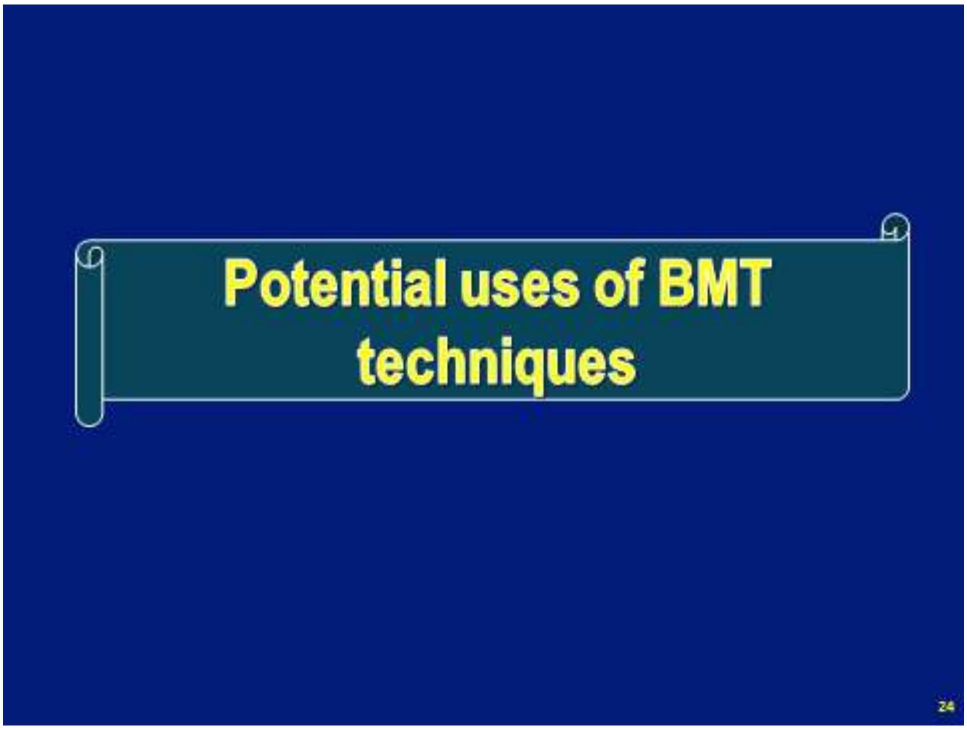
Variety	Locus 1					Locus 2				Locus 3				Locus 4					......
	A1	A2	A3	A4	A5	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4	A5	......
1	182		197		203	235	241				248	253	276		289		298	301	......
2	182				203	235	241				248	253	276		289		298	301	......
3	182	189	197	201			241	247	256	238			276	287	289	291		301	......
4	182	189		201			241	247	256	238			276	287	289	291			......
......																			

Variety 1 and variety 2: S=0.94, DL=1; Similar or EDV
Variety 3 and variety 4: S=0.88, DL=2; Similar or EDV
Variety 1 and variety 3: S=0.31, DL=4; Distinct

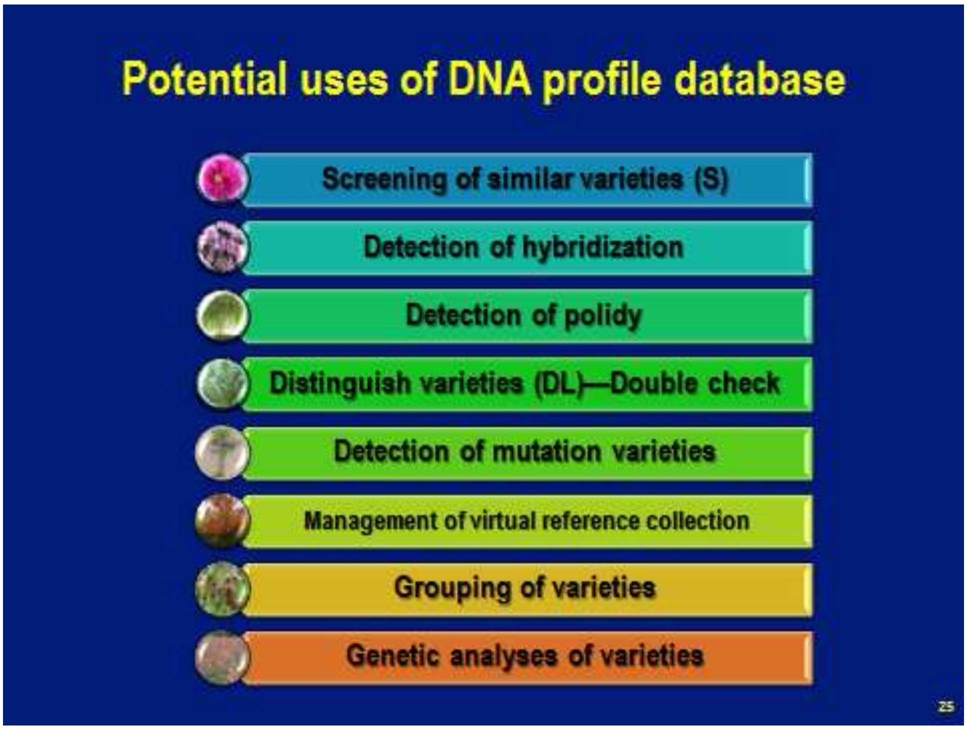


Detection of identical varieties

- ✓ Three pairs of Rose varieties shared the same allelic genotype at 40 SSR loci, indicating identical/mutation varieties;
- ✓ The divergence of SSR DNA profiles among hybrid varieties was obvious;
- ✓ Mutation (Bud mutant) not distinguishable, AFLP markers used



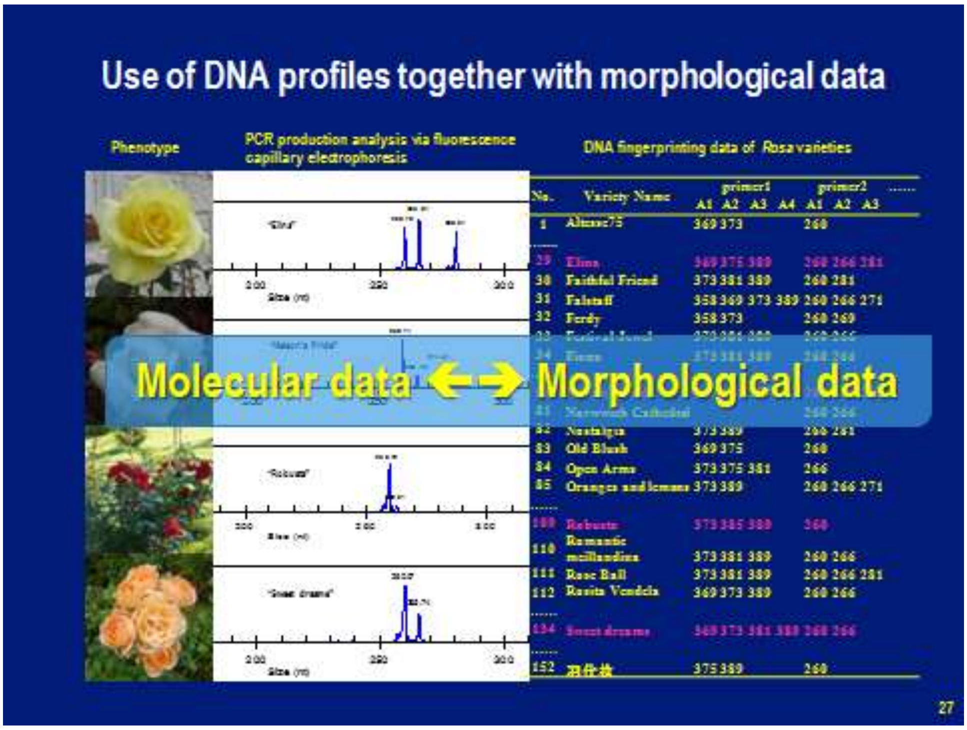
Potential uses of BMT techniques



Potential uses of DNA profile database

- Screening of similar varieties (S)
- Detection of hybridization
- Detection of polidy
- Distinguish varieties (DL)—Double check
- Detection of mutation varieties
- Management of virtual reference collection
- Grouping of varieties
- Genetic analyses of varieties

Discussions



Use of DNA profiles together with morphological data

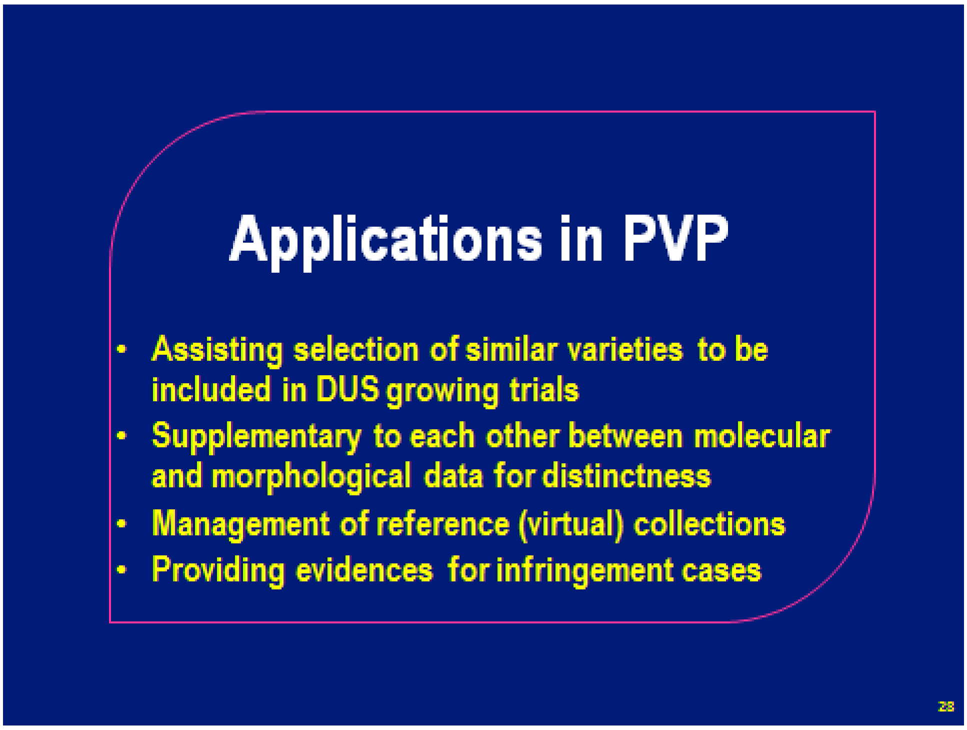
Phenotype

PCR production analysis via fluorescence capillary electrophoresis

Molecular data ←→ Morphological data

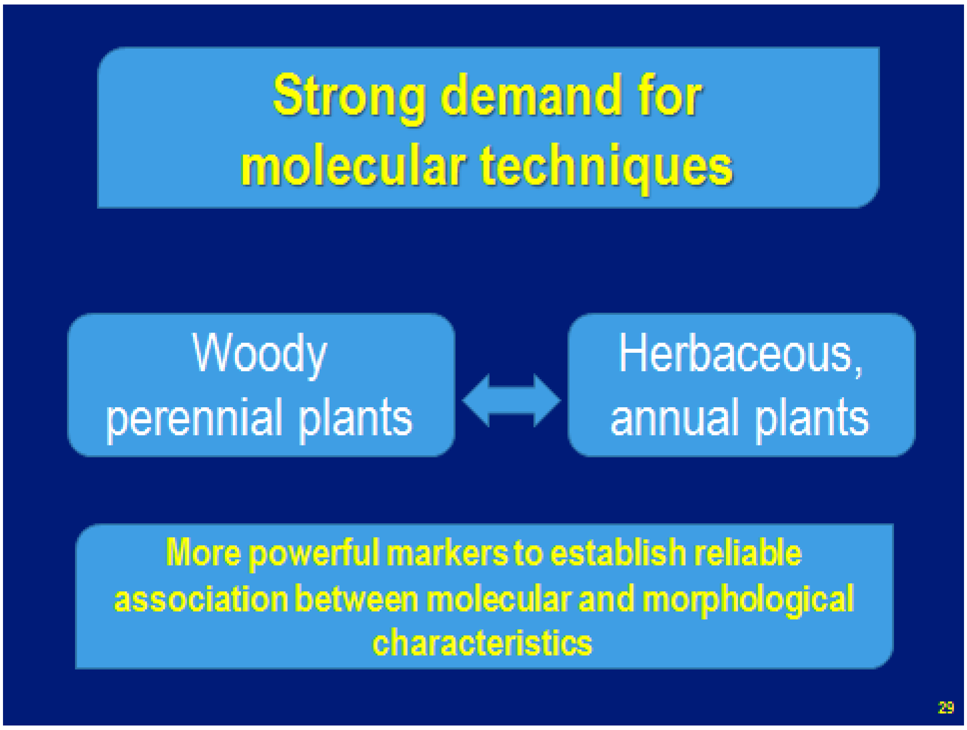
DNA fingerprinting data of Rosa varieties

No.	Variety Name	primer1				primer2			......
		A1	A2	A3	A4	A1	A2	A3	
1	Altesse75	369	373			260			
......									
29	Elina	369	375	389		260	266	281	
30	Faithful Friend	373	381	389		260	281		
31	Falstaff	358	369	373	389	260	266	271	
32	Ferdy	358	373			260	269		
33	Festival Jewel	373	381	389		260	266		
34	Fiona	373	381	389		260	266		
81	Norwich Cathedral					260	266		
82	Nostalgia	373	389			260	281		
83	Old Blush	369	375			260			
84	Open Arms	373	375	381		266			
85	Oranges and lemons	373	389			260	266	271	
......									
109	Robusta	373	385	389		260			
110	Romantic meillandina	373	381	389		260	266		
111	Rose Ball	373	381	389		260	266	281	
112	Rosita Vendela	369	373	389		260	266		
......									
134	Sweet dreams	369	373	381	389	260	266		
......									
152	[illegible]	375	389			260			



Applications in PVP

- Assisting selection of similar varieties to be included in DUS growing trials
- Supplementary to each other between molecular and morphological data for distinctness
- Management of reference (virtual) collections
- Providing evidences for infringement cases



Strong demand for molecular techniques

Woody perennial plants ↔ Herbaceous, annual plants

More powerful markers to establish reliable association between molecular and morphological characteristics

Future plan

- Complete all rose varieties
- Collection of 574 Peony varieties at test station
- Collection of more than 300 Rhododendron varieties
- >300 Willow varieties
- >100 Poplar varieties

Molecular characteristics (Description of DNA profile) on voluntary basis

Thanks for
Your Attention

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