TWC/31/2 Add.
ORIGINAL: English
DATE: June 14, 2013

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

Thirty-First Session
Seoul, Republic of Korea, June 4 to 7, 2013

ADDENDUM

MOLECULAR TECHNIQUES

Document prepared by experts from China

The Annex to this document contains a copy of a presentation on the research on the construction of the DNA fingerprint database in Maize that was made at the Technical Working Party on Automation and Computer Programs (TWC), at its thirty-first session.

[Annex follows]

Research on the Construction of DNA Fingerprint Database in Maize

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Beijing Academy of Agriculture and Forestry Science, China

Overview

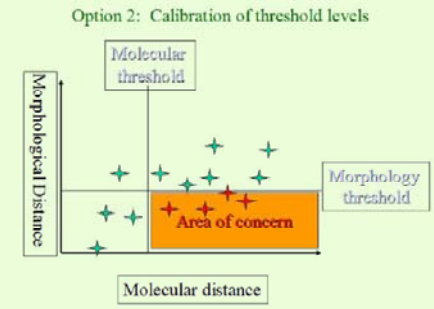
1. Possible Use of Molecular Markers in the DUS Test
2. Lab Preparation & Demand Analysis
3. Plant DNA Fingerprint Database Design & Implementation
4. Maize DNA Fingerprint Database Management System Architecture & Functional Module
5. Construction of DNA Fingerprint database of China Maize Varieties

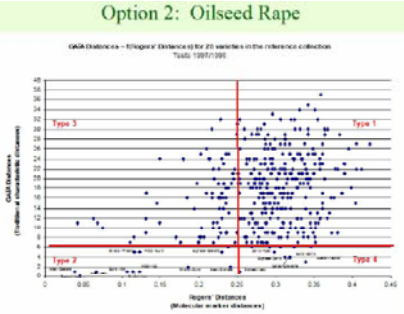
1. Possible Use of Molecular Markers in the DUS Test

- **Option 1: Molecular characteristics as a predictor of traditional characteristics**
  - **Option 1 (a): Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)**
  - **Option 1 (b): Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics**

1. Possible Use of Molecular Markers in the DUS Test

- **Option 2: Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics**





It is divided into four areas by molecular threshold and morphology threshold

1. Possible Use of Molecular Markers in the DUS Test

- **Option 3: Development of new system**
  - **This approach would mean that clearly distinguishable differences in molecular characteristics would be considered as threshold levels for judging distinctness.**
- **Our choice**
  - **DNA fingerprint database, combined with morphologic character: Option 2**
  - **Function marker: Option 1**

Overview

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3. **Plant DNA Fingerprint Database Design & Implementation**
4. **Maize DNA Fingerprint Database Management System Architecture & Functional Module**
5. **Construction of DNA Fingerprint database of China Maize Varieties**

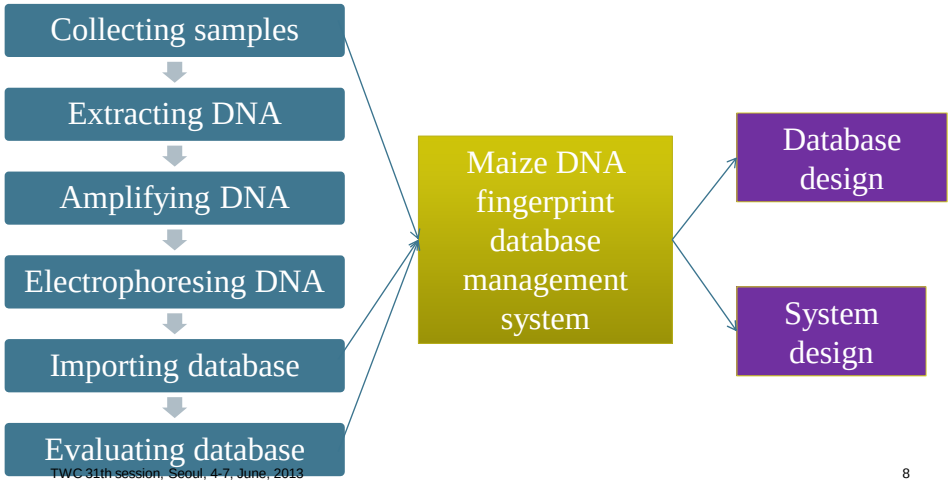
2.Lab Preparation & Demand Analysis

❖ **2.1 The following nine aspects were standardized, which would be helpful for collaboration among different laboratories to construct DNA fingerprint database in maize.**

1. molecular marker
2. detection platform
3. reagent
4. sample
5. evaluation procedure
6. data conformity
7. pattern database
8. extended database
9. blind test

2. Lab Preparation & Demand Analysis

2.2 The main process of maize DNA fingerprint database construction



2. Lab Preparation & Demand Analysis

2.3 The basic demand of maize DNA fingerprint database

- Storing multi-source data from the specific types of molecular marker
- Recording complex relationship between variety information, morphological characters and genetic characteristics
- Searching mass data from many varieties which obtained by various molecular marker methodology

2. Lab Preparation & Demand Analysis

2.4 The basic demand of maize DNA fingerprint database management system

- Importing data quickly
- Storing data standardly
- Sharing data analysis easily

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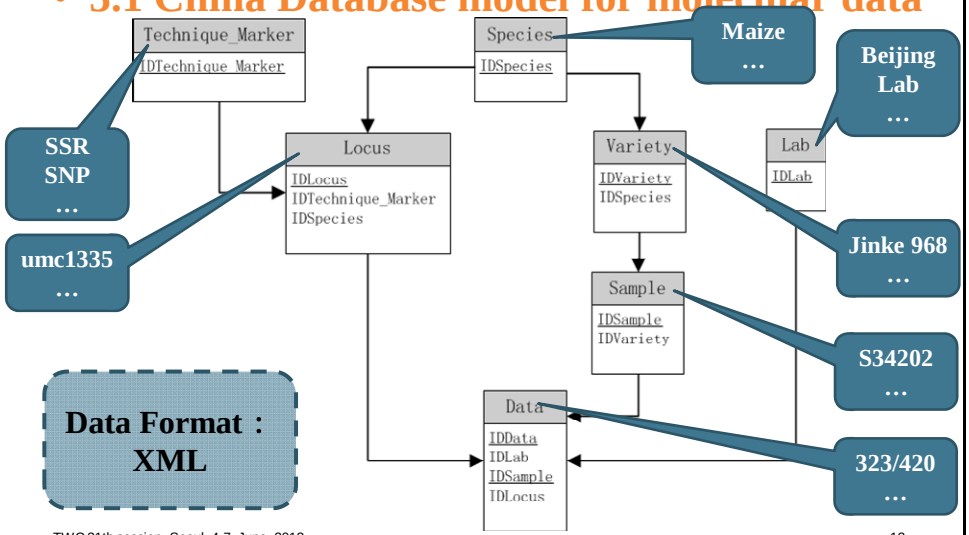
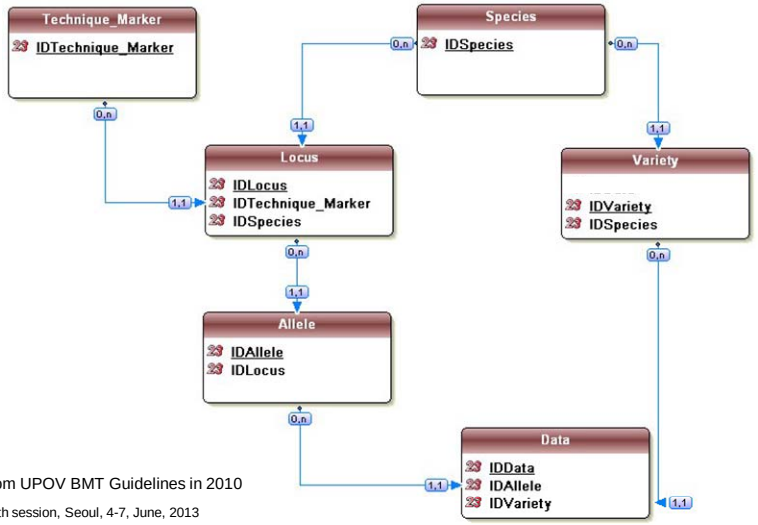
3. Plant DNA Fingerprint Database Design & Implementation

- 3.1 China Database model for molecular data



3. Plant DNA Fingerprint Database Design & Implementation

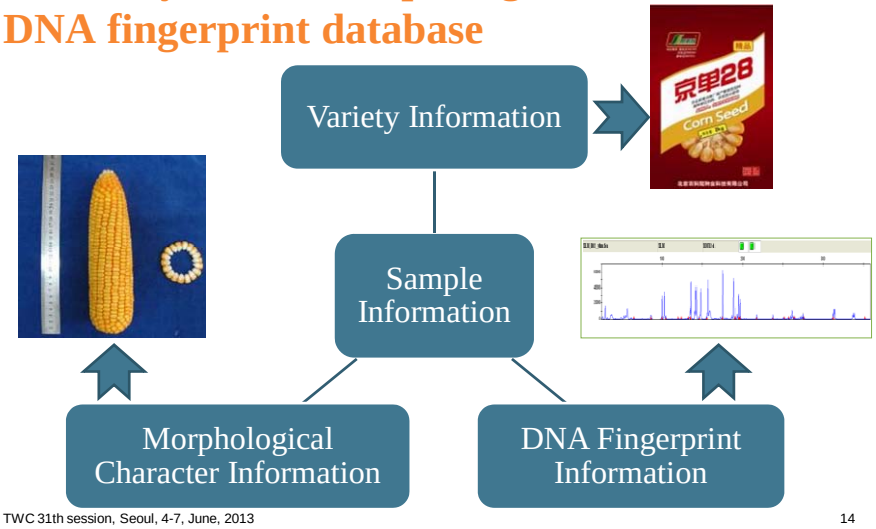
3.2 BMT Database model for molecular data



Cited from UPOV BMT Guidelines in 2010

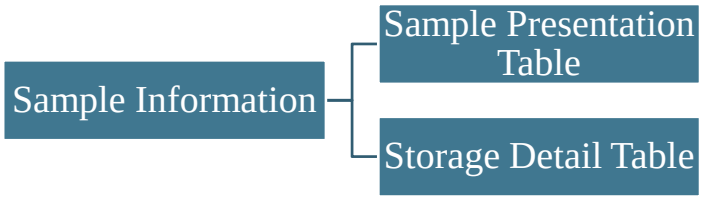
3. Plant DNA Fingerprint Database Design & Implementation

3.3 Entity Relationship Diagram of maize DNA fingerprint database



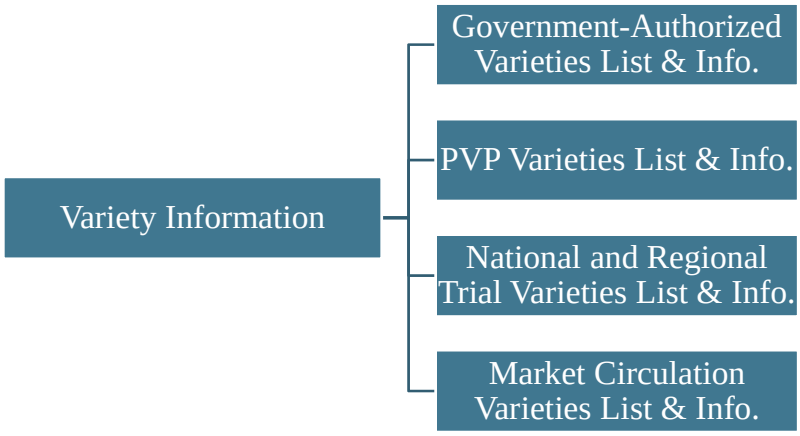
3. Plant DNA Fingerprint Database Design & Implementation

3.4 Entity Relationship Diagram of maize DNA fingerprint database



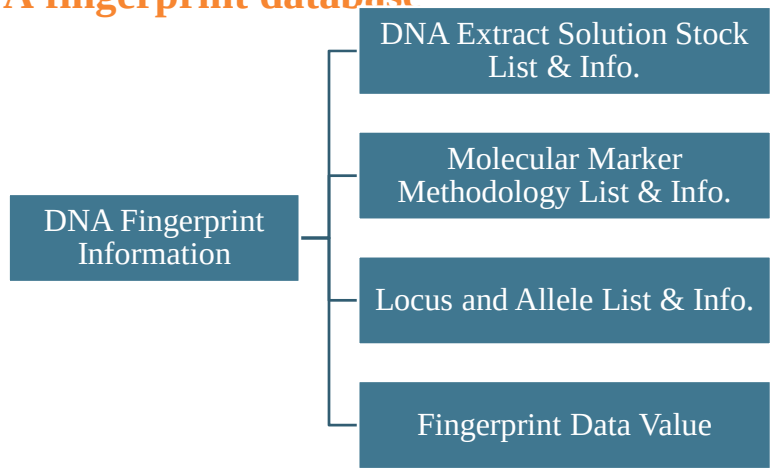
3. Plant DNA Fingerprint Database Design & Implementation

3.4 Entity Relationship Diagram of maize DNA fingerprint database



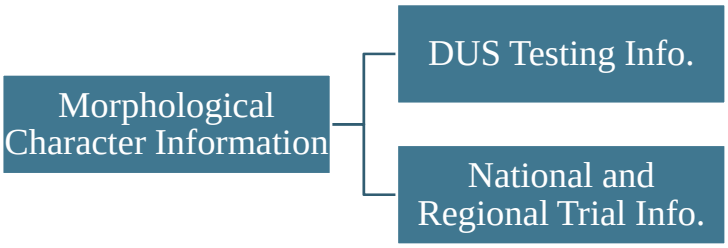
3. Plant DNA Fingerprint Database Design & Implementation

3.4 Entity Relationship Diagram of maize DNA fingerprint database



3. Plant DNA Fingerprint Database Design & Implementation

3.4 Entity Relationship Diagram of maize DNA fingerprint database



3. Plant DNA Fingerprint Database Design & Implementation

- **Information of the 20 basic core primers**

No.	primer	BIN	Forward Primer Sequence (5' 3')	Reverse Primer Sequence (5' 3')	Alleles	Range
N01	bnlg439w1	1.03	AGTTGACATCGCCATCTTGGTGAC	GAACAAGCCCTTAGCGGGTTGTC	12	319-369
N02	umc1335y5	1.06	CCTCGTTACGGTTACGCTGCTG	GATGACCCCGCTTACTTCGTTTATG	4	333-357
N03	umc2007y4	2.04	TTACACAACGCAACACGAGGC	GCTATAGGCCGTAGCTTGGTAGACAC	14	233-300
N04	bnlg1040k7	2.08	CGTTTAAGAACGGTTGATTGCATTCC	GCCTTTATTTCTCCCTTGCTTGCC	13	324-388
N05	umc2105k3	3.00	GAAGGGCAATGAATAGAGCCATGAG	ATGGACTCTGTGCGACTTGTACCG	6	280-350
N06	phi053k2	3.05	CCCTGCCTCTCAGATTCAGAGATTG	TAGGCTGGCTGGAAGTTTGTTGC	4	333-363
N07	phi072k4	4.01	GCTCGTCTCCTCCAGGTCAGC	GAGCTTCTTTTGCACCACAAGGTC	4	408-432
N08	bnlg2291k4	4.06	GCACACCCGTAGTAGCTGAGACTTG	CATAACCTTGCCTCCCAAACCC	6	362-421
N09	umc1705w1	5.03	GGAGGTCGTCAGATGGAGTTCG	CACGTACGGCAATGCAGACAAG	10	254-349
N10	bnlg2305k4	5.07	CCCCTCTTCCTCAGCACCTTG	CGTCTTGTCTCCGTCCGTGTG	12	240-312
N11	bnlg1611k8	6.00	TCTCAGCTCCTGCTTATTGCTTTCG	GATGGATGGAGCATGAGCTTGC	14	154-216
N12	bnlg1702k1	6.05	GATCCGCATTGTCAAATGACCAC	AGGACACGCCATCGTCATCA	13	260-347
N13	umc1545y2	7.00	AATGCCGTTATCATGCGATGC	GCTTGCTGCTTCTTGAATTGCGT	7	180-249
N14	umc1125y3	7.04	GGATGATGGCGAGGATGATGTC	CCACCAACCCATACCCATACCAG	5	149-175
N15	bnlg240k1	8.06	GCAGGTGTCGGGGATTTTCTC	GGAACTGAAGAACAGAAGGCATTGATAC	7	220-239
N16	phi080k15	8.08	TGAACCACCCGATGCAACTTG	TTGATGGGCACGATCTCGTAGTC	6	202-238
N17	phi065k9	9.03	CGCCTTCAAGAATATCCTTGTGCC	GGACCCAGACCAGGTTCCACC	4	391-415
N18	umc1492y13	9.04	GCGGAAGAGTAGTCGTAGGGCTAGTGTAG	AACCAAGTTCTTCAGACGCTTCAGG	4	270-290
N19	umc1432y6	10.02	GAGAAATCAAGAGGTGCGAGCATC	GGCCATGATACAGCAAGAAATGATAAGC	7	211-269
N20	umc1506k12	10.05	GAGGAATGATGTCCGCGAAGAAG	TTCAGTCGAGCGCCCAACAC	6	163-196

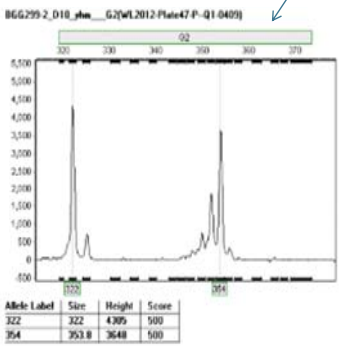
3. Plant DNA Fingerprint Database Design & Implementation

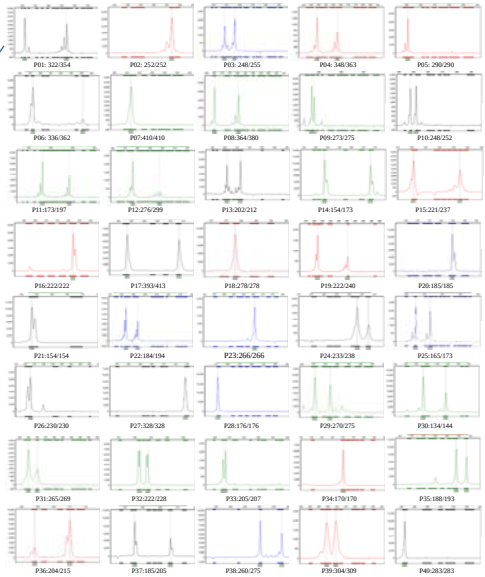
- **Example of PDNAF Database Record, like ×××, One Sample contain two origin data and one standard data**

引物编号	合并标准基因型	一组原始基因	二组原始数据
P01	322/354	322/354	322/354
P02	252/252	252/252	252/252
P03	248/255	248/255	248/255
P04	348/363	348/363	348/363
P05	290/290	290/290	290/290
P06	336/336	336/336	336/336
P07	410/410	410/410	410/410
P08	364/380	364/380	364/380
P09	273/275	273/275	273/275
P10	248/252	248/252	248/252
P11	173/197	173/197	173/197
P12	276/299	276/299	276/299
P13	202/212	202/212	202/212
P14	154/173	154/173	154/173
P15	221/237	221/237	221/237
P16	222/222	222/222	222/222
P17	393/413	393/413	393/413
P18	278/278	278/278	278/278
P19	222/240	222/240	222/240
P20	185/185	185/185	185/185
P21	154/154	154/154	154/154
P22	184/194	184/194	184/194
P23	266/266	266/266	266/266
P24	233/238	233/238	233/238
P25	165/173	165/173	165/173
P26	230/230	230/230	230/230
P27	328/328	328/328	328/328
P28	176/176	176/176	176/176
P29	270/275	270/275	270/275
P30	134/144	134/144	134/144
P31	265/269	265/269	265/269
P32	222/228	222/228	222/228
P33	205/207	205/207	205/207
P34	170/170	170/170	170/170
P35	188/193	188/193	188/193
P36	204/215	204/215	204/215
P37	185/205	185/205	185/205
P38	260/275	260/275	260/275
P39	304/309	304/309	304/309
P40	283/283	283/283	283/283

3. Plant DNA Fingerprint Database Design & Implementation

- **Fingerprint Map of One Sample**





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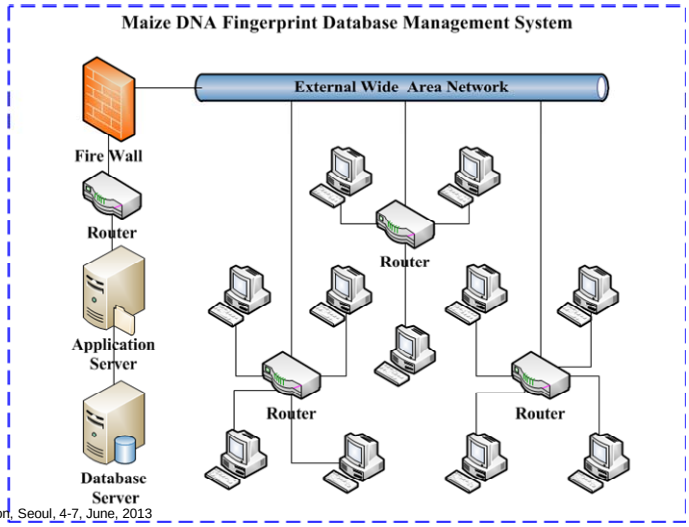
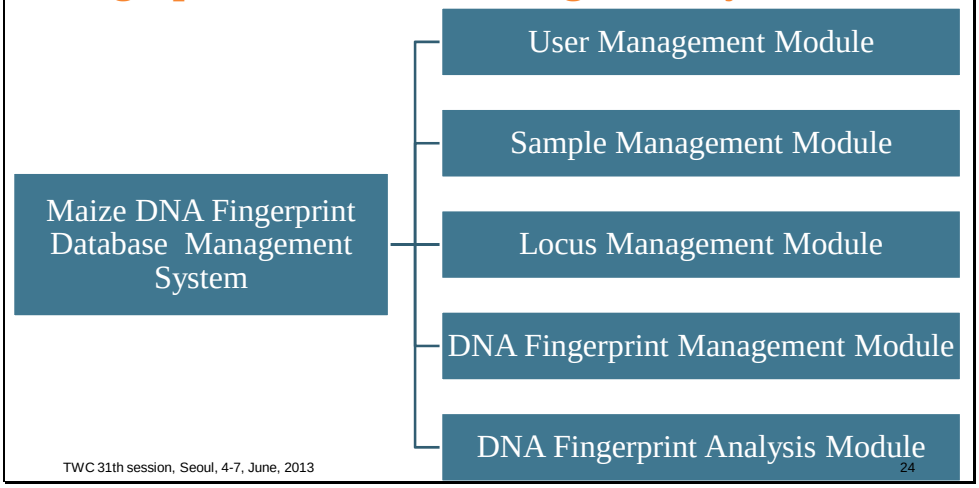
4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.1 Network topology structure - Browser/Server



4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.2 Overall function diagram of maize DNA fingerprint database management system



4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.3 User Management Module

4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.4 Sample Management Module

4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.5 Locus Management Module

4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.6 DNA Fingerprint Management Module

4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.7 DNA Fingerprint Analysis Module

- Cultivar Genuineness Test
- Outputting DNA Fingerprint Map
- Varietal Paternity Test
- Varieties Consistency Test
- Varietal Purity Test

4. Maize DNA Fingerprint Database Management System Architecture & Functional Module

4.8 DNA Fingerprint Analysis Module

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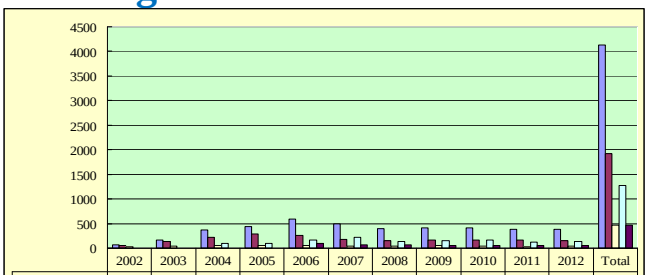
5. Construction of DNA Fingerprint database of China Maize Varieties

- **5.1 Database Construction of Varieties in National Regional Test**

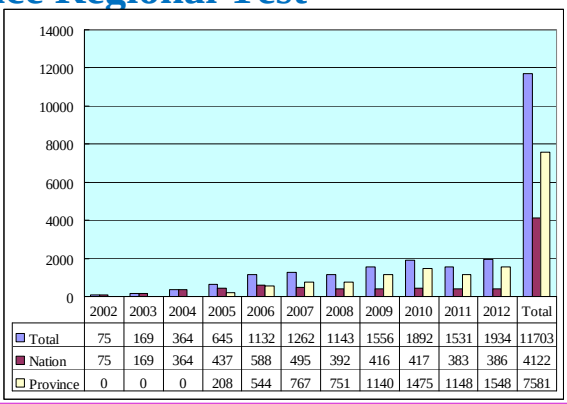


	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Total	75	169	364	437	588	495	394	416	417	383	386	4124
BAAFS	52	131	213	282	265	185	146	167	163	164	155	1923
Sichuan AAS	23	38	53	58	55	35	42	49	35	33	41	462
CAU			98	97	167	213	132	147	161	125	137	1277
Yangzhou U					101	62	74	53	58	61	53	462

Since 2002, organized by the variety manage department of Ministry of Agriculture, DNA fingerprint database of more than 4000 maize varieties in national regional test established.

5. Construction of DNA Fingerprint database of China Maize Varieties

- **5.2Database Construction of Varieties in Province Regional Test**

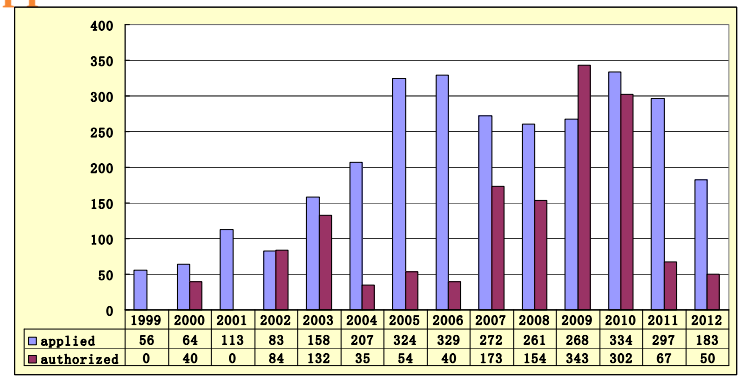


	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Total	75	169	364	645	1132	1262	1143	1556	1892	1531	1934	11703
Nation	75	169	364	437	588	495	392	416	417	383	386	4122
Province	0	0	0	208	544	767	751	1140	1475	1148	1548	7581

Up to 2012, the database has included 11703 varieties in regional test, including nearly all the corn-produced provinces (23 provinces) .

5. Construction of DNA Fingerprint database of China Maize Varieties

- **5.3 Database Construction of Varieties Applied for Plant Varieties Protection**



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
applied	56	64	113	83	158	207	324	329	272	261	268	334	297	183
authorized	0	40	0	84	132	35	54	40	173	154	343	302	67	50

- **By 2012, the DNA fingerprint database including 1587 maize PVP varieties has been constructed.**
- **In the future, the number of PVP varieties will expand annually.**

5. Construction of DNA Fingerprint database of China Maize Varieties

- **5.4 Database Construction of Maize Registered Varieties**
  - **Under the deployment of Ministry of Agriculture, the standard sample of all maize registered hybrids have been collected since August 2008.**
  - **Until 2012, the standard database of 3500 maize registered hybrids have been established.**

Total Varieties Count(Until 2012)：16790

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Thanks for your attention!

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[End of Annex and of document]