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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



Jiuran ZHAO, Fengge WANG, Yang YANG, et al.

Maize Research Center,

Beijing Academy of Agriculture and Forestry Science,
China



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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

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1. Possible Use of Molecular Markers in the DUS Test

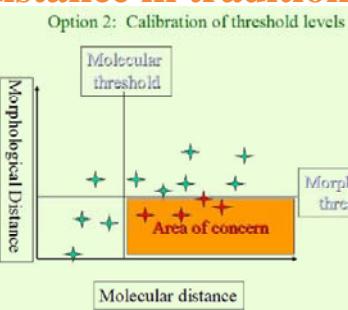


- **Option 1: Molecular characteristics as a predictor of traditional characteristics**
 - Option 1 (a): Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)
 - Option 1 (b): Use of a set of molecular characteristics which can be used reliably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

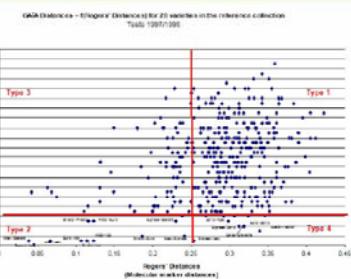
1. Possible Use of Molecular Markers in the DUS Test



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



Option 2: Oilseed Rape



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

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

2. Lab Preparation & Demand Analysis



2.2 The main process of maize DNA fingerprint database construction

Collecting samples

Extracting DNA

Amplifying DNA

Electrophoresing DNA

Importing database

Evaluating database

Maize DNA
fingerprint
database
management
system

Database
design

System
design

2. Lab Preparation & Demand Analysis



2.3 The basic demand of maize DNA fingerprint database

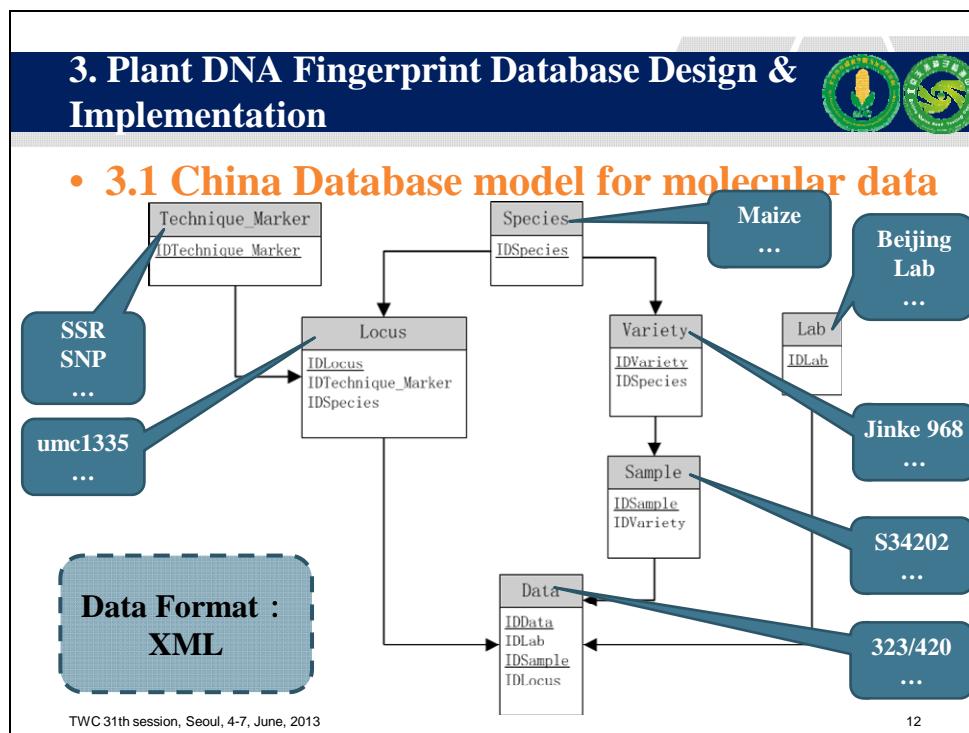
- Storing multi-source data from the specific types of molecular marker
- Recording complex relationship between variety information, morphological characters and genetic characteristics
- Searching mass data from many varieties which obtained by various molecular marker methodology

2. Lab Preparation & Demand Analysis



2.4 The basic demand of maize DNA fingerprint database management system

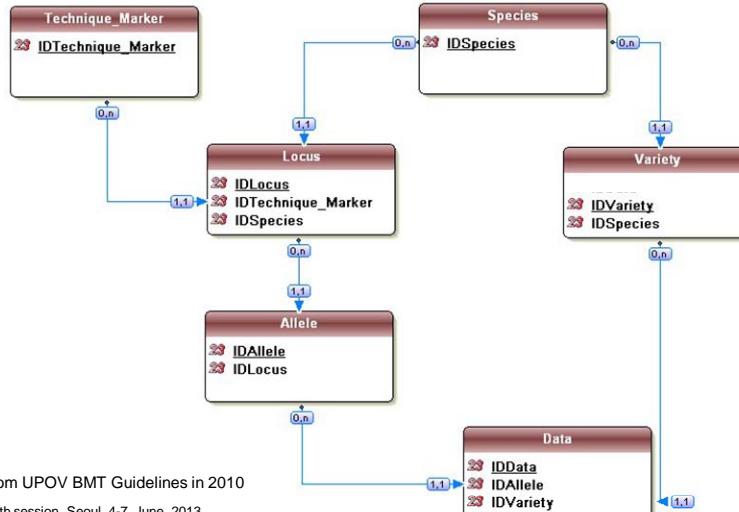
- Importing data quickly
- Storing data standardly
- Sharing data analysis easily



3. Plant DNA Fingerprint Database Design & Implementation



3.2 BMT Database model for molecular data



Cited from UPOV BMT Guidelines in 2010
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3. Plant DNA Fingerprint Database Design & Implementation



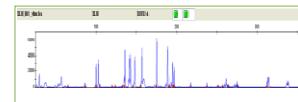
3.3 Entity Relationship Diagram of maize DNA fingerprint database



Variety Information



Sample Information



Morphological Character Information

DNA Fingerprint Information

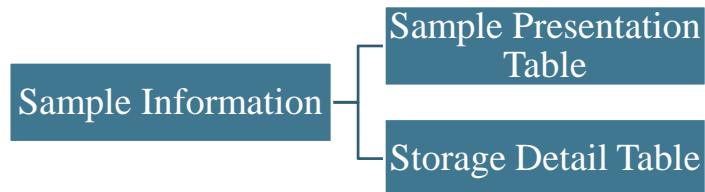
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3. Plant DNA Fingerprint Database Design & Implementation



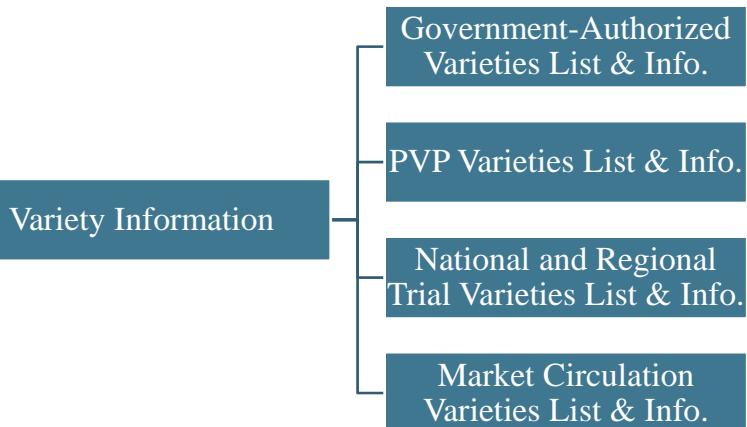
3.4 Entity Relationship Diagram of maize DNA fingerprint database



3. Plant DNA Fingerprint Database Design & Implementation



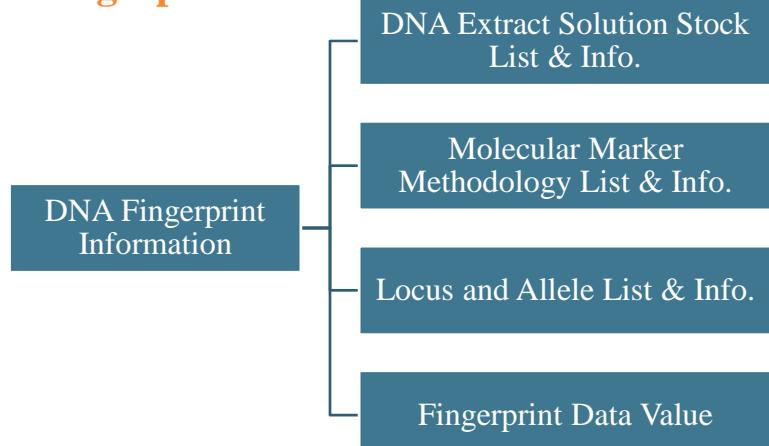
3.4 Entity Relationship Diagram of maize DNA fingerprint database



3. Plant DNA Fingerprint Database Design & Implementation



3.4 Entity Relationship Diagram of maize DNA fingerprint database



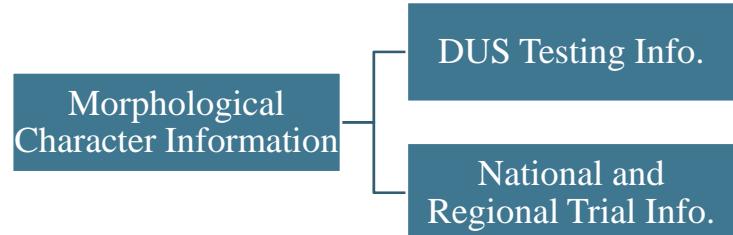
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3. Plant DNA Fingerprint Database Design & Implementation



3.4 Entity Relationship Diagram of maize DNA fingerprint database



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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	bulg439w1	1.03	AGTTGACATCGCCATCTGGTGAC	GAACAGGCCCTAGCGGGTGTTC	12	319-369
N02	ume1335y5	1.06	CCTCGTTACCGTTACCGCTGCTG	GATGACCCCGCTTAATTCGTTATG	4	333-387
N03	ume2007y4	2.04	TTACACAACCGAACACGAGGC	GCTTAGGCCCTAGCTTGGTAGACAC	14	233-300
N04	bulg1040k7	2.08	CGTTTAAGAACCGTTGATTCATTCC	GCCCTTATTCCTCCCTGGCTGCC	13	324-388
N05	ume2105k3	3.00	GAAGGGCAATGAATAGAGCCATGAG	ATGGACTCTGTCGGACTITGACCG	6	280-350
N06	phl053k2	3.05	CCCTGCGCTCTCAGATTAGAGATG	TAGGCTGGCTGGAAGTTGTTGC	4	333-363
N07	phl072k4	4.01	GCTCGTCTCCCTCCAGCTCAGG	GACCTCTTTGCCACCAAGGTC	4	408-432
N08	bulg2291k4	4.06	GCACACCCGTTAGTAGCTGAGACTTG	CATAACCTCCCTCCCAAACCC	6	362-421
N09	ume1705w1	5.03	CGAGCTCGTCAGATGGAGCTCG	CACGTACGGCAATGAGACAAG	10	254-349
N10	bulg2305k4	5.07	CCCCCTCTCTCAGCACCTTG	CGCTCTGCTCCGTCGTG	12	240-312
N11	bulg161k8	6.00	TCTCACCTCTCTTATTCCTTCC	GATGGATCGACCATGACCTTGC	14	154-216
N12	bulg1702k1	6.05	GATCCGCAATTGCAAAATGACAC	AGGACACGCCATGTCATCA	13	260-347
N13	ume1548y2	7.00	AATGCCCTTATCATGCCATGCC	GCTTCCTGCTCTTGAATTGCGT	7	180-249
N14	ume1125y3	7.04	GGATGATGGCGAGGGATGAATGTC	CCACGGTACCCATACCCATACCCAG	5	149-175
N15	bulg240k1	8.06	GGAGGTGTCGGGATTTCTC	GGAAGTGAAGAACAGAAGGCATTGATAC	7	320-339
N16	phl080k15	8.08	TGAACCCACCGATGCACTTG	TTGATGGGCACGATCTGCTAGTC	6	202-238
N17	phl068k9	9.03	CGCCCTCAAGAAATATCCTTGCCC	GGACCCAGACCAAGCTTCCCAC	4	391-418
N18	ume1492y13	9.04	GCGGAAGAGTAGTICGTAGGGCTAGTGTAG	AACCAAGTCTTCAGAGCTTCAGG	4	270-290
N19	ume1432y6	10.02	GAGAAATCAAGAGTGGCAGGATC	GGCCATGATAACAGAACAAATGATAAGC	7	211-269
N20	ume1506k12	10.05	GAGGAATGATGTCCGGGAAGAAG	TTCAAGTCGAGCGCCCAACAC	6	163-196

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3. Plant DNA Fingerprint Database Design & Implementation

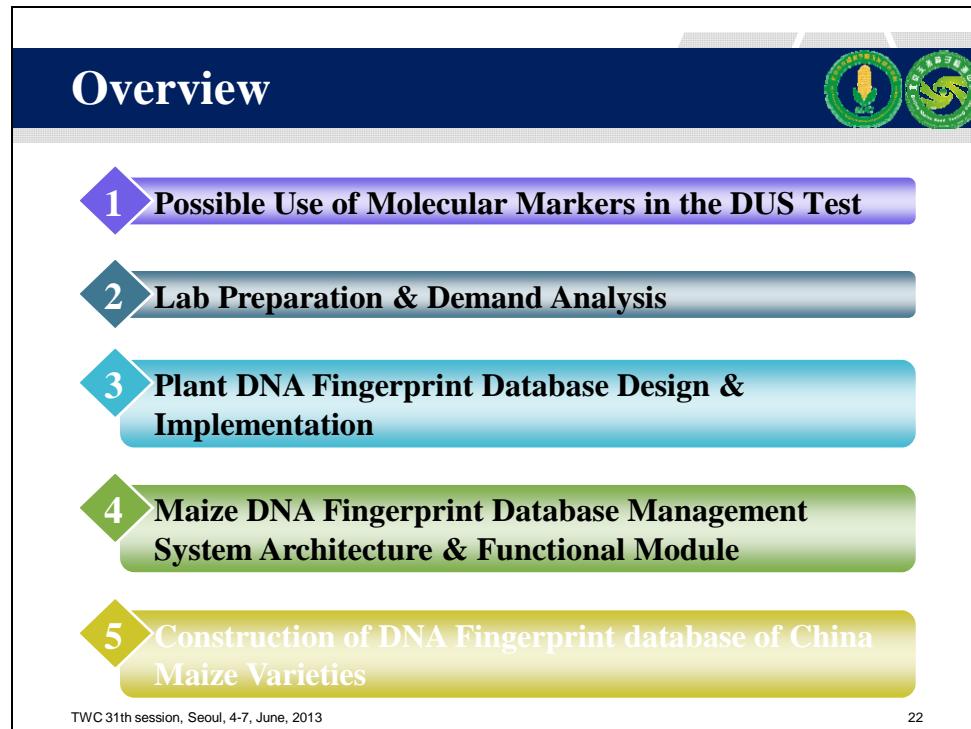
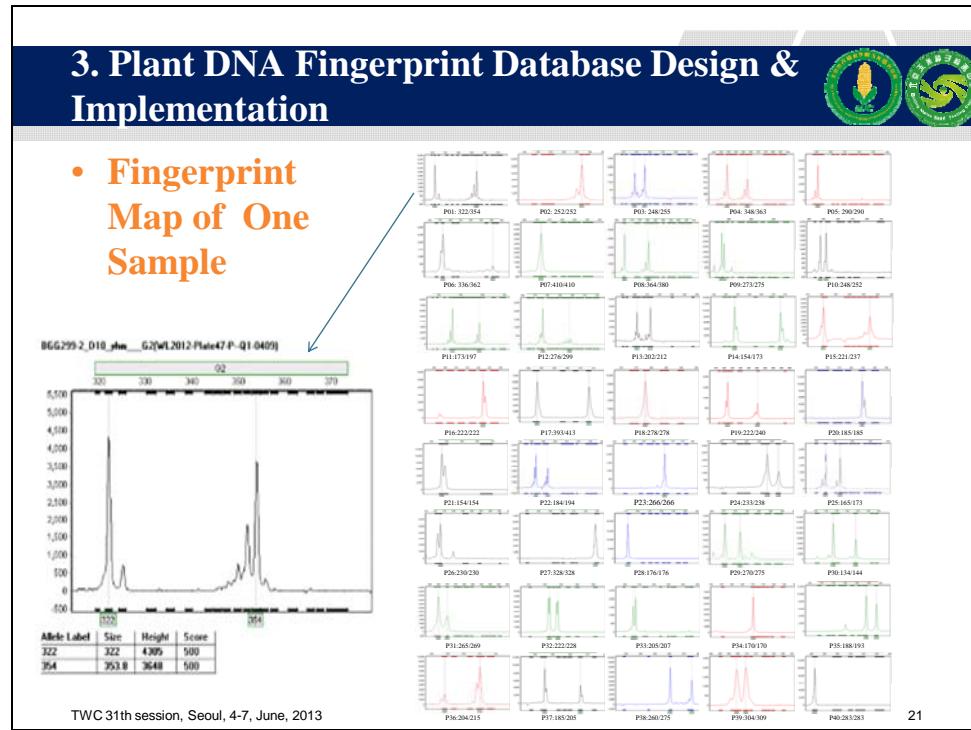


- Example of PDNAF Database Record, like xxx, One Sample contain two origin data and one standard data

单株编号	一单株数据	二单株数据
P01	322/354	322/354
P02	252/252	252/252
P03	248/265	248/265
P04	348/363	348/363
P05	290/290	290/290
P06	336/336	336/336
P07	410/410	410/410
P08	364/380	364/380
P09	273/275	273/275
P10	248/252	248/252
P11	173/197	173/197
P12	276/299	276/299
P13	202/212	202/212
P14	154/173	154/173
P15	221/237	221/237
P16	222/222	222/222
P17	393/413	393/413
P18	278/278	278/278
P19	222/240	222/240
P20	185/185	185/185
P21	154/154	154/154
P22	184/194	184/194
P23	266/266	266/266
P24	233/238	233/238
P25	165/173	165/173
P26	230/230	230/230
P27	328/328	328/328
P28	1/6/1/6	1/6/1/6
P29	270/275	270/275
P30	134/144	134/144
P31	265/269	265/269
P32	222/228	222/228
P33	205/207	205/207
P34	170/170	170/170
P35	188/193	188/193
P36	204/215	204/215
P37	185/205	185/205
P38	260/275	260/275
P39	304/309	304/309
P40	283/283	283/283

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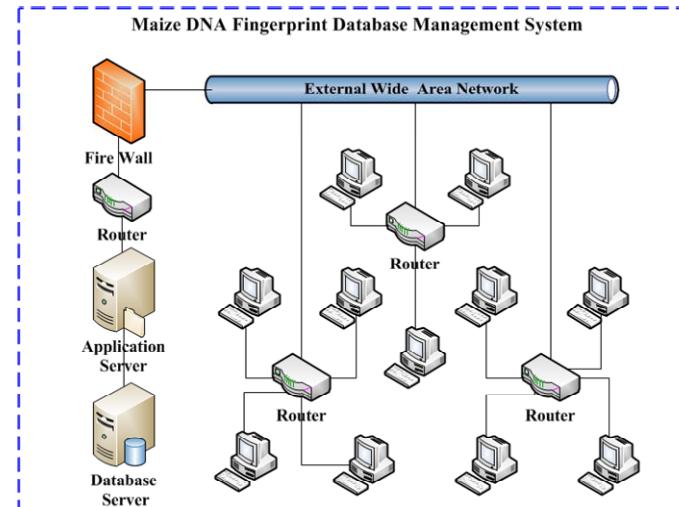
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4. Maize DNA Fingerprint Database Management System Architecture & Functional Module



4.1 Network topology structure - Browser/Server



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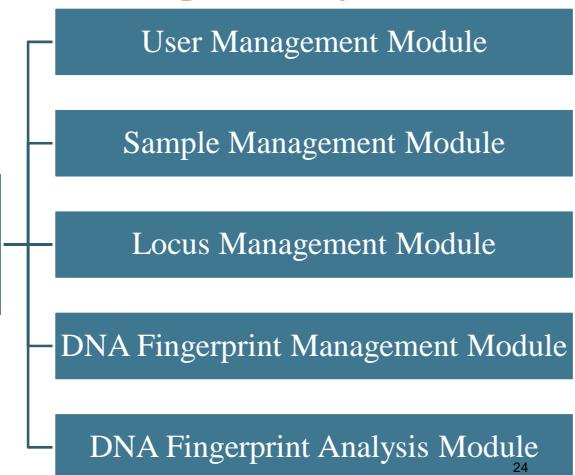
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4. Maize DNA Fingerprint Database Management System Architecture & Functional Module



4.2 Overall function diagram of maize DNA fingerprint database management system

Maize DNA Fingerprint Database Management System



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

4.3 User Management Module

The screenshot shows the 'User Management' section of the maize DNA database system. It displays a list of roles with their names, Chinese names, descriptions, and operations. A modal window is open for the 'ROLE_CUSTOMER' role, showing its details: Chinese name '外部客户' (External Customer), description '远程客户' (Remote Client), and a save button. A large yellow arrow points from the 'User Setting' label at the bottom right towards the modal window.

User Setting

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

4.4 Sample Management Module

The screenshot shows the 'Sample Management' section of the maize DNA database system. It displays a list of samples with their IDs, codes, and other details. A modal window is open for sample ID '100-06-00004', showing its details: flow number '100-06-00004', variety code 'ND4', name '488', entry date '2011-12-28', weight '185', and a note. A blue arrow points from the 'Variety Info. Detail' label at the bottom right towards the modal window.

Search Condition

Variety Info. List

Variety Info. Detail

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

4.5 Locus Management Module

The screenshot shows the Locus Management Module. On the left, there is a sidebar with a list of loci (P01-P10). In the center, a detailed view of P01 is shown with fields for引物编号 (Primer No.), 引物名称 (Primer Name), 染色体位置 (Chromosome Position), 正向引物序列 (Forward Primer Sequence), 反向引物序列 (Reverse Primer Sequence), 等位基因型 (Allele Type), and 测定导入区间 (Measurement Import Range). On the right, there is a list of operations (Edit, Delete, etc.) for each locus.

Search Condition

Locus Info. List

Locus Info. Detail

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

4.6 DNA Fingerprint Management Module

The screenshot shows the DNA Fingerprint Management Module. On the left, there is a table titled "DNA Fingerprint Data" with columns for行号 (Line No.), 图像 (Image), 引物编号 (Primer No.), 引物名称 (Primer Name), 节点编号 (Node No.), and 纹带 (Band Pattern). The table lists 16 entries from 1 to 16. On the right, there is a section titled "DNA Fingerprint Info. List" which contains a table with columns for操作 (Operation), 引物编号 (Primer No.), 引物名称 (Primer Name), 节点编号 (Node No.), and 纹带 (Band Pattern).

Upload DNA Fingerprint Data

DNA Fingerprint Info. List

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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

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

4.8 DNA Fingerprint Analysis Module

The screenshot shows a web-based application for DNA fingerprint analysis. At the top, there are two circular logos: one for 'Maize DNA Fingerprint Database Management' and another for 'National Research Institute of Horticultural and Herbal Science'. Below the logos, the title '4. Maize DNA Fingerprint Database Management System Architecture & Functional Module' is displayed.

The main content area is titled '4.8 DNA Fingerprint Analysis Module'. It features a table with data from a comparison analysis. The table has columns for '样品信息' (Sample Information), '比对结果' (Comparison Result), and '遗传相似度' (Genetic Similarity). The data includes sample names like 'YET01' and 'BGG156', their types ('杂交种'), sources ('品种权保护'), and various comparison statistics.

To the right of the table, there are three vertical panels:

- DNA Fingerprint Compare Condition:** A blue panel containing the text 'DNA Fingerprint Compare Condition'.
- Similar Varieties List:** A blue panel containing the text 'Similar Varieties List'.
- Locus-Allele Detail:** A blue panel containing the text 'Locus-Allele Detail'.

A large blue arrow points from the 'Locus-Allele Detail' panel towards the bottom left of the table, indicating a detailed view of specific locus-allele data.

At the bottom of the screenshot, the text 'TWC 31th session, Seoul, 4-7, June, 2013' is on the left, and the number '30' is on the right.

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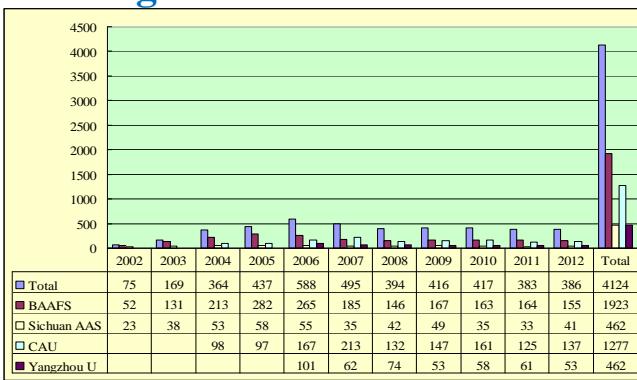
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5. Construction of DNA Fingerprint database of China Maize Varieties



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



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

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.

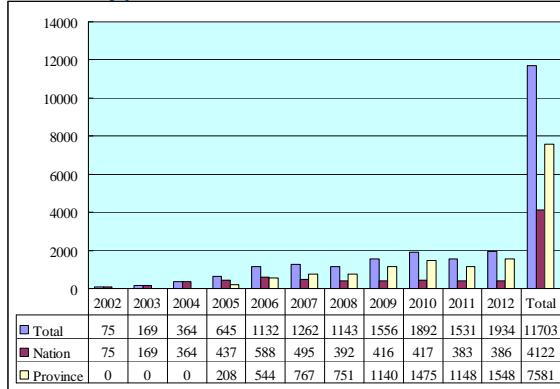
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5. Construction of DNA Fingerprint database of China Maize Varieties



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



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

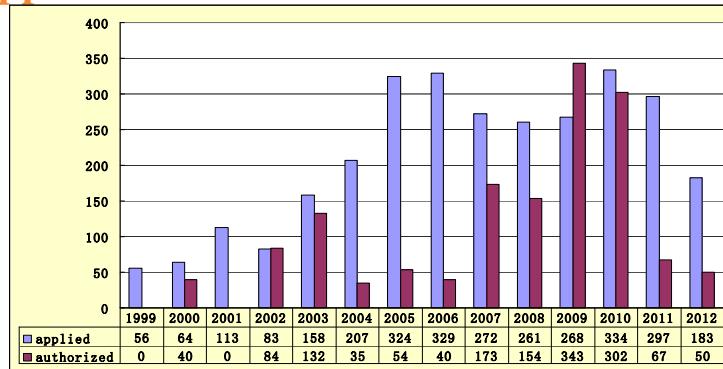
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5. Construction of DNA Fingerprint database of China Maize Varieties



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



- 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.

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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

**Thanks for your
attention!**

