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ASSESSMENT OF THE UNIFORMITY OF CHINESE MAIZE VARIETIES BY
A SET OF SSR MARKERS

Document prepared by experts from China

1.   The BMT agreed that, where agreed by the relevant experts, the presentations made at the meeting should be made available in the BMT document section of the UPOV website, as addenda to the relevant documents. This document contains a copy of the presentation made by Ms. Fengge Wang (China), for document BMT/9/5.

Assessment of the uniformity of Chinese maize varieties by a set of SSR markers

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Plant material-from national VCU in 2004

Region	No. of accessions	No. of subgroup
Northeast Early-mature	32	2
Northeast & North	70	4
Huanghuaihai	66	4
Jingjintang	17	1
Northwest	12	1
Extremely Early-mature	16	1
total	213	13

213 varieties (single hybrids) from six groups of Chinese National Regional Trials in 2004 , of which 16 were duplicated

- ten primers P1-P10 for uniformity analysis , other three primers P6-1,P8-1,P8-2 for correlation analysis.
- 20 individual seeds per variety were randomly taken

Selection of SSR primers

No.	Locus	Bin	No. of alle	PIC
P1	bnlg439	1.03	6	0.58
P2	bnlg125	2.02-2.03	5	0.72
P3	phi053	3.05	4	0.56
P4	phi072	4.01	4	0.66
P5	umc1822	5.05	5	0.7
P6	Bnlg161	6.00	8	0.85
P6-1	phi126	6.00	8	0.82
P7	umc1944	7.04	6	0.65
P8	bnlg162	8.05	6	0.68
P8-1	bnlg240	8.06	5	0.77
P8-2	Phi080	8.08	6	0.79
P9	phi065	9.03	4	0.52
P10	umc1084	10.07	6	0.72

Selection criteria:
➢high degree of polymorphism
➢robust single-locus amplification
➢easily scored products
➢One primer per chrom

Band types in uniformity analysis

- Seven types of variation between normal and off-type bands appeared
- types 1, 3, 5 , whose normal and anomalous bands shared one common allele appeared more frequently than types 2, 4, 6, 7 , that shared none common allele.

Distribution of variety uniformity among different varieties and different SSR loci

- 1. Distribution of uniformity ratio was quite uneven at different varieties and different SSR loci
  - Some varieties had high uniformity ratio at all loci (for instance, K1, K103);
  - some varieties had high uniformity ratio at most loci and low ratio only at one or two loci (for instance, K76, K153);
  - some varieties had low uniformity ratio at most loci (for instance, K29, K185)

Uniformity ratio of the maize varieties (part)

No.	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	R
K1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.000
K2	0.95	1.00	1.00	1.00	0.95	0.95	1.00	0.95	0.95	1.00	0.975
K8	0.90	0.55	0.95	0.80	0.95	0.95	1.00	0.55	0.84	1.00	0.849
K12	0.95	1.00	1.00	0.95	1.00	0.90	1.00	0.58	0.95	1.00	0.933
K27	1.00	0.80	0.60	0.95	1.00	1.00	0.95	0.90	1.00	1.00	0.920
K29	0.74	0.85	0.83	0.70	0.85	0.50	1.00	0.85	0.95	1.00	0.827
K33	1.00	1.00	0.77	1.00	0.75	1.00	1.00	0.67	1.00	1.00	0.919
K36	0.90	0.70	0.90	0.95	0.95	1.00	1.00	0.95	0.90	1.00	0.925
K50	1.00	0.81	0.78	1.00	0.75	0.94	0.90	0.80	1.00	0.94	0.892
K75	0.95	1.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00	0.85	0.960
K76	1.00	1.00	1.00	1.00	1.00	0.60	1.00	1.00	1.00	1.00	0.960

Distribution character of variety uniformity among different varieties and different SSR loci

- 2. To many accessions, no-uniformity were detected at more than one locus, but the anomalous individuals among these loci were usually different
- 3. To different SSR loci, the amount of accessions distributed in different uniformity ratio interval varied much

Amount of accessions distributed in different SSR loci and different uniformity ratio interval

SSR loci	Different uniformity ratio interval													
	1	0.95	0.9	0.85	0.8	0.75	0.7	0.65	0.6	0.55	0.5	0.45	0.4	0.35
P1	144	32	11	7	3	1	1	3	4	5	0	1	0	0
P2	147	28	6	10	7	4	3	1	1	1	2	1	1	0
P3	148	20	8	9	10	6	3	0	2	5	0	0	0	1
P4	164	27	7	3	6	2	1	1	0	1	0	0	0	0
P5	154	19	8	8	6	5	4	1	0	3	2	2	0	0
P6	143	29	12	7	2	6	5	0	3	2	3	0	0	0
P7	180	14	7	2	3	2	1	1	1	0	0	0	1	0
P8	129	32	18	7	8	4	1	1	3	7	2	0	0	0
P9	160	22	9	5	3	4	4	0	2	3	0	0	0	0
P10	159	22	14	8	5	0	2	0	1	1	0	0	0	0

Distribution character of variety uniformity
among different varieties and different SSR loci

- 4. The number of no-uniformity loci in the
  different individuals of each accession
  varied greatly from 0 to 7 and the number of
  no-uniformity individuals of each accession
  also varied much from 0 to 20

Distribution of individuals in different number of no-uniformity loci for each accession (part results)

Acc. No.	Sum of no-uniform loci							No-std indiv	Std. indiv	Prop. of std indiv
	1	2	3	4	5	6	7			
K1	0	0	0	0	0	0	0	0	20	1.00
K2	1	0	0	1	0	0	0	2	18	0.90
K26	12	5	0	1	0	0	0	18	2	0.10
K55	7	0	0	0	0	0	0	7	13	0.65
K60	6	3	0	0	0	0	0	9	11	0.55
K108	4	0	0	0	0	0	0	4	16	0.80
K116	10	4	0	0	0	0	0	14	6	0.30
K120	8	4	0	0	0	1	0	13	7	0.35
K128	6	1	1	0	0	0	0	8	12	0.60
K147	12	5	0	0	0	0	0	17	3	0.15
K150	10	0	0	0	0	0	0	10	10	0.50
K183	6	2	0	0	1	1	1	11	9	0.45
K184	0	1	0	0	0	0	0	1	19	0.95
K185	3	7	4	5	0	1	0	20	0	0.00
K187	1	4	0	1	0	0	0	6	14	0.70

the standard for evaluating uniformity of
maize variety was suggested as follow:

(1) Uniformity at single SSR locus (r): high (r? 95%);
middle (85%< r< 95%; low (r? 85%) ;

(2) Average uniformity at all of the ten SSR loci (R):
high (R? 95%); middle (85%< R< 95%; low
(R? 85%) ;

(3) After taking account of both the criteria above, a
comprehensive standard for uniformity of maize
variety were brought forward, in which the uniformity
were divided into five levels (table below).

Standard for evaluating uniformity level of maize varieties

Level	Standard
First (highest)	(i) R? 99%; or (ii) r? 95% at all of the ten SSR loci
Second (higher)	95%? R＜ 99% and no more than 2 SSR loci with r? 85%
Third (middle)	(i) 95%? R＜ 99% and no less than 3 SSR loci with r? 85%; or (ii) 90%? R＜ 95%; or (iii)85%＜ R＜ 90% and no more than 2 SSR loci with r? 85%
Fourth (lower)	85%＜ R＜ 90% and 3-4 SSR loci with r? 85%
Fifth (lowest)	(i)R? 85%; or (ii) no less than 5 SSR loci with r? 85%

Summary of uniformity levels of each group of the 213 accessions

Group	Level					Sum on group
	1	2	3	4	5	
Northeast Early-mature	10(31%)	12(38%)	5(16%)	2(6%)	3(9%)	32
Northeast & North	17(24%)	34(49%)	15(21%)	4(6%)	0(0%)	70
Huanghuaihai	30(45%)	19(29%)	12(18%)	4(6%)	1(2%)	66
Jingjintang	10(59%)	3(18%)	3(18%)	1(6%)	0(0%)	17
Northwest	3(25%)	6(50%)	3(25%)	0(0%)	0(0%)	12
Extremely Early-mature	5(31%)	3(19%)	4(25%)	1(6%)	3(19%)	16
Sum on level	75(35%)	77(36%)	42(20%)	12(6%)	7(3%)	213

the uniformity levels varied among different groups
Extremely Early-mature group was the lowest
the next the Northeast Early-mature group

Discussion-1

- Comparison of different sampling way
  - bulked sampling : mixing several individuals into one sample
  - single individual sampling : selecting one individual with 'typical' band types of a variety
  - multiple individual sampling (recommended): selecting several individuals (usually at least 5) with typical band types of a variety.

Discussion 2

-About standard for uniformity testing by SSR markers

Why the SSR loci used for uniformity testing should be evenly distributed over the whole genome?

Only when the loci are evenly distributed, interlock among the loci could be reduced in most extent. Therefore, if 10 loci are used, one locus per chromosome is the best selection in maize.

Discussion 2

– Why both uniformity at single locus and average uniformity at all of the SSR loci should be considered when evaluating variety uniformity?

breeders did not go on a long-term selection for uniformity at SSR loci before. Residual variation at some loci may retain and result in low uniformity, which may not reflect directly in morphological traits.

variations at SSR loci are neutral and not affected by human selection, and they have no direct interlock with morphological traits. Therefore, rational evaluation on variety uniformity would not be drawn if only based on uniformity at single locus.

we may get a comprehensive evaluation to variety uniformity by randomly scanning evenly distributed SSR loci in the whole genome to detect both uniformity at single loci and average uniformity at all the loci.

Discussion 2

Why the suggested standard for uniformity testing by SSR markers is different from the standard currently applied for DUS testing by morphological traits?

Since SSR loci do not suffer long-term selection for uniformity and some of them may have high mutation, the suggested standard by SSR markers is lower than that used in morphological traits. Of course, after gathering mass related information, we could fix a set of SSR markers for uniformity identification by gradually discarding SSR markers with high mutation. These markers may use the same criteria as morphological traits at single loci by now.

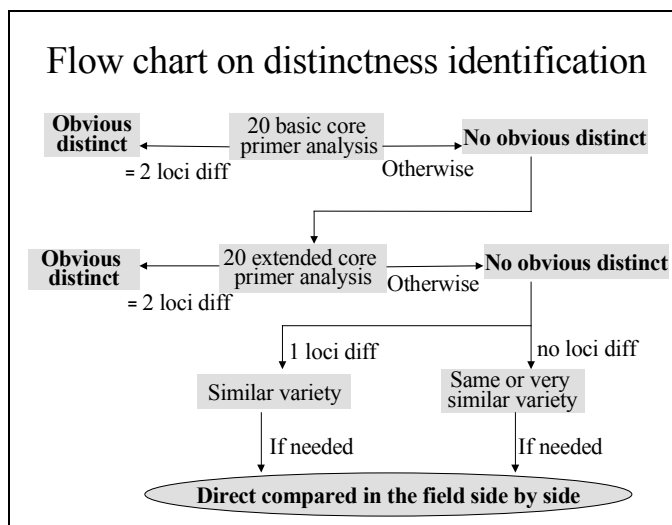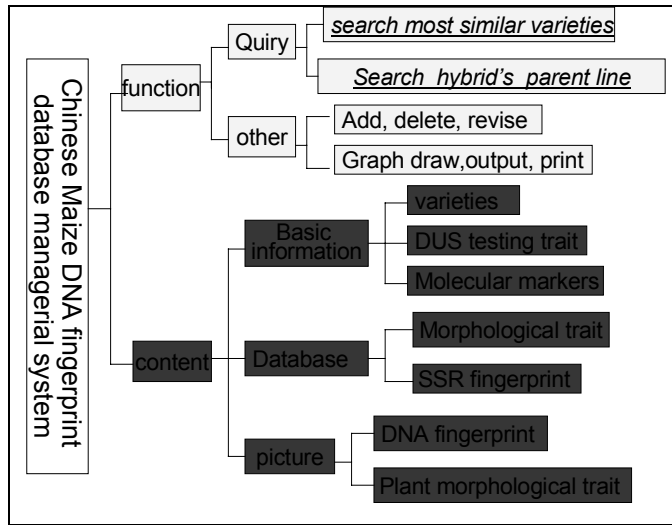About molecular marker selection
and database construction

Evaluation criterion of core primer used in maize DNA fingerprint database

- ❖ accordance with mendelian inheritance
- ❖ Have been localized at fixed chromosome
- ❖ No or low linkage between primer loci
- ❖ Easy amplification
- ❖ Accurate and easy band typing
- ❖ Sufficiently low mutation rate
- ❖ Coincidence among different tissues of the same individual
- ❖ High polymorphism
- ❖ Specific among different species
- ❖ Potential of multiplex amplification
- ❖ Known Allele frequency distribution in maize

Determination of core SSR primers in maize

From maize database   1900

Screened by polymorphic
information on line   300

Screened with 60 key
inbred lines and 100   40
Hybrids in china

Basic core primers   20   10 ┼ 10   Extended core primers

| 2 primers per chrom (long arm, short arm) | 1 primers near centromere per chrom, another 1 no fixed loci |

Flow chart on distinctness identification
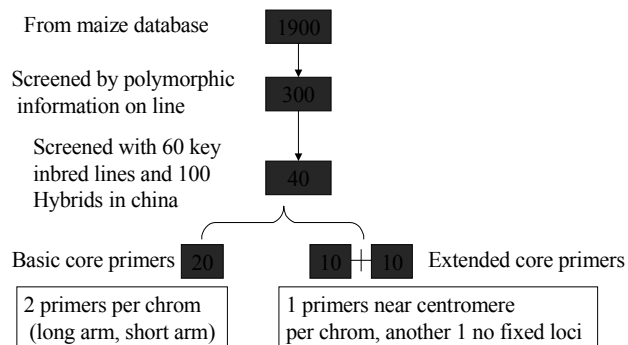
Application

➢ A DNA fingerprint database of about 500 maize varieties has been preliminarily constructed with the 20 core primers, which will be useful for <u>screening most similar variety of a candidate variety</u> in DUS testing

➢ has taken 91 maize infringing cases entrusted by court . These DNA fingerprint was as the main basis for judgement.

➢ Provide service of purity identification: more than 4000 samples by now.

Advice about BMT guidelines

➢ On page 4 2.1 general criteria ©
 add: evenly distribution throughout the
 genome, no or low linkage between markers.
 Delet: which whilst not being essential, is
 useful information.

➢On page 6   3.3.2 self-pollinated and mainly
 self-pollinated varieties.

 Recommended: analyze at least 5 individuals
 per variety.

Thank you!

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