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APPLICATIONS OF MNP MARKER IN PLANT VARIETY PROTECTION

Document prepared by an expert from China

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The annex to this document contains a copy of a presentation on "Applications of MNP marker in plant variety protection", to be made at the eighteenth session of the BMT.
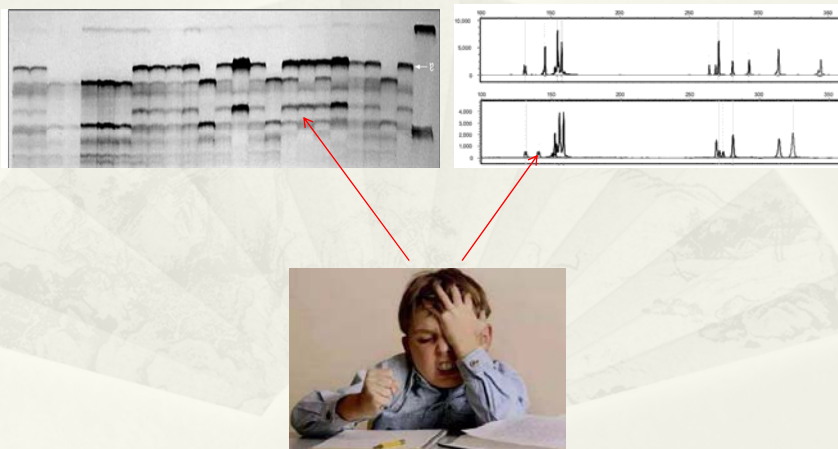
[Annex follows]

Applications of MNP marker in plant varieties protection

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Problems of existing technology

SSR marker method



Nucleic Acids Research, 2017   1
doi: 10.1093/nar/gkx093

An accurate and efficient method for large-scale SSR genotyping and applications

Although electrophoresis can discern the differences of SSR amplicon lengths, it cannot distinguish base changes or base differences. Among the 449.71 differential SSRs, 33.68 (7.07%) had different bases but the same amplicon lengths between two varieties so that they were deemed to be mistaken as identical on electropherograms. For example, amplicon AMPL1141969 has the same amplicon length but distinct SSR genotypes in varieties F and G, which could be clearly identified by AmpSeq-SSR (Figure 2C) but not by electrophoresis (Figure 2D). Furthermore, when the differences of amplicon lengths were below the resolution, they might also be indiscernible on electropherograms (e.g. Figure 2E). Among the 449.71 differential SSRs, 221.32 (51.72%) had amplicon length differences no >5 bp (Figure 2B and Supplementary Table S7), which was the resolution of CE (Figure 1E), the most accurate electrophoresis technique.

SNP maker method
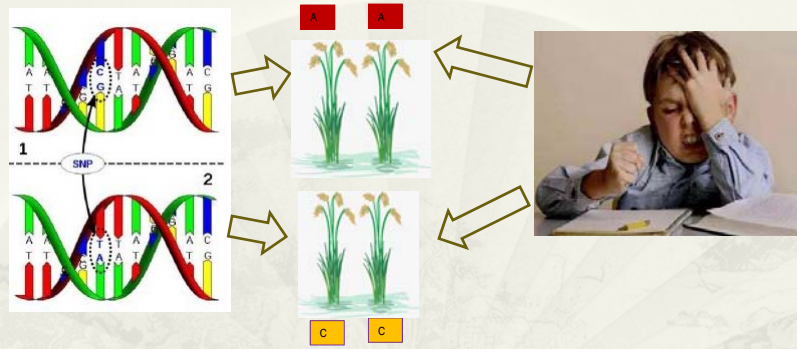

RESEARCH ARTICLE   **Open Access**

A powerful tool for genome analysis in maize: development and evaluation of the high density 600 k SNP genotyping array

Sandra Unterseer[1], Eva Bauer[1]*, Georg Haberer[2], Michael Seidel[2], Carsten Knaak[3], Milena Ouzunova[3], Thomas Meitinger[4], Tim M Strom[4], Ruedi Fries[5], Hubert Pausch[5], Christofer Bertani[6], Alessandro Davassi[6], Klaus FX Mayer[2] and Chris-Carolin Schön[1]*

99.76% to 99.84%. Furthermore, two lines (DK105 and EP1) were represented by two samples each comprised of a single plant and a pooled sample, respectively, showing 99.51% and 97.73% concordance. Some lack of concordance here can be explained by residual heterozygosity in the pooled samples. For determination of stable Mendelian inheritance, 23 trios with both parental lines as well as the corresponding F1 hybrid were analysed. These trios revealed stable Mendelian inheritance between parental lines and their offspring in 94.3% of the variants. After excluding the trio with the lowest call rate
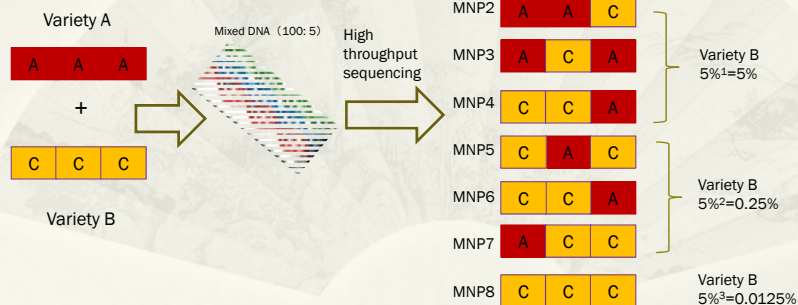
Inventions

MNP marker
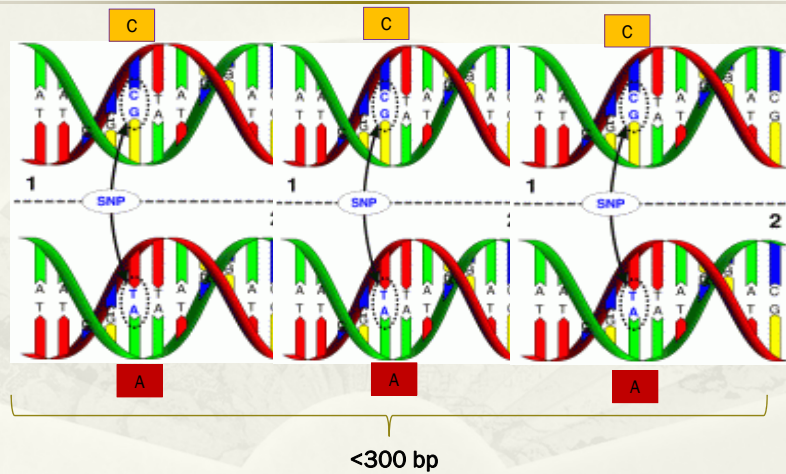
High efficiency
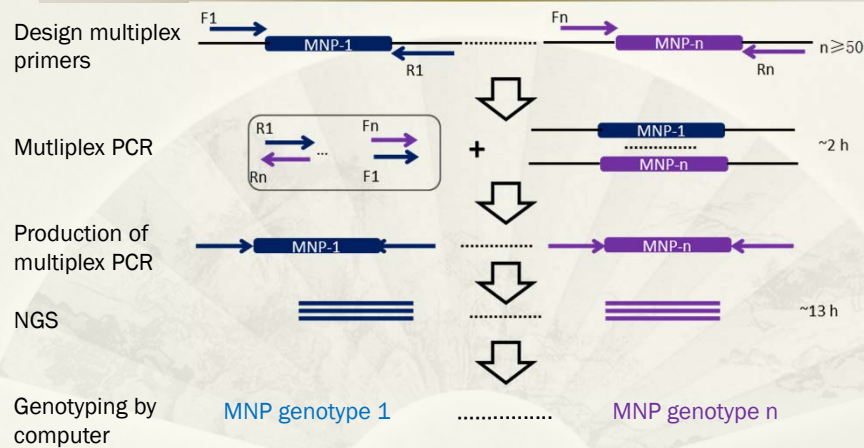
Theoretical accuracy

Actual accuracy

Species	Experiment Steps		Compared MNP markers	With different genotypes		Reproducibility ratio	Accuracy
	Sequencing Library	Sequencing		Number	Ratio		
Rice	Repetitive experiments	Repetitive experiments	306,651	10	0.0033%	99.997%	99.998%
	The same Libraries	Reproducibility experiment	155,231	10	0.0064%	99.994%	99.997%
	Reproducibility experiment	Reproducibility experiment	306,528	35	0.0114%	99.989%	99.994%
Cotton	Repetitive experiments	Repetitive experiments	295,975	68	0.0230%	99.977%	99.989%
	The same Libraries	Reproducibility experiment	150,284	54	0.0359%	99.964%	99.982%
	Reproducibility experiment	Reproducibility experiment	295,957	111	0.0375%	99.962%	99.981%

* Reproducibility condition
* Different times
* Different locations (Beijing and Wuhan)
* Different laboratory staff
* Different reagents
* Different instruments

* Variety authenticity（is it me？）
* Variety identity（Who is it?)
* Variety rights authorization (Do I exist?)
* EDV（have my relatives?）

Theoretical polymorphism
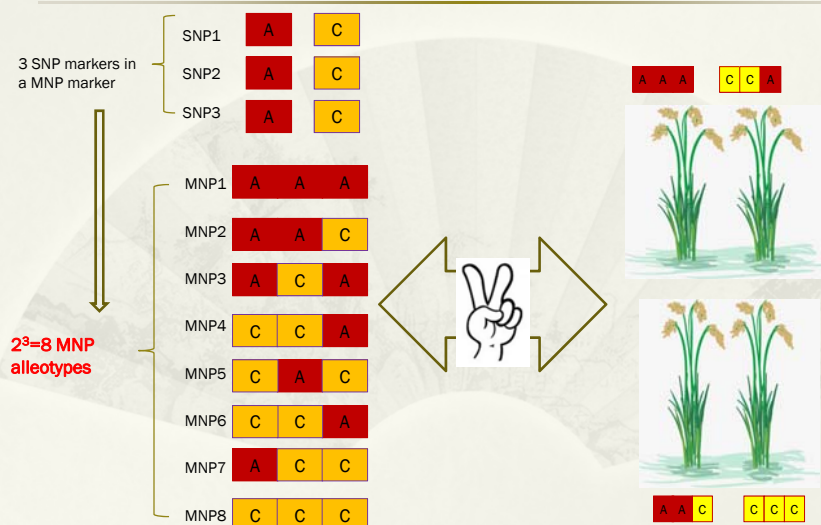
Actual polymorphism
(Compared to SSR markers)

Average alleles per marker in 1500 rice varieties

+ 1.1 alleles per marker
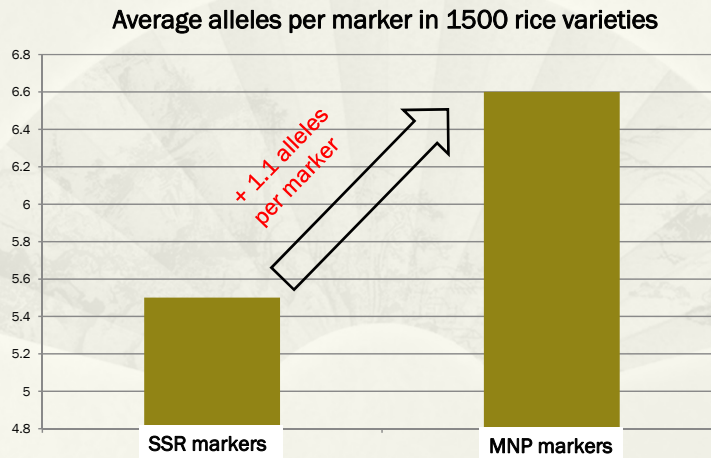
Actual polymorphism
(Maize varieties)

Distribution of Distance Between 621,056 Pairs of Maize Varieties

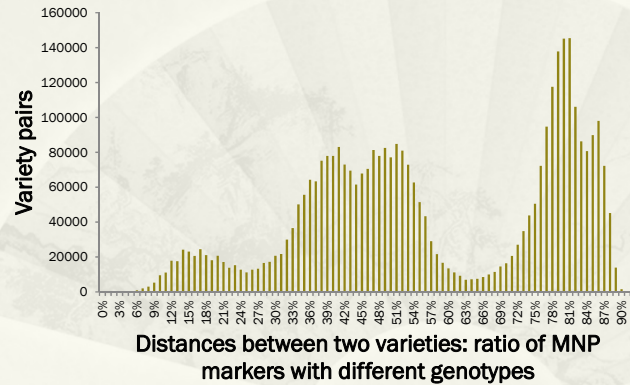Variety pairs

Distances between two varieties: ratio of MNP markers with different genotypes
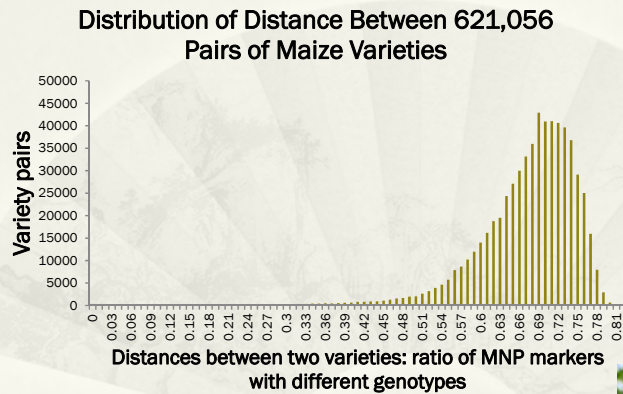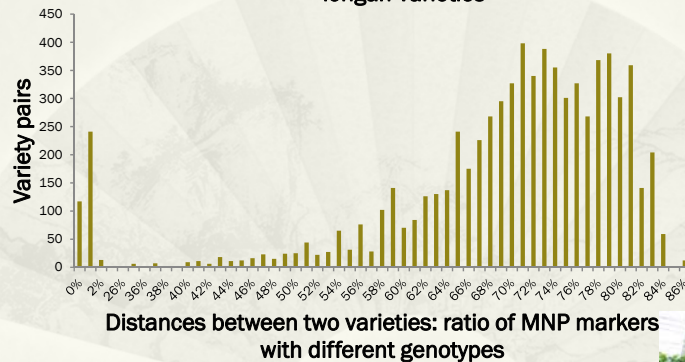
Actual polymorphism
(Rice varieties)

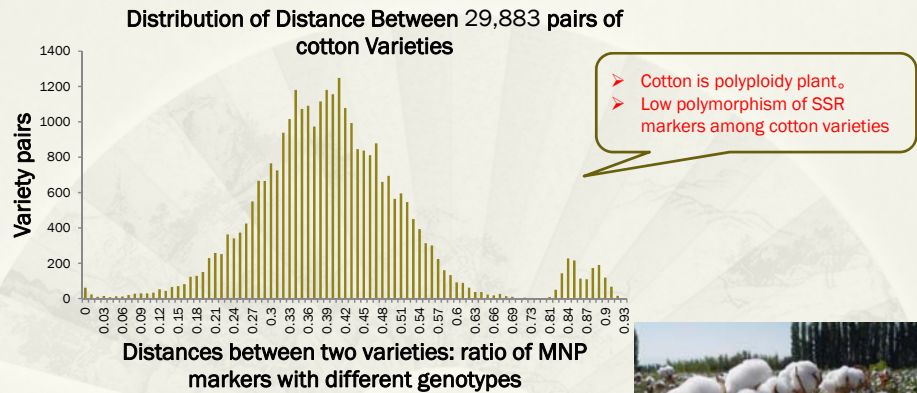Distribution of Distance Between 3,730,438 pairs of Rice Varieties

Actual polymorphism
(longan varieties)
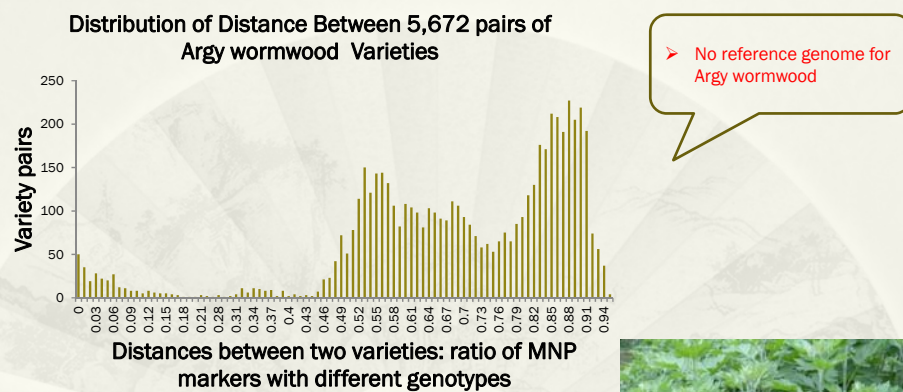
Distribution of Distance Between 7, 503 pairs of longan Varieties

Actual polymorphism
（Cotton varieties）

Actual polymorphism
（Argy wormwood varieties）

Establishment of National Standards for Plant Variety Identification

ICS 65.020.01
B 04

GB

中华人民共和国国家标准

GB/T XXXXX—201X

植物品种鉴定　MNP 标记法

Identification of plant varieties—MNP marker method

（报批稿）

➢ 16 species
➢ Authenticity Identification
➢ EDV Identification

High Resolution DNA Fingerprint Database of Authorized Plant Varieties

* Plant species: rice, maize, cotton and other 16 species
* Number of varieties: ~10,000
* The number of MNP markers per variety: 600-1,000
* Accuracy: Nearly 100%

> ➢ Variety authenticity（is it me？）
> ➢ Variety identity（Who is it?)
> ➢ Variety rights authorization (Do I exist?)
> ➢ EDV（have my relatives?)

Identification of rice variety name for XX Company

Blind sample	Name from identification (<1% distance from varieties in database)	Real Name	Right?
JHU1	Wuyoudao 4, Daohuaxiang 2	Wuyoudao 4	√
JHU2	Longyang 05-16	Longyang 05-16	√
JHU3	Yanfeng 47, Jinfeng 1, Tianfeng 202, Tianfeng 201, Liaohan 109,	Yanfeng 47	√
JHU4	Meixiangzhan No. 2, Lianxiangzhan, Zhongguangxiang No. 1	Meixiangzhan No. 2	√
JHU5	Xudao 9, Jinlangjing 2, Longjing 968	Xudao 9	√
JHU6	Taiyou 390	Taiyou 390	√

➢ Compared with the database records of more than 4000 varieties, the identities of the 6 blind samples were 100% correctly identified。

Identification of rice varieties in the mixed rice flour for XX Company

Blind sample	Included varieties		Identified ratio	Real ratio
	Identification	Right？		
JHU8	JHU1	√	92.17%	95%
	JHU2	√	7.83%	5%
JHU9	JHU1	√	48.77%	50%
	JHU2	√	10.28%	10%
	JHU3	√	40.38%	40%
JHU10	JHU2	√	19.34%	20%
	JHU3	√	27.79%	30%
	JHU4	√	34.53%	30%
	JHU6	√	17.31%	20%

➢ The varieties in the mixed rice flour were 100% correctly identified.
➢ The correlation between quantitative identification results and real values is R2=99.62%.

Screening for the similar varieties before DUS test in field

Varieties under test	Similar varieties		MNP marker number	
	Name	Detected	Number of markers with different genotypes	Ratio of markers with different genotypes
01555a	LongJing 4302A	524	0	0.00%
1604a	ZhongXianHuangZhang	460	0	0.00%
1543a	QuanXian 9A	568	2	0.35%
1616a	Chan 9S	652	8	1.23%
1665b	XianLong No.5	571	30	5.25%
1653a	GuiJingShiMiao	509	37	7.27%
1663a	LiangYouPeiJiu	579	60	10.36%
1686a	R555	400	66	16.50%
1655a	YuXian 203	361	89	24.65%

➢ Speed: 600 varieties in 10 days
➢ Important findings: Genetically modified ingredients and very similar varieties among the same batch of applications

Rapidly settle disputes over the variety rights

∗ Variety A and Variety B are under dispution over PVP, and they are in our MNP fringprinting database ;

Marker types	No. of markers	No. of different markers	Ratio of different markers
SSR	205	6	2.93%
MNP	376	12	3.19%
Total	581	20	3.10%

∗ Conclusions : they are similar but different varieties

Variety A

Variety B

➢ It only took about 5 minutes to get the conclusions

Paternity Identification of Plant Varieties

Varieties under test	Suspected parent 1	Suspected parent 2	Detected MNP alleles	Alleles which is not from suspected parents		Paternity
				number	Ratio	
DYou 13	ShuHui 527	D702A	353	0	0.00%	Yes
HongGuangGeng NO.1	HuangJingQing	YuGen NO.7	456	0	0.00%	Yes
ShuHui 368	MingHui 63	PeiAi 64	321	18	5.60%	No

➢ Written Examination of Application for Variety Right Authorization
➢ Exchange and Benefit Sharing of Genetic Resources

Applications of MNP marker methods beyond PVP

Detection of *XOO* Races in Leaves of Bacterial Blight from Hainan Province

XOO Races	Ratios (Before calibrationg)		Ratios (After calibrationg)	
	Soaking	Griding	Soaking	Griding
P1	0.37%	0.38%	0.71%	0.73%
P2	-	-	-	-
P3	-	-	-	-
P4	0.57%	1.18%	0.60%	1.24%
P5	2.09%	2.16%	4.68%	4.83%
P6	0.94%	0.92%	1.40%	1.37%
P7	-	-	-	-
P8	49.33%	56.18%	52.26%	59.51%
P9	-	-	-	-
P10	39.50%	75.28%	19.75%	37.64%

* Simultaneously detect 10 *Xoo* races
* The pathogenies included 6 races, instead of 1 race
* The strong pathogenicity of P6 did not lead to its growth advantage

➢ The investigation of the epidemic pathogens is no longer arduous.

Identification of contributors in the mixed forensic samples

Blind samples	No.of contributors detected		Contributors detected	
	Results from detection	Right	Results from detection	Right
Mix1	2	√	326,361	√
Mix2	3	√	303,316,361	√
Mix3	3	√	316,324,326	√
Mix4	3	√	308,316,361	√
Mix5	3	√	316,326,344	√
Mix6	3	√	308,324,344	√

➢ Competency tests organized by Shanghai Academy of Forensic Expertise, using blind samples.
➢ Ror the first time, realize the identification of contributors in the mixed forensic samples
➢ 50% of the forensic samples are mixed samples for the serious crimes

Monitoring after stem cell transplantation

Receptor Ratio in Blind Samples	No. of MNP markers Detected	The receptor ratio calculated
50.00%	72	50.07%
25.00%	72	25.36%
12.50%	72	11.04%
6.25%	71	5.38%
1.00%	30	0.97%
0.50%	22	0.51%
0.10%	17	0.14%
0.01%	5	0.05%
0%	25	0.00%

➢ Competency tests organized by Henan Cancer Hospital, using blind samples.
➢ The correlation coefficient between the real and detected values amounted to as high as 99.98%.
➢ For the first time, realize the accuraty detection of receptor ratio for any patient.
➢ Our method has been apllied for over 1000 patients

Thanks

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