



**MNP marker
and its
applications**

**Hai Peng, Jiangnan university
18971601772, November 2020**

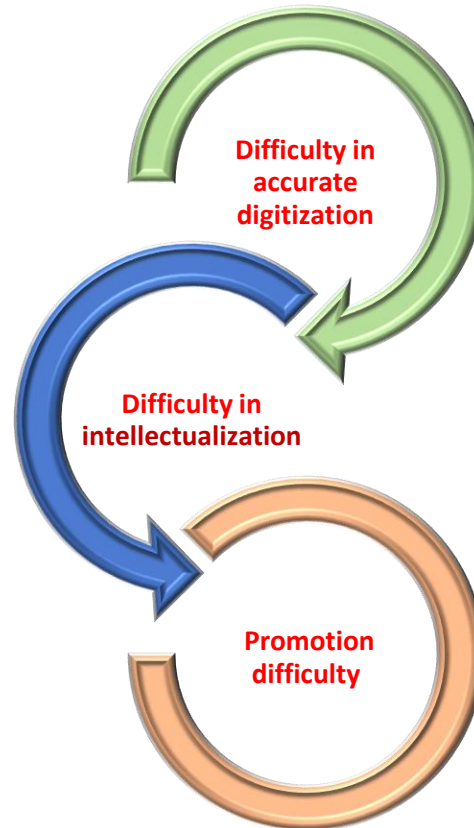
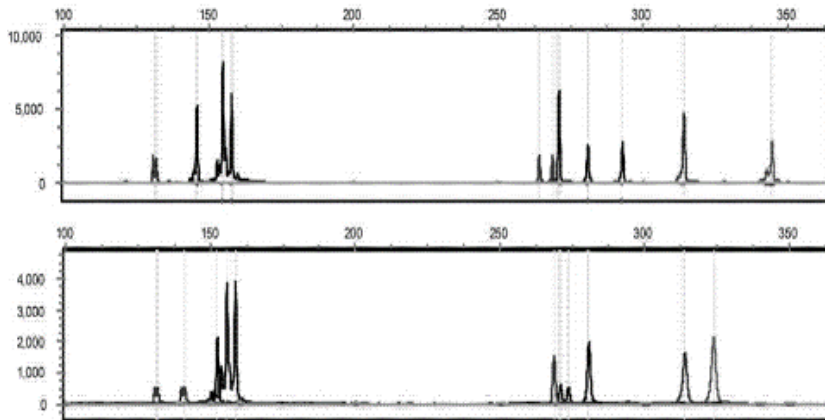
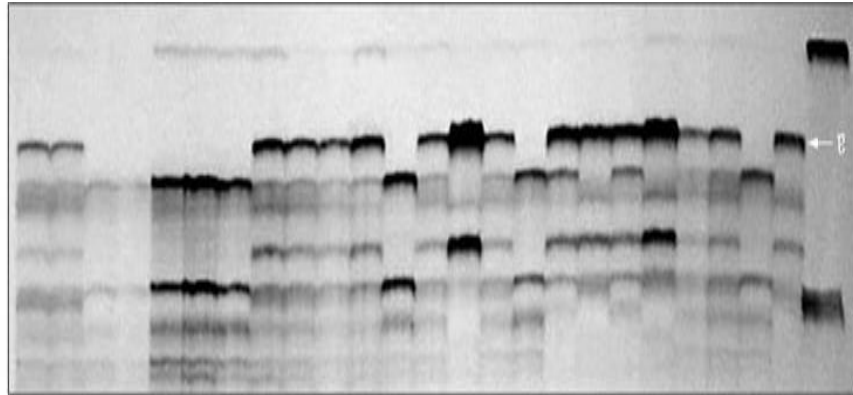
01

Limitations of existing DNA marker methods



SSR marker methods

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.

Industrial standards of SSR marker methods

中华人民共和国农业行业标准

NY/T 1433—2014
代替 NY/T 1433—2007

水稻品种鉴定技术规程 SSR标记法

试验样品为种子时，其质量应符合 GB 4404.1 中对水稻种子纯度的要求。

8.4 PCR产物检测

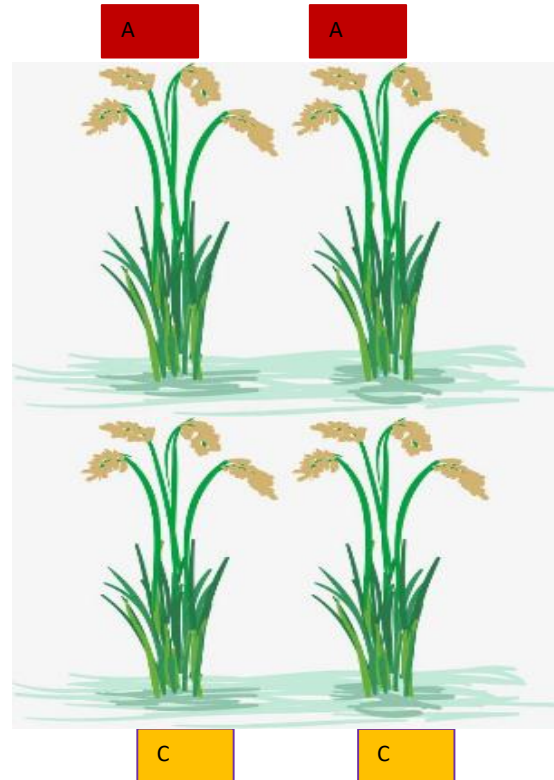
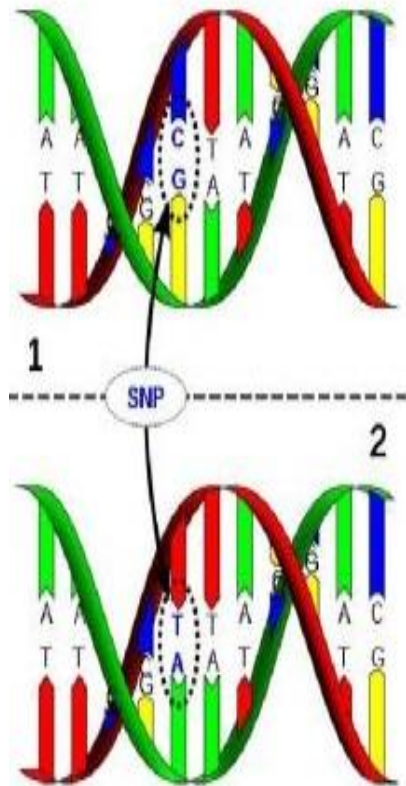
8.4.3 毛细管电泳荧光检测

8.4.1 非变性聚丙烯酰胺凝胶电泳银染检测

对于利用附录 C 中 48 对引物仍未检测到 ≥ 2 个差异位点数的样品，

- Purity
 - Artificial mixtures, such as adulterated seeds
 - Natural mixtures, such as rape seeds.....
- Limited markers
 - Dispute of court judgments
 - Identification of essential derived varieties
- Accurate identification requires parallel experiments with standard samples
 - Standard samples are difficult to obtain
 - Screening for the similar varieties, the infringed varieties and the essential derived varieties

SNP markers (detection by microarray)



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

Sandra Unterseer¹, Eva Bauer^{1*}, Georg Haberer², Michael Seidel², Carsten Knaak³, Milena Ouzunova³, Thomas Meitinger⁴, Tim M Strom⁴, Ruedi Fries⁵, Hubert Pausch⁵, Christofer Bertani⁶, Alessandro Davassi⁶, Klaus FX Mayer² 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

Industrial standards of SNP marker method

中华人民共和国农业行业标准

NY/T 2745—2015

水稻品种鉴定 SNP标记法

Protocol for identification of rice varieties—SNP marker method

8.1 样品准备

试验样品为种子时,其质量应符合 GB 4404.1 中对水稻种子纯度的要求。

种子样品的分样和保存,应符合 GB/T 3543.2 的规定。

每份样品检测至少 50 个个体(种子、叶片)的混合样。

➤ Purity

- Artificial mixtures, such as adulterated seeds
- Natural mixtures, such as rape seeds.....

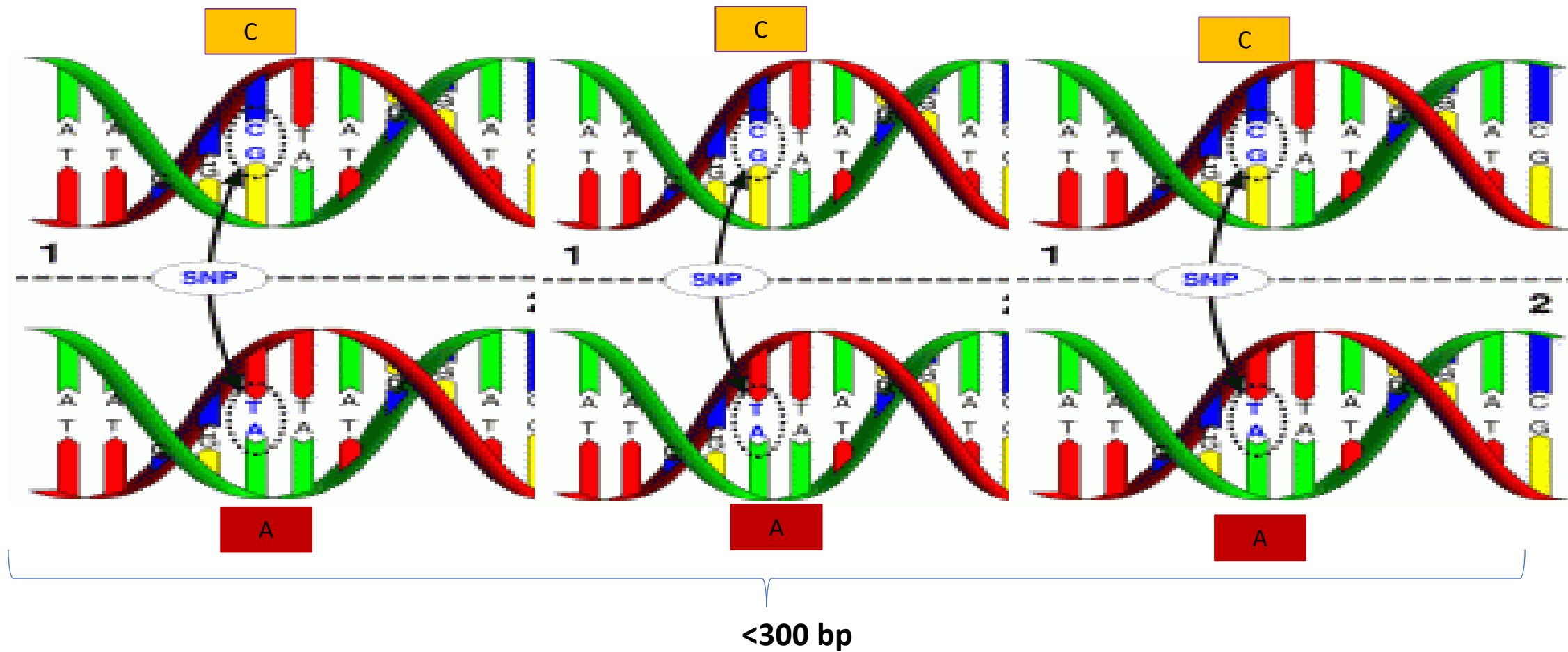
➤ Flexibility

02

Technical inventions



MNP marker

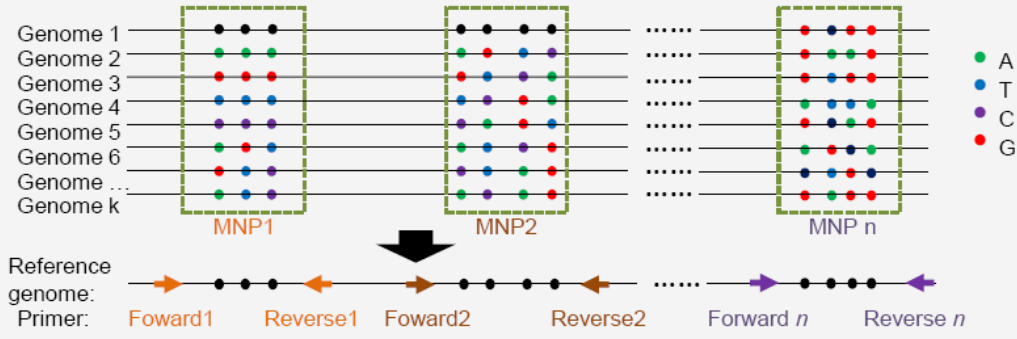


MNP marker method

A MNPs design:

MNPs selection

- a) ≥ 3 dispersed SNPs
- b) No missing genotype
- c) Discriminative power ≥ 0.2



B MNP-Seq:

Multiplex PCR

S_1 - S_n : n samples

Ligated barcode

One barcode for one sample

Mix and sequencing

Mixture of n samples and high throughput sequencing

partitioned by barcodes

Genotyping

Reads filter → Mapping → MNP genotyping

- Accurate
- Efficient
- Digital
- Intelligent

Fig 1. The procedure of MNPs design and MNP-Seq.

High efficiency

农业农村部植物新品种测试

(广州)分中心文件

[2019]02号

应用证明

Application prove

2016年和2017年,我单位委托江汉大学开展鉴定水稻DUS测试品种中的近似品种。江汉大学利用MNP标记技术检测了600个水稻的DNA身份,并与全国3000多个授权水稻品种DNA身份证数据库进行了比对,筛选出了每个品种的近似品种,鉴定速度快、等位基因型分辨率达到单碱基水平,筛选出来的近似品种与田间表现基本一致,很好地辅助了水稻品种授权审查工作。

特此证明。

农业农村部植物新品种测试(广州)分中心

2019年5月19日



Stages of DNA identification	National standard of MNP marker method (GB/T 38551)	Industrial standard of SSR marker methods	Efficiency promotion (Folds)
PCR amplifications	317 to 1042 markers in a single PCR reaction	1 to 10 markers in a single PCR reaction	31-1042 folds
PCR production detection	10 thousands to 1 million markers once	1 to 10 markers once	10 thousands to 1 million folds

One person identified 1,032 markers of 600 rice cultivars in 10 days and compared them with 3000 cultivars in database

Theoretical accuracy



Variety 1

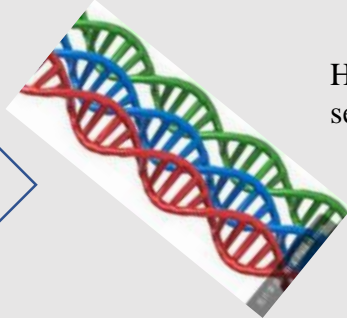
A A A

+

C C C

Variety 2

Mixed DNA (100: 5)



High-throughput sequencing

MNP1

A A A

MNP2

A A C

MNP3

A C A

MNP4

C C A

MNP5

C A C

MNP6

C A A

MNP7

A C C

MNP8

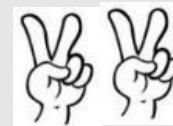
C C C

Error rate

$5\%^1=5\%$

$5\%^2=0.25\%$

$5\%^3=0.0125\%$



Actual accuracy

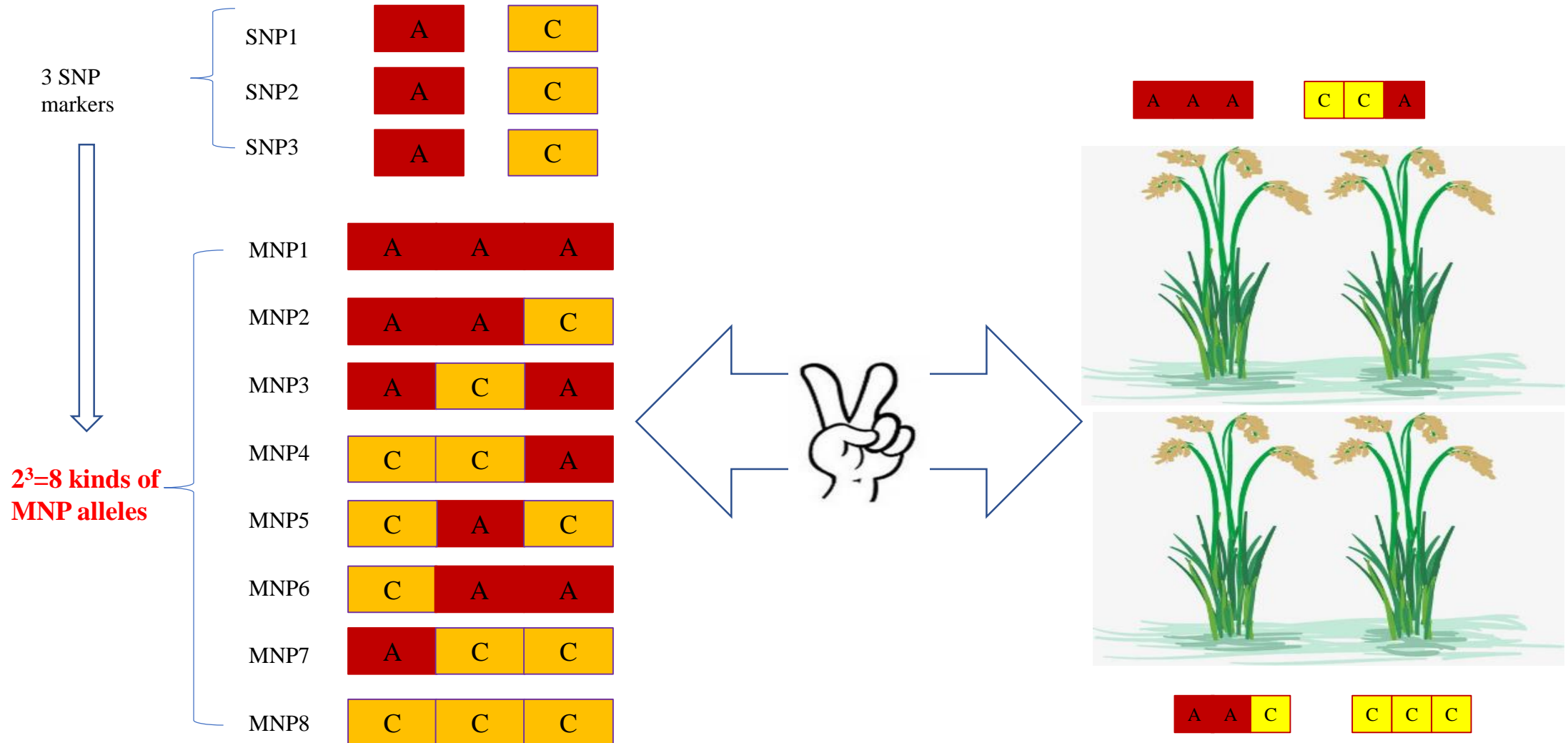
Species	Experiment steps		Markers compared	Distinct		Reproducibility rate	Accuracy rate
	Library construction	Sequencing		Number	Ratio		
Rice	Repeatability experiment	Repeatability experiment	306,651	10	0.0033%	99.997%	99.998%
	Same library	Reproducibility experiment	155,231	10	0.0064%	99.994%	99.997%
	Reproducibility experiment	Reproducibility experiment	306,528	35	0.0114%	99.989%	99.994%
Cotton	Repeatability experiment	Repeatability experiment	295,975	68	0.0230%	99.977%	99.989%
	Same library	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 conditions

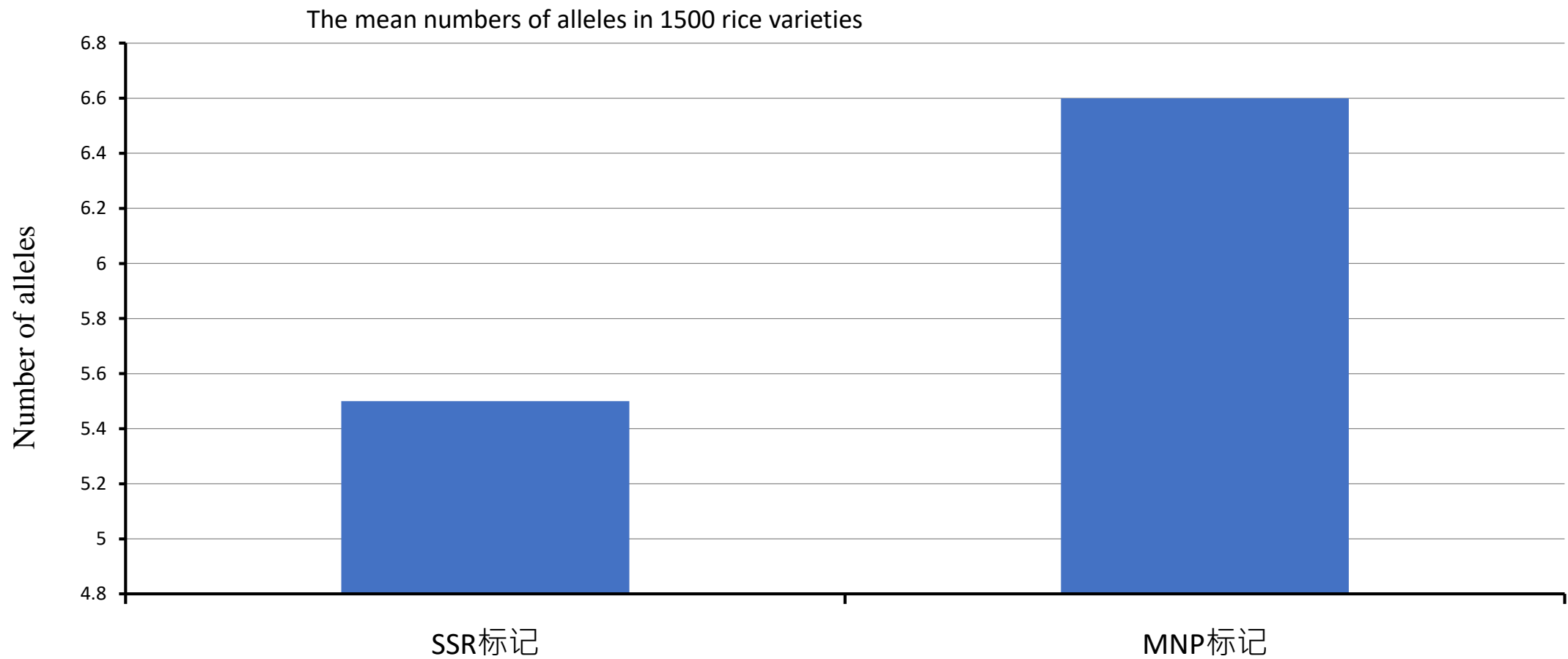
- Different time
- Different labs
- Different persons
- Different reagents
- Different instruments

- * Solve problems of standard samples
 - * Difficult to obtain
- * To solve the problem of accurate comparison with a large number of varieties
 - * EDV identification
 - * Identification of the infringed varieties
 - * Identification of the similar varieties
 - * Screening for duplicated authorized varieties

Theoretical polymorphism

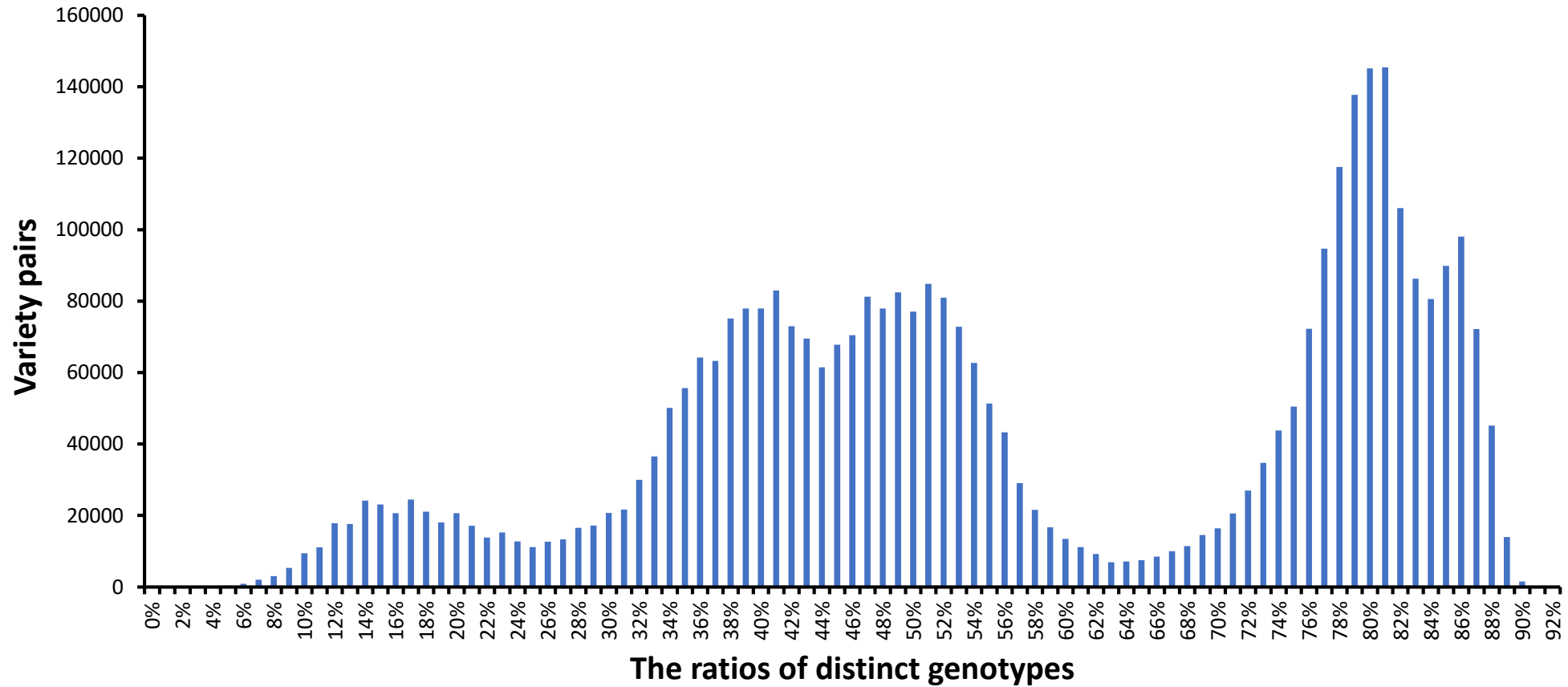


Actual polymorphism

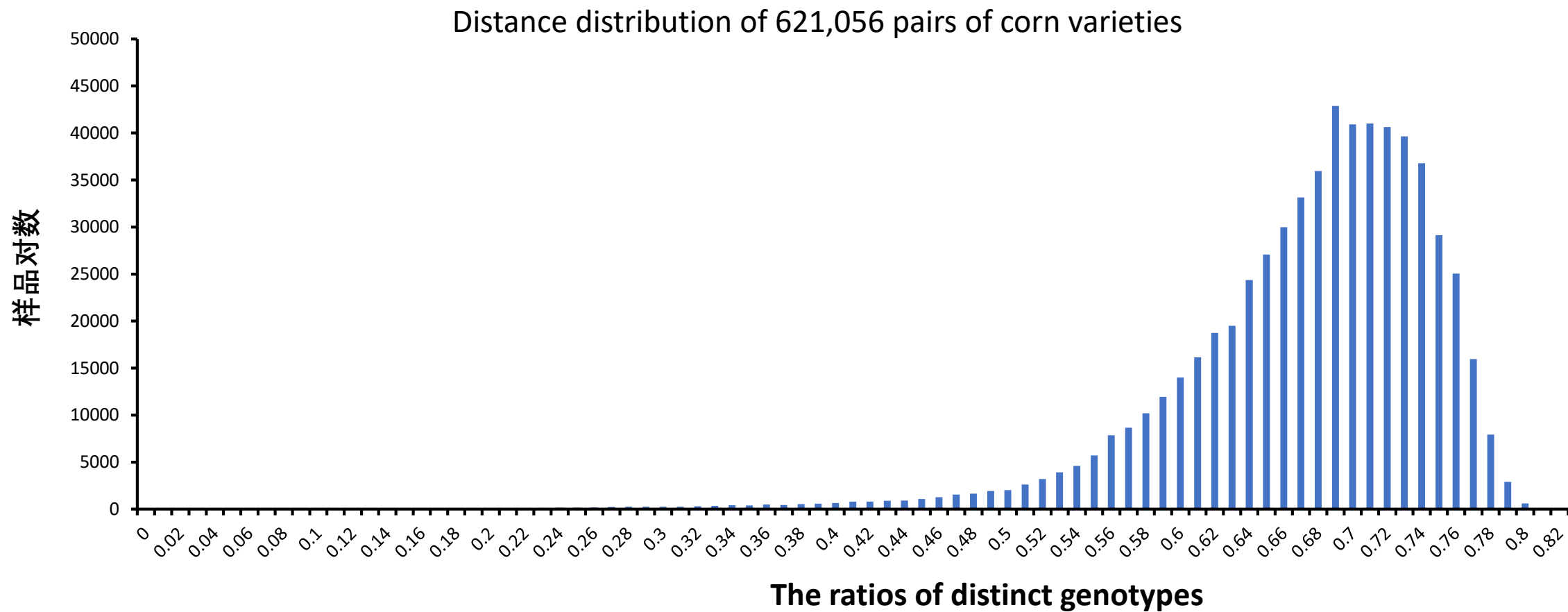


Varieties identification: Rice

Distance distribution of 3,730,438 pairs of rice varieties

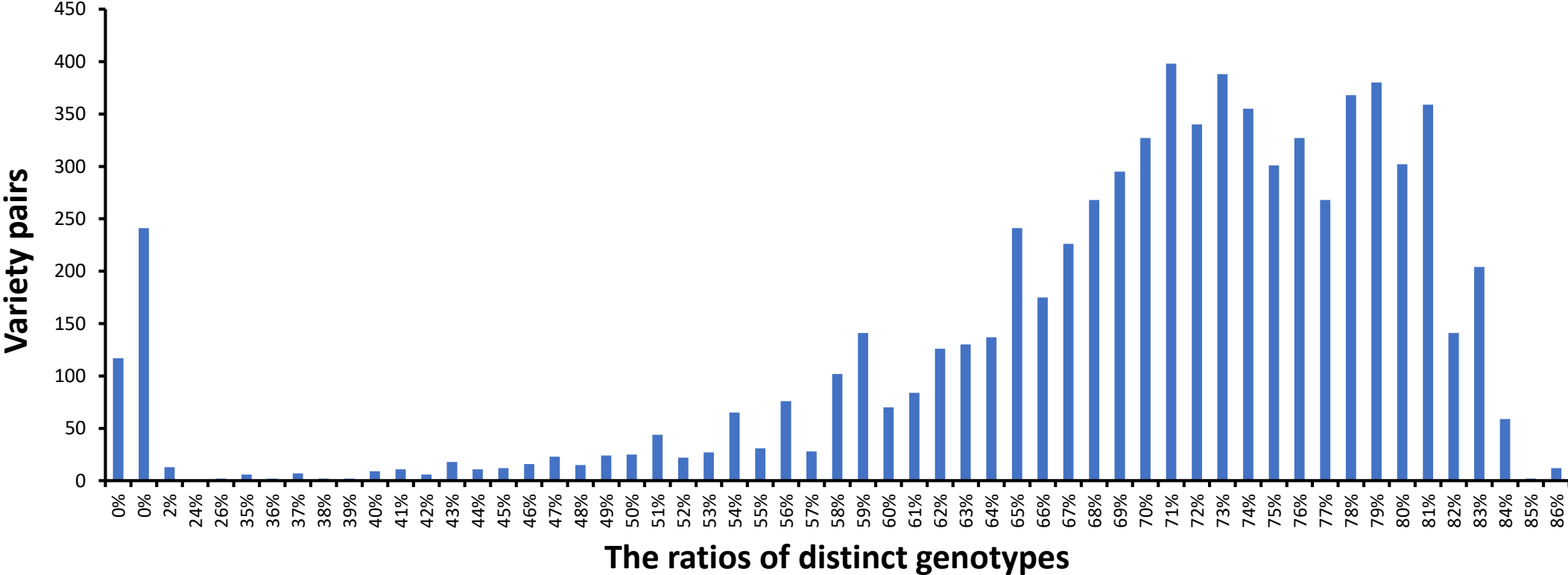


Varieties identification: Corn



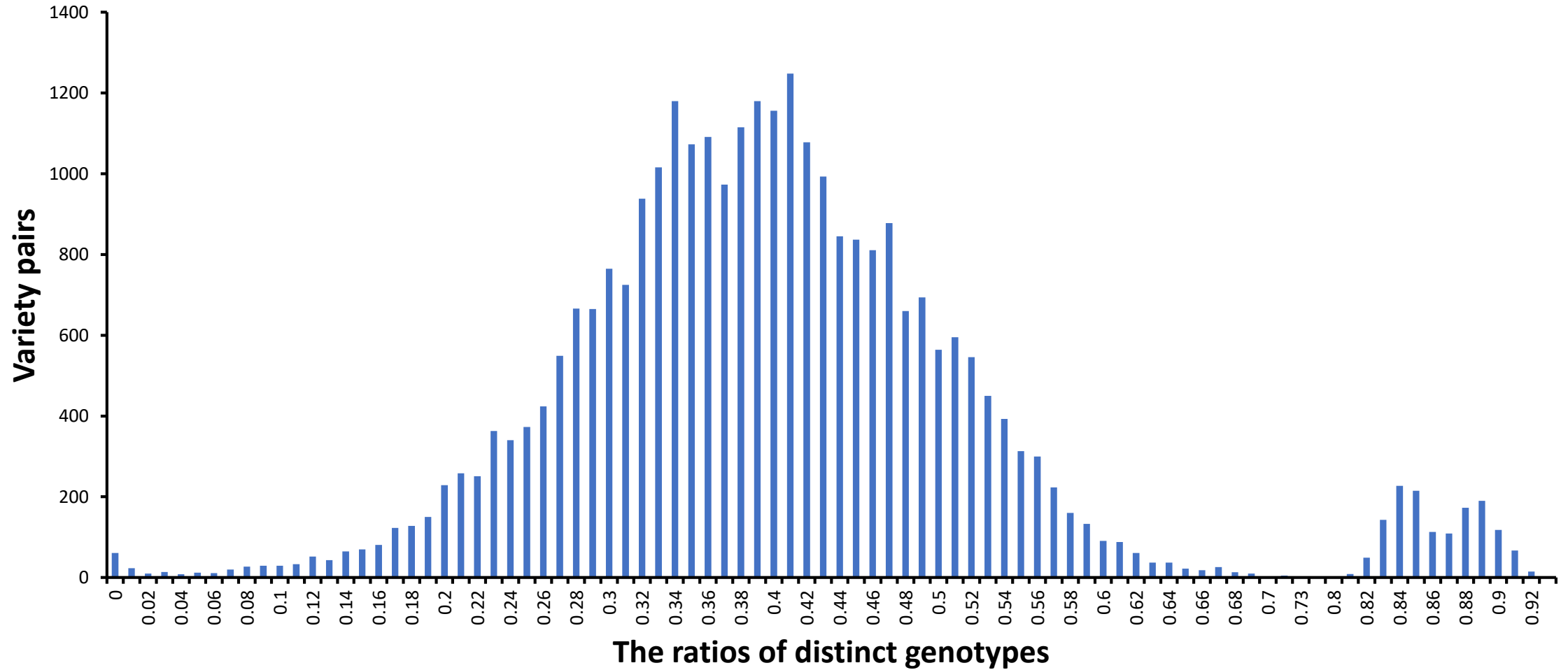
Varieties identification: Longan

Distance distribution of 7, 503 pairs of longan varieties



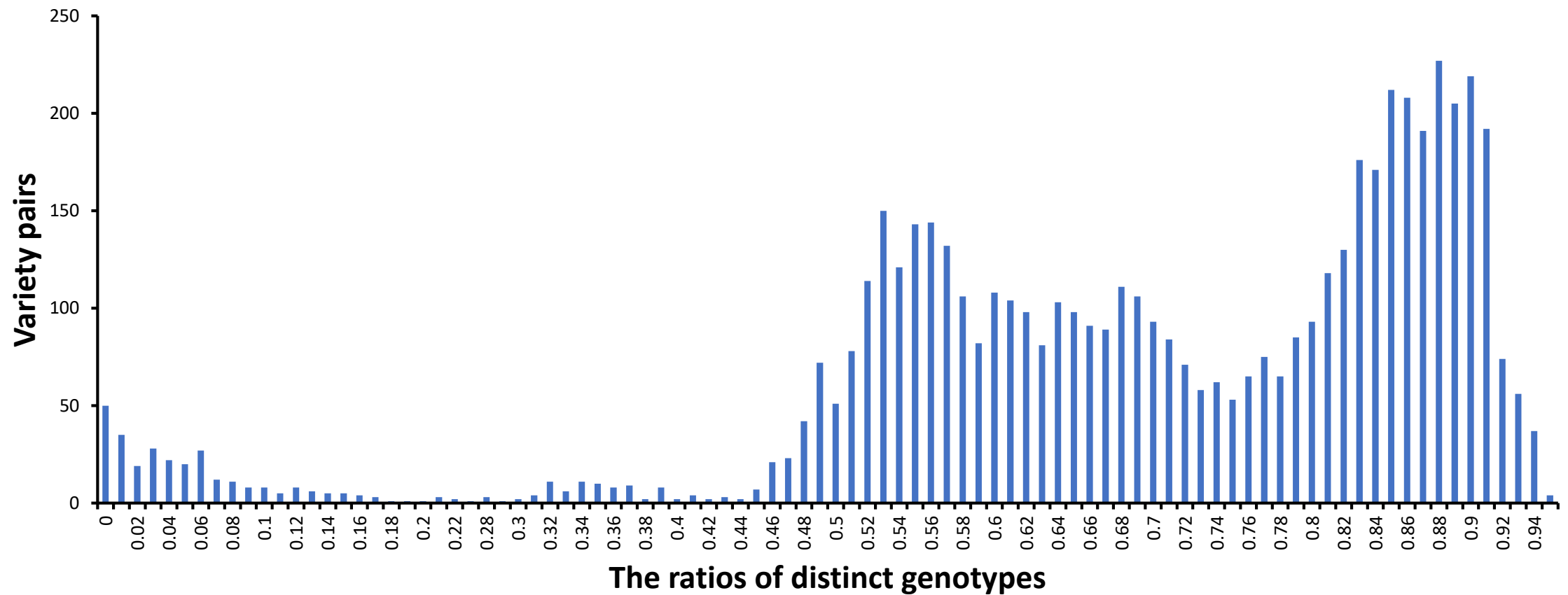
Varieties identification: Cotton

Distance distribution of 29,883 pairs of cotton varieties



Varieties identification: wormwood

Distance distribution of 5, 672 pairs of wormwood varieties



Patent groups

证书号第 2818438 号

发明专利证书

发明名称: 一种测试植物品种实质性派生关系的方法

发明人: 彭海;张静;陈红;张继

专利号: ZL 2015 1 0148657

专利申请日: 2015年03月31日

专利权人: 江汉大学;农业部

授权公告日: 2018年02月13日

本發明經過本局依照中華人民共和國專利法及實施條例的規定，在專利登記簿上予以登記。專利權的期限為二十年，自公告之日起計算。本專利的年費應自公告之日起繳納。專利證書記載專利權登記時的專利權人的姓名或名稱、國籍、地址。

局長
申長雨

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APPLICATION NUMBER	FILING or PRIORITY DATE	UR/PART UNIT	FE.FEE REC'D	ATTY DOCKET NO	TOT CLAIMS	IND CLAIMS
16/073,395	07/27/2018		860	C1367.70028US00	9	1

CONFIRMATION NO. 6756
FILING RECEIPT

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

Date Mailed: 11/06/2018

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

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62 authorized patents



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

Lun Li^{1,†}, Zhiwei Fang^{1,†}, Junfei Zhou¹, Hong Chen², Zhangfeng Hu¹, Lifeng Gao¹, Lihong Chen¹, Sheng Ren^{3,4}, Hongyu Ma⁵, Long Lu^{1,3}, Weixiong Zhang^{1,6,7,*} and Hai Peng^{1,*}

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中国农业科学 2018,51(6):1013-1019
Scientia Agricultura Sinica

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IF:11.561

Received December 05, 2017

ABSTRACT

Accurate and efficient method for large-scale SSR genotyping and applications

利用概率估算提高植物品种分子标记鉴定的准确率

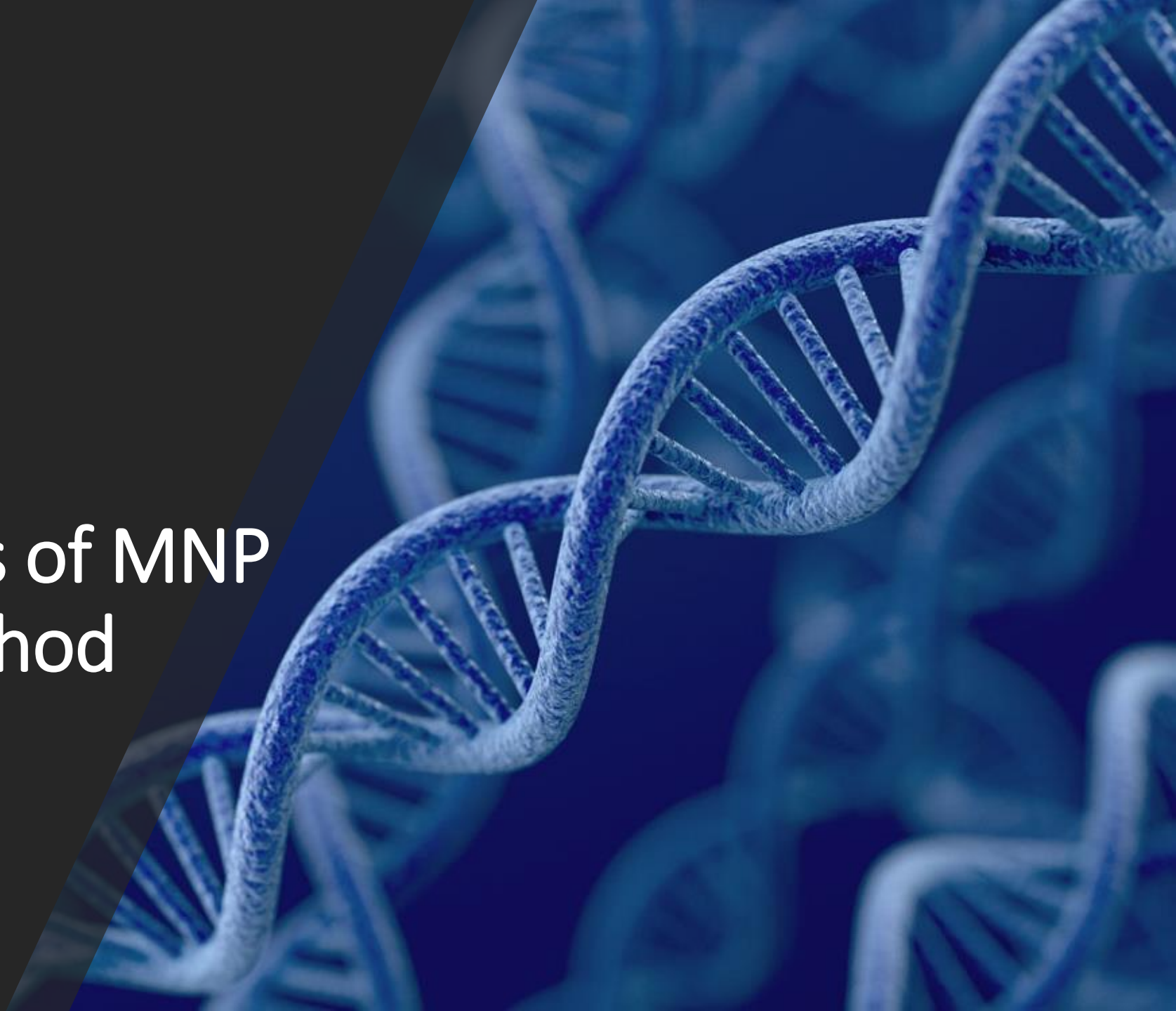
周俊飞¹, 崔野韩², 唐浩², 李论¹, 陈红², 温雯², 韩瑞奎², 黄思思², 方治伟¹, 彭海¹

(¹江汉大学系统生物学研究院, 武汉 430056; ²农业部科技发展中心, 北京 100122)

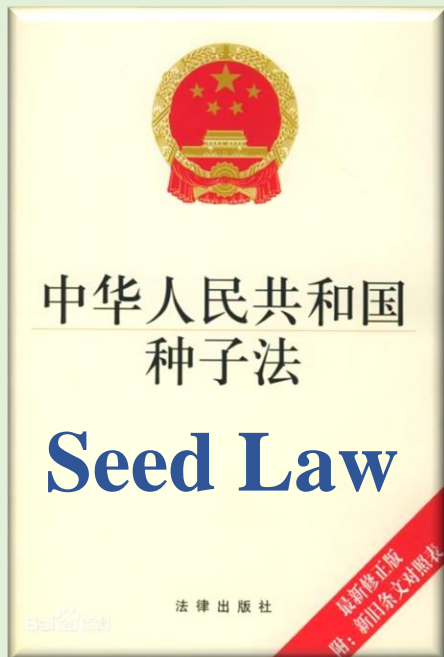
摘要:【目的】植物品种分子标记鉴定标准只抽检了基因组上部分标记位点, 存在抽样误差, 鉴定结论常常因此被质疑。估计植物品种分子标记鉴定位点的抽样误差与鉴定结论的可靠性, 为品种分子鉴定标准的应用提供科学依据。【方法】以 2 个品种间观察到的差异位点的数目为条件, 根据贝叶斯公式建立品种间真实差异位点数目的条件概率模型。根据观察到的差异位点的数目服从于二项分布, 且真实差异位点数的先验概率近似于均匀分布的特点, 实现条件概率模型的计算。根据概率模型获得的概率保障的大小, 将品种间的关系划分为红区、绿区和黄区, 对应相同或近似品种、不同品种和待定品种。利用 8 个水稻品种的 3 205 个 SSR 分子标记位点的分型结果, 估计 2 个品种组合间的真实差异水平, 进而判定品种间关系的真实值。对每一对品种进行 10 000 次的分子

03

Applications of MNP
marker method



Support the implementation of the Seed Law



Seed Law clauses which implementations required DNA identification

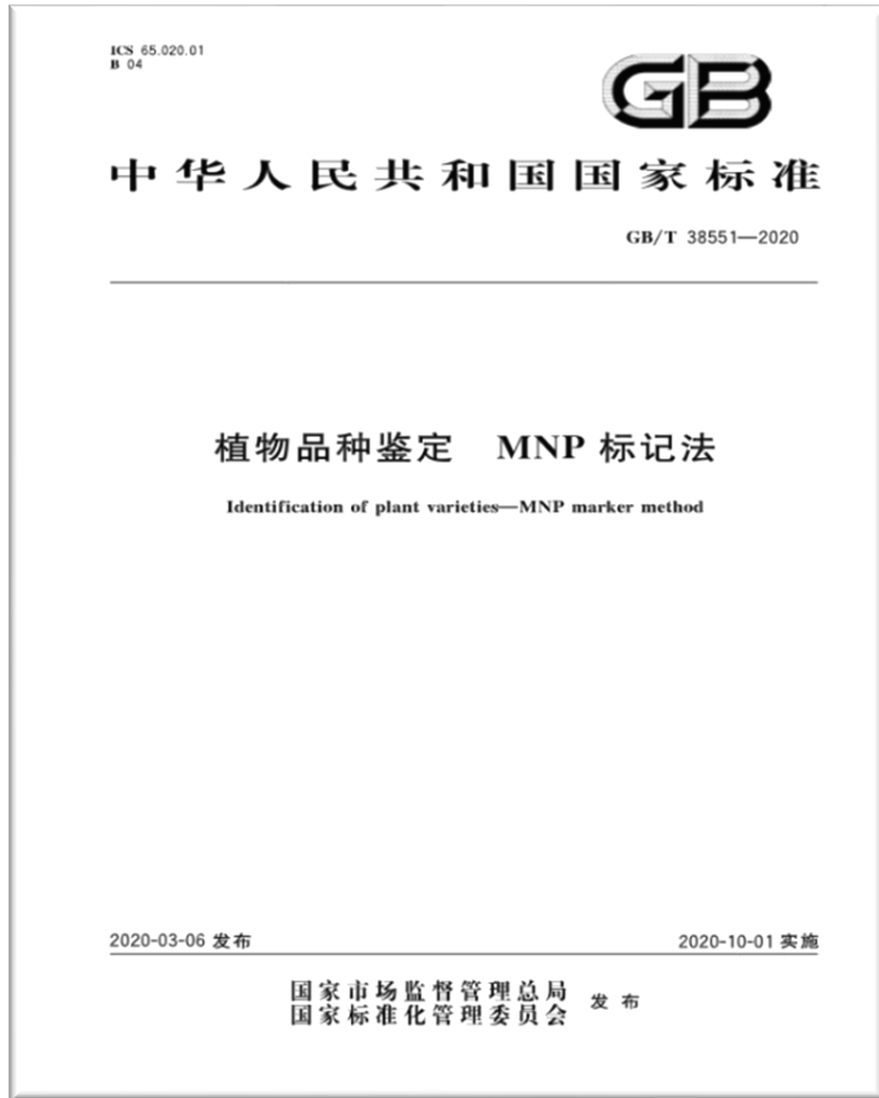
Examination and approval	Registration	Protection
60 points	37 points	34 points
Authorization	Infringement	Variety rights
11 points	10 points	33 points

Developed MNP marker methods for 34 kinds of plant species



- Developed 317-1042 MNP markers for each species ;
- The detection rates were over 95% for all species ;
- The detection accuracies were over 99.9% for all species

National standards for variety DNA identification



- The first national standard for the identification of multiple (16) plant varieties
- The first national standard for the identification of the essential derived varieties;
- Co-construct and share DNA fingerprints
- Little dependence on standard varieties
- Elimination of aerosol pollution
- Can be implemented by ordinary operators

Establish MNP fingerprint database of authorized varieties

项目委托协议书

project contract

项目名称：中国授权水稻品种 DNA 身份证构建

甲方：农业农村部科技发展中心

乙方：江汉大学
合同专用章

二〇一八年五月

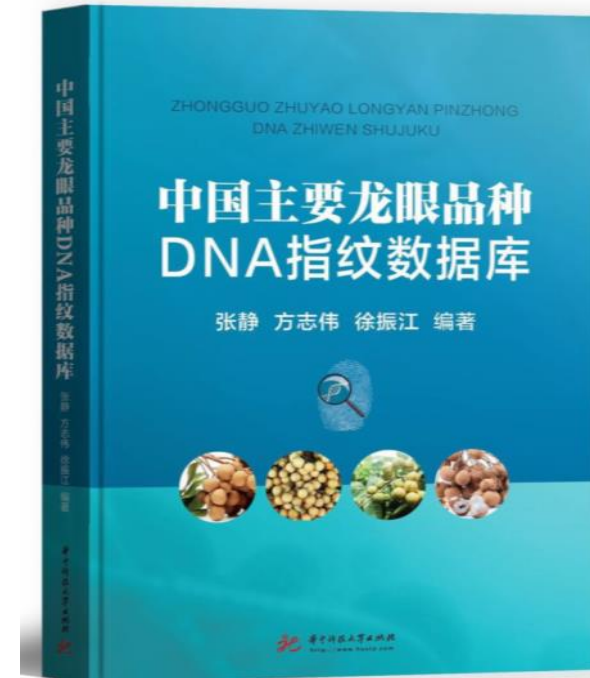
农业部科技发展中心

Application prove

证明

我单位于2015年-2017年与江汉大学开展合作，委托其拟定实质性派生品种审查指南和技术规范，分别鉴定了518671份、181503份和42486份常规水稻、籼稻和粳稻品种组间的实质性派生关系，在此过程中还建立了4000多份中国授权水稻品种的DNA身份证数据库。江汉大学出色完成了委托任务，鉴定速度快、结果准，为品种审查、鉴定与维权打假奠定了坚实的基础。

特此证明！



The database contains MNP fingerprints of over 10,000 rice and maize varieties

ASLIGIG2

Authorization prospect judgment of varieties

Variety under test	Similar variety			Compared markers		
	Acceptation code	Acceptation code	Name	Total number	Distinct Num.	Distinct ratio
15006	200235	25274	R9194	305	24	0.079
15006	200235	28534	27占	169	25	0.148
15006	200235	25246	泰恢187	350	62	0.177
15006	200235	13777	成恢3203	354	63	0.178
15006	200235	21856	启元S	111	21	0.189
15006	200235	23751	湘恢59	347	66	0.19
15026	200236	25274	R9194	314	36	0.115

Authorization prospect judgment of varieties

Alleles	Detection rate		
	Rapidly dehydrated varieties	Other varieties in database	Folds
1	31.25%	1.20%	25.97
2	26.67%	2.38%	11.19
3	37.50%	3.88%	9.66
4	25.00%	2.73%	9.15
5	43.75%	6.36%	6.88

The marker screening for rapidly dehydration

应用证明

Application prove

2018年11月,我单位委托江汉大学开展《水稻品种亲缘关系鉴定》项目。江汉大学利用MNP标记技术检测了112个水稻的DNA身份,对其中110个水稻品种进行了聚类分析,结果与品种表现基本一致。对其中2个品种进行了真实性鉴定,没有发现差异标记位点,鉴定为同一个品种。鉴定工作在7天内完成,平均检测的MNP标记数量为386.87个,等位基因型分辨率达到单碱基水平,表明MNP标记技术在辅助育种与品种真实性鉴定中具有检测位点多、检测效率高、速度快和标记分辨率高的优势。

特此证明。



 湖南杂交水稻研究中心

 2018年11月28日

Variety authorization review

Variety under test	Similar varieties		Detected markers	
	Name	Total number	Distinct Num.	Distinct ratio
01555a	隆晶4302A	524	0	0.00%
1604a	中香黄占	460	0	0.00%
1543a	荃香9A	568	2	0.35%
1616a	常9S	652	8	1.23%
1665b	先农5号	571	30	5.25%
1653a	桂晶丝苗	509	37	7.27%
1663a	两优培九	579	60	10.36%
1686a	R555	400	66	16.50%
1655a	渝香203	361	89	24.65%



农业农村部植物新品种测试 (广州)分中心文件

[2019]02号

Application prove 应用证明

2016年和2017年,我单位委托江汉大学开展鉴定水稻DUS测试品种中的近似品种。江汉大学利用MNP标记技术检测了600个水稻的DNA身份,并与全国3000多个授权水稻品种DNA身份证数据库进行了比对,筛选出了每个品种的近似品种,鉴定速度快、等位基因型分辨率达到单碱基水平,筛选出来的近似品种与田间表现基本一致,很好地辅助了水稻品种授权审查工作。

特此证明。

农业农村部植物新品种测试(广州)分中心

2019年5月19日



品种权打假维权

政府购买服务合同

服务事项：MNP 标记法在植物品种权打假维权中的能力测试

购买主体：农业农村部种业管理司（甲方）

承接主体：江汉大学（乙方）

签订时间：2019 年 5 月 28 日

农业农村部计划财务司 制



政府购买服务合同

服务事项：利用 MNP 标记法进行植物品种的打假与维权

购买主体：农业农村部种业管理司（甲方）

承接主体：江汉大学（乙方）

签订时间：2020 年 7 月 2 日

- 从100份水稻品种DNA指纹库中，100%正确鉴定了50份盲样水稻品种的名称

Crack down on counterfeits and protect variety rights

Variety under test	Standard variety	MNP marker method (GB/T 38551-2020)				SSR marker method (NY/T 1433-2014)		
		Compared makers	Distinct markers	Genetic similarity (GS)	Conclusion	Compared makers	Distinct markers	Conclusion
SD044	两优56	1027	335	67.38%	Distinct variety	48	11	Distinct variety
SD071	宜优29	1030	441	57.18%	Distinct variety	48	9	Distinct variety
SD075	隆两优1146	1022	433	57.63%	Distinct variety	48	12	Distinct variety
SD084	德优8258	1032	15	98.55%	Similar variety	48	0	Extremely similar or identical variety
SD151	德优8258	1031	15	98.55%	Similar variety	48	0	Extremely similar or identical variety
SD107	荣优淦3号	1028	499	51.46%	Distinct variety	48	13	Distinct variety
SD131	奥富优383	1014	548	45.96%	Distinct variety	48	15	Distinct variety
SD009	徽两优粤农丝苗	1030	12	98.83%	Similar variety	No standard sample	/	/
SD031	荃优727	955	304	68.17%	Distinct variety	48	10	Distinct variety
SD082	荃优华占	1010	14	98.61%	Similar variety	No standard sample	/	/
2020B16	华两优2821	1033	556	46.18%	Distinct variety	48	13	Distinct variety
	两优1208	1028	154	85.02%	Distinct variety	48	5	Distinct variety
	中9优2040	1030	499	51.55%	Distinct variety	48	14	Distinct variety

- The 99 rice and 68 maize variety samples were received on 25 April 2020 and after 14 days, the results of MNP marker method were delivered on 8 May.
- MNP and SSR marker methods were respectively implemented by Jiangnan University and Hubei Seed Management Station, and the cross-validation of the methods was passed

Seed quality control for adulteration

技术应用证明

Application prove

我单位于2017年-2019年委托江汉大学系利用其发明的MNP标记法与目标区域测序法，对公司出口至巴基斯坦的水稻种子进行品种真实性鉴定和转基因成份鉴定（详见附件说明）。MNP标记法与目标区域测序法鉴定速度快，而且结果准确可靠，与欧盟最权威鉴定机构的鉴定结果一致。利用江汉大学的技术，杜绝了种子收购与进出口贸易过程中的质量风险，对公司稳健发展意义重大。公司进一步委托了江汉大学利用其专有技术建立巴基斯坦国水稻品种与资源的DNA身份证数据库，以实现种子的精准进出口贸易和公司品种知识产权的市场维护。

特此证明

武汉庆发禾盛农业发展有限公司

2019年4月20日



技术服务合同

项目名称：巴基斯坦国水稻品种及资源DNA身份证数据库建设

委托方（甲方）：武汉庆发禾盛农业发展有限公司（盖章）

受托方（乙方）：江汉大学系统生物学院（盖章）

项目负责人：朱晓波（签字）

项目技术负责人：彭海（签字）

签订时间：2019年 月 日

签订地点：武汉

有效期限：2019年01月7日—2020年01月7日

中华人民共和国科学技术部印制

➤ The seeds to be exported to Pakistan was found to have adulteration;

Quality control for rice counterfeit and adulteration

Blind sample	Identified variety name	Real name	Right(✓) or wrong (X)
JHU1	五优稻4号, 稻花香2号	五优稻4号	✓
JHU2	龙洋05-16	龙洋16号	✓
JHU3	盐丰47, 锦丰1号, 田丰202, 田丰201, 辽早109	盐丰47	✓
JHU4	美香占2号, 连香占, 中广香1号	美香占2号	✓
JHU5	徐稻9号, 金廊粳2号, 隆粳968	徐稻9号	✓
JHU6	泰优390	泰优390	✓

Blind sample	Included varieties	Determined ratios	Real ratios
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%

项目委托协议书
project contract

项目名称: 水稻混合 DNA 样本测试

甲 方: 世界500强公司

乙 方: 江汉大学系统生物学院

项目起止日期: 2018年9月1-2019年3月1

➤ Big companies are afraid of buying fake or adulterated rice and damaging their brands

Identification for the essential derived variety

ICS 65.020.01
B 04



中华人民共和国国家标准

GB/T 38551—2020

植物品种鉴定 MNP 标记法

1 范围

本标准规定了植物品种鉴定多核苷酸多态性(MNP)标记法的原理、试剂或材料、仪器设备、测定步骤、结果分析。

本标准适用于水稻(*Oryza sativa* L.)、玉米(*Zea mays* L.)、大豆[*Glycine max* (Linn.) Merr.]、棉花(*Gossypium* spp.)、花生(*Arachis hypogaea* L.)、谷子(*Setaria italica*)、西瓜(*Citrullus lanatus*)、甜瓜(*Cucumis melo* L.)、黄瓜(*Cucumis sativus* L.)、艾草(*Artemisia argyi*)、番茄(*Lycopersicon esculentum* Mill.)、辣椒(*Capiscum annuum* L.)、白菜[*Brassica pekinensis* (Lour.) Rupr.]、龙眼(*Dimocarpus longan* Lour.)、荔枝(*Litchi chinensis* Sonn.)、猕猴桃(*Actinidia Chinensis*)的原始品种鉴定、实质性派生品种鉴定和品种真实性鉴定。其他植物品种鉴定可参考本标准。



意见》《商标侵权判断标准（征求意见稿）》《水稻实质性派生品种鉴定 MNP 法》。研究修改《商标档案管理暂行办法》。

制定出台《关于依法加强对境外著作权认证机构常驻中国代表机构管理的意见》。

We published the first national standard and seven technical specifications to identify the essential derived varieties.

One technical specification was included in the white paper on the state of Intellectual Property Protection in China in 2019

Develop national standards for GMO testing



- Test GMO ingredients of all plants at one time
- Detect all known GMO elements and their events at one time
- Completely avoid microbial contamination and aerosol pollution, can be carried out in ordinary laboratory
- No need for extensive experience, can be implemented by ordinary operators



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