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The development and application scenarios of molecular technology in PVP system

分子技术在PVP系统中的发展及应用场景

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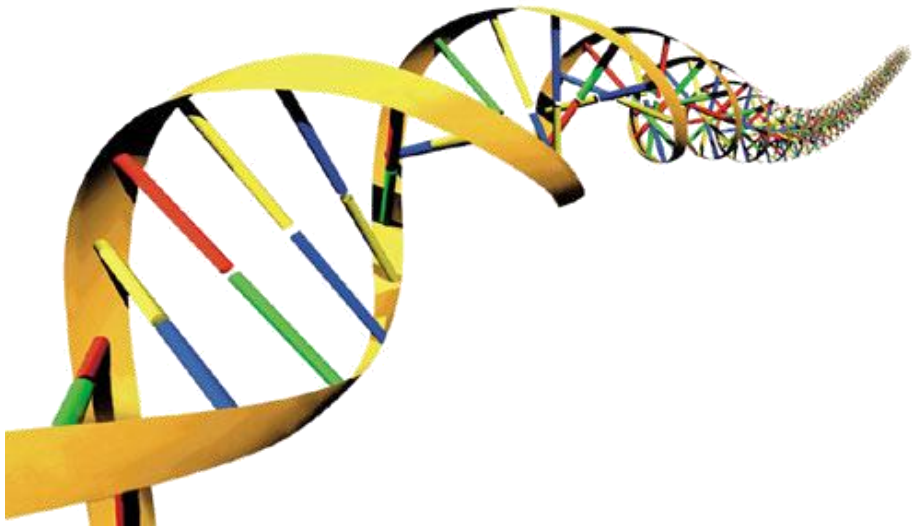
Advantages of DNA in PVP sytem

PVP系统中DNA的优势

PVP system based on morphology

基于形态学的PVP系统



- DNA databases
- DNA数据库
- DNA markers are suitable for harmonization
- DNA标记适合校对
- No phytosanitary rules apply for DNA
- 没有植物检疫规定适用于DNA
- DNA profiling is faster and cheaper then growing trial
- DNA分析比生长试验更快更便宜

DNA in PVP system

PVP系统中的DNA

UPOV-BMT

The Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular (BMT) *生物化学和分子技术工作组，特别是DNA分析工作组（BMT）*

MODEL 1: Characteristic-specific molecular markers

模式1：特征特异性分子标记

MODEL 2: Combining phenotypic and molecular distances in the management of variety collections

模式2：结合表型和分子特征进行品种收集管理

UPOV - Model 1

UPOV-模式1

DNA Markers as Predictors of 'Traditional' DUS Characteristics 作为"传统"DUS特征的预测因子的DNA标记

- Gene-specific **markers** for predicting individual phenotypic characteristics.
- 预测个体表型特征的基因特异性标记
- Examples:
  - *Flower or Fruit colour (red or yellow bell pepper)*
  - *花色或果色（红或黄甜椒）*
  - *Cytoplasmatic Male Sterility in Brassicaceae (sterile or fertile flowers)*
  - *十字花科植物细胞质雄性不育（不育或可育花）*
  - *Disease resistances in tomato (resistance or susceptibility against Fol infection)*
  - *番茄的抗病性（抗或感病性）*

- Reliable linkage between the **marker** and the expression of the characteristic required.
- 标记与表达所需特征之间的可靠联系。

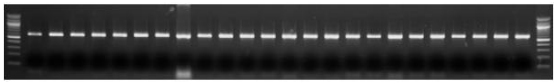
Resistances in DUS test tomato

番茄的抗性DUS试验

- Resistance / Susceptibility for the obligatory diseases (information on the TQ) is used to select relevant reference varieties (grouping characteristics)
- 对强制性疾病的抗性/易感性(TQ信息)用于选择相关参考品种(分组特征)
- The breeder must claim in the TQ 'real' resistance or 'real' susceptibility, as morphology is always leading.
- 育种者必须在TQ中声明“真正的”抗性或“真正的”易感性，因为形态学总是优先的
- Information on TQ for a candidate variety must be confirmed
- 必须确认候选品种的TQ信息
- Confirmation is done by bio-assay or by a DNA marker test
- 通过生物测定或DNA标记试验进行确认
- The DNA resistance marker needs to be predictive for the bioassay results (reliable correlation)
- DNA抗性标记需要预测生物测定结果（可靠相关性）
- If this is the case, the UPOV guideline gives a protocol of the bio-assay and of the DNA marker test as alternative
- 如果是这种情况，UPOV指南给出了生物测定和DNA标记测试的方案作为替代

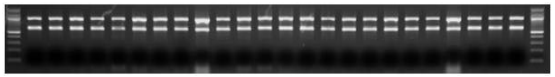
DNA marker for Resistance in DUS test tomato

番茄DUS抗性的DNA标记



#24 纯合子
抗性等位基因

#24 homozygous
Resistant allele



#24 heterozygous
both alleles

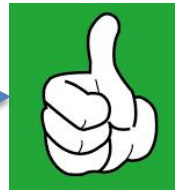
#24 杂合子
两个等位基因

resistant

抗性的

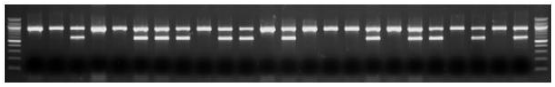
T
Q

TQ = RES
DNA = RES
Confirmed



T
Q

TQ = SUSC
DNA = RES
Contradiction



#12 plants (heterozygous) resistant allele
#12 plants (homozygous) susceptible allele

- #12 植物（杂合子）抗逆性
- #12 植物（纯合子）易感性

Genetically NOT uniform
遗传不均一

Bio-assay生物测定

Strategy when using model 1

模式1的使用方法

	Dominant marker		**Co-dominant marker**	
1. PCR result PCR结果	Resistance marker present 抗性标记	Resistance marker absent 无抗性标记	Homozygous resistant or heterozygous抗性纯合或杂合	Homozygous susceptible 易感纯合
2. Conclusion DNA DNA结论	Resistant 抗性的	Susceptible, or a PCR reaction failed, or Resistant (based on a different gene) 易感，或PCR反应失败，或抗性(基于不同的基因	Resistant 抗性的	Susceptible, or Resistant (based on a different gene)易感的，或抗性的（基于不同的基因）
3a. TQ info **RES** TQ信息	Okay: conclusion resistant 抗性结论	Not okay: bioassay 生物测定	Okay: conclusion resistant抗性结论	Not okay: bioassay 生物测定
3b. TQ info **SUSC** TQ信息	Not okay: bioassay 生物测定	Confirmation by bioassay (# plants) 生物测定确认（植物）	Not okay: bioassay生物测定	Okay: conclusion susceptible 抗性结论

Model 2 (Morphology + DNA)

模式2（形态学＋DNA）

AIM（目标）：

- Increase reliability: expend the collection of reference varieties by the use of DNA databases when living reference collections are limited.
- 增加可靠性：当活体参照品种收集有限时，通过使用DNA数据库来增加参照

- Minimize the risk of incorrectly excluding reference varieties from the trial by setting thresholds for minimal distances.
- 通过设置最终状态的阈值，降低从试验中不正确排除参照品种的风险

- Safely exclude varieties of common knowledge from the trial to reduce workload and costs without decreasing quality of the DUS test.
- 在不降低DUS测试质量的情况下，从试验中排除熟知的品种，减少工作量和成本

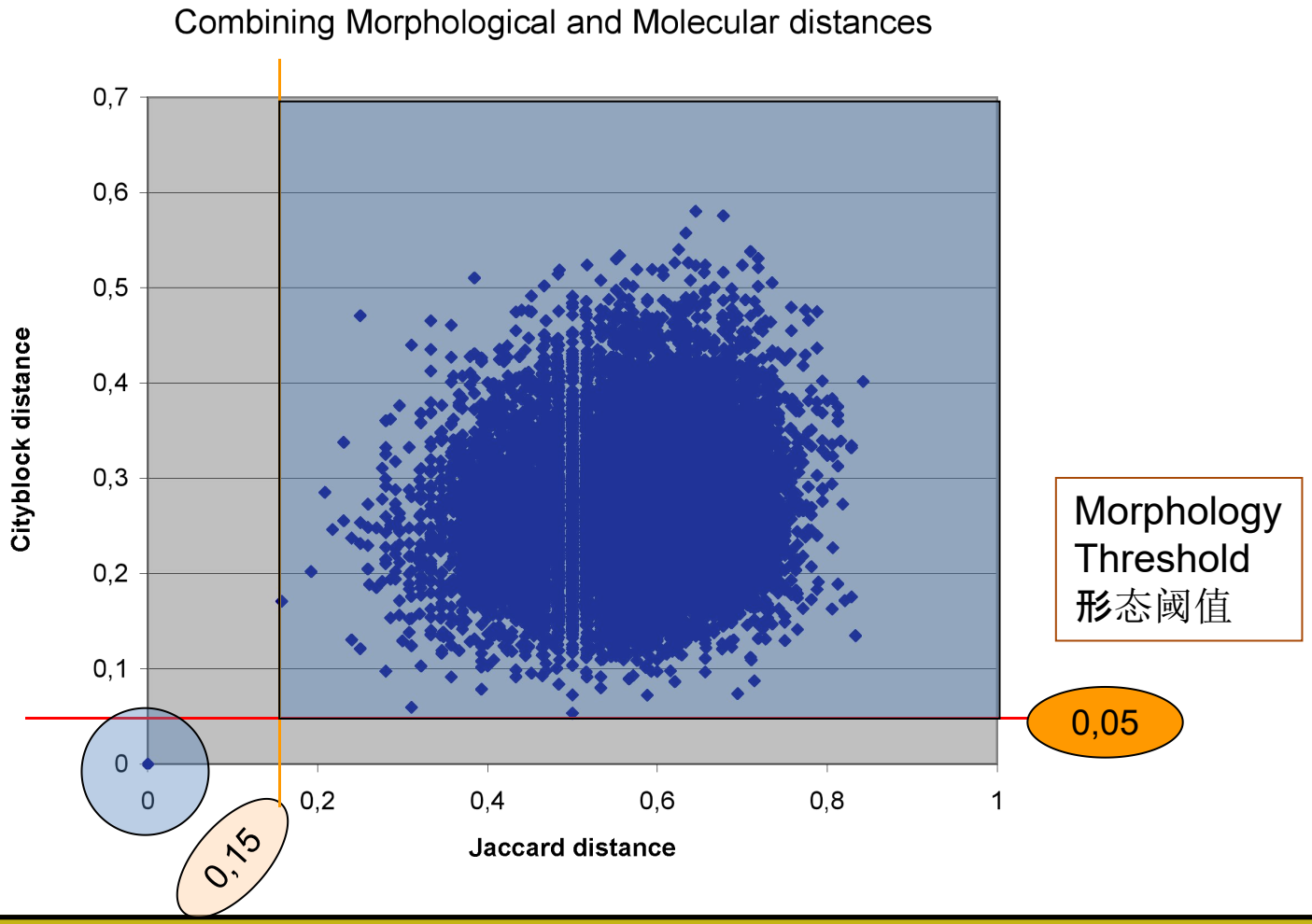
Example (NL): Potato
示例（NL）：马铃薯

Limitations of current system (without DNA)
当前系统的局限性（无DNA）

- Limited (living) reference collection
- 有限的（活体）参照物
- Limitations due to Phytosanitary risks and costs
- 植物检疫风险和成本的限制
- Limited coverage of database
- 数据库覆盖有限
- Variation of morphological data
- 形态数据的变化

Example: Potato (NL)

示例：马铃薯（NL）



NL: use in POTATO

NL：用于马铃薯

DUS test based on morphology ***and*** DNA:

基于形态和DNA的DUS测试：

- **First year**（第一年）:
  - start of season: lightsprout test (photo) and DNA profile
  - 季节开始:光芽试验(图)和DNA图谱
  - DNA results, molecular distance and matches are provided for all candidates before the planting of the DUS trial, impact on selection of similar varieties
  - 在DUS试验前，提供所有候选品种的DNA结果、分子量和匹配物，影响相似品种的选择
  - Growing season: morphological observations as usual
  - 生长季节：形态学观察
- **End first year**（第一年结束）:
  - DUS decision based on morphological description and DNA profile compared with the databases
  - 基于形态描述和DNA图谱的决策与数据库进行比较
- **Second year test for candidate varieties only when**（候选品种的第二年测试仅在以下情况下进行）:
  - Candidate/reference pair below threshold for morphological distance (cityblock distance < 0,05)
  - 候选/参考对低于形态距离的阈值（cityblock distance<0.05）
  - Candidate/reference pair below threshold for molecular distance (Jaccard < 0,15).
  - 候选/参考对低于三个最佳分子量（Jaccard<0.15）

NL: use in POTATO

NL：用于马铃薯

Advantages（优势）:

- Significant expansion of reference collection (potentially cooperative DNA labs) reduces the risk of granting right for varieties that are already common knowledge in other parts of the world.

•参照品种收集的显著增加（潜在的合作DNA实验室）降低了在世界其他地区已经为人们所熟知的各种物种争取权利的风险

- Detection of most similar reference varieties (Jaccard distance < 0,15)

•大多数相似参照品种的检测（Jaccard distance<0.15）

- In most cases the duration of DUS trial can be reduced (cost reduction) without affecting the reliability of the DUS test.

•在大多数情况下，可以缩短DUS试验的持续时间（降低成本），而不影响DUS试验的可靠性

- The DNA database can also used to check stability when renewing reference material

•在更新参照品种时，DNA数据库可以用来检查稳定性

- Spin-off: the DNA database can be used for other purposes than DUS.

•衍生产品：DNA数据库可以用于其他目的

Example (NL): French bean
示例（NL）：四季豆

Problem: Many varieties in same group (TG/12/9 Rev. 2). Based on grouping characteristics 15-20 reference varieties are selected for every candidate.
问题：同一类群中有许多品种(TG/12/9 Rev. 2)，根据分组特点，每个候选品种选择15-20个参考品种。

Too expensive 太昂贵
No efficient side-by-side comparison
没有有效的并行比较

Aim: improve efficiency in DUS testing for French bean and other crops with normally two growing cycles
目的：提高四季豆DUS检测效率，
并且其他作物通常有两个生长周期

Genetic selection of similar varieties for the first growing cycle
相似品种第一生长周期的遗传选择

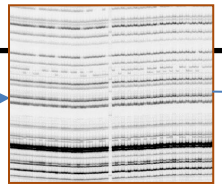
Start

seed
种子

T
Q

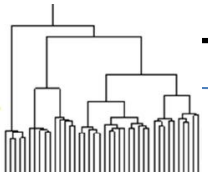
DNA profiling

DNA分析



DNA similarities

DNA相似性

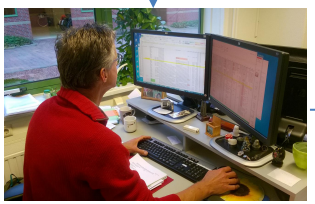


first selection

第一选择

check morphological database

形态数据库



discard on QL grouping chars only

只放弃QL分组特征

Fönix	afwezig	
Hansi	afwezig	
Bravo	afwezig	
Selma	afwezig	z
~~Dipinto~~	aanwezig	z
~~Geivert~~	aanwezig	l
~~Bruine Ko~~	aanwezig	r
~~Stokkievit~~	aanwezig	r

(short) list of varieties to be put in field trial (1st cycle)
（短）田间试验品种表（第1周期）

1st growing cycle

第一生长周期

Side-by-side comparisons and complete description

并排比较和完整描述

'paper check' morphological database

"paper check"
形态数据库

discard on all chars

放弃所有分组特征

Extra similar varieties needed?

需要额外的相似性吗？

Clearly D and no extra similar varieties: positive conclusion after 1st cycle
很明显，没有额外相似的品种：第1个周期后的肯定结论

Not clearly Distinct and/or extra similar varieties needed: normal 2nd growing cycle
不明显的区分和/或额外相似的品种需要:正常的第二生长周期

2nd growing cycle

第二生长周期

Candidate
候选品种

Closest similar variety from 1st growing cycle
与第一个生长周期最接近

Extra similar varieties from 'paper check'

'paper check'的额外相似性

Knowing that these are genetically Distinct
知道这些是遗传上的区别

并列比较

Side-by-side comparisons

Positive decision on Distinctness
对差异性的积极决定

French bean example
四季豆的例子

Advantages（优势）:

- The selection of reference varieties is not based on the information in TQ but ONLY on DNA profile.
- 参照物的选择不是基于TQ中的信息，而是基于DNA图谱
- Reduce costs and workload.
- 减少成本和工作量
- Selection of similar varieties based on morphology using *own reliable* description. So, relevant refs that might be missed by DNA selection first year are identified in second year.
- 基于形态特征的相似样本的选择，采用完全可靠的描述，因此，在第二年确定了第一年DNA选择可能遗漏的相关参照品种

Thank you for your attention

Quality in Horticulture