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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR POTATO

Second Session
Quimper, France, April 17, 2007

ADDENDUM TO DOCUMENT BMT-TWA/POTATO/2/4

USE OF SSR MARKERS FOR VARIETY IDENTIFICATION AND CERTIFICATION OF SEED POTATOES IN FRANCE

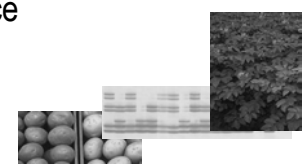
Document prepared by experts from France

This document is an addendum to document BMT-TWA/Potato/2/4 "Use of SSR Markers for Variety Identification and Certification of Seed Potatoes in France" and contains a copy of the presentation made by Mrs. Sylvia Marhadour, France, at the second session of the *Ad Hoc* Subgroup on Molecular Techniques for Potato.

FNPPPT

INRA

SSR to identify and certificate seed potatoes in France

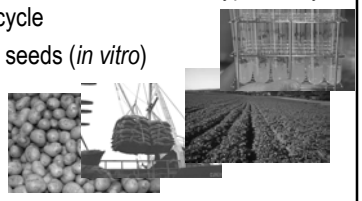


S. Marhadour

April 2007

Use of molecular tools

- To reinforce the guaranties for varieties identity on certified seeds
- To check quickly the varietal trueness-to-type at any stage in the plant cycle
- To check prebasic seeds (*in vitro*)



Five SSR markers have been chosen

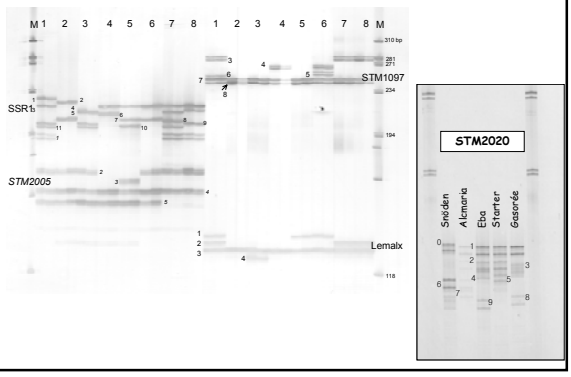
Moisan-Thiéry,M., Marhadour et al 2005 Potato Research 48

- 30 primer pairs tested
  - Provan et al (1996), Kawchuk et al (1996) and Milbourne et al (1998)
- 5 primer pairs chosen based on
  - Number of alleles amplified
  - Size of the PCR products
  - Polymorphism revealed between cultivars
- Construction of a database
  - SSR profiles of more than 400 cultivars

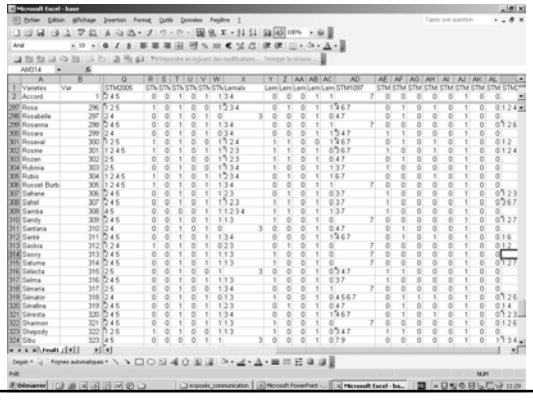
Characteristics of the 5 markers

Locus	repeats	Reference	size (bp)	Number of alleles
SSR1	$(TCAC)_n$	Kawchuk (1996)	210-252	11
STM2005	$(CTGTTG)_n$	Milbourne (1998)	160-193	6
Lemalx	$(ATT)_n$	Milbourne (1998)	126-135	4
STM1097	$(CGTTT)_n$	Milbourne (1998)	252-307	8
STM2020	$(TAA)_n$	Milbourne (1998)	162	10

The 5 Markers



Database : illustration



Technical characteristics of the procedure

- Two independent DNA extraction/sample
- Multiplex PCR and/or post PCR multiplexing
- Silver-staining revelation
  - Standard laboratory equipment

Current Applications in France

- Routine application in *4* laboratories
  - Common procedure and database
- Control of *in vitro* collections
- Tests in case of commercial dispute or fraud
- Genotyping of hybrids in selection

Inter-laboratory tests

- Organized each year since 2003 by the Official Inspection Service (SOC)
- Aims :
  - Determine variety identity
  - Test reproducibility over labs

	# labs	# samples	results
2003	2	30	28 cultivars identified/ 2? Not registered
2004	4	20	20/20 identified in the 4 labs
2005	4	10	10/10 identified in the 4 labs
2006	4	15	15/15 identified in the 3 labs*
2007	4	5	in progress

** Results for 4th lab not available*

Limits (1/2)

- Mutants
  - Field test trial each year to check varietal identity and trueness-to-type of all first generation lots

Limits (2/2)

- 5 pairs of varieties with the same profile using 5 markers
  - 3 differentiated using at least one additional marker
  - 2 pairs remain identical even after testing 14 additional markers
- Additional markers in the procedure are needed

Conclusions

- We have an efficient set of markers to
  - Identify varieties provided its profile has been registered in the database
  - Verify identity of any seed lots to a reference in case of doubts
- But visual observations remain essential to check the conformity

Prospects

- In progress
  - Choice of additional markers (from Ghislain et al 2004 TAG 108)
- We propose to use SSR markers
  - as a complementary tool for producing rapid results for the protection of breeders against unauthorized exploitation of their protected varieties
  - To characterize seed potatoes in the certification scheme
- We are open to exchange on results and database and participate to a European inter-laboratories test

Acknowledgements

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