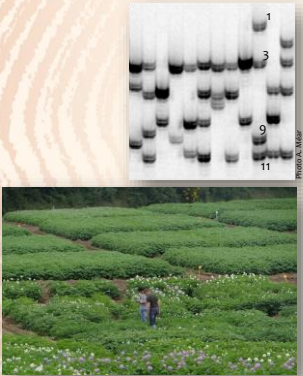


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## Varietal seed potato identification using SSR markers in France : organization, methods and database

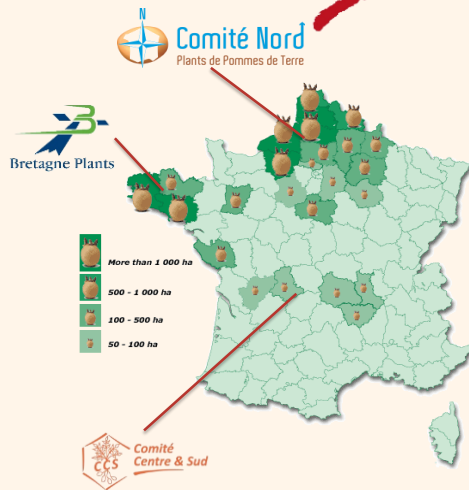
Marhadour, S. , Méar, A. , Dargier, C., Laversin, N.,  
Perramant, M., Pavy, V., Meytraud, F., Lesage, M.L.  
, Bronsard, G., Esnault, F. , Le Hingrat, Y.

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## Organization of the seed potato sector in France

- France ranked 2<sup>nd</sup> for seed potato exportation
  - 1000 producers
  - 18 000 ha of production
  - ~450 000 t of certified seeds produced each year
  - 450 cultivars multiplied
- Seed inspection and certification verify
  - Health quality and
  - Varietal identity
  - along the multiplication process



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## Microsatellites markers (SSR) are a tool of choice to check cultivar identity

**cultivar A**  
# repeats: 3, 5, 5 and 7

**cultivar B**  
# repeats: 2, 5, 5 and 5

PCR

Repeat unit ex: (AT)

PCR primers

A B

Electrophoresis

7 5 3 5 2

- **Single Sequence Repeats:**
  - PCR amplification of repetitions units
- **An essential tool to fingerprint cultivars, check their identity and manage collections**
  - High reproducibility (Jones et al., 1997, McGregor et al., 2000)
  - High level of heterozygosity (Powell et al., 1996)
  - Environmental stability, reasonable costs, simplicity, rapidity (Schneider & Douches, 1997, Sosinski & Douches, 1996)

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## Numerous kits of markers have been published for potato

- **Varying in**
  - the type and number of genotypes tested
  - in the number of markers involved
    - Ghislain et al 2004 & 2009, Moisan-Thiéry, Marhadour et al 2005, Reid and Kerr 2007, Reid et al 2011, Karaagac et al 2014, Spanoghe et al, ...
  - In relation with their use: certification, management of genetic resources, ...
- **However, SSR can only be used as a complementary tool to usual phenotyping**
  - mutants can not be detected by SSR.

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## Aims of the talk

- **Give some information about**
  - the kit of markers we are routinely using in the scheme of certification
  - the database we set up
- **Show how these tools help to strengthen the seed potato production in France**



## A 5 markers' kit recently upgraded with 2 additional ones

- **2001: FN3PT proposed to implement SSR markers in the French certification scheme**
  - Research program in collaboration with Inra
- **2005: publication of the first kit (5 markers), 286 cultivars identified**
  - Moisan-Thiéry M., Marhadour S., et al (2005). Potato Research 48:191-200.
- **2013: Two markers added, 1193 cultivars identified (and 415 hybrids) and an interactive database set up**
  - Marhadour S., Dargier C., et al (2014) Innovations Agronomiques 35:161-172.

### Six French labs are using the same kit

- Around 1500 tests performed each year
- to control the *in vitro* collections managed by regional seed producers' organizations.
- to test
  - first steps of the multiplication procedure
  - complementary to the phenotypic controls made in the field

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### Composition of the upgraded kit

| Marker*   | Repeat motif          | Chromosome | # Alleles | Size range (pb) | Number of allelic phenotypes | PIC value |
|-----------|-----------------------|------------|-----------|-----------------|------------------------------|-----------|
| ➔ SSR1    | (TCAC) <sub>n</sub>   | VIII       | 18        | 200-230         | 110                          | 0,93      |
| ➔ stm2005 | (CTGTTG) <sub>n</sub> | XI         | 8         | 150-200         | 23                           | 0,80      |
| Lemalx    | (ATT) <sub>n</sub>    | V          | 5         | 120-140         | 17                           | 0,84      |
| stm1097   | (CGTTT) <sub>n</sub>  | VII**      | 9         | 230-280         | 28                           | 0,82      |
| stm2020   | (TAA) <sub>n</sub>    | I          | 12        | 160-200         | 95                           | 0,96      |
| stgbss    | (TCT) <sub>n</sub>    | VIII       | 13        | ~120            | 50                           | 0,88      |
| ➔ stm5136 | (AGA) <sub>n</sub>    | I          | 13        | 210-250         | 48                           | 0,92      |

➔ In common with Reid & Kerr 2007, Reid et al 2011

\* Kawchuk et al 1996, Milbourne et al 1998, Ghislain et al 2004, <http://www.ars.aphis.usda.gov/ARS/Handlers/Download.aspx?id=15577>  
 \*\*modified from Moisan-Thiery, Marhadour et al 2005

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## 7 ring tests have been organized for 10 years

| Year | # Labs involved | #samples to identify | Results                                       |
|------|-----------------|----------------------|---|
| 2003 | 2               | 30                   | 28/30*  |
| 2004 | 2               | 20                   | 20/20   |
| 2005 | 4               | 10                   | 10/10   |
| 2006 | 4               | 15                   | 15/15**                                       |
| 2007 | 4               | 5                    | 5/5   |
| 2010 | 6               | 8                    | 8/8 in 3 labs<br>7/8 in 1 lab<br>6/8 in 1 lab |
| 2013 | 7               | 8                    | 8/8   |

\* Two cultivars not registered  
\*\* in 3 labs

- Official Certification Body involved (SOC)
- 2010: first participation of Agroscope Changins, Switzerland (E. Droz)
  - Marhadour S., Droz E., et al (2011) EAPR 2011, Oulu, Finland.
- 2013: first participation of Centre & Sud Regional Organization
- No miss identification but
  - the need of a common database shared by users and updated regularly is reinforced



## Construction of an internet platform named IdeALE

- The need to exchange updated data between the 6 labs involved in control and certification
- FN3PT IT department constructed the platform
- Login and password needed, data encrypted, secured platform
- Profiles of 1193 cultivars and 415 hybrids entered
  - Cultivars multiplied in France
  - 350 varieties of the Inra worldwide collection described in Esnault et al (2013) included
- 30 markers described
  - 7 of the routinely used kit for seed certification
  - 23 used to describe the Inra Collection



Esnault F., et al (2013) Plant Genetic Resources : Characterization and Utilization 16:15.

## Can the markers of the kit be used in another system of revelation ?



- System in use: acrylamide followed by silver staining
  - Profiles contained in the database
- How can the number of analysis managed in our labs be increased ?
  - Tests of higher throughput systems: Licor and ABI
  - Constraint: to be able to use information of the profiles priority entered in the database as extensively as possible
- Previous positive experiences
  - on a limited number of markers/cultivars concerning ABI system (Reid, SASA)
  - More extensive on Licor system (FN3PT, Droz Agroscope)

## Analysis of transferability

- 184 cultivars
- 7 markers of the kit
- Comparison of profiles
  - Already available in the database (acrylamide + silver staining)
  - Obtained on Li-cor system by Inra team
  - Obtained on ABI system by Inra team

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## Transferability depends on the markers and on the systems

Acrylamide, silver staining  
Photo: N. Laverdin

'Rosine' profiles:  
1 3 9 11 on both gel-based systems  
224:216:200:196 on ABI PRISM®

ABI PRISM® analyzer  
Photo: F. Lemaire

LI-COR® analyzer  
Photo: N. Laverdin

- Transfer to Licor system is generally easier than on ABI system in the conditions we used
- Depends on the marker and sometimes on allele within marker

Marhadour et al (2013) EAPR Breeding section Hungary;  
Marhadour et al, in prep

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## Summary and prospects

- A molecular tool used to strengthen the certification scheme relatively to varietal identity
  - In complement to visual inspection
- A network of labs well trained to use a powerful kit of markers
- An important database coupled to an internet platform and allowing easier exchanges of information between labs
- Possibilities to transfer the system on other technological platform evaluated
- Prospects
  - To get additional experience about the kit developed by Reid and Kerr (2007) and others
  - To validate our kit using the ISO 13495 standard (in progress)

14 EAPR 2014 S. Marhadour



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- Eric Droz Agroscope Changins Switzerland (Scientific and profiles exchanges)

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