Potato variety identification using SSR in France and Switzerland

FÉDÉRATION NATIONALE DES PRODUCTEURS DE PLANTS DE POMME DE TERRE

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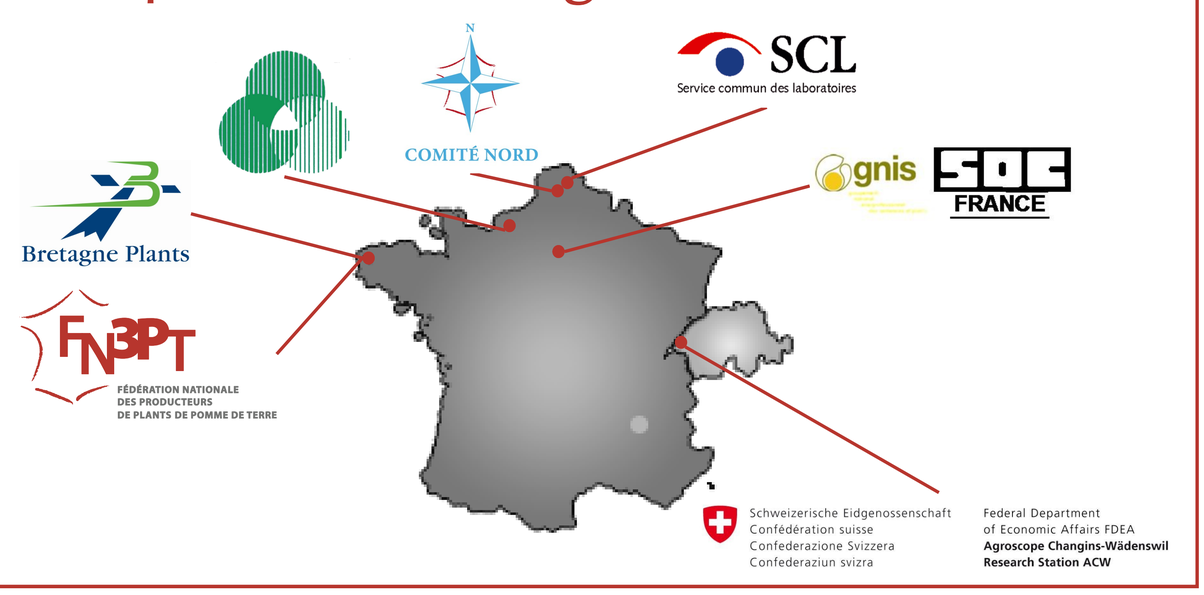
Context

Six years ago, we published a set of 5 SSR markers usable to identify 286 potato varieties (Moisan-Thiéry et al., 2005). The database has been increased and now more than 500 potato varieties have been genotyped.

In 2010 a ring test was organized by the GNIS/SOC and FN3PT for the French labs involved in the certification of seed potato, genebanks, *in vitro* multiplication and associated partner i.e. SCL (official control on ware potato and selling points). It included for the first time a laboratory from Switzerland (ACW) and an additional French site was also included increasing the total number of participants to six. Here we present the results of the ring test

Participants to the ring test



Markers used in the ringtest

	Chromosomal location	Number of alleles observed in our database	Source of the primers
SSR1	VIII	14	Kawchuk et al (1996) . Am.J. of Pot. Res. 73:325-335
STM2005	XI	7	Milbourne et al (1998) M.G.G. 259:233-245
STM1097	XII	9	Milbourne et al (1998) M.G.G. 259:233-245
Lemalx	V	5	Milbourne et al (1998) M.G.G. 259:233-245
STM2020	I	11	Milbourne et al (1998) M.G.G. 259:233-245
STM5136	I	11	https://research.cip.cgiar.org/IPD/
STGBSS	VIII	9	Ghislain et al (2004) . T.A.G. 108:881-890.
STM5140	IV	-	Bradshaw et al (2006) T.A.G. 113:943-951

Markers of the current procedure

Markers being implemented to the procedure

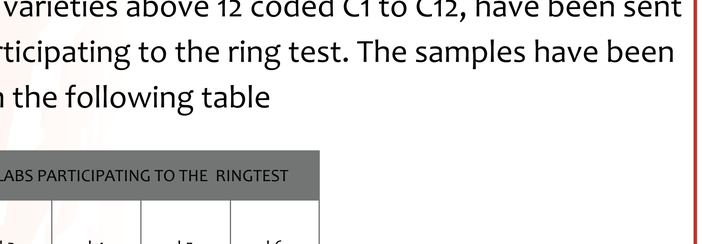
Markers SSR1, STM2005, STM1097 and STM2020 were used in the 6 labs. Lemalx was used in 5 labs. STGBSS was used in 1 lab only. 3 labs used STM5136 or STM5140 as a 6th marker to the standard procedure.
One lab used 13 additional markers (not shown).

Distribution of the samples to the labs

Samples, i.e. 5 tubers of 8 varieties above 12 coded C1 to C12, have been sent to the 6 labs (L1 to L6) participating to the ring test. The samples have been distributed as described in the following table

	IDENTIFICATION OF THE LABS PARTICIPATING TO THE RINGTEST					
CODES OF THE VARIETIES TO IDENTIFY	L1	L2	L3	L4	L5	L6
C8	C25301	C25370	C25324	C25330	C25333	C25341
C12	C25309	C25302	C25308	C25317		
C2	C25318	C25310			C25327	C25336
C6	C25325	C25319	C25313	C25304	C25312	C25331
C10	C25340		C25320		C25305	C25316
C1	C25347	C25335	C25328	C25321	C25315	C25306
C4	C25351	C25346	C25345	C25329	C25322	C25323
C7			C25348	C25344	C25337	C25334
C3	C25360	C25355	C25353	C25349		
C5		C25364		C25369	C25362	C25357

Preparation of the samples received by FN3PT participant for DNA extraction



Results of the ring test

None of the samples were misidentified
- 3 labs identified precisely the 8 samples received
- 1 lab identified 7 out of 8 samples received
- 1 lab identified 6 out of 8 samples received

		IDENTIFICATION OF THE LABS PARTICIPATING TO THE RINGTEST					
VARIETIES	CODES OF THE VARIETIES TO IDENTIFY	L1	L2	L3	L4	L5	L6
INNOVATOR	C8	INNOVATOR	INNOVATOR	INNOVATOR	INNOVATOR	INNOVATOR	INNOVATOR
BINTJE	C12	BINTJE	BINTJE	BINTJE	BINTJE		
ALLIANS	C2	ALLIANS	ALLIANS			?	?
GOURMANDINE	C6	GOURMANDINE	GOURMANDINE	GOURMANDINE	GOURMANDINE	GOURMANDINE	GOURMANDINE
FRANCELINE	C10	FRANCELINE		FRANCELINE		FRANCELINE	FRANCELINE
CHARLOTTE	C1	CHARLOTTE	CHARLOTTE	CHARLOTTE	CHARLOTTE	CHARLOTTE	CHARLOTTE
MANON	C4	MANON	MANON	MANON	MANON	MANON	?
SATURNA	C7			SANDY/ SATURNA	SATURNA	SATURNA	SATURNA
AMANDINE	C3	AMANDINE	AMANDINE	AMANDINE	AMANDINE		
CELTIANE	C5		CELTIANE		CELTIANE	CELTIANE	CELTIANE

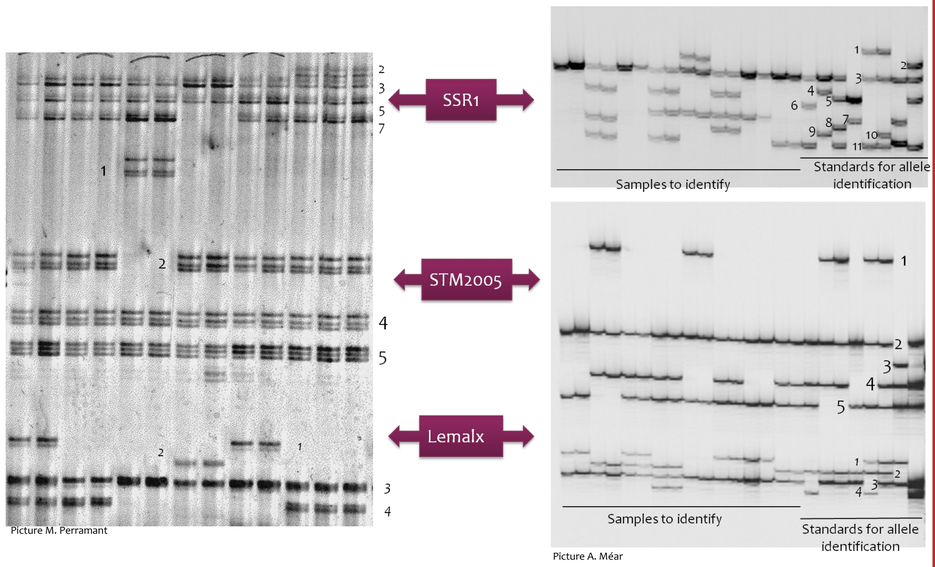
Lab 3 proposed 2 varieties for one sample as both varieties have the same profiles for the 5 markers commonly used

An additional allelic profile obtained using another marker is needed in this case

2 samples could not be identified in Lab 6
1 sample could not be identified in Lab 5

Absence of the reference profile in the labs

Two systems of revelation were used



Examples of polymorphism observed using the Silver staining system (left) and *LI-COR*® system (right) for SSR1, STM2005 and Lemalx markers. Alleles observed are indicated (samples amplified are not the same in both gels)

Picture M. Perramant

Picture A. Méar

Prospects

The lack of identification was due to the absence of the reference profiles in the database of the labs concerned. The problem of Sandy/Saturna emphasized the need to implement the procedure with additional markers.

These results also reinforce the need of a common database shared by the users and updated regularly. The development of an informatics tool is in progress in France in a project partly funded by the French Ministry of Agriculture and led by the FN3PT. In addition, the set of markers is currently updated and we plan to make the experiments necessary to fit the French standard AFNOR V03-45 (2009) which international extension is currently under discussion.

References

Moisan-Thiéry, M., S. Marhadour, M.C. Kerlan, N. Dessenne, M. Perramant, T. Gokelaere, and Y. Le Hingrat. 2005. Potato cultivar identification using simple sequence repeat markers (SSR). Potato Research 48:191-200.

NF V03-045, AFNOR, mars 2009. "Principes de sélection et critères de validation des méthodes d'identification variétale par analyses d'acides nucléiques spécifiques", Standard/Norme AFNOR. 27p.

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