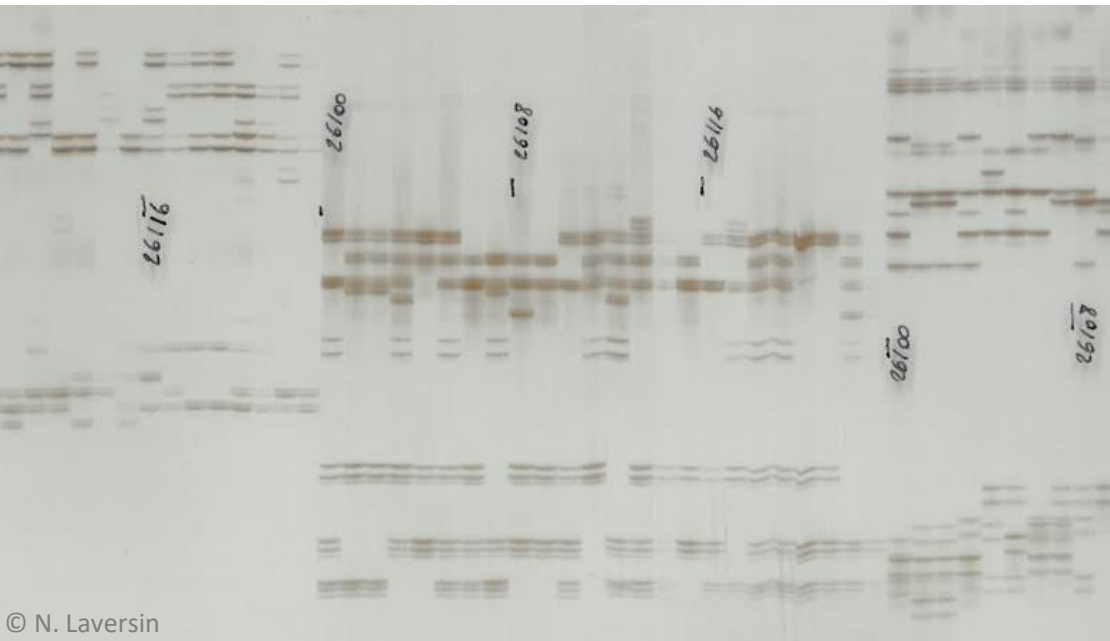




**inov3PT**  
SEED POTATO  
FOR THE FUTURE

## Genetic fingerprints using SSR markers to secure seed potato production in France: method and database improvement.

Marhadour, S., Méar, A., Dargier, C., Laversin, N., Le Bechenec, S., Madruga, F., Passeret, C., Bronsard, G., Wambre, V., Droz, E., Esnault, F., Lesage, M.L., Le Hingrat, Y.



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EAPR 2020 Krakow 04/07/2022

RECHERCHE – DEVELOPPEMENT – INNOVATION DES PRODUCTEURS DE PLANTS DE POMME DE TERRE

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## Outline of the talk

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- How we are organized in France to check varietal identity of seed potatoes using molecular markers
  
- New project to make the electrophoresis step of the procedure evolve
  - Preliminary results of an ongoing project named IdEvol

Avec  
la contribution  
financière du compte  
d'affectation spéciale  
développement  
agricole et rural  
CASDAR



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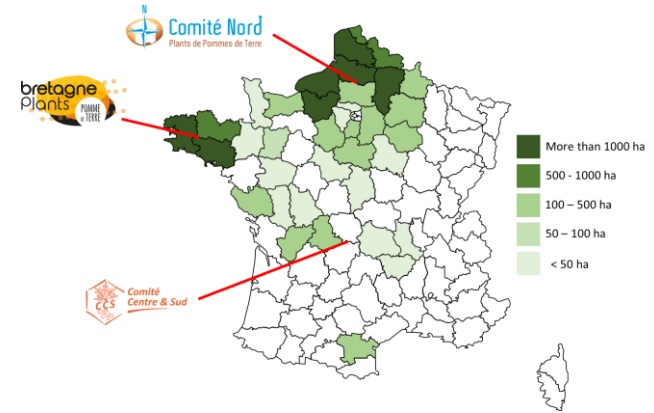
# Varietal identification using SSR

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*Our organisation in France for the seed potato  
sector*

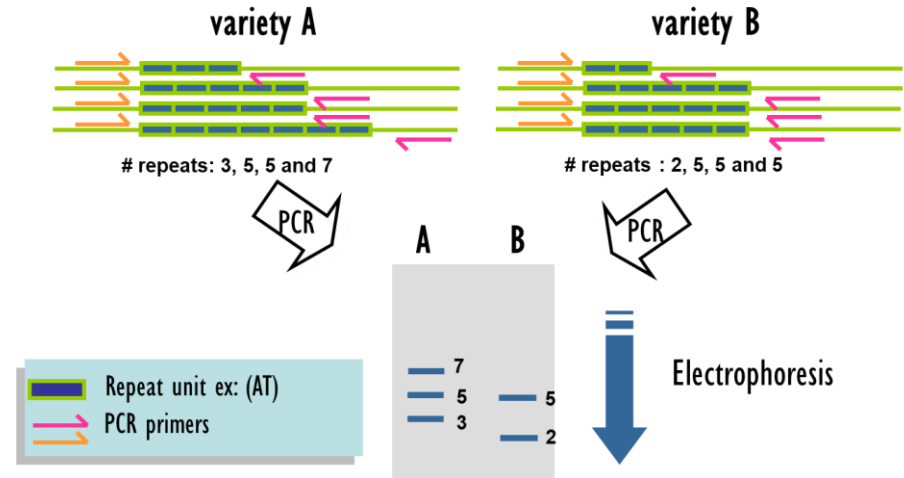
## Organization of the seed potato sector in France

- France ranked 2<sup>nd</sup> for seed potato exportation
  - 650 000 t of certified seed potatoes produced in 2021
  - 24 300 ha of production (2021)
  - 850 growers/ structures of production
  - 460 varieties multiplied each year
  
- Seed inspection and certification
  - health quality and
  - varietal identity and purity
  - along the multiplication process



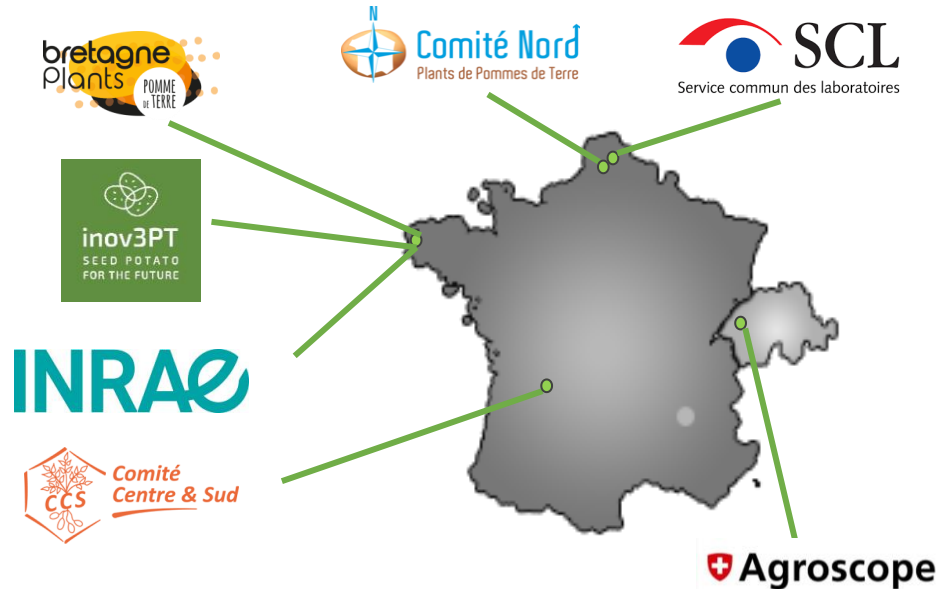
## SSR\* markers : still a tool of choice to profile potato varieties

- Huge variability of SSR polymorphism in potato varieties
  - Only few markers needed to draw specific molecular fingerprints
  - Balance their moderate throughput
- Portability over a large diversity of potato panels
  - A lot of markers available
- Technical advantages
  - Stability, reproducibility of the profiles
  - ~ simple to set up



\*Single sequence repeat

## Six French Partners are using the same procedure and kit of markers



- ~3000 tests (↑) performed each year
- **80% by the seed potato sector** (Bretagne Plants, Comité Nord and Comité Centre et Sud) to control
  - 1230 official tests on seed potatoes (combined with field inspection)
    - *in vitro* collections (starting material)
    - *ad hoc* tests in case of doubt
- 20% :
  - Official tests on ware potatoes (SCL)
  - Management of BRC BrACySol collections (INRAE)
- Partnership with Agroscope Changins

## Features of the current kit



Marker*	Repeat motif	Chr	Size range (pb)	# Alleles	#allelic phenotypes	#data	PIC value
➔ SSR1	(TCAC) <sub>n</sub>	8	200-230	17	177	2307	0,93
➔ STM2005	(CTGTTG) <sub>n</sub>	11	150-200	8	29	2306	0,81
LEMALX	(ATT) <sub>n</sub>	5	120-140	5	16	1957	0,83
STM1097	(CGTTT) <sub>n</sub>	7**	230-280	9	42	2300	0,83
STM2020	(TAA) <sub>n</sub>	1	160-200	12	132	2240	0,96
STGBSS	(TCT) <sub>n</sub>	8	~120	13	86	1881	0,84
➔ STM5136	(AGA) <sub>n</sub>	1	210-250	13	79	2074	0,92
STM5140	(AAT) <sub>n</sub>	4	180-219	6	42	1663	0,91

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

\* Kawchuk et al 1996, Milbourne et al 1998, Ghislain et al 2004,

<https://research.cip.cgiar.org>

\*\*modified from Moisan-Thiery, Marhadour et al 2005; Marhadour et al 2017

## IdeAle: our common database to manage the profiles

- Built during a previous project financed by the French Ministry of Agriculture\*
- Secured internet platform
  - authenticated access
  - updated in real time for every partner
- Content:
  - 4105 molecular profiles
  - 2200 specific varieties' profiles + 900 hybrids' profiles
  - Updated in 2020 with 310 profiles from Agroscope Changins
- Different electrophoresis systems



\*Marhadour S, et al (2014) Innovations agronomiques 35:161-172



## Ring tests are regularly organized

- 9<sup>th</sup> ring test organised in 2019 under the aegis of SOC\*
  - 6 French partners + Agroscope Changins (CH)
  - 8 unknown samples to identify using profiles and database
- 100% identification in 100% of the labs
  - Power of the kit confirmed
  - Content of the database validated
  - Qualifications of the partners confirmed



Essai interlaboratoires pour l'identification de variétés de Pomme de Terre EIL IV PDT2019

Variétés prélevées		Codes laboratoires et variétés identifiées					
		L1	L2	L3	L4	L5	L6
FONTANE	V 1	FONTANE	FONTANE	FONTANE	FONTANE	FONTANE	FONTANE
AMYLEA	V 2	AMYLEA	AMYLEA	AMYLEA	AMYLEA	AMYLEA	AMYLEA
LUTINE	V 3	LUTINE	LUTINE	LUTINE	LUTINE	LUTINE	LUTINE
AGATA	V 4	AGATA	AGATA	AGATA	AGATA		AGATA
CHEYENNE	V 5	CHEYENNE	CHEYENNE	CHEYENNE		CHEYENNE	CHEYENNE
SPUNTA	V 6		SPUNTA	SPUNTA	SPUNTA	SPUNTA	
INSTITUT de BEAUVAIS	V 7	INSTITUT de BEAUVAIS		INSTITUT de BEAUVAIS	INSTITUT de BEAUVAIS	INSTITUT de BEAUVAIS	INSTITUT de BEAUVAIS
TENTATION	V 8		TENTATION	TENTATION	TENTATION		TENTATION
CHARLOTTE	V 9	CHARLOTTE		CHARLOTTE	CHARLOTTE	CHARLOTTE	CHARLOTTE
DALIDA	V 10	DALIDA ?	DALIDA			DALIDA	

\*SOC: official body for seed certification in France (SEMAE)



# Evolution of our process

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*Make the wet lab work easier*

## A new project to change the electrophoresis step

- The electrophoresis step is long, sensitive and requires the use of toxic reagents
  - Need to use an automated approach but
    - *Maintain the use of our markers and data!*
    - *Use an affordable equipment (medium size labs)*
  
- Since 2019, a new project (IdEvol) set up with the financial support of the French Ministry of Agriculture
  - AMS-IdEvol for « Technological evolution to identify potato varieties using microsatellites markers in support of the seed certification process ».
  - Analysis of the transferability of the kit on the sequencing platform Qiaxcel Advanced
  - 2022: last year of the project



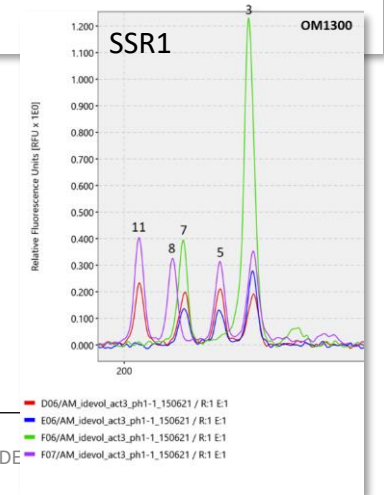
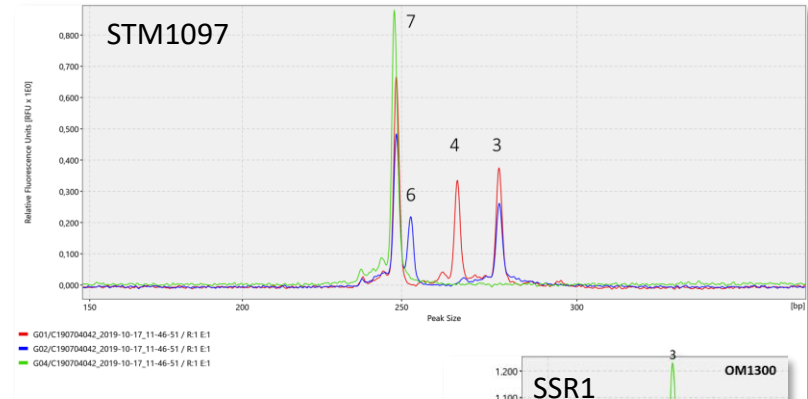
Avec la contribution financière du compte d'affectation spéciale développement agricole et rural CASDAR



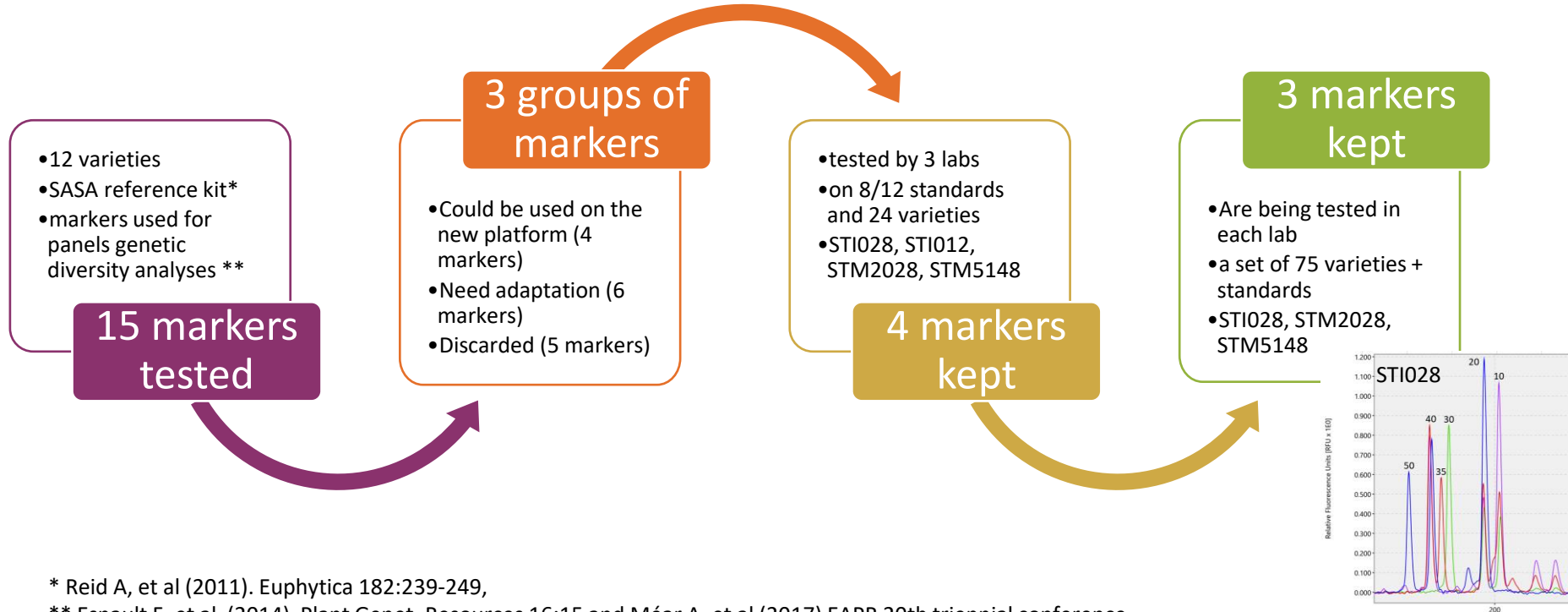
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## Some main results of the IdEvol project

- Adaptation of the PCR conditions to
  - standardize the profile intensities and limit the dimers to make automatic scoring easier
  
- 8 markers tested on 75 varieties on the new platform in each of the labs
  - 5 markers can be used on the new platform
  - 3 markers can not for the moment
    - *insufficient resolution*
    - *not denaturing electrophoresis ?*



## Other results of the IdEvol project: Update of the kit to replace markers not suitable to the new system



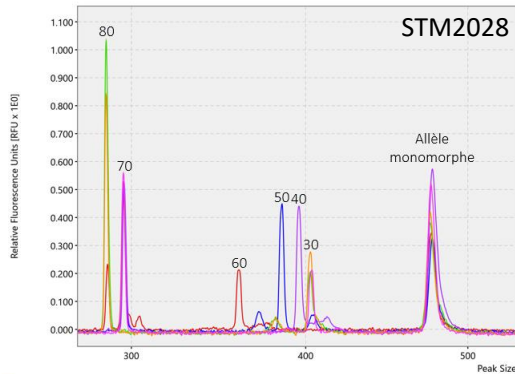
\* Reid A, et al (2011). Euphytica 182:239-249,

\*\* Esnault F, et al (2014) Plant Genet. Resources 16:15 and Méar A, et al (2017) EAPR 20th triennial conference

## Take home messages



- A network of 6 partners using the same kit, procedure and database
  - Secure the seed certification process in addition to BRC BrAcySol management and *ad hoc* + official tests on ware potatoes
- A robust and efficient method based on 8 markers and a significant database:
  - 9 ring tests since 2004
- Evolution of the electrophoresis step in our procedure to make lab work easier and more rapid
  - A new instrument was selected
    - *most of the markers can be transferred provided some adaptations*
    - *new markers identified to be implemented in our new procedure*



■ A08/C210309031\_2021-10-12\_STM2028 / R1 E1  
 ■ A11/C210309031\_2021-10-12\_STM2028 / R1 E1  
 ■ B05/C210309031\_2021-10-12\_STM2028 / R1 E1  
 ■ B09/C210309031\_2021-10-12\_STM2028 / R1 E1  
 ■ C05/C210309031\_2021-10-12\_STM2028 / R1 E1  
 ■ C10/C210309031\_2021-10-12\_STM2028 / R1 E1

Thank you for your  
attention

