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SRAP polymorphisms associated with Phytophthora Root Rot (PRR) tolerance in alfalfa

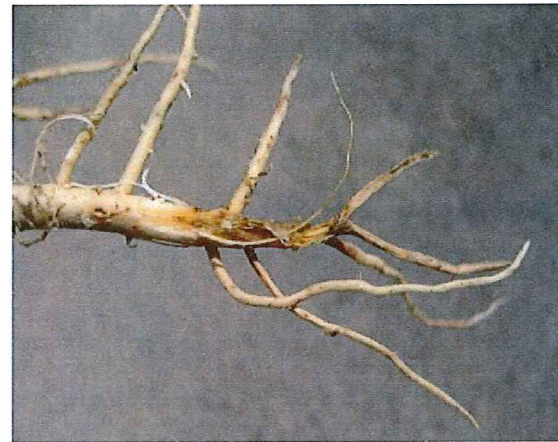
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Phytophthora root rot (PRR)

- *Phytophthora medicaginis*
- Survive in organic debris and become active in wet soil.
- Young seedlings particularly vulnerable
- Can cause significant losses (70%)
- Affect regain vigor after cuts (yield)
- Similar symptoms: Aphanomyces root rot (*Aphanomyces euteiches*)



Objectives

- **Develop PRR-tolerant alfalfa populations by successive cycles of recurrent selection**
- **Identify DNA polymorphisms associated to PRR-tolerance in improved Apica and Caribou populations developed by recurrent selection.**
 - **Bulk segregant analysis (BSA) of PRR-tolerant genotypes**
 - **SRAP (sequence related amplified polymorphism) technique**

Recurrent selection for the identification of DNA polymorphisms

- **Requires long-term investment for population development**
- **Takes advantage of extensive genetic diversity in allogamous species**
- **Exploits gene diversity among populations derived from an initial genetic background**
- **Allows selective enrichment of low frequency alleles**
- **Generates gene interactions (epistatic effects) with adaptive value**

Recurrent selection

- Plant material
 - Two alfalfa populations derived from the PRR-sensitive Apica (A) and PRR-moderately resistant Caribou (C).
 - These two populations underwent three cycles of recurrent selection for superior tolerance to freezing*.
 - ATF3 (S) and CTF3 (MR)

% resistant plants	Classe	Abbreviation
< 6	Susceptible	S
6-15	Low resistance	LR
16-30	Moderately resistant	MR
31-50	Resistant	R
> 50	Highly resistant	HR

*Castonguay et al, 2009

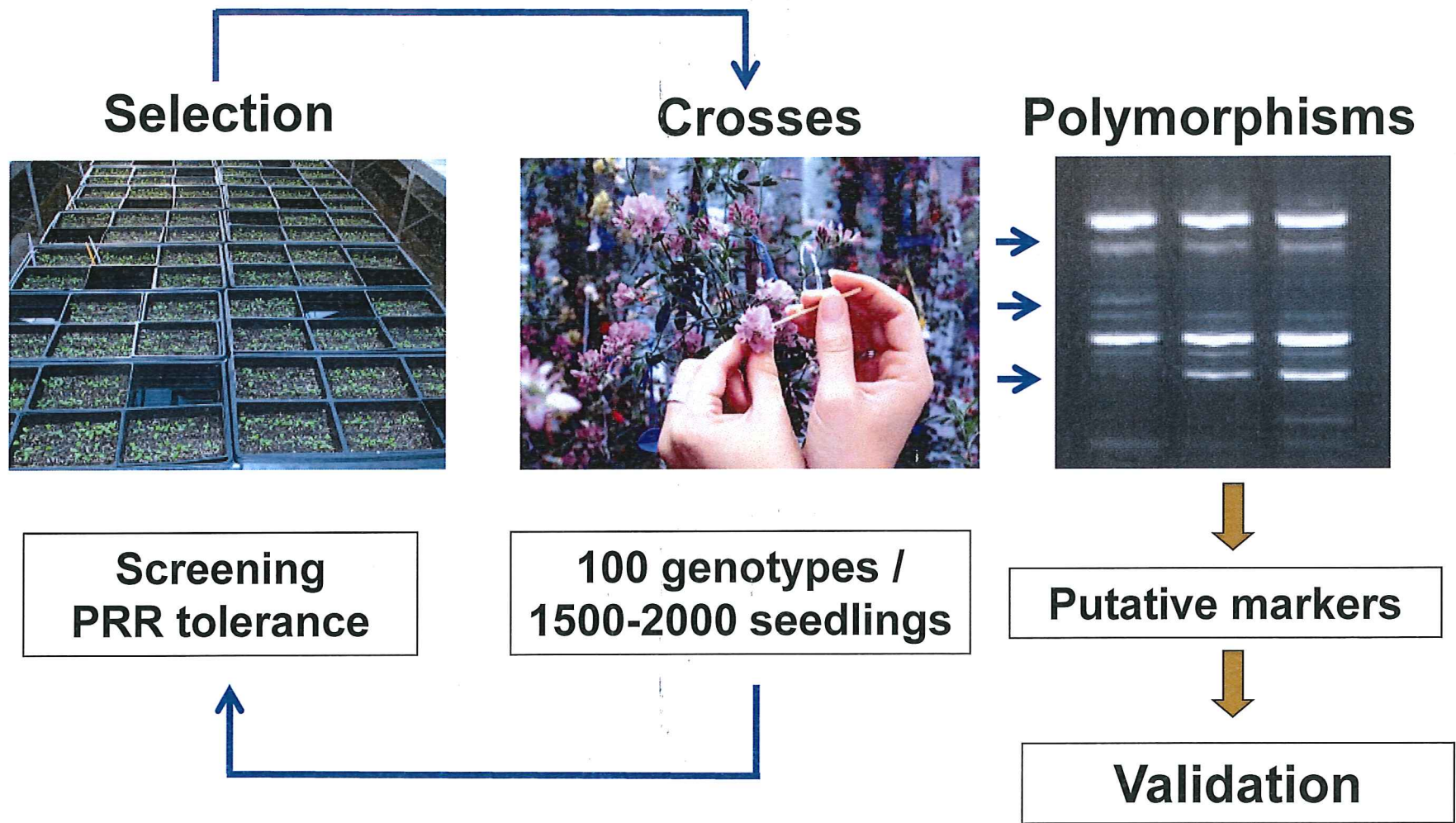
PRR-tolerance screening

- **1500 to 2000 seeds**
 - **Inoculum: A blend of 4 PRR isolates**
 - **One-week old culture on nutritive media**
 - **Spores harvested and pooled**
 - **> 1000 spores per ml**
 - **Inoculation on 10-day old seedlings (20-24°C)**
 - **Flooding condition during 3 days**
 - **Control: Saranac (S) and Amerigraze (HR)**
 - **Plantlet selection: 2 weeks post inoculation**
 - **Most vigorous plantlets (100) were kept for markers assessment and crossed for the next cycle of the recurrent selection.**

Selection of PRR-tolerant phenotypes



Development of PRR-tolerant populations by recurrent selection



Selection of PRR-tolerant phenotypes

- **Initial populations: ATF3 (Apica) and CTF3 (Caribou)**
- **Six populations (3 cycles of recurrent selection)**
 - **ATF3-PRR1, ATF3-PRR2, ATF3-PRR3**
 - **CTF3-PRR1, CTF3-PRR2, CTF3-PRR3**
- **Three more populations (1 cycle of selection)**
 - **Brador (R), Maska (R), Melodie (MR)**

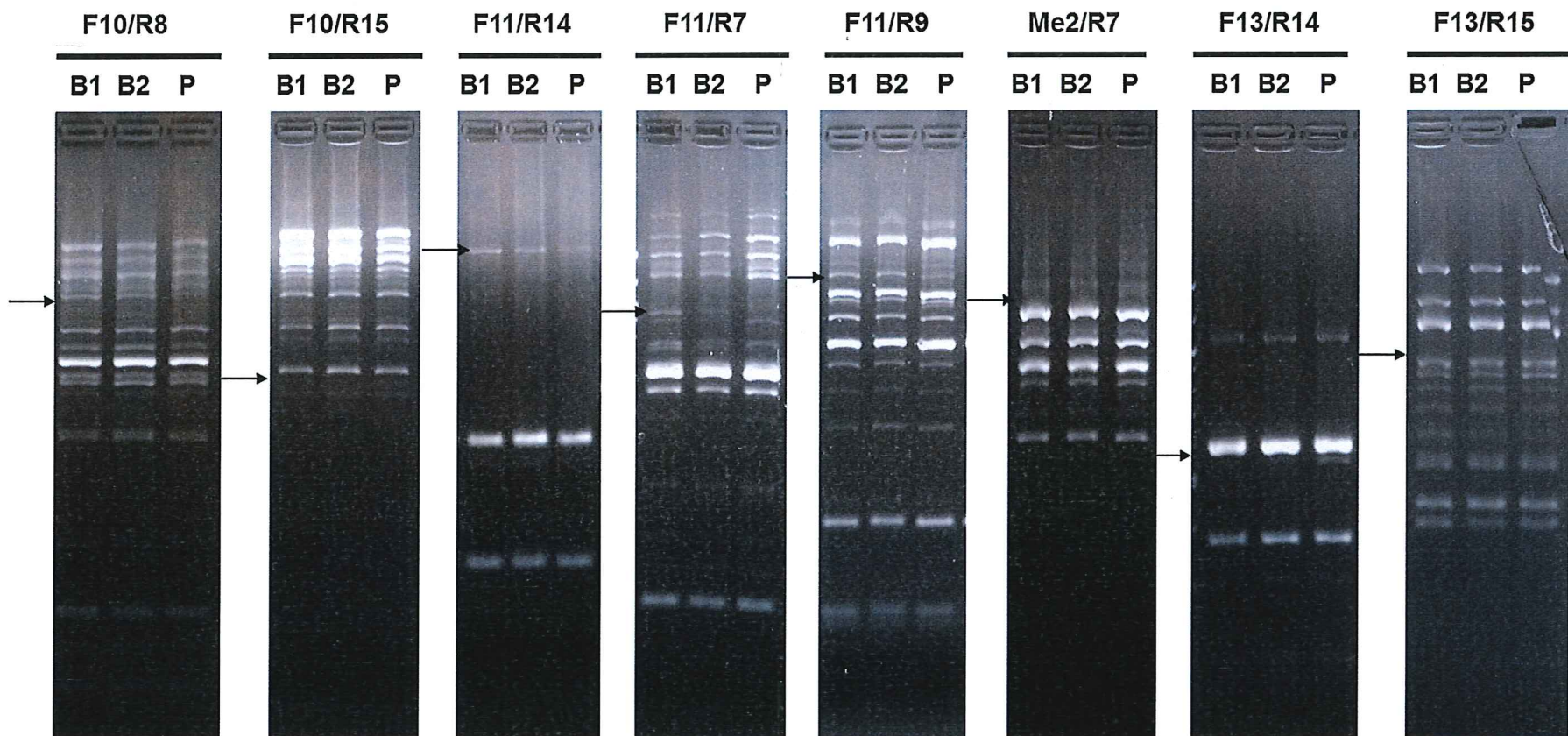
Bulk segregant analyses

- **Random pools of genotypes (about 50) with contrasted phenotypic traits**
- **Identification of polymorphic DNA fragments between pooled genotypes from the original and PRR-improved populations**
- **These polymorphisms between pools are potential indicator of the frequency of genotypes linked to PRR-tolerance**
- **SRAP (sequenced-related amplified polymorphisms)**
 - **Preferentially amplified coding regions**
 - **270 primer-pair combinations (18 X 15)**

SRAP analyses – first cycle of selection

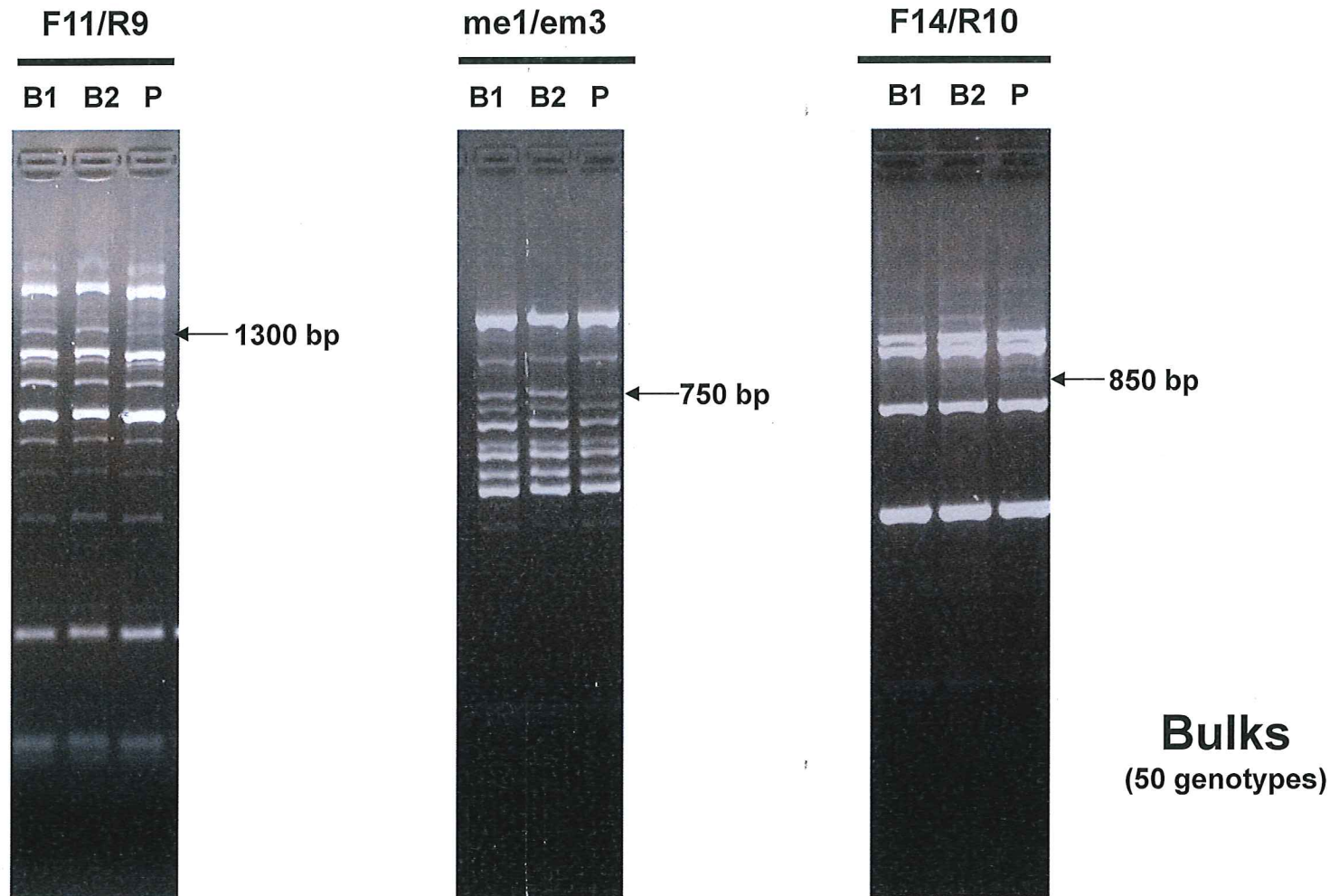
Caribou PRR1+

Apica PRR1+



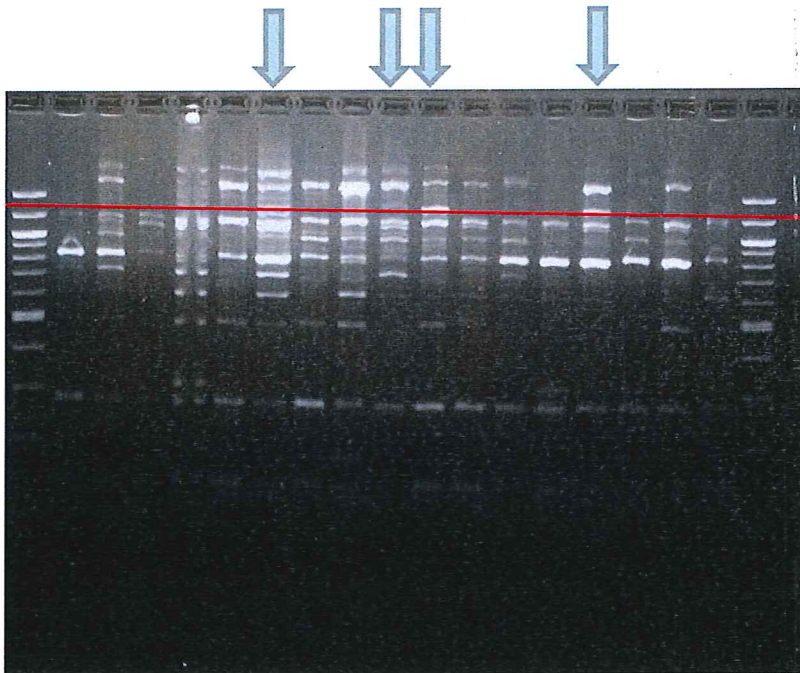
- Several polymorphic DNA fragments following the recurrent selection
- Some polymorphisms are positively-linked, other negatively.

SRAP identification of potential PRR-related amplicons in Apica

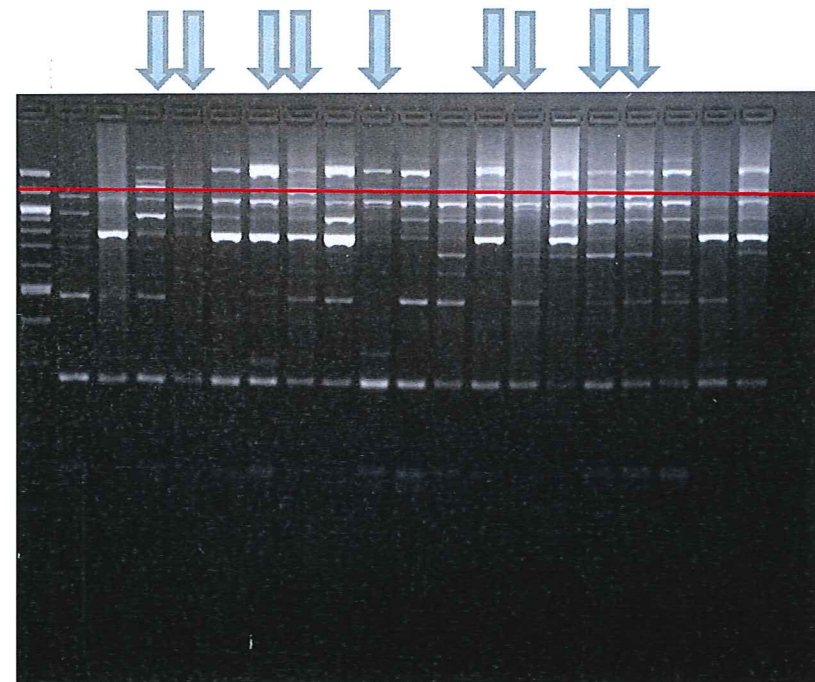


F11-R9 fragment frequency in Apica-derived populations

ATF3-PRR1 individual genotypes



ATF3-PRR3 individual genotypes



- The frequency of the polymorphic F11-R9 fragment increased with recurrent selection for PRR-tolerance

F14-R10 fragment frequency in Apica-derived populations

ATF3 individual genotypes



ATF3-PRR1 individual genotypes



ATF3-PRR3 individual genotypes



- The frequency of the polymorphic F14-R10 fragment decreased with recurrent selection for PRR-tolerance

Potential SRAP markers for PRR-tolerance

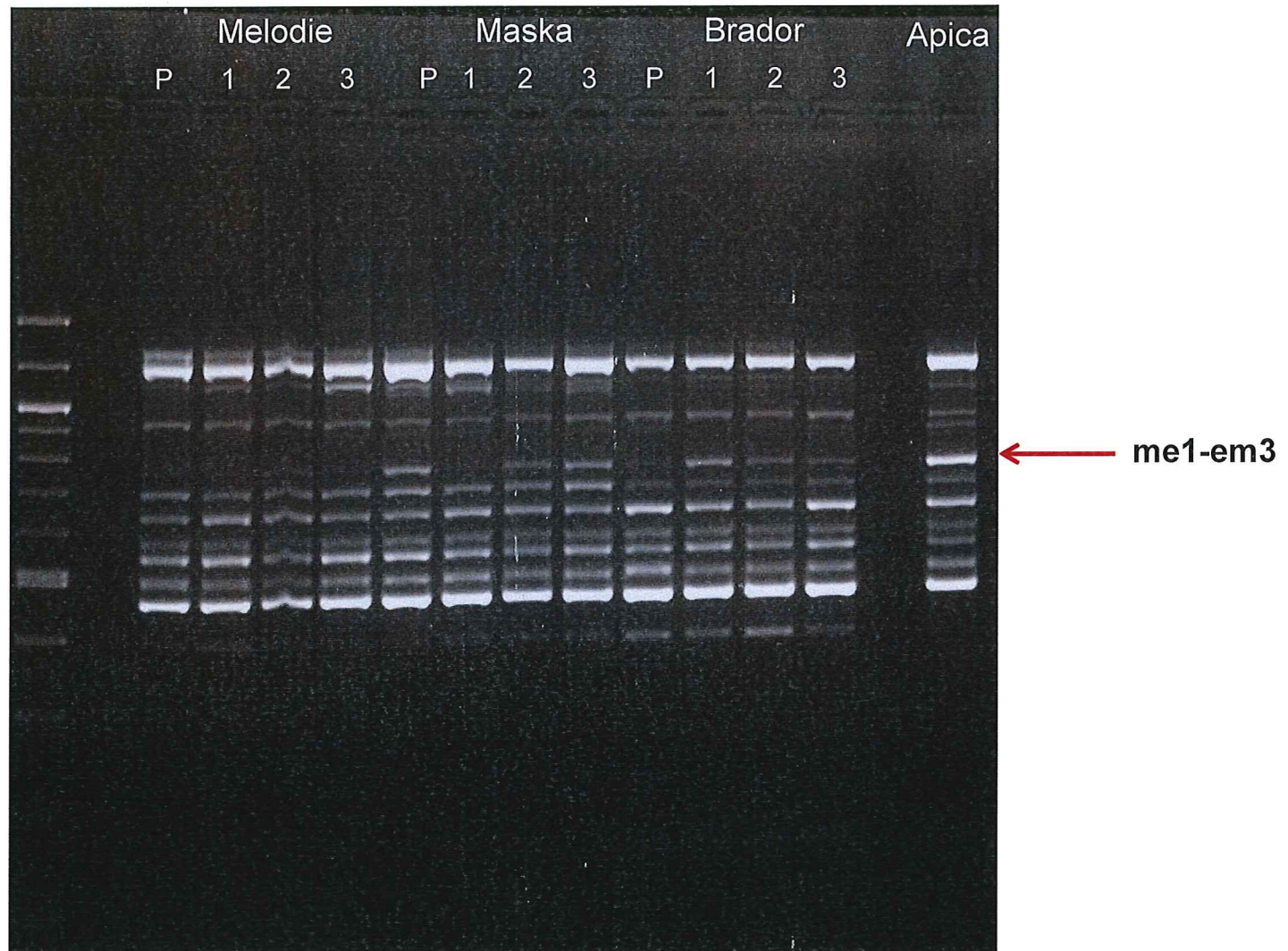
APICA	SRAP primer pair			PRR-tolerant
	me1-em3 %	F14-R10 %	F11-R9 %	14 days p.i 200 genotypes %
ATF3 50 genotypes	20.0	74.0	16.0	10.6
ATF3-PRR1 114 genotypes	26.8	60.6	30.0	22.5
ATF3-PRR3 115 genotypes	38.3	53.9	53.4	54.0

SRAP me1-em3 DNA fragment in Apica and Caribou

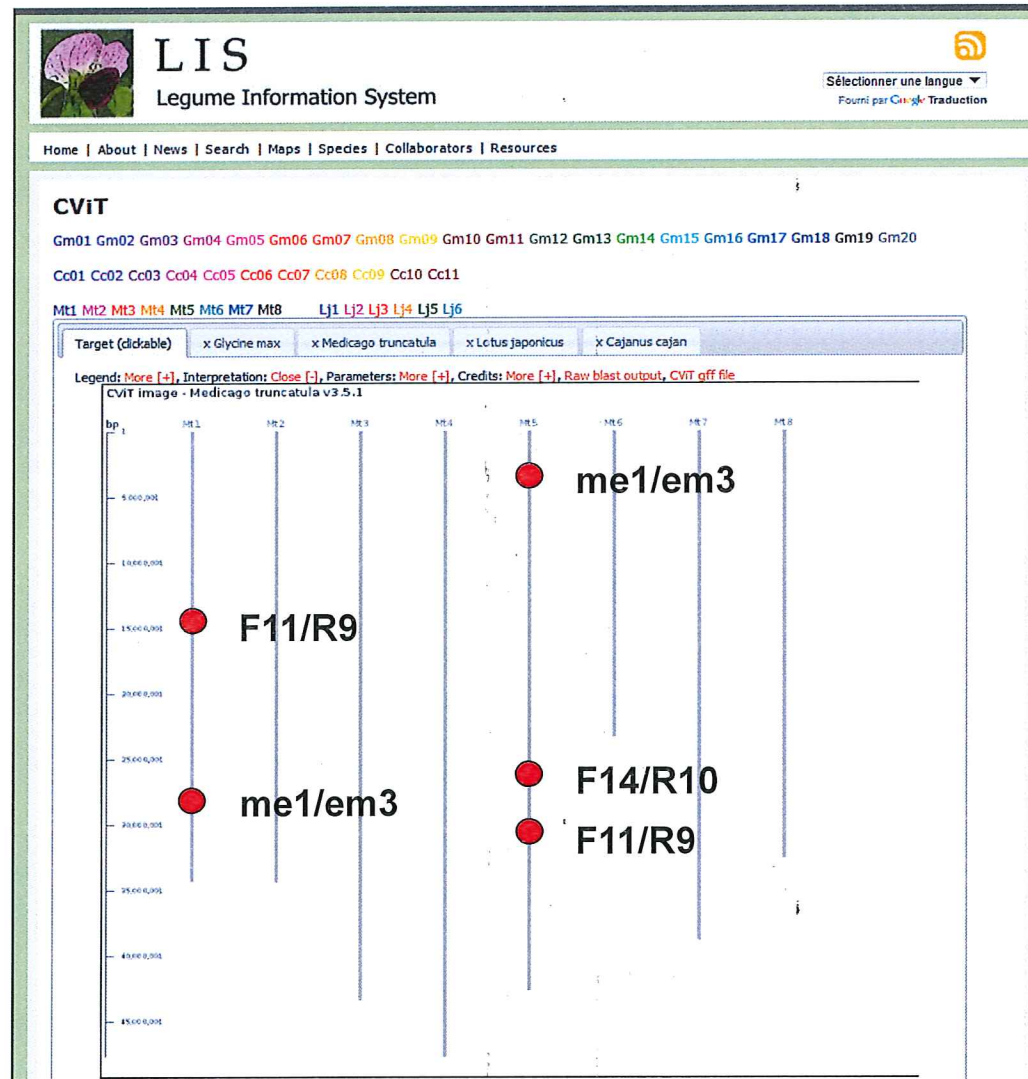


Caribou	me1-em3 %	Apica
CTF3 40 genotypes	7.5	(20.0)
CTF3-PRR1 85 genotypes	8.2	(26.8)
CTF3-PRR3 99 genotypes	22.2	(38.3)

SRAP me1-em3 DNA fragment in Brador, Maska and Melodie



Localisation of SRAP amplicons on *M. truncatula*



Conclusion

- **SRAP polymorphic fragments linked to PRR tolerance were identified using bulk segregant analyses on alfalfa populations developed by recurrent selection.**
- **The F11-R9 and me1-em3 amplicon frequency increased in advanced PRR-tolerant Apica-derived populations while the frequency of the F14-R10 amplicon decreased.**
- **These three polymorphic fragments were also found in other alfalfa genetic background; Caribou, Brador, Maska and Melodie.**
- **Search for DNA polymorphisms between recurrent selections is a powerful marker discovery strategy.**
- **Validation of these markers are in progress (TRAP, amplicon sequence analyses)**

Research team

Scientists

Patrice Audy	Molecular plant pathology
Yves Castonguay	Molecular physiology
Annie Claessens	Plant breeding

Research assistants

Réjean Desgagnés	Molecular biology
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Tommy Gerez	Molecular biology