Genetic and Molecular Characterization of Host-Plant Resistance to Root-knot Nematodes and Fusarium Wilt in Cotton

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Project Objectives

1. Determine the genetic basis of resistance to root-knot nematode (RKN) and Fusarium wilt (FW); uniqueness of NemX v. other R genes
2. Develop linked molecular markers to RKN and FW resistance genes;
3. Localize RKN *R* genes to chromosome genome of cotton;
4. Develop a high resolution linkage map around the *R* genes.

The Phenotype of Parents for Reaction to RKN and to FOV

Genotype	*M. incognita* (RKN)*	*F.o. vasinfectum* (FOV)**	RKN +FOV*
SJ-2	S	S	S
NemX	**R**	S	R
Pima S-7	S	**R**	MR

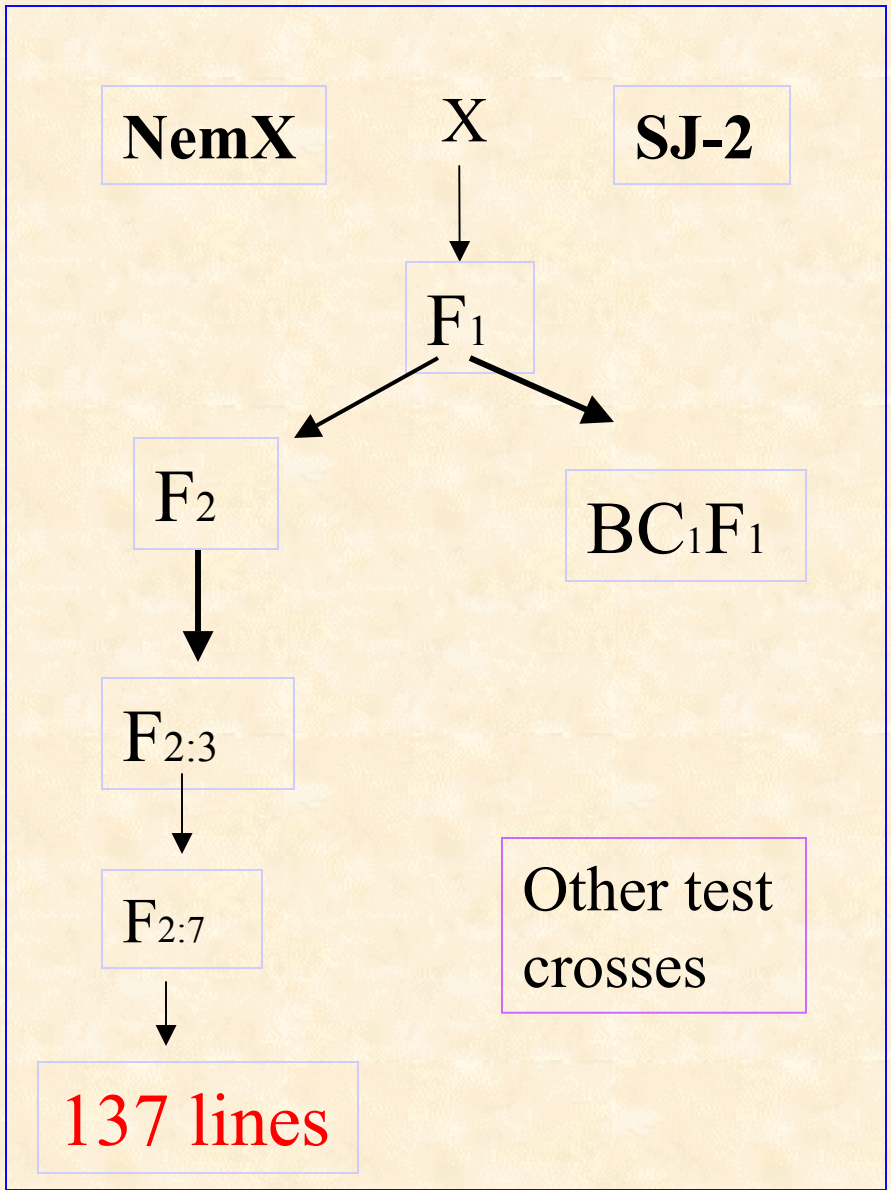
*I. Seedling inoculation method

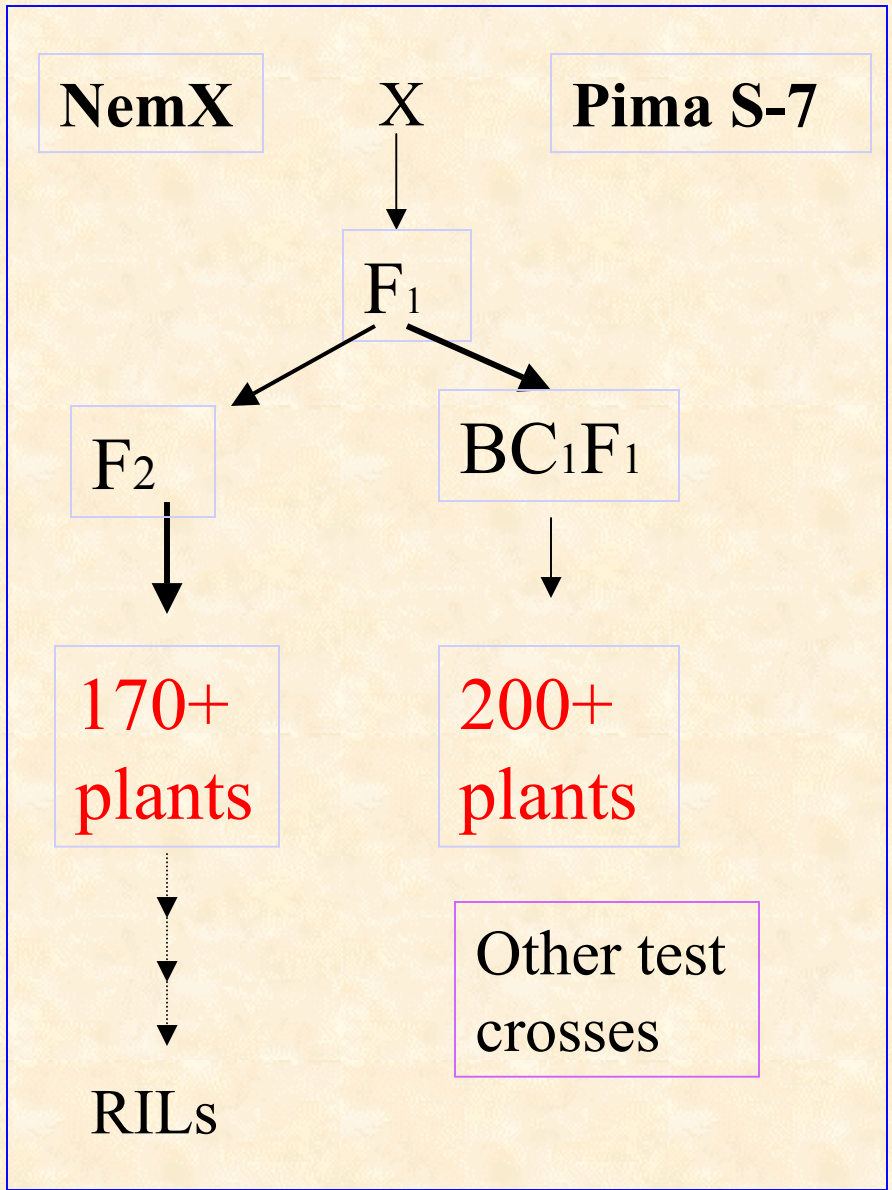
**II. Cut-root-dip method

SJ-2 ← Root-knot Nematode → Pima S-7

NemX

Plant Populations





F_2 and BCF_1 plants

F_2

BCF_1

NemX x Pima S-7

The Phenotype of Parents and F1 for Reaction to *M. incognita*

Genotype	Gall rating	Total eggs (in 1000s)	Eggs/g root
SJ-2	5.58a	259.4a	4,129a
NemX	1.17b	14.8c	291b
N901	1.35b	8.0c	175b
F1			
SJ-2 x NemX	5.37a	186.9ab	2,295a
Sj-2 x N901	5.06a	147.4b	2,487a

Summary of Segregation Data for *Meloidogyne incognita* Resistance in Cotton Cultivar Acala NemX

Parent or generation	Total plants or families	Observed R: I: S ratio	Expected R: I: S ratio	χ^2	*P* value
SJ-2	10	0 : 0:10	All S	0	>0.995
NemX	10	10 : 0: 0	All R	0	>0.995
F_1	10	0 : 0: 10	All S	0	>0.995
BC_1	346	91 : 172: 83	86.5 : 173: 86.5 (1:2:1)	0.382	0.75-0.90
		Observed R: Seg: S ratio	**Expected R: Seg: S ratio**		
$F_{2:3}$	84 (families)	7 : 45: 32	5.3 : 42: 36.8	1.386	0.50-0.75
$F_{2:3}$	132 (families)	9 : 71: 52	8.3 : 66: 57.7	1.001	0.50-0.75

R - Resistant; S - Susceptible; I – Intermediate ; Seg – Segregating.

Multiplexed AFLP Protocol

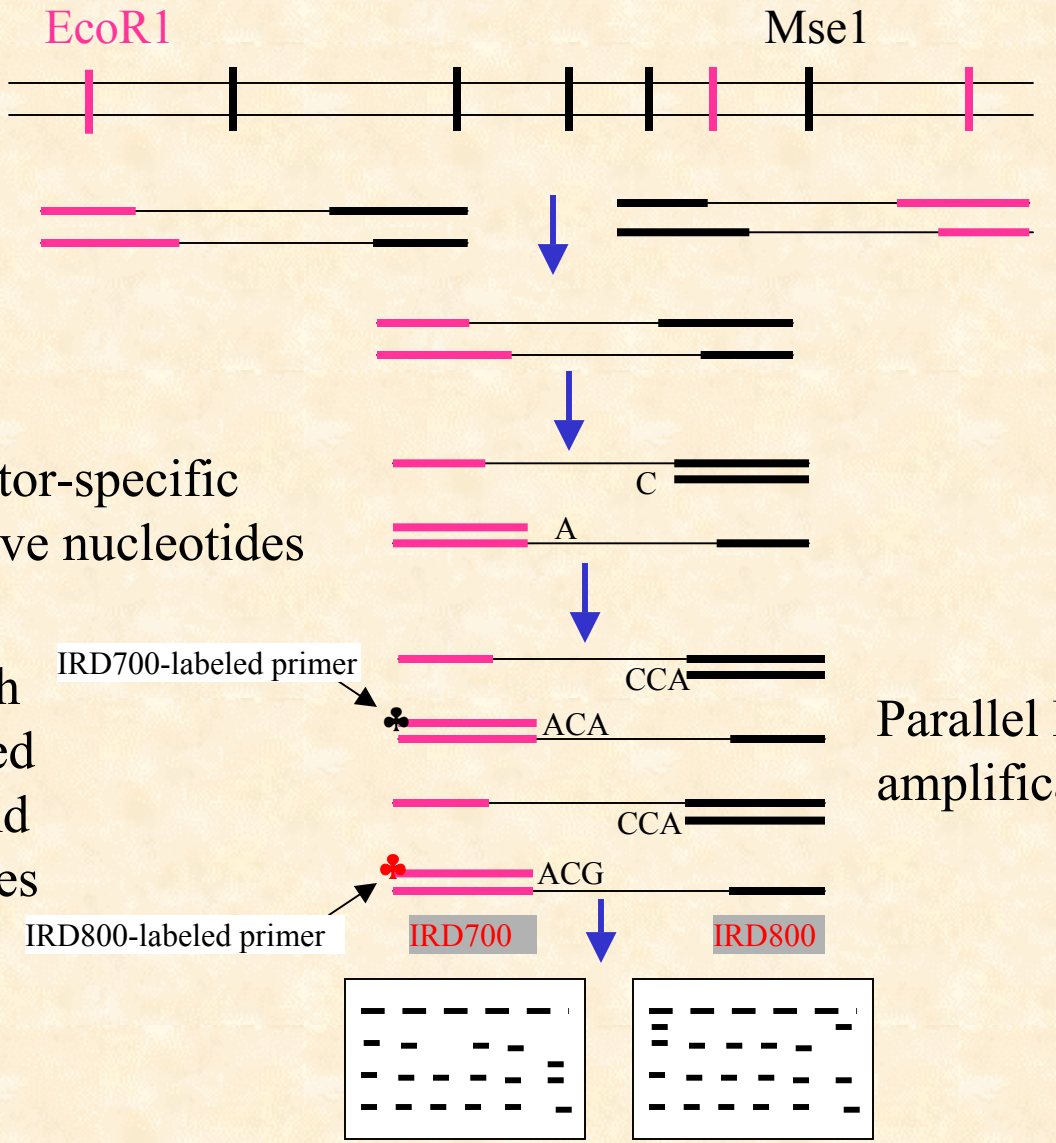
Genomic DNA

Restriction digestion and ligation of adaptors to the fragment ends

Preamplification with adaptor-specific primers and (+1/+1) selective nucleotides

Selective amplification with IRD800 and IRD700 labeled adaptor-specific primers and (+3/+3) selective nucleotides

Gel electrophoresis



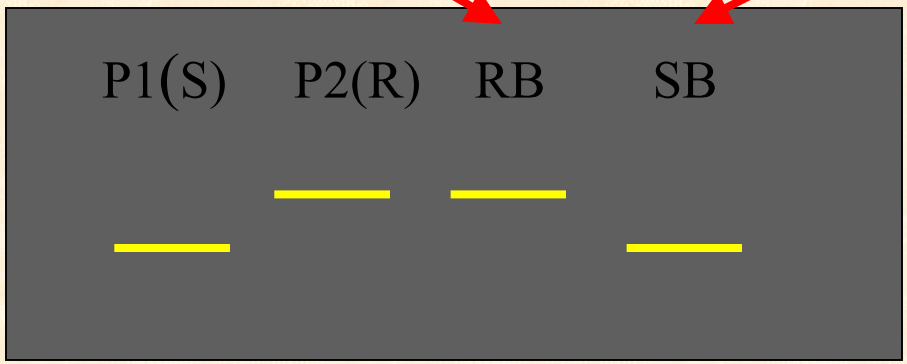
Parallel PCR amplification

Bulked Segregant Analysis (BSA)

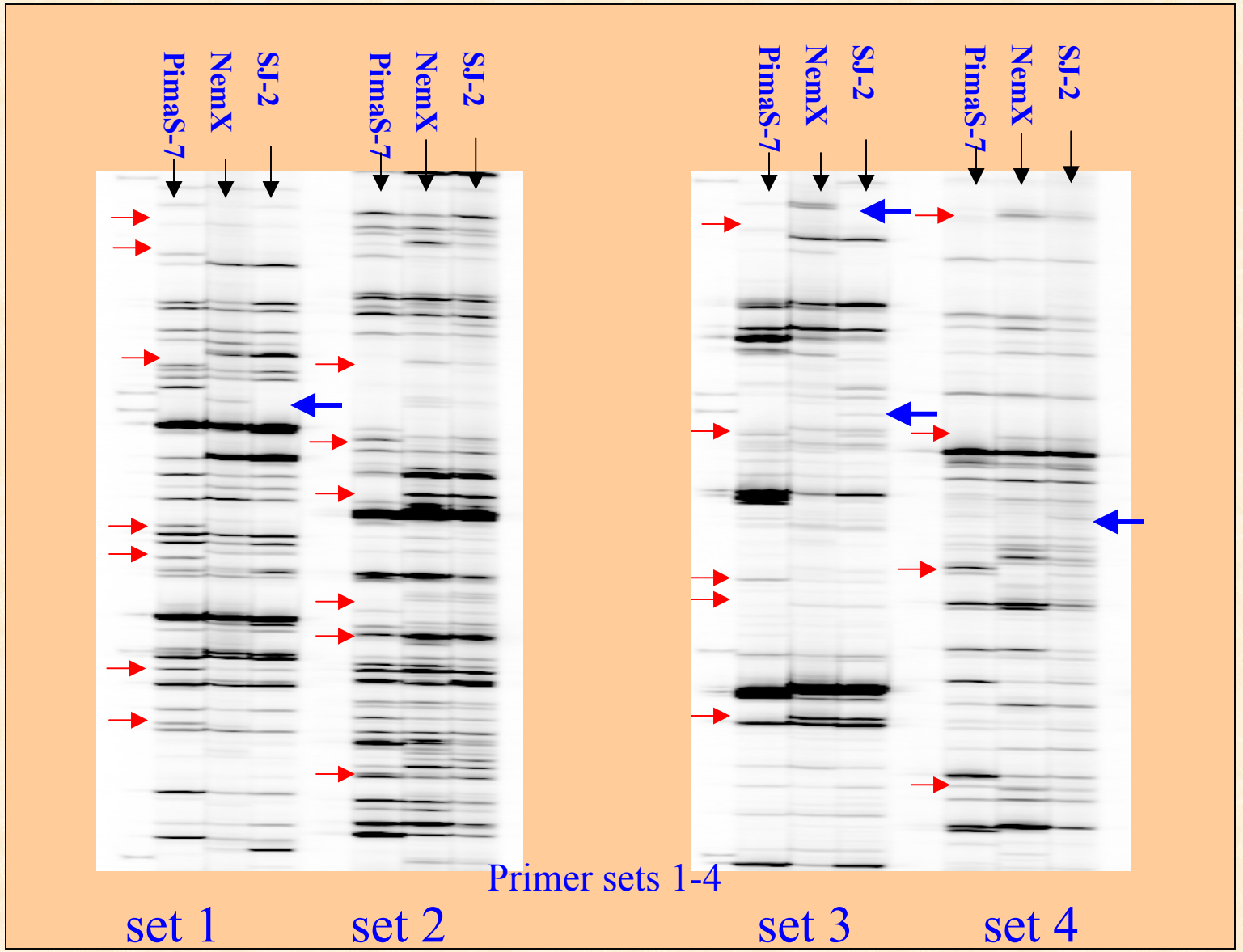
- BSA is used to make resistant plant DNA pools and susceptible DNA pools to obtain markers that correlate with each resistance trait.
- BSA has proved to be a powerful technique to identify markers linked to resistance genes.

Individual homozygous F_2 or $F_{2:3}$ resistant plants (7-10)

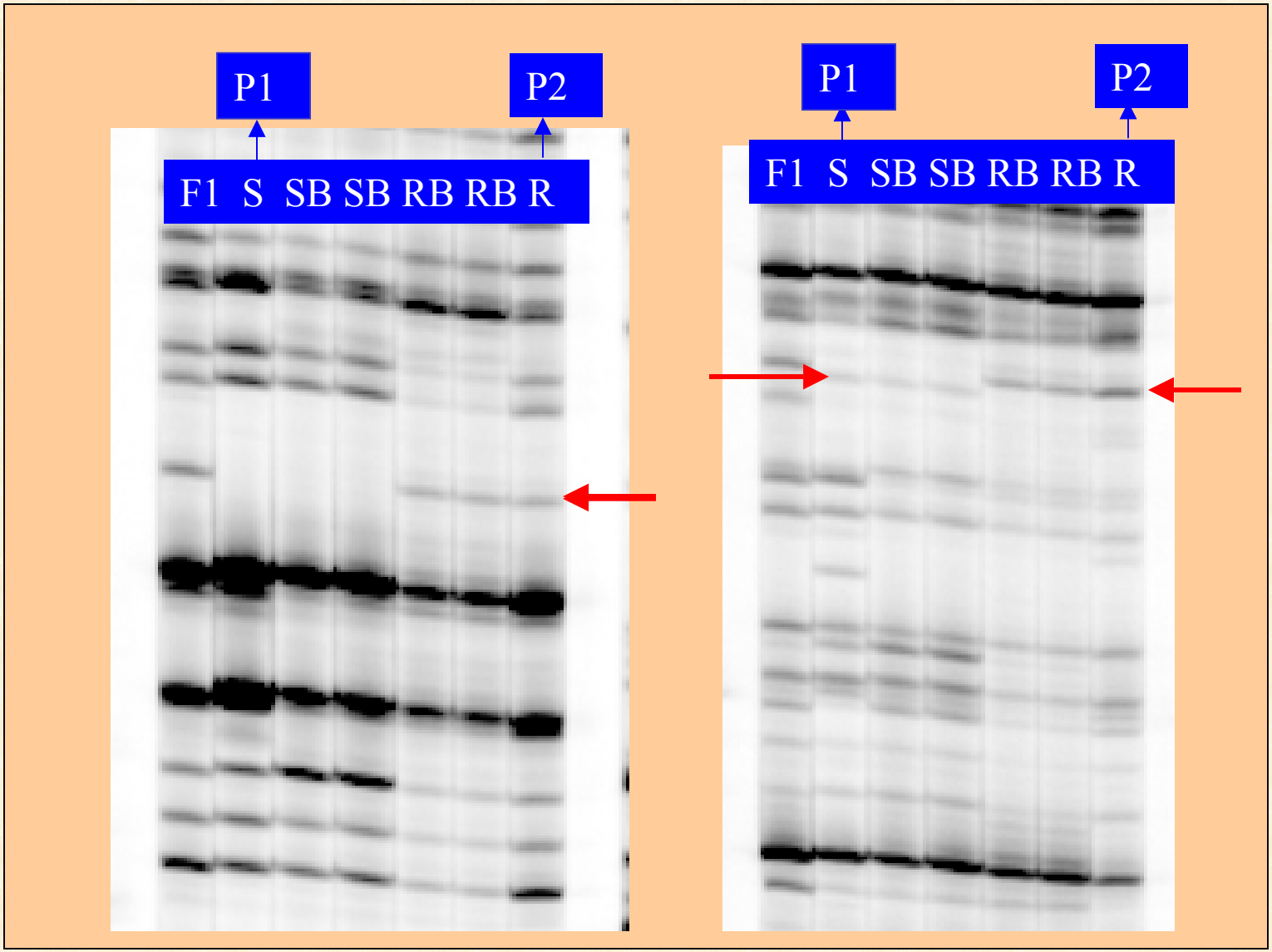
Individual homozygous F_2 or $F_{2:3}$ susceptible plants (7-10)



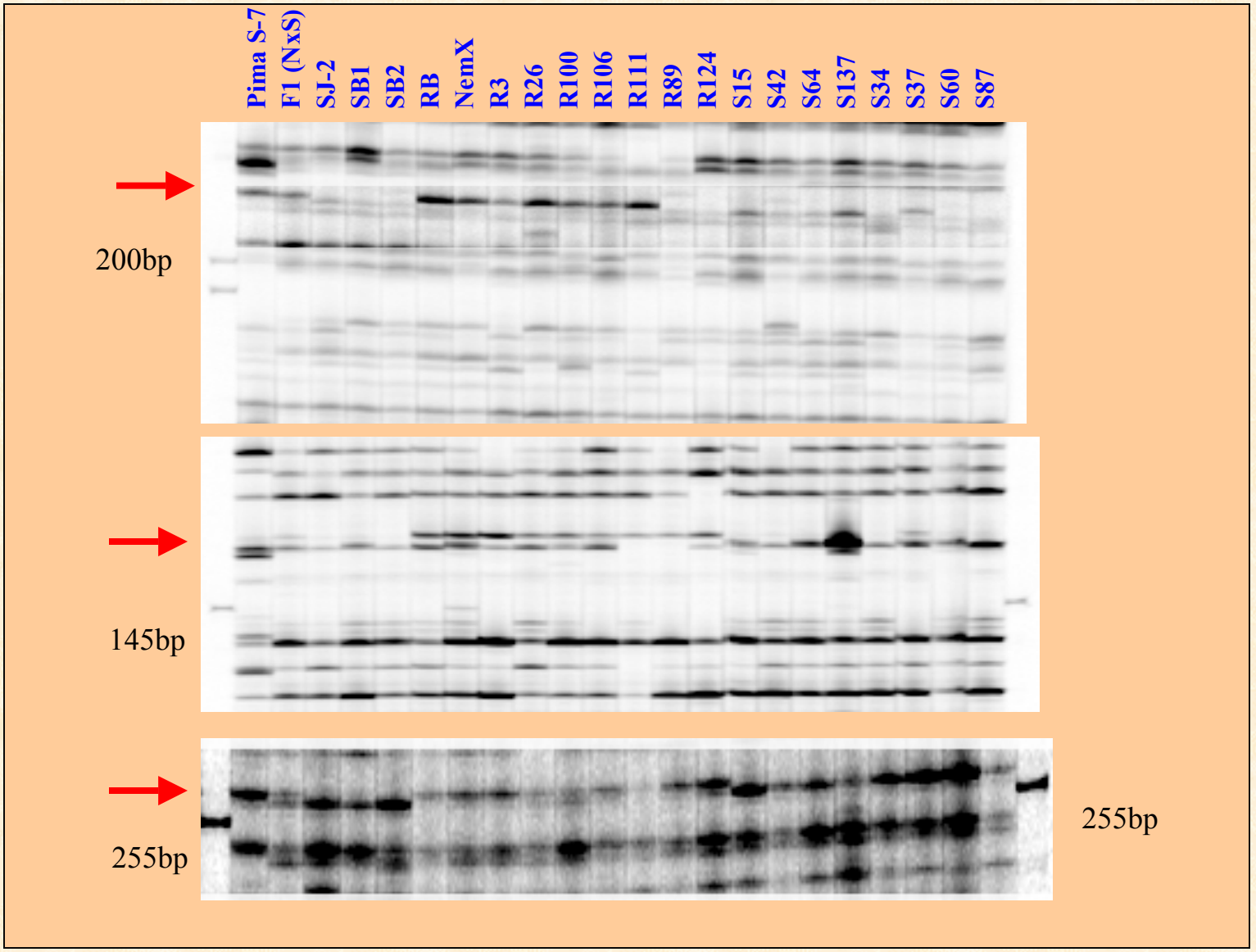
AFLP (amplified fragment length polymorphism)
Screening of Parental Polymorphism



AFLP and Bulked Segregant Analysis (BSA)



Potential Markers



Future Work

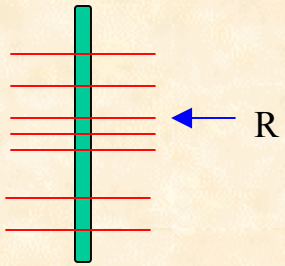
1 Screen potential markers for linkage to nematode and wilt resistance traits;

2 Use markers to confirm gene interactions that determine resistance phenotype;

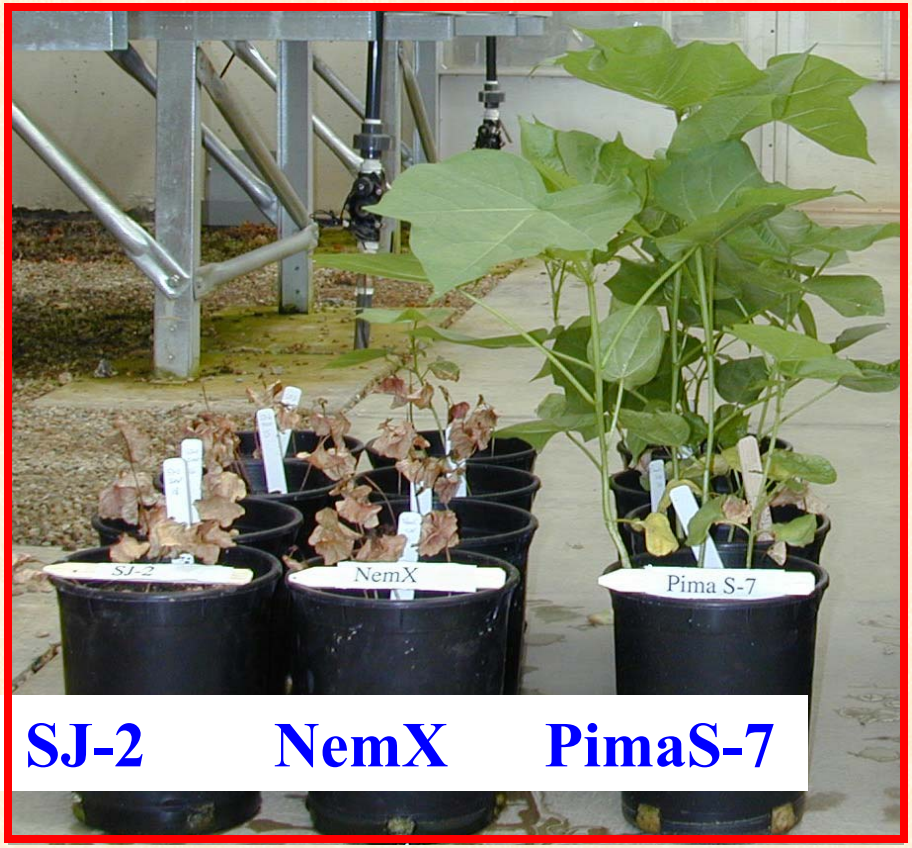
3 Convert markers to STS/SCAR forms;

4 Localize resistance genes to the chromosomes;

5 Fine mapping of resistance genes

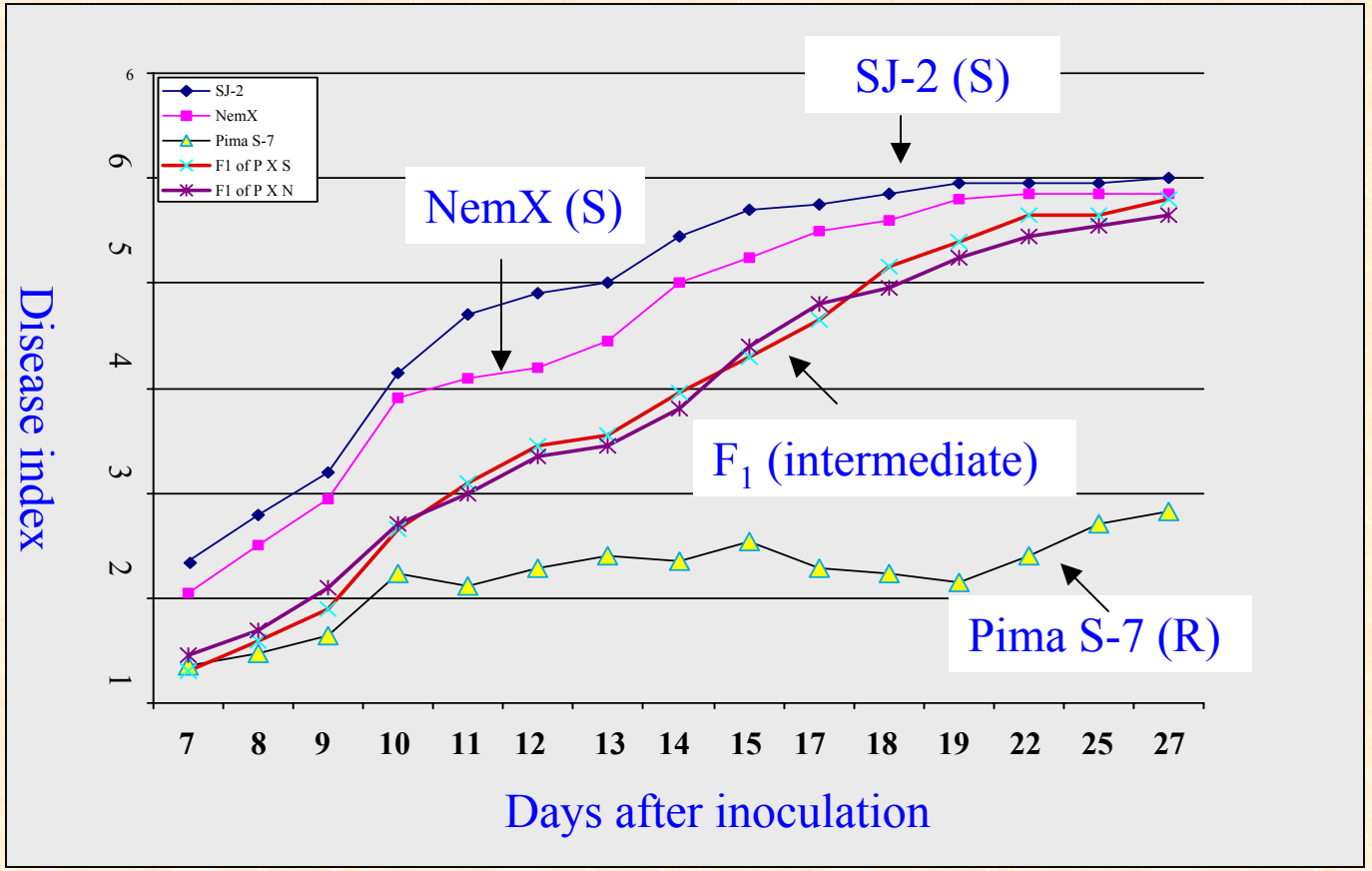


Reaction to FOV



Cut-root-dip method
4 weeks after inoculation

The development of Fusarium wilt Disease in Parents and F_1 plants



F_1 Pima S-7 x NemX
Pima S-7 x SJ-2

Cut-root-dip method