

# Phenotypic and genotypic characteristics of *Phytophthora infestans* from mating: Determination of inheritance or recombination

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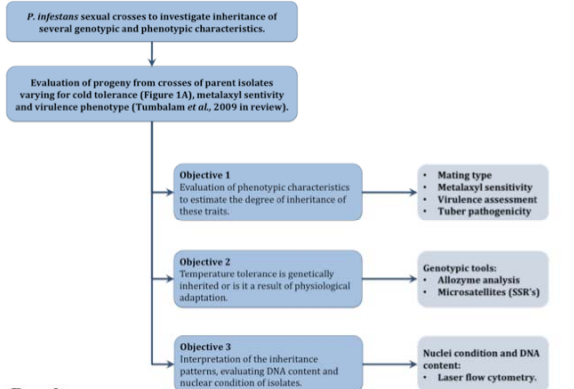
## Introduction

The heterothallic species *Phytophthora infestans*, the causal agent of potato late blight is bisexual and capable of self-mating and producing oospores.

Sexual crosses have been made in vitro for *P. infestans* in order to investigate inheritance of several phenotypic and genotypic characteristics such as resistance to metalaxyl (Shattock 1998; Lee *et al.*, 1999); allozyme markers (Spielman *et al.*, 1990); mating type (Judelson *et al.*, 1995) and for avirulence genes (Al-Kherb *et al.*, 1995).

Single oospore progeny (n = 300) from the in vitro crosses (Parent isolates in table 1) were analyzed individually for compatibility type, metalaxyl resistance, virulence assessment, allozyme analysis, and tuber pathogenicity.

## Materials and Method



## Results

### Objective 1

#### Mating type

Progeny from each cross had a different segregation in compatibility type, and some self-fertile progeny were also observed. Also, the number of oospores germinated in matings were significantly different in each cross (Figure 1A).

The number of self-fertile oospores in the progeny isolates increased with time. It was quite difficult to understand if the progeny isolates were self fertile or hybrid, as they have a tendency to change their compatibility type, from being heterothallic (A1 or A2) to homothallic.

#### Metalaxyl sensitivity

Parents in this study were either resistant, intermediate in sensitivity or sensitive to metalaxyl (Figure 1C, and Table 1). Variation was observed in the progeny isolates in metalaxyl phenotype, with 117 isolates determined as hybrids out of total of 285 progeny isolates.

#### Virulence assessment

Virulence testing was completed for all the parents and several progeny isolates were randomly selected from the crosses. The parental isolates chosen were similar in race structure and had common virulence genes.

All the isolates were shown to be pathogenic by infecting the R0 differential and were able to overcome at least one virulence factor. Variation was observed in the progeny isolates, by having different R genes.

About 80% of the isolates were pathogenic on all 11 R - gene differentials. 76% progeny isolates have not inherited the virulence phenotype but were more aggressive than parents.

Table 1. *P. infestans* parental isolates.

Isolate	Mating Type	Cold Tolerance	Metalaxyl Sensitivity
Pi 41-02	A1	T	R
Pi 02-007	A2	T	R
Pi S1-3	A2	T	R
Pi 98-1	A2	I	I
Pi 62-02	A1	T	I
Pi 95-3	A1	S	S
Pi 4-19	A1	S	I
Pi Atlantic 2N	A2	S	I

\* Tolerance (T, R); Resistant (R, R), Intermediate (I, R) and Sensitive (S, R).

Figure 1. Number of isolates of *P. infestans* progeny for different phenotypic characteristics: (A) Cold Tolerance, (B) Mating Type, and (C) Metalaxyl sensitivity, resulting from oospores generated by crossing parental isolates with different compatibility types and tolerance to temperature.

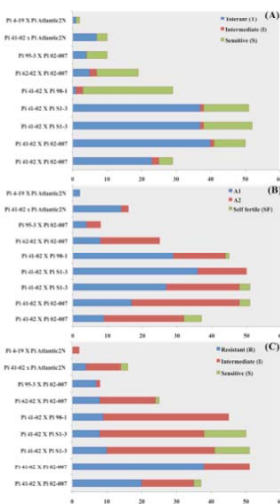


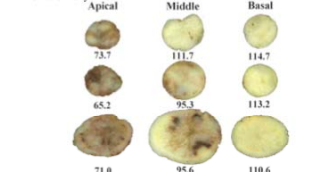
Table 2. Presumed genotypes at the Glucose phosphate isomerase (GPI) locus in parental and single oospore progeny isolates of *Phytophthora infestans*.

Parents	Genotype	Progeny
Pi 41-02 X Pi 02-007	100 / 100	13
Pi 41-02 X Pi 02-007	100 / 111 / 122	24
Pi 41-02 X Pi 02-007	100 / 111 / 122	31
Pi 41-02 X Pi 98-1	100 / 100	42
Pi 41-02 X Pi 98-1	100 / 111 / 122	8
Pi 41-02 X Pi 98-1	100 / 100	45
Pi 41-02 X Pi 98-1	100 / 111 / 122	5
Pi 41-02 X Pi 98-1	100 / 100	38
Pi 41-02 X Pi 98-1	100 / 122	7
Pi 02-007 X Pi 62-02	100 / 100	18
Pi 95-3 X Pi 62-007	96 / 100	0
Pi Atlantic 2N X Pi 41-02	100 / 111 / 122	0
Pi Atlantic 2N X Pi 41-02	100 / 100	16
Pi Atlantic 2N X Pi 4-19	100 / 111 / 122	0
Pi Atlantic 2N X Pi 4-19	100 / 100	2

Table 3. Different isolates of *Phytophthora infestans* showing variation in levels of nuclear condition, karyotype and sexuality

Isolates	GPI	Asexupl	Diploid	Heterodiploid	Nuclear size	Nuclear condition	Sexuality
Pi Atlantic 2N	100/112	87%	47%	-	2N = 2N	Standard	Heterothallic
Pi 02-007	100/112	100%	-	-	2N = 2N	Standard	Heterothallic
Pi 41-02	100/100	100%	-	-	2N = 2N	Standard	Heterothallic
4-19	100/114	100%	-	-	2N = 2N	Standard	Heterothallic
3-08	100/100	100%	-	-	2N = 2N	Standard	Heterothallic
3-08	100/100	100%	-	-	2N = 2N	Standard	Heterothallic
A-07	100/100	100%	-	-	2N = 2N	Standard	Heterothallic

Figure 2. Late Blight Development in FL1879 tuber tissue caused by *Phytophthora infestans* (isolate Pi S-16), incubated at 10°C for 30 days.



## Tuber Pathogenicity

A digital image analysis technique was used to assess tuber tissue infection similar to the method described for estimation of growth of cultures of *P. infestans* on Petri dishes (Tumbalam *et al.*, 2009, in review). The progeny isolates from the different thermal phenotype crosses were pathogenic (Figure 2). The variability in pathogenicity and virulence of both parental and progeny isolates of *P. infestans* with different thermal tolerance phenotypes was clear from this study. About 50% progeny isolates have got inherited.

## Objective 2

To determine whether the phenotypic character of temperature tolerance was genetically inherited or occurred as a result of physiological adaptation, by using genotypic tools like Allozyme analysis and Microsatellites (SSR's).

The electrophoretic patterns at the GPI loci were identified for all the parents and progeny (n=300) isolates from the nine crosses. There were no progeny with recombinant or hybrid patterns, which could be a result of selfing (Table 2).

Of the two SSR markers (Pi 4B and Pi 02) used to characterize parental isolates, marker Pi 4B differentiated well between the isolates. Parents selected for crosses in this study have heterozygous alleles using SSR markers (Figure 3).

Using the marker Pi 4B, 8 out of 25 progeny isolates tested, showed patterns of alleles that were recombinants (hybrids), differing from the pattern of either parent and the other 17 had alleles identical to the parents, and therefore many of these offspring may have resulted from apomixis (selfing).

## Objective 3

To clarify interpretation of inheritance patterns, DNA content of nuclei and nuclear condition of isolates were examined with laser flow cytometry (Table 3).

Examination of field isolates of *P. infestans* using laser flow cytometry revealed that of 16 isolates tested, 10 contained one nuclear population (G1 peak) that was presumably diploid. The other 6 isolates were heterokaryotic, containing more than one nuclear population in their thallus (Figure 4).

Laser flow cytometry revealed that the nuclear populations present in the heterokaryotic parents were not inherited equally by the single-zoospore derived isolates (asexual progeny).

Our data supports the conclusion that *P. infestans* has the potential to be homothallic and have the ability to self under certain conditions.

## Conclusions

Phenotypic and genotypic evaluation of the progeny isolates from nine different crosses have shown that in total, 139 isolates have attained phenotypes that are not inherited from either parent (Figure 5). 49% progeny were recombinants (139 / 284 isolates).

In the evolution of field isolates and genetic diversity of populations, not only outcrossing but also selfing should be considered. Sexual recombination will produce new genetic combinations, creating the possibility of unusually fit or pathogenic individuals or individuals with novel ecological characteristics.

Oospores formed at the beginning of epidemics could therefore contribute to the emergence of novel phenotypes within a growing season as well as between seasons.

## References

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