DNA polymorphism in the pepper-*Phytophthora capsici* pathosystem

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Introduction

Phytophthora capsici is a pathogen on several economically important crops, affecting tomatoes, eggplants, squash, and melons, and it is one of the most damaging pathogens currently affecting pepper (*Capsicum annuum*) worldwide (Tyler 2002, Oelke *et al*. 2003). The species *Phytophthora capsici* was first reported in 1922 in New Mexico and spread to vegetable production areas in Colorado and Florida in the 1930´s and 1940´s. This oomycete attacks the roots, stems, leaves and fruit of the plant. Resistance in *Capsicum annuum* is genetically complex. In order to integrate genetic linkage maps of peppers and *P. capsici*, a series of SSRs, RAPD, CAPS and COSII markers and candidate genes for virulence (glucanase, pectinase, chitinase and cutinase) have been tested in a panel of 6 different *C. annuum* (American and European genotypes) and in 2 *P. capsici* selected because they are the parents of a segregating cross used for linkage mapping. We are interested in finding polymorphisms in the pepper-*P. capsici* system because these could serve as potential genetic markers for the construction of linkage maps.

Materials and Methods

Population
Six pepper (*C. annuum*) genotypes were used: "Padrón 124", "Couto 12B", "CM334", "PI201234", "PSP-11" and "*NuMex Joe E. Parker* (JEP)", with different levels of resistance to *P. Capsici*. "CM334" is the most resistant according to previous research (Guerrero and Laborde 1980, Pochard et al. 1983, Gil Ortega et al. 1992).
Two *P. capsici* isolates, Ppc3 and GSP1-1, selected for different levels of virulence on pepper, were used.

DNA extractions
DNA genomic was extracted following the procedures of Prince *et al*. 1997, and using the "Mini-Plant Genomic DNA Isolation Kit " (Metabion)

Molecular markers
RAPD: were generated using 20 different decamers (Operon Technology, Alameda, Calif.)
CAPS: primer pairs have been obtained based on previously sequenced and mapped tomato RFLP clones, chosen because of their even distribution throughout the genome of tomato.
COSII: chosen because of their distribution in tomato and *Arabidopsis*. Sequences available at ftp://ftp.sgn.cornell.edu/COSII/pepper_mapping/
SSRs: *P. capsici* EST sequences from the *Phytophthora* Functional Genomics Database (Gajendran *et al*. 2006) were screened for SSR sequences using Sputnik (Abajian 1994).
Candidate genes for virulence: Primers sets have been designed from previously cloned genes that we are investigating as potential candidate genes for virulence: glucanase, pectinase, chitinase and cutinase.
We used various of the above molecular markers to detect polymorphism on C. *annuum* and *P. capsici*.

PCR amplicons were analyzed on 1- 1.5% (w/v) agarose gel.

Results

More than 75 molecular markers have been designed and used to analyze polymorphism in the *P. capsici*- pepper pathosystem, so far.
Fifteen polymorphisms were found in pepper plants and nineteen between the two isolates of *P. capsici* studied (see Tables 1 and 2).
We detected the highest levels of polymorphism (75%) in *P. capsici* using candidate genes for virulence and in pepper plants using RAPD markers (>65%).
Polymorphisms in the peppers seem to be slightly more common among JEP and CM334 than between the rest of the pairs of mapping parent genotypes (data not shown).
(Figures 1 and 2 show examples of polymorphism found using the different types of molecular markers).

	Phytophthora capsici	
Type of Marker	**Number Available for screening**	**Number Polymorphic**
SSR	13	2
RAPD	20	10
Virulence genes	4	3
TOTAL	37	**15**

	Capsicum annuum	
Type of Marker	**Number Available for screening**	**Number Polymorphic**
COSII	32	10
RAPD	9	6
CAPS	6	3
TOTAL	47	**19**

Table 1 and 2. Molecular markers being used in *P. capsici* and *Capsicum annuum* screening.

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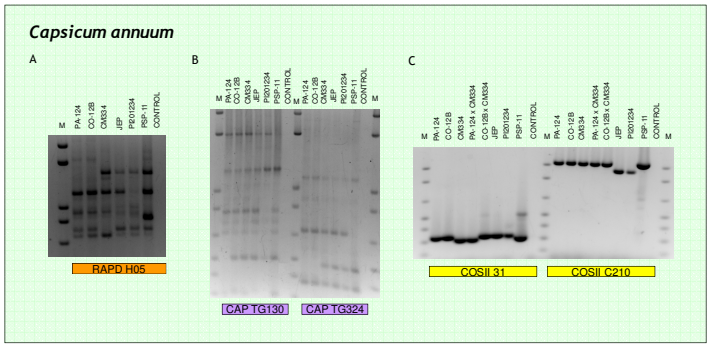

Figure 1. Different genotypes of *C. annuum* tested with RAPD (A), CAP (B) and COSII (C) markers. The lane marked M contained 0.8 μg of a 1 kb plus ladder DNA marker (Invitrogen).

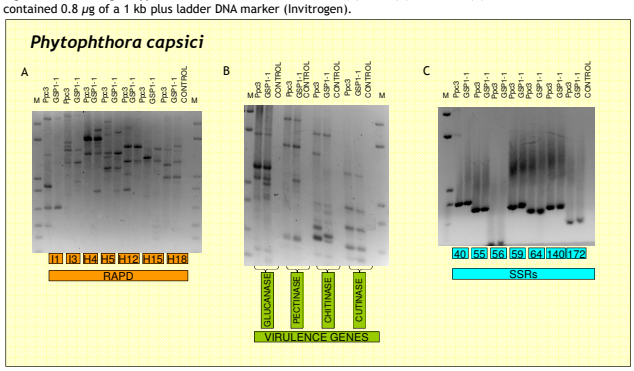

Figure 2. Different genotypes *P. capsici* tested with RAPD markers (A), candidate genes for virulence (B) and COSII markers (C). The lane marked M contained 0.8 μg of a 1 kb plus ladder DNA marker (Invitrogen).

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