



isolates is associated with variation in the *SIX1* sequence.

Molecular phylogeny of *Phytophthora* species; impact of reticulation and ecological parameters

Laurens. P. N. M. Kroon¹, F.T. Bakker²,
G.B.M. van den Bosch¹, P.J.M. Bonants¹, and
W.G. Flier^a

¹Plant Research International, P.O.Box 16,
6700 AA Wageningen, The Netherlands ²National Herbarium
Netherlands, Wageningen University Branch, P.O.Box 9101,
6700 HB Wageningen, The Netherlands
Contact: laurens.kroon@wur.nl

A molecular phylogenetic analysis of the genus *Phytophthora* was performed, based on both nuclear and mitochondrial DNA sequence data. Emphasis in our study was on species collected from the Toluca Valley in central Mexico, the presumed center of origin of *Phytophthora infestans* and other closely related species. A total of 113 isolates from 48 *Phytophthora* species and two *Pythium* species were used in this analysis. Phylogenetic analyses were performed for combined mitochondrial sequences, for combined nuclear sequences and for all sequences combined and between-data set congruence was tested. Results indicate that the classical taxonomic grouping as described by Waterhouse (1963) does not reflect true phylogenetic relations. *Phytophthora* species were redistributed into eight clades, providing a more accurate representation of phylogenetic relationships within the genus *Phytophthora*. The evolution and transition of morphological, pathogenic and reproductive traits was inferred from the cladogram

generated in this study. Incongruence was found between phylogenies for nuclear and mitochondrial DNA, a possible indication for reticulate evolution in *Phytophthora* species.

Characterisation of the signal transduction pathway resulting in the hypersensitive response in planta

Iris Stulemeijer

Laboratory of Phytopathology, Wageningen University,
Bode 45, Postbus 8025, 6700EE Wageningen,
The Netherlands
Contact: Iris.Stulemeijer@wur.nl

The hypersensitive response (HR) is an efficient, active defence response in plants based on a resistance (*R*) gene in the plant that mediates resistance against a pathogen that contains the corresponding avirulence (*Avr*) gene. In tomato, the resistance gene *Cf-4* mediates specific recognition of the corresponding elicitor AVR4 produced by the pathogen *Cladosporium fulvum*. To study the signal transduction pathways resulting in HR, we have generated tomato seedlings that express both *Cf-4* and AVR4. Since HR resulting from AVR4 recognition is suppressed at elevated temperatures (33°C), systemic HR in *Cf-4*/AVR4 tomato seedlings can be synchronised by a shift from high to low (20°C) temperature (De Jong *et al.*, 2002). This system will be further referred to as 'dying seedlings'.

In the past, several studies have been done to identify parts of the signal transduction pathway in cell suspensions. However, the dying seedlings give us a nice tool to study the signal transduction pathway in intact plants. The system allows studies on cell death, H₂O₂ production, callose formation, MAP kinase activity and alkalization of the leaves.

Furthermore, protein phosphorylation events that play a key role in signal transduction pathways can be studied in these dying seedlings. Several phosphorylation enzymes, such as Pto, Xa21, MAPKs and CDPKs are specifically activated during HR. To search for target proteins of phosphorylation enzymes in general, we aim to study changes in the phosphoproteome during HR in the dying seedlings. Differentially phosphorylated samples can be identified on Western blot by specific antibodies, whereas proteins can be isolated for further analysis by immunoprecipitations.

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