



General data	
PPP-number	KV 1406-056
Project title	An integrated genomics and effectoromics impulse for potato wart resistance management and breeding
Roadmap/Cluster	T&U, Meer met Minder
Executive research institution(s)	-
Project leader research institute (name and e-mail address)	Jack Vossen, jack.vossen@wur.nl
PPP coordinator (on behalf of private parties involved)	Stan Oome
Government contact person	-
Starting date	01-04-2015
End date	01-10-2019
Brief description of project content	Wart disease in potato is caused by the obligate biotrophic fungus <i>Synchytrium endobioticum</i> and is subject to quarantine containment. Unlike other diseases, the deployment of resistant varieties is the only disease management strategy. In this project we will acquire insights in the way polygenic disease resistance against obligate biotrophic pathogens is build up. This knowledge will be used to develop simplified, unambiguous functional and molecular tools for pathogen race typing for resistance based quarantine management. Furthermore, the newly developed tools will be deployed to tag wart disease <i>R</i> genes in breeding programs, and to identify complementary wart resistance sources in breeding and germplasm material. This combined public private research effort will create a major breakthrough in potato wart disease resistance management and breeding.

Highlights
<p>Workpackage 1. <i>S. endobioticum</i> genomics</p> <p>A. Identification of effector candidates: A draft genome sequence of <i>S. endobioticum</i> was available as background to this project. In 2015 the genome assembly was further polished and annotated. <i>S. endobioticum</i> genome version 1.0 comprises 21.6 Mb which were build up from 804 contigs. 8408 genes were predicted of whom 505 were predicted to be secreted (secretome). Comparative analyses of the <i>S. endobioticum</i> and other Chitridiomycetes secretomes revealed 319 <i>S. endobioticum</i> specific proteins. Successive comparisons between pathotypes revealed 24 candidate Avr's. Sequencing of additional <i>S. endobioticum</i> genomes have/will be performed to refine or identify additional Avr candidates. But also to validate diagnostic markers in the mitochondrial genome.</p> <p>B. Preparations were made for the functional testing of the Avr candidates. First we confirmed that <i>S. endobioticum</i> resistance was expressed in above ground tissue (shoots and leaves). This opened the door to further study Avr responsiveness in leaves. Plants from the segregating populations (see point 2A) were screened for</p>

an appropriate response to control agroinfiltrations. At least 20 “agrocompetent” individuals per population have been selected. Finally, all 24 candidate AVR genes were inserted through the Golden gate cloning system, into plant expression vectors. Agroinfiltration of 24 Avr candidates (with and without signal peptides) is planned in spring 2017

- C. Comparative analysis of the Mitochondrial genome sequences for diagnostic purposes. Sequence data of additional *S. endobioticum* strains have revealed SNPs that allows classification of *S. endobioticum* isolates. New insights emerge about the history of isolates and pathotypes.
- D. A manuscript on the *S. endobioticum* genome has been compiled with the collaborating research group from Canada. The message of this paper is structured around obligate biotrophy in the *Synchytrium* group. The manuscript will be submitted to the consortium for publication clearance in April 2017.

Workpackage 2. Potato genomics

- A. Phenotyping of populations. Four potato populations segregating for resistance were available as background to this project. These populations have different donors of resistance and a first round of phenotyping was performed in three out of four populations in 2015. In 2016 a selection of individuals from the first three populations and the entire fourth population were phenotyped for resistance against summer spores and winter spores from pathotypes 1, 2, 6 and 18. These populations were used for GWAS validation and SNP discovery (points 2B and 2C)
- B. GWAS analyses have been performed in 2015 in order to identify 969 SNPs from a 20k SNP array that were associated with and potentially linked to wart disease resistance. A representative set of 263 SNPs have been tested using KASP and CAPS marker assays. 60 of these markers indeed showed association with pathotypes 1, 2, 6, or 18 resistance in independent populations. Three major QTLs and three minor QTLs for wart disease resistance were discovered.
- C. In parallel a SNPs discovery approach was pursued to find markers linked to wart resistance. For this purpose we sequenced the genomes of 18 potato varieties, including the resistant and susceptible parents of the four populations. In addition a pool of resistant and a pool of susceptible offspring from one of the populations were sequenced in order to mine for associated SNPs using a bulked segregant analysis.
- D. SNPs that were identified using this bulked segregant analysis were validated using KASP assays in the segregating populations. One major QTL and one minor QTL were identified in this way. This successful approach was elaborated on by sequencing 6 additional bulks. In 2017 we aim to identify the remaining QTLs for pathotype 2, 6 and 18 resistance to summer spores and winter spores in the four populations.
- E. A manuscript has been prepared that describes the identification of a haplotype that is responsible for pathotype 1 resistance in the vast majority of pathotype 1 resistant potato varieties. The manuscript will be submitted to the consortium in June 2017 and after clearance it will be submitted for publication.

3. Oral and poster presentations:

Poster presentation: B.T.L.H. van de Vossen, M. van Gent-Pelzer, E.G.W.M. Schijlen, S. Warris, H.C. van de Geest, P. Bonants, C.A. Lévesque, J. Cullis, C.T. Lewis, J.T. Chapados, W. McCormick, K. Dadej, Z. Adam, G.J. Bilodeau, M. Gagnon, D.S. Smith, R.F.G. Visser, J.H. Vossen, T.A.J. van der Lee,, Unravelling potato wart disease; determination of the draft genome of the obligate biotrophic fungus *Synchytrium endobioticum*, EEC-EPPO Workshop on Euphresco, 26-29-7-2016, Moscow, Russia

Oral presentation: B.T.L.H. van de Vossen, H. Nguyen, Z. Adam, L.V. Bakker, G.J. Bilodeau, P. Bonants, J.T. Chapados, J. Cullis, K. Dadej, M. Gagnon, H.C. van de Geest,

M. van Gent-Pelzer, C.T. Lewis, W. McCormick, D.S. Smith, R.G.F. Visser, J.H. Vossen, S. Warris, C.A. Lévesque and T.A.J. van der Lee, Unravelling potato wart disease; a joint effort of Canadian and Dutch partners, CFIA seminar, Ottawa, Canada

4. Communication: Phenotypic data and molecular markers have been shared with the consortium members using a password protected teamsite