

the 19th century. Downy mildew caused by *Plasmopara viticola* is a common disease present in all regions where vines are cultivated. *P. Viticola* is an obligate biotrophic pathogen of grape that uses one of the most specialised infection strategies of plant pathogens. This oomycete obtains its nutritional resources from living cells of its host and is completely dependent on living plant tissue for its growth and propagation.

In Europe, the application of copper fungicides is widely used in vineyards in order to control the development of *P. viticola*, however the replacement of copper-based fungicides by synthetic fungicides has promoted the development of resistant isolates of *P. viticola*. For this reason alternative ways of combating downy mildew are badly needed. Genomics approaches are likely to have particular value for grape improvement because they have the potential to identify transcriptional, biochemical and genetic pathways that contribute to agronomic properties including disease resistance (e.g. specific resistance genes and downstream transcriptional pathways).

Many processes are still unknown such as which defence mechanisms are delayed or repressed in susceptible grapevine varieties to downy mildew. For this reason by screening differentially expressed genes in infected and non-infected susceptible grapevine, we hope to obtain novel genes involved in the defence pathway against downy mildew. Since many defence genes are found in low abundance, a Suppression Subtractive Hybridization (SSH) library was constructed in order to specifically amplify low expressing defence genes.

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TOWARDS CISGENIC LATE BLIGHT RESISTANT POTATO VARIETIES; CLONING AND CHARACTERIZATION OF RELEVANT R GENES FROM WILD SOLANUM SPECIES

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The necessity to develop potato varieties that possess durable resistance against the oomycete pathogen *Phytophthora infestans* is increasing as more virulent and pesticide resistant strains of the pathogen are rapidly emerging. Isolation of resistance (*R*) genes from wild *Solanum* species and subsequent introduction of a combination of these genes as cisgenes into existing potato varieties using marker free plant transformation technologies is currently the fastest means of exploiting potentially durable resistance present in the *Solanum* gene pool. This approach opens the way to efficient *R* gene pyramiding or polyculture strategies. We have identified high levels of resistance in several wild *Solanum* species. Based on resistance assays with a diverse set of *P. infestans* isolates and apparent 1:1 segregation of resistance in initial intraspecific F1 mapping populations (n=50) we are currently pursuing map-based cloning and candidate gene approaches to isolate 10 novel late blight *R* genes from a diverse set of wild *Solanum* species. Marker saturation of the different *R* loci is being achieved through BSA and NBS profiling. Markers that cosegregate with resistance in large intra- or interspecific F1 mapping populations (n=2500) are being used to screen BAC libraries that have been generated for the relevant parental genotypes. Candidate RGA's derived from BAC clones spanning the individual *R* loci are subjected to stable complemen-

tation analyses in a susceptible potato variety or transiently in *N. benthamiana*. Ultimately, the cloned *R* genes will be used to develop cisgenic late blight resistant potato varieties.

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EFFECTOR GENOMICS OF PHYTOPHTHORA INFESTANS ACCELERATES IDENTIFICATION, FUNCTIONAL CHARACTERIZATION AND CLONING OF RESISTANCE GENES IN POTATO

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The best strategy to genetically manage the devastating late blight disease in potato requires rapid identification and functional characterisation of resistance (*R*) genes. Here we show that a functional genomics approach which combines mining effectors from the genome sequence of *Phytophthora infestans* with screening for responses in wild *Solanum* species accelerates the cloning of *R* genes. We cloned a collection of RXLR effectors representing candidate avirulence (*Avr*) genes in a *Potato virus X* expression vector, and screened resistant wild *Solanum* for necrotic responses. A large variation of responses to various RXLR effectors became evident in a large number of *Solanum* clones. Sexual crossing of these plants resulted in populations, which we examined for resistance to *P. infestans* and response to effectors. In several populations resistance to *P. infestans* co-segregated with the effector response, suggesting an *R-AVR* interaction. Genetic studies with *R* gene specific markers indicated positional and sequence homology with a late blight *R* gene previously cloned from an unrelated *Solanum* species. Co-infiltration of *Agrobacterium tumefaciens* strains containing the candidate *Avr* gene and the *R* gene in *Nicotiana benthamiana* leaves resulted in a specific hypersensitive response. This prompted us to follow a candidate gene approach and clone specific full-length *R* gene analogues (RGAs) from the selected wild *Solanum* genotypes. An extensive number of RGAs were obtained, yet we could efficiently identify the functional homologues that displayed the specific effector response in transient functional assays. These experiments prove that novel *R-AVR* combinations can be identified using functional profiling with RXLR effectors, and that availability of interacting effectors enables exceptionally fast cloning of *R* genes. Moreover, it demonstrates how conserved *R* genes with identical recognition specificities can efficiently be identified and cloned from unrelated *Solanum* species.