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Exploring the Natural Biodiversity of Potato for Late Blight Resistance

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Abstract This paper gives an overview of the activities in Sub-project 4 of the BIOEXPLOIT programme. The European genebank collections of potato need to be narrowed down into (customized) core collections by focusing on loci associated with disease resistance. Therefore, new and more efficient molecular methods are being developed to identify these loci, extensive genotyping of resistance to *Phytophthora infestans* and signalling loci is being performed, and genotyped selections of accessions are phenotypically analysed for their resistance specificities. The results of these activities are being assembled in an integrated database.

Keywords Genetic resources · *Phytophthora infestans* · Potato · *Solanum*

Introduction

The genetic variation in wild accessions is still largely unexplored. Only a fraction of the natural biodiversity in disease resistance is currently included in the genetic bases of commercial varieties of crop species. The major goals of Sub-project 4 (SP4) of BIOEXPLOIT are:

1. To explore the genetic resources in preferably EU genebanks of potato and wheat;
2. To identify the relevant genes for the most important pathogens of these two crops; and
3. To make these genes available for designing new varieties of these two crops via Sub-projects 2, 5 and 6.

For potato, BIOEXPLOIT concentrates on late blight, a severe potato disease caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. This paper gives

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an overview of the activities in SP4 for potato and shows some first results. Other results may be confidential, are waiting for publication by the researchers involved or are at the stage of writing this paper still preliminary.

Sub-project 4 includes three Work Packages (WP4.1, WP4.2 and WP4.3):

1. The goal of WP4.1 is to perform extensive genotyping of allelic variance related to disease resistance;
2. The goal of WP4.2 is to phenotype selected clones and accessions for their resistance to late blight. The results will lead to so-called customized core collections;
3. The goal of WP4.3 is to establish an Integrated Database which will make the information from WP4.1 and WP4.2 accessible.

The research groups working on potato in SP4 are listed in Table 1. The leader of Sub-project 4 is Patrick Schweizer from the Leibnitz Institute of Plant Genetics and Crop Plant Research (IPK, Germany).

Sub-project 4 will interact with other sub-projects within BIOEXPLOIT. The genetic variation present at disease resistance loci identified in Sub-project 2 will be mined in wild material. The outputs will be fed back into Sub-project 2 for cloning of novel disease resistance alleles, will be fed forward into Sub-project 5 for marker-assisted breeding and will ultimately be prepared for introducing into elite clones (Sub-project 6). Moreover, Sub-project 4 will facilitate the phenotypic screening during intermediate breeding phases based on the germplasm.

Table 1 Research groups working on potato in the three work packages of Sub-project 4 of BIOEXPLOIT

Partner ID	Institution	Researcher	Work Package
P01HvE	Wageningen University, NL	Herman van Eck	4.1
P02JEC	Institut National de Recherche Agronomique, F	Jean Eric Chauvin	4.1 & 4.2
P02VL	Institut National de Recherche Agronomique, F	Veronique Lefebvre	4.1 & 4.2
P03DM	Scottish Crop Research Institute, UK	David Marshall	4.3
P03GB	Scottish Crop Research Institute, UK	Glenn Bryan	4.1
P06AF	University of Dundee, UK	Andy Flavell	4.3
P09RH	Centre for Genetic Resources the Netherlands, NL	Roel Hoekstra	4.1 & 4.3
P10CG	Max-Planck Institute for Plant Breeding Research, GER	Christiane Gebhardt	4.1
P14BV	Plant Research International, NL	Ben Vosman	4.1 & 4.2
P15EZ	Plant Breeding and Acclimatization Institute, POL	Ewa Zimnoch-Guzowska	4.2
P19ER	NEIKER—Basque Institute for Agricultural Research and Development, ESP	Enrique Ritter	4.1
P30JT	National Institute of Agricultural Botany, UK	Jane Thomas	4.2
P35TG	National Federation of the French Seed Potato Growers (FNPPPT), F	Thiery Gokelaere	4.1
P40AC	APPACALE, ESP	Ana Carrasco	4.1
P43JL	SaKa Forschung GmbH, GER	Jens Lübeck	4.2
P44BT	ARC Seibersdorf research GmbH, AUT	Bodo Trognitz	4.1

Below we will describe the three work packages of Sub-project 4 in detail.

Work Package 4.1

Preliminary Core Collection

Genebanks worldwide hold collections of the genetic resources of crop plants for long-term conservation and utilization. Some collections have become very large and their size may hinder conservation and the use of the germplasm they hold. Frankel and Brown (1984) proposed to establish "core collections", a limited sample representing the available genetic diversity with a low similarity between the accessions. Such a sample can be managed better than the whole collection. Worldwide about 15,000 wild potato accessions are being maintained in large collections located in Russia, Western Europe, USA, Peru, Bolivia, Argentina and Chile. Due to redundancy within and between collections the amount of unique accessions is probably around 7,500. For BIOEXPLOIT the material to be used for the creation of the preliminary core collection is restricted to the 7,224 wild and cultivated potato accessions maintained within the EU at the collections of the following three institutes: Centre for Genetic Resources the Netherlands (CGN; The Netherlands), the Scottish Crop Research Institute (SCRI; United Kingdom), and the Leibnitz Institute of Plant Genetics and Crop Plant Research (IPK; Germany). This is done to assure easy access to high quality material. In these collections the plants are tested on quarantine diseases according to the requirements of Council Directive 2000/29/EC and its amendments. Table 2 shows the overlap within and between these three collections.

The first deliverable of WP4.1 was the establishment of a preliminary core collection for potato using the genebank data gathered in WP4.3 (partner P09RH). It is based on passport data only and will be refined later using the genotypic and phenotypic characterisation data from Sub-project 4. Instead of determining a fixed core collection, the core selector tool (van Hintum 1999) was implemented in Excel, using path, weight and priority descriptors. The domain is flexible, because the researcher may choose to work with specific species or material originating from a specific region (e.g., Mexico) only. A 'weight' was appointed to each group, indicating the relative importance of this group including its subgroups. It reflects a

Table 2 Number of accessions and probable redundancy within and between the three main potato collections in the EU

Potato collection	Number of accessions	Number of probable duplicates within collection	Probable redundancy between collections		
			CGN	CPC	GLKS
CGN	2,720	4		206	457
CPC (SCRI)	1,482	123	206		393
GLKS (IPK)	3,022	126	457	393	

general idea about the genetic diversity in the groups. If available, it is possible to compare marker diversity within the groups and base the weight on this comparison. The priority is assigned to the individual accessions within the endgroups (e.g., tested on quarantine diseases). Results from molecular characterisations can be plugged in later to adjust and improve the structure of the overall collection and the path of individual accessions.

Known Molecular Markers

Existing marker and sequence information was collected from available databases and literature, resulting in a list of 116 molecular markers linked to *P. infestans* (PI) resistance located on all 12 chromosomes. The number of markers varies from 3 to 16 per chromosome. The focus is on PCR-based markers. Other markers were included when considered important (partners P01HvE, P02JEC, P03GB, P10CG, and P14BV). This compiled marker information will serve as a basis for the development of efficient molecular methods for the evaluation of potato genebanks in WP4.1. A set of these markers will be tested for applicability across a wide range of germplasm and for mining of new resistance sources. The list is also useful for Sub-project 5 of BIOEXPLOIT, where selected markers might be used to develop robust PCR or high-throughput markers.

Development of more Efficient Genotyping Methods

Many cloned *R* genes are related in sequence. The most prevalent group is classified as NBS-LRR (see Slootweg et al. 2009). In order to make genotyping of allelic variance in resistance of potato more efficient, easy-to-use markers of different types (SSRs, SNPs, CAPS) for *R* gene regions and for Resistance Gene Analogue (RGA) profiling as well as new primers for amplification of R-gene related sequences are being developed (partners P02JEC, P02VL, P03GB, P14BV, and P44BT). This task is essential for haplotype analysis and allele mining work in Sub-project 4 and is connected with work in Sub-project 2 of BIOEXPLOIT. Primers were designed that amplify *Rpi-blb1*-like, *Rpi-blb2*-like and *R1*-like genes. Furthermore, primers were developed for RGA profiling by amplifying the NBS-region of resistance genes. RGA markers are of interest because they can be closely linked to disease resistance genes. For example the *Rpi-blb1* gene is part of a cluster on chromosome VIII consisting of three RGAs, where RGA1 and RGA3 are flanking the *Rpi-blb1*. Because of the sequence relationships between *Rpi-blb1*, RGA1-blb and RGA3-blb it was suggested that the *Rpi-blb1* gene most likely evolved from intragenic recombination between the ancestral genes of RGA3-blb and RGA1-blb (van der Vossen et al. 2003).

To tag *R* genes and RGAs partner P14BV uses NBS-profiling. Nearly 50 taxa were tested with specific primers. RGA1 is probably very ancient, because homologues were found in all taxa including non tuber-bearing species. RGA3 was found in half of the material. Candidates for a likely functional allele of *Rpi-blb1* were found in *Solanum bulbocastanum* and *S. stoloniferum*. No homologues of *Rpi-blb2* were detected (Wang et al. 2008). Partner P44BT developed primer sets for NBS profiling, which were tested on late blight resistant and susceptible genotypes

of *Solanum caripense*, a non-potato species (Trognitz and Trognitz 2005). DNA fragments were isolated from late blight resistant *S. caripense*, some with similarity to known resistance genes. Additionally, primers were designed for the amplification of *R1*-like sequences in wild potato species, to selectively amplify single SNP-characterized alleles of one locus.

Late blight resistance Quantitative Trait Loci (QTLs) are associated with durable resistance. QTLs have been mapped on most of the potato chromosomes (Gebhardt and Valkonen 2001). Partner P02JEC is testing published markers in two segregating populations for the late blight resistance QTL on chromosome X. Two of the 29 tested markers are polymorphic between the parents. They are being tested for association with the resistance QTL. Partner P03GB is developing new SNP markers for interesting QTLs on chromosomes IV and V. Partner P02VL is mapping new PCR-based markers that are linked to late blight resistance QTLs.

Allelic Diversity of Cloned Resistance Genes and Haplotype Analysis

The sequencing of major resistance (*R*) genes and the development of molecular markers allow the analysis of the genetic variability of R loci and also allow searching for potentially favourable alleles and haplotypes.

Partner P14BV found sequence variation in the *Rpi-blb1* gene in *S. bulbocastanum* and *S. cardiophyllum*. For the putitative resistant (*PR*) allele 28 sequences were classified into 19 haplotypes, containing maximally eight out of 34 different SNPs. Several haplotypes were identified only once, so there are probably more. For the susceptible (*PS*) allele only one haplotype was found so far (Wang 2007).

Partner P19ER analysed candidate genes from Sub-project 2 which are co-located with QTLs for late blight resistance or differentially expressed after late blight infections in a set of wild species. Some partial cDNAs showed allelic variation of up to three alleles. No clear association between resistance behaviour and specific alleles was observed. In clones of Andean cultivated potato species some cDNAs showed 3–4 alleles in diploid material, indicating a second locus for the same gene. High resolution sequencing gels do not detect small SNPs. The sequencing of co-migrating bands revealed the true allelic variability. Efficient association analysis is hampered by co-migrating alleles being scored as identical.

Work Package 4.2

Collections of *Phytophthora infestans* Isolates

To phenotype allelic variance in resistance traits, representative collections of isolates of *Phytophthora infestans* are required. Partner P15EZ tested 41 PI isolates from Poland for mating type, metalaxyl resistance and virulence on Black's differentials as well as an additional seven potato genotypes that represent various resistance sources. Furthermore they were characterised by means of ten SSR markers. Six isolates were chosen for core collection and further studies of aggressiveness. They have been made available to the BIOEXPLOIT consortium. The two most aggressive ones will be proposed for the consortium to use in late

blight resistance. The Eucablight (www.eucablight.org) screening protocols were used.

Phenotyping Methods for Plants Derived from In Vitro Plantlets

Plants from in vitro plantlets have a different architecture and physiological status compared to the ones growing from seed tubers. Standard late blight resistance tests are not suitable for those plants. Partner P43JL validated existing phenotyping schemes for resistance to foliage blight to be able to screen potato plants derived from in vitro plantlets (e.g., transgenic plants). In the in vitro test, plantlets got quickly infected regardless of field resistance and maturity class. This test is therefore not suited. The detached leaf assay on greenhouse grown plants fitted better to the known field resistances. For all maturity groups, a good correlation with field derived data was obtained by using whole potato plants grown and infected in the greenhouse. Screening protocols are available at the Eucablight website (www.eucablight.org).

Phenotyping Promising Potato Material

Partner P02JEC phenotyped more than 400 clones belonging to 35 potato species for resistance to late blight. The material was screened in the field under natural conditions favourable to late blight. First results show highest resistance levels in clones of *S. berthaultii, S. bulbocastanum, S. hougasii, S. brachistotrichum, S. trifidum* and *S. stoloniferum*. Other sources of resistance might be specific clones from *S. tarijense* and the cultivated diploids *S. phureja* and *S. stenotomum*. The most resistant genotypes were re-tested in the greenhouse with well characterized isolates of *Phytophthora infestans*. The used detached leaflet test enables to distinguish four components of resistance: infection efficiency, lesion growth rate (the strongest component), latent period and sporulation capacity. Lesion growth rate was also determined in a stem assay. For each of the five components, genotypes carrying single component resistance have been detected. Partner P15EZ tested wild species, interspecific hybrids, breeding lines and some varieties using detached leaflets (based on the Eucablight protocol). High foliar resistance levels were detected in specific clones of *S. sparsipilum, S. vernei, S. simplicifolium* and *S. okadae*. For some material also tuber slices were tested. Some clones of *S. michoacanum, S. pinnatisectum, S. sparsipilum* and *S. chacoense* showed resistance in the tuber. Partner P35TG is screening clones for resistance to three different PI isolates by means of a stem test, in order to identify *R* genes.

Functional Analysis of New Homologues of *R* genes

Partner P14BV carries out allele mining experiments within Work package 4.1. He showed the presence of *Rpi-blb1* homologues in *S. stoloniferum, S. papita, S. polytrichon* and *S. fendleri*. The genes were denoted: *Rpi-sto1, Rpi-plt1, Rpi-pta1* and *Rpi-pta2*. Progenies of a cross between a *S. stoloniferum* clone containing the *Rpi-blb1* homologue and a susceptible *S. tuberosum* clone were evaluated for resistance. The results indicated a single dominant *R* gene. Further analysis showed that *Rpi-sto1* is located on chromosome VIII, like the *Rpi-blb1* gene in *S. bulbocastanum* (Wang et al.

2008). Offsprings of the populations Pta 04-323, Pta 04-325 and Plt 04-281 were tested on resistance. Almost 75% of the offsprings were resistant, indicating the presence of two genes. One of them, *Rpi-plt1*, is highly homologous to *Rpi-blb1* as indicated by the use of four specific primer pairs for *Rpi-blb1*. It is also located on chromosome VIII. The other gene is an unknown *R* gene. Based on partial sequences of the gene, three haplotypes were discovered. *Rpi-plt1*, *Rpi-pta1* and *Rpi-pta2* are highly homologous to *Rpi-blb1*. This supports the suggested synonymy of *S. papita* and *S. polytrichon* to *S. stoloniferum* (Spooner et al. 2004). Furthermore, the data suggest that *S. bulbocastanum* most probably is one of the progenitors of *S. stoloniferum* (Wang et al. 2008). *S. verrucosum* is considered to be the other progenitor (Pendinen et al. 2008).

Work Package 4.3

The first task in this work package was to help gathering available passport and evaluation data from preferred EU genebanks to support WP4.1 in the establishment of preliminary core collections (Partner P09RH). These data sets are also the starting point for the integrated database. For the subsequent development and deployment of the database, WP4.3 utilized the experience of participating groups, the EU project GENEMINE and the national UK project GERMINATE (Partners P03DM and P06AF). The GERMINATE scheme for example is able to cover many of the needs of the project, including the incorporation of sequence and map data. Developments in the Generation Challenge Programme (www.generationcp.org) on similar aspects are being closely observed. As a result, an integrated database has been constructed of passport, phenotypic and genotypic data of accessions carrying alleles associated with disease resistance, for the accumulation and dissemination of background information on the lines/accessions utilized in the project and the genotypic and phenotypic information generated by partner organisations. The basic structure of the GERMINATE model (http://bioinf.scri.ac.uk/germinate/) has been adapted to incorporate information for potato (and wheat). Access to the database is provided through a web interface. The public frontpage is http://germinate.scri.ac.uk/bioexploit, containing 7.2k tuber bearing *Solanum* accessions in the EU, including 28k phenotypic characterisation data (15.4% on late blight). A password protected frontpage has been created as a medium to share unpublished and confidential data within BIOEXPLOIT.

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