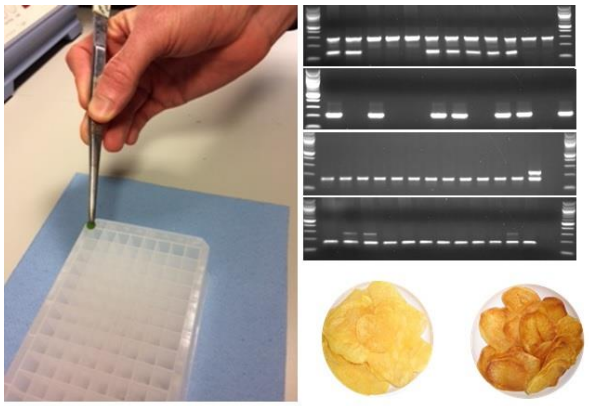
Project number: 6232
Funding source: Teagasc & IPM Potato Group
Date: Nov, 2018
Project dates: June 2012-June 2016

Biotechnology-based approaches for improved disease resistance in potato breeding



Key external stakeholders:
Plant Breeders, Potato Growers

Practical implications for stakeholders:
Late blight and potato cyst nematode (PCN) are major diseases of potato. Breeding resistant varieties is important to augment and/or replace existing chemical-based (?) control measures. This project provides the foundation to develop genome-based methods such as marker assisted selection (MAS) for resistance breeding in potato.

Main results:
- A major resistance locus to the PCN *Globodera pallida* pathotype Pa2/3 was precisely located on chromosome 4
- The use of this locus in combination with other loci in breeding PCN resistant potato was demonstrated
- Areas of the genome governing resistance to tuber blight were identified
- Tuber blight resistance was shown to be heavily influenced by environmental factors
- Insights were gained into the importance of the different layers of the tuber in resistance to late blight.

Opportunity / Benefit:
The results of the project will be exploited to develop varieties exhibiting better resistance to major pests and pathogens of potatoes – these will benefit Irish growers by augmenting or replacing other control methods

Collaborating Institutions:
UCD

Teagasc project team:
Dr. Dan Milbourne (PI)
Dr. Denis Griffin
Dr. Emmet Dalton
Dr. Marialaura Destefanis
Dr. Brian Rigney
Dr. Joseph Mulhare

External collaborators:
Dr Paul McCabe (UCD)
Dr Claire Cave (UCD)
Dr Vivian Blok (James Hutton Institute, UK)

Contact Dan Milbourne Email: dan.milbourne@teagasc.ie.

1. Project background:

This project sought to develop an understanding of specific sources of resistance to potato cyst nematodes (PCN) and late blight that would enable the development of genome based strategies for breeding resistant potato varieties.

PCN Resistance

The potato cyst nematode *Globodera pallida* pathotype Pa2/3 is one of the most significant soil-borne pests in the UK and the Mediterranean region. The ability of the cysts to remain dormant in the soil for decadal period's renders crop rotation less effective and chemical control is becoming less prevalent due to legislation limiting the use of nematicides. Because of these factors, developing potato varieties exhibiting high levels of genetically encoded resistance to PCN is a high priority for many potato breeding programmes, especially those targeting continental Europe. Unfortunately, no single resistance gene confers complete resistance to the most prevalent species of PCN in Europe, *G. pallida pathotype* Pa2/3. Resistance to this strain of the pest is mediated by genomic regions (quantitative trait loci or QTLs) conferring partial resistance to the pest. Developing varieties harbouring several partially effective QTLs (a process called "pyramiding") has been demonstrated as a viable strategy for achieving near complete resistance towards *G. pallida*. Over time, it has emerged that, in many cases, partially effective QTLs for disease and pest resistance are encoded by the same types of resistance (R-) genes that confer complete resistance to other pests and pathogens. The short arm of potato chromosome 4 harbours a complex locus for disease resistance where both QTLs as well as R-genes conferring resistance to late blight and potato cyst nematodes have been found. Significantly, previous work at Teagasc found a large effect QTL (called $GpaIV^{s}_{adg}$) for resistance to *G. pallida* Pa2/3 in this region of chromosome 4, in potato breeding material at Teagasc.

Blight Resistance

Upon release in 2004, the Teagasc bred variety Setanta displayed high levels of resistance in both foliage and tubers to infection from *Phytophthora infestans*. The arrival in Ireland and the UK in 2008 of the 13_A2 genotype of *P. infestans* has since defeated this foliage resistance. Preliminary experiments suggested resistance in the tubers of Setanta remains intact and is therefore independent of foliar resistance. Thus Setanta may remain a valuable source of tuber blight resistance for breeding.

2. Questions addressed by the project:

PCN Resistance

- Identify whether the region harbouring the $GpaIV^{s}_{adg}$ QTL also harbours "classical" R-genes?
- Is it possible to narrow down the region on the genome to identify the most likely candidates underlying the partial resistance to PCN?
- Can pyramiding $GpaIV^{s}_{adg}$ with another large effect resistance QTL provide resistance to multiple populations of *G. pallida* Pa2/3?

Blight Resistance

- What regions of the potato genome are involved in resistance to tuber blight (to older and newer, more aggressive, strains of blight)
- What is the relationship between different tuber layers in tuber blight resistance?
- What is the genetic basis of the "defeated" resistance to foliar blight in Setanta?

3. The experimental studies:

PCN Resistance

In previous studies we identified the presence of genes similar to the blight resistance gene *R2* in the $GpaIV^{s}_{adg}$ region. In order to better characterise the structure of this region, we took existing sequence information and improved the quality of the published potato genome sequence for this area, identifying a large cluster of genes exhibiting similarity to *R2*. We refer to these as *R2* gene homologues (R2GHs). We used this information to develop sequence-based genetic markers spanning the R2GH containing region. These markers were subsequently used in two genetic mapping populations involving crosses between the advance potato breeding line C1992/31 (containing $GpaIV^{s}_{adg}$) and *G. pallida* susceptible potato varieties. The same populations were also challenged by inoculation with a *G. pallida* population in order to identify

Contact Dan Milbourne Email: dan.milbourne@teagasc.ie.

resistant and susceptible individuals. The resistance phenotyping information was combined with the genetic mapping information to produce a high resolution genetic map of the region on which it was possible to identify a candidate region for the genes underlying the QTL. We created a genomic library that allowed us to isolate large DNA fragments from this region in a *GpaIV$^{s}_{adg}$* containing plant. Two of these fragments were subsequently sequenced to create a catalogue of candidate genes for *GpaIV$^{s}_{adg}$*.

In a final experiment, we used molecular markers to help create a population in which the QTLs *GpaIV$^{s}_{adg}$* (this study) and another QTL, *Gpa5* were segregating. This population contained equal quantities of individuals (25 plants each) with either *GpaIV$^{s}_{adg}$*, *Gpa5*, both QTLs and neither QTLs. We tested these for their ability to resist four UK-derived field populations of *G. pallida* pathotype Pa2/3.

Blight Resistance

To understand the genetic and physiological basis for Setanta's tuber resistance, we made a cross between Setanta and the Teagasc breeding clone, C1992/42 from which, 183 F_1 genotypes were generated. The population was genotyped using the Illumina Infinium 8303 Potato Array to construct a genetic linkage map of Single Nucleotide Polymorphism markers (SNP) to identify quantitative trait loci (QTLs) for resistance to blight.

To determine tuber resistance, field grown tubers of the population were inoculated in a range of assays over two consecutive years and also assessed for physiological traits. In the first year isolates of the 13_A2 genotype of *P.infestans* were used as inoculum and in the second year isolates of the 13_A2 and the previously predominant *P.infestans* 5_A1 population were used. Tuber assays assessed the periderm (skin), cortex (under the skin) and the medulla (middle) layers of tubers separately for resistance. In the second year, the 5_A1 population was also used to inoculate the foliage of the mapping population in an attempt to identify regions of the genome (QTLs) involved in the "defeated" foliar resistance of Setanta

4. Main results:

PCN Resistance

We identified and assembled a region spanning over 1 million nucleotides on potato chromosome 4 in the reference genome sequence of potato carrying a large cluster of R2GHs. These were organized into 4 distinct sub-clusters containing between 2 and 26 individual R2GHs. We demonstrated that this assembly was more accurate than the existing assembly in the potato genome reference sequence available at the time. This more accurate reference sequence was used as the basis to create genetic markers spanning the region. Utilising the two mapping populations described above, we developed a high-resolution genetic linkage map of the region. Combining this with phenotypic data allowed us to identify a region of approximately 250K nucleotides as the region most likely to contain the gene or genes underlying *GpaIV$^{s}_{adg}$*. We created a genomic library that allowed us to isolate two fragments of DNA spanning the majority of this region from a plant containing the QTL. We sequenced these fragments and identified the genes contained in the fragments. Two intact *R2* gene homologues (R2GHs) capable of producing NBS-LRR proteins of the type associated with pest and pathogen resistance were identified as the most likely candidates for the gene/s underlying the QTL. Further work is required to validate these candidates.

In the population segregating for both *GpaIV$^{s}_{adg}$* and *Gpa5*, we found that plants with both QTLs were extremely resistant, plants with either QTL showed intermediate resistance, whilst plants with neither QTL were susceptible. Some plants with both QTLs exhibited complete resistance, and in general, *Gpa5* was seen to have a stronger partial effect than *GpaIV$^{s}_{adg}$*. Interestingly, no differences in the relative effect of the QTLs were found across four different *G. pallida* field populations, indicating that the underlying resistance should be effective against a broad range of natural populations in the field.

Blight Resistance

Analysis found all resistance traits to be highly variable between years which indicated a strong interaction with the environment on the expression of these traits. No relationships between tissue layers for resistance were observed in 2013 but in 2014 a small significant correlation between periderm and medulla resistance was found. These resistance traits were associated with the physiological traits of dry matter and maturity. The periderm was the only layer that consistently exhibited resistance to both the 5_A1 and 13_A2 populations of blight.the only lay. Foliar resistance against a 5_A1 isolate of *P.infestans* was shown to remain in Setanta and segregate in the population in a detached leaflet assay in 2013 and 2014. The foliage results of the population were more consistent than the tuber results over years. A small but significant correlation between resistance in the tuber periderm layer to 5_A1 isolates and foliage resistance to a single

Contact Dan Milbourne Email: dan.milbourne@teagasc.ie.

5_A1 isolate in 2014 was found to be the only relationship between tuber and foliage resistance in this population.
QTL analysis found a number of small and variable QTLs across a number of chromosomes for all tuber traits, with some of these co-locating with physiological QTLs. Little consistency of QTL location across years was found, mirroring the low correlation of the observed phenotypes across years. Larger, more consistent QTLs for foliage variation were also found but they generally did not co-locate with the small effect tuber QTLs. This highlights the polygenic nature of each tuber resistance component in Setanta, and indicates that they are independent from Setanta's foliage resistance. Surprisingly, foliage resistance QTLs were also found to segregate from the C1992/42 parent but they also did not co-locate with any small effect tuber QTLs.

5. Opportunity/Benefit:

PCN Resistance

The location of the gene/s underlying *GpaIV*$^{s}_{adg}$ was narrowed down to a relatively small genomic region (~250K nucleotides). This will allow the development of genetic markers very closely linked to the underlying gene/s, which can be used in MAS strategies for selection for the QTL in breeding. Two potential gene candidates for the QTL were identified – further work may allow the cloning of the specific gene involved in the resistance phenotype for use in resistance breeding strategies. The finding that the QTLs *GpaIV*$^{s}_{adg}$ and *Gpa5* exhibited the same effect across multiple nematode field populations indicated their utility individually and in pyramiding strategies as an approach for broad spectrum resistance to *G. pallida* Pa2/3 in real world potato production.

Blight Resistance

The observations on the lack of relationship in resistance levels of different tuber layers will be beneficial for breeding programmes for screening and breeding for tuber blight resistance. The results demonstrated that different tuber layers react differently to blight, meaning care must be taken to choose the most relevant assay when assessing tuber blight resistance. Current assays frequently focus on the cortex – this may not be the most appropriate approach since the disease must first penetrate the outer layers. Unfortunately, because of the year to year variation, no targets for the development of markers for tuber blight resistance that could be deployed in MAS were identified.

6. Dissemination:

The results were disseminated via two PhD theses and a number of peer reviewed journal publications and at domestic and international scientific meetings. During the course of the project, the two Walsh Fellows also disseminated their results directly to Teagasc stakeholders by presenting their work to numerous visitors to the potato breeding programme (est. >200 persons pa), and at Oak Park Crops Open Days. Brian Rigney collaborated with the Teagasc KT programme to present a series of poster boards on good practice to prevent the spread of PCN to potato growers at the 2016 Potato Conference.

Main publications:

B Rigney, V Blok, D Griffin, E Dalton, D Milbourne (2016) Consistent action of two partially effective loci conferring resistance to *Globodera pallida* Pa2/3 across multiple nematode field populations. Plant Pathology 66 (6), 1031–1040

M Destefanis, I Nagy, G Bryan, D Griffin, B Rigney, I Hein, K Mclean, D Milbourne (2015) A disease resistance locus on potato and tomato chromosome 4 exhibits a conserved multipartite structure displaying different rates of evolution in different lineages. BMC Plant Biology 15 (1), 255

E Dalton, D Griffin, TF Gallagher, N de Vetten, D Milbourne (2013) The effect of pyramiding two potato cyst nematode resistance loci to *Globodera pallida* Pa2/3 in potato. Molecular Breeding 31, 4, 921-930.

B Rigney (2016) Characterization of the genetic architecture and interactions of loci conferring partial resistance to *Globodera pallida* Pathotype 2/3 in potato breeding germplasm. PhD Thesis, University College Dublin.

J Mulhare (2016) Differential expression of tuber and foliage resistance in the potato variety Setanta to the 5_A1 and 13_A2 genotypes of *Phytophthora infestans*. PhD thesis,University College Dublin.

Contact Dan Milbourne Email: dan.milbourne@teagasc.ie.

7. Compiled by: Dan Milbourne, Denis Griffin

Contact Dan Milbourne Email: dan.milbourne@teagasc.ie.