The Search For Nematode Resistance

Mapping The *H2* Gene

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Introduction

- British *Globodera pallida* populations consist of three pathotypes and unlike the *H1* gene in *G. rostochiensis*, no single gene has been discovered which confers resistance to all pathotype present.
- The best method to combat this parasite is to incorporate multiple potato cyst nematode resistance (*R*) genes into a single cultivar.
- The *H2* gene from the wild potato species *Solanum multidissectum* confers a high level of resistance to the Pa1 pathotype of *G. pallida*[1,2].
- The location of *H2* in the potato genome is unknown, the aim of this project is to map its location in order to create markers for breeding.

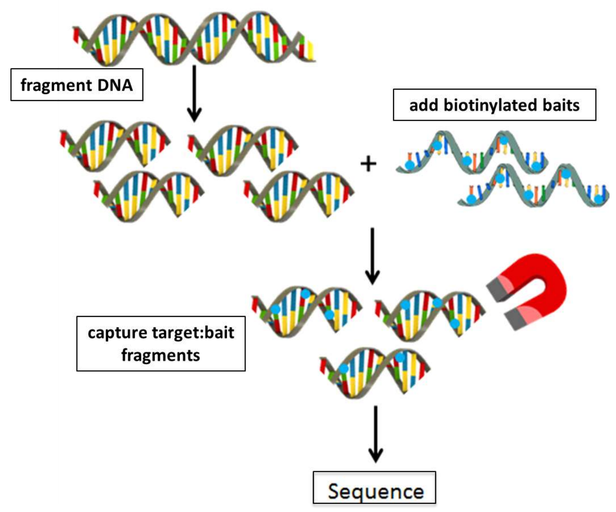
Methods

Bulk Segregant Analysis

Progeny from a Picasso (susceptible) x P55/7 (resistant) cross were infected with Pa1 cysts. Plants were grown for 8 weeks, then plant roots were scored for presence/absence of females

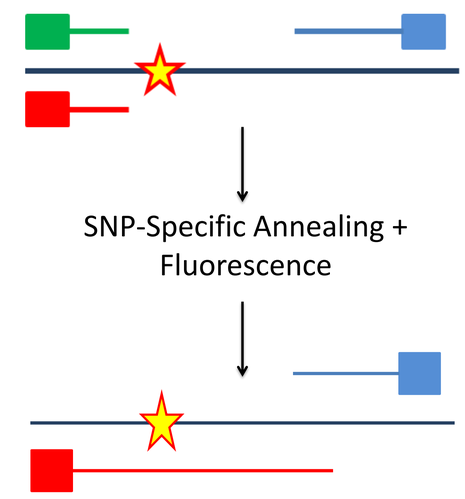
Gene Enrichment

DNA was extracted from the 20 most resistant and susceptible progeny, as well as Picasso and P55/7 plants, creating 4 pooled samples. Sequencing libraries were produced using a Target Enrichment technique (SureSelect^{XT})



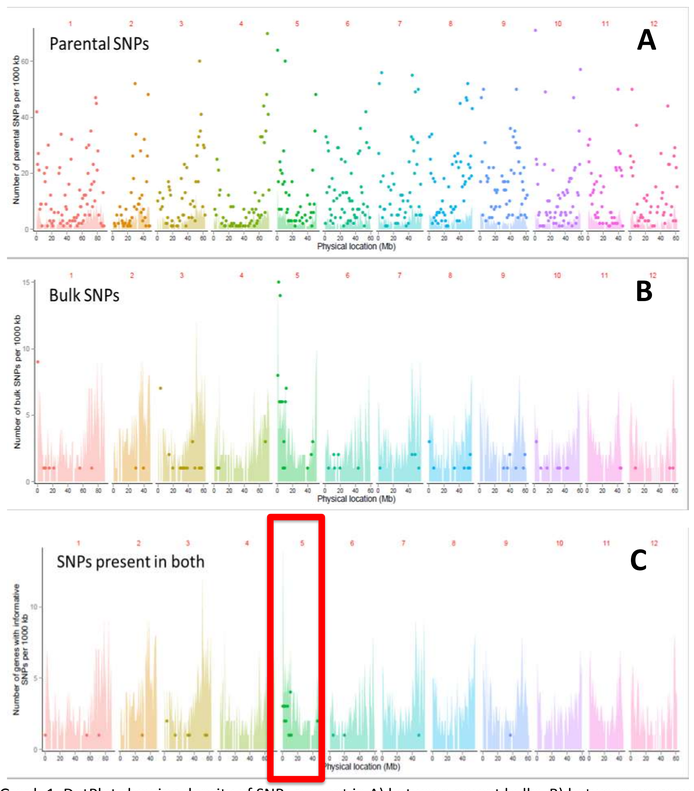
KASP Markers

KASP (Kompetitive Allelic Specific Primer) markers were designed based on the 12 informative SNPs (single nucleotide polymorphism) found on chromosome V.

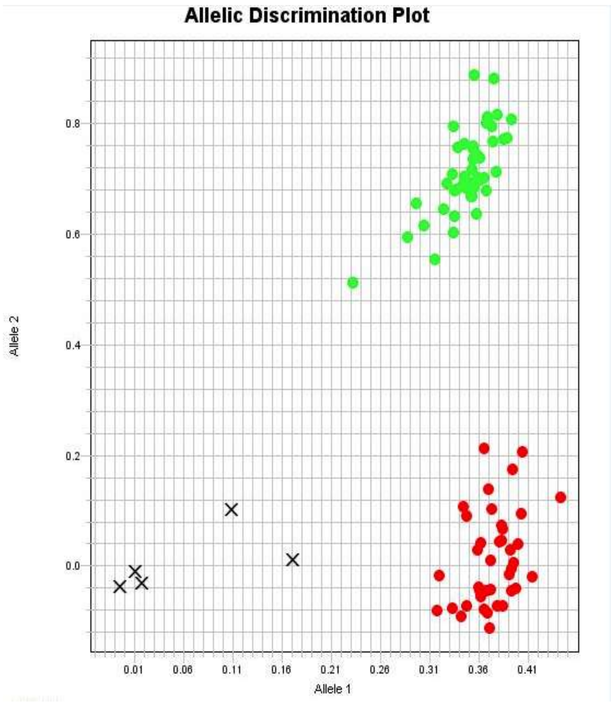


Results

Resistant progeny and parent were compared to susceptible progeny and parent (C, Graph 1). The highest number of informative SNPs were present on chromosome V, and so it can be confidently hypothesised that the *H2* gene resides within this chromosome.



Graph 1. DotPlot showing density of SNPs present in A) between parent bulks, B) between progeny bulks, and C) between resistant and susceptible. Each dot represents how many SNPs there are in a 1Mb area of the chromosome. The background spikes show the density of the enrichment target genes, also in 1Mb areas.



Graph 2. Allelic Discrimination Plot showing the clustering of resistant and susceptible samples based on the presence/absence of a single SNP

KASP markers were synthesised for each of the SNPs on chromosome V. Graph 2 depicts a typical allelic discrimination plot. Depending whether a SNP is present/absent dictates how samples cluster.

KASP Discrimination Plots were transformed into a more readable format (Table 1). As hypothesised resistant progeny show resistant alleles, and susceptible progeny show susceptible alleles. Progeny demarked with a star highlight that the *H2* gene must be in this 4.7Mb area where all susceptible progeny contain the susceptible allele.

	Picasso	P55/7	Resistant Bulk																Susceptible Bulk																
			11	66	84	87	91	107	108	110	130	132	138	139	152	155	157	158	180	181	6	7	8	17	40	45	47	55	57	65	72	93	100	104	131	135	168
ST04_03ch05_1416331	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
ST04_03ch05_1437827	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
ST04_03ch03_1438531	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
ST04_03ch05_3000757	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ST04_03ch05_4491040	-	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
ST04_03ch05_4737653	-	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	0	0	-	0	0	0	0	0	0	0	0	0	0	0	-	0
ST04_03ch05_6079232	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
ST04_03ch05_10615824	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1
ST04_03ch05_11253634	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1

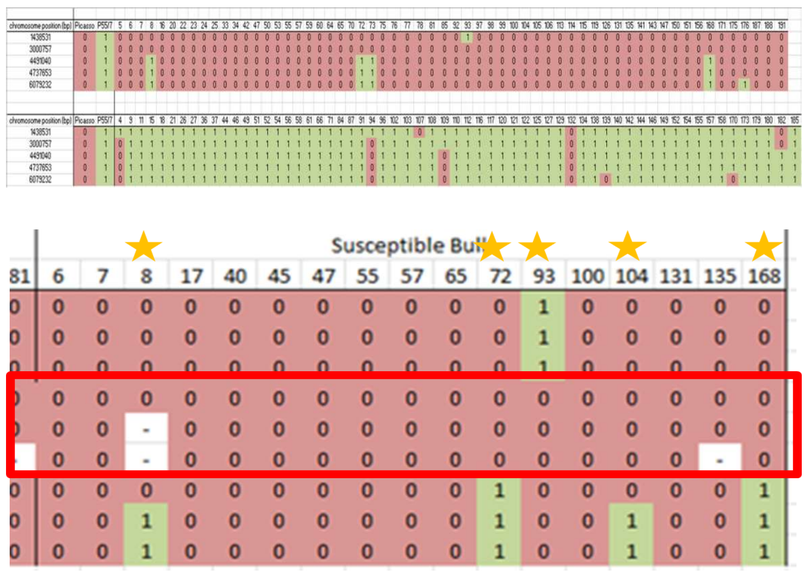


Table 1. Graphical representation of the distribution plots, from Graph 2, for each of the 12 SNPs from chromosome V. Samples labelled pink contain the susceptible allele, while samples labelled green contain the resistant allele.

Conclusions

- Based on the results in Table 1 the *H2* gene can be mapped to a 4.7Mb region of chromosome V
- Analysis of a further 1000 progeny plants from the Picasso x P55/7 cross will allow the area of interest to be narrowed further
- Candidate *R* genes identified in this region will be taken forward for knock-out experiments to determine whether they control the *H2* resistance phenotype
- Markers which are generated through this research will be able to determine whether a potato cultivar contains the *H2* gene, making them useful tools for commercial breeding

References
[1]Bryan *et al* (2002), Mapping QTLs for resistance to the cyst nematode *Globodera pallida* derived from the wild potato species *Solanum vernei*, Theor Appl Genet. [2]Bradshaw *et al* (1998), Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* in tetraploid potato, Theor Appl Genet.

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