Use of the Pyrosequencing technique to differentiate multiplex H1 status within potato breeding germplasm

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The H1 gene is extremely effective at reducing populations of the potato cyst nematode *Globodera rostochiensis* throughout the UK and Europe. An important breeding objective is to assess the copy number of the H1 gene in potato breeding lines, permitting clones possessing high dosages of the H1 gene to be identified and employed as breeding parents.

Triplex and quadruplex parental material give rise to 100% H1 resistant progeny. The identification of genotypes that are triplex and quadruplex for H1 is difficult and time consuming, requiring extensive resources during the lengthy process of phenotyping derived progenies from crosses between H1-bearing and susceptible parents.

Nematicide treated field strips

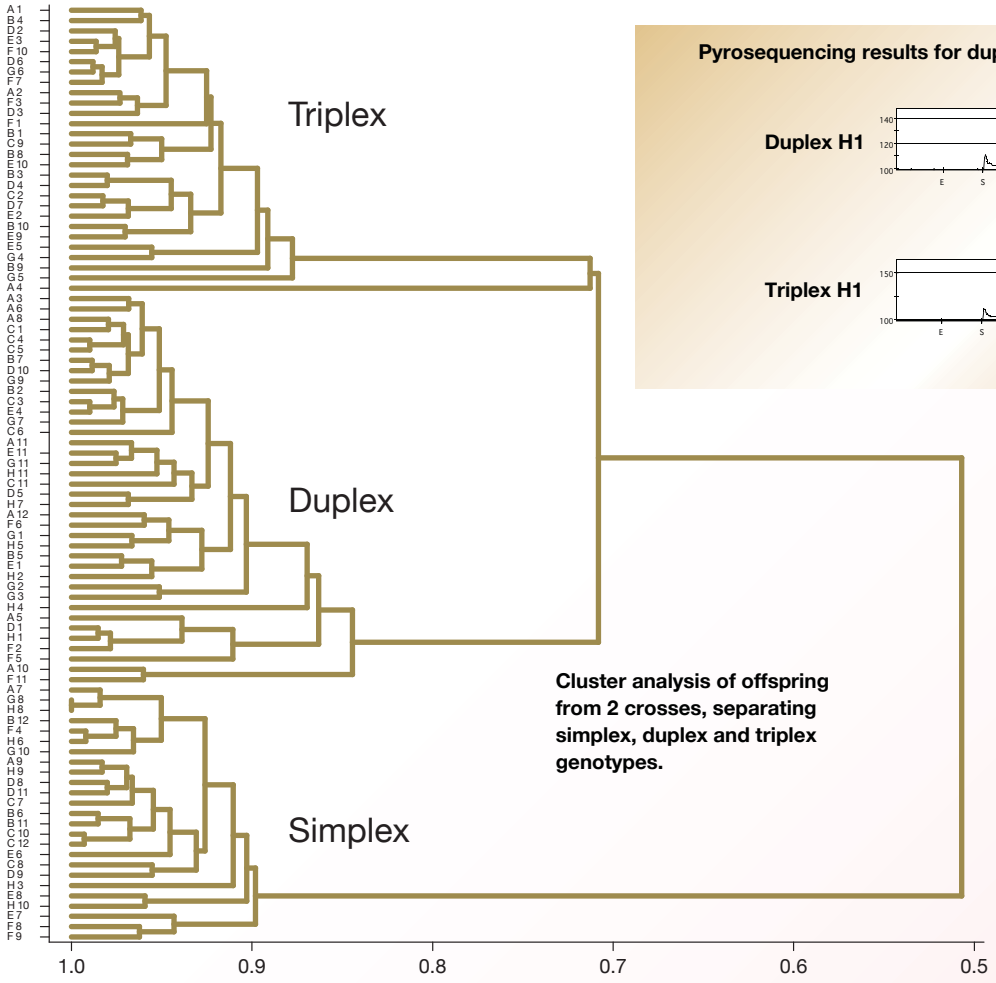
Method

Male parent - crossed to:	Susceptible parent	Genotypes of progeny	Phenotypes of progeny
Simplex = 1 H1gene	Nulliplex = 0 H1 genes	½ Susc, ½ Simpl	½ Susc., ½ Resistant
Duplex = 2 H1genes	Nulliplex = 0 H1 genes	⅙ Dup, ⅔ Simp,⅙ Susc	⅙ Susc., ⅚ Resistant
Triplex = 3 H1genes	Nulliplex = 0 H1 genes	½ Duplex, ½ Susc.	All resistant
Quadruplex = 4 H1genes	Nulliplex = 0 H1 genes	All Duplex	All resistant

We have utilised markers from previous H1 mapping and cloning efforts to develop quantitative single nucleotide polymorphism (SNP) markers flanking the H1 gene that can be used indirectly to measure gene dosage*. PCR products were sequenced from different H1 resistant and susceptible genotypes and comparison of sequence polymorphism data has been used to develop SNP markers whose dosage can be measured. We have applied a Pyrosequencing method to quantify relative levels of different sequence variants in a DNA sample to a range of material with known dosages of the H1 gene and to unknown seedling progenies to measure H1 gene dosage.

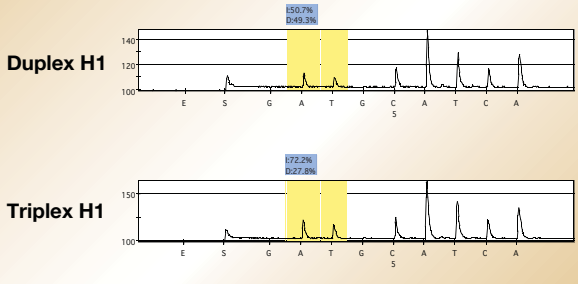
* Primer based on RFLP marker CP113, a 2bp deletion closely linked to the H1 gene (Gebhardt *et al* 1993. TAG 85 p541-544)

Results



Cluster analysis of offspring from 2 crosses, separating simplex, duplex and triplex genotypes.

Pyrosequencing results for duplex and triplex genotypes.



Results demonstrate:

- Potential to select multiplex parental material.
- Eliminates lengthy backcross programmes to test gene status.
- Accuracy will improve as further H1 gene sequence data becomes available.
- Potential to apply pyrosequencing to other major genes of resistance to viruses Y and X.

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