Efficiency of molecular markers associated to *H1*, a major gene to control *Globodera rostochiensis*

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Key messages

Several published markers are available for the marker assisted selection (MAS) of the *H1* gene. Using a panel of INRAE breeding lines (see presentation n°117), a SolCAP marker also diagnostic for *H1* was identified. In the Geconem French research project C-2018-07 (see presentation n°401), we tested the available *H1* markers on several potato panels to update our MAS toolbox. Two markers (solcap_snp_c2_55239 and 57R) outperformed the others in characterizing the presence of *H1*. Both markers flanked the gene and can be confidently used in MAS.

6 markers were tested for their suitability for MAS

The 57R and TG689 markers were used as references and were therefore screened in the different panels we used.

Tab.1: Markers diagnostic for *H1* compared in this study.

MARKERS	TECHNOLOGY	SOURCE
Kasp H1_mlb	PACE/KASP	Meade *et al.* (2020). Potato Res. 63: 57-73
Solcap_c2_55239	PACE/KASP	Geconem project C-2018-07
TG689_1P & 57R_1P	HRM	Meiyalaghan *et al.* (2018). Mol. Breed. 38: 79
TG689	Standard PCR	Schultz *et al.* (2012). Plant Breed. 131: 315-321
57R	Standard PCR	Finkers-Tomczak *et al.* (2011). TAG 122: 595-608

Results obtained using HRM

57R_1P and TG689_1P (Tab. 1) were sequencially tested using the 3 commercial HRM kits (see M&M) on the polymorphic set of 16 varieties. We searched for polymorphism (curve shapes) corresponding to the *H1* presence in the positive controls (Fig. 1).

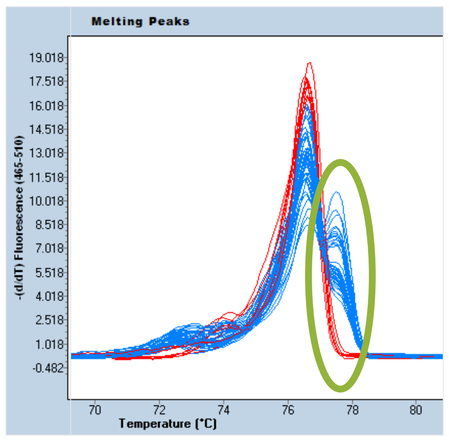


Fig. 1 Genotyping results obtained using the 57R_1P marker (Tab. 1) on 48 genotypes of the GWAS panel (see M&M) using the HRM Qiagen kit. Red curves correspond to susceptible genotypes whereas blue ones correspond to resistant ones. Green ellipse figures the polymorphism we can use to detect the resistant genotypes.

A strong impact of the kit was observed which confirmed already communicated results (Méar *et al.,* 2018) (Fig. 2).

For the marker TG689_1P, the Agilent kit performed best (not shown), whereas for the 57R_1P marker, the Qiagen kit was the easiest to score (Fig. 2).

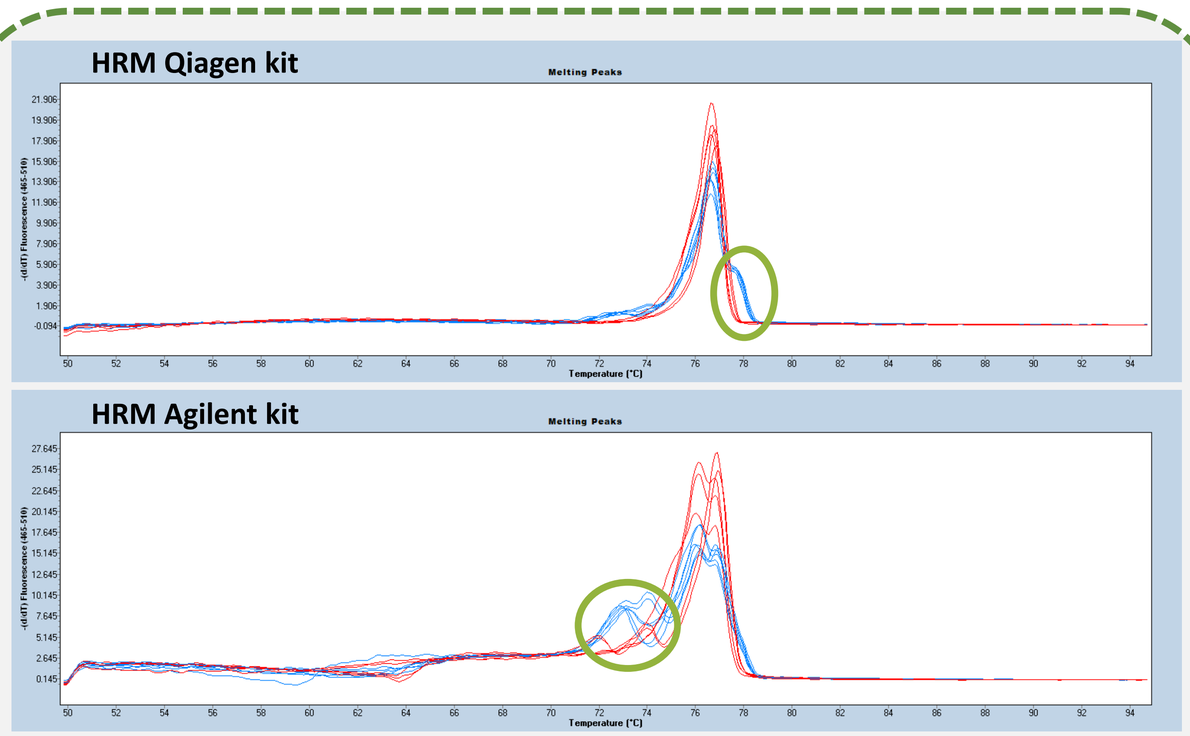


Fig.2 Impact of the HRM commercial kit on the 57R_1P marker. Green ellipses highlight the polymorphism we use to detect the resistant genotypes.

Material and Methods

Plant material

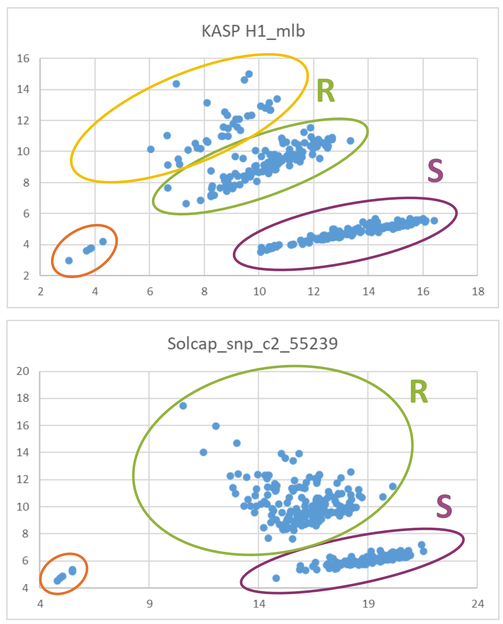
- 16 resistant or susceptible varieties were used for the initial steps of the tests.
- 284 genotypes of a GWAS panel (see presentation n°120) were genotyped with the most interesting markers. The phenotype was known for 207 of these genotypes.

Genotyping procedures and reagents

- HRM: Qiagen Type-it HRM PCR kit, Agilent Technologies Brilliant HRM Ultra-Fast Loci Master Mix and Roche Lightcycler 480 High Resolution Melting Master kit.
- PACE Genotyping Master Mix Low ROX – 25nM (3CR Bioscience).
- Standard PCR: GoTaq G2 Hot Start Polymerase (Promega).

HRM and PACE genotyping were performed using a Lightcycler 480 Roche whereas standard PCR products were revealed using a Qiaxcel Advanced (Qiagen).

Results obtained using PACE (KASP analog) technology



Both PACE/KASP markers performed quite well on our GWAS panel (Fig. 3).

Solcap_snp_c2_55239 and 57R are the closest and flanking *H1* (Fig. 4).

Fig. 3 Results from the 2 tested PACE/KASP markers in the GWAS panel. S = resistant allele of the marker absent, R = resistant allele present in at least one copy.

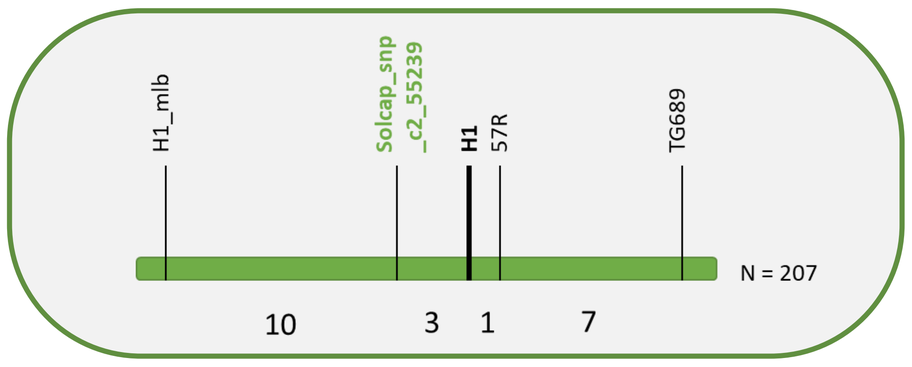


Fig. 4 Genetic map of the *H1* locus in 207 genotypes of the GWAS panel. Numbers below refer to the number of recombinants.

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References
Méar, A., A. Barbary, H. Corre, J. E. Chauvin, Y. Le Hingrat and S. Marhadour (2018). Comparison of HRM Derived Techniques and Standard PCR Diagnostic Markers Used in MAS for Resistance Genes in Tetraploid Potato. 19th triennial meeting of the EAPR section "Breeding and varietal assessment", Rostock, Germany.