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# Marker-assisted selection of QTL *PiXspg* in potato diploid backcross populations

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**SUMMARY**
Using a stem assay, a major QTL originating from the wild potato relative *S. spegazzinii* has been identified and mapped to chromosome X. Named *PiXspg*, it explains between 30 to 40% of the phenotypic variation for the stem resistance component. A fine mapping of *PiXspg* has been carried out, leading to the development of two SSR markers (SSR223 and SSR74) and three CAPS markers (P10c8, TG403F1 and P8h11) that are closely linked to the QTL. The usefulness of these markers in a marker-assisted selection program has been evaluated in four diploid backcross populations. A good correlation between the phenotypic data and the genotypic data has been observed for three backcross populations. However, as the presence of *PiXspg* is not always correlated with stem resistance, it is likely that other genomic regions and/or epistatic interactions are involved in the expression of this trait.

**KEYWORDS**
Potato, Late blight resistance, Quantitative Trait Loci, *S. spegazzinii*, Marker-assisted selection

## INTRODUCTION

As most of the R-genes identified in *Solanum* wild species have been overcome by *Phytophthora infestans*, the UMR APBV team aims at identifying and studying quantitative trait loci (QTL) involved in late blight resistance.

A segregating population (96D32) obtained by crossing a susceptible dihaploid *S. tuberosum* clone (Rosa H1) with a resistant clone belonging to the wild potato relative *S. spegazzinii* has been studied for late blight resistance using a stem assay. A major QTL originating from the wild species has been identified. Using genetic map developed by Caromel (2004), this QTL has been mapped to chromosome X and is named *PiXspg*. It explains between 30 to 40% of the phenotypic variation for the stem resistance component. A fine mapping of *PiXspg* has been carried out, leading to the development of two SSR markers (SSR223 and SSR74) and three CAPS markers (P10c8, TG403F1 and P8h11) that are spread over a 15 cM region including the QTL (Quélennec *et al*., 2009). The objective of this study is to evaluate the usefulness of these markers in a marker-assisted selection program.

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## MATERIALS AND METHODS

Among the 96D32 population, four clones (96D.32.6, 96D.32.26, 96D.32.66 and 96D.32.124) which carry *PiXspg* and show a good level of resistance in field assay were selected. They were crossed with a susceptible dihaploid *S. tuberosum* (Caspar H3), leading to the obtention of four backcross populations (06D.23, 06D.24, 06D.27 and 06D.29 respectively). These populations, which comprise 137, 149, 136 and 140 clones respectively, were evaluated for stem resistance using a stem assay as described by Danan (2009). The following variables were studied: L32 (Necrosis length 32 days after inoculation), REC (Receptivity) and IND (Inductibility).

The parents and 6 clones of each of these populations were genotyped with the SSR markers SSR223 and SSR74 (SGN database) and the CAPS markers P10c8/TaqI, TG403F1/CfoI and P8h11/HaeIII. When the markers were polymorphic, they were used on the entire population. For the CAPS markers, specific primer pairs were designed based on the DNA sequence available in the PoMaMo database using Primer3 program. PCR amplifications were performed in a PTC200 thermal cycler (Bio-Rad).

Concerning the SSR markers, each 10µl PCR reaction volume contained 1X buffer (Promega), 2 mM MgCl2, 150 µM dNTP, 0.1 µM M13-tailed forward primer, 0.2 µM reverse primer, 0.1 µM IRD 700-labeled M13 tail, 0.35 U Taq DNA polymerase (Promega) and 20 ng of genomic DNA as template. The cycling protocol consisted of an initial denaturation step at 94°C for 4 min, then 12 cycles of denaturation for 30 s at 94°C, annealing for 1 min from 65°C to 54°C (-1°C per cycle) and extension for 30 s at 72°C, then 25 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 53°C and extension for 30 s at 72°C, followed by a final 10 min extension step at 72°C. Fluorescence labeled fragments were separated on a LI-COR DNA Analyser using 5.5% acrylamide gels.

Concerning the CAPS markers, each 17µl PCR reaction volume contained 1X buffer (Promega), 2 mM MgCl2, 150 µM dNTP, 0.3 µM of each of the primers, 0.03 U Taq DNA polymerase (Promega) and 20 ng of genomic DNA as template. The cycling protocol consisted of an initial denaturation step at 94°C for 3 min, then 30 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 55°C and extension for 1 min 30 s at 72°C, followed by a final 10 min extension step at 72°C. After digestion with restriction enzymes, ethidium bromide-stained PCR fragments were visualized on 1.5%-agarose gels.

Goodness-of-fit between observed and expected segregation ratios at marker loci was tested by a chi-square analysis. A one-way analysis of variance (GLM procedure, SAS software) was used to test the correlation between the phenotype and the genotype.

## RESULTS AND DISCUSSION

As the CAPS marker P10c8/TaqI is linked to the susceptible allele of the *S. spegazzinii* parent, it can not be used in backcross populations. The CAPS marker TG403F1/CfoI was monomorphic in the four backcross populations. The CAPS marker P8h11/HaeIII was polymorphic in two populations: 06D.24 and 06D.27. The two SSR markers were polymorphic in all four populations. According to chi-square tests, the segregation of these molecular markers in the backcross populations did not deviate significantly from to the 1:1 expected ratio (Figure 1).

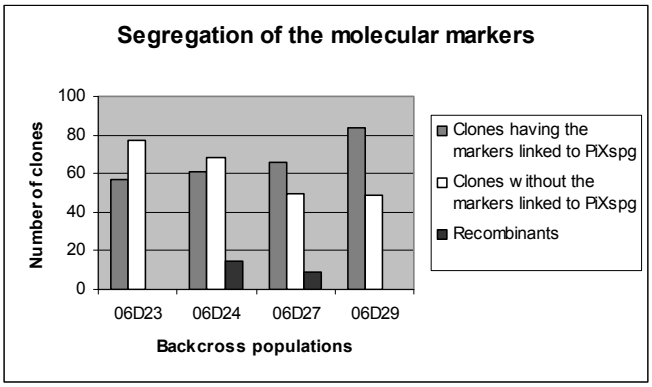


**Figure 1:** *Segregation of the molecular markers in the backcross populationfor 06D.23 and 06D.29, segregation of SSR74; for 06D.24 and 06D.27, segregation of SSR74 and P8h11*

The analysis of variance showed that in populations 06D.23 and 06D.24, the clones having the markers linked to *PiXspg* have significantly lower values of L32 and IND than the clones without these markers (Table 1). In population 06D.29, this is observed only for L32. In population 06D27, no significant correlation is observed.
In population 06D24, the presence of the markers is associated with lower REC values whereas no QTL for REC was detected in the 96D32 population.

**Table 1:** *Mean values of L32 (cm), IND (cm/day2) and REC (cm/day) according to the presence or not of the markers linked to PiXspg and results of the ANOVA. ***, **, *: significant at the 0.001, 0.01, 0.05 probability level respectively*

| Backcross population | Variable | Clones having the markers linked to PiXspg | Clones without the markers linked to PiXspg | ANOVA | |
|---|---|---|---|---|---|
| | | | | F | $R^2$ |
| 06D23 | L32 | 5.21 | 7.58 | **11.67**** | 0.06 |
| | IND | 0.008 | 0.017 | **8.70**** | 0.05 |
| | REC | 0.07 | 0.06 | 0.05 | 0 |
| 06D24 | L32 | 4.25 | 6.04 | **16.36***** | 0.12 |
| | IND | 0.007 | 0.012 | **2.95*** | 0.02 |
| | REC | 0.05 | 0.1 | **15.88**** | 0.12 |
| 06D27 | L32 | 4.91 | 5.68 | 1.7 | 0.02 |
| | IND | 0.008 | 0.011 | 0.83 | 0.01 |
| | REC | 0.07 | 0.07 | 0.03 | 0 |
| 06D29 | L32 | 6.32 | 7.47 | **4.27*** | 0.02 |
| | IND | 0.013 | 0.017 | 2.13 | 0.01 |
| | REC | 0.07 | 0.07 | 0.45 | 0 |

## CONCLUSIONS

In 3 out of the 4 studied backcross populations, the molecular markers linked to *PiXspg* appear to be useful to predict the late blight stem resistance of the clones. The usefulness of these markers will also be evaluated for marker-assisted selection at the tetraploid level.

However, as the presence of *PiXspg* is not always correlated with stem resistance, it is likely that other genomic regions and/or epistatic interactions are involved in the expression of this trait.

## REFERENCES


Caromel B., 2004. Cartographie génétique et étude de QTL conférant la résistance au nématode à kyste *Globodera pallida* (Stone) chez la pomme de terre (*Solanum tuberosum ssp. tuberosum* L.). PhD Thesis, Université de Paris XI Orsay, France.

Danan S., 2009. Diversité structurale des locus de résistance à *Phytophthora infestans* chez la pomme de terre et synténie chez les Solanacées. PhD Thesis, Supagro Montpellier, France.

Quélennec R., Esnault F., Mauger S., Pellé R., Dantec J.P., Chauvin J.E., 2009. Marker-assisted selection of quantitative late blight resistance originating from potato wild species. Scientific BioExploit Meeting, 31 March – 1 April, Wageningen, The Netherlands. (Poster).