Development of markers for potato genetics and breeding

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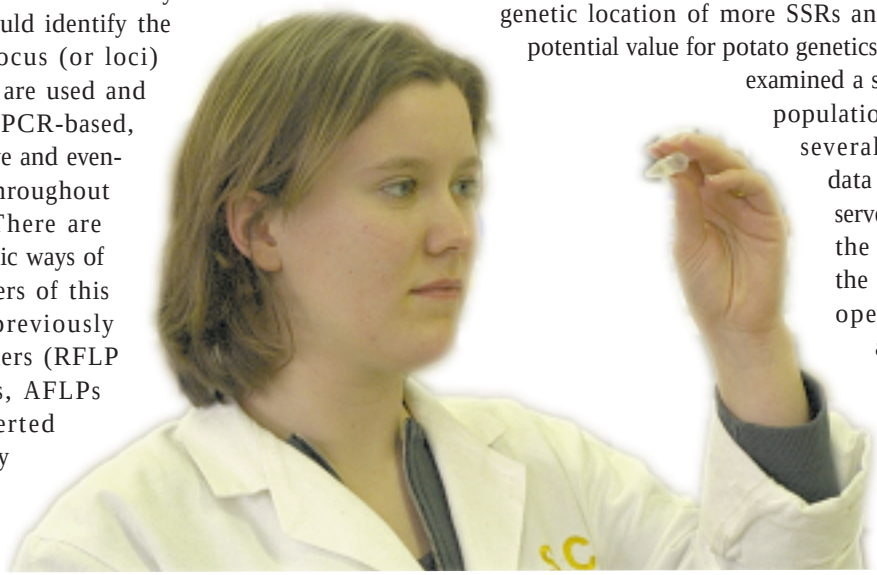
In practical terms, the adoption and more efficient application of DNA-based molecular markers in marker assisted selection (MAS) schemes, particularly by non-molecular biologists, would be increased if the markers exhibited a combination of features which made them more user-friendly. Ideally, they should identify the same defined locus (or loci) every time they are used and they should be PCR-based, highly informative and evenly distributed throughout the genome. There are presently two basic ways of obtaining markers of this type. Either previously identified markers (RFLP probes, RAPDs, AFLPs etc.) are converted into such an assay (usually called CAPs), or a new set of markers, with all of these attributes, is developed *de novo*. If polymorphic, these could then be used in any population to monitor the inheritance of a specific chromosomal segment in crossing programmes or to evaluate the variation available in the genepool at that particular locus. If that chromosomal segment was associated with a trait, then the marker could be used to indirectly follow the inheritance of the trait. As the level of polymorphism associated with CAPs markers is usually low and the assay involves a post-PCR enzymatic cleavage step, we chose the *de novo* option by developing a class of markers from the potato genome which are known as microsatellites or simple sequence repeats (SSRs). SSRs are a tried and tested marker assay. They are the principal assay used in human and animal genetics, largely because they are abundant, and have a high information content. As such they have a high subsequent value for genetical analyses. Their adoption in plant genetics has been slow because, until recently, they have been difficult to characterise from plant genomes in sufficient numbers to make them really useful for the majority of potential applications.



Previously, we reported the genetic mapping of 47 SSR loci in a reference diploid potato mapping population (*Ann. Rep. 1996/97*, 96-98). To determine the genetic location of more SSRs and assess their potential value for potato genetics, we have now examined a second diploid population which had several years trait data scored. This served to confirm the usefulness of the already developed SSRs and allowed associations with desirable characters in potato breeding programmes to be identified. In this case, the traits examined included partial resistance to leaf and tuber blight, tuber characteristics, maturation type, yield and other characteristics. Segregation data were obtained for 67 SSR loci and analysed alongside those derived from other marker types. Twenty-four of these, which were also mapped in the reference population, were used for linkage group assignation and orientation. In total, 90 discrete SSR loci have now been mapped. They are located on all 12 potato linkage groups and provide a significant and convenient alternative to RFLPs for future linkage studies in potato. By combining SSR-based assays with multiplex assays such as AFLPs, chromosome-designated and orientated-linkage maps can quickly be produced using only PCR-based markers. The genetic locations of the SSRs in the two populations are shown in Figure 1.

Having constructed a linkage map, a quantitative trait locus (QTL) analysis was performed using the map

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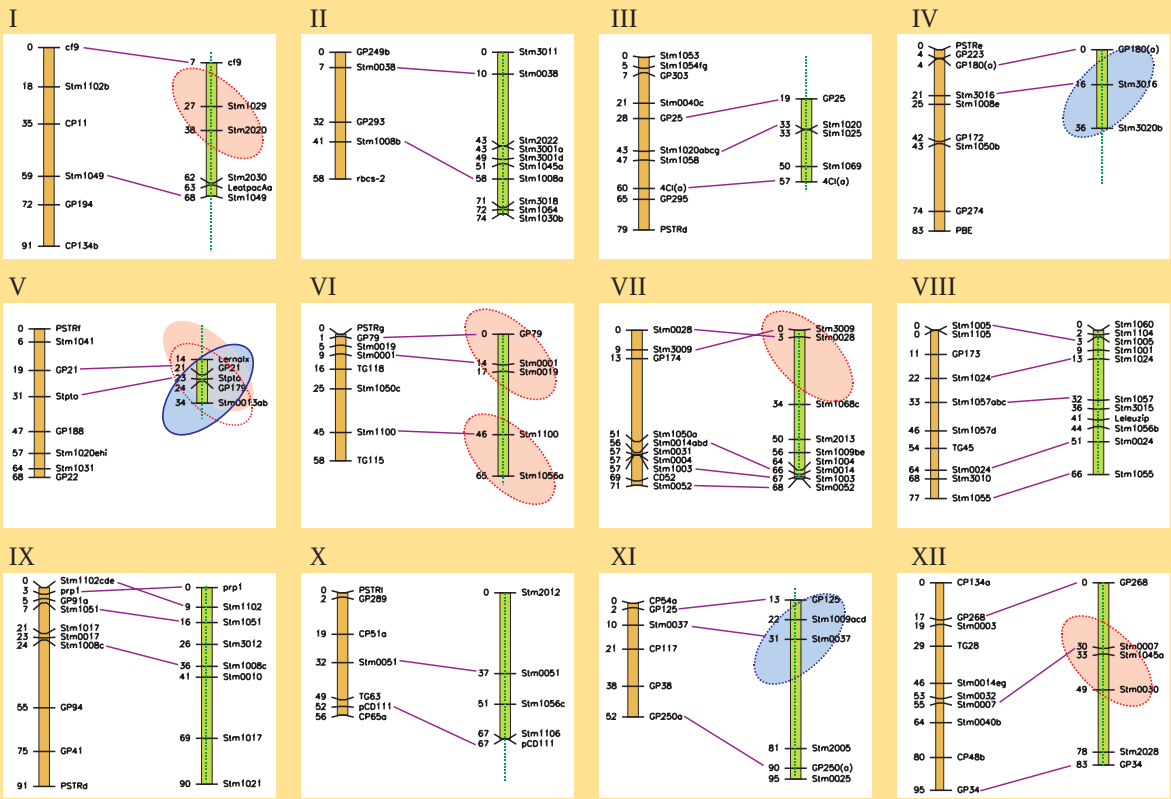

Figure 1 Location of SSRs on a genetic linkage map of potato. LG's I - XII are indicated. The maps on the left of each pair are the 'reference' population. Only a few of the >400 loci mapped on this population are shown. The markers designated PSTR are sub-telomeric repeats indicating the ends of linkage groups. The maps on the right show the position of the SSRs on the QTL mapping population. Only the SSRs (from >360 markers in total) are shown. Dotted lines show the extent of the maps of this population when all markers are included. Arrows indicate the position of the common loci mapped in both populations.
The regions of the genome associated with resistance to late blight in the QTL population are highlighted. Solid ellipses are QTL-detected in all seasons, dotted ellipses in a single season. Those originating from the male parent (susceptible) are in blue, and the female (resistant) parent in red.

and the assembled trait scores. Loci affecting resistance levels to leaf and tuber blight, earliness, plant vigour and other characters were detected on a number of linkage groups. The most significant QTLs for leaf blight were detected on linkage groups V and VI from both the male and female parents. The QTL from the male parent was significant over all three seasons in which data were collected. Interestingly, major QTLs for plant vigour and earliness were coincident with the major QTL (from the susceptible parent) for late blight on linkage group V. Informative SSR markers spanning these regions have been identified.

The major QTLs were at approximately the same position as those detected in previous studies on diploid populations[1], suggesting a fairly robust association of these regions of the genome with quantitative resistance to late blight. The most significant QTL for LB resistance is in the same region of the genome as the R1 resistance gene which is effective against pathotypes of *P. infestans* expressing *avr1*. The coincidence of QTLs for LB resistance with those for earliness and plant vigour may suggest that the mechanism of resistance in this population at the locus on LG V is a consequence of genes controlling physiological factors which affect the ability of the pathogen to develop a successful lesion. This conclusion is consistent with the well-known correlation between earliness and susceptibility to natural infection by late blight in the field. While this is potentially contributing to the overall quantitative resistance level of any given line, the data are also consistent with the presence of additional resistance factors (e.g. on the top of LG's IV, V and VI). Our studies on partial resistance to late blight at the tetraploid level (the ploidy level at

which the majority of potato breeding is practised) have not, to date, identified a QTL associated with resistance on chromosome V - even though the population exhibits a spectrum of maturity types. Rather, the major component of resistance is located on linkage group IV, in the same location as an environmentally sensitive QTL found here. In the tetraploid studies, the SSR markers have been particularly useful in identifying specific chromosomal segments and providing multi-allelic bridges between linkage groups assembled using mono-allelic single dose markers (AFLPs). This has allowed us to identify the location of components of $H3_{(adg)}$ derived horizontal resistance to *G. pallida* (Pa2/3) at the tetraploid level and has provided a platform for developing strategies ultimately to clone the component genes.

Thus, SSR markers are a particularly attractive tool for examining allelic variation. They have a high probability of directly detecting polymorphism in any potato cross and, as such, can be used simply and effectively to evaluate allelic composition or haplotypes at mapped SSR loci. Their potential for use in MAS in diploid and tetraploid potatoes is currently being evaluated. The results so far look encouraging.

Reference

[1] Leonard-Schippers, C., Gieffers, W., Salamini, F. & Gebhardt, C. (1994). *Genetics* 137, 67-77.