

CHAPTER 45

APPLYING MOLECULAR GENETIC MAPS TO GERM PLASM ENHANCEMENT OF POTATO

M. Bonierbale*, O. Pineda†, and C. Yencho†

Comparative Genome Mapping

Genome mapping in potato took advantage of the similarity between two related crops in the Solanaceae family. Tomato is a diploid species with a long history of genetic studies, a wealth of genetic stocks, and saturated maps of classical and molecular markers. Potato is a heterozygous tetraploid, subject to inbreeding depression, and, as such, has undergone limited classical genetic analysis. The tomato and potato share a basic chromosome number of 12, and their karyotypes are very similar.

Similarities between tomato and potato at the DNA level allowed a comparative study of their genome organization and the efficient development of a potato genetic map. Restriction fragment length polymorphism (RFLP) analyses of 150 DNA probes from the tomato genetic map were used to map the potato genome. Nearly all single-copy tomato probes were homologous and polymorphic in the interspecific potato mapping population used. Thus, a potato genetic map could be based on tomato markers, bypassing the need to construct a potato DNA library. Comparative mapping showed a very high degree of linkage conservation between the two genomes. The chromosome contents are entirely conserved, with only five apparent differences—each an inversion of a chromosome segment.

Relationships among Genetic Resources

The homology of molecular markers across species and genera permits assessment of similarity among genetic resources. The first application of the potato map was to compare 200 germ plasm accessions, representing 18 species, used in potato improvement. Evaluation of the proportion of shared restriction fragments leads to a similarity index, used to construct a dendrogram. Analysis of 30 loci led to the depiction of relationships within and among species. The resulting information is useful for formulating strategies for germ plasm

* CIAT, Cali, Colombia. Now at the Centro Internacional de la Papa (CIP), Lima, Peru.

† Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY.

conservation and use. The similarity between tomato and potato also points out that the genetic resources of one crop may be considered as available to the other.

The basic demands for a potato genetic map were, however, more practical. Originally, there were two, dealing with constraints to the applied improvement programme being conducted by the breeder, entomologist, and pathologist at Cornell University. One was the breeder and pathologist's interest in screening for resistance to a quarantine pathogen (the golden nematode) in the absence of the pest. The second came from complications encountered during introgression of a complex type of insect resistance from wild germ plasm.

Gene Localization

The golden nematode is a serious pest with restricted distribution in North America and Europe. Host-plant resistance the most effective control is conferred as immunity by the single dominant gene H_1 from Latin American germ plasm. This gene is used in all breeding programmes where the pest is present and in fact is legally required as a component of new cultivars for the New York State. Deployment of the H_1 gene in potato cultivars significantly reduces nematode populations, and it provides a level of crop protection equivalent to the application of pesticides. In association with breeding and research programmes, the pest is handled only under strict quarantine conditions, and screening for resistance is slow and expensive.

We used a breeding population segregating for resistance to tag the H_1 gene with a molecular marker. This marker can be used to discriminate resistant from susceptible plants without introducing the pest. A population of 80 individuals from the experimental population was screened for resistance by exposure to the nematode, resulting in the expected 1:1 ratio of susceptible to immune plants. The parents and groups of resistant and susceptible individuals were then characterized for RFLPs, using markers from the potato molecular map, until one was found that showed polymorphism between resistant and susceptible types. This marker and the restriction enzyme *Dra*I were then used to compare the original donor (CPC 1673) with the immediate parents and screened progeny individuals. A good correlation between the resistant phenotype and the molecular genotype was found. This marker from chromosome 5 shows tight linkage to the resistance gene. Publication of this work led to interest by the New York State Department of Agriculture and Markets, which enforces the deployment of the H_1 gene. The Department is now funding the development of a PCR-based assay for the resistance marker.

To date, more than five major genes for resistance have been tagged with RFLP markers in potato. Applications of markers for major genes include (1) screening in the absence of a pest or disease agent; (2) identifying parents with the greatest genetic value either with high allelic plex levels in the case of polyploids, or with limited amount of linkage drag; and (3) checking the independence or identity of similar genes from different germ plasm sources. The latter is useful in efforts to pyramid different genes towards durable resistance.

Quantitative Trait Analysis

The second leading pest of potato of local importance is the Colorado potato beetle (CPB). As is common in many regions of the world, the principal control method for this and other potato insects is the use of systemic pesticides. Both in production fields and experimental plots, damage can be severe, yet the use of pesticides is restricted because they contaminate ground water. In important potato-growing regions of New York State, the use of the leading insecticide is no longer permitted. Host-plant resistance is a more viable solution but, in this case, not a simple one.

Very effective resistance was identified in wild germ plasm, including the Bolivian diploid *Solanum berthaultii*. Resistance from this source is conferred largely by glandular trichomes on the foliage, giving this germ plasm the nickname of "the hairy potato". Characterization of the resistance mechanism by entomologists and biochemists has defined the chemicals involved and their effects on insect biology. Two types of trichomes (A and B) produce distinct secondary compounds: a sticky exudate and an enzyme that results in an oxidative browning reaction. The resistance reduces fecundity in the CPB and entraps small insects such as aphids. Quantitative biochemical assays have been developed for each compound and are used in the breeding programme to enhance screening efficiency.

Trichome-mediated insect resistance results in broad-spectrum resistance at a level equivalent to that provided by the systemic insecticide. However, because this resistance is genetically complex, it has been difficult to use it fully in the breeding programme. Two principal difficulties have been (1) the low frequency of recovery of the entire mechanism(s) in a single clone, and (2) an undesirable linkage between the sucrose droplet of B trichomes and the short-day requirement of the donor species. Neither the donor species nor early generation resistant selections from the breeding programme are adapted to tuberization under the long days of temperate growing seasons. For these reasons it was decided to attempt dissecting the resistance mechanism into simpler components by genetically mapping and tagging important chromosome segments for a more efficient incorporation of resistance into adapted germ plasm.

Two segregating diploid populations were developed from a hybrid between *S. tuberosum* and *S. berthaultii*. One was a backcross to potato (*S. tuberosum*) and the other to the resistance donor (*S. berthaultii*). The progenies of 150 and 300 individuals, respectively, were characterized for trichomes, tuberization, and insect resistance phenotypes. A subset of 150 clones from each cross was selected to include phenotypic extremes and the two subsets characterized for RFLPs, using 80 markers from the potato map.

The process of interval mapping with "Mapmaker QTL" was used to identify associations between marker and biological phenotypes. Polymorphisms for molecular markers on six of the 12 potato chromosomes explained 34, 51, 63, 65, and 100 per cent of the phenotypic variation measured for trichome densities, the enzymatic browning reaction

associated with type A trichomes, levels of sucrose after production by B trichomes, and the presence or absence of sucrose droplets on the B trichomes. One important outcome was the discovery of linkage, on chromosome 5, between a single gene controlling the presence or absence of sucrose droplets and the requirement of short days for tuberization.

Current efforts are dedicated to verifying results in independent tetraploid populations and determining the basis of additional components of insect resistance that are not associated with current measurements of trichome properties. RFLPs will be used in continuing cycles of the breeding programme to identify the insect resistant clones that promise the most genetic advance, based on their content of only the minimal desired segments of the wild species genome.

Molecular Genetic Mapping of Cassava

A similar approach is being applied to the development of a genetic map for cassava, also reported at this meeting (Ch. 9, this volume). A hybrid population between two heterozygous cassava cultivars has been used to follow the segregation of DNA markers to define linkage groups. The DNA markers are of two types: random amplified polymorphic DNA markers (RAPDS) and genomic probes used as RFLPs from DNA libraries constructed at CIAT. The intraspecific mapping population is currently being screened for agronomic traits of interest, and analyses will be conducted to identify markers that indicate the location of genes controlling desirable traits. These markers will help define the genetic control of complex traits and may be used in the selection process.

Homology of DNA clones among related species of Euphorbiaceae [cassava (*Manihot esculenta*) and rubber (*Hevea brasiliensis*)] has been demonstrated (M Bonierbale and M Seguin, 1994, unpublished data), and suggests that similar efficiency to that experienced in tomato and potato may be gained through the use of a common set of probes for these crops and others in the genus.

Bibliography

- Bonierbale MW; Plaisted RL; Tanksley SD. 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* 120:1095-1103.
- Bonierbale MW; Plaisted RL; Pineda O; Tanksley SD. 1994. QTL analysis of trichome-mediated insect resistance in potato. *Theor Appl Genet* 87:973-987.
- Gebhardt C; Ritter E; Barone A; Debener T; Walkemeier B; Schachtschabel U; Kaufmann H; Thompson RD; Bonierbale MW; Ganai MW; Tanksley SD; Salamini F. 1991. RFLP maps of potato and their alignment with the homologous tomato genome. *Theor Appl Genet* 83:49-57.
- Gómez R; Angel F; Rodríguez F; Tohme J; Bonierbale M; Roca WM. 1995. Selection of heterozygous parents

Proceedings of the Tenth Symposium of the International Society for Tropical Root Crops,
held in Salvador, Bahia, Brazil, October 23-29, 1994

and single-dose markers for genetic mapping in cassava. In: Thro AM, ed. Third Scientific Meeting of the Cassava Biotechnology Network, 20-24 Aug, Bogor, Indonesia. CIAT, Cali, Colombia. p 113-124.

Pineda O; Bonierbale MW; Plaisted RL; Brodie BB; Tanksley SD. 1993. RFLP markers linked to the H1 gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. *Genome* 36:152-156.

Tanksley SD; Ga MW; Prince JP; de Vicente MC; Bonierbale MW; Broun P; Fulton TM; Giovannoni JJ; Grandillo S; Martin GB; Messeguer R; Miller JC; Miller L; Patterson AH; Pineda O; Roder M; Wing RA; Wu W; Young ND. 1992. High-density molecular linkage maps of the tomato and potato genomes. *Genetics* 132:1141-1160.