**Portfolio • First Phase (1999-2004)**

**Network:** Wheat Disease Resistance

**Development of Genomic Tools for the Isolation of Genes of Agronomic Interest in Wheat**

**Project ID:** WN7

**Project Duration:** 1 April 2000 – 31 August 2004

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**RATIONALE**

This research group is interested in the development of genomic tools to support the isolation of agronomically important genes. Their first approach, the establishment of a transposon tagging system in a diploid wheat species (*Triticum monococcum*), represents basic research. Transposons are mobile DNA elements, which excise and reintegrate randomly in the genome. The advantage of transposons over mutagenesis induced chemically or by radiation is that the DNA sequence flanking the transposon insertion site can be conveniently isolated and characterised. This should allow the isolation and characterisation of interesting genes for future transformation and the development of improved wheat lines.

*T. monococcum* – a close relative of the A-genome donor of wheat - is used as a model system; hexaploid wheat is not the best system for such an approach, since only few mutations might result in observable phenotypes. Prof. Keller's group has shown that subgenomic map based cloning of genes can be made feasible by using comparative genetic approaches between *T. monococcum* and wheat. In a collaborative effort with Dr. Christof Sautter's group at the Swiss Federal Institute of Technology (Project WN9) a transformation / regeneration system for *T. monococcum* has been established. Currently, transgenic plants are being analysed for their transposition activity and gene tagging efficiency. In a second step, lines containing active transposons will be propagated to allow the transposon to integrate at numerous positions in the genome.

The group’s second approach is aimed at the development of new molecular markers for adult plant resistance genes, which have so far been recalcitrant to detailed analysis due to the lack of polymorphisms between the parents used for the mapping populations. With the newly available tools for wheat genomic research (e.g. bacterial artificial chromosome library of *T. tauschii*), however, it should be possible to design specific primers for Adult Plant Resistance (APR) genes, e.g. for the Lr34 gene. APR genes have been proven to be the basis of durable resistance in many cases.
SUMMARY OF THE ACHIEVEMENTS OF THE FIRST PROGRAM PHASE
adapted from the summary provided by the project partners

In the frame of the wheat network, it was proposed to conduct a project concerning the development of new and more efficient methods for the isolation of agronomically important genes in wheat. The proposed strategies were on the one hand the development of molecular markers for an adult plant resistance gene \textit{Lr34}, which would directly be used by the Indian breeders to support the introgression of single genes or QTL associated with slow rusting genes to their elite varieties, and on the other hand, the establishment of a transposon-mediated mutagenesis system in a diploid wheat species, \textit{Triticum monococcum}, which would allow the isolation and the characterisation of interesting genes for future transformation and the development of improved wheat lines.

In the first approach, QTL mapping of leaf rust resistance was performed using a new marker developed by CIMMYT in a population of a cross between the Swiss winter wheat resistant variety “Forno” and the susceptible cultivar “Arina”. This revealed a strong QTL at the \textit{gwm295} locus on chromosome 7DS. There is probably an allele of \textit{Lr34} conferring durable resistance to the winter wheat variety “Forno”. The durable leaf rust QTL was narrowed to a 7 cm chromosomal segment. A high-resolution map is being established around the QTL in near-isogenic lines of the background Arina using the rice-wheat synteny in the region of interest.

As a first step in the second approach, a transformation protocol for \textit{T. monococcum} had to be established and this was done in collaboration with Dr. Christof Sautter. The method developed did not produce any transgenic plants. In a complementary approach, a highly efficient transformation system for wheat was developed (\textit{Triticum aestivum}, cv. Bobwhite), which allowed to extend the establishment of a transposon-mediated gene tagging system based on the maize \textit{En-1} element to hexaploid wheat. Although several transgenic lines were identified to have integrated the entire \textit{En-1} transposon-coding sequence, only one line showed a transposition activity in the T\textsubscript{1} generation. However, no transposition activity could be observed in the following generations, suggesting that a transposon-mediated gene tagging approach is not the method of choice for hexaploid wheat. Considering the inbuilt genetic redundancy in hexaploid wheat, RNAi might block the three homoeologous genes at once and thus may present an alternative approach to the characterization of gene function. Experiments to test the feasibility of this approach in wheat have been initiated. An RNAi construct targetting the phytoene desaturase (PDS) gene was transformed into wheat, where it caused a reduced expression level of PDS and the development of albino plants.