Network: Wheat Disease Resistance

Identification of Molecular Markers Linked to Leaf Rust Resistance Genes

Project ID: WN5

Project Duration: 1 April 2000 – 31 August 2004

Project Partners:
- Dr Vidya S. Gupta, Dr Prabhakar K. Ranjekar
  National Chemical Laboratory (NCL)
  Pune – 411 008, INDIA
  E-mail: vidya@ems.ncl.res.in
  pkr@ems.ncl.res.in

- Dr V.S.P. Rao
  Division of Plant Sciences
  Agharkar Research Institute (ARI)
  Pune – 411 004, INDIA
  E-mail: arimacs@pn2.vsnl.net.in

RATIONALE

Development of genetic resistance to rust is the most efficient, cost effective and ecofriendly approach to overcome the losses caused by rust epidemics. However, due to selection pressure and evolution, new and virulent races of the fungus appear, which increase the need to develop durable resistance. This can be achieved by pyramiding seedling and adult plant resistance genes, which are difficult to monitor in the field for expression of individual resistance genes against the background of other resistance genes. With the advent of molecular marker technology it is now possible to tackle such complex problems.

The objective of this project is to identify molecular markers linked to leaf rust resistant genes, which can later be incorporated into molecular breeding programmes for marker assisted selection of genotypes with pyramided resistance genes.

In the first phase of the project, various activities were initiated simultaneously in order to identify molecular markers linked to leaf rust resistance genes Lr15, Lr22A, Lr23, and Lr34 (if feasible also Lr46). Mapping populations (F2 plants) of crosses from susceptible parent Thatcher Tc and different rust resistant, near isogenic lines were created or are currently under development. The seeds were procured from various sources. So far, F2 populations for Lr15 have been obtained. For other crosses work is in progress. The seeds from the F2 populations will be used for disease scoring, while F2 tissue harvested from individual plants will be used for molecular analysis. The parental genotypes were tested for the polymorphism using different types of already available markers (ISSR, RAPD, AFLP, RGA primers; partly obtained from Prof. Keller’s lab and DWR). Out of the tested markers, 14 primers revealed polymorphism in parental screening. The results, however, need to be confirmed in the course of the ongoing parental screening. The polymorphic markers will subsequently be used with F2 mapping population to study segregation of polymorphic markers. The work on Lr15 will be carried out in close collaboration with Prof. Keller’s group (Project WN7).

SUMMARY OF THE ACHIEVEMENTS OF THE FIRST PROGRAM PHASE
The wheat disease resistance network was launched in 1999 with joint support from SDC, Switzerland and DBT, New Delhi. The overall goal of this network was to focus the efforts on the improvement of wheat through the production of agronomically useful wheat lines for India with higher endogenous disease resistance, especially to leaf rust resistance, using molecular markers. This included the development of pyramided varieties by combining both seedling as well as adult plant resistance genes. Molecular markers linked to rust resistance genes are useful tools for indirect selection of such pyramided genotypes. The joint project of the National Chemical laboratory and the Agharkar Research Institute aimed at the identification of molecular markers linked to various leaf rust resistance genes. The specific objective of the ARI project was to develop mapping populations and to phenotype the population for specific rust genes, whereas the NCL project was to identify molecular markers linked to the leaf rust resistance genes Lr15 and Lr23 as seedling resistance genes and Lr22a and Lr34 as adult plant resistance genes. Finally, efforts were concentrated on Lr15 and Lr34 genes. The entire project was executed in collaboration with DWR, Karnal and PAU, Ludhiana.

For tagging Lr15 gene, 148 F2 lines of Tc X Tc Lr15 were analyzed using ISSR, STMS and RGA markers. The segregation data for ISSR markers and phenotypic data for the Lr15 gene were used for linkage analysis. In addition, 20 STMS primers specific for chromosome 2D (location of Lr15) were investigated. One marker (Xgdm5) was found to be polymorphic: Unfortunately, no close linkage could be established with any of the polymorphic markers.

Differential gene expression studies with pathotype-challenged-and unchallenged- Tc Lr15 revealed a total of 80 differentially expressed bands which were gel excised, reamplified, cloned in pGEMT-E vector and sequenced. In order to detect early defense responsive transcripts with the Suppression Subtractive Hybridization approach (SSH), a total of 96 clones were sequenced on MegaBACE1000. Most of the transcripts showed homology to Glutathione S transferase, Sm-D1 and 14-3-3importin alpha chain. Unique clones identified through suppressive subtraction library were used for isolating the recombinant plasmids. Further investigation of the clones did not indicate any linkage or direct relation to resistance gene Lr15. The mapping of SSH clones on chromosome deletion stocks is still in progress.

For tagging Lr34 gene, using RN2491 x WL711 population, 6 microsatellite primer pairs available from advanced microsatellite map and Bin deletion lines for 7D/7DS/7DS4 were attempted. One marker (Xgwm295) was found to be closely linked (4cM) to the Lr34 locus and segregating in the population.

In order to map novel leaf rust (Lr) and stripe rust (Yr) genes in T. monococcum x T. boeoticum a population of 129 RILs was analysed using various molecular markers viz ISSR, RGA as and AFLPs (in collaboration with project WN6). Six ISSR primers were polymorphic. However, no linkage could be established when attempted on complete population. With AFLP analysis, 584 polymorphic loci could be detected using 11 EcoRI-Msel primer combinations. These are being used with the population.