Medicago genomics and its use to study plant-pathogen interactions in alfalfa

G.B. KISS, J. JAKAB, K. MIRÓ, Á. DALMADI, T. PETROVICS and M. BALOGH

Structural, comparative and functional genomics studies of model legume species have taken a spectacular turn in the last few years. Progress in genome sequencing (1950 BAC clones have been sequenced generating 279 Mbp sequence of which 190 Mbp non-redundant of the cc. 500 Mbp total genome size), chip technology, genetic mapping (see *Figure 1*), and map based cloning (see *Figure 2*) contributed tremendously to our understanding of the molecular bases of symbiotic nitrogen fixation, endomycorrhiza (see *Figure 3* and *4*), plant pathogen interactions, and other aspects of legume species.

In our laboratory besides the study of symbiotic nitrogen fixation we focused on Fusarium disease, which is one of the most damaging fungal pathogen of alfalfa (see *Figure 5*). In order to grow alfalfa (*Medicago sativa* L.) more safely and economically we have identified resistant determinants against the fungal pathogen *Fusarium oxysporum* f.sp. *medicaginis* and try to transfer some of the genes into alfalfa cultivars by introgression. To this end, different Medicago lines (diploid and tetraploid *M. sativa*) were tested in biological Fusarium test. Resistant individuals were selected and in sexual crosses between resistant and susceptible individuals F1 and F2 progenies were generated and dominant-recessive nature and segregation ratios were determined. In a diploid *M. sativa* F2 population the recessive Fusarium determinant (fms1) was mapped and map based cloning was started. The progress of the genetic mapping and molecular cloning work is shown in *Figure 6*.

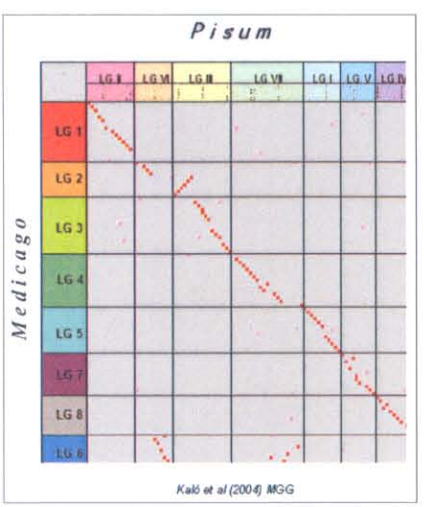

Figure 1: **Comparative genetic mapping of *Medicago* and *Pisum***

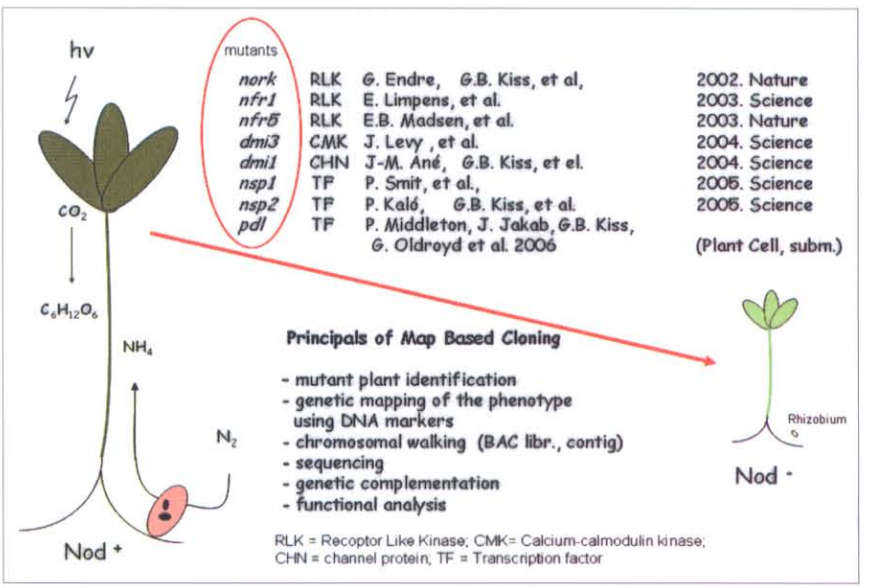

Figure 2: **Map based cloning of Early Symbiotic Genes**

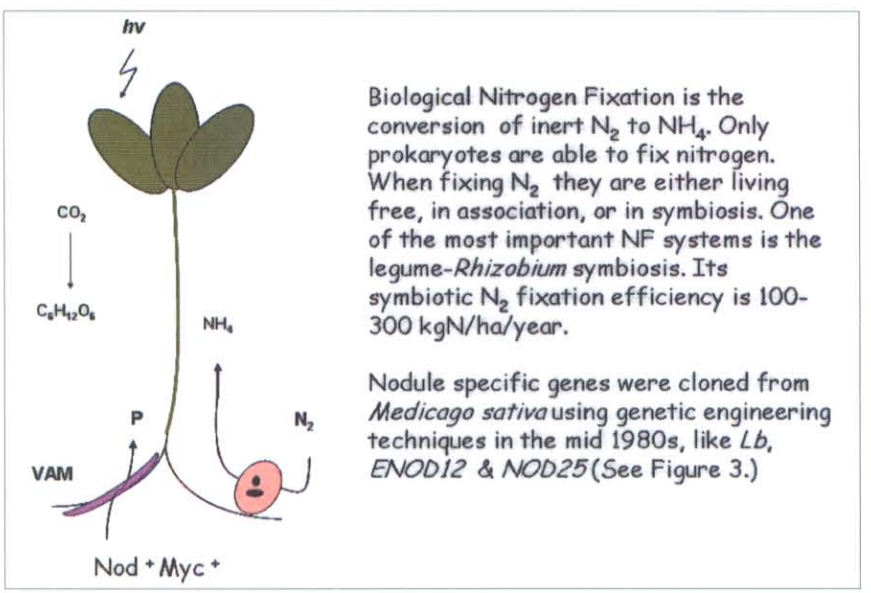

Figure 3: **Schematic representation of symbiotic nitrogen fixation and endomycorrhiza**

Autoren: György B. KISS, Júlia JAKAB, Krisztina MIRÓ, Ágnes DALMADI, Tünde PETROVICS and Márta BALOGH, Agricultural Biotechnology Center, Szent-Györgyi A. u. 4. H-2100 GÖDÖLLŐ

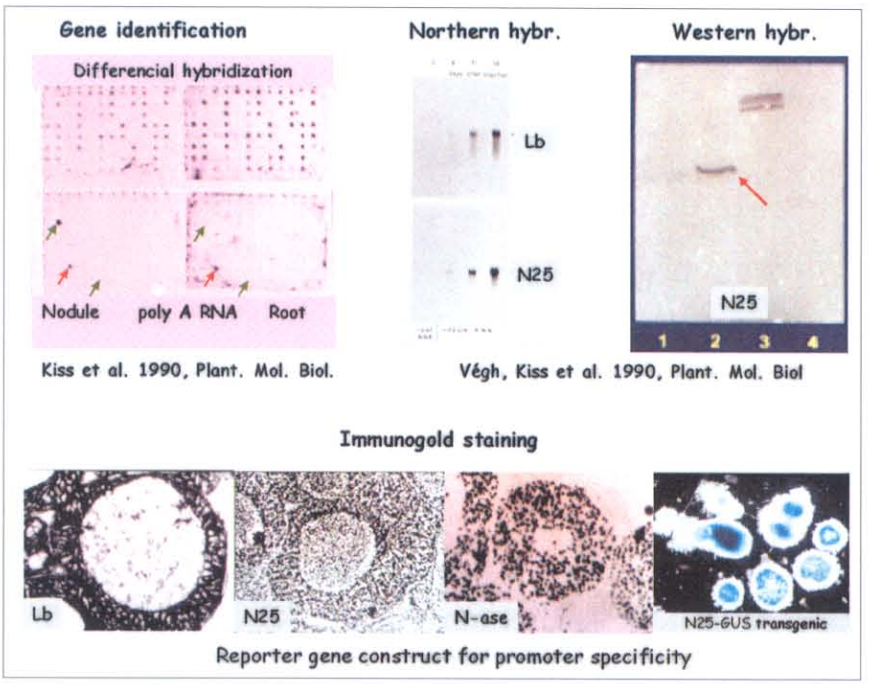

Figure 4: **Molecular cloning of nodulin-25**

Fusarium wilt	• serious disease of many plants
	• of alfalfa described in 1927 in the US
	• the causal agent is a fungus, *Fusarium oxysporum* f. sp. *medicaginis* & *Fusarium solani*
	• a vascular disease: a fungal pathogen blocks the xylem and disrupts the water transport
	• *Fusarium* toxins are additional factors
Infection	• favored by high temperature
	• the pathogen survives in soil for ~ 10 y (rotation does not help)
Symptoms	• biotic stress shoots become yellow and reddish, then wilt
	• sections of the root show brownish discoloration of the xylem

Biological test for *Fusarium*

- *Fusarium* strains are maintained on Czapek-Dox medium
- freshly formed spores are washed off
- the tip of the roots are cut off
- plants are soaked in *Fusarium inoculum* for 24 hours (10^6 conidiums/ml)
- treated plants are put into soil and grown for more than 2 months in fitotrons (26 °C, 75%-os humidity)

Figure 5: **Fusarium oxysporum as an alfalfa fungal disease**

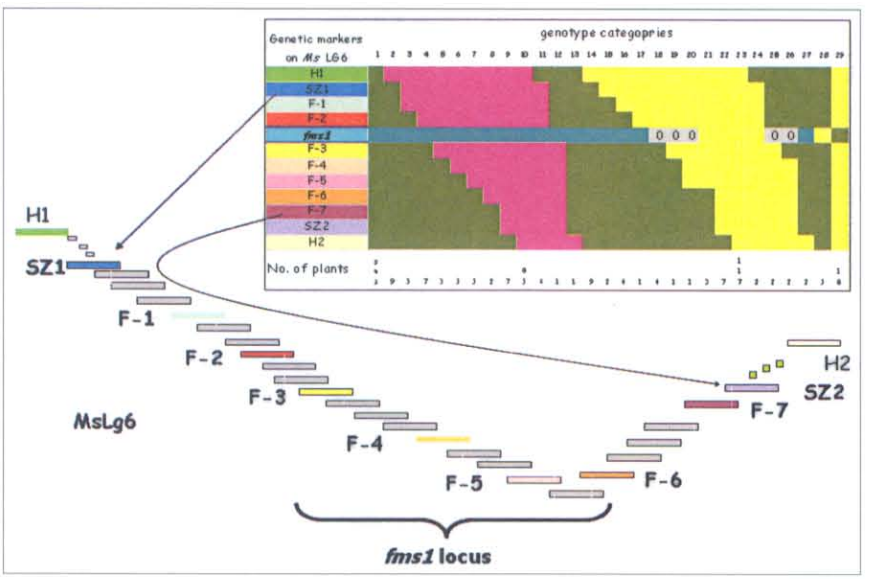

Figure 6: **Map based cloning of *fms1***

To test the feasibility of the introgression of the fms1 locus from diploid to tetraploid plants the recessive Fusarium determinant identified in diploid alfalfa was introduced into a susceptible tetraploid line by the 4x-2x crosses. F1 hybrids were self-pollinated and homozygotes for the recessive trait have been looked for. These derivatives if appropriate will be used in breeding programs to develop Fusarium resistant cultivars.

In parallel experiments Fusarium resistant *M. truncatula* accessions were screened for and genetic test have been performed to learn more about the nature and genomic position of the determinants. To this end two dominant resistant determinants were identified in the model plant *Medicago truncatula*.