Genetic Basis for the Improvement of Symbiotic Nitrogen Fixation in Legume-Rhizobium Associations

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Thirty five years ago FLOR /1955/ elaborated his gene to gene hypothesis for the explanation of the genetic basis of host-parasite interactions. The root of the matter is, that in the course of infection the genotypes of the host and the pathogen are practically in conversation and genetic signalization with each other. If the speculation of VANCE /1983/ that Rhizobium is a beneficial pathogen is true, then FLOR's hypothesis is adaptable to the relation of host /legume/-Rhizobium symbiosis. Indeed, a cascade system is perceptible in which, from infection to nitrogen fixation the genes of Rhizobium and host plant induce each other.

We can follow this during the development of the whole nitrogen fixation system. The first signal comes from the host when, by means of root exudates, the plant attracts the mobile Rhizobia by chemotaxis. Then a colonization takes place on the root to where the bacteria attach themselves in special places by adhesion. It is not impossible that in compliance with the hypothesis of DAZZO /1985/ this adhesion takes place on patches containing lectin with lectin-polysaccharide links. It is true that exopolysaccharides /EPS/ have a role in the infection, invasion processes and in the formation of nodules. Mutants of Rhizobia with non-succinylated EPS produce empty nodules /LEIGH and REED, 1987/.

After adhesion, another signal comes from the host. The host, with the help of flavonoids, induces the common *nod* genes of bacteria /DJORDJEVIC, 1987/ which are carried on a symbiotic megaplasmid /in case of fast growing Rhizobia/. As the effects of this, the first symptom of invasion, the curling of the root hairs appears, then infection thread formation and cortical cell divisions also start. It is known from the research of KONDOROSI et al. /1988/ that in case of *Rhizobium meliloti* the *nod* D_1 gene product is induced by the host produced luteolin and this initiates on the common *nod* promotor /nod box/ the transcription of *nod* ABC genes. At the same time the transcription of another gene cluster, the *hsn* is coordinately induced also with the D_2 gene products+flavonoid, which determines host specificity, and after that the host specificity genes also express themselves. These two gene clusters determine the early part of nodule formation and host specificity.

I should like to mention our experiment which was made by Rhizobia isolated from *Medicago sativa*, *M. falcata* and from their natural hybrids, the M. varia. In combinations of these isolates we infected the *M. sativa*

host. Reisolating and bacteriophage typing the nodule initiating bacteria we learned that the isolates from the hybrids and M. falcata infected the M. sativa host in a significantly decreasing proportion /SZENDE et al., in press/. With the evolutionary divergence of hosts the signals or the receivers changed. The same phenomenon was observable in the opposite direction, too.

The nodulins form in the developing nodules, in their tissues and specific proteins /VANCE et al., 1988/. Some 30 nodulins have been demonstrated till now but their function became known in five cases only. The others are possibly enzyme proteins, auxins cytokinins, tubulins etc. The best-known nodulins are the apoproteins of leghaemoglobins, the globins. They are determined by the host genes and they comprise 30% of all the soluble proteins in the nodule. The enzymes are among others, glutamine synthetase /GS/, glutamate synthase /GOGAT/ and uricase.

When the infection thread reaches the newly produced nodule cells the bacteria are released into the cytosol, through the cell wall except the tip of it, but remain within by the peribacteroid membrane /PBM/. At the same time the bacteria are going through a morphogenetic process, turn pleomorphic, sometimes grow bigger, and transform into bacteroids. After these events the expression of the nif and fix genes starts. The signal for this is the release of bacteria.

In case of Bradyrhizobia /HENNECKE, 1987/ the NtrA product, a protein, is activated then activates the FixR NifA operon. In case of low O_2 tension the activated NifA protein, with the same NtrA product, activates the other nif/fix operons, among them the nif DKEN operons, which are the structure genes of the nitrogenase enzyme.

In the process of nitrogen fixation by nitrogenase several host functions participate, transferring the organic acids /photosynthate+nod CO_2 fixation products/, which are necessary for the working of nitrogenase and the respiration of bacteroids and which transform the ammonia produced into amino acids and ureides.

It is necessary to mention the Hup system of bacteria which recuperates the H_2 released during nitrogen fixation, so it is an energy sparing process /ROBSON and POSTGATE, 1980/.

Let us look at our problem now from the host's side. VANCE et al. /1988/ mentions 45 host genes in 8 species which influence nodulation and nitrogen fixation. Such are the non-nodulation /nodulation resistant/, inefficient and supernodulation genes. In case of alfalfa such genes are: in_1, in_2, in_3 and in_4 in_5 system. Any of the R. meliloti strains in combination with these mutants are inefficient. Non-nodulating phenotypes are the results of the expression of nn_1 and nn_2 genes.

In soybean the r_{j1}, the R_{j2}, R_{j3}, R_{j4} nodulation genes are well-known for 30 years. The first is a recessive gene, the others are dominant ones. From the Bragg cultivar with ethyl methane sulfonate /EMS/ mutagenesis supernodulation mutants and nitrate tolerant mutants were produced with 20 times more nodules. This supernodulation is in correlation with nitrate tolerance. It is possible that this trait is determined by a Mendelian recessive gene. In case of pea, LIE /1984/ described that Pisum sativum cv. Afghan is restrictive against the European R. leguminosarum strains but nodulates well with the R. leguminosarum Tom. This strain originates from the Middle East. This trait is determined in the host with the sym-2 gene. For the explanation of this nodulation resistant phenotype there are two hypotheses:

1. The gene reduces the nodulation frequency, or
2. Inhibits the normal process of nodulation /PUEPPKE and PAYNE, 1987/.

It raises the question: how are these nodulation resistant lines able to promote the improvement of nitrogen fixing systems? A widely known phenomenon is the competition of Rhizobia for nodulation. This occurs between the non-adapted but valuable bacteria of the inoculum, introduced into the soil, in an unfamiliar environment and the indigenous, mostly valueless population. This can be avoided by two means. One of them is to produce by breeding if it is possible, such a host line which is able to produce increased nodulation and a good quality of nitrogen fixation with all individual bacteria of the indigenous population. The other possibility is to develop an association in which the host genes inhibit the invasion of all bacteria except those which form the inoculum. A model for such a system is the above mentioned Pisum sativum cv. Afghan - R. leguminosarum Tom system /WINARNO and LIE, 1979/.

Of course, this is not the only way to increase the nitrogen fixing potential of the host.

Breeding, on the side of the host, utilizes the natural variation of it, and on the other side the speeding of this variation by induced mutagenesis. In many cases /Phaseolus, Vigna, Pisum, Vicia/ the natural variation of the host was used for selection /NEUHAUSEN et al., 1988; DUC et al, 1988/. Generally, the parameters of selection are: yield, nodule number and weight, nitrogen content etc. but it is possible to select for the enzymes of N- and C-metabolism. JESSEN et al. /1988/ demonstrated that the selection in alfalfa for the GS and for phospho-enol pyruvate decarboxylase activity correlate with the increase of acetylene reducing activity.

NUTMAN /1984/ improved the nitrogen fixing ability of Trifolium pratense and T. subterraneum by breeding. He used many germ plasms of T. pratense in combination with one strain of R. trifolii. The heredity of the effective phenotypes was complex and was determined by many major and minor genes. In the case of T. subterraneum selection was unsuccessful.

As regards environmental stress effects, the breeding is directed mostly to the elimination of the combined nitrogen effect. The above mentioned supernodulation mutants of Glycine hispida cv. Bragg are tolerant against nitrate /CAROLL et al., 1985/. MYTTON and RYS /1985/ selected Trifolium repens for 0, low, and high frequency of nodulation, then checked them in the presence or absence of combined nitrogen. In tube experiments a heritable factor was demonstrated which determines the nodule number but the presence of nitrogen suppresses the expression.

This was a short evaluation of the role of host plants in legume-Rhizobium associations in connection with the genetic background of the improvement in nitrogen fixation.

Another possibility is to improve the bacterium. The usual technologies for the isolation of superior nitrogen fixing Rhizobium strains are well known. In many cases it produces outstanding strains which are generally used for inoculation. The genetic basis of these improvements are not known. It is not excluded that it is the result of a change of any one gene in the symbiotic process from the chemotaxis till the nif, fix functions.

The present investigations cover the nodulation /nod/, the nitrogen fixation /nif, fix/ and the host specificity /hsn/ genes, their localization by molecular means and their regulation. It is necessary to examine the whole system because a breeding program without the knowledge of causes is impossible. The system is very complicated, so the task will be very hard to accomplish.

The population biology, and ecological genetics of Rhizobia are very promising fields for examination. The main subjects of these disciplines are the laws of competition between the inoculated and indigenous populations, the host preference of Rhizobia /HARRISON et al., 1989; THURMAN and BROMFIELD, 1988; SZENDE, 1987/ and the adaptation of Rhizobium populations to the stress effects of the physical, chemical and biological environment.

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