TROPICAL PASTURES

1. Genetic Transformation of *S. guianensis*

Genetically transformed plants of the forage legume *Stylosanthes guianensis*, CIAT 184, were obtained by regeneration of leaf-disc explants inoculated with the disarmed strain of *Agrobacterium tumefaciens* EHA101 and with the wild strain R1000 of *Agrobacterium rhizogenes*, both containing the plasmid pGV1040 in a binary disarmed vector system (7). The T-DNA region of this vector contains the selectable gene neomycin phosphotransferase II (npt II), which confers resistance to the antibiotic Kanamycin; the reporter gene ß-glucuronidase (uid A) and the herbicide resistance gene (bar) which confers resistance to the herbicide commercially known as "Basta" (Hoechst). Minimum lethal doses were determined for both Kanamycin and the herbicide (Basta) and used for selection during regeneration of transformed plants.

The GUS assay was carried out on leaves of transformed plants as described by Jefferson (1987). Percentage of transformation with the disarmed strain was about 10% but only 5% could be regenerated, while with the wild strain transformation was rate 5% and regeneration 2% respectively. In a second experiment carried out with the *A. tumefaciens* strain EHA101, transformation percentage was 11% and regeneration 5%. Regenerated plants were planted in the glasshouse.

DNA extracted from leaves was hybridized to a portion of the plasmid pGV1040, stemming from the region between the T-DNA border sequences. Results of this test provided evidence of physical DNA transfer for each of the transformation events of the first experiment.

As in the *in vitro* step, minimum lethal dosis of the herbicide was determined for use under glasshouse conditions; the results showed that a concentration of 0.5 l/ha produced death of rooted cuttings of non-transformed check-plants after 5 days. Rooted cuttings of transgenic plants were challenged with herbicide concentrations of 1 and 2 l/ha without severe damage. Work is underway to determine the inheritance of the bar gene to the next generation.

2. Somaclonal Variation for Plant Adaptation to Acid Soils

Fourteen *S. guianensis* CIAT 2243 derived somaclonal lines were selected from our early work on somaclonal variation generated through *in vitro* culture. Some of these lines displayed superior agronomic performance than the check line and others showed special morphological characteristics such as dwarf (bushy) plant habit, chlorotic foliage, 1-2 leaflet leaves, and tetraploidy. This material was grown

in the field for two generations and in the greenhouse for one generation. The observed variations remained throughout the four generations.

The objective of this work is to select somaclonal lines with plant characteristics (shoot-root biomass) suitable as tools in studies of plant adaptation to acid soils.

The results of this research have been presented and discussed in the TPP Annual Report, Section on Plant-Soil Relations and Nutrient Cycling (I. Rao).

3. Construction of a *Brachiaria* molecular linkage map

The overall goal of the proposal is to construct a highly saturated molecular map for *Brachiaria*, using random genomic and cDNA clones and random primers. The map will be used to tag important agronomic traits (simple or quantitatively inherited traits), to study the genetics of diploid-tetraploid species, to estimate the level of variability and heterozygosity within and between gene pools, to understand the evolution of the species, as for Salamini's group is working on the mapping of potato.

A random genomic library will be constructed from leaf tissue. Preliminary work will be initiated with random primers to look at the feasibility of using the random amplified polymorphic DNA (RAPD) technique (Williams et al., 1991) for mapping, in addition to the random genomic clones. If promising, a set of arbitrary primers (200 primers presently available at CIAT) would be used to initiate the mapping.

The random genomic library and arbitrary primers will be screened on genotypes involved in controlled interspecific crosses at the diploid level to detect polymorphisms among the parental genotypes. As polymorphic markers are identified, appropriate crosses would be carried out. Once probing with a number of RFLP clones or arbitrary primers has been completed, results will be used in the segregation analysis.

While most of the work could be carried out at CIAT it is suggested to write a joint project proposal with the Max-Planck Institute in Cologne (Dr. Salamini's group). This collaboration will provide CIAT with complementary expertise in genome mapping of diploid-tetraploid species.

4. DNA Fingerprinting of *Colletotrichum gloeosporoides*

Considerable pathogenic variability exists in populations of *Colletotrichum gloeosporoides*, particularly in Brazil, the center of diversity of *Stylosanthes capitata*.

Both the importance of *S. capitata* and the variability of *C. gloeosporoides* make it important to seek better biological characterization of this pathogen.

Genetic fingerprinting is nowadays the method of choice for taxonomic and phylogenetic studies in bacteria or fungi, the advantages being accurateness, independence from growth status of the pathogen, and the biological implications concerning variability and stability of the pathogen. This has been well demonstrated through work done in the Rice Program together with M. Levy and J.M. Hamer at Purdue University on *Pyricularia*, and the work on *Xanthomonas campestris* pv phaseoli in the Bean Program and the University of Wisconsin.

The necessary molecular techniques have been fully taken over by the programs. Bean Pathology is working now also on *Phaeoisariopsis griseola* and *Colletrotrichum lindemuthianum* the causative agents of angular leaf spot disease and anthracnose, respectively. This gave us the opportunity to establish a collaborative link between Bean Pathology, Pastures Pathology and the BRU. We are basically involved in the planning and conduction of initial experiments. We consider it a good investment to introduce the Pastures Pathology personnel into such widely applicable techniques.

Preliminary experiments with a few contrasting isolates have shown a good level of polymorphism using heterologous DNA probes, such as ribosomal DNA and a DNA fragment stemming from the M13 phage which has already shown high levels of polymorphism in different organisms. Our aim is to isolate a homologous semirepetitive DNA fragment from a *C. gloeosporoides* genomic library, which is presently being constructed.

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5. Identification of the Spittlebug Resistance Factor in *Brachiaria jubata* and Development of a Screening Procedure

A phytoecdysteroid has been postulated as the resistance principle against spittlebug infestation in *Brachiaria jubata*. This stems from S. Lapointe's observations of the effects on the insect of feeding on resistant accessions. The interference with morphogenesis of the insect strongly supports some kind of hormonal disturbance, the picture favoring the production of a phytoecdysteroid. This has also been partially corroborated by HPLC analysis of spittlebug excreta by J.C. Steffens at Cornell University. Steffens is also working on aminoacid contents of different *Brachiaria* accessions with varying degrees of resistance to splittlebug.

As an alternative project we have initiated the production of antibodies against 20-hydroxyecdysone (ß-ecdysone). The immunological approach would lead us first to the identification of the resistance factor. If the hypothesis is correct, the antibodies would then be used for the immunological detection of phytoecdysteroid producing plants in a breeding program using ELISA techniques. Antisera have

successfully been used in the transfer of arcelin into bean commercial cultivars for bruchid resistance.

To generate antibodies, ecdysone has to be linked to bovine serum albumin (BSA) to make it immunologically active, as the molecule alone is too small to show good immune stimulation in rabbits. This will be achieved by binding a succinate bridge to the steroid using the hydroxyl groups in the A-ring. After activation with ethyl chloroformate, the link with BSA can be formed. This complex will then be used in an immunization scheme. After purification of the IgG fraction by affinity chromatography through Protein G Agarose Beads, the IgGs will be used to develop an ELISA test for ecdysone as an alternative to the RIA described in the literature, thus obviating the use of radioactivity.

Some comparative studies between resistant and susceptible *Brachiaria* accessions will be performed using HPLC as to complement Steffens' work. Different groups of substances can be monitored using this technique.