Veit Schubert

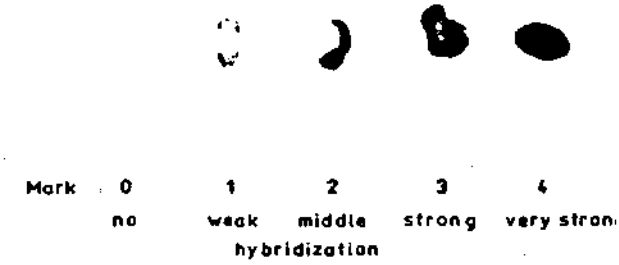

Fig. 1. Hybridization strenght scale for squash dot tests

Triticum and some *Aegilops* species but evident in *Secale* also support this conclusion.

In the laboratory of M. Metzlaff, genome specific DNA probes from *Hordeum vulgare* were cloned and characterized by H. Junghans. Regarding repeated DNA sequences our further investigations will concentrate on the proof of alien chromatin in the wheat - *Ae. markgrafii* crossing material and on the enlargement of the investigation to their distribution in Poaceae species.

cDNAs of a wheat tetrameric inhibitor of α-amylases

Proteinaceous inhibitors of digestive enzymes from herbivorous insects are widespread in plants and might be involved in their protection. Recently F. Garcia-Maroto, C. Marana, M. Mena, F. Garcia-Olmedo and P. Carbonero (53) at the Universidad Politecnica de Madrid in Spain, have cloned and characterized cDNAs corresponding to the three types of subunits of a wheat tetrameric inhibitor which is active against alpha-amylases from insects.

The genes for two of the subunit types are within a few kb of each other in the short arms of chromosomes 4A, 4B and 4D of hexaploid wheat, whereas the third type of subunit is encoded in the short arms of chromosomes 7A, 7B and 7D.

Comments of Pilar Carbonero: The alluded subunits are part of a protein family that also includes trypsin inhibitors and monomeric and dimeric a-amylase inhibitors. The reported work is part of an ongoing project which involves not only the characterization of this complex protein family but also the molecular cloning and transgenic expression of the corresponding genes, so that their potential protective properties can be tested.

An immediate precedent of the report has been the characterization and *in vitro* reconstitution of the inhibitor from its purified protein components carried out by L. Gomez, R. Sanchez-Monge, F. Garcia-Olmedo and G. Salcedo (PNAS 1989, 86: 3242-3246) in our department.

The cloning of the cDNAs has been important in two aspects. First of all, it has provided the whole deduced amino acid sequence of the three

Montana Mena, Garcia-Olmedo, Pilar Carbonero, and Federico Garcia-Maroto.

subunits of the inhibitor (only a short stretch of the amino terminal sequence had been obtained by direct protein sequencing for each of them), thus facilitating the evolutionary studies of this family in wheat and other species.

The second aspect, is that using the cDNAs as probes in Southern experiments we have found that genes for two of the subunits are linked within a few kilobases of each other and that probably genes corresponding to the A genome have been silenced (pseudogenes?), since protein subunits encoded by chromosomes 4 and 7 from the B and D genomes have been identified while the proteins corresponding to the A genome are not detected.

We have already obtained transgenic plants expressing genes for other members of the family and have been able to show lethal effects on insect larvae feeding on them. We are now going to undertake the more complex task of carrying out similar experiments with the tetrameric inhibitor.

Maize and Wheat tRNA^{Pro}

The nucleotide sequences of two maize mitochrondrial DNA regions containing tRNA^{Pro} genes have been compared with the corresponding regions of wheat mitochondrial DNA by Abdourahamane Sangare, Jacques-Henry Weil, and Jean-Michel Grienenberger (54) at the Universtite Louis Pasteur.

Comments of Jean Michel Grienenberger: This report describes experiments which have been undertaken during the PhD thesis of A. Sangare, now at the ABIDJAN University, Ivory Coast. Our aim was to study the sequence and the localization of the tRNA genes which are encoded by the maize mitochondrial (mt) genomes of fertile (F) and male sterile (T: Texas) lines. A previous report from our lab has shown that a tRNA^{Pro} gene is located downstream of the ribosomal 5S-18S locus (Runeberg-Roos *et al.*, 1987, Plant Mol. Biol., 9:237-246) in the wheat mt genome. A second copy of this gene has been reported to be present in another location of the wheat mt genome (Joyce *et al.*, 1988, Plant Mol. Biol., 11:833-843). This gene can be shown to originate from the first tRNA^{Pro} gene after intragenomic and site-specific rearrangements.

Using a synthetic oligodeoxynucleotide homologous to the wheat mt tRNA^{Pro} gene sequence, we have located the corresponding genes on the maize mt genomes. In the fertile line of maize mt genome, there are two sequences which are homologous to the probe, the first one being the genuine tRNA^{Pro} gene, the second one being a pseudo-gene corresponding to only a part of the same gene. The sequence analysis of the flanking regions of these two genes and their comparisons with the corresponding wheat sequences have shown that all these sequences derived from the same ancestor sequence but that the different organization of their flanking regions in wheat and maize mitochondria is probably the result of a number of subsequent recombination events specific for each plant. These recombinations occurred directly at the tRNA^{Pro} gene locus, confirming the idea that tRNA genes can be involved as landmarks for the structural organization and the expression of mitochondrial genomes (Ojala *et al.*, 1981, Nature 290:470-474).

The localization and organization of all tRNA genes on the mitochondrial genomes of the fertile and male-sterile lines of maize has now been published (Sangare *et al.*, 1990, Mol. Gen. Genet., 223:224-232) and it has been shown that these two tRNA^{Pro} gene sequences are also present in the T cytoplasm albeit on other locations.

This is only one example of the general features of the organization of plant mt tRNA genes which we have shown to be very conserved in their sequences, present in the same local environment but with a very different overall genomic organization due to the number of rearrangements and recombinations which occurred between the two lines as already described (Fauron and Havlik, 1989, Curr. Genet.,15:149-154).