

persuasion and at last send him what he has been asking for over three ANL numbers!

No, my task is more modest - I want to show that point in works by Petrov et al., that beginning of divergence between their views and the actual material, if you please, that beginning of falsity which, in my opinion, was removed in proper time and this is just that very thing that subsequently led to the known final. I was persuaded of necessity to do this by no other arguments but immediatly Dr. Savidan himself, who emphasized fairly that it's no use pointing out a negative result without taking an attempt to explain the failure. And he himself is convinced that the main reason for the "failure" of Petrov's program lies in his wrong idea about the nature of apomixis as a system of elements ("his wrong philosophy on the nature of apomixis", he remarks).

For the present it is early to speak whose philosophy is more correct here as well as premature to declare the failure of the entire program, though. One can only say with certainty that the mistake by Petrov is not strategical but, rather, a tactical kind; it's much simpler, "more prosaic", so to say, than that Dr. Savidan tries to convince us of. It is as follows: the invalid assumption about the possibility for genetic material in maize x *Tripsacum* hybrids to be transferred easily (rapidly) from chromosomes of *Tripsacum* to maize chromosomes.

Let's, however, appeal to the original sources. While analyzing the causes of a higher apomixis frequency in 38-chromosome *Zea* x *Tripsacum* hybrids as compared with 56-chromosome ones, which they produced from, Petrov, Fokina and Belousova wrote in 1976: "We consider transfer of loci with apomixis genes from chromosomes of *Tripsacum* to chromosomes of maize to be the most probable cause of the appearance of an increases tendency to regular apomictic reproduction" (In: Apomixis and its significance for evolution and selection. Novosibirsk, Nauka, 1976, pp.85-95).

Is this a hypothesis? Yes, it is, for the time being. But in the same work, namely on the next page, we are reading as follows: "In addition, such 38-chromosome apomicts are very interesting as, having chromosomes of maize with loci comprising genes controlling separate elements of apomixis, they can successfully be used for obtaining maize-like hybrids with separate elements of apomixis and even immediatly maize-like hybrids with regular apomixis". As we can see, this not simply a hypothesis already; this is half an assertion.

Further I could successively, in chronological order, call quite a number of publications by Petrov and others in which the hypothesis about "transfer of loci" is gradually growing from the semi-assertion into full assertion. the authors are not in the least embarrassed by the contradiction to the information available that *Zea* and *Tripsacum* are distantly related genetically

because of which spontaneous segmental exchanges as well as genic recombinations in intergeneric *Zea* x *Tripsacum* hybrids occur in actual fact rarely (C.A. Newell and J.M.J. de Wet Can.J.Genet.Cytol.15:763-778, 1973; M.P. Maguire Genetics, 45:195-209, 1960). They might also have noted, not without good for themselves, that these observations have been made on sexual forms of hybrids, of which it follows that in apomictic hybrids probability of such exchanges is evidently still lower.

But, be that as it may, the assertion has been made. And, though it is not at all being supported by new findings, in the consciousness of its authors it is getting firmly established from year to year. In 1978 there appears the next paper in turn whose title directly speaks for itself: "Insertion of sites of *Tripsacum* chromosomes into maize chromosomes in connection with the transfer of elements of apomixis" (In: Apomixis in plants and animals. Novosibirsk, Nauka, 1978, pp.45-74). The question about transfer of genes for apomixis and insertion of them into chromosomes of maize appears here before the readers as a fact proved; the authors not only once and for all have come to believe in their version - they have got used to it.

Well, and further everything is simple. The maize produced after all from such forms and released from chromosomes of *Tripsacum* simply "could not", but have turned to be apomictic..... On summing up the above one involuntarily comes to the thought: a banal case, in general, from the point of view of the science historian - an assumption, once advanced, through being often used, becomes a "theory" later on.

P.S. On reading this final part of the paper Dr. Savidan is most likely to be indignant again. "It's unethical!", he will exclaim - "Demolished!.. Destructive!"... and so on. And, possibly, he will perceive in this a plausible excuse in order not to publish my comment.

Of course, that is his business. Though taking of such a decision will in fact mean a new quality - "playing a game into one goal". According to my standards.

Boris F. Yudin

From Mexico, Mexico:

New maize x *Tripsacum* hybridizations and the feasibility of apomixis transfer.

There are two points to consider in the preceding story. One is the research and the data which have been obtained. The other one is what has been published from these results. Yudin's comment

Fonds Documentaire ORSTOM



010011156

Fonds Documentaire ORSTOM

Cote: B* M 156 Ex: 1

focuses on this second point. Although I continue to disagree on the manner he chose to make his point, I have to agree with his willingness to make the truth. But for me, discussing the first point - research itself - is what is most important. My main recollections of Moscow and Novosibirsk have never been cocktails or manifestations of friendship - although everyone appreciates to be well received when travelling abroad - and I made it clear in my preceding comment: the most important thing I brought back with me in my luggage in 1978 was the conviction that (1) transferring apomixis to maize was possible, and (2) Petrov's group was very unlikely to succeed in this matter, although some of his results were extremely encouraging. As a consequence, and less than one year later, I submitted a proposal for a new maize x *Tripsacum* program to the Director of my Institute.

My only serious point of disagreement with Yudin's preceding comments is when he says that we should hardly discuss about philosophies at this point. A great deal of progress have been achieved since 1978, in apomixis research as in other fields which are taking a larger place each day in our research, as molecular biology and biotechnology (see Peacock's article above), which make that we can indeed discuss about it. Moreover, we need to. Or how will we decide the pathways we are going to follow from now on?

I agree with Dr. Yudin, and everybody agrees, I guess, that publishing assertions that are not confirmed by the data is no good. I could, however, mention several papers on apomixis research, published by other American or European scientists, in which the interpretation of the data is at least highly controversial. It does not make that these persons are not highly respectable. The real point is, knowing about the progress which have been achieved since these works were published, we must ask ourselves about what went wrong in these studies that we can avoid in our new attempts, and what are the elements we can add to make these new attempts more likely to be successful.

The two pillars on which we should, in my opinion, build these new attempts, are (1) adequate genetic resources and (2) adequate knowledge of all possible applications of molecular markers and biotechnological tools available. But I will first discuss about current knowledge of apomixis.

Petrov - after Powers and others - considered apomixis as something like a kit: you put one element above the other, and then another one, and the final result will be an apomictic plant. I do not see any convincing evidence, in the literature, for the existence of two or more separate (unlinked) genic systems, like one for the failure of meiosis and one for the failure of fertilization. I know there is such a claim in *Taraxacum*, but the data I saw, so far, do not look convincing at all to me. The one dominant gene model that fits with our data in *Panicum* and *Ranunculus* (Gian Nogler's

program) does not please all members of our apomixis fan club. But it is based on the only two analyses which were built on a large series of hybridizations within a single agamic complex. Once again, I claim that all other data may be interpreted in more than one single way, and cannot seriously be used to reject the one dominant gene hypothesis. I am still using the word hypothesis because what is true for *Panicum* and *Ranunculus* may not be true for *Tripsacum* or *Pennisetum*. We have to make other genetic analyses. And this is the first part of our studies for which we imperatively need to establish adequate germplasm collections.

As I discussed previously, such analyses should be based on using **diploid sexual** accessions and apomicts from the same species, or at least, the same agamic complex. The only two studies I know about at this time which fit with such requisites are made in Brasil on *Brachiaria*, and in Argentina on *Paspalum*. Other should be started, including on diplosporous materials. We plan one in *Tripsacum*.

Now an adequate germplasm is also a must in the transfer program itself, whatever the pathway - conventional, through backcrosses, or more modern through plant transformation - we want to follow. Was Petrov lucky to get F1s, BC1s, etc, when he had only one *Tripsacum dactyloides* accession to start with? Was Wayne Hanna very lucky to succeed, having only a few wild relatives to pearl millet in his introduction garden when he started his transfer attempt? Reality is, in both cases, *Tripsacum* and *Pennisetum*, there are plenty of species, races, or ecotypes from these two genera which would have probably given the same result. Apomixis is probably present in more than one hundred species of *Pennisetum*, and probably present in all polyploid ecotypes from the 16 known species of *Tripsacum*. The point is: is the introduction of *Tripsacum dactyloides* used by Petrov or the introduction of *Pennisetum squamulatum* used by Hanna the best potential donors of apomixis for transferring to these crops? There is just no reason they should be, or it would be an incredible luck: we crossed 90 different accessions of *Tripsacum* (from different species) with maize in 1991; almost all produced F1 hybrids.

Now, although we started conventional crossings anew, I believe that we are closer than ever to be able to link apomixis with markers and possibly to manipulate this trait as discussed above by Jim Peacock. In the last couple of years it has been shown that transformation of grain crops was perfectly possible, using *Agrobacterium*, which may help getting apomixis in crops with no apomictic relative, from an apomictic maize or an apomictic pearl millet. This means that we should also, beside our classical approaches, invest in molecular studies, to find the markers we need, and that is just what we started in our maize x *Tripsacum* program some months ago. Such a transfer,

from a wild species to a crop is not simple, but we can surely make the assertion that it is more and more likely to be successful. This justifies all current efforts towards this goal. And our willingness to cooperate, through a networking system, with all other labs working to achieve the same goal.

From last year's paper in ANL, we continued our collection efforts in Mexico, to reach over 2,250 accessions from 149 different populations. Emphasis is now put on producing tetraploid sexual plants from selected diploids. This is being done two ways: 1) by colchicine treatment of calli, 2) by colchicine treatment of isolated tillers. Tetraploid sexual *Tripsacum* might also be obtained from 2x-x-4x interspecific crosses (Harlan and deWet got their first 4x sexual plant this way in the *Bothriochloa-Dichanthium* complex) or directly within the wild populations. For the latter, we put some hope in a detailed study of two populations, one of *T. dactyloides* and one of *T. zopilotense*, which look to be entirely diploid and sexual, save one exceptional tetraploid which might be a recently produced autotetraploid (at least in the case of the *T. dactyloides*, the population is completely isolated from any other *Tripsacum*). We also started a cytoembryological study of a sample of *Tripsacum* accessions, completed by an isozyme analysis (as progeny-test). We will report on that in the next ANL issue. Progress in the study of *Tripsacum* diversity is reported in the following paper.

Yves Savidan

Progress in the study of *Tripsacum* diversity using RFLPs

A collection of wild populations of *Tripsacum* from Mexico has been assembled and established at the CIMMYT experimental station of Tlaltizapan, Morelos, Mexico (Berthaud & Savidan, ANL3:29-30, 1991; Savidan et al., ANL3:30-31, 1991). Such a collection allows us to carry out a study of the genetic diversity of *Tripsacum* species using molecular markers. The objectives of the study are:

- to group the *Tripsacum* accessions in genetic clusters corresponding or not to the two sections and the 16 described diploid and tetraploid species;
- to study the relationships between diploid species, between tetraploid species, and between the diploid and tetraploid compartments;
- to raise information on the extent of genetic diversity available in the populations;
- to know more about the gene flows within the agamic complex(es). For this specific objective, populations have already been identified to include several ploidy levels and morphotypes (Savidan et al., 1991, op cit.).

To study the organization of the diversity within the *Tripsacum* genus, 175 accessions belong-

ing to more than 100 populations are analyzed for RFLPs using conserved maize nuclear probes (15 UMC probes):

- 100 tetraploid plants (1 plant/morphotype in each tetraploid population);
- 14 triploid plants from 7 populations;
- 57 diploid plants from 23 populations.

A first look at the data, prior to any multivariate analysis that will be run when data are completed, shows the diploid species to be well separated. With the probes we used, they all exhibit species-specific bands and do not show any common band. This will not allow us to cluster diploid species. The genetic clustering of accessions within the tetraploid compartment is much less clear at this point. It is very difficult to distinguish clusters without a computer analysis, although three groups can be seen, based on common bands of probes that are diagnostic for diploid species. These three groups correspond roughly to three geographical origins (Tables 1 and 2).

These results are not surprising, knowing that although the morphological distinction of the described diploid species is quite clear, there are serious taxonomical problems at the tetraploid level. For example, *T. dactyloides* is a very heterogeneous species, with different subspecies, described from northern United States to South America. In Mexico, and more specifically in the states of Jalisco and Nayarit, it is common to find plants with morphological characters from *T. pilosum*, *T. maizar* and *T. dactyloides* within one tetraploid population, sometimes grouped in one same plant. This makes interesting to study in more detail the gene flows within these complex populations.

Table 1. Clusters observed in diploid accessions of *Tripsacum*

diploid accessions
<i>T. pilosum</i> (2 populations) and diploids of pop#39
<i>T. maizar</i> (5 populations)
<i>T. laxum</i> (3 populations) and diploids of pop#99
<i>T. bravum</i> (1 population)
<i>T. latifolium</i> (5 populations)
<i>T. zopilotense</i> (3 populations)
<i>T. intermedium</i> (population #95)

Table 2. Clusters observed in tetraploid accessions of *Tripsacum*

tetraploid accessions
<i>T. pilosum</i> - <i>maizar</i> type, Jalisco and Nayarit (37 populations)
<i>T. intermedium</i> , Chiapas (7 populations)
<i>T. dactyloides</i> type, Mexico, Michoacan, Guerrero (25 populations)

APOMIXIS NEWSLETTER

4

April 30, 1992

The data presented here are not to be used in
publications without the consent of the authors

Apomixis Newsletter is a publication of
APONET
International Network for
Apomixis Research

Yves H. Savidan and Charles F. Crane (Eds.)
ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération,
and Department of Soil & Crop Sciences, Texas A&M University, College Station