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BMT/5/12

ORIGINAL: English

DATE: August 31, 1998

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR
TECHNIQUES AND DNA-PROFILING IN PARTICULAR**

Fifth Session

Beltsville, United States of America, September 28 to 30, 1998

PRESCREENING OF VARIETIES (WITH THE HELP OF ELECTROPHORESIS):
PROGRESS REPORT OF A CASE STUDY IN *POA PRATENSIS* AND
SOLANUM TUBEROSUM (TWA/26/10 AND TWA/27/20) AND
REPORT OF THE DISCUSSION IN THE TECHNICAL
WORKING PARTY FOR AGRICULTURAL CROPS

Document prepared by experts from the Netherlands and the Office of the Union

I. Report of Discussion on Prescreening of Varieties held during the Twenty-Seventh Session of the Technical Working Party for Agricultural Crop (extracted from TWA/27/27 Prov., paragraphs 33-40)

The twenty-seventh session of the Technical Working Party for Agricultural Crops (hereinafter referred to as "the Working Party") noted that the Technical Committee had rediscussed the question of prescreening and noted the different views of the various Working Parties. In order to make progress in the discussions, the Technical Committee agreed that some concrete cases would have to be selected and the whole problem further investigated on the basis of them. It proposed to ask all Technical Working Parties to rediscuss the question of prescreening and to cite examples that would support their positions. For the TWA, the species *Poa* and potato were mentioned as possible examples and, for the TWO, roses. For roses there was already a good deal of additional information that would be helpful. In addition, it would underline the importance of ornamental varieties and the international trade in them. For the TWF, the species peach was mentioned. The Technical Committee also agreed that, in addition to developing models for the prescreening of varieties, it was very important to have an intensive exchange of information between the testing stations and the offices of member States. Only if they knew what varieties were protected or tested in other member States would they be able to check a complete collection of varieties to find all similar varieties which should be compared with a candidate variety. The expert from the United States explained that in his country there existed a large list of descriptive data which would be available on the Internet.

The expert from the Netherlands introduced document TWA/27/20 (reproduced in the section II-2 of this document) on the Prescreening of Varieties: Progress report on a Case Study in *Poa pratensis* and *Solanum tuberosum*. He recalled that *Poa pratensis* is an apomictic species in which it is not possible to select certain bands and in which all plants within a variety have the same genotype. In the prescreening, electrophoretic characteristics not in the Test Guidelines had been combined with seedling characteristics which could be observed before planting in the field. The electrophoresis method was not robust enough over the years, laboratories, gels and experts applying the method. Therefore it could not be used alone. In potato, electrophoresis characteristics were combined with light sprout characteristics to compare a candidate variety in a computer database with descriptions from other varieties. Also in this case, the electrophoretic characteristics could not be trusted alone; they were only used to confirm the morphological differences between varieties, based on those routine Test Guidelines characteristics stored in the database which could be observed and compared before the planting or sowing of the trials.

The Working Party repeated all the arguments in favor and against the use of characteristics not included in the UPOV Test Guidelines and the need to reach a balance of the different risks involved. Some experts repeated that it was too risky to accept a difference in the electrophoresis band without having checked the uniformity as the difference could be caused by an off-type only and one would in this case wrongly not plant the variety in question for comparison in the field. It was also necessary to fix the minimum difference and to do that it was important to know the genetic control of the bands used. There had to be a possibility to limit the risk of taking a wrong decision. In the past, a regional reference collection had not involved a big risk but nowadays, especially in the ornamental species, a world reference collection was needed which required some screening to keep the number of varieties planted

reasonable. The Working Party finally accepted the idea of prescreening and agreed that UPOV had to search for a good system to select all similar varieties to be grown.

Several experts insisted that the electrophoresis method was not robust enough to be used alone. Therefore it should only be applied together with other characteristics. Some experts were of the opinion that all information should, however, be collected on the same testing station or trial field in order to be applicable. In the prescreening, a larger difference had to be required to avoid eliminating a very similar variety. The comparison was therefore different from that of testing DUS where a small difference might be enough.

Characteristics less affected by the environment were preferable, for example, the characteristics resulting from protein electrophoresis. If there was hesitation in using electrophoresis alone, it should be combined with other characteristics. However, the whole screening process should be clearly defined and laid down in the description of the testing for the species concerned, e.g. in the Test Guidelines or in an annex to them.

Some experts considered that, in addition to traditional (morphological) characteristics for prescreening, other methods could be also envisaged as for example image analysis or even DNA methods.

However, before being able to do so, it was necessary in looking at particular cases to establish certain basic principles for prescreening irrespective of which methods were used. Only thereafter should it be decided where the rules were to be reproduced in the Test Guidelines. In order to make progress during its next session, the Working Party asked the expert from the Netherlands to prepare a draft for a protocol for the prescreening of *Poa pratensis* varieties and the experts from France for maize varieties. The two documents should be prepared before the end of 1998 and circulated by the Office of UPOV for comments before the end of March 1999, for documents to be distributed in May 1999.

In connection with the discussions on prescreening, the Working Party noted that prescreening was mainly a question for the system of government growing tests and to a lesser extent for the system of testing by the applicant or breeder where the possibility of six months' opposition after publication of the description before the final granting would allow correction by third parties of oversights during the testing. For the description, breeders were recommended to use as many characteristics as possible as that would increase their possibility of defining their rights. In case of opposition, it might be possible that additional tests were required, if need be, with additional varieties.

II. PRESCREENING OF VARIETIES: PROGRESS REPORT OF A CASE STUDY IN *POA PRATENSIS* AND *SOLANUM TUBEROSUM*

1. Report presented at the twenty-sixth session of the Technical Working Party for Agricultural Crops (TWA/26/10)

Referring to the document TWA/25/7 and the discussion during the TWA meeting in 1996 (TWA/25/7, paragraphs 17-24), the following remarks can be made:

1. The testing of the electrophoretic database for *Poa* has been delayed due to technical problems. Only recently all 250 varieties have been stored in the database.
2. The first experience shows a major problem. The comparison of similar lanes on different gels is not accurate enough. Although each gel contains three reference lanes, the positioning of the important main bands is in some cases problematic.
3. Another difficulty is the low intensity of some bands. This is a well known problem in electrophoresis but it complicates the task to identify an unknown sample, by comparing it with the electrophoresis database.
4. A possible improvement may be reached by using a computer system in which the conformity of the electrophoretic patterns is calculated. Using a threshold value gives a reliable chance to find the similar variety. This system may be tested next spring.

According to paragraph 20 of the report of the 1996 TWA meeting, morphological seedling characteristics might be used in combination with electrophoresis.

The following procedure may be tested next spring:

1. The candidate varieties are put in the electrophoretic database.
2. Seedling characteristics are recorded and fed into a database, which contains the characteristics of all varieties.
3. The candidate varieties are compared on the basis of the seedling characteristics with all varieties in the database. Similar reference varieties, of which the differences are not clear or doubtful, are printed on a list.
4. The electrophoretic lanes of these close reference varieties are compared with the candidate varieties. If the electrophoretic differences are clear and support the (small) differences recorded in the seedling characteristics, the reference variety may be omitted in the spaced plant trial.

In this way the "grouping" will be based on the seedling characteristics. The electrophoretic characteristics may be regarded as "supportive" or "complementary" characteristics. This approach may prevent the complication of using non-guideline or non-routine characteristics for grouping.

A similar approach may be tested for potatoes, using lightsprout characteristics in combination with electrophoresis.

2. Progress report presented at the twenty seventh session of the Technical Working Party for Agricultural Crops (TWA/27/20)

A. *Poa pratensis*.

The database for *Poa pratensis* contains the morphological description and the electrophoretic pattern of 270 varieties. The prescreening procedure is as follows:

1. In the beginning of January the candidate varieties are seeded in a seedling trial in the greenhouse, together with 15 standard varieties.

At the same time the electrophoretic pattern is established by the IEF method on the seed.

2. After 6 weeks the plants are scored for 6 seedling characteristics, number 1-6 of TG/33/6.
3. The scores for each variety are compared with the scores of all varieties in the database, by a special computer program. Those varieties are printed of which the difference in two of the six characteristics is only one class or less.
This greenhouse trial shows sufficient reliability from year to year in order to compare the results from different years.
4. The electrophoretic pattern of the candidate variety is compared with the electrophoretic pattern of these close reference varieties.
5. If there is no clear difference, the reference variety is planted in the spaced plant trial adjacent to the candidate variety.
If there is a clear difference, the reference variety is to be regarded sufficiently distinct from the reference variety on the basis of the difference(s) of the seedling characteristic(s). On this basis the reference variety can be left out of the spaced plant trial.
6. All 270 varieties will be sown in the row trial, as a living database. This offers the opportunity of a field comparison of the candidate variety with all reference varieties. In the unlikely event, that a reference variety has been overlooked, it can be planted in the second year.

The results with the candidate varieties in 1998 have shown 10 varieties with an unclear difference on the basis of seedling characteristics. 4 of these varieties were also not different in their electrophoretic pattern. The other 6 varieties appeared to have an electrophoretic pattern clearly different from the printed reference varieties. All other candidate varieties in test, 15 in total have also been screened on electrophoretic resemblance. They have all been found clearly different from all reference varieties. This confirms the differences found between these candidate varieties and the reference varieties on the basis of the seedling characteristics which are stored in the morphological

descriptor database. This means, that in principle, only 4 reference varieties, on top of the 15 standard varieties, have to be planted in the spaced plant trial.

B. *Solanum tuberosum*

The database for potato contains the morphological description, the photograph of the lightsprout and the electrophoretic pattern of respectively 725, 542 and 670 varieties.

The prescreening procedure is as follows:

1. The candidate and 25 standard varieties are put in the lightsprout trial, at the beginning of January. Characteristic 47 of TG/23/5, the tuber color of skin, is directly scored from the tuber.
At the same time the electrophoretic pattern is established by the IEF method on the tuber.
2. After 6 weeks the expression of the 12 lightsprout characteristics, number 1-12 of TG 23/5 can be scored.
3. The scores for each variety of the 13 characteristics are compared with the scores of all varieties in the database, by a special computer program. Those varieties are printed of which the difference in 3 characteristics is only one class or less.
The lightsprout trial, performed in a closed room at a controlled temperature and low light intensity, has shown very reliable results over the different years.
4. The lightsprout photographs and the electrophoretic patterns of the printed varieties are compared with the photograph and the electrophoretic pattern of the candidate variety.
5. If there is no clear difference, the reference variety is planted in the spaced plant trial adjacent to the candidate variety.
If there is a clear difference, the reference variety is to be regarded sufficiently distinct from the reference variety on the basis of the difference(s) of the lightsprout and tuber characteristic(s). On this basis the reference variety can be left out of the spaced plant trial.
6. The complete set of scores of all 50 characteristics is compared with the full set of all varieties in the descriptive database after the first observation year. In case of doubt an extra comparison can be made between the lightsprout photographs and the electrophoretic patterns of the candidate variety and the relevant reference varieties.

The results of 1998 comprise a few example cases. Only a few closely similar varieties have been selected on the basis of the 13 characteristics. The differences in the photographs are sometimes difficult to see. The difference in the electrophoretic pattern however can be very clear. It is to be expected that in potato only a few problem cases, mainly mutants or possibly GMO's, will occur.

The varieties not printed in step 3 and the varieties having a sufficient difference according to step 5, may be left out of the trial.

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In this way the electrophoresis can be used to confirm the morphological differences between varieties, based on the routine guideline characteristics stored in the database, which can be observed and compared before the planting or seeding of the trial.

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