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GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

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DRAFT REPORT ON THE SUBGROUP MEETING ON ELECTROPHORESIS IN CROSS-FERTILIZED CROPS HELD IN GENEVA, SWITZERLAND, ON APRIL 3, 1998

prepared by the Office of the Union

Opening of the Meeting

1. The meeting was held in the headquarters of UPOV in Geneva, Switzerland. It was opened by Mr. Aubrey Bould, United Kingdom, Chairman of the Technical Working Party for Agricultural Crops. The list of participants appears in the Annex to this report.

Background Information

2. The Subgroup based its discussions on documents TWA/24/5, TWA/25/5 and TC/34/6 and Circular U 2636.

3. The Subgroup recalled the discussions held in the TWA on the possible inclusion of electrophoretic characteristics in the Test Guidelines for Ryegrass, on the lines of what had already been done for some species, and the problems encountered. It noted the questions raised in document TWA/25/5 and during the last session of the TWA, which had led to the convening of the present Subgroup meeting, namely the need (a) to present the legal questions

regarding possible additional requirements for the breeder of the similar earlier variety to the Technical Committee and to the Administrative and Legal Committee (CAJ); (b) to engage in further discussions on uniformity, as uniformity could not be applied but only stability of frequencies; (c) to ask the Technical Working Party on Automation and Computer Programs (TWC) for advice on the number of plants in tetraploid varieties to be observed and whether the chi-squared test was at all applicable; (d) to obtain the opinion of breeders; (e) to rediscuss the question of example varieties and of a ring test.

4. It also recalled that, even though quite an amount of technical information existed, there was still a need to check that consistent results were obtained by different laboratories. It noted moreover that another problem to be solved was the checking of uniformity in a bulk sample and in frequencies of alleles.

Report from ASSINSEL

5. Mr. Le Buanec (ASSINSEL) introduced document TC/34/6, which reproduces the discussions at the meeting of the ASSINSEL Fodder Crop Section held at Merelbeke, Belgium, on February 26, 1997, and which had also been unanimously adopted during the ASSINSEL Congress in Stockholm in May 1997. He stressed that the document represented ASSINSEL's position at the present stage of knowledge of the methods. In it ASSINSEL reported that it could not accept electrophoretic characteristics in parallel to traditional phenotypic analysis even as a last resort. It agreed that electrophoretic characteristics were very useful, but not for studying the distinctness of synthetic forage varieties. It therefore also rejected their use for the assessment of uniformity. It did not agree that the breeder of an earlier variety should be forced to maintain his variety fixed in the future for new electrophoretic characteristics, as the variety was not selected and fixed for those characteristics. Electrophoretic characteristics should not be used to assess the drift of variety A towards variety B, as that was a problem of stability in a characteristic for which the variety was not selected. A difference in the frequency of alleles should not be used to establish distinctness. ASSINSEL was at present studying the assessment of essential derivation on ryegrass, and some further remarks might be made as a result of the outcome of those discussions. ASSINSEL was prepared to accept that electrophoretic characteristics could be used for pure lines. They should only be used for synthetic varieties if both varieties were homozygous for the alleles of a locus, which was highly improbable. Electrophoretic characteristics provided useful additional information, but should not be used for DUS testing of cross-pollinated forage varieties, as it would be too easy to accumulate slight differences and achieve distinctness. This would lower the barriers of DUS and therefore weaken the protection and open the door to piracy and plagiarism.

Criteria for the Acceptance of Distinguishing Characteristics

6. Most experts agreed that, because of the many possibilities for plagiarism, it was not desirable to introduce electrophoretic characteristics for distinctness in cross-fertilized varieties without taking some precautions at the same time. The same criteria as were applied at present to the introduction of any new morphological characteristics would also have to be applied to the use of electrophoresis, however. It was therefore necessary to study the reason

why the use of electrophoretic characteristics should not be accepted or to investigate means of limiting the risk of plagiarism.

7. The previous day there had been a discussion in the Administrative and Legal Committee (CAJ) on the use of molecular markers. The problems in the use of electrophoretic characteristics of isoenzymes were rather similar, so an approach similar to that adopted by the CAJ in recommending solution two might have to be agreed upon. The main difference between molecular markers and electrophoresis, however, was that isoenzymes were part of the phenotype.

Difference with Regard to Wheat, Barley and Maize

8. The Subgroup recalled that for wheat, barley and maize UPOV had agreed to attach electrophoretic characteristics in an annex to the Test Guidelines:

> "thereby creating a special category of characteristics, because the majority of the UPOV member States are of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should be used not as routine characteristics but at the request or with the agreement of the applicant of the candidate variety."

The question therefore was whether one could apply the same procedure for ryegrass, and if not why not.

9. All experts agreed that there were additional problems with cross-fertilized varieties, where the variety consisted of a population with several genotypes, and also that so far mainly bulk samples had been analyzed. A further problem was the testing of uniformity in characteristics of frequencies.

Testing of Frequencies

10. The Subgroup discussed at length whether there was any difference between the use of a morphological characteristic or electrophoretic characteristics. For morphological characteristics, UPOV had accepted differences in frequencies for distinctness. Other experts responded that it would depend on the minimum distance between varieties and the ease with which changes could be made. It was much easier to alter an allele frequency than to change a morphological characteristic like earliness. For earliness the genetic control was very complex and a change would also change several other characteristics. In electrophoretic characteristics, often controlled by a single gene, a change was very easy to make if the difference referred to frequencies and the distribution in a population.

11. A much larger minimum distance therefore had to be required than in the case of morphological characteristics. While all agreed that distinctness could in principle be based

on differences in proportion, several experts insisted that each characteristic had to be studied individually for each species with regard to its complications and consequences. For electrophoresis in cross-fertilized varieties, the use of frequencies should not be allowed because it would transfer all problems to the criteria of essential derivation.

12. Several experts reported that there were differences between testing at the Plant Variety Rights (PVR) Offices for the granting of rights and later on in the post-control tests. Test findings showed that while the varieties seemed to remain stable in the tests at PVR stations, a test on four different alleles had shown changes in the frequencies of two of them during certification in row trials or plots. This meant that even if a variety had been bred correctly, the frequency might change in the post-control tests and therefore might not be stable. It was also hardly possible to check whether a given allele frequency was obtained through original breeding or just through a mechanical mixture of two existing varieties.

13. Breeders also reported that differences in the multiplication of the same variety were often greater than differences between varieties. If electrophoretic characteristics were accepted, breeders would be forced to undertake a large amount of laboratory work, which would prevent them from applying for protection until much later than at present.

Testing of Uniformity

14. The Subgroup noted that so far mainly bulk samples had been tested, which would not allow uniformity within a variety to be judged. In addition, in the case of observing allele frequencies, there was by definition no uniformity either. The problem was how to solve this difficulty. Should one accept any variety as long as the frequency was kept stable? Did one have to analyze each individual plant in the population separately? In ryegrass measurements would serve only to describe an imaginary medium plant of the variety.

15. In this connection the Subgroup also discussed whether a difference in the level of uniformity could be used for establishing distinctness. All experts agreed that a difference in uniformity would not be sufficient to establish distinctness. Some experts recalled that a characteristic could only be used for distinctness if both varieties were uniform in that characteristic. If one of them (normally the earlier one) lacked uniformity, the characteristic was excluded from use for testing distinctness between the two. If a candidate were only more uniform than an existing variety, that candidate would no longer be considered new, as uniformity was only a quality, not a distinguishing characteristic.

Advantages or Goals of Using Electrophoretic Characteristics

16. The Subgroup agreed that electrophoretic characteristics should only be used if their use offered a clear advantage. One main advantage of electrophoretic characteristics of isoenzymes would be that they were less influenced by the environment. Another, in grasses with few characteristics, would be that there was no correlation between isoenzymes and morphological characteristics. Some experts considered that without that new technique a point might be reached in the future at which the species had to be closed for new varieties as they could no longer be distinguished. Electrophoretic characteristics could become a second

target for breeding, and their use would therefore provide a new way of distinguishing varieties.

17. A prerequisite for their use, however, was that there should be less variation within a variety than between varieties. Many post-control tests had shown that there were greater differences in allele frequencies within varieties than between varieties, making the application of electrophoretic characteristics useless. Another question was whether the breeder would be able to keep his variety stable in that limited range of frequencies, or at what cost he would be able to do so. If electrophoretic characteristics were accepted, breeding schemes would be restricted and more selection would be required of the breeder before he could ask for plant variety protection.

Disadvantages of the Use of Electrophoretic Characteristics

18. The Subgroup agreed that electrophoresis should only be used if its use were not accompanied by too many disadvantages and did not ultimately create more problems than it solved. All agreed that one of the main problems with accepting electrophoretic characteristics was that of reducing minimum distance and the ease with which changes could be made in the allele frequency in an existing variety. The question was therefore whether and if so how plagiarism could be prevented.

19. The ideal solution would be if it were possible to fix a clear minimum difference in such a way as to safeguard against misuse. If this were not possible but the method were nevertheless accepted, all problems would be concentrated on the judgement of essential derivation which, however, was applicable only to some of the varieties involved (as long as the original variety was not itself an essentially derived variety). It was necessary to maintain the integrity of the PVR system and of the DUS tests. A system that regarded any difference as acceptable and led to a new variety would not, whatever the safeguards introduced through essential derivation criteria, be a good solution for the PVR system as a whole.

Problems of the Methods

20. The Subgroup noted that, although the methods had already been thoroughly studied, work was still necessary to improve and standardize them with a view to achieving harmonized results between different laboratories. It noted a report from breeders on the sensitivity of the method to small differences in the protocol on isoenzymes, which would lead to completely different results. In a ring test organized by breeders on the same plant material, four different conclusions were obtained. A mere difference in the water used or a slight change in the pH value led to different results. Therefore many efforts would still have to be made to reach an agreed, standardized and reliable method leading to repeatable results.

Problems of Interpretation of Results

21. The Subgroup agreed that it was important not only to reach a harmonized handling of the method but also to agree on and harmonize the interpretation of results. Should one only accept a clear absence or presence of a given band and omit different intensities? There too,

however, were different personal interpretations of the point from which a certain band was considered present. For that reason there was a need to conduct further ring tests to study the outstanding problems of the method and to come to an agreed common interpretation of the results.

Statistical Questions

(a) Sample size for tetraploid varieties

22. The Subgroup noted that one still unresolved item was the required sample size for tetraploid varieties. The answer to this, and to the question whether the chi-square test or the AMOVA were the right statistical method for the testing, had to be awaited from the Technical Working Party on Automation and Computer Programs (TWC). The problem at present was that mainly bulk samples had been tested. This would not allow the testing of uniformity, which can only be judged by testing single plants.

(b) Fixing of the higher required difference

23. Several experts stated that the possible use of electrophoretic characteristics depended on the basis on which a decision on distinctness would be taken. At present the experts lacked the information on which to fix that basis. Some wondered whether a larger minimum distance could be preset using special statistical methods. This would make it more difficult to change the frequency of the alleles to obtain a new variety. Other experts stated that statistics would merely tell whether there were two populations and whether the results were significant. One could increase the number of observations or raise the significance level to make sure that there really was a new variety. Whatever level was fixed, however, it would not be sufficient to discourage someone who really intended only to change the frequency. It would only require a little more work. The TWC should be asked for advice.

24. Some experts were of the opinion that it might be possible to consider using electrophoretic characteristics in combination with other characteristics, and requiring clear differences in at least two or more characteristics. Others imagined them being used in the case of differences in morphological characteristics below the significance level. Some experts, however, wondered how to determine the difference in a morphological characteristic below its significance level and–even more difficult–how to check uniformity of an expression that existed only below the significance level.

25. The Subgroup finally came to the conclusion that electrophoretic characteristics in cross-fertilized varieties should never have an independent function in DUS testing. A difference in an electrophoretic characteristic alone should not be sufficient to establish distinctness. It should only have a supporting function and only be used in addition to another difference in a morphological characteristic. The question of how large that difference and that different requirement had to be was left open, however. Another possibility could be a difference in a characteristic not used so far, like yield, but that raised the question of distance and the means of checking uniformity or stability of yield. In general it should only be used if the crop expert was convinced that the candidate variety was a different variety, in which case the characteristic would only support what had been observed in other traditional characteristics, but at a level that alone might not have been sufficient to establish

distinctness. It might also support differences in other morphological or physiological characteristics, or characteristics like yield, which in many cases had not yet been accepted for DUS testing.

Program

26. The Subgroup finally realized that too many questions were still outstanding. It also noted that the introduction of electrophoretic characteristics raised many additional questions which still had to be solved. Other Technical Working Parties should therefore also study the use of electrophoresis in cross-fertilized varieties. Especially the Technical Working Party for Vegetables (TWV) and Technical Working Party for Ornamental Plants and Forest Trees (TWO) (for seed-propagated varieties) should give their opinion, and the Technical Working Party on Automation and Computer Programs (TWC) should deal with the questions of sample size, the best method for establishing distinctness and whether, and how much higher, minimum distances could be preset to discourage plagiarism. Moreover, the effect of its possible use in the certification and national listing system should be considered, and also whether VCU results could be used in combination with electrophoretic characteristics to establish distinctness. However, no system would be acceptable if it did not allow the checking of uniformity and stability in the characteristics eventually used to establish distinctness.

27. The Subgroup reminded experts that in all studies it should be kept in mind that the advantage achieved from the method should be weighed against the effect it might have on the varieties, on the breeder and on the whole PVR system. If it was liable to create more problems than it solved, it should not be accepted and discussions should not be pursued further.

[Annex follows]

ANNEX

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