Disclaimer: unless otherwise agreed by the Council of UPOV, only documents that have been adopted by the Council of UPOV and that have not been superseded can represent UPOV policies or guidance.

This document has been scanned from a paper copy and may have some discrepancies from the original document.

———

Avertissement: sauf si le Conseil de l'UPOV en décide autrement, seuls les documents adoptés par le Conseil de l'UPOV n'ayant pas été remplacés peuvent représenter les principes ou les orientations de l'UPOV.

Ce document a été numérisé à partir d'une copie papier et peut contenir des différences avec le document original.

———

Allgemeiner Haftungsausschluß: Sofern nicht anders vom Rat der UPOV vereinbart, geben nur Dokumente, die vom Rat der UPOV angenommen und nicht ersetzt wurden, Grundsätze oder eine Anleitung der UPOV wieder.

Dieses Dokument wurde von einer Papierkopie gescannt und könnte Abweichungen vom Originaldokument aufweisen.

———

Descargo de responsabilidad: salvo que el Consejo de la UPOV decida de otro modo, solo se considerarán documentos de políticas u orientaciones de la UPOV los que hayan sido aprobados por el Consejo de la UPOV y no hayan sido reemplazados.

Este documento ha sido escaneado a partir de una copia en papel y puede que existan divergencias en relación con el documento original.

UPOV

TWA/21/9

ORIGINAL : English

DATE : January 22, 1993

0429

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

Twenty-first Session

Menstrup Kro, Denmark, June 16 to 19, 1992

REPORT

adopted by the Technical Working Party for Agricultural Crops

Opening of the Session

1. The twenty-first session of the Technical Working Party for Agricultural Crops (hereinafter referred to as "the Working Party") was held in Menstrup Kro, Denmark, from June 16 to 19, 1992. The list of participants is reproduced as Annex I to this report.

2. Miss J. Rasmussen, Director of the Department of Plant Variety Testing, welcomed the participants to Denmark. The session was opened by Dr. M.S. Camlin (United Kingdom), Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party adopted the agenda of its twenty-first session as reproduced in document TWA/21/1 after having agreed to discuss also under item 3 the important decisions taken during the twentieth session of the Working Party.

Important Decisions Taken During the Twenty-Seventh Session of the Technical Committee

4. Dr. Camlin (United Kingdom) gave a brief report on the important decisions taken during the previous session of the Working Party, referring to document TWA/20/9 and highlighting several paragraphs of that document. Dr. M.-H. Thiele-Wittig gave a brief report on the important decisions taken during the previous session of the Technical Committee, referring to the full report on that session reproduced in document TC/27/9.

0430

UPOV Central Computerized Data Base

5. Dr. Thiele-Wittig briefly related the history of the discussions in the different Technical Working Parties which finally led to the establishment of document TWC/10/2 as a result of a questionnaire issued by the Office of UPOV. He also reported on the discussions held on the same subject by the Technical Working Party on Automation and Computer Programs (TWC) and their proposal to set up a small subgroup which could comprise one or two experts from the TWC, as well as a crop expert and an expert with administrative and legal knowledge, to develop a minimum proposal for the checking of variety denominations and another proposal with further items useful for inclusion in a future UPOV data base. The Working Party had a lengthy discussion on the usefulness of such a data base, on its possibilities and on what should be stored in it. It was important to create an efficient system for all member States and too much parallel work should be avoided between UPOV and the EC, which had simultaneously planned to develop a data base for the future EC Plant Variety Protection Office, in addition to the already existing Common Catalogue of Varieties for agricultural plants and vegetables. Many experts were worried about the cost which the setting-up and running of such a data base would require.

6. The Working Party finally agreed to support the proposal of the TWC to set up a small subgroup to discuss the minimum content of such a data base. It proposed that, at the beginning, the data base should be limited to administrative information and to the checking of variety denominations. At present, no technical information should be included. To advance the discussions, it set up a small subgroup to prepare a list of minimum information to be included in the data base. As a result of the work of that subgroup and the discussions in the Working Party on the proposed list, it finally proposed the list of items to be included in the UPOV data base as reproduced in Annex II to this report. As the Working Party had mainly concentrated its discussions on the items to be included and as lack of time did not allow further discussion on the subject during the present session, it asked the other Technical Working Parties to put more emphasis on how fast it wanted the information to be distributed, on which medium, in which format and how to extract the data from the data base as this would also influence the cost of the overall system. If a subgroup were to be convened to discuss final items together with experts from WIPO, it proposed that Mr. Bar-Tel (Israel) be made a member of that subgroup, or, if he should be prevented from attending, Mr. Ghijsen (The Netherlands). The Working Party insisted that in addition to the discussions on the setting-up of a data base, parallel discussions should take place to standardize the national gazettes, and especially the information contained in the different sections, to facilitate a future exchange of information or, once a UPOV data base had been established, the inclusion of that information directly in the UPOV Data Base.

General Discussion on the Use of Electrophoresis in the Examination of Varieties

7. Dr. Cooke (United Kingdom) introduced documents TWA/21/2, TWA/21/8, as well as further information distributed during the session and reproduced in Annex IV to this report. He further referred to the report on the last session of the Working Party, of which paragraphs 28 and 29 recorded the decisions of the Working Party and the task given to the Subgroup on Electrophoresis. He reported that with respect to wheat and oats, the task given to the Subgroup had been carried out, but that with respect to barley, as could be seen from

the paper distributed during the session, no agreement on a single method could be reached. For barley, two methods would be proposed in parallel (the acid PAGE method recommended by ISTA, as well as the SDS-PAGE method which was widely used). There was no rational criterion which would help to decide which method should be used and which be eliminated.

8. The Working Party appreciated the work of the Subgroup on Cereals. It noted, however, that at present the discussions had mainly centered on the methods to be used rather than on the interpretation of the results or on the definition of the characteristics, although it was recognized that considerable comparative work had been carried out in respect of Relative Electrophoretic Mobility (REM) values for protein bands. The Working Party agreed that it should not be left to the individual member States to decide how to interpret the results.

9. In a survey, taken during the meeting, on which of the member States already used electrophoresis in the testing of distinctness or which member States planned to use it, it appeared that most member States were very cautious with respect to characteristics obtained with the help of electrophoresis especially as most breeders would be afraid of the obligation to make all the varieties homogeneous with respect to characteristics obtained with the help of electrophoresis. However, several member States reported that where the variety could not be distinguished by other traditional characteristics, and if the breeder agreed, they would accept a characteristic obtained with the help of electrophoresis as the final resort for taking a decision and declaring the variety distinct. In that case, however, a prerequisite was that the old variety from which the new variety was otherwise not distinct had also to be homogeneous in that characteristic and that the breeder of the old variety maintained his variety homogeneous in that characteristic. As some States would therefore accept characteristics obtained with the help of electrophoresis, UPOV had to advance in its discussions to prevent being overtaken by events.

10. The Working Party agreed to make a survey on how far member States already used electrophoresis in other species. Dr. Camlin (GB) would prepare a questionnaire to be circulated via UPOV with answers to be sent by April 1, 1993, for preparation of a document by August 30, 1993.

11. The Working Party therefore reconfirmed what it had already agreed during its last session, namely that

(i) electrophoretic characteristics should be included in the Table of Characteristics and not in an annex to the Test Guidelines;

(ii) the characteristics should not have an asterisk;

(iii) it had to be studied further whether the characteristics could be used alone or only in combination with a traditional characteristic and whether a difference in one of the characteristics alone would be sufficient to establish distinctness.

12. With respect to the definition of the characteristics obtained with the help of electrophoresis, the Working Party finally decided that the characteristic should be defined as the absence or presence of a given allele.

13. Having taken that decision of principle on the use and definition of the characteristics obtained with the help of electrophoresis, the Working Party then went back to the proposals of the Subgroup on Electrophoresis to find out how that decision could be applied to the testing of wheat, barley and oats

with the help of electrophoretic characteristics. It noted that genetic knowledge differed for the different species, for example there was good knowledge with respect to hordeins in barley and glutenins in wheat, but there was less knowledge with respect to gliadins in wheat and with respect to avenins in oats.

14. With respect to the methods for barley, the Working Party finally recommended that the SDS-PAGE method be used. However, it would be stated in the Test Guidelines that, if only B-hordeins and C-hordeins were analysed, the acid PAGE method was an acceptable alternative. Therefore, the Working Party proposed that the Subgroup on Electrophoresis should meet again to prepare the following proposals:

(a) For hordeins: characteristics on the absence or presence of alleles
(b) For glutenins: characteristics on the absence or presence of alleles
(c) For gliadins: if possible characteristics on the absence or presence of alleles (otherwise study the question further)
(d) For avenins: characteristics with band patterns and an agreed method (to be proposed for an intermediate period before more genetic information is available)

In the Test Guidelines, the method would have to be included and, in the case of barley, the full SDS-PAGE method together with a table establishing the correspondance with the Acid-PAGE method.

15. With respect to the consequences of the introduction of electrophoresis in the Test Guidelines, the Working Party agreed that Mr. Guiard (France) would prepare for September a document which would restrict itself not only to electrophoresis but also enlarge to new sophisticated methods used for identification and for distinctness purposes. That document would include three chapters, namely:

(i) Current developments in the field of description of varieties
(ii) Consequences of the distinctness of varieties and breeding activities
(iii) Solutions which should be used to keep a good protection system.

16. With respect to the current developments, the document would discuss the tendency to search increasingly for smaller and smaller differences, to use ever more precise methods to describe varieties and research into new characteristics independent of environment. It would mention the pressure on breeders to go further in developments and the fact that breeders would always think that their variety was new. It would take into account the possibility of reading variability which was not available before and the rapid evolution of the methods and tools. As there was a wish for an exchange of descriptions, there was a need to have characteristics independent of environment which would lead to the introduction of characteristics closer to the expression of the gene. The offices would have no choice and indeed no reason to reject characteristics of this kind. With respect to the consequences of distinctness, the document would mention the existence of increasingly closer varieties, the fact that if no limit was placed there would always be a possibility of finding differences, that there was no reason to reject a new characteristic, the risk of moving towards ever more uniform varieties, the decrease in protection for the breeder which would result from that fact and the transfer of the exercise of protection to the dependency stage. With respect to the solutions for maintaining good protection, the document would start with the correct interpretation of the new version of Article 1(iv) and Article 7 of the Convention of 1991, the study of the question species by species, the use of the genetic background of the characteristics and would promote a multivariate approach to distinctness, as there was a need to introduce the notion of weighting of characteristics.

0433

17. Having noted the outline of the document given by Mr. Guiard, some experts fully agreed with the intended document, while others warned that not just any new characteristic should be acceptable as one should be aware that the introduction of a new characteristic would be an act to the detriment of the breeder of the existing variety. Sophisticated characteristics should only be used once general agreement had been reached within UPOV. Others stated that, in general, when testing distinctness a set of several small differences was observed and it should be discussed whether that set of differences should be considered or other sophisticated methods which would help to find distinctness in the presence of the set of small differences. When looking for small differences, it should always be kept in mind that when accepting such small differences for distinctness, the breeder should also be able to keep his variety stable within this small range. On the other hand, when testing varieties, it should always be kept in mind that true breeding should be rewarded. When accepting certain methods also, it should be kept in mind that it should be possible to define the method with accuracy and the effort should remain reasonable.

18. The breeder present as an observer in the meeting stated that, in his personal opinion, many breeders were not in favor of too many varieties on the market as that would reduce the value of protection for each variety. The commercial advantage of each variety would be larger if fewer varieties were admitted. Therefore, not just any variety should be admitted to the market and the introduction of the system of essential derivation would work in that direction. On the other hand, however, authorities should be less reluctant to admit new, modern methods in the testing of varieties. If these methods are used in the breeding of varieties, they should also be admitted in testing by the authorities. Their application could be an important cost-saving factor.

19. The Working Party finally agreed that it would await with interest the paper prepared for the Technical Committee by the experts from France and would continue the discussion during its coming session on the basis of that paper and any results of the discussions which were expected to take place in the Technical Committee.

20. The Working Party agreed, following an invitation by the expert from the United Kingdom, that the next session of the Subgroup on Electrophoresis in Cereals would take place in Cambridge on March 9 and 10, 1993.

21. The Working Party furthermore agreed that, in future, the question of electrophoresis would not be discussed in separate subgroups to be set up on electrophoresis, but by the Subgroup for the species concerned when it discussed revision or establishment of a new Test Guidelines document.

22. The Working Party discussed again the consequences when an existing variety, from which a new variety was distinct in electrophoretic characteristics only, which was uniform at the time of the decision and later became heterogeneous. The Working Party noted that there was only a very small chance of an existing variety already uniform in an electrophoretic characteristic later becoming heterogeneous. Therefore, there was no need to consider that case in particular. However, the discussion also led to the question of the legal situation if, with the introduction of new characteristics, the description of existing varieties which had not been tested for those characteristics had to be extended. This led to the question whether the protected plant material would define the description, or whether the description was only defined by the description established on paper at the time of granting protection, and also whether the breeder would also have to keep his variety uniform in those characteristics that had not been observed at the time of granting the breeder's right, but which had been uniform, in order to keep his variety material in conformity with the deposited material.

Color Measurements

23. The Working Party noted document TWO/25/2, prepared by the Technical Working Party for Ornamental Plants and Forest Trees, on the measuring of color and document TWA/21/7 on the measurement of leaf color in ryegrass, prepared in the United Kingdom by experts of the Working Party. Dr. Camlin (United Kingdom) introduced document TWA/21/7. He concluded that the results of the comparison of visual rating with measured color characteristics were very encouraging, since color measurements were likely to be utilized in cases where color differences were observed via recorders in the field trials. It was, however, wrong to use the machine data to establish distinctness and, in that respect, he would support the proposal, mentioned in document TWO/25/2, to use the measurements only as support, as otherwise there was a risk that one would use machine data to create differences that did not exist. In visual observations, the observer would view each plant and would form a final impression of the color of that plant which would include the actual leaf color of all leaves including diseased and senescent leaves. The observation would be dependent on the environment and, in general, would be a relative observation rather than an absolute one. Measurements by the machine should only be used to confirm observed differences and be of additional help in establishing the conformity of the variety. For registration purposes, however, there was still a need for the adoption of a standardized method before it could be used for the observation of uniformity. Dr. Camlin finally pointed to the fact that the document had been prepared on the basis of data from one year and therefore the results needed to be checked again.

24. The Working Party, having discussed the possibilities of measuring color for agricultural species, confirmed the position taken by the document that it would only use differences seen by the eye. It could not see much interest in using the measurement of color for agricultural species. However, it would keep an eye on the progress made in the Technical Working Party for Ornamental Plants and Forest Trees and it asked the Office of UPOV to submit document TWA/21/7 to the TWO for information.

Statistical Methods

25. Mr. Kristensen (Denmark) introduced document TWC/10/5, explaining the approach in selecting the right tables for the testing of uniformity of vegetatively propagated and truly self-pollinated varieties, as listed in document TC/XXV/8. He emphasized that it was imperative that the technical experts, when preparing Test Guidelines and discussing the maximum number of off-types admitted, should first fix the population standard, i.e. the maximum percentage of off-types allowed in varieties of that species if the whole population were examined.

26. Mr. Ghijsen (The Netherlands) introduced document TWC/10/9, explaining certain consequences where the sample size chosen was too small. He emphasized that there should be equilibrium between the risk of erroneously accepting a heterogeneous variety as uniform and the risk of rejecting a uniform variety as heterogeneous. The decrease in the risk of committing the one error would automatically mean an increase in the risk of committing the other. The Working Party also noted the certification standards in The Netherlands (see Annex V). For DUS testing for plant variety protection purposes, the population standard could not be as strict as for certification. In a small sample the chances of admitting a heterogeneous variety were rather big. In that respect, the Working Party also noted a remark by the expert from the

United Kingdom that not all off-types could be observed with the same ease, some could be observed very easily, others with much more difficulty. The tables in document TC/XXV/8 would, however, be based on the condition that all off-types were observable with equal ease. The Working Party further agreed that the tables listed in document TC/XXV/8 would lead to a small risk for the breeder, but to a higher risk for the consumer (meaning that a heterogeneous variety was still accepted as uniform). The tables were, however, the best approximation to reality at present and the experts would always take into account certain exceptions. In order to study the subject further, the Working Party proposed to the TWC that it discuss during its next session whether sequential sampling, that means taking subsamples from the samples, could reduce the workload of the offices without unnecessarily increasing the risk of accepting heterogeneous varieties.

27. The Working Party noted that the General Introduction to the Test Guidelines did not explicitly deal with clear off-types in qualitative characteristics of cross-fertilized plants. There was, moreover, no unanimous opinion on the standards to be applied in this case and whether or not the tables in document TC/XXV/8 could be used in the same way. The Working Party agreed that this situation should be clarified at the next revision of the General Introduction to the Test Guidelines. Having noted the decision of the Technical Committee, taken during its last session, that the table of maximum numbers of off-types, as reproduced in paragraph 28 of document TG/1/2, was to be replaced by the tables reproduced in document TC/XXV/8 and having noted the doubt expressed with respect to this replacement by the TWC and the latter's request for clarification by the Technical Committee, a subgroup of the Working Party prepared a proposal for a text to replace paragraph 28 of document TG/1/2. After having amended the proposal in the meeting, it proposed to the Technical Committee for final approval the text reproduced in Annex III to the present report.

28. The Working Party also noted certain criticisms of the high number of documents produced by the TWC, many of which seemed too theoretical and were difficult for the crop experts to understand and apply. It noted at the same time that, without the help of the TWC and the establishing of the COYD analysis, national offices would be at a loss with respect to the testing of, for example, varieties of grasses and would have to reject many varieties to the detriment of the breeder. It also noted, as concerns the testing of TWV and TWO varieties, that the methods developed by the TWC were hardly used as the TWO very seldom applied statistics and statistics were at any rate not applicable to most of the vegetable species. As to the criticism concerning the difficulty of understanding the TWC documents, the Working Party was informed that the TWC had issued a document (TWC/10/3) in which the different methods were explained and that a revised version of that document containing a much simpler introduction to each of the methods was under preparation for the coming session of the TWC.

29. The Working Party also discussed problems connected with the low sample size in the case of electrophoretic characteristics. It noted that at present the sample size of the gel varied between 10 and 80. To justify a low sample number in electrophoretic characteristics it was stated that electrophoresis was a very precise tool which was not influenced by environment, which used the same standard as, for example, that used in ear rows. It was necessary, however, to study the risk involved via the sequential analysis and especially to give a better description of the risk when reducing the sample size. The experts in favor of a low sample size were ready to accept a higher consumer risk because of the very precise method. The Working Party agreed that it would discuss the sample size and the study of subsampling on the basis of the paper on sequential analysis which the experts from The Netherlands offered to prepare.

Cooperation With Breeders in the Testing of Varieties

30. The Chairman referred to the report on the last session of the TWA, which reported on the excellent discussion on cooperation with breeders in the testing of varieties which had taken place last year and on the different ways of having the breeder participate in the testing of varieties selected in the different member States. The Working Party further reviewed the participation of breeders in the discussion in the Technical Working Parties and Subgroups which had taken place in the past years and the need for help in the testing by the breeder because of the opening up of plant variety protection to the whole plant kingdom. As the technical experts from the breeders had been nominated to attend the session of the Working Party rather late this year, the experts looked for possibilities of increasing the participation of the breeders in their discussions. In this respect, it also pointed to the decision of the Council and the Technical Committee to invite breeders to selected items to be discussed in a given Working Party or Subgroup meeting. It therefore agreed that at the time of planning the coming session, it would take more time to select the items to which breeders would be especially invited to send experts.

31. Mr. Ghijsen (The Netherlands) informed the meeting that they were currently reviewing their system and the principles for testing vegetables and the participation of breeders in the testing. He promised to prepare a document by April 1993 for discussion during the coming session of the Working Party.

Report from the Subgroup on Electrophoresis in Cereals on the Test Guidelines for Wheat, Barley, Oats

32. The results of the discussions of the Subgroup and of the Working Party are reported under item 5 of this report. With respect to the individual documents for revised Test Guidelines for Wheat (TWA/20/2), for Barley (TWA/20/3) and for Oats (TWA/20/4), the Working Party discussed whether there was a need for further changes. It noted that several example varieties may now have to be replaced. In this connection it noted that the example varieties given in the three above-mentioned documents were mainly example varieties for the European region. The question would arise whether separate sets of example varieties would have to be established for different areas. The Working Party finally agreed that comments on the three above-mentioned documents for revision of example varieties or other comments should be sent to Miss Rasmussen (Denmark) before November 1992 in order to draft proposals for the next session of the Working Party. These comments could also include proposals for separate sets of example varieties for regions other than those in the present draft. The separate lists of example varieties should include the expressions of the grouping characteristics for each example variety. The whole question of example varieties should be discussed by the Technical Committee.

Discussion on Working Papers on Test Guidelines

Test Guidelines for Maize (Revision)

33. Mr. Guiard (France) introduced document TWA/21/3, reporting on the meeting of the Subgroup which took place on February 16 and 17 in La Minière, France.

34. In the discussion following the introduction, the Working Party discussed at length the differences between the member States as to the use of one or two testing places and their influence on the decision on distinctness. There were mainly two approaches, the first using only one location for the decisions on distinctness, but keeping a second testing place as a backup in case of unfavorable climatic or other conditions, and the second to use both testing places and obtain an average for the description of the variety. The Working Party therefore asked the Subgroup to address the question again and come back with further remarks. At the same time, it asked the Technical Committee to discuss the problem for other species.

35. The Working Party noted the report on the testing of distinctness of hybrids on the basis of the inbred lines and the formula. It noted that this testing was only considered to be a preliminary grouping of the varieties to find out those lines and hybrid varieties which would have to undergo a more precise test. Thus the testing of the inbred lines would not replace the testing of the hybrid itself.

36. The Working Party also noted the results of the testing of electrophoresis in maize and the proposal to start with the testing of isozymes. It invited all other member States to join in the planned ring test as laid out in paragraph 21 of document TWA/21/3. Any member State interested in joining the test, should contact the French experts.

37. The Working Party noted the weighting of characteristics in the screening of inbred lines. It noted that the threshold for screening was higher than that applied for distinctness purposes. However, the Working Party did not take any definite decision on the weighting of characteristics and would leave that to further discussion during its coming session or to the Technical Committee.

38. The Working Party did not find time to discuss in detail the Table of Characteristics, but asked all experts to send their comments on document TWA/20/8 to France. It asked the Subgroup to produce a new draft during its coming session, which would also contain characteristics obtained with the help of electrophoresis.

Test Guidelines for Soya Bean (Revision)

39. Mr. Strachan (United States of America) introduced document TWA/21/4. The Working Party noted that the draft differed from other drafts, and contained a lot of information which was addressed less to the official testing authorities than to breeders doing tests. This resulted in a discussion on who was the primary addressee of the UPOV Test Guidelines. As a result of these discussions, the Working Party agreed that the addressees of the Test Guidelines were the national offices of the member States. These national authorities would then either use the Test Guidelines in the given form or modify them to suit the national situation. They may, if applicable, add additional information addressed to the breeder to ensure thorough testing by the breeder.

40. The Working Party therefore agreed that the draft would need rewording and that this would best be done in the Subgroup on Soya Bean which was to meet the day before the next session of the Working Party. The Subgroup should also discuss the possibility to introduce isozyme characteristics in the document. With respect to the characteristics of diseases, the Working Party confirmed that only those characteristics would be included for which there was a standardized method, which would also have to be included, for which the race

was defined, and for which example varieties were given for each state of expression. In the case of resistance characteristics, the Working Party had so far only used the states "absent" and "present" for distinctness purposes. With respect to the practical procedure, the Working Party invited all member States to send their comments, by the end of November, to Mr. Strachan (United States of America), who would prepare a revised version of the document by the end of April 1993 for circulation for comments.

Test Guidelines for Peas (Revision)

41. The Working Party noted document TWV/24/9 and the fact that the expert in the Technical Working Party for Vegetables had been asked to prepare a new draft. It agreed to leave it to the TWV to approve the new draft before presentation to the professional organizations for comments.

Test Guidelines for Flax (Revision)

42. The Working Party referred to its discussion on document TWA/20/5. It asked all experts to send further comments to Mr. Guiard (France), who would prepare by the end of April 1993 a new amended document for discussion during the coming session of the Working Party.

Test Guidelines for Rape (Revision)

43. Dr. Fuchs (Germany) introduced document TWA/21/5, containing a report on the second meeting of the Subgroup on Rape held at La Minière, France, on October 23 and 24, 1991. He referred to the discussions on the different groups of varieties, inbred lines and narrow populations, hybrid varieties and synthetic varieties. The question whether the components of hybrids should be included in the test and whether unthreshed plants should be tested would have to be presented to the Technical Committee for discussion. At present it was premature to include electrophoretic characteristics in the Test Guidelines. There had been discussions on the different approaches with respect to off-types depending on whether one looked at distinctness, where some experts would admit a difference in several characteristics in contrast to the off-type in uniformity where an off-type in one characteristic would be sufficient to reject the variety. With respect to uniformity also standards would still have to be established as in some countries not all characteristics were tested for uniformity and for hybrids so far no standards existed.

44. The Working Party, having discussed the future procedure for establishing revised Test Guidelines for Rape, agreed to request a further meeting of the Subgroup. The Subgroup would meet in Menstrup Kro (Denmark) on April 27 and 28, 1993. In order to meet at the optimal time of development of the plant, the exact meeting date would only be confirmed about 3 weeks before the session. For that meeting the Subgroup would prepare a ring test with unthreshed plants.

Test Guidelines for Fodder Beet

45. Time did not allow a detailed discussion of document TWA/21/6. The Working Party therefore asked the member States to send their comments on that document to Miss Rasmussen (Denmark) before January 1, 1993, to enable her to prepare an amended document by April 1, 1993, to be forwarded to the members of the Working Party via UPOV.

Status of Test Guidelines

46. The Working Party agreed to rediscuss the revision of the Test Guidelines for Maize (Revision), Rape (Revision), Flax (Revision), Fodder Beet and Soya Bean (Revision) at its next session. It also agreed to rediscuss the draft Test Guidelines for Wheat (Revision), Barley (Revision) and Oats (Revision) at its next session if the inclusion of electrophoretic characteristics had been completed.

Future Program, Date and Place of Next Meeting

47. At the invitation of the expert from New Zealand, the Working Party agreed to hold its twenty-second session in Lincoln, near Christchurch, New Zealand, from November 23 to 27, 1993. On November 22, 1993, the Subgroup on Soya Bean would meet at the same place. At the invitation of the expert from Australia, the Working Party would spend three days of official visits at the PVR Office in Canberra and breeding institutions nearby from November 29 to December 1, 1993, after its session in New Zealand. The Working Party planned to discuss or rediscuss the following items at its coming session:

(i) Important decisions taken during the twenty-eighth session of the Technical Committee;

(ii) UPOV Central Computerized Data Base

(iii) General discussion on the consequences of the introduction of new characteristics in the Test Guidelines;

(iv) Survey on the use of electrophoresis by the UPOV member States (GB to prepare a document);

(v) DNA techniques (FR to prepare a document);

(vi) Statistical methods;

(vii) Cooperation with breeders in the testing of varieties (NL to prepare a document);

(viii) Report from the Subgroup on Electrophoresis in Cereals on the Test Guidelines for:

- Wheat (TWA/20/2)
- Barley (TWA/20/3)
- Oats (TWA/20/4)

(ix) Discussion on working papers on Test Guidelines for:

- Peas (Revision)(TWV/24/9, GB to prepare a document)
- Maize (Revision)(TWA/21/3, FR to prepare a document)
- Rape (Revision)(TG/36/3, TWA/XIX/2 Rev., TWA/21/5, Subgroup report)
- Flax (Revision)(TG/57/3 ,TWA/20/5 FR to prepare a document)
- Fodder Beet (TWA/21/6,DK to prepare adocument)
- Soya Bean (Revision)(TG/80/3 ,TWA/21/4US to prepare a document).

48. The Working Party agreed that the Subgroups should meet as follows:

(i) Subgroup on Cereals in Cambridge, United Kingdom, on March 9 and 10, 1993;

(ii) Subgroup on Rape in Menstrup Kro, Denmark, on April 27 and 28, 1993 (date to be confirmed three weeks before the session);

(iii) Subgroup on Maize in Hanover, Germany, on February 16 and 17, 1993;

(iv) Subgroup on Soya Bean in Lincoln, New Zealand, on November 22, 1993.

49. The Working Party agreed to especially invite technical experts/breeders to the above-mentioned subgroup meetings and to the discussions which will take place on November 24 during its coming session in Lincoln on the following items:

- General discussion on the consequences of the introduction of new characteristics in the Test Guidelines;
- DNA techniques;
- Cooperation with breeders in the testing of varieties.

Technical experts would, however, also be welcome to participate in the discussions on other items on the agenda.

Visits

50. In the afternoon of June 17, the Working Party visited the Department of Variety Testing with its trial fields at Tystofte. In the same afternoon, it also visited Borrevy castle with its farm of more than 600 ha in the neighborhood of Tystofte.

51. This report has been adopted by correspondence.

[Five annexes follow]

LIST OF PARTICIPANTS AT THE TWENTY-FIRST SESSION OF THE TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS MENSTRUP KRO, DENMARK, JUNE 16 TO 19, 1992

I. MEMBER STATES

AUSTRALIA

Mr. M. KETHRO, Plant Variety Rights Office, Department of Primary Industries and Energy, G.P.O. Box 858, Canberra, ACT 2601 (tel. (06) 271 6476, telex 61289, fax 06 272 3650)

CANADA

Ms. V. SISSON, Plant Breeders Rights Office, Plant Products Division, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ontario, K1A OC6, (tel. (613) 995-7900, fax (613) 992-5219)

DENMARK

Mrs. J. RASMUSSEN, Director, Department of Variety Testing, Statens forsoegsstation, Teglvaerksvej 10, Tystofte, 4230 Skaelskoer (tel. 53-596141, fax 53-590166)

Mr. K. KRISTENSEN, Afdeling for Biometri og Informatik, Lottenborgvej 24, 2800 Lyngby (tel. 45 93 09 99, telefax 42 87 08 76)

Mr. G. DENEKEN, Department for Variety Testing, Teglverksvej 10, Tystofte, 4230 Skaelskoer (tel. 53 59 61 41, fax 53 59 01 66)

Mr. O. FALKENBERG, Department for Variety Testing, Teglverksvej 10, Tystofte, 4230 Skaelskoer (tel. 53 59 61 41, fax 53 59 01 66)

Ms. B. BOESEN, Department for Variety Testing, Teglverksvej 10, Tystofte, 4230 Skaelskoer (tel. 53 59 61 41, fax 53 59 01 66)

Ms. B. HOEGH, Department for Variety Testing, Teglverksvej 10, Tystofte, 4230 Skaelskoer (tel. 53 59 61 41, fax 53 59 01 66)

FRANCE

Mr. J. GUIARD, GEVES, La Minière, 78285 Guyancourt Cedex (tel. 30.83.35.80, fax 30.83.36.29)

Mrs. J. LALLEMAND, GEVES, Domaine du Magneraud, B.P. 52, 17700 Surgères (tel. 46 68 30 33, fax 46 68 30 87)

GERMANY

Dr. G. FUCHS, Bundessortenamt, Osterfelddamm 80, 3000 Hannover 61 (tel. 0511-57041, tx. 9 21 109 bsaha d, fax (0511) 56 33 62)

0442

ISRAEL

Mr. B. BAR-TEL, Plant Breeders' Rights Council, Agricultural Research Organization, The Volcani Centre, P.O.B. 6, Bet Dagan 50250 (tel. and fax 00972 3 9683492)

NETHERLANDS

Mr. H.C.H. GHIJSEN, Head of DUS Department, CPRO-DLO, P.O. Box 16, 6700 AA Wageningen (tel. 08370-76800, fax 08370-22994)

NEW ZEALAND

Mr. G.A. SPARKS, Plant Variety Rights Office, Canterbury Agricultural and Science Centre, Ellesmere Junction Road, Lincoln, P.O. Box 24, Lincoln (tel. (03) 253-011, fax (03) 252-946))

SPAIN

Mr. L. SALAICES SANCHEZ, Instituto Nacional de Semillas y Plantas de Vivero, Carretera de La Coruña, Km. 7,5, 28040 Madrid (tel. (1) 3476900 or 3474162, tx. 48226 INSM, fax 4428264 or 3474168)

SWEDEN

Mr. G. SALLVIK, Statens Utsädeskontroll, Box 33, 221 00 Lund (tel 046 124520)

SWITZERLAND

Mr. D. VALENGHI, Swiss Federal Research Station for Agronomy, Postfach, 8046 Zürich (tel. 01-377 72 68, fax 01-377 72 01)

UNITED KINGDOM

Dr. A. BOULD, Technical Adviser, Plant Variety Rights Office, White House Lane, Huntingdon Road, Cambridge CB3 OLF (tel. 0223 - 342384, fax 0223/342386)

Dr. M.S. CAMLIN, Department of Agriculture for Northern Ireland, Plant Testing Station, 50 Houston Road, Crossnacreevy, Belfast BT6 9SH (tel. 0232 448121/2/3, fax 0232 448353)

Dr. R.J. COOKE, National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE (tel. 0223 276381, fax 0223 277602)

UNITED STATES OF AMERICA

Dr. J.L. STRACHAN, Examiner, Plant Variety Protection Office, NAL Building, Room 500, 10301 Baltimore Blvd., Beltsville, MD 20705-2351 (tel. 301-504-5489)

0443

II. NON MEMBER STATES

AUSTRIA

Mrs. B. FUERNWEGER, Bundesanstalt für Pflanzenbau, Postfach 64, 1201 Wien (tel. 0222-23 01 78/13, fax 2162056)

GREECE

Dr. G. APOSTOLATOS, Hellenic Ministry of Agriculture, 2, Acharnon Street, 101-76 Athens (tel. 01-5243722, fax. 01-5243162)

III. OBSERVER ORGANIZATION

COMMISSION OF EUROPEAN COMMUNITIES (CEC)

Dr. M. VALVASSORI, Commission des Communautés Européennes, Administrateur principal, Direction générale de l'Agriculture, VI B II 1, (Loi 84 1/7), 200, rue de la Loi, 1049 Bruxelles (tel. 2/2356971, fax 2350165)

IV. TECHNICAL EXPERT

Mr. A. KLEINHOUT, Danish Plant Breeding Inc., P.B. 19, Hojerupvej 31, 4660 Store Heddinge, Denmark (tel. 45 53 703023, fax 45 53 703524)

V. OFFICER

Dr. M.S. CAMLIN, Chairman

VI. OFFICE OF UPOV

Dr. M.-H. THIELE-WITTIG, Senior Counsellor, 34, chemin des Colombettes, 1211 Geneva 20, Switzerland (tel. 022 7309152, tx. 412 912 ompi ch, fax. (041-22)7335428)

[Annex II follows]

ANNEX II

LIST OF MINIMUM INFORMATION TO BE INCLUDED IN THE UPOV CENTRAL COMPUTERIZED DATA BASE

- Latin Name
- Application Number
- Registration Number
- Country of Application for PBR
- Country of Application for National Listing
- Place of Publication (non protected, non listed varieties)
- Breeder
- Maintainer
- Breeder's Reference
- Approved Variety Denomination
- Proposed Variety Denomination
- Date of Application for PBR
- Date of Application for National Listing
- Date of Proposed Denomination
- Date of Approval of Denomination
- Date of Rejection of Denomination
- Date of Grant of Protection
- Date of Inclusion in National List
- Source of Information
- Remarks

[Annex III follows]

0445

ANNEX III

TESTING OF HOMOGENEITY OF VEGETATIVELY PROPAGATED AND TRULY SELF-POLLINATED VARIETIES

Proposal of the Technical Working Party for Agricultural Crops for the replacement of paragraph 28 of document TG/1/2

For vegetatively propagated and self-fertilized species the sample size and the maximum number of off-types will be given in the individual guidelines and are based on the tables of document TC/XXV/8. The crop experts choose the appropriate table when preparing the guidelines by first fixing the population standard, i.e. the maximum percentage of off-types that is allowed if the whole population could be examined. Then the acceptance probability--i.e. the probability that a variety having P% of off-types is correctly considered uniform--and the sample size are chosen. Small sample sizes increase the risk of accepting heterogeneous varieties.

Examples:

Population standard "P"	Acceptance probability	Sample size	Maximum number of off-types allowed	Risk of erroneously accepting a heterogeneous variety with, for instance, x% off-types	x
1%	95%	10	0	60%	5
1%	95%	20	0	36%	5
1%	99%	100	3	26%	5
0.1%	99%	1000	3	1%	1
0.1%	99%	2000	5	0.1%	1

[Annex IV follows]

ANNEX IV

UPOV TWP AGRICULTURAL CROPS - JUNE 1992

THE USE OF ELECTROPHORESIS FOR THE DISTINCTNESS TESTING OF VARIETIES OR BARLEY

1. INTRODUCTION

The TWP for Agricultural Crops has been actively examining the possible use of electrophoresis for DUS testing of barley since 1988/89. One difficulty has been that the participating members of the TWP have not been able to reach agreement regarding the most appropriate methodology for the electrophoretic analysis of barley. Although in all cases, the alcohol-soluble seed storage proteins (hordeins) are taken, there exist two widely used and effective electrophoresis methods - SDS-PAGE and acid PAGE - for the analysis of hordeins. The methods utilise different physical parameters of hordeins to effect the electrophoretic separation. SDS-PAGE separates on the basis of the molecular weight (size) of the proteins, whereas acid PAGE separates largely on the basis of the charge density of the proteins, although there is also a molecular sieving effect which is size-dependent. However, both methods have been successfully used for barley variety identification in different laboratories throughout the world.

This paper attempts to compare the two methods with the objective of enabling the TWP to decide on an appropriate course of action in this matter.

2. BACKGROUND

The difficulties raised by the existence of these two methods of hordein analysis were recognised at the meeting of the Cereals sub-group (Hannover, April 1989). The report of that meeting, subsequently endorsed by the TWA in June 1989 (Belfast), suggested the use of either SDS-PAGE or acid PAGE for hordein analysis. This view was also held by the Electrophoresis sub-group (Surgeres, October 1990) who agreed a detailed description of the two methods and undertook an exercise, using the variety Atem, to 'calibrate' gels with a view to being able to use the calibration (REM) data as a means of identifying individual hordein bands and hence describing varieties. The results of this exercise were presented at the sub-group meeting in 1991 (October, Hannover) and are reproduced in Table 1. Also at that meeting, the UK presented diagrams of C- and B-hordein alleles (patterns or groups of bands), with REM numbers based on the Atem calibration system and tables of reference varieties and descriptions, to illustrate how a band nomenclature system might operate (see TWA/XIX/3 (1990) for background information on interpretation of barley electrophoretic data). As an example, the patterns for the C-hordeins are reproduced in Table 2. Similar information could be made available for hordein patterns following SDS-PAGE.

The 1991 sub-group meeting also discussed the two methods, but could not reach an agreed position. It was thus decided that a paper should be presented to the next TWA, pointing out the advantages and disadvantages of each method and providing examples, if possible, of the type of protein separations achievable.

3. COMPARISON OF THE METHODS

3.1 Introduction

Electrophoresis has been in use for barley variety identification for

0447

almost 15 years and a wide range of methodologies have been utilised (for instance Cooke, 1988, listed 16 different methods and more have been added since then). Versions of both SDS-PAGE and acid PAGE were published at about the same time (Shewry et al 1979, Marchylo and La Berge 1980) and several variants of both procedures have been subsequently developed.

The acid PAGE method suggested by the Electrophoresis sub-group is the standard reference method recommended by ISTA and derives from collaborative ring tests organised by both ISTA and the European Brewery Convention (EBC) over the period 1982-86. In all, six ring tests of the method were carried out by ISTA/EBC, involving a total of 22 laboratories in 13 different countries. The method has been subsequently incorporated into both the 'Analytica EBC' and the ISTA International Rules (both in 1986). It is widely used in grain trading and is accepted for this purpose by the appropriate trade organisations (eg GAFTA in the UK). It is also used in different countries for varietal identification work and in seed testing and breeding laboratories. At the NIAB, we have examined over 400 different varieties of diverse origins over the past 6-8 years and have an extensive catalogue of all of the varietal patterns. Overall, we have examined in excess of 3000 different samples by acid PAGE (equivalent to over 150,000 individual separations) since 1985.

The recommended SDS-PAGE method is a version of that used by Montembault et al (1983), which in turn is a variant of the widely used 'Laemmli' system. As far as I am aware there have been no systematic ring tests of this method, but it is commonly used by breeders and testing organisations in various parts of the world.

3.2 Analytical Aspects

It is difficult to compare the two methods in terms of their ease of use or in cost/time terms, since laboratories utilise staff and resources in different ways and design their modes of operation around the particular methods of choice. Perhaps the only way to approach this is to take the view of an uncommitted laboratory with staff familiar with electrophoresis, but not especially trained in any one particular method.

i) Sample preparation - in both cases, single grains have to be crushed. In SDS-PAGE, centrifuge tubes with caps or lids are commonly used to contain the samples, whereas in acid PAGE, it is usual to put the crushed seed meal into the wells of a microtiter plate.

ii) Sample extraction - SDS-PAGE requires a two-step extraction process (mixing with freshly made extractant for 2 hours and then boiling) prior to centrifugation; acid PAGE employs a rather more simple single step extraction procedure and no centrifugation.

iii) Gel preparation - in SDS-PAGE, a two phase gel system (separating gel and stacking gel) is used, which means that gel preparation requires two stages as opposed to the single step required in acid PAGE. Also, because of the catalysts used and the nature of the gels, polymerisation is slower with SDS-PAGE. As an example, at NIAB, assuming that gel cassettes and solutions etc are already

available, it is possible to prepare 32 acid PAGE gels in about 15 minutes; to make the equivalent number of SDS PAGE gels would take 4-5 hours.

iv) Gel running time - this will vary depending on the nature of the electrophoresis equipment used, size of gels etc. In general, however, acid PAGE gels are run at higher power and hence will take less time to run than equivalently sized SDS PAGE gels. Again as an example from NIAB, acid PAGE separations will be completed in about 2 hours, whereas SDS-PAGE gels in the same type of equipment take about 5 hours to run.

v) Gel staining - SDS-PAGE gels are usually fixed and washed prior to being stained, whereas acid PAGE gels are fixed and stained in a single step. In both cases, the staining time at room temperature is normally allowed to be overnight.

vi) Overall consumables costs - at least in the UK, SDS-PAGE is somewhat more expensive in terms of consumable chemicals costs than acid PAGE, primarily due to the relatively high cost of the electrophoresis buffer (tris/glycine/SDS).

3.3 Gel Interpretation

The Electrophoresis sub-group has suggested that three aspects of gel interpretation should be considered with regard to these two methods:

i) the number of loci examined

ii) the number of clear allelic types at each locus

iii) the number of hordein bands which can be clearly recognised and identified (by REM number).

i) In both SDS-PAGE and acid PAGE, the hordein proteins are analysed. It is generally agreed that there are three groups of hordein proteins, usually termed B, C and D-hordeins in decreasing order of electrophoretic mobility and which are encoded by a series of loci on chromosome 5. As far as I am aware, the so-called 'D/C' hordeins seen in SDS-PAGE are not thought to represent products of a discrete locus (confirmed by Prof. Peter Shewry, LARS). Rather, some of them represent B-hordeins of lower than normal mobility (in SDS-PAGE systems) and some represent C-hordeins. The hordein composition of the reference variety Atem as separated by SDS-PAGE and acid PAGE is shown in Figure 1. SDS-PAGE clearly resolves the D-hordeins, which are not properly evident in acid PAGE separations, as well as the C- and B-hordeins which are also seen on acid PAGE gels.

ii) The number of different alleles recognised by the methods at each locus is not an especially useful consideration, since it will depend on the range of genotypes examined. This is clearly shown in the literature where the number of C-hordein alleles reported, for example, varies from 7 to 23, which is entirely dependent on the material examined. As far as I know, there has been no systematic comparison of the number of alleles detectable by different techniques in a given collection of genotypes. It is, however, unlikely that any one method is 'better' than the others in distinguishing hordein alleles. Examination of catalogues of variety patterns produced by different laboratories generally results

0449

in a high degree of agreement as to allelic types (cf. varietal classifications produced by NIAB and GEVES).

iii) It is also very difficult to compare the number of clearly recognisable hordein bands produced by the two methods. Although line diagrams of allelic patterns can be drawn, doubts can arise as to whether bands are really 'clearly separable'. In the end, this is largely a subjective opinion and decision. Again, there has been no direct comparison using the same collection of varieties, although in this case this would only be of limited value, as it does not remove the subjective element. The difficulty is illustrated by Figure 2, which presents patterns of a range of varieties separated by SDS-PAGE (GEVES) or acid PAGE (NIAB). How can one judge what is 'clearly separable'? It is possible to describe each allelic pattern in terms of the presence/absence of hordeins of particular REM (see Table 2), but this still relies on decisions as to whether particular bands are present or not.

4. DISCUSSION

The foregoing illustrates the enormous difficulty in trying to make a rational, balanced and informed decision between two perfectly satisfactory analytical methods. Instead of trying to make such a decision, which is inevitably going to be somewhat arbitrary and which could have serious financial consequences for laboratories (replacement of equipment, re-training of staff, revision of catalogues of varietal data, etc), we should perhaps consider whether or not it is a necessary decision.

i) why have a single method? For instance, in TWA/21/4 (Working Paper on Revised Test Guidelines for Soya Bean), it is suggested that a single method (for isozyme analysis) is not proposed, but rather that analysts choose a proven method from the literature and receive permission to use that method from the competent authorities. For barley, it might be possible for the authorities to advise the possible use of either SDS-PAGE or acid PAGE - both clearly defined so as to avoid potential minor variations.

ii) are data produced by one or other of the methods acceptable to other laboratories? I would suggest that the answer to this must be yes. Speaking personally, I would have no hesitation in accepting data from a laboratory which demonstrated, for instance, that a candidate variety differed from an already listed variety in its hordein composition as revealed by SDS-PAGE. An accompanying photograph of the two varieties would verify the difference. In addition, it would be extremely surprising if this difference could not be demonstrated by acid PAGE analysis of hordeins.

iii) there is still the question of how to record hordein banding patterns and how, for instance, to record such a difference as that mentioned above between two particular varieties. The sub-group has previously concluded that it is necessary to be able to identify each protein band individually and that only those 'clearly separable' bands can be used in DUS testing. However, there is now beginning to be a shift in the emphasis of this philosophy. It has been agreed that for HMW glutenins in wheat, we will utilise allelic patterns of bands even though some of the individual bands in some alleles cannot be separated from one

0450

another. Dr Ohms has recently suggested something similar for gliadins, although this has still to be discussed. Hordeins are ideally suited for this type of approach, as was pointed out in the paper TWA/XIX/3 (1990) since the D, C and B-hordein patterns represent a series of alleles at different loci. Each allele (pattern) could thus be described by a reference variety and at the same time given a description based on the absence/presence of particular bands, measured using the REM system already devised. An illustration of this for some C-hordeins analysed by acid PAGE is given in Table 3. A similar catalogue could be readily devised for hordein alleles revealed by SDS-PAGE.

In conclusion, I suggest that it is not possible to make an informed and rational decision as to which of the two suggested electrophoresis methods is better. Both have advantages and disadvantages and both are widely and successfully used. Further, I suggest that such a decision need not be necessary and that for DUS testing in barley UPOV should recognise the character as 'Hordein Composition' and allow analysis by either SDS-PAGE or acid PAGE, with the methods clearly defined and the interpretation based on the recognition of hordein alleles, with appropriate reference varieties and descriptions.

REFERENCES

Cooke, R J (1988) Advances in Electrophoresis, 2, 171-261.

Shewry, P R et al (1979) J.Nat.Inst.Agric. Bot. 1, 34-50.

Marchylo, B A and LaBerge, D E (1980) Can.J.Plant.Sci. 60, 1343-50.

Montembault, A et al (1983) J.Inst.Brewing, 89, 299-302.

0451

UPOV TWA (1992)

TABLE 1

BARLEY HORDEINS - GEL CALIBRATION EXERCISE (Atem)

(1) ACID PAGE

Band No.	Mean REM (4 results)	SE	UPOV REM
1	27.8	1.0	27
2	30.2	0.8	30
3	33.0	0.7	32
4	36.8	1.5	37
5	39.3	1.7	39
6	71.7	0.8	71
7	100	-	100

(2) SDS-PAGE

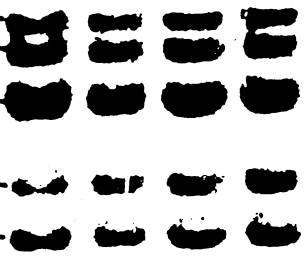
Band No.	Mean REM (4 results)	SE	UPOV REM
1	36.4	3.7	36
2	51.4	3.8	51
3	54.8	3.9	54
4	62.4	4.5	62
5)			
6)	66.9	3.2	66
7	69.9	3.5	69
8	80.6	2.3	80
9	100	-	100

UPOV TWA (1992)

TABLE 2

BARLEY - C-HORDEINS (Acid PAGE)

Hordein Band UPOV REM No.	Reference Variety
25	Berta
27	Atem
30	Atem
31	Ilka
32	Atem
34	Klaxon
35	Odin
37	Atem
38	Digger
39	Atem
41	Igri
42	Digger
43	Marko



	25	27	30	31	32	34	35	37	38	39	41	42	43
Atem	1	9	9	1	9	1	1	9	1	9	1	1	1
Berta	9	9	1	1	9	9	1	9	1	9	1	1	1
Ilka	1	1	1	9	1	9	1	9	9	1	9	1	1
Klaxon	1	1	1	1	9	9	1	9	1	1	1	1	1
Igri	1	1	1	1	9	1	1	9	1	9	9	1	1
Digger	1	1	1	1	1	1	1	1	9	1	9	9	1
Marko	1	1	1	1	9	1	1	9	1	1	9	1	9
Odin	1	1	1	1	9	1	9	9	9	1	1	1	1

0453

UPOV TWA (1992)

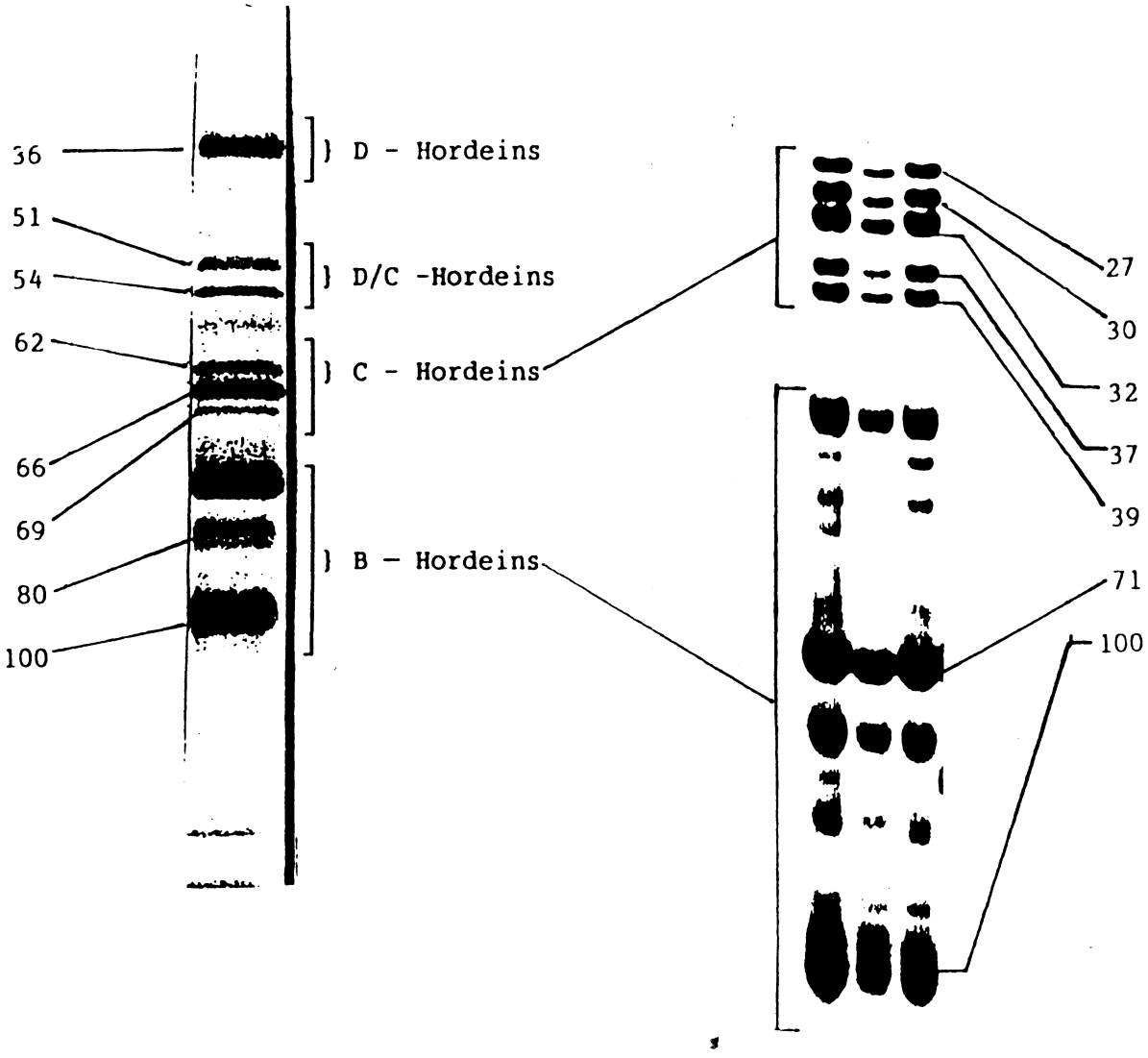
TABLE 3

Some C-Hordein Patterns following Acid PAGE - Description of Reference Varieties and Alleles.

		Band No. (REM)												
NIAB Group	Variety	25	27	30	31	32	34	35	37	38	39	41	42	43
1	Pirate	1	9	9	1	9	1	1	9	1	1	1	1	1
2	Igri	1	1	1	1	9	1	1	9	1	9	9	1	1
3	Marko	1	1	1	1	9	1	1	9	1	1	9	1	9
4	Digger	1	1	1	1	1	1	1	1	9	1	9	9	1
5	Goldspear	1	1	9	1	9	1	1	9	1	1	1	1	1
6	Camargue	1	1	1	1	9	1	1	9	9	1	1	1	1
7	Capri	1	1	1	1	1	1	1	1	9	1	9	1	1
8	Ilka	1	1	1	9	1	9	1	9	9	1	9	1	1
9	Klaxon	1	1	1	1	9	9	1	9	1	1	1	1	1
10	Atem	1	9	9	1	9	1	1	9	1	9	1	1	1
11	Natasha	1	1	1	1	9	1	1	9	1	1	9	1	1
12	Odin	1	1	1	1	9	1	9	9	9	1	1	1	1
13	Berta	9	9	1	1	9	9	1	9	1	9	1	1	1
14	Albacete	1	1	1	1	1	9	1	1	9	1	9	9	1

FIGURE 1

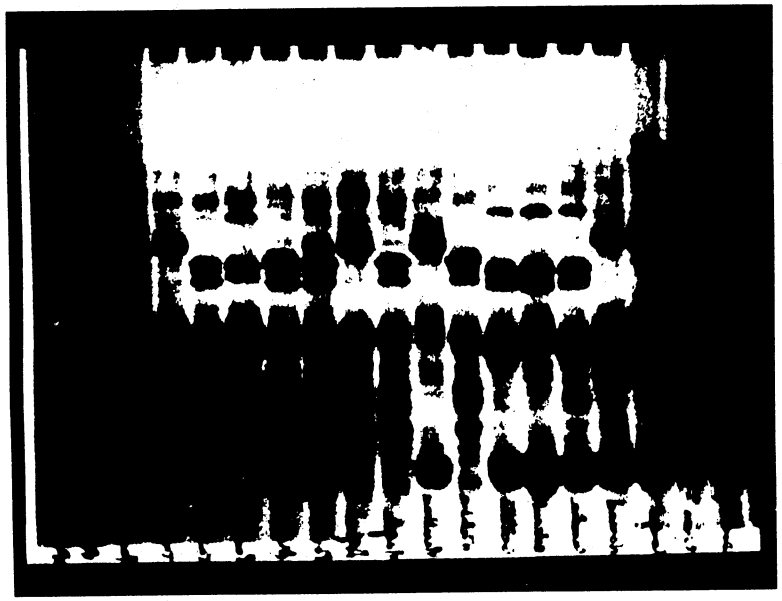
The Hordein Composition of the Reference Variet Atem, separated by SDS-PAGE (left) and Acid PAG (right).

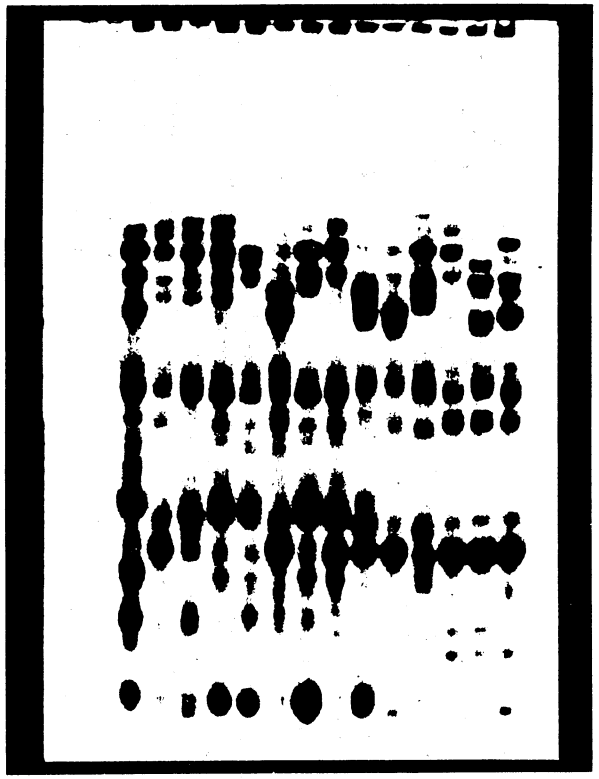


UI TWA (1992)

IGURE 2

ne Electrophoretic Patterns of a Range of Varieties
eparated by SDS-PAGE (left) or Acid PAGE (right).





UPOV TWA (1992)

The Use of Gliadin Alleles for Wheat DUS

1) Dr Ohms (letter 8 May 1992) has suggested that instead of looking at individual gliadin bands following electrophoresis (EP), in the way that has been pursued thus far, we should consider gliadin alleles (or blocks). It is well known that gliadins are encoded at six loci on the group 1 and 6 chromosomes of *T. aestivum* and that there are a considerable number of alleles at each of these loci.

2) Metakovsky *et al* have published a catalogue of gliadin alleles at the different loci, as separated by an acid PAGE method (Theor Appl Genet 67, 559, 1984); the nomenclature has recently been revised (J Genet Breeding 45, 317, 1991). The loci are now called Gli A1,B1, D1; A2, B2, D2 instead of Gld 1A,1B,1D; 6A,6B,6D respectively and the various alleles are now designated by letters instead of numbers.

3) In theory then, it should be possible to descibe the gliadin composition of a given variety of wheat in terms of the gliadin alleles present following EP. A list of reference varieties could also be agreed which would represent each of these alleles. This would be analogous to the glutenins in wheat, which occur as a series of alleles, the products of three loci on the group 1 chromosomes, and to the situation in barley, where the hordein proteins are the products of two or three loci on chromosome 5 and are revealed as a series of distinct patterns on EP gels.

4) There are a number of difficulties, however :

i) there is no list of gliadin alleles compiled using the acid PAGE method which the UPOV EP sub-group has recommended;

ii) there is no list of gliadin alleles for varieties from most UPOV member states;

iii)gliadin alleles are much more difficult to handle than glutenins or hordeins, since the products of the various loci can be rather complex and overlap on gels, making correct interpretation a problem;

iv) for DUS purposes, it would presumably still be necessary to be able to identify clearly the difference between various alleles, in terms of the presence or absence of particular bands. This position has been strongly advocated by the Bundessortenamt previously and has in no small way influenced the way in which the EP sub-group has approached its task (cf discussions on interpretation of barley and oat gels).

v)adoption of this system would undoubtedly take considerable time and would delay further the revision of the Guidelines.

5)On the other hand, the use of such an allelic system would allow UPOV to adopt a consistent approach across all species and protein types (if the general philosophy were extended to barley and oats). Also, the description of EP gels would be more genetically based, which might make it more acceptable to breeders.

0457

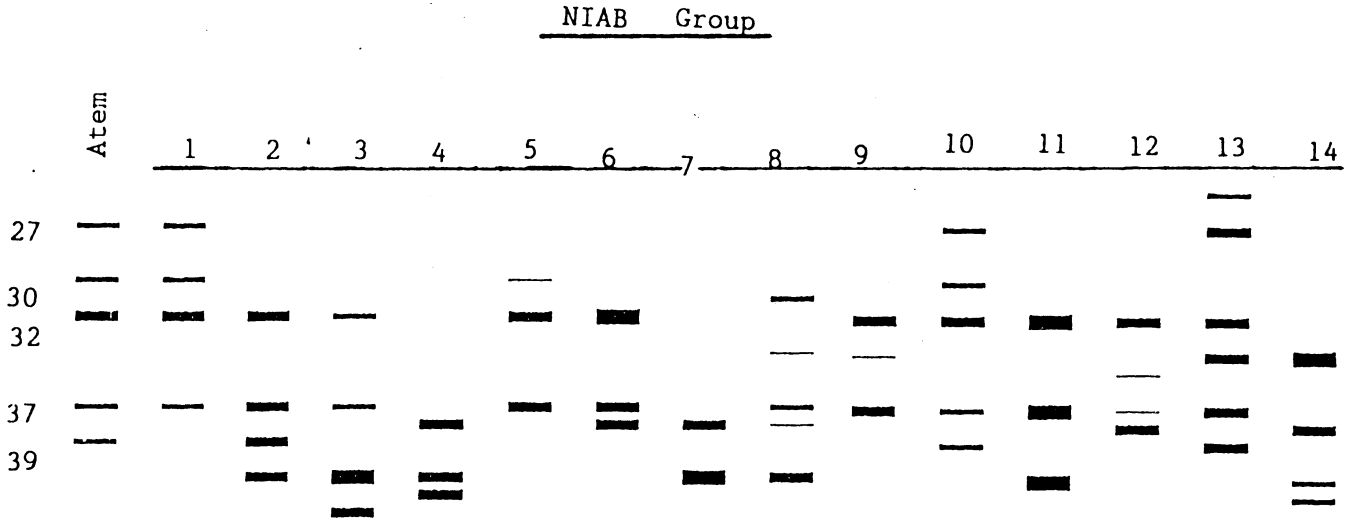
UPOV TWA (1992)

Correspondance between some Hordein alleles as revealed by SDS-PAGE or Acid PAGE

SDS-PAGE (GEVES)	Acid PAGE (NIAB)
C1 B1	10.3
C1 B4	10.14
C2 B7	10(a).10
C2 B6	10(a).9
C3 B6	1.9
C8 B6	2.9
C8 B2	2.4
C8 B8	2.7
C4 B2	11.4
C7 B1	9.3
C7 B3	9.13
C10B12	5.11
C6 B1	6.3
C16B6	7.9

Note - preliminary data only; not all alleles recognised by either method can be easily matched from the currently published information.

BARLEY - C-HORDEINS (Acid PAGE)



[Annex V follows]

ANNEX V

0459

DUTCH CERTIFICATION STANDARDS
FOR THE MAXIMUM NUMBER OF OFF-TYPES PER HA.

	Outbreeding grasse s	Poa	Cereals	Beans	Potatoes (in %)
Pre-Basic	0	500	200	200	0
Basic	10	500	700	500	0,05
Certified 1	1000	6000	1500	1500	0,1
Certified 2	-	-	3000	3000	-
Number of plants / ha (x 1000)	+/- 1000	+/- 100	2000	300	-
Population standard in % (certified)	0,1	6	0,15	1,0	0,1
P [illegible]	[illegible]	[illegible]	1%	1%	?

[End of annex and of document]