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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

Nineteenth Session
Wageningen, The Netherlands, May 15 to 18, 1990

REPORT

adopted by the Technical Working Party for Agricultural Crops

Opening of the Session

1. The nineteenth session of the Technical Working Party for Agricultural Crops (hereinafter referred to as "the Working Party") was held in Wageningen, The Netherlands, from May 15 to 17, 1990. The list of participants is reproduced in Annex I to this report.

2. Mr. C.A.A.A. Maenhout, Deputy Director of the Center for Variety Research and Seed Technology (CRZ) welcomed the participants to the CRZ. He gave a short explanation of the CRZ that was established on February 1, 1990, after a reorganization of the RIVRO (Government Institute for Research on Varieties of Cultivated Plants) and the RPvZ (Government Seed Testing Station). 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 nineteenth session, as reproduced in document TWA/XIX/1, after having agreed to delete item 9, Definition and examination of hybrid varieties, and to add the item "Cooperation with Breeders in the Testing of Varities".

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

4. The Chairman gave a short report on the important decisions taken during the last session of the Technical Committee, referring to the full report on that session reproduced in document TC/XXV/11 for further information.

5. Access to Data of Other Member States. The Working Party shortly discussed the question of access by authorities of member States responsible for plant variety protection and testing to data held by an authority of another member State. It noted that this matter should be studied carefully in the light of the confidentiality of the data. A start could be made with the list of varieties under test, which contained very useful information which would not cause any problems for the authorities.

6. Homogeneity in Self-Fertilized Species. The Working Party agreed to rediscuss the testing of homogeneity of self-fertilized and vegetatively propagated species during its next session on the basis of document TC/XXV/8, which should be distributed again to ensure all experts had a copy.

New Methods, Techniques and Equipment in the Examination of Varieties

7. Dr. T.A. Salt (United States of America) reported briefly on the Workshop on the Examination of Varieties of Soya Bean held in New Carrollton and Queenstown in Maryland, United States of America, from September 27 to 29, 1989, referring to the summary report on that Workshop, reproduced in document TC/XXV/7 Add. Mr. J. Guiard (France) also reported on the Workshop on the Examination of Varieties of Maize held at the INRA station in Versailles, France, on October 2 and 3, 1989, referring to the same document. In both workshops electrophoresis and other new methods had played an important part during the discussions. Full reports on both Workshops are in preparation. (The discussions of the Working Party on Electrophoresis are reported upon in paragraph 14).

Statistical Methods

8. The Working Party reminded its experts to send the information on their existing methods for the identifying of similar varieties to Mrs. Campbell (United Kingdom) before the coming session of the Technical Working Party on Automation and Computer Programs.

Minimum Distances between Varieties

9. Dr. M.-H. Thiele-Wittig reported on the technical issues arising in relation to the revision of the UPOV Convention, which was at present being discussed by the Administrative and Legal Committee, referring to document TWA/XIX/8. He especially explained the concept "essentially derived variety" in relation to the minimum distance between varieties.

10. Most experts in the Working Party welcomed that new concept as it would exclude plagiarism of a protected variety. After having shortly discussed examples of essentially derived varieties contained in Annex II to that document, the Working Party asked its experts to study at home whether those examples covered all foreseen cases or whether further examples were needed. They were furthermore requested to inform their countries' participants at the coming sessions of the Administrative and Legal Committee of the outcome of the study.

Cooperation with Breeders in the Testing of Varieties

11. The expert from Denmark reported on the opinion expressed by breeders in her country that if the results of the test of a variety for the first year were sufficient for an authority to grant a plant breeder's right, the test for the second year was no longer necessary, as breeders wished to save costs and time. The expert from France reported that, as far as maize was concerned, if the data of an applicant on his candidate variety was obtained in conformity with a protocol which the French authority provided and if the results agreed with the results of the competent authority, only one year's official test was needed. Some experts mentioned that whether one year test was sufficient or not would depend on characteristics and crops. It would be more likely for qualitative characteristics than quantitative ones, for self-pollinated crops than cross-pollinated ones, because the former characteristics or crops had less fluctuation depending on the year. It would be also more likely for major crops than minor ones because the experience of cultivation of major crops was greater than that of minor ones.

12. The expert from the United States of America reminded the Working Party that in his country cooperation with breeders was a basis of the plant breeders' system and that a collaborative system among breeders on the variety description was established. He showed an example in which three breeders including the applicant actually made a DUS test of a candidate variety for at least two years and would describe independently that candidate variety. The authority therefore received three independent descriptions of one candidate variety.

13. The Working Party finally agreed to study this subject again at its next session. The experts from France and the United States of America will prepare information papers on their relevant systems before the next session.

Report from the Subgroup on Electrophoresis in Cereals

14. The Working Party noted the report on the Subgroup Meeting on Electrophoresis in Cereals held at the same place on May 14, 1990. A summary report of the meeting is reproduced in Annex II to this report. The Working Party confirmed the decisions of the Subgroup to continue its discussions with the aim of introducing electrophoresis as a group of non-routine characteristics for cereals, the use of which could be requested by the applicant if other characteristics failed to establish distinctness, and to set up a further Subgroup of the meeting to facilitate the study. That Subgroup will meet on October 16 and 17, 1990, at Le Magneraud, Surgères, France. The result of that meeting will be presented to the Working Party for discussion during its coming session. Depending on the results, the chairman may also decide to present them to the professional organizations for comments.

Final Discussion on Draft Test Guidelines

Test Guidelines for Bent (Revision) (TG/30/4(proj.))

15. The Working Party noted that no comments had been received in writing on the draft Test Guidelines for Bent as reproduced in document TG/30/4(proj.)). It therefore only made the following main changes in that document:

(i) Material Required: The recommended quantity of seed to be supplied should be 400g;

(ii) Literature: The expert from The Netherlands will prepare literature.

(iii) Technical Questionnaire: Subitems (i) and (ii) of item 7.2 should be deleted.

Test Guidelines for Ryegrass (Revision) (TG/4/5(proj.))

16. The Working Party noted that no comments had been received in writing on the draft Test Guidelines for Ryegrass as reproduced in document TG/4/5(proj.). It therefore only made the following main changes in that document:

(i) Subject of these Guidelines: In the last sentence of paragraph 3, the word "protein" should be inserted before "electrophoresis."

(ii) Table of Characteristics:

Characteristics	
2	transfer the words "of year of sowing" to the explanations
6	add the words "in spring" after "habit" and transfer the phrase "4 weeks after spring growth has started in earliest variety in second year" to the explanations
7	add the words "in spring" after "height" and transfer the phrase "4 weeks after spring growth has started in earliest variety in second year" to the explanations

(iii) Literature: The experts from The Netherlands will indicate additional literature.

(iv) Technical Questionnaire: In subitems (i) and (ii) of item 1, the words "Annual" "Biennial" and the brackets should be deleted and in subitem 7.3 the word "(Hybrid)" should be inserted after "type" and "biennial" should be replaced by "Italian."

Test Guidelines for Kentucky Bluegrass (Revision) (TG/33/4(proj.))

17. The Working Party noted that no comments had been received in writing on the draft Test Guidelines for Kentucky Bluegrass as reproduced in document TG/33/4(proj.). It therefore only made the following main changes in that document:

(i) Subject of these Guidelines: The sentence should be amended to read: "These Test Guidelines apply to all varieties of Kentucky Bluegrass/Smooth Stalked Meadow Grass (Poa pratensis L.).

(ii) Conduct of Tests: In the third sentence of paragraph 3 and in the first sentence of paragraph 4, the phrase "for apomictic varieties and 60 plants for non-apomictic varieties" should be inserted after the words "spaced plants."

(iii) Methods and Observations: In paragraph 2, the phrase "for apomictic varieties and 60 plants or parts of 60 plants for non-apomictic varieties" should be added at the end.

(iv) Table of Characteristics:

Characteristics

1, 2	delete the example variety "Orna"
2, 3, 5, 6	add the words "density of" before "hairs" or "fringe"
3, 4, 5, 11, 12, 13	delete the example variety "Apart"
5	add the example variety "Barsweet (1)"
7	delete the example variety "Bluebell" and correct the spelling of "Sydsport"
10	add the example variety "Dormie (7)"
11	replace the example variety "Gruber" by "Kenblue" and add "Merpona (3)"
13	add the example variety "Ampellia (7)"
19	replace the example variety "Bluebell" by "Dormie"

(v) Technical Questionnaire: In item 1, the words "(apomictic varieties)" should be deleted and in subitem 7.2 the question "apomictic variety Yes [ ]/No [ ]" should be included.

Test Guidelines for Safflower (TG/134/1(proj.))

18. The Working Party noted that no comments had been received in writing on the draft Test Guidelines for Safflower as reproduced in document TG/134/1(proj.). It therefore only made the following main changes in the table of characteristics of that document:

In characteristics 7, 9 and 10, the word "low" should be replaced by "short" and an asterisk (*) should be added to characteristic 24. The expert from Spain will prepare example varieties for characteristics 2, 15, 21, 24, 25, 26, 28 and 29 by the beginning of July 1990.

Discussions on Working Papers on Test Guidelines

19. As far as the Test Guidelines for Wheat, for Barley and for Oats were concerned, the Technical Working Party agreed to discuss parts of those Test Guidelines other than those on electrophoresis, pending the outcome of the discussions by the Subgroup on Electrophoresis in Cereals.

Test Guidelines for Wheat (Revision)

20. The Working Party noted document TWA/XIX/4 containing a working paper on the revision of the Test Guidelines for Wheat prepared by the experts of the Subgroup on Cereals as well as document TWA/XIX/4 Rev. containing a revised version of that document prepared by the Office of UPOV. The Working Party finally made the following main changes in document TWA/XIX/4 Rev.:

(i) Material Required: The fourth sentence of paragraph 1 should be amended to read "If requested by the competent authority, in addition at least 150 ears for winter wheat and 100 ears for spring wheat should be submitted."

(ii) Table of Characteristics:

Characteristics

5	replace the example varieties "Etoile de Choisy (1)", "Renard (3)", "Hildur (9)" by "Aubaine, Prinqual (1)", "Etoile de Choisy (3)", "Kosack (9)" respectively.
9	amend the example varieties to "Sprint; Briscard (1)", "-; Arkas (3)", "Arminda (5)"
10, 15, 16	amend the stage to "80-92"
10	replace the word "observed" by "per plant should be recorded" with explanations
11	place the state "fusiform" before "tapering"
12	replace the words "assessed as" by "observed either visually or by" in the explanations
13	add the words "(excluding awns and scurs)" after "length"
14	delete the words "(at maturity)" and place this characteristic after characteristic 16
16	delete all example varieties, and experts of the Subgroup to prepare those which have short scurs for Note 1 and long awns for Note 9
19	delete the example variety "Avalon"
21, 24	amend the Notes to "1, 2, 3, 4, 5"
23	delete
25, 26	delete the asterisk (*) and delete characteristic 25 from the grouping characteristics and from the Technical Questionnaire
26	delete the item "Equipment" in the explanations
27	amend the first sentence in the explanations to read "The seasonal type should be assessed on one or several plots sown in springtime" and after this sentence, insert the sentences "Example varieties should always be included in the plots. When the example varieties behave according to their description, other varieties can be classified."

Test Guidelines for Barley (Revision)

21. The Working Party noted document TWA/XIX/5 containing a working paper on the revision of the Test Guidelines for Barley prepared by the experts of the Subgroup on Cereals as well as document TWA/XIX/5 Rev. containing a working paper on the revised version of that document prepared by the Office of UPOV. The Working Party finally made the following main changes in document TWA/XIX/5 Rev.:

(i) Material Required and (ii) Conduct of Tests: The same amendments as made for Wheat should be made (see subitems (i) and (ii) of paragraph 20 above).

(ii) Table of Characteristics:

Characteristics

11	amend the word "drooping" to "recurved"
16	add the words "(excluding awns)" after "length"
17	amend the states to "very short, short, medium, long, very long" and add in the explanations the sentence" "medium" means that the length of awns is equal to that of ears."
25	delete the asterisk (*) and delete the characteristic from grouping characteristics and from the Technical Questionnaire
28	add in the explanation "The color of the aleurone layer should be assessed visually after the kernel is put in the water for 12 hours and, if necessary, a magnifying-glass can be used."
29	make amendments in the explanations comparable to those made for Wheat (see characteristic 27 in subitem (iii) of paragraph 16 above)

Test Guidelines for Oats (Revision)

22. The Working Party noted document TWA/XIX/6 containing a working paper on the revision of the Test Guidelines for Oats prepared by the experts of the Subgroup on Cereals, as well as document TWA/XIX/6 Rev. containing a revised version of that document prepared by the Office of UPOV. The Working Party finally made the following main changes in document TWA/XIX/6 Rev.:

(i) Subject of these Guidelines: The words "and Avena nuda L." should be added after "sativa L."

(ii) Material Required and (iii) Conduct of Tests: The same amendments as for Wheat should be made (see subtims (i) and (ii) of paragraph 20 above).

(iii) Table of Characteristics:

Characteristics

1	amend the stage to "25-29"

2	add in the explanations the sentence "This characteristic will be more easily observed in a greenhouse."
14	amend the stage to "70-75"
25	delete

Test Guidelines for Maize (Revision)

23. Mr. J. Guiard (France) reported that the French authorities were revising the DUS testing system concerning maize in view of the implication of electrophoresis on isoenzyme methods. He will prepare a document concerning electrophoresis methodology for DUS purposes of maize before the end of 1990, and a working paper on Test Guidelines for Maize including electrophoresis characteristics before March 1, 1991. The Working Party agreed to discuss the Test Guidelines for Maize at its next session on the basis of the above-mentioned papers.

Test Guidelines for Rape (Revision)

24. The Working Party noted document TWA/XIX/2 containing a working paper on the revision of the Test Guidelines for Rape prepared by experts from Germany, as well as document TWA/XIX/2 Rev. containing a revised version of that document prepared by the Office of UPOV. It noted that there were different notions of rape varieties among member States. For some member States rape varieties covered pure line varieties, synthetic varieties (under certain conditions) as well as hybrid varieties (in future). For other member States rape varieties covered partly cross-pollinated varieties. The Working Party agreed to set up a Subgroup on Rape in order to discuss this matter. The Subgroup will consist of experts from Germany, the Netherlands, France, Denmark and Spain. It will meet in Hanover (Scharnhorst), Germany, on April 3 and 4, 1991. The Test Guidelines should be established in such a way as to allow member States to follow one or the other notion of variety.

25. The Working Party only looked at the beginning of the draft for revised Test Guidelines for Rape and made the following changes in document TWA/XIX/2 Rev.:

(i) Subject of these Guidelines: The document to apply to "Brassica napus L. oleifera"

(ii) Material Required: Paragraph 1 to receive an additional sentence reading: "If required by the competent authority, 50 unthreshed plants should be submitted."

(iii) Conduct of Tests: The words "single spaced" in the second sentence of paragraph 1 to be replaced by "individual."

(iv) Methods and Observations: To have the words "in general" inserted in paragraph 1 after "should" and to have the word "6th" deleted in paragraph 3.

Possible further changes in the document will be discussed by the Subgroup.

Status of Test Guidelines

26. The Working Party agreed that the draft Test Guidelines for Bent (Revision), for Ryegrass (Revision), for Kentucky Bluegrass (Revision) and for Safflower should be sent to the Technical Committee for final adoption.

27. The Working Party agreed to rediscuss the draft Test Guidelines for Wheat (Revision), for Barley (Revision) and for Oats (Revision) at its next session in view of the inclusion of electrophoresis characteristics which might be proposed by the Subgroup on Electrophoresis in Cereals. The documents would not be finalized if the Subgroup did not complete its work.

28. The Working Party agreed to rediscuss the draft Test Guidelines for Rape (Revision) at its next session in the light of the proposals expected from the Subgroup on Rape.

29. The Working Party agreed to rediscuss the revision of the Test Guidelines for Maize at its next session.

Future Program, Date and Place of Next Session

30. At the invitation of the expert from the United States of America, the Working Party agreed to hold its twentieth session in the United States of America. [After the session it was decided to hold it in Beltsville, Maryland, from May 13 to 17, 1991] The Working Party plans to discuss or rediscuss the following items at its next session:

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

(ii) New methods, techniques and equipment in the examination of varieties

(iv) Access to data in the data bases of UPOV member States

(v) Statistical Methods (TC/XXV/8)

(vi) Cooperation with breeders in the testing of varieties

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

- Wheat (TWA/XIX/4 Rev.)
- Barley (TWA/XIX/5 Rev.)
- Oats (TWA/XIX/6 Rev.)

(viii) Final Discussion on draft Test Guidelines for Peas (United Kingdom to prepare a working paper)

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

- Maize (Revision) (TG/2/4 and France to prepare a document on technical aspects and details of present testing in France and working paper on revised Test Guidelines)

- Rape (Revision) (TG/36/3, TWA/XIX/2, TWA/XIX/2 Rev. and report from the Subgroup on Rape)

- Flax (Revision) (TG/57/3 and France to prepare a working paper)

- Fodderbeet (Denmark to prepare a working paper)

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

(i) Subgroup on Electrophoresis in Cereals, at Le Magneraud, Surgères, France, on October 16 and 17, 1990:

(ii) Subgroup on Rape, in Hanover, Germany, on April 3 and 4, 1991.

Visits

32. On May 16, the Working Party visited facilities of the CRZ concerning electrophoresis, image analysis and isoenzyme analysis as well as its trial fields of grasses.

33. This report has been adopted by correspondence.

[Annex I follows]

ANNEX I

LIST OF PARTICIPANTS AT THE NINETEENTH SESSION OF THE TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS WAGENINGEN, THE NETHERLANDS, MAY 15 TO 17, 1990

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[Annex II follows]

ANNEX II

Summary Report on the Subgroup Meeting on Electrophoresis in Cereals

1. The Subgroup Meeting on Electrophoresis in Cereals was held in the afternoon of May 14, 1990, at the CRZ in Wageningen, The Netherlands. After having received the following two reports concerning the methodologies of electrohporesis made by Dr. R.J. Cooke (NIAB, United Kingdom) and Dr. J.P. Ohms (Bundessortenamt, Germany), the Subgroup discussed the inclusion of electrophoresis characteristics in the Test Guidelines for Wheat, Barley and Oats.

2. Dr. Cooke introduced papers on "Interpretation of Electrophoresis Data for Use in Distinctness Testing of Varieties of Barley and Oats" (document TWA/XIX/3 and "Interpretation of Electrophoresis Data for Use in Distinctness Testing of Wheat Varieties" (document TWA/XIX/7), as well as a discussion paper on "Interpretation of Electrophoresis Data" which was distributed during the meeting and is reproduced in Annex III to document TWA/XIX/9. He reported as follows:

(i) There are basically two ways of handling the data of electrophoresis on Cereals, namely (1) a way of considering each band individually and determining its position on the gel ("each band system") and (2) a way of recognizing groups or patterns of bands by treating the information in terms of overall groups of bands ("band pattern system").

(ii) The "each band system," however, has the following problems in measuring the position of individual bands:

(a) what should be measured exactly, e.g. absolute mobility or relative electrophoretic mobility (REM) and whether the bands should be numbered sequentially from 1 upwards or given REM value,

(b) how each band should be measured, e.g. from the leading or trailing edge or the middle,

(c) how many standard errors between measurements should be needed to differ two bands,

(d) the fact that closely associated bands would be difficult to separate based on measurements of the relative electrophoretic mobility (REM)$\pm$ standard error may lead to the decisions to ignore certain bands for distinctness purposes,

(e) the fact that measurements are inevitably affected by the type of equipment used and that laboratories use realistically different equipment may cause problems in comparing or exchanging the information,

(f) because of sequent numbering system, the each band system is not so flexible as to allow the incorporation of additional bands or groups of bands.

(iii) The "band pattern system" can minimize many of above problems as long as it is used along with a list of reference varieties exhibiting each particular pattern. However, the following points still have to be decided:

(a) the identity of the groups, i.e. what constitutes a difference between two groups of bands,

(b) the denomination of the groups, e.g. 1, 2, 3, ... or A, B, C, ... etc.,

(c) the reference varieties, in order to ensure that all groups from the National List are covered initially.

(iv) For barley and oats, the "band pattern system" can be devised along with a list of reference varieties. For wheat, however, the situation is more complicated. Therefore, a compromise can be suggested, in which groups of bands are recognized along with reference varieties, but which also involves a means of labelling individual bands.

Dr. Cooke further noted that the possible disadvantage of the "band pattern system" would be that each band was not considered as a separate characteristic.

3. Dr. J. Ritz reported, to the contrary, on the advantages of the "each band system" which the Bundessortenamt had adopted. His discussion paper was distributed during the meeting as reproduced in Annex IV to document TWA/XIX/9. He explained as follows:

(i) As the "each band system" modified by the Bundessortenamt defines groups of bands by their REM value and identifies each band within a particular group as being either absent (1) or present (9), it can describe the groups of bands more precisely than by the "band pattern system".

(ii) It can describe the difference between two groups, while the "band pattern system" cannot describe it.

(iii) As it can describe each group of bands precisely, it does not need many example varieties for those groups, while the "band pattern system" needs example varieties for individual groups.

(iv) It can easily describe minimum distances between varieties by using numbers of bands.

4. The Subgroup further noted views of experts from professional organizations, who participated in the meeting, that the breeders were opposed, at the present time, to the introduction of electrophoresis in the Test Guidelines as a routine test for the following reasons:

(i) Technical Aspects:

(a) Its technique has not yet been stabilized;

(b) The interpretation of its results has not yet been standardized;

(c) The wording of the electrophoresis characteristics has not yet been defined.

(ii) Non-technical Aspects:

(a) It would not be a fingerprinting method;

(b) It could not replace morphological characteristics;

(c) It would slow down the speed of breeding;

(d) It would change the type of varieties (100% electrophoretic purity to be required);

(e) It would increase the cost of breeding, seed certification and seed production.

5. The above reports and views led to a discussion on possible inclusion of electrophoretic characteristics in the Test Guidelines. The Subgroup finally concluded as follows:

(i) Breeders are not against the inclusion of electrophoretic characteristics in Test Guidelines as a group of non-routine characteristics, possibly either in the Table of Characteristics without an asterisk (*) or as an Annex to the Test Guidelines.

(ii) The electrophoretic characteristics should be used as non-routine characteristics, the use of which could be requested by the applicant if other characteristics failed to establish distinctness. Only in these cases would homogeneity be required in those characteristics.

(iii) The approach to the introduction of electrophoresis within member States should be standardized.

(iv) Standardized methods and techniques should be fully described.

(v) Electrophoresis characteristics should be precisely defined e.g. absence or presence of bands or patterns of bands.

(vi) Interpretation of results should be standardized.

(vii) Example varieties for the standard characteristics should be studied.

6. The Subgroup agreed to continue its study with the aim of introducing electrophoresis considering the above-mentioned points. It further agreed to set up a further Subgroup to facilitate that study. That further Subgroup consisting of experts from the Federal Republic of Germany, Denmark, Spain, France, The Netherlands and the United Kingdom will meet on October 16 and 17, 1990, at Le Magneraud, Surgères, France.

[Annex III follows]

ANNEX III

UPOV TWP AGRICULTURAL CROPS

INTERPRETATION OF ELECTROPHORETIC DATA

1) There are basically two ways of handling the data (banding patterns, electrophoregrams, 'fingerprints') arising from the electrophoresis of cereal storage proteins:-

   a) Consider each band individually and determine its position on the gel;
   b) treat the information in terms of overall groups of bands.

2) Problems can arise with measuring the position of individual bands:-

   a) what exactly is measured - absolute mobility or relative electrophoretic mobility? Are bands simply numbered sequentially from 1 upwards or given 'REM' values?

   b) how is each band measured, from the leading or trailing edge or the middle?

   c) once measurements are made, then the recognition of standard errors of the measurements become inevitable. Decisions then have to be made as to the criteria for describing two bands as the same or not. How many SE's between measurements are needed for two bands to differ? How are these decisions to be made and on what basis?

   d) closely associated bands may be difficult to separate based on REM $\pm$ SE measurements. This may lead to the decision to ignore certain bands for distinctness purposes. Can such a position be defended scientifically?

   e) measurements are inevitably affected by the type of equipment used, in particular the dimensions of the gel. Given the realistic position that laboratories are going to use different equipment, this is going to cause problems in comparing or exchanging information.

   f) any system must be sufficiently flexible as to allow the incorporation of additional bands or groups of bands - this could be difficult with sequentially numbered systems.

3) The recognition of groups or patterns of bands, along with a list of reference varieties exhibiting each particular pattern, can minimise many of these potential problems. Decisions still have to be made as to:-

   a) the identity of the groups (ie. what constitutes a difference between two groups of bands);

   b) what the groups are called (1,2,3... A,B,C... etc);

   c) the reference varieties, to ensure that all groups from National collections are covered initially.

   A possible disadvantage of this approach is that each band is not treated as a separate character.

4) For oats and barley, a grouping system can be devised, along with a list of reference varieties. For wheat, the situation is more complicated and a compromise system can be suggested, in which groups of bands are recognised, along with reference varieties, but which also involves a means of labelling individual bands.

R J COOKE
8 May 1990

[Annex IV follows]

Bundessortenamt

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(1)

UPOV electrophoresis test 1989

Species: Oat Method: PAGE pH 3.1 Gene locus: B-,C-Ave

Classification: NIAB

NIAB - group 1 3 1

REM	Leanda	Condor	Cabana A
63 →	++	++	++
	++	++	++
	+++	+++	+++
	++	++	++
	++	++	++
		(+)	(+)
	+++	+++	+++
	+	(+)	+
107 →	(+)	(+)	

NIAB - group 6 8 9 9

Perona	Pinto	Milo A	Milo B
++	++		
+++	+++	+++	+++
		+	+
++	++	+	+
++	+	++	++
+	+++	+	+
(+)	(+)		
			+
+++	+++	+++	+
+	+	+	(+)
		(+)	

NIAB-group 2 2 2 7

Condor	Cabana B	Orlando B	Elen
++	++	++	++
++	++	++	++
+++	+++	+++	+++
++	++	++	++
++	++	++	++
(+)	(+)		
		+	+
+++	++	+	
(+)			+
(+)	+	+	++

TWA/XIX/9
ANNEX IV

5

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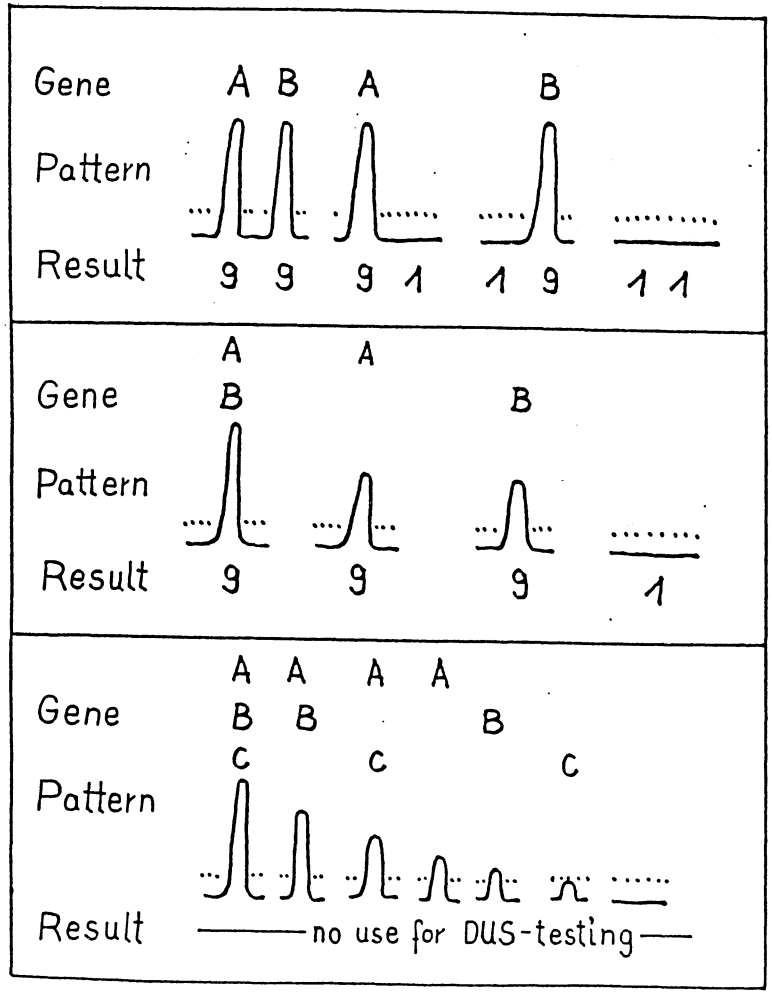
(2)

UPOV electrophoresis test 1989

Preconditions for the use of polymorphisms for DUS-testing

Postulate: qualitative expression of bands



Postulate: clear discrimination of bands

Model	A		B						
Variety	1	5	1	2	3	4	5	6	
Pattern	—	—	—	—	—	—	—		} no use for DUS-testing
	—	—	—		—		—	—	
	—	—	—			—	—	—	
MW	100	92	100	98	96	94	92		± 2 } no use for DUS-testing
	83	83	83		83		83	83	± 1
	76	76	76			76	76	76	± 1

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(3)

UPOV electrophoresis test 1989

Species: Oat Method: PAGE pH 3.1 Gene locus: B-,C-Ave

Classification: BSA

BSA group	REM (NIAB) 63	67	69 - 100	NIAB group
01	9	9	1 - 9	1+2+3
02	9	9	1 - 1	7
03	9	1	9 - 9	-
04	9	1	9 - 1	-
05	9	1	1 - 9	6+8+12
06	9	1	1 - 1	-
07	1	9	9 - 9	4
08	1	9	9 - 1	13
09	1	9	1 - 9	-
10	1	9	1 - 1	11
11	1	1	9 - 9	5+9
12	1	1	9 - 1	10
	C		- B	
		Ave		

Clear discrimination between the BSA-groups

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UPOV electrophoresis test 1989

Species: Wheat Method: SDS-PAGE Gene locus: HMW-Glu

Band nomenclature: PAYNE

Classification: BSA

BSA-group	Band-nr. 1 · 2 · 4 · 5 (2*)	+	BSA-group	Band-nr. 6 · 7 · 14 · 15	+	BSA-group	Band-nr. 8 · 9 · 12 (10)
01	1 · 1 · 1 · 9		01	1 · 1 · 9 · 9		01	1 · 1 · 9
02	1 · 9 · 1 · 9		02	1 · 9 · 1 · 1		02	1 · 9 · 9
03	9 · 1 · 1 · 9		03	9 · 1 · 1 · 1		03	9 · 1 · 9
04	1 · 1 · 9 · 1					04	1 · 9 · 1
05	1 · 9 · 9 · 1					05	9 · 9 · 1
06	9 · 1 · 9 · 1						
07	1 · 9 · 1 · 1						
08	9 · 9 · 1 · 1						
	A + D Genome			B Genome			B + D Genome

Clear discrimination between the BSA-groups

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(5)

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Suitability of protein polymorphisms for DUS-testing

Examination points: Qualitative expression of bands (A) and clear discrimination of bands (B)

Species	Method	Gen locus	A	B
oat	PAGE pH 3.1	B-Ave	+/-	+/-
		C-Ave	+/-	+/-
barley	SDS-PAGE	B-Hor	+/-	+/-
		C-Hor	+ +	+
		D-Hor	+ +	+
wheat	PAGE pH 3.1	α-Glia	(+)	(+)
		β-Glia	+/-	+/-
		γ-Glia		
		ω-Glia	+	+
wheat	SDS-PAGE	HMW-Glu	+ +	+ +
		LMW-Glu	+ +	+ +

Results: HMW-Glu, LMW-Glu, ω-Glia and after an additional test α-Glia can be used in DUS-testing.
B-Hor (only a few bands), C-Hor, D-Hor can be used in DUS-testing.
B-Ave (only a few bands), C-Ave (only a few bands) can be used in DUS-testing.

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Strategies for the use of protein polymorphisms in DUS-testing

	Methods Evaluation of pattern	Results Description of variety
NIAB	Association with specific band groups described by reference varieties	Name of the band group
BSA	Evaluation of bands defined by their REM-value	Sequence of defined bands
NIAB + BSA	Definition of band groups as sequence of bands defined by their REM-value	Name of the band group

Group description		Group definition		Description of the difference between two groups		Reference variety	
NIAB	BSA	NIAB	BSA	NIAB	BSA	NIAB	BSA
1	9·1·9·1·9·1	by reference variety	by REM {calculated by reference variety}	no	yes	A	A
2	9·1·1·9·9·1					B	
3	1·9·9·1·9·1					C	
4	1·9·1·9·1·9					D	D
5	1·9·1·1·1·9					E	
6	1·1·9·1·1·9					F	
7	1·1·1·9·9·9					G	
8	1·1·1·9·1·9					H	
9	1·1·1·1·9·1					I	
10	1·1·1·1·1·1					J	
	90 87 83 79 66 61 REM					n = 10	n = 2

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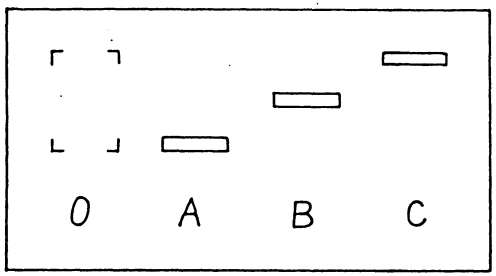
Protein polymorphism and definition of the minimum distance between two varieties

There isn't any difference between polymorphism containing only one band per allel (1) and polymorphism containing two and more bands per allel (2).

(1)

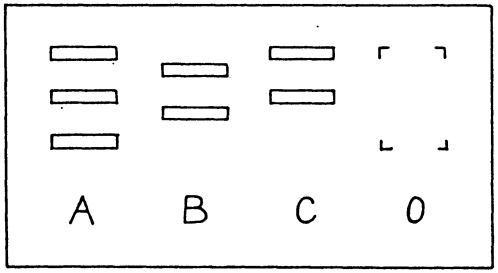


Minimum distances

1 band : O - A
O - B
O - C

2 bands: A - B
A - C
B - C

(2)



Minimum distances

1 band : A - C
2 bands: O - B
O - C
4 bands: B - C
5 bands: A - B

Always the distance between two varieties is equal or greater than one band.
The minimum distance is one band.

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(8)

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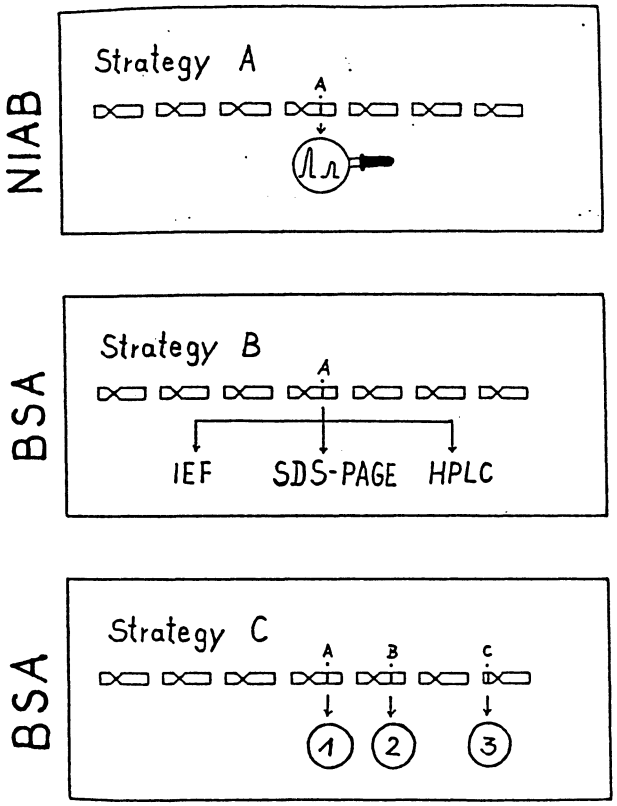
Co-operation of gene locus and protein polymorphism



Co-operation of gene locus and polymorphism in oat (47 varieties)

Proteins	Method	Group
Peroxydases	IEF	13
Prolamins	PAGE	15
Prolamins	PAGE SDS-PAGE	30
Prolamins Peroxydases	PAGE IEF	39
Prolamins Peroxydases	PAGE SDS-PAGE IEF	45

The use of protein polymorphism from different gene loci produces the best results in the variety discrimination

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