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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

## TECHNICAL COMMITTEE

### Twenty-fifth Session
### Geneva, October 5 and 6, 1989

## NEW METHODS, TECHNIQUES AND EQUIPMENT IN THE EXAMINATION OF VARIETIES

Document prepared by the Office of UPOV

1. In 1988 several Technical Working Parties discussed the question of the possible introduction of new methods, techniques and equipment in the examination of varieties for the purpose of plant variety protection.

2. Following the proposal to establish a separate Technical Working Party on new technology, which would study that possible introduction, the Technical Committee and the Council of UPOV agreed that such a new working party should not be established and that work on the use of new technology in the examination of varieties should be intensified and carried out on an ad hoc basis.

3. As a first start, the Technical Committee decided on two steps to be taken:

(a) It invited the Technical Working Parties to investigate and compile an inventory of the species and methods in connection with which the application of the above new technology was being researched at the national level in each member State. Furthermore, they should discuss and try to reach a consensus on whether characteristics obtained by such methods could or should replace certain less important characteristics in the present UPOV Test Guidelines, or only be used in the same way as any other additional characteristic. In this context, special attention should be given to the question of the homogeneity of the new characteristics.

(b) For a selected number of species, some experts should prepare clear draft proposals on how to integrate new technology (for the present electrophoresis and image analysis) most efficiently and cost-effectively into the present UPOV Test Guidelines for the species concerned. Those draft proposals should be discussed at the forthcoming sessions of the Technical Working Parties and their Subgroups. The Technical Working Parties should prepare final proposals for presentation to the next session of the Committee. The species and member States selected for the preparation of the above proposals were as follows:

| | | |
|---|---|---|
| France | Maize, Pea | Electrophoresis |
| Netherlands | Kentucky Bluegrass | Electrophoresis |
| South Africa | Brassica | Electrophoresis |
| United Kingdom | Wheat, Barley, Oats, Ryegrass | Electrophoresis |
| France | Carnation | Image analysis |
| United Kingdom | Wheat, Onion | Image analysis |

4. The present document contains in its annexes the information received, at its request, by the Office of UPOV concerning the subjects mentioned in subparagraphs 3(a) and (b), as well as some further information given during the recent sessions of the Technical Working Parties and considered useful for the discussions.

5. Additional information from member States and from the Technical Working Parties, as well as the views and requested final proposals of the Technical Working Parties on the way the above-mentioned characteristics should be used, will be presented in a separate document.

6. The annexes to this document are listed below:

Annex I A list of species for which member States study the possible use of electrophoresis in the examination of varieties for distinctness: Information received in compliance with the request of the Office of UPOV made in Circular U 1384 of January 12, 1989.

Annex II A list of new methods, other than electrophoresis, under study in the UPOV member States.

Annex III A list of species for which, besides wheat, barley and oats, electrophoresis has been investigated as a means for variety identification, copied from Annex III of document TWA/XVII/9.

Annex IV Proposal for the integration of electrophoresis and machine vision (wheat only) into the Test Guidelines for cereals, prepared by experts from the United Kingdom for the meeting of the Subgroup of Grasses in April 1989 (proposals from NIAB only).

Annex V Proposal for the integration of electrophoresis into the Test Guidelines for Ryegrass and possibly other Test Guidelines, prepared by experts from the United Kingdom (only personal proposal from the expert).

Annex VI Report on the experiences in the use of electrophoresis in Poa pratensis L. made in The Netherlands.

Annex VII Proposal for the incorporation of image analysis into DUS testing of onions, prepared by experts from the United Kingdom (only proposals from NIAB).

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Annex VIII Introduction to the various applications of electrophoresis at the biochemistry laboratory of G.E.V.E.S. in connection with variety registration and seed certification, given during the last session of the Technical Working Party for Agricultural Crops (copied from Annex IV of document TWA/XVII/9).

Annex IX Extract from the report on the last session of the Technical Working Party for Agricultural Crops, dealing with electrophoresis (copied from document TWA/XVII/9, paragraphs 21 to 30).

7. The Technical Committee is invited to note the information contained in this document and its annexes, as well as in the additional document to be prepared after the sessions of the Technical Working Parties will have taken place, and to decide on the necessary action to be taken.

[Nine annexes follow]

Species for which member States study the possible use of electrophoresis in the examination of varieties for distinctness purposes

| SPECIES | COUNTRY | TEST PLANNED | UNDER STUDY | APPLIED FOR IDENTIFICATION | FOR DISTINCTNESS | FOR HOMOGENEITY | ORGAN | PROTEIN | METHOD |
|---|---|---|---|---|---|---|---|---|---|
| Apple | FR | | X | X | | | Pollen | Isoenzymes | |
| Apple | FR | | X | | | | Leaf | Acid phosphatase | Polyacrylamid gel |
| Apple | FR | | X | | | | | Endopeptidase | Polyacryl.gel |
| Apple | FR | | X | | | | | Esterase | Polyacryl.gel |
| Apple | FR | | X | | | | | Phosphoglucoisomenase | Starch gel |
| Apple | FR | | X | | | | | Superoxyd dismutase | Polyacrylamid gel |
| Apple | FR | | X | | | | | Peroxydase | Polyacryl.gel |
| Apple | GB | | X | | X | | | Isoenzymes | |
| Asparagus | DE | | X | | | | Phylloclades | Albumine, Globuline | PAGE, pH 7,9 |
| Asparagus | DE | | X | | | | Phylloclades | Peroxydase | IEF, pH 3-10 |
| Barley | DE | | | X | X | | Endosperm | Prolamine | IEF pH 4-8 |
| Barley | DE | | | X | X | | Endosperm | Prolamine | SDS-PAGE |
| Barley | DE | | | X | X | | Endosperm | Albumine, Globuline | PAGE pH 8,9 |
| Carnation | NL | | X | | X | | | | |
| Edible Mushrooms | JP | | X | | | | | Isoenzymes | |
| Forest trees | JP | | X | | | | | Isoenzymes | |
| Hazelnut | FR | X | | | | | | | |
| Maize | CH | X | | | X | | | | |
| Maize | DE | | X | | | | Endosperm | Zeine | IEF pH 4-8 |
| Maize | DE | | X | | | | Embryo | MDH | PAGE pH 8,9 |
| Maize | DE | | X | | | | Embryo | Esterase | PAGE pH 8,9 |
| Maize | DE | X | | | | | Embryo | Peroxydase | PAGE pH 8,9 |
| Maize | DE | X | | | | | Cotyledon | Isoenzyme | |
| Maize lines | HU | | | X | | X | | Isoenzymes | |
| Oats | DE | | | X | X | | Endosperm | Prolamine | PAGE pH 9,1 |
| Oats | DE | | | X | X | | Endosperm | Prolamine | SDS-PAGE |
| Oats | DE | | | X | X | | Embryo | Peroxydase | IEF pH 3-10 |
| Peach | FR | | X | | | | | Zimylase | PAGE |
| Peach | FR | | X | | | | | Alcooldeshydrogenase | |
| Peach | FR | | X | | | | | Malate deshydrogenase | |
| Pears | GB | | X | | | | | | |
| Pelargonium | DE | | X | | | | Leaf or root tips | Isoenzymes | IEF or PAGE |
| Potatoe | DE | | | X | X | | Tuber | Albumine, Globuline | PAGE pH 7,9 |
| Potatoe | DE | | | X | X | | Tuber | Peroxydase | PAGE pH 7,9 |
| Potatoe | DE | | | X | X | | Tuber | Esterase | PAGE pH 7,9 |

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| SPECIES | COUNTRY | TEST PLANNED | UNDER STUDY | APPLIED FOR IDENTIFICATION | | FOR DISTINCTNESS | FOR HOMOGENEITY | ORGAN | PROTEIN | METHOD |
|---|---|---|---|---|---|---|---|---|---|---|
| Quinces | GB | | X | | | | | | | |
| Rape | DE | | X | | | | | Seedling | Peroxydase | IEF pH 4-8 |
| Rape | DE | | X | | | | | Seedling | Acid Phosphatase | IEF pH 3-10 |
| Rice | HU | | | X | | X | X | Seed | Polypeptides | |
| Strawberries | GB | | X | | | | | | | |
| Triticale | DE | | | X | X | | | Endosperm | Prolamine | PAGE pH 3,1 |
| Triticale | DE | | | X | X | | | Endosperm | Gluteline | SDS-PAGE |
| Triticale | DE | | | X | X | | | Endosperm | Amylasen | PAGE pH 8,9 |
| Walnut | FR | X | | | | | | | | |
| Wheat | CH | X | | | X | | | | | |
| Wheat | HU | | | X | | | X | Seed | Storage protein | |
| Wheat | DE | | | X | X | | | Endosperm | Prolamine | PAGE pH 3,1 |
| Wheat | DE | | | X | X | | | Endosperm | Gluteline | SDS-PAGE |
| Wheat | DE | | | X | X | | | Endosperm | Amylasen | PAGE pH 8,9 |

[Annex II follows]

New Methods Other than Electrophoresis Under Study in the UPOV Member States

| COUNTRY | START OF STUDY | METHOD | SPECIES | CHARACTERISTIC |
|---|---|---|---|---|
| NL | | Image analysis | Carnation | Shapes (crenellation)<br>Color patterns |
| NL | 1989 | Somaclonal variation | Gerbera | |
| ZA | 1988 | Numerical comparison (same principle as image analysis) | Mango | Shapes |
| DE | 1988 | Hand colorimeter + microprocessor with liquid cristal indication | Elatior Begonia | Flower color |
| JP | 1983 | Ultraviolet absorption, capsaicinoid content | Sweet pepper, chili | Bitterness |
| JP | 1983 | Gas-chromatography, fragrance components | Tea | Fragrance |
| JP | 1983 | Gas-chromatography, aroma components | Melon | Aroma |
| JP | 1984 | Gas-chromatography, fragrance components | Rose | Fragrance |
| JP | 1984 | Gas-chromatography, aroma components | Vine | Aroma |
| JP | 1984 | Gas-chromatography, allicin content | Garlic | Allicin (stink) |
| JP | 1985 | Gas-chromatography, fragrance | Common stock (Matthiola incana (L.) R. Br.) | Fragrance |
| JP | 1986 | Gas-chromatography, aroma components | Apple | Aroma |
| JP | 1986 | Gas-chromatography, aroma components | Strawberry | Aroma |
| GB | | Image analysis | Wheat | Shapes |
| GB | | Image analysis | Onion | Shapes |

[Annex III follows]

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# Electrophoresis and Variety Identification: A Summary of Species Which Have Been Investigated (Other than the Self-Pollinating Cereals Wheat, Barley and Oats)

| Species | Protein/enzyme(s) analysed |
|---|---|
| Pisum sativum (pea) | seed globulins |
| | seed albumins/globulins |
| | seedling Prx<br>shoot/root Est,Lap,Prx<br>seed Amy, Est,Got<br>seed legumin/vicilin |
| | SDS-soluble seed proteins |
| | buffer-soluble seed proteins |
| | water-soluble seed proteins<br>urea-soluble seed proteins |
| Phaseolus vulgaris (French bean, common bean, snap bean, kidney bean, field bean (US)) | water-soluble seed proteins<br>isozymes (10 systems) from seeds and seedlings<br>leaf, stem root Est, Prx, Acp<br>acid salt-soluble proteins<br>seed globulins |
| | SDS-soluble seed proteins |
| Glycine max (soybean) | buffer-soluble seed proteins<br>seed Est<br>isozymes (9 different systems) from seeds and leaves |
| | seed $\beta$-Amy, urease<br>isozymes (11 different systems) from seedlings<br>seed Adh,Amy,Acp,trypsin inhibitor<br>buffer-soluble seed proteins |
| Arachis hypogaea (peanut, groundnut) | buffer-soluble seed proteins<br>seed Est,Cat,Lap,Acp,Adh, 'INT oxidase' |
| Trifolium subterraneum (subterranean clover) | seed globulins<br>isozymes (15 different systems) from seeds root Est |
| Lolium perenne, L.multiflorum (perennial and Italian ryegrass) | leaf Pgi,Got (and other enzymes).<br>SDS-soluble seed proteins |
| Solanum tuberosum (potato) | soluble proteins from tubers<br>Est from tubers |

| | |
|---|---|
| Oryza sativa (rice) | buffer-soluble seed proteins<br>seed prolamins<br>urea-soluble seed proteins |
| Allium spp. (onions) | seed Est |
| Zea mays (maize) | seed zeins (prolamins)<br>isozymes (12 systems) from coleoptiles |
| Medicago sativa (lucerne, alfalfa) | buffer-soluble seed proteins<br>leaf Prx,Est,Acp<br>root Prx, root or leaf Est, seed or pod Lap,<br>seed Adh |
| Festuca rubra (fescue) | seed Est<br>SDS-soluble seed proteins |
| Dactylis glomerata (cocksfoot) | SDS-soluble seed proteins |
| Bromus spp. (bromegrass) | SDS-soluble seed proteins |
| Agrostis palustris (creeping bentgrass) | buffer-soluble leaf proteins |
| Digitaria spp. (digitgrass) | leaf Est |
| Phleum spp. (timothy) | seedling Est |
| Vicia faba (broad, field or faba bean) | water-soluble seed proteins<br>seed globulins<br>water- or buffer + urea- soluble seed proteins<br>SDS-soluble seed proteins<br>urea-soluble seed proteins |
| Psophocarpus tetragonolobus (winged bean) | buffer-soluble seed proteins |
| Secale cereale (rye) | seed Prx, Alp, seedling $\alpha$-Amy<br>seed secalins (prolamins)<br>germinated seed $\alpha$-Amy |
| Beta vulgaris (sugar beet) | leaf Pgi, Mdh, Pgm |
| Brassica spp. (Brussels sprouts, cabbage etc) | seed Acp<br>seedling Acp<br>seedling Pgm,Lap,Adh,Got |
| Coffea spp. (coffee) | water- or SDS-soluble seed proteins |
| Lens spp. (lentil) | leaf Got, Pgm, Pgd, Adh |

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| | |
|---|---|
| Olea europea (olive) | salt-soluble pollen Est, Me, Lap |
| Persea americana (avocado) | leaf Prx, Mdh, Lap, Pgm |
| Carya illinoensis (pecan) | pollen Got, Est |
| Rosea spp. (rose) | leaf proteins, Prx, Est, Mdh (and others) |
| Dianthus spp. (carnation) | leaf Pgi, Lap, Est, Pgm, Sdh |
| Lycospericon spp. (tomato) | leaf Acp, Est, Got, Prx |
| Capsicum spp. (pepper) | buffer-soluble seed proteins |
| Raphanus sativus (radish) | root Ldh, Pgm, Pgd, Est, Lap |
| Apium spp. (celery) | seed (or seedling) Adh,Mdh,Pgi,Pgm,Sdh |
| Lactuca sativa (lettuce) | seed protein, Est |
| Cucumis spp. (cucumber) | buffer-soluble seed/cotyledon proteins, Pgi, Pgd, Pgm (and others) |
| Malus spp. (apple) | leaf Pgd, aspartate aminotransferase |
| Pyrus spp. (pear) | leaf Prx |
| Prunus spp. (peach) | buffer-soluble proteins of woody tissue |
| Fragaria spp. (strawberry) | fruit Est, Prx, Adh |
| Vitis spp. (grape) | buffer-soluble fruit proteins, Est, Prx, Mdh<br>fruit Est, Acp, Adh, Lap (and others) |

[Annex IV follows]

# The Incorporation of Electrophoresis into DUS Testing of Cereals

## 1. INTRODUCTION

1.1 The UPOV Technical Committee has recently requested that the NIAB prepare proposals on how to integrate 'New Technologies' into the UPOV Test Guidelines for Wheat, Barley and Oats in the most efficient and cost-effective manner.This paper suggests ways in which electrophoresis can be incorporated into schemes for wheat, barley and oats and also proposes the use of machine vision for wheat.

With regard to electrophoresis (EP), there would appear to be two approaches that can be taken:

A. EP may be regarded as an additional character, to be added to the current list of morphological characters; EP could possibly replace some of the morphological characters rather than merely being used as an adjunct.

B. The incorporation of EP can be regarded as an opportunity for a radical reassessment of the DUS system. This is likely to be the only way to make significant cost reductions.

1.2 A proposal for a re-assessment of DUS procedures should satisify the following requirements :

1.2.1 Comparability to existing system

Any new scheme would need evaluation to confirm that the discrimination between varieties would be at the desired level. To a large extent this could be done retrospectively, using existing data. A period of parallel running may also be needed.

1.2.2 Cost of new proposals

Realistically, any revised proposal which is to gain widespread acceptance must be no more costly than the existing procedures. We must, therefore, attempt to devise the most cost-effective solution compatible with our scientific integrity and with the aims of DUS testing.

1.2.3 Use in seed certification

It is important to devise a system which can be adopted or adapted by the seed certification agencies. The use of chemotaxonomic tests might be inappropriate if they could not be carried out rapidly enough for seed certification purposes.

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1.2.4 Uniformity

New varieties must be shown to be uniform for the characters used to establish distinctness. They should also appear uniform to the grower, who might react adversely to a variety which displayed morphological non-uniformity even if it performed well in other respects. Uniformity for important characters such as plant height and time of ear emergence and ripening is also essential.

1.3 The following background information should also be noted :

1.3.1 Electrophoresis of seed storage proteins is probably the single most powerful discriminator available for cereal varieties.

1.3.2 The examination of ear and grain morphology also provides a large number of discriminating characters.

1.3.3 With any system, the direct cost of the technical work comprises only a portion of the overall costs, the remainder being administrative costs and overheads.

1.3.4 There is a minimum number of field characters which are required for an adequate varietal description for seed certification purposes. This set is probably of no more than 30 characters.

1.3.5 There are no characters used in current cereal DUS testing which are recorded as truly continuous data. Distinctness decisions therefore do not depend on having two years of statistically analysed data.

1.3.6 Earlier procedures in the U.K. required a 2-year system, but recent changes make it possible to arrive at decisions in one year in some cases.

Bearing in mind all of these points, a revised DUS procedure for winter barley, incorporating EP as a primary distinctness character, is proposed below. A similar system could be used for spring barley and oats. A scheme for wheat is discussed separately.

## 2. CURRENT SYSTEM FOR DUS TESTING OF WINTER BARLEY

Candidate varieties are grown at two centres in each of two years. For distinctness purposes, they are compared with the U.K. National List. For uniformity, candidates are grown in ear-row beds. Stability is assumed in the absence of evidence to the contrary. Observations are made on all varieties during the growing season, such that about 40 characters are recorded. The electrophoretic pattern of the variety is not taken into account. DUS testing is concurrent with, but separate from, VCU trials.

Number of winter barley varieties in U K. DUS tests, 1984-86.

| Year of sowing | N List | Candidates Year 1 | Year 2 | Total |
|---|---|---|---|---|
| 1984 | 39 | 24 | 13 | 76 |
| 1985 | 42 | 26 | 15 | 83 |
| 1986 | 37 | 23 | 21 | 81 |

## 3 SUMMARY OF PROPOSED DUS PROCEDURE FOR WINTER BARLEY

3.1 The proposed scheme uses EP and ear and grain morphology as primary characters for discrimination between varieties. A reduced scale field test is employed as a source of additional essential information. These plots are sown at one centre (plus one as a reserve) in a single year. With most candidate varieties there is no second year of DUS testing.

3.2 Varieties received from breeders would be examined by EP (it is suggested that the ISTA standard reference PAGE method for storage protein analysis be used). Initially, only 14 grains would be tested so that results would be available prior to sowing. Ear and grain morphology would also be examined during this pre-sowing period.

3.3 The EP and ear/grain results would be used to group the candidate varieties (it is assumed that the current National List varieties have already been classified) and from the groupings a field sowing plan would be devised. The field plots would comprise the NL varieties classified into morphological groups, these being sub-divided according to EP pattern. The candidate varieties would be inserted into these groups/sub-groups based upon their ear/grain morphology and EP records.

3.4 All of the minimum set of characters from 1.3.4 above are recorded for all plots.

3.5 During the winter period after sowing a much larger number (eg 2 x 50) of grains would be examined by EP and detailed ear and grain morphology recorded. Varieties must be adequately uniform for all recorded characters. It is proposed that variation in EP pattern would not be permitted. Varieties exhibiting electrophoretic lines or biotypes would be refused.

3.6 Candidate varieties shown to be distinct by morphological characters or EP patterns and achieving the accepted level of uniformity would be reported as being DUS. Rules for distinctness and uniformity by morphology already exist. For EP, distinctness would require the presence or absence of at least one clear band. Uniformity requirements would permit the presence of one 'variant' banding pattern in the 100 (2 x 50) seeds analysed. The variety would then await the successful completion of VCU trials before being placed on the National List.

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## 4 OTHER SPECIES

The scheme proposed above could also be used for the DUS testing of spring barley and winter and spring oats.

## 5 PROPOSED DUS PROCEDURE FOR WHEAT

The above scheme could be used for the DUS testing of spring and winter wheat. However, in this case , in addition to the candidate varieties being examined by EP and their ear/grain morphology recorded, they would also be subject to examination by machine vision (image analysis). This would provide additional morphological characters of the grain which could be used both for distinctness purposes and for the variety description. The field plots would be sown as in 3.3 above. All other details of the procedure would be as described.

## 6 CONCLUSIONS

6.1 The proposed scheme would appear to be a technically feasible and defensible way of incorporating electrophoresis and machine vision techniques into the DUS testing of self-fertilised cereal species.

6.2 The scheme would in most cases be more rapid than the existing procedure and no more costly.

6.3 It is seen as a significant advantage of the proposed scheme that it formally incorporates into DUS testing at an early stage the methods which are most important in the subsequent commercial trading of the crop, ie electrophoresis of grain proteins and machine vision. New varieties would be granted PBR and released along with their descriptions based as now on morphological characters and ,additionally, their EP and machine vision characteristics.

6.4 The scheme can be viewed as a first step to the possibility of radically revising DUS procedures, to incorporate 'New Technologies' more fully.

6.5 An important feature of the proposed scheme is the identification of the minimum character set of morphological characters which will provide both adequate distinctness and allow for proper certification procedures (see 1.3.4). Work is in progress at the NIAB to identify this set.

[Annex V follows]

# Integration of Electrophoresis into UPOV Test Guidelines

Proposals for Ryegrass (Prepared by Dr M Camlin, UK)

A first draft of the proposed new Ryegrass guideline has been prepared and is due for consideration at the next meeting of the Technical Working Party for Agricultural Crops (TWA). This supplementary paper sets out possible means for future incorporation of electrophoretic methods into the guideline, as requested by the Technical Committee and the Council.

## INTRODUCTION

It has, up until now, been generally accepted by UPOV that, because of possible implications for minimum distances, electrophoresis should be restricted to providing supportive evidence of distinctness in cases where, despite agronomic advantages, a variety cannot be found distinct using standard UPOV characteristics. It has also been accepted that distinctness would normally be based upon the agronomic feature rather than on electrophoresis alone. However, UPOV now wishes to consider possible strategies for the introduction of electrophoresis into Test Guidelines for various crops.

For ryegrass, a cross-fertilized crop, there are inherent problems with lack of uniformity (homogeneity) of the electropherograms due to the presence of genetic differences between member plants of the same variety. This means that electrophoretic characteristics cannot yet be examined with respect to DUS in full accordance with the UPOV Convention. In addition, breeders still have a mixed reaction to the extensive routine use of electrophoresis for the ryegrasses because of the additional problems which this disuniformity may bring with respect to possible plagiarism and erosion of PBR protection.

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These serious difficulties must be addressed before the introduction of electrophoretic characteristics for this crop is actively considered by UPOV. Because of these additional problems with ryegrasses and similar cross-fertilized crops it may be difficult to make as rapid progress as may be possible with other vegetatively propagated or self-fertilized crops. It is felt that involvement of the breeders is essential at an early stage in consideration of the use of electrophoresis for DUS testing. This will be particularly important when addressing the problems associated with ryegrasses and other out-breeding crops. Only if this involvement is achieved as a first step and agreement is reached can UPOV hope to embark on consideration of individual electrophoretic characters for DUS testing in their own right.

TECHNICAL PROPOSALS

These proposals will require active breeder participation. They are mainly of a technical nature and are intended to allow for the the possible future use of electrophoresis within UPOV guidelines for test procedures for ryegrasses. Research needs are identified and provision is made for further investigative work into methods and techniques before the final consideration of the use of electrophoretic characteristics.

(1) A definitive set of electrophoretic methods and characters for ryegrass should be established within UPOV. For methods to be acceptable they must be proven to allow useful discrimination between varieties and be able to produce repeatable results, unaffected by environmental and developmental factors. To date, of the isozyme systems, only PGI has been proven to fully satisfy these criteria but others including ACP, IDH and GOT are under active investigation. Useful preliminary results have also been obtained by ISTA with seed

globulins using PAGE. Initially it is therefore suggested that starch gel electrophoresis on only four isozyme systems, PGI, ACP, IDH and GOT be investigated, together with PAGE on seed globulins. Standardised methodology should be agreed and described.

(2) The number of plants examined to produce electrophoretic descriptions should be standardised. This will ensure that all laboratories are working to similar levels of discrimination and will help standardise 'minimum distances', even though these may be reduced overall. However, it is also suggested that, where practical, in order to limit the reduction of 'minimum distances', a similar number of plants should be examined for each variety as is presently used for morphological characteristics.

(3) Once the methodology and sample numbers have been agreed and standardised, ring-tests should be organised to see if participating laboratories can produce the same results and are capable of achieving similar levels of discrimination between varieties.

(4) The problem of inherent lack of uniformity of electrophoretic characteristics within varieties of most cross-fertilized crops must be addressed. In order for DUS testing to be conducted in accordance with the UPOV Convention some modification of the concept of uniformity (homogeneity) may be required for electrophoretic characteristics. PAGE on seed globulins, under investigation by ISTA, uses bulk samples and, unlike examination of isozymes, does not exploit plant-to-plant variation within varieties. It may therefore be a more acceptable method from the point of view of uniformity

(homogeneity) as inherent varietal disuniformity is not directly observed.

(5) A comprehensive data-base, authenticated by check testing of different samples of each reference variety should be assembled for existing varieties in common knowledge, using the agreed UPOV electrophoretic characteristics.

(6) To allow future development of a complete data-base, the descriptions of new candidate varieties should in future include electrophoretic information, assuming breeder's consent can be obtained.

(7) Until electrophoretic methods have been studied and agreed by UPOV, and a complete and authenticated data-base of electropherograms and genotype frequencies established, there should be no reduction in the 14 morphological characteristics listed in the draft guideline or in those listed with asterisks. However, no new morphological characteristics should be added until the decision has been taken on how to use electrophoretic characteristics.

(8) The draft UPOV guideline for ryegrass should be amended under Para III Conduct of Tests and Para IV Methods and Observations to include reference to electrophoretic tests and methods, for example, as follows:

Para III Conduct of Tests

Add (Para 7) "Electrophoresis Electrophoretic descriptions of varieties may be produced using methods approved by UPOV".

Para IV Methods and Observations

Add (Para 7) "Electrophoretic methodology is currently under investigation".

Also, a modification of the draft Technical Questionnaire to seek breeders' approval for production of electrophoretic descriptions may be required.

Individual electrophoretic characteristics should not be included in the Table of Characteristics until the methodology to be adopted has been standardised and UPOV has taken a positive decision to use electrophoretic characteristics for the ryegrasses.

SUMMARY

TECHNICAL PROPOSALS

(1) Standardised methods and characteristics to be adopted.

(2) Standardised discrimination and 'minimum distances' to be agreed.

(3) Ring-test procedures to be established for checking results.

(4) Uniformity criteria for electrophoretic characteristics to be considered.

(5) Authenticated data-base to be produced for the common knowledge collection.

(6) Electrophoretic descriptions to be produced for future candidate varieties.

(7) Existing characters in guideline to be retained at present.

(8) Guideline to include some reference to electrophoretic tests.

M S CAMLIN
7 December 1988

[Annex VI follows]

# Experiences on the Use of Electrophoresis in Poa Pratensis L.

## Introduction

The perspectives of the use of electrophoretic techniques for the rapid variety identification and for DUS-purposes in Kentucky bluegrass (Poa pratensis L.) look very promising at forehand, since Kentucky bluegrass is generally considered as an apomictic species.

During 1987 and 1988, several investigations were started to find out the power of electrophoresis in discriminating between varieties. These investigations will be continued/expanded in 1989, whereas the results obtained so far will be published soon.

The present paper gives - briefly - the most important information concerning these matters.

## Seed

The most simple way is to apply electrophoretic techniques on an amount of seed. Isoelectric focusing with esterase on 60 mg grinded seed (about 200 seeds) gives many sharply outlined, but closely situated bands. Varietal differences with this method are clear. The interpretation of the results is rather difficult, but can be done easier after grouping according to the first esterase band from the cathode

A disadvantage of this method is that mixtures of varieties might not be recognized as such and might result in a different pattern compared with its constituents. When varieties that are quite different in their patterns are mixed, the effect of the mixing rate (25 to 75%) is neglectable; the mixture shows the bands of both varieties with equal intensity. For varieties that resamble each other, it is very hard to find mixtures.

The application of storage protein electrophoresis on individual seeds with silver staining did show many, clear bands but did not show variety differences.

## Plantlets

PAGE was applied on individual seedlings. A first investigation was based on 3-4 month old seedlings grown in the greenhouse and carried out with 8 enzyme systems viz. esterase, GOT, GPI, MDH, PGM, ACP, tyrosinase and peroxidase. From these, esterase, peroxidase and GPI showed the best combination of enzyme-activity, sharpness of bands and differentiation between varieties. To establish varietal homogeneity, 8-12 plants were sampled; to estimate within-plant-variation, 4-8 sprouts per plant were sampled.

Altough the investigated varieties could be identified very clearly by their electrophoretic patterns, both within variety and within plant variation was shown. Therefore in a following experiment the growing conditions and the sampling techniques were investigated. The method was restricted to PAGE with esterase and peroxidase. Furthermore, the variation on electrophoretic pattern in the varieties studied were related to the variation in morphological characters established in the greenhouse. The electrophoretic homogeneity of plants grown in the growth chambers was improved compared to the greenhouse. The growing conditions, the age of the leaves and the sampling technique strongly influenced the homogeneity of the patterns and the between variety variation. The used isozymes interfered with these results. There was no association between electrophoretic heterogeneity and morphological heterogeneity.

**Conclusions**

IEF of the esterase enzyme system on seed samples as a whole gives quite good patterns. Their interpretation however, might be obstructed by impurity of the seedsample concerned. For a quick reference this method can be very powerful provided information on all varieties is available and adequately stored for appropriate comparison. At the moment, data on 120 varieties of Kentucky bluegrass is available at RIVRO.

The characterization of individual seeds is technically possible, but gives no varietal differences.

For the use of electrophoresis in DUS testing of Kentucky bluegrass, the PAGE method on individual plants with esterase and peroxidase looks quite promising when optimum sampling methods can be developed and adequate growing conditions of plantlets can be formulated.

As in other crops, the discussion on how to deal with electrophoresis in DUS testing on Kentucky bluegrass has to be started, taking in account the results of the TWA of 1988 (TWA/XVII/9 par. 21 trough 29)

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[Annex VII follows]

# Incorporation of Image Analysis into DUS Testing of Onions (Allium Cepa L.)

## Proposal

It is proposed to replace by image analysis, conventional measurement protocols for UPOV Characters 9, 10, 11, and for additional UK characters 'bulb top shape', 'bulb base shape', and 'bulb base to widest point'.

At a later date the UPOV Technical Working Party will be asked to consider the use of additional novel characters made possible by the use of image analysis.

## Background

The use of Image Analysis in the DUS testing of onions at NIAB Cambridge has been under investigation since the harvest of 1987. For the purposes of this report the work on only one small group of onions is dealt with and is related solely to the bulbs of that group not the foliage.

The group comprises seven varieties together with two selections (approved maintenance) of two of those varieties, all of which have flask-shaped bulbs. The report refers only to 1987 data.

## Methods

Normal recording was carried out on 15 harvested bulbs per plot in four replications. In order to allow a flexible approach to the investigation silhouette 35 mm photographs of these bulbs were taken at harvest and the negatives used for image analysis. Standard illumination and focal length was used in photography.

Bulb photographs were made to allow the investigation to be conducted at leisure and to allow various and repeated estimations to be made.

The methods for scanning the photographs have been described elsewhere, (Keefe and Draper, 1988)

## Characters recorded

The characters recorded manually are listed below. The same characters are recorded using image analysis.

UPOV character 10 (bulb diameter), UPOV character 9 (bulb height) and UK character 58 (bulb distance from base to widest point), are measurements (mm).

UPOV character 11 (bulb shape), UK character 54 (bulb top shape) and UK character 55 (bulb base shape), are scores, nominally on a 1-9 scale. UPOV character 11 utilises the shape diagrams in the UPOV Onion Guideline TG/46/3. The characters UK-54 and UK-55 are components of UPOV character 11. They are not on TG/46/3 but are part of the UK test procedure. Similarly character 58 is only included in the UK test procedure.

Results and discussion

The results are given in Annex I and represent mean values for 15 bulbs recorded per plot in four replicates. Manual data are compared with image analysis data variety by variety.

The data acquired by image analysis are amenable to analysis in the way that manual data are presently analysed both for distinctness and uniformity purposes.

It is considered that the image analysis data is more accurate than that obtained manually but more importantly the characters recorded are those that already exist in the UPOV Guidelines or UK Test Procedure. In this investigation therefore image analysis is no more than a method of data capture.

A more extensive evaluation of image analysis is underway covering all short overwintered onions (1987/1988) and all long day onion (1988). In the future it may be possible to introduce more bulb characters but this would need investigations to evaluate their value before they might be suggested for inclusion in any revision of the UPOV Guidelines.

References

P.D. Keefe and S.R. Draper (1988). An automated machine vision system for the morphometry of new cultivars and plant genebank accessions. Plant Varieties and Seeds, 1, 1-11.

S.R. Draper and P.D. Keefe (1989). Machine vision for the characterisation and identification of cultivars. Plant Varieties and Seeds, (in press).

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Annex 1

Variety: De Mulhouse type Auxaine

| UPOV/UK Char.No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 80 | 80 | 90 | 84 | 84 | 82 | 81 | 91 | 82 | 84 |
| UPOV 9 | Bulb: height (mm) | 103 | 104 | 111 | 102 | 105 | 109 | 112 | 120 | 104 | 111 |
| UK 54 | Bulb: top shape (score) | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 6 | 7 |
| UK 55 | Bulb: base shape (score) | 6 | 6 | 5 | 5 | 6 | 6 | 6 | 5 | 5 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 40 | 41 | 42 | 40 | 41 | 46 | 48 | 49 | 43 | 46 |
| UPOV 11 | Bulb: whole shape (score) | 4 | 4 | 4 | 4 | 4 | 3 | 2 | 3 | 3 | 3 |

Variety: Ailsa Craig

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 100 | 108 | 103 | 111 | 106 | 116 | 109 | 106 | 116 | 112 |
| UPOV 9 | Bulb: height (mm) | 116 | 125 | 118 | 128 | 122 | 137 | 136 | 127 | 141 | 135 |
| UK 54 | Bulb: top shape (score) | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 6 | 5 |
| UK 55 | Bulb: base shape (score) | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 5 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 55 | 55 | 53 | 53 | 54 | 62 | 63 | 60 | 61 | 62 |
| UPOV 11 | Bulb: whole shape (score) | 4 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Variety: The Kelsae

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 111 | | 115 | 124 | 120 | 128 | | 129 | 127 | 128 |
| UPOV 9 | Bulb: height (mm) | 140 | | 146 | 157 | 152 | 172 | | 171 | 170 | 171 |
| UK 54 | Bulb: top shape (score) | 6 | | 6 | 6 | 6 | 6 | | 6 | 6 | 6 |
| UK 55 | Bulb: base shape (score) | 7 | 6 | 7 | 6 | 6 | 7 | | 7 | 6 | 7 |
| UK 58 | Bulb: base to widest point (mm) | 68 | | 70 | 67 | 69 | 78 | | 78 | 75 | 77 |
| UPOV 11 | Bulb: whole shape (score) | 3 | | 3 | 4 | 4 | 3 | | 3 | 3 | 3 |

Variety: Beacon

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 120 | 121 | 108 | 116 | 116 | 124 | 124 | 119 | 124 | 123 |
| UPOV 9 | Bulb: height (mm) | 158 | 150 | 135 | 153 | 149 | 172 | 160 | 157 | 174 | 166 |
| UK 54 | Bulb: top shape (score) | 6 | 5 | 5 | 6 | 6 | 6 | 5 | 6 | 6 | 6 |
| UK 55 | Bulb: base shape (score) | 7 | 7 | 7 | 7 | 7 | 8 | 7 | 7 | 7 | 7 |
| UK 58 | Bulb: base to widest point (mm) | 74 | 69 | 67 | 69 | 70 | 82 | 77 | 76 | 80 | 79 |
| UPOV 11 | Bulb: whole shape (score) | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 |

Variety: Mammoth - approved maintenance Mammoth Improved

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 121 | 117 | 112 | 109 | 115 | | 121 | 117 | 122 | 120 |
| UPOV 9 | Bulb: height (mm) | 141 | 133 | 138 | 124 | 134 | | 143 | 153 | 143 | 146 |
| UK 54 | Bulb: top shape (score) | 5 | 4 | 5 | 4 | 4 | | 5 | 5 | 5 | 5 |
| UK 55 | Bulb: base shape (score) | 7 | 7 | 7 | 7 | 7 | | 6 | 7 | 6 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 64 | 62 | 64 | 64 | 64 | | 70 | 74 | 68 | 71 |
| UPOV 11 | Bulb: whole shape (score) | 4 | 5 | 5 | 4 | 4 | | 4 | 3 | 4 | 4 |

Variety: Mammoth

| UPOV/UK Char. No | Character | Manual Report | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diamter (mm) | 124 | 123 | 114 | 120 | 120 | 130 | 131 | 119 | 122 | 126 |
| UPOV 9 | Bulb: height (mm) | 138 | 143 | 137 | 133 | 138 | 153 | 162 | 151 | 143 | 152 |
| UK 54 | Bulb: top shape (score) | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |
| UK 55 | Bulb: base shape (score) | 6 | 7 | 7 | 6 | 6 | 6 | 6 | 7 | 6 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 62 | 65 | 64 | 62 | 63 | 71 | 76 | 73 | 70 | 72 |
| UPOV 11 | Bulb: whole shape (score) | 5 | 5 | 5 | 5 | 5 | 4 | 3 | 3 | 4 | 4 |

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Variety: Monkston

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 109 | 120 | 115 | 111 | 114 | 125 | 124 | 119 | 117 | 121 |
| UPOV 9 | Bulb: height (mm) | 130 | 146 | 143 | 135 | 138 | 154 | 159 | 154 | 150 | 154 |
| UK 54 | Bulb: top shape (score) | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 |
| UK 55 | Bulb: base shape (score) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 60 | 64 | 62 | 59 | 61 | 68 | 72 | 68 | 68 | 69 |
| UPOV 11 | Bulb: whole shape (score) | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 3 |

Variety: Ailsa Craig - approved maintenance Crosslings Seedling

| UPOV/UK Char. No | Character | Manual Report | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 103 | 102 | 98 | 106 | 102 | 104 | 103 | 101 | 112 | 105 |
| UPOV 9 | Bulb: height (mm) | 113 | 114 | 115 | 116 | 114 | 118 | 121 | 124 | 129 | 123 |
| UK 54 | Bulb: top shape (score) | 4 | 4 | 5 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |
| UK 55 | Bulb: base shape (score) | 6 | 5 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 53 | 50 | 52 | 54 | 52 | 58 | 56 | 58 | 61 | 58 |
| UPOV 11 | Bulb: whole shape (score) | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 3 | 4 | 4 |

Variety: Lancastrian

| UPOV/UK Char. No | Character | Manual Record | | | | | Image Analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Mean | Rep1 | Rep2 | Rep3 | Rep4 | Mean |
| UPOV 10 | Bulb: diameter (mm) | 106 | 111 | 86 | 100 | 101 | 111 | 116 | 92 | 105 | 106 |
| UPOV 9 | Bulb: height (mm) | 111 | 114 | 87 | 108 | 105 | 118 | 124 | 99 | 116 | 114 |
| UK 54 | Bulb: top shape (score) | 3 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 |
| UK 55 | Bulb: base shape (score) | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 6 |
| UK 58 | Bulb: base to widest point (mm) | 51 | 52 | 47 | 53 | 51 | 59 | 60 | 51 | 60 | 58 |
| UPOV 11 | Bulb: whole shape (score) | 6 | 6 | 5 | 6 | 6 | 4 | 5 | 4 | 4 | 4 |

[Annex VIII follows]

[English translation]

# Introduction to the Various Applications of Electrophoresis at the Biochemistry Laboratory of G.E.V.E.S. in Connection with Variety Registration and Seed Certification

## Introduction

Electrophoresis is a technique that is widely used for any research relating to genetics. It enables proteins, which are the primary expression of genes, to be separated.

The proteins contained in a fresh sample are made to migrate in a gel (starch or polyacrylamide) subjected to an electric field. The proteins, which are charged molecules, may be separated according to their electric charge and size. Another method, SDS electrophoresis, enables proteins to be separated according to their size alone.

Research is conducted on a number of species, namely maize, pea, wheat, ryegrass and soya.

## Maize

We carry out electrophoresis of isoenzymes on a starch gel, using coleoptiles of five-day old plantules, as described by Cardy, Goodman and Stuber.

19 enzyme loci, 16 of them polymorphic, are analyzed. The genetic pattern and chromosomic location is known for each locus.

Up to the present, isoenzymatic analysis has been carried out on 330 lines: 60% can be identified in only one way. Residual variability has been observed in 16% of the lines.

In DUS testing, isoenzymatic analysis is used to verify the accuracy of a hybrid's parental formula. Depending on the types of hybrid (simple and three-way), the number of individuals tested is different (Table 1). P denotes the probability of detecting only one genotype instead of two possible genotypes.

Another application is for the testing of hybrid purity on commercial samples. A hundred grains are analyzed in each sample for each hybrid. The enzyme loci are selected according to the "fingerprint" of the parent lines, with those for which the allele contribution of the parent lines is not the same being selected. A earlier test performed on one hybrid, which will be continued later on other hybrids, has revealed close correspondence between the percentage of impurities estimated by electrophoresis and the percentage estimated on the basis of morphological characteristics. The bibliography also gives a description of work resulting in the same conclusions.

One future application that has already been tested on one particular case is for distinctness in parent lines in connection with the registration of hybrids.

Wheat

The varieties in the French catalogue are described according to their electrophoretic gliadin patterns. About 60 band mobilities have been listed, which enables 72 varieties to be identified out of the 78 described by a standard electrophoregram.

Every year, the standard pattern is drawn up for newly-registered varieties, and the identification key is brought up to date. The homogeneity of 50 grains is also tested. If a variety presents two standard patterns, the breeder is invited to select one of the two.

Even though electrophoretic characteristics are not officially recognized in DUS research, electrophoresis can constitute an additional aid for varieties showing very small morphological differences.

Essentially electrophoresis is used for identification of a variety or of a mixture of varieties.

Another application has to do with the purity of wheat hybrids (tracing of self-pollinating grains). The method used is SDS electrophoresis of reserve proteins, which enables sub-units of gliadins and glutenins to be separated. The genetic patterns of sub-units of glutenins of high molecular weight is known, and enables an electrophoregram of a hybrid to be made on the basis of those of the parents.

Pea

Eight polymorphic enzyme loci and the polymorphism of the reserve proteins enable the French varieties of proteaginous pea to be identified. Of the 28 varieties analyzed, 24 can be distinguished in one way alone.

Electrophoresis is not used for registration but serves as an aid in decision-making by complementing the morphological characteristics (used in the case of Maxi and Calypso).

The main use is for the purity testing of seed samples for certification. 80 grains are analyzed, which allows a 4% impurity rate to be detected with a 5% margin of error. This check, which now is made a posteriori, will soon be made a priori, before sowing, for the purposes of initial sorting of samples.

Barley

The hordeins of barley have been studied in order to permit the identification of varieties on the basis of the grain (a problem for maltsters among others). Electrophoresis (acrylamide gel, SDS method) gives four clearly-separated zones A to D. Zone A is not interpreted. Zones B, C and D remain, for which 5, 20 and 20 types respectively are observed on the material studied (about 250 registered varieties in the European catalogue).

These results have made it possible to classify the 250 varieties in 80 groups, thereby adding to the distinguishing power of the morphological characteristics alone.

Other biochemical methods (isozymes, RFLP) could improve results. For the moment, the possible applications are the following:

- use of the electrophoretic characteristics for variety description;

- detection of heterogeneous elements or impurities in seed samples.

Ryegrass

As ryegrass is a cross-pollinated plant, there is considerable intracultivar variability, and it is sometimes difficult to distinguish between two cultivars. In such cases electrophoretic characteristics can be very useful. Three enzymatic systems (PGI, ACP, IDH) are routinely used in the G.E.V.E.S. Biochemistry Laboratory for the following applications:

- DUS: distinctness, comparison of varieties between themselves;

- checking: verification of the composition of mixtures;
  detection of genetic drift in the course of seed multiplication;
  detection of aberrant samples;

- a priori proposal of sowing plan.

The electrophoretic characteristics are given for 100 individuals of each variety. Other information may be obtained from those results: genetic structure of varieties (heterozygocity percentage, frequency of di, tri and tetragenicity in tetraploids), distinction between English and Italian ryegrass, detection of tetraploids in a sample of diploids, etc.

Soya

A certain number of enzymatic systems (20) have been tested for the description of varieties in the French catalogue. Nine have given satisfactory results. The others were either monomorphic in our sample or difficult to use. Moreover, the use of the total proteins of the grain also revealed polymorphism, albeit slight.

The figures obtained on enzymes and total proteins combined have made it possible to work out a key for the determination of varieties in the French catalogue that leaves only three couples undifferentiated.

Another result of electrophoresis on soya has been the revelation of a lack of homogeneity of varieties: one-third of the varieties in the catalogue had too or even three different types.

Finally, electrophoretic analyses extended to foreign varieties have shown a higher degree of variability, which is in line with expectations in view of the narrow genetic bases of French breeding practice.

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Applications of Electrophoresis

(1) Plant Improvement:

. Description of genetic make-up;
Estimation of genetic distances;

. Early verification of the success of hybridization or of forced self-pollination;

. Early detection of a characteristic that interests the breeder in the case of a link with an electrophoretic marker;

. Estimation of loss of variability in the course of generations in dynamic gene banks.

(2) DUS:

. Supplementary characteristic for identification or distinctness;

. Verification of the homogeneity of candidate varieties;

(3) Verification

. Identification of seed samples;

. Verification of hybrid formulae;

. Detection of impurities.

Conclusion

In many of its applications to variety registration and seed sample certification, electrophoresis has the advantage of being more rapid; it can serve to avoid implantation (verification of the parent formula of maize hybrids), or to provide optimum testing facilities for the comparison of varieties. Moreover, the response time is very short compared with orthodox testing.

As electrophoretic markers are independent of environmental conditions, the reference collections of the various countries could be readily described, and common data bases could be set up.

In order to conclude whether or not a variety is new, it is important to define a minimum genetic distance. Isoenzymatic analysis seems to be a highly suitable way of achieving that end:

. because the genetic pattern of the enzyme loci is often known or can be readily found out (monogenic mendelian determinism);

. because of their chromosomic location.

This genetic information makes it possible to describe genetic variability accurately and to calculate genetic distances. It will be possible to weight those distances according to the distribution of the various loci on the chromosomes.

| MAIZE | OFFSPRING | No oF INDIVIDUAL |
|---|---|---|
| Single hybrid | 100% | 2 |
| Three-Way hybrid $\left(\frac{a}{a} \times \frac{a}{a}\right) \times \frac{b}{b}$ | $\rightarrow$ 100% $\frac{a}{b}$ | 2 |
| $\left(\frac{a}{a} \times \frac{b}{b}\right) \times \frac{a}{a}$ | $\rightarrow$ 50% $\frac{a}{b}$, 50% $\frac{a}{a}$ | 6 |
| $\left(\frac{a}{a} \times \frac{b}{b}\right) \times \frac{c}{c}$ | $\rightarrow$ 50% $\frac{a}{c}$, 50% $\frac{b}{c}$ | 6 |

$$\text{Probability} = \left(\frac{1}{2}\right)^n + \left(\frac{1}{2}\right)^n = 2\left(\frac{1}{2}\right)^n$$

For $n = 5$ $\quad p = 0,06$

$n = 6$ $\quad p = 0,03$

$n = 7$ $\quad p = 0,015$

[Annex IX follows]

TABLE 1

Extract From the Report on the Seventeenth Session
of the Technical Working Party for Agricultural Crops
(Document TWA/XVII/9, paragraphs 21 to 30)

Electrophoresis Tests on Wheat

21. Dr. R.J. Cooke (United Kingdom) introduced a discussion paper on electrophoresis tests on wheat which was distributed during the session and is reproduced in Annex III to this report. This discussion paper was prepared in order to reply to the problems raised at the last session of the Technical Committee (see document TC/XXIII/6, pargraphs 37 and 38). Dr. Cooke provided the following information:

(i) The results of electrophoresis are independent of environmental conditions inasmuch as the same protein profile is obtained. The laboratory procedure itself is inevitably affected by the quality of the chemicals and by the design of the equipment used, but that influence can be eliminated by precise specification of the method, the sources of the chemicals and the equipment.

(ii) The application of electrophoresis to DUS testing may be useful for comparing the "discrimination power" of characteristics. The suitability of characteristics for detecting heterogeneity within a variety needs to be investigated. In any case, the method of electrophoresis needs to be strictly defined.

(iii) Electrophoresis of protein and enzyme for identification has been reported in many species. Electrophoresis of seed storage protein is a very successful way of distinguishing between varieties of self-pollinated cereals. Vegetatively propagated species can also be fairly readily distinguished. However, cross-pollinated species present more problems with respect to the way of achieving the discrimination between varieties. Pages 3 to 5 of Annex III contain a list of species for which the application of electrophoresis has been studied. It was supplied by Dr. Cooke after the session.

22. Mrs. M. Greneche (France) and Mrs. J. Lallemand (France) explained in detail their studies on the application of electrophoresis to maize and wheat and to barley, soybean and ryegrass. A summary of those explanations is reproduced in Annex IV to this report. Depending on the species concerned the study would comprise the possible use of electrophoresis for: variety control; checking seed lots; help in registration; test for purity; identification; detection of mixtures; checking of inbred lines to determine whether they are true parents; control of genetic shifts; stability; preliminary test for an optimum layout of the field test; grouping of varieties; distinguishing parental lines of hybrids; help in distinguishing varieties. Also, depending on the species concerned, efforts would concentrate more on the seed proteins or more on the enzymes.

23. The above papers and reports given led to a detailed discussion of the different electrophoresis methods and their possible use for purposes of plant variety protection. The results of the discussion could be summarized as follows:

24. Technical Aspects: It seemed possible to solve without great difficulty the problems of the technical nature of the electrophoresis method for distinctness purposes. Results were very similar even if the gels looked different when different equipment and chemicals were used. A solution had to be found species by species. For wheat, the method selected by the International Seed Testing Association (ISTA) seemed to be a good, stable and repeatable method for seed storage protein. However, to reach an agreed analytical method, some further parameters of the method should be defined and the nomenclature of the bands should be harmonized by giving them agreed numbers. Also the question of homogeneity would require further study.

25. Non-Technical Aspects: The reluctance to use electrophoresis for distinguishing varieties for plant variety protection purposes was due not so much to shortcomings of the technique as to the consequences such use would have for the whole system of plant variety protection. The main obstacle was that very small differences could be detected which, if accepted, could destroy the breeding work or lead to an erosion of the whole system of plant variety protection. Therefore it was not enough to have a good and reliable method (as the ISTA method for wheat) which worked for the seed trade, but UPOV had to agree on more, especially on how to interpret the results, on what differences would be sufficient to justify a separate protection right that would be legally defensible, and on what difference the breeder would be able to maintain.

26. Definition of Required Difference for Distinctness: The Working Party agreed that the most important and most difficult task was that of interpreting the results and of defining the required difference. It agreed that differences in the quantity of a certain band were not enough, neither was the absence or presence of one single band, for example in the case of wheat. All depended on the knowledge of the genetic background for each band. Some alleles would count for certain groups of bands. So before being able to fix a certain difference, for example an agreed combination of bands, the genetic difference shown by that combination should be known. This would require still quite detailed studies. Electrophoresis could only be used for plant variety protection purposes if it presented an objective measurement of a sufficient genetic difference.

27. Replacement of Other Characteristics: Not all traditional characteristics present in the UPOV Test Guidelines were an objective measurement of genetic difference. Some of them showed a higher variation than some of those obtained by means of electrophoresis. Once the remainder of the above requirements were fulfilled, some minor characterstics of doubtful importance in the present UPOV Test Guidelines could be replaced by characteristics obtained by means of electrophoresis.

28. Views of Breeders: Before introducing electrophoresis for distinguishing purposes for PVR, the views of the breeders should also be heard. The grass breeders present at the session declared themselves against the use of electrophoresis for distinctness purposes, at least at the present time, even though in grasses there was a lack of good distinguishing characteristics. Other breeders present preferred resistance characteristics to those of electrophoresis, despite the more complex and costly tests.

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29. Conclusion: The Working Party agreed that electrophoresis was a useful means of testing varieties for distinctness if it could be ensured that sufficient minimum differences between varieties were maintained either by a clear definition of the method and the interpretation of the results themselves or otherwise. How to reach that assurance depended on the case and the species concerned. For varieties of species which had to pass a VCU test before they could be commercialized the risk of too small differences was already considerably reduced.

30. Proposal to the Technical Committee: Having noted the studies made in the different member States with respect to electrophoresis, and being aware of the fact that in a few years it would no longer be possible to refuse electrophoresis as a tool for observing varieties for distinctness purposes, and in order to avoid a situation where different member States developed different methods and different interpretations of the results, the Working Party proposed to the Technical Committee that UPOV should study this question in more detail and give it higher priority. One possibility could be to create an additional Technical Working Party on New Technology (see also paragraph 35) which would deal with the harmonization of the application of electrophoresis for DUS purposes and try to reach an agreed interpretation of the results with respect to minimum distances. In the meantime, however, member States should not use characteristics obtained with the help of electrophoresis as the only means of establishing distinctness for the purpose of granting a new plant variety right.

[End of Annex IX
and of document]