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Wheat — Identification of varieties by electrophoresis

Blé — Identification des variétés par électrophorèse

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 8981 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 4, *Cereals and pulses*.

Annex A forms an integral part of this International Standard. Annex B is for information only.

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Introduction

The protein composition of wheat results from direct genetic control and in general is not affected by environmental conditions (e.g. location or year of growth). In addition, because wheat is essentially a self-pollinating plant, the protein composition of the different varieties of wheat remains stable for several plant generations. Therefore, the protein composition of a wheat can be used to characterize and thus to identify its variety.

Protein profiles can be obtained by carrying out polyacrylamide gel electrophoresis (PAGE) separations of the wheat gliadins. The polyacrylamide gels are stained to make the separated protein components visible. If such protein profiles are established for all wheat varieties which can be expected to occur in a particular region (i.e. a variety catalogue is prepared), the identification of an unknown variety of wheat can be established by reference to such a catalogue. Such a practice has been thoroughly characterized and is in common use in numerous countries.

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Wheat — Identification of varieties by electrophoresis

1 Scope

This International Standard specifies a method for the identification of the variety of a given lot of soft or hard wheat, in the form of individual ground kernels, flour farina or semolina, by the separation of gliadin proteins.

2 Principle

The separation of gliadin wheat proteins by polyacrylamide gel electrophoresis (PAGE) in slab gels containing aluminium lactate buffer, pH 3,1.

3 Reagents

Use only reagents of recognized analytical grade unless otherwise specified. The water used shall be deionized water having a resistance greater than 10 MΩ.

3.1 Extraction solution, ethanol, 70 % (*V/V*).

Dilute 700 ml of ethanol (absolute) with 300 ml of water.

3.2 Buffer solution, aluminium lactate, 2,5 g/l solution.

Dissolve 15,0 g of aluminium lactate in 5,5 litre of water. Adjust the pH to 3,1 with lactic acid. Make up to 6 litre with water. Vacuum filter through a filter of pore size 0,45 µm.

Store at 4 °C.

NOTE 1 The conductivity of the solution should be approximately 950 µS/m.

3.3 Sample dilution buffer

Dissolve 60,0 g of sucrose in 50 ml of buffer solution (3.2).

Store at 4 °C.

3.4 Acrylamide, 60,0 g/l gel solution.

WARNING — Acrylamide monomer is a neurotoxic substance which can be absorbed through the skin. Caution should be taken when handling either the crystalline powder or the gel solution.

Dissolve, in a 100 ml one-mark volumetric flask, 6,0 g of acrylamide (electrophoresis grade), 0,300 g of *N,N*-methylene bisacrylamide (electrophoresis grade) and 0,020 g of ascorbic acid in the buffer solution (3.2).

Make up to the mark with the buffer solution.

Store at 4 °C.

3.5 Initiator solution, iron(II) sulfate heptahydrate, 10 g/l solution.

Dissolve, in a 10 ml one-mark volumetric flask, 0,1 g of iron(II) sulfate heptahydrate in water and make up to the mark with water.

Prepare this solution shortly before use.

3.6 Catalyst solution, hydrogen peroxide, 0,99 % (*m/m*) solution.

Dissolve, in a 10 ml one-mark volumetric flask, 0,33 ml of hydrogen peroxide [30 % (*m/m*)] in water.

Make up to the mark with water.

Prepare a fresh solution daily and store at 4 °C.

3.7 Trichloroacetic acid, 9,6 % (*m/m*) solution.

Dissolve, in a 1 000 ml one-mark volumetric flask, 96 g of trichloroacetic acid in water.

Make up to the mark with water.

3.8 Brilliant blue R250, 5,0 g/l stock solution.

Dissolve 5,0 g of Brilliant blue in 1 litre of ethanol, stir for 1 h, and then filter through a filter paper (4.10) to remove any inorganic salts present.

3.9 Staining solution: Brilliant blue, 0,25 g/l solution.

Mix 5,0 ml of stock solution (3.8) with 95 ml of trichloroacetic acid solution (3.7).

3.10 Marker or **tracking dye**, e.g. Pyronin G, Methyl green.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.1 Vertical electrophoresis unit[1)]

4.2 Slot-formers, appropriate for the equipment used.

4.3 Power supply

A d.c. power supply which provides stable and constant voltages up to 1 000 V and constant current up to 300 mA (300 W min.).

4.4 Cooling bath, thermoregulated.

A circulating water bath to maintain the electrophoresis unit at 20 °C ± 1 °C.

4.5 Water purification system

A deionization system to provide high quality water having a resistance greater than 10 MΩ.

4.6 Analytical balance

4.7 pH-meter, with an accuracy of 0,05 pH units.

4.8 Conductivity meter (optional).

4.9 Vacuum filter unit, with cellulose nitrate filters, pore size 0,45 µm.

4.10 Filter paper, Whatman No. 1, 15,0 cm.

4.11 Magnetic stirrer, with PTFE-coated[2)] stirring bars, 2,5 cm and 5 cm long.

4.12 Hammer and **steel plate**, for crushing individual kernels.

4.13 Sample grinder, capable of yielding a particle size of 500 µm.

4.14 Sample tubes, with caps.

4.15 Micropipettes

Hand-held repeater pipettes with 2,5 ml disposable tips to deliver volumes of 50 µl to 250 µl. Adjustable micropipettes to deliver 10 µl to 100 µl and 100 µl to 1 000 µl.

4.16 Microcentrifuge, with minimum centrifugal force of 10 000 *g*.

4.17 Centrifuge tubes, of capacity 1,5 ml to 2,0 ml.

4.18 Vortex stirrer

4.19 Microsyringes, of capacity 25 µl.

4.20 Rigid polyethylene containers, approximately 6 cm × 17 cm × 17 cm.

4.21 Pyrex glass trays, 33 cm × 23 cm × 5 cm, for gel washing, viewing and photography.

4.22 Variable-speed shaker, capable of a shaking speed of approx. 50 rpm.

4.23 Light box, equipped with a fluorescent light source and a minimum viewing surface area of 30 cm × 60 cm for gel examination and photography.

4.24 Camera

Single-lens reflex 35 mm camera, loaded with fine-grain black and white or colour film.

4.25 Camera stand

Any stable stand for photography with a camera using slow shutter speeds.

5 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. Recommended sampling methods are given in ISO 950, ISO 2170 and ISO 6644.

1) Numerous commercial vertical electrophoresis units are available.

2) PTFE = polytetrafluoroethylene

6 Procedure

6.1 Preparation of test samples and extraction

6.1.1 Single kernel samples

NOTE 2 The number of individual seeds which need to be analysed depends on the accuracy of determination required. Confidence limits based on the examination of various numbers of kernels are listed in annex A. Analysis of larger sub-samples yields proportion estimates of greater accuracy.

Place the kernel between folded paper (5 cm × 5 cm) on a metal plate and pulverize with the hammer (4.12). Transfer the crushed material to the sample tube (4.14). Add 200 µl of extraction solution (3.1) using a repeater pipette (4.15). Mix the contents with the vortex mixer (4.18) for 10 s and allow to stand at room temperature (20 °C) for at least 1 h. Add 100 µl of sample dilution buffer (3.3) and remix. Allow mixture to settle for 10 min before sampling for analysis.

If the kernel extract is to be kept for more than 24 h, transfer extract into a centrifuge tube (4.17) and centrifuge for 3 min at 10 000 *g* in the microcentrifuge (4.16). Decant the supernatant into a sample tube and replace the cap.

Store at 4 °C for up to 10 days.

NOTE 3 The volumes used are for an average weight of 30 mg. Generally, most laboratories do not weigh the individual kernels, but, if desired, one could weigh the kernel and then add volumes equivalent to a total of 10 µl per milligram of wheat. Using kernel weights results in better electrophoregrams but it increases sample preparation times.

6.1.2 Bulk samples

Grind the sample in the electric grinder (4.13). Weigh 0,2 g of ground meal into a centrifuge tube (4.17) and add 600 µl of extraction solution. Mix the contents with the vortex mixer (4.18) for 10 s and allow to stand at room temperature (20 °C) for at least 1 h. Centrifuge at 10 000 *g* for 5 min. Transfer 200 µl of the supernatant into a sample tube containing 400 µl of sample dilution buffer (3.3) and replace the cap. Mix for 10 s.

Store at 4 °C for up to 10 days.

6.2 Preparation of standard extract

Proceed as described in 6.1 but use kernels of the pure reference variety (Neepawa)[3].

6.3 Preparation of gels

Assemble gel cassettes with 1,5 mm-thick spacers, as described in the manufacturer's instructions.

NOTE 4 To aid in pouring the gel solution and inserting the slot-former into the cassette, tape a piece of plastic (e.g. Plexiglas[4], 140 mm × 25 mm × 3 mm) to the top edge of one of the cassette glass plates.

To prepare each gel, combine 30 g of gel solution (3.4) and 75 µl of initiator solution (3.5) in a 125 ml filtering flask with a side arm. Degas the solution under a vacuum of at least 50 mmHg for 2 min with constant mixing. Add 120 µl catalyst solution (3.6). Swirl the solution gently by hand for 10 s, taking care not to incorporate air bubbles. Quickly pour the solution into the cassette in a continuous stream to minimize the introduction of air. Immediately after the cassette is filled, insert the slot-former (4.2). Polymerization is complete in 5 min to 10 min.

NOTE 5 A casting temperature of 20 °C to 22 °C has been found to give satisfactory results.

6.4 Sample loading

Carefully remove the slot-former and fill the sample wells with buffer solution (3.2) to prevent dehydration of the gel. Using a microsyringe (4.19), deposit the sample extract (6.1.1 or 6.1.2) in the bottom of the wells. The amount of sample extract depends on the equipment used. (The dense sample extract forms a layer under the buffer solution in the wells). Place standard extract (6.2) in any well except the two outer wells.

NOTE 6 As a guide, deposit a sample of 2 µl in each well of a 30-well, 1,5 mm-thick gel.

6.5 Assembly of electrophoresis unit

Attach the upper buffer chamber to the tops of the gel cassettes. Assemble the components of the lower chamber according to the manufacturer's instructions. Place the upper chamber/gel cassette assembly into the lower chamber which has been filled with buffer solution (3.2) (or enough buffer to cover the gel cassettes). Fill the upper chamber with buffer solution, place the safety lid onto the unit and connect the electrodes to the power supply (4.3). Ensure that the upper chamber electrode is the anode and that it is

3) Kernels of the pure reference variety (Neepawa) can be obtained from the Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, R3C 3G8 Canada.

4) This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

connected to the positive (+) pole of the power supply, while the lower chamber electrode is the cathode and is connected to the negative (–) pole of the power supply.

6.6 Electrophoresis

Set the water bath (4.4) to maintain the electrophoresis unit at 20 °C during the run. Use a constant current of 90 mA per gel for electrophoresis. Use a marker or tracking dye (3.10) to determine the optimum run-time for different types of equipment and sizes of gels. When the run is finished, turn off the power supply, disconnect the safety lid, remove the upper chamber assembly and pour off the upper layer of buffer.

WARNING — The high voltages are potentially lethal. Ensure that the electrophoresis apparatus is used safely according to the manufacturer's instructions and that no electrical leaks are present.

6.7 Staining

Detach the gel cassettes from the electrophoresis unit. Open the cassettes, remove the gels and place each in a plastic container (4.20) containing 100 ml of staining solution (3.9). Place the covered containers on the shaker (4.22) and agitate the contents gently at approximately 50 rpm for 4 h to 18 h. Destaining is not required, but place the gels in glass trays containing water and wipe them carefully with a cotton swab to remove precipitated stain.

7 Photography

Mount the camera (4.24) on the stand (4.25) above the light box (4.23). Place the cleaned gel in a glass tray (4.21) and position it under the camera. Using illumination from below only, photograph the gel.

8 Pattern interpretation

8.1 Pattern characterization

The electrophoretic pattern of a variety of wheat (see figure 1) can be characterized visually by the relative mobility and intensity values of its bands, as described in reference[4]. The pattern (or patterns, due to the different biotypes present) obtained from analysis of 100 kernels of each variety is recorded in this manner, as well as photographically, in the variety pattern catalogue. These pattern characterization data are used to create an identification key based on the absence or presence of bands of particular relative mobilities and/or intensities.

8.2 Identification of varieties

The following procedure shall be followed to identify an unknown variety of wheat by its electrophoretic pattern.

a) Examine visually the gel or its photograph and note the position and intensities of its bands relative to specific bands in the reference variety pattern which has also been analysed.

b) Consult the key prepared as in 8.1 to identify the variety.

c) Verify the identification by comparing the observed pattern with that documented in the variety pattern catalogue.

9 Expresssion of results

Statistical considerations shall be taken into account when conclusions about the varietal composition of a sample are made on the basis of analysis of individual kernels.

Confidence intervals are given in annex A.

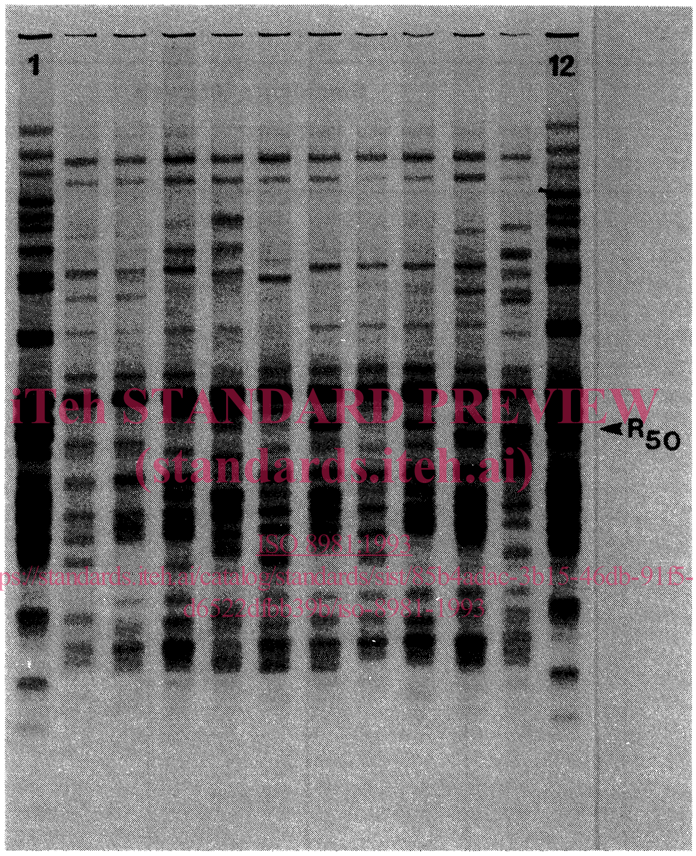

Figure 1 — PAGE patterns of gliadins of Neepawa (1 and 12), Slejpner (2), Rektor (3), Prinqual (4), Mercia (5), Maris Huntsman (6), Galahad (7), Champlein (8), Camp Remy (9), Beauchamp (10) and Avalon (11) wheats (R_{50} is a reference band used to determine relative mobilities)