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THE HARMONIZED PRESENTATION AND DOCUMENTATION OF PROTEIN AND DNA-POLYMORPHISMS AS PRE-CONDITION FOR THE INTRODUCTION OF BIOCHEMICAL AND MOLECULAR BIOLOGICAL METHODS FOR DUS-TESTING

Document prepared by experts from Germany

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THE HARMONIZED PRESENTATION AND DOCUMENTATION OF PROTEIN AND DNA-POLYMORPHISMS AS PRE-CONDITION FOR THE INTRODUCTION OF BIOCHEMICAL AND MOLECULAR BIOLOGICAL METHODS FOR DUS-TESTING

Introduction

The fundamentals of every use of biochemical and molecular biological methods for DUS-purposes are the following:

- All laboratories involved must produce identical banding patterns from the same genotype at any time.

- The difference between two polymorphisms scored as different must be defined very exactly.

- Unavoidable laboratory-made effects must be compensated. Such effects can be caused by different qualities of solvents used for protein extraction or by different qualities of polymerases used for DNA-amplification.

Ring tests are useful instruments for controlling the accuracy of biochemical and molecular biological methods. Specially regarding the incorporation of new polymorphisms as new states of expression in the table of characteristics ring tests are of great influence. Concerning the storage protein polymorphisms in barley and wheat and the isoenzyme polymorphisms in maize we have experiences with ring tests within UPOV over several years. This report summarizes these experiences and should help to define the requirements for the presentation and documentation of new polymorphisms.

Description of a new polymorphism

Polymorphisms obtained by use of biochemical or molecular biological methods are visible as banding patterns of proteins, DNA-fragments or amplification products of DNA-sequences. They are characterized by the position in the gel (electrophoretic mobility) and by the quantity (intensity) of their individual bands.

There will always be bands, which show only extremely small differences in their electrophoretic mobility. These band must be scored as identical. Also they get the same REM-value (Table 1). The mobility of new bands is estimated by the comparison with a band with defined mobility. If several bands characterized by the same REM-value, but showing small differences in their mobilities in reality are used as standard, it is possible to calculate different REM-values-dependent on the choice of the individual standard band (Table 1). Therefore it is necessary to use always the same band for REM-calibration purposes.

A polymorphism qualified for calibration should show only a few bands. Their intensities should be clear, but not too strong. Additionally the calibration bands should be located in the gel at regular intervals. This is the basis for an equally accurate estimation of the mobility over the whole REM-range.
Fig. 1 shows the allele "B2 (Aramir)" of the gene locus Hor 2 in barley as an example for a polymorphism fit for calibration purposes.

Band	real REM-value	Calibration band	estimated REM-value
Band 100 in variety A	99,7		100
Band 100 in variety B	100,0		100
Band 100 in variety C	100,4		100
Unknown band in variety D	99,4	Band 100 of variety A	100
		Band 100 of variety C	99

Table 1: Influence of the calibration bands on the REM-estimation

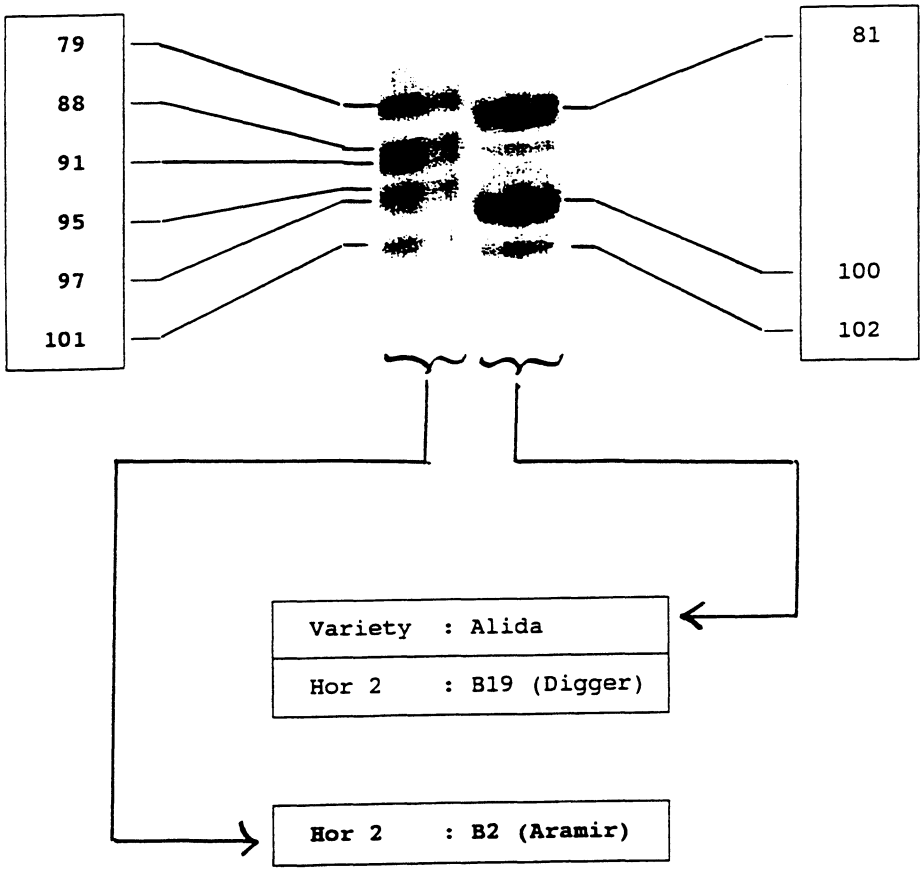

Fig. 1: Use of allele B2 (Aramir) as standard for calibrating B-hordein polymorphisms in barley

Grouping of polymorphisms

If a gene locus contains only 2 - 6 alleles it is easy to overlook the spectrum of alleles and to recognize an individual allele. However, with increased number of alleles in a gene locus the recognition of individual alleles is more and more difficult. For gene loci existing as a gene cluster the number of polymorphisms is extremely high. Then the formation of groups containing only a small number of polymorphisms has the aim to minimize the number of polymorphisms which must be compared with one another. The grouping helps to avoid miss-interpretations. The number of bands used for grouping should be limited to 2 or 3. They should be characterized by a clear expression and have an exposed position in the gel. Table 2 shows the grouping of B-hordein-polymorphisms as an example for the procedure and the result of grouping.

Group	Criterion of grouping	Number of alleles
Atem	strong band in position 100	4
Aramir	no strong band in position 100 strong single band in position 78 or 79	8
Valerie	no strong band in position 100 strong double band in position 78 or 79	6
Igri	no strong band in position 100 no strong single band in position 78 or 79 no strong double band in position 78 or 79	7

Table 2: Grouping of B-hordein polymorphisms detectable in German barley varieties

Establishing differences between similar polymorphisms

It is clear, that a large number of available polymorphisms involves the existence of similar polymorphisms. It is often difficult to discriminate these, and the risk of miss-interpretation is higher. For a correct interpretation of a banding pattern obtained by biochemical or molecular biological methods the knowledge of similar polymorphisms is important. The grouping as illustrated in table 2 is the first step for achieving this knowledge. The second step is the assortment of the similar polymorphisms side by side (Table 3).

Finally the differences between two similar polymorphisms are fixed exactly. This fixation of differences as illustrated in figure 2 by the B-hordein polymorphisms B9 (Grit) and B27 (Aura) allows the unambiguous assignment of varieties to one of the polymorphisms established in the table of characteristics or the formation of a new state of expression.

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Group	Similar alleles	Group	Similar alleles
Atem	B1 and B10	Valerie	B3 and B23
Aramir	B2 and B5		B3 and B7
	B2 and B11		B12 and B14
	B5 and B11	Igri	B15 and B17
	B9 and B27		

Table 3: Assorting of similar B-hordein polymorphisms detectable in German barley varieties

Differences between B9 (Grit) and B27 (Aura)
1.) In B9 (Grit) the band 100 is present, the band 101 is absent. 2.) In B27 (Aura) the band 100 is absent, the band 101 is present.

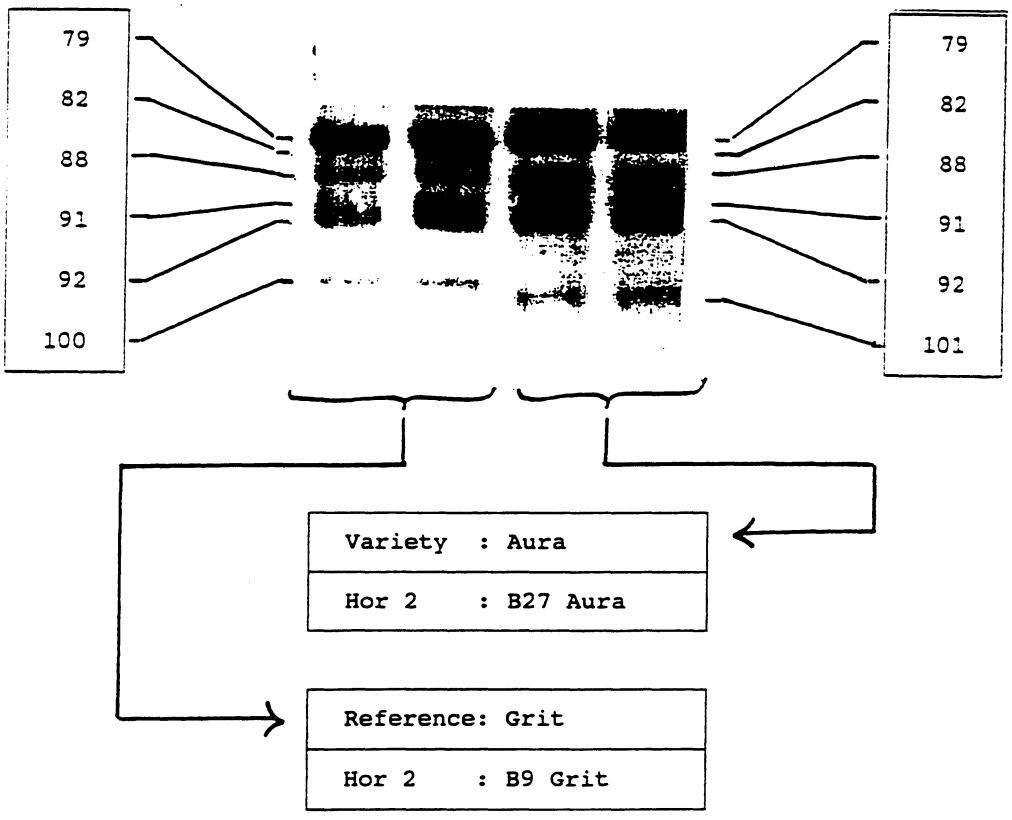

Fig. 2: Discrimination between the B-hordein polymorphisms B9 (Grit) and B27 (Aura)

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The culmination of similarity of two or three polymorphisms is their score as undistinguishable. Fig. 3 shows the B-hordein patterns of the barley varieties Alida and Digger. The standard samples of Alida and Digger are distinct by the expression of the B-hordein in position 102: This hordein is present in Alida and absent in Digger. Nevertheless the result of the UPOV-ring test in 1996 concerning these two varieties was the following:
"Alida shows the same allele as Digger. Alida and Digger are not distinguishable by their B-hordein polymorphisms".
Of course there were good reasons for this decision. In several varieties assigned to the allele B19 (Digger) a weak band in position 102 is visible. There is not any clear difference between these varieties and Alida. In the future it is possible that a second variety, identical with Alida, will be registered. Then the risk exists, that this B-hordein-type will be interpreted as a new allele and consequently as different from Alida. In order to avoid such miss-interpretation it is necessary to describe both sub-types of allele B19 (Digger) and to explain the reason why the sub-type Alida showing a clear band in position 102 is assigned to the allele B19 (Digger) described in the UPOV-guidelines without the band 102.

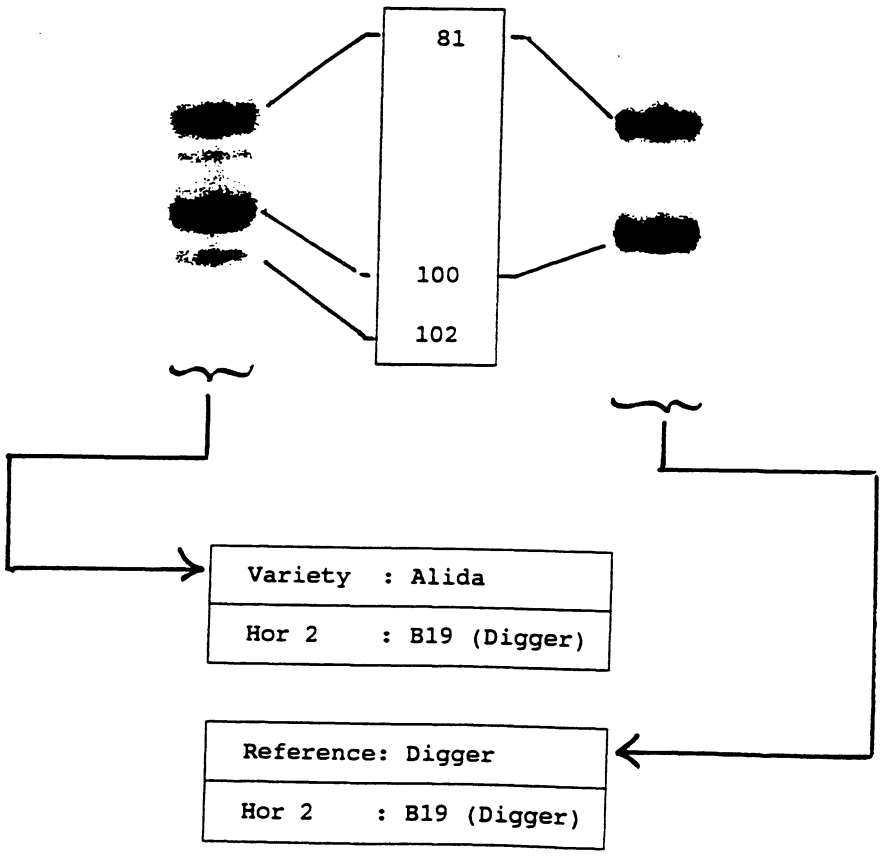

Fig. 3: Comparison of two sub-types of the B-hordein polymorphism B19 (Digger)

Summary

The fundamentals of every use of BMT's for DUS-purposes are the following:
All laboratories involved must produce identical banding patterns from the same genotype at any time.
The difference between two polymorphisms scored as different must be defined very exactly. Specially unavoidable laboratory-made effects, caused by different qualities of solvents used for protein extraction or of polymerases used for DNA-amplification e.g., must be compensated.
Ring tests are in general useful instruments for these purposes. Concerning the storage protein polymorphisms in barley and wheat and the isoenzyme polymorphisms in maize we have experiences with ring test within UPOV over several years. The report summarizes these experiences and helps to define requirements for the presentation and documentation of new polymorphisms:

- Exact description of the positions of all bands of a new polymorphism by a comparison with a very well defined calibration polymorphism.

- Alignment of new polymorphisms into the pool of existent polymorphisms.

- Definition of similar polymorphisms and definition of the differences between the new polymorphism and the similar polymorphisms.

- Explanation of the reasons for scoring two polymorphisms as undistinguishable.

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