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THE EFFECT OF NON-UNIFORMITY AND NON-STABILITY ON THE CORRECTNESS OF THE VARIETAL IDENTIFICATION OF SEED AND COMMERCIAL LOTS IN CEREALS

Document prepared by experts from Germany

THE EFFECT OF NON-UNIFORMITY AND NON-STABILITY ON THE CORRECTNESS OF THE VARIETAL IDENTIFICATION OF SEED AND COMMERCIAL LOTS IN CEREALS

Introduction

The introduction of characteristics used for identification purposes only as a new class of characteristics is discussed seriously in UPOV. For granting Plant Breeders Rights or for National Listing varieties have to be uniform and stable in all characteristics used for the assessment of distinctness. For such a new class of identification characteristics the varieties will not be subject to these strong conditions, there will not be a requirement of uniformity and stability. Nevertheless the user of identification characteristics presupposes that the varieties are stable and - in so far as they are autogamous - uniform in these characteristics also.
Since 20 years storage protein and isoenzyme polymorphisms are used as identification characteristics routinely for controlling the varietal purity of seed lots and commercial lots of autogamous cereals.
This report summerizes these experiences and should help to define the requirements for the use of biochemical and molecular biological markers as identification characteristics.

Uniformity of varieties in identification characteristics

Since 20 years and more the electrophoresis of storage proteins and isoenzymes is used as the main instrument for varietal identification of seed lots and commercial lots of the autogamous cereals soft wheat, durum wheat, barley, oats and sometimes triticale. Everybody, who performs this method routinely, knows, that in the autogamous cereals a considerable number of varieties exists showing two or more different banding patterns. Different factors influence the rate of varieties not uniform in these characteristics. The number of inhomogenous varieties is mainly influenced by the number of gene loci used for identification purposes. Table 1 demonstrates this by means of the analysis of three storage protein loci and two isoenzyme loci in 390 German barley varieties. In average one can calculate that per gene locus a rate of 3 % non-uniform varieties will be found. This result in the identification of barley varieties is confirmed by identification of German wheat varieties. The percentage of non-uniform varieties is the following:

for omega-gliadin-loci : 9 %
for HMW-glutenin-loci : 6 %
for alpha-gliadin-loci : 5 %
for Albumin-loci : 4 %

For a clear identification the number of analysed gene loci should be nearly ten. Such a standard set of identification characteristics makes a rate of 20 - 30 % inhomogenous varieties unavoidable.

Gene loci	Number of non-uniform varieties	
	winter barley	spring barley
Hor 3	10 (5%)	0 (0%)
Hor 3 + Hor 1	19 (9%)	16 (9%)
Hor 3 + Hor 1 + Hor 2	29 (13%)	23 (13%)
Hor 3 + Hor 1 + Hor 2 + Prx	37 (17%)	26 (15%)
Hor 3 + Hor 1 + Hor 2 + Prx + Est 3	40 (18%)	28 (16%)
	217	174
	Total number of varieties	

Table 1: Influence of the number of gene loci used for identification purposes on the uniformity of German barley varieties

Not only the number of gene loci, but also the number of detectable alleles per locus influences the rate of non-uniform varieties. As table 2 illustrates, the use of loci containing a high number of alleles like Hor 1 and Hor 2 produces a large number of non-uniform varieties. In isoenzyme loci, which have a small number of alleles, varieties are only in a few cases non-uniform.

Gene locus	Number of alleles	Number of non-uniform varieties	
		winter barley	spring barley
Hor 1	19	9 (4%)	16 (9%)
Hor 2	27	14 (6%)	18 (10%)
Hor 3	3	10 (5%)	0 (0%)
Prx	6	10 (5%)	6 (3%)
Est 3	3	5 (2%)	4 (2%)
		217	174
		Total number of varieties	

Table 2: Influence of the number of alleles per locus used for identification purposes on the uniformity of German barley varieties

In addition to the number of gene loci and the number of alleles per locus the frequencies of individual alleles are of importance concerning the degree of non-uniformity. The loci Prx, Hor 1 and Hor 2 can illustrate this. At present the locus Prx contains six alleles in German barley varieties. In spring barley one certain allele is by far the most frequent allele. In winter barley on the other side three alleles share the pool position. Therefore the rate of varieties non-uniform in Prx is higher in winter barley than in spring barley. In the loci Hor 1 and Hor 2 in spring barley several alleles with equal frequencies are found. However in winter barley two alleles in Hor 1 and Hor 2 are marked by their high frequencies. That is the reason why the rate of varieties non-uniform in Hor 1 and Hor 2 is lower in winter barley than in spring barley. In spring barley the locus Hor 3 is monomorphic, so all varieties are uniform in this locus.

Stability of varieties in identification characteristics

Non-uniform varieties have a clear tendency to non-stability as is demonstrated by table 3. One of three winter barley varieties containing two biotypes in the ratio 50:50 or 75:25 shows a dramatic fluctuation in the frequencies from the first sample to the standard sample:
from 50:50 to 100:0 or from 75:25 to 25:75.

First sample	Standard sample				
	80 A 0 B	60 A 20 B	40 A 40 B	20 A 60 B	0 A 80 B
40 A + 40 B	5	1	6	÷	÷
60 A + 20 B	1	1	4	1	0
	Number of varieties				

Table 3: Stability of non-uniform winter barley varieties

These results obtained by analysis of winter barley varieties are supported by analysis of winter wheat varieties. The fluctuation of the frequencies of the different genotypes can be small as in the wheat varieties 1 and 2 (table 4) or extremely strong, (e.g. from 50:50 to 100:0 in variety 3 or from 25:25:25:25 to 100:0:0:0 in variety 4 (table 4)).

Variety	Genotypes	Breeders samples in test Number of grains in 80 grains DUS-sample 1990	VCU-sample 1991	Standard sample 1992
1	A	50	44	37
	B	30	36	42
	X	0	0	1
2	A	17	45	23
	B	15	0	13
	C	14	0	0
	D	10	33	34
	E	8	0	5
	F	7	0	4
	X	9	2	1
3	A	46	76	73
	B	32	1	6
	X	2	3	1
4	A	27	0	
	B	21	79	
	C	18	0	
	D	14	0	
	X	0	1	

Table 4: Stability of German wheat varieties in identification characteristic

Identification of varieties by means of electrophoresis is associated with the fixation of an authentic sample for the individual variety. The seed sample or the commercial sample which should be analysed concerning its varietal relationship is compared with the authentic sample. The authentic sample can be taken from different productions. In non-uniform varieties this may result in quite different authentic samples. Therefore the conclusion drawn from the comparison between the seed sample or commercial sample with the authentic sample is influenced by the choice of the authentic sample (table 5). For identical data obtained by electrophoresis the conclusions are extremely different:

" Assessment of the varietal purity"

or

"Manifestation of a mixture of several varieties".

Therefore the beginning of establishing identification characteristics must be the fixation of varieties in this class of characteristics.

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	authentic sample				
Variety	**First sample**	**Standard sample**	**Seed sample**		
			Result	**Interpretation based on DUS-sample**	**Standard sample**
1	52 A 10 B 9 C 4 D 2 E 3 X	75 A 0 B 0 C 0 D 0 E 5 X	52 A 6 B 5 C 12 D 1 E 4 X	Variety confirmed	Mixture of several varieties
2	29 A 19 B 18 C 13 D 1 X	1 A 79 B 0 C 0 D 0 X	29 A 39 B 2 C 10 D 0 X	Variety confirmed	Mixture of several varieties

Table 5: Influence of the choice of the authentic sample on the result of varietal identification (example winter barley)

Summary

Concerning the use of characteristics only for identification purposes varieties are not subject to the conditions of uniformity and stability. Experience in routine use of identification characteristics in cereals over 20 years illustrates the following:

- In general varieties can not be expected to be uniform in identification characteristics. The degree of non-uniformity is dependent from the number of gene loci used for identification purposes and from the number and frequency of the alleles detectable in the gene loci used for identification purposes. Non-uniformity of varieties on the other hand does not automatically destroy the possibility for varietal identification.
- In general varieties can not be expected to be stable in identification characteristics. Mainly non-uniform varieties show a strong tendeny to non-stability. Non-stability makes a correct varietal identification impossible. Biochemical and molecularbiological markers can be used successfully as identification characteristics only if the varieties are fixed in these characteristics.

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