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USE OF PATANIN POLYMORPHISMS FOR DISTINGUISHING POTATO VARIETIES

Document prepared by experts from Germany

USE OF PATANIN POLYMORPHISMS FOR DISTINGUISHING POTATO VARIETIES

Introduction

Methods for identifying genotypes by use of biochemical or molecular biological markers should fulfill generally the following requirements:

1.) They should be qualified for recognizing individual gene loci.

2.) They should be robust and precise.

3.) They should be powerful concerning the discrimination of genotypes.

As everyone knows molecular biological methods are very powerful, but very expensive instruments for identifying genotypes. Their use for potato is profitable only, if their costs are compensated by high performance concerning the discrimination of varieties and by high level of robustness and precision. Since 25 years patanin polymorphisms are used on large scale for identification purposes in the German commercial potato market. Since several years they are also used routinely by the BUNDESSORTENAMT for DUS-purposes. Therefore a lot of information is accumulated which is useful for checking the fulfilment of the above-mentioned requirements by electrophoresis of patanins.

Qualification of electrophoresis of patanins for recognizing individual gene loci

Patanins are encoded by a family of genes containing 60 - 80 members per genome. This patanin gene family is located as a single locus on the long arm of chromosome 8.

Robustness and precision of electrophoresis of patanins

The robustness and precision can be influenced in a negative sense by different factors:
- changement of the physiological status of the tubers caused by their treament during the harvest or in the storage-house.
- changement of the equipment used for electrophoresis
- changement of the accuracy for the interpretation of the patanin banding patterns.

Patanins are extracted from tubers. Depending from harvest and postharvest conditions the physiological status of potato tubers can be different: Immaturity, sprouting, damage, fungal and bacterial infections and at last rottenness are possible. Theoretically a shift in the physiological status can induce dramatic fluctuations in the expression of patanins.

Sometimes it is necessary to replace the equipment. New equipment adapted to the technical progress can be changed in its technical parameters. One result of changed technical equipment can be the non-identity of banding pattern obtained from identical samples.

Patanin genes exist as a gene cluster. Generally the product of a gene cluster is a complicated banding pattern. Overlapping of bands is the normal case. The consequence of overlapping is the following: Only a part of the bands can be assigned to individual alleles.

Potato is an allogamous species. A large number of heterozygous genotypes must be expected. The consequence is the same as for the clustering of patanin genes: Strong overlapping of bands and therefore imperfect assignment of bands to individual alleles. Additionally potato is an autotetraploid species. A strong gene-dosis-effect is observed. Genotypes can be different only by differences in the band intensities.

The existence of patanin genes as a gene cluster, the allogamy and the tetraploidy of the potato make the interpretation very difficult and can limit the precision and also the power concerning the discrimination of genotypes.

For identification purposes in the German commercial market over 20.000 samples were analysed between 1972 and 1997. In this space of time a number of varieties have a remarkable position in the market. These varieties are investigated very often: more than 20 samples per annum. These varieties are the following:

Hansa	- Sieglinde	- Nicola
Linda	- Grata	- Desiree
Atica	- Gloria	- Hela
Bintje	- Saturna	

The individual samples of each of these varieties are different concerning the storage time and the storage conditions.

For all of the above-mentioned varieties the results were the same:
Modifications induced by storage factors, which could cause miss-interpretations, were not detectable. However, two effects were visible:
Long time storage at unnatural conditions and sprouting result in more or less indistinct patanin patterns. Extreme sprouting and strong rottenness result in extremely weak patanin pattern. In the first case very good experience is necessary for the correct interpretation. In the second case an interpretation of the patanin patterns is not possible. These results are confirmed by analyses of the BUNDESSORTENAMT for DUS-purposes from 1987 to 1996 (Table 1). For new varieties three different samples of plant material are investigated and additionally compared with the harvest material from these samples. Plant material and harvest material show identical patanin patterns.

Table 1: Testing of tubers for DUS-purposes

Age of tubers	
4 months	Plant material for DUS-testing
6 months	Plant material for VCU-testing
8 months	Plant material for testing the resistence to nematodes
0 months	Harvested material from VCU-trials
	Harvested material from DUS-trial

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A strong progress concerning the technical performance of the equipment is visible in the last 25 years (Table 2). Although the technical parameters were changed dramatically patterns of the same variety produced by different generations of equipment are identical (Fig.1).

	Laboratory	Gel size
1974 - 1979	BBA Braunschweig (Stegemann, Löschke	170x130x3 mm: 8 lanes
	LUFA Hameln (Ohms)	110x110x1,5 mm: 12 lanes
1982 - 1984	LBP Freising (Fritz)	110x110x1,5 mm: 12 lanes
	LUFA Hameln (Ohms)	110x110x1,5 mm: 12 lanes
1990 - 1996	BSA Hannover (Ohms)	60x110x1mm: 24 lanes
	LUFA Hameln (Paradies)	110x110x1,5 mm: 12 lanes

Table 2: Comparing investigations for analysing patanin polymorphisms

Year	1974	1994
Gel thickness	3 mm	1 mm
Capacity per electrophoresis	8 tubers	48 tubers
Pattern of "Hansa"		

Fig. 1: Influence of equipment

Performance of the electrophoresis of patanins concerning the discrimination of genotypes

The existence of an individual locus as a gene cluster produces the same effect as the polyploidy. The number of genotypes increases strongly (Table 3). An extremely strong increase of the number of genotypes must be expected if in a tetraploid crop like potato a gene cluster is used for identification of genotypes. Such conditions require an extremely clear system of grouping of genotypes.

	Species			
	Maize (Hybrids)	Rye-grass (4n)	Potato	Barley
Ploidy	2n	4n	4n	2n
Locus	Acp	Pgi	Pat	Hor 2
Gene cluster	–	–	+	+
Alleles	4	4	?	25
Genotypes	10	35	134	25
Notes for DUS-testing	6	?	85	25
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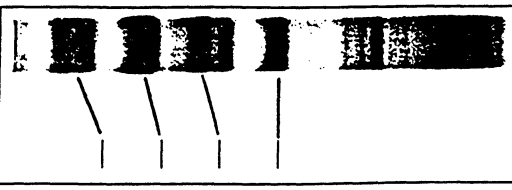
Table 3: Influences of polyploidy and clustering of storage protein genes to the number of genotypes

For DUS-purposes in potato the BUNDESSORTENAMT uses a three-step-system of grouping. The first step is identical with the grouping by STEGEMANN and LÖSCHKE. The different states of expression (absent or present) of four main bands, called A-, B-, C- and D-band, allow a classification of the varieties into 8 groups (Table 4). A few varieties are characterized by the absence of the B-band. These varieties form the additional group "9".



	A	B	C	D
1		+		
2		+		+
3		+	+	
4		+	+	+
5	+	+		+
6	+	+		
7	+	+	+	
8	+	+	+	+

Table 4: Use of main patanin bands for grouping of potato genotypes

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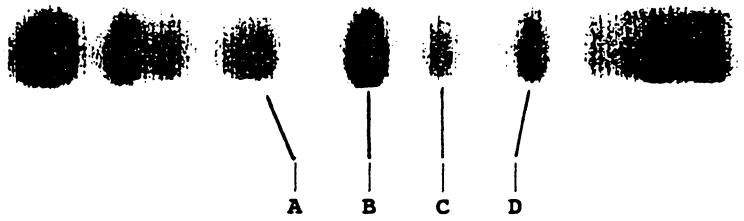
The second step has recourse to differences of the electrophoretic mobility within the A-, B-, C- and D-zone. Table 5 shows the subgroups within the group 7 as an illustration for the effect of incorporating the REM-values into the system of grouping. Each of the groups in table 4 are classified in 5 - 9 subgroups as far as the number of varieties in the groups is sufficienty large.



7	+	+	+
	33	42,5	51
	33	43	49,5
	33	43	50
	33	43	51
	33	43	52
	36	43	50
	36	43	52

Table 5: Use of REM-values of the main patanin bands for grouping of potato genotypes

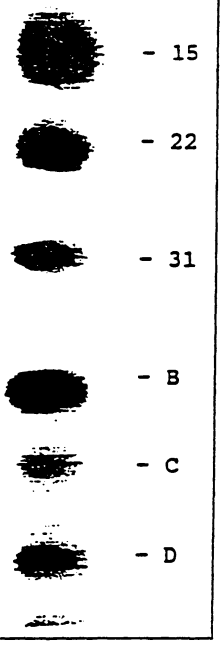
High-pK-Patanins			15 26	15 31	15	15 22 31	15 22	13 17	13	
7	33	42,5 51						X	X	
	33	43 49,5				X				
	33	43 50	X	X	X		X			
	33	43 51		X						
	33	43 52	X		X					
	36	43 50			X					
	36	43 52	X		X					

Table 6: Use of High-pK-Patanins for grouping of potato genotypes

For the third step of grouping it is necessary to make the range of patanins broadly detectable by electrophoresis. This is possible by changing the pH-value of PAGE from pH 7.9 to pH 8.9 or changing the method from PAGE pH 7.9 to IEF pH 4-9. PAGE pH 8.9 and IEF pH 4 - 9 make additional patanins visible characterized by high pK-value (> 7.9) (Fg. 2 and 3). These additional patanins form new sub-classes (Table 6). In 1996 the total number of sub-classes amounted to 85.

Fig. 2: Influence of the electrophoretic method on the localisation of the main bands A, B, C and D

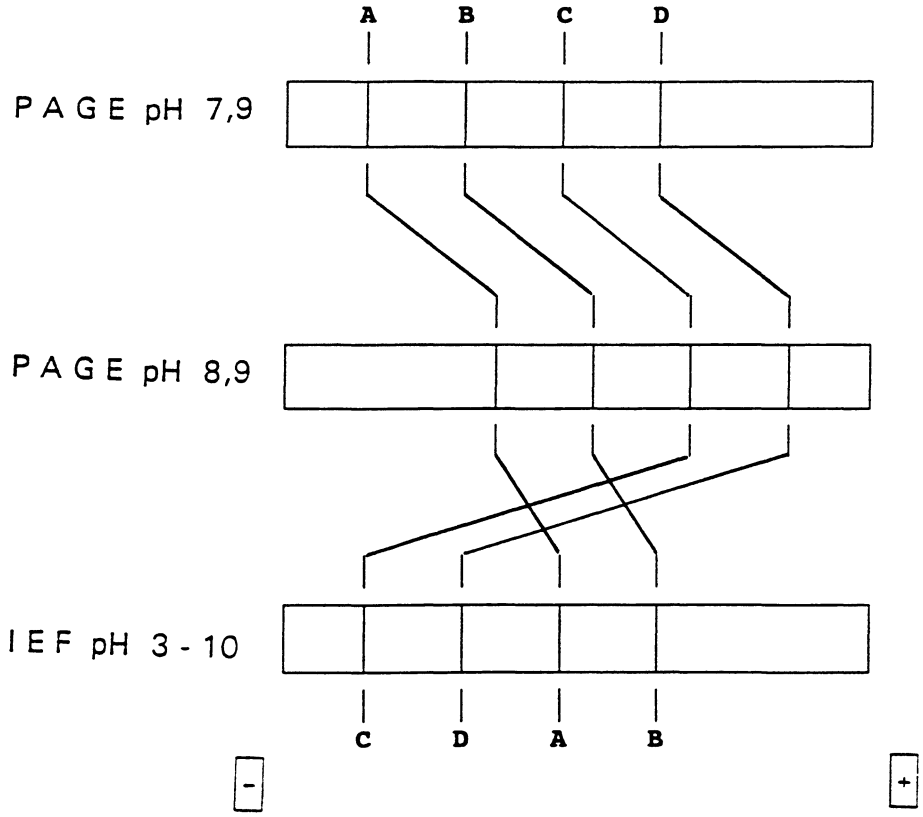


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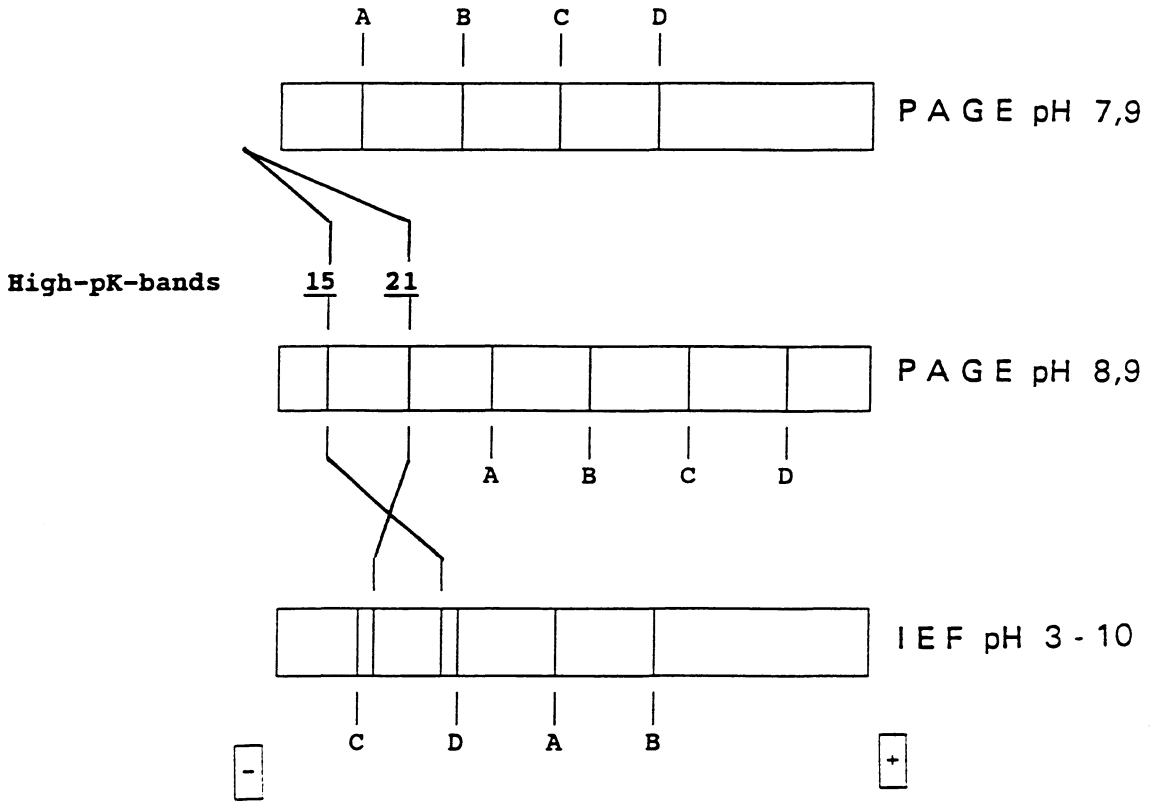
Fig. 3: Influence of the electrophoretic method on the detection of High-pK-bands (REM < 32)



Summary

Regarding the use of patanin polymorphisms for identification or DUS-purposes comparative investigations over a period of more than 20 years exist. The results of these investigations are the following:

- All patanin patterns of an individual variety are identical. They are independent from the equipment used by the different laboratories and from the physiological status of the tubers influenced by harvest and postharvest conditions.
- The large variability of the patanin polymorphisms caused by clustering of patanin genes and by autotetraploidy and allogamy of potato can be used in DUS-testing by an adequate system of grouping. The result of the grouping is the establishing of more than 80 notes in 1996 for the characteristic "Patanin composition: Allele expression at locus Pat".

As mentioned in the introduction the molecular biological methods are very powerful, but very expensive instruments for identifying genotypes. Their use for potato is profitable only, if their high costs are compensated by high performance concerning the discrimination of varieties. The patanin electrophoresis should be the standard for checking the performance of molecular biological methods. Additionally it should be guaranteed, that the molecular biological methods are not less robust and precise than the patanin electrophoresis.

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