RAPESEED MYROSINASE ISOENZYMES STUDIES USING ISOELECTRIC FOCUSING ON ULTRA THIN GELS

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Introduction

Myrosinase /thioglucoside glucohydrolase, EC.3.2.3.1/ as the enzyme hydrolising glucosinolates is known to occur in all glucosinolate containing plants in multiple isoenzymatic forms. Myrosinase isoenzymes from rapeseed were purified /Lönnerdal and Janson, 1973; Buchwaldt et al. 1986/ and studied electrophoretically /Vaughan et al. 1968; Klepacka et al. 1983/. Our attitude to this enzyme studies differs from the previous ones in some respects:

- isoenzyme patterns in a number of single seeds from each cultivar were recorded,
- isoelectric focusing of proteins on ultra thin gels was used to separate isoenzyme bands,
- Brassica napus isoenzyme patterns were compared to these of its "parental" species - Brassica campestris and B. oleracea.

Materials and Methods

Seed samples of winter rape cultivars and of summer rapes were kindly provided by Dr. W. Brzeziński - Research Center for Testing Cultivars, Słupia Wielka, Poland and Dr. H. Sørensen - Royal Veterinary and Agricultural University, Frederiksberg, Denmark respectively. Other Brassica accessions were obtained from different seed supliers.

Sinigrin was purified in this laboratory, Ampholine pH 4 - 6 was from LKB, Sweden, other chemicals were of analytical or electrophoresis grade from Serva, FRG.

Generally, 45 single seeds from each of the studied cultivars were analysed. Every seed was ground with 40 µl of water containing 2-mercaptoethanol /3mM/. The extracts were soaked directly onto squares /4x4 mm/ of Whatman 3MM paper and applied at the cathodic side of 0.15 mm thick polyacrylamide slab gels /7.5% T, 2.5% C/ containing 2% of Ampholine pH 4 - 6. Isoelectric focusing was carried out at 200 volts for 1 hr and thereafter at 1500 volts for 3 hr. The myrosinase isoenzyme patterns were developed using solution of sinigrin /2 mM/, ascorbic acid /1 mM/ and barium acetate /3 mM/.

Results

The Ampholine pH range and the electrofocusing parameters for the myrosinase isoenzyme separations had been optimized in a series of preliminary experiments. No additional myrosinase bands were recorded when broader pH range carrier ampholytes were used. Protein focusing in a prolonged time and the Ampholine concentration increase did not result in the isoenzyme bands sharpening.

Proteins with the myrosinase activities were detected in polyacrylamide gels immediately after electrofocusing according to the following chemical reactions scheme:

$$\text{sinigrin} + H_2O \xrightarrow{\text{myrosinase}} \text{different products} + SO_4^{--}$$

$$SO_4^{--} + Ba^{++} \longrightarrow BaSO_4\downarrow$$

White bands of barium sulfate precipitates were clearly visible on a transparent background of the unstained gels.

Three major myrosinase isoenzyme patterns were found in all the studie cultivars and breeding forms of both winter and summer rape /Fig. 1./. These patterns, referred to as A, B and C, consisted of 3 bands / denoted as a, b and d/, 3 /a, c, f/ and 6 bands /a, b, c, d, e, f/ respectively. All the bands in each pattern had near the same intensity. The pattern A dominated in almost all the studied rapes /Table 1./ and in some cultivars it was the unique one. On this basis protein extracts of Jet neuf cultivar were applied to each gel as an internal standard of the pattern A. Apparent myrosinase activities were very similar in all the seeds used and did not depend upon the glucosinolate contents of the corresponding cultivars.

Since we had neither an efficient surface pH electrode nor samples of proteins with isoelectric points close to 5, it was impossible to set apparent pIs to the individual bands in each pattern.

Myrosinase patterns other then A, B or C were found in several cultivars. An example of these "additional" isoenzymes is shown in figure 2. However, their incidence was very low: they were found in only 18 out of more then 1600 analysed seeds.

The Brassica napus myrosinase patterns were compared to some examples of both B. campestris and B. oleracea enzymes /Fig. 3./. As a rule the B. campestris myrosinase isoenzymes were focused in a lower pH range and the B. oleracea ones in a higher pH then their B. napus counterparts.

Discussion

All the authors of myrosinase studies published so far agree that this enzyme occurs in rape seeds in multiple isoenzymatic forms. However, this is the first report demonstrating this enzyme heterogenicity within single seeds. At least three protein bands having the myrosinase activity were observed in each of more then 1600 analysed seeds. From our results /Table 1./ it appears that one isoenzymatic set /pattern A in Fig. 1./ is the essential one for B. napus. In 18 cultivars per 38 analysed we found only this myrosinase isoenzyme composition. What is more, in additional 13 cultivars at least 90% of seeds contained the myrosinase isoenzymes giving pattern A after isoelectrofocusing. Only in 4 analysed rapes this pattern was found in less then 50% of seeds. We have not observed any interdependence between the myrosinase isoenzymes and glucosinolate contents or origin of the rape cultivar.

The nature of "additional" myrosinase isoenzyme patterns /Fig. 2./ can not be explained on the basis of our experimental results. The number of seeds containing them was to low to discuss this problem. It can not be ruled out that these patterns come from some contaminations in the seed material.

Since Brassica napus is regarded to be an amphidiploid of B. oleracea and B. campestris /U,1935/ it is interesting to compare myrosinase isoenzymes of all these taxa. As can be seen from Fig. 3. there are some interesting common features in the corresponding patterns. Isoenzyme bands a and b can be found in the B. campestris myrosinases and the band d is frequent in the B. oleracea myrosinase patterns. Taking these observations into account one may conclude that the Brassica napus myrosinase is a sum of isoenzymes coming from the two primary genoms. However, the myrosinase genetics is not clear yet and needs some more additional studies.

Literature:

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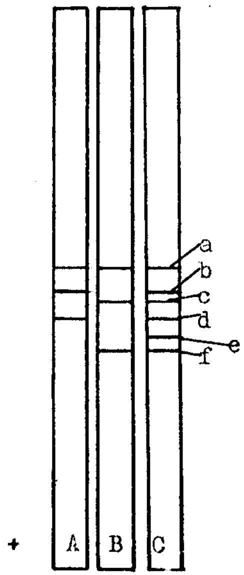

Fig. 1. Myrosinase isoenzyme patterns from rape seeds.

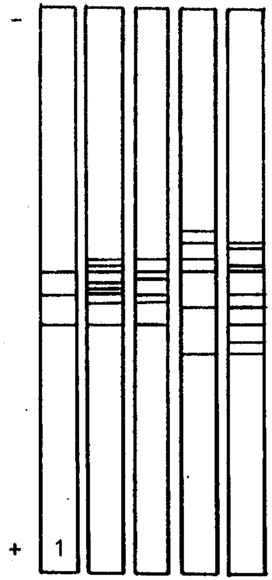

Fig. 2. The rarely found myrosinase isoenzyme patterns from rape seeds. Line 1 shows the pattern A for comparison.

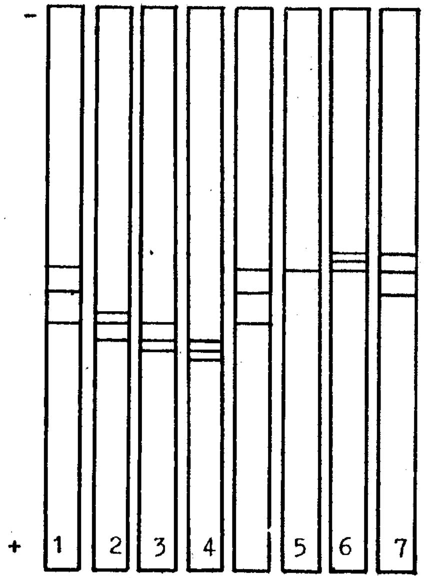

Fig. 3. Myrosinase isoenzymes from B. campestris and B. oleracea. Line 1: pattern A from B. napus; 2: white cabbage; 3: Brussels sprout; 4: broccoli; 5: Yellow srson; 6: cv. Candle; 7: Chinese cabbage.

Table 1.
Myrosinase isoenzyme patterns in single seeds of rape cultivars.

Cultivar	Country of origin	Type[§]	Myrosinase pattern /number of seeds/ A	B	C	other
Górczański		T	25	5	12	2
Beryl		E	31	6	8	-
BKH 284		E	42	-	3	-
BKH 385		E	38	-	-	3
BOH 283	PL	G	44	-	1	-
BOH 384		G	21	8	14	2
BOH 484		G	41	-	4	-
POH 185		G	8	22	10	-
POH 285		G	2	34	5	-
POH 385		G	36	1	6	-
Marinus	DDR	E	40	3	2	-
Jupiter		E	22	13	10	-
Ww 956	S	E	38	-	-	4
Ww 957		E	42	1	-	1
Belinda		E	43	-	1	-
Tamara		E	44	1	-	-
Mirander	D	E	41	-	3	-
Lirakotta		E	43	-	-	2
Liglandor		G	43	-	-	2
Lirabon		G	43	-	-	2

Cultivars in which only myrosinase pattern A was found:

BOH 183	PL	G
BOH 585		G
Ww 970	S	E
Gundula		E
Doral		E
Ridana		E
Licantara	D	G
Lindora		G
Liropa		G
Rubin		G
Santana		G
Jet Neuf		E
Bienvenue	F	E
Tandem		G
Darmor		G
JN 404		G
DP 11-15	DK	G
SV 2233		G

[§]Abbreviations: T - high erucic acid and glucosinolates
E - low erucic acid
G - "double low" forms