ANALYSIS OF THE MYROSINASE ENZYME SYSTEM IN DOUBLE HAPLOIDS AND RESYNTHESIZED *BRASSICA NAPUS*.

V. BEISVÅG, J. DAVIK# AND A. M. BONES*

UNIGEN Center for Molecular Biology, Department of Botany, University of Trondheim, MTFS, N-7005 Trondheim, and # Planteforsk, Kvithamar Research Station, N-7500 Stjørdal, Norway, *Fax: 47-73-598705, E.mail: Atle.Bones@Unigen.Unit.No

ABSTRACT

***Brassica napus* resynthesized from *B.* oleracea and *B. campestris*, double haploids of *Brassica napus* and plants obtained by sexual crosses between resynthesized *Brassica napus* and double haploids have been used to analyse the expression of components belonging to the myrosinase enzyme system.**
The expression of polypeptides and active myrosinase complexes were determined by immunoblots, detection of active myrosinase complexes after separation by isoelectric focusing in polyacrylamide gels and by determination of total and specific myrosinase activity.
Different parts of the plants at seedling and early flowering stages were examined. Immunoblots after SDS-PAGE gave patterns directly reflecting the classes of myrosinase subunits. Examination of the active myrosinase complexes showed a high degree of variation which probably reflect that different combinations of polypeptides are expressed.

INTRODUCTION

In *Brassica napus* the number of genes belonging to the myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) gene family have been estimated to be approximately 20 (Thangstad et al. 1993). The high number of genes is also reflected in the number of polypeptides which can be detected on e.g. Western blots or by detection of polypeptide complexes with myrosinase activity after electrophoresis or isoelectric focusing in polyacrylamide gels (see e.g. Bones et al. 1994).
Earlier studies of different cultivars of *Brassica napus* and other species have shown that the expression of components belonging to the myrosinase enzyme system varies. The aim of the present and ongoing experiments have been to examine the variation of the expression of the myrosinase enzyme system in sexual crossings and resynthesized plants. In the present report results from resyntehesised *Brassica napus* and crosses between these and double haploid lines are included.

EXPERIMENTAL

Double haploid lines of *Brassica napus* were obtained from Svalöf Weibull AB. Resynthesized *Brassica napus* and sexual crosses were obtained from Kvithamar Research Station. Immunoblots, myrosinase activity assay and the barium sulphate assay were performed as described by Bones et al. (1994). Myrosinases were detected by an anti-myrosinase antibody (3D7) followed by rabbit anti-mouse Ig(G+A+M) conjugated with horseradish peroxidase and visualised by chemiluminescence.

Expression of myrosinase polypeptides

Immunoblots after SDS-PAGE of extracts from permanent leaves of resynthesized, double haploid lines and crosses between them show that 2-3 classes of myrosinase subunits can be found (Fig.1A). Two of these classes (here collectivly named 68 kDa group of subunits) migrate close together on 12 % polyacrylamide gels and appear as one broad band on immunoblots. Partial purification and examination by isoelectric focusing revealed that the 68 kDa group of polypeptides can be separated into at least 11 components with different isoelectric points (data not shown). Separation on a 9% polyacrylamide gel will increase the resolution of the 68 kDa class of polypeptides so that 2 subunit classes can be seen. Isoelectric focusing followed by barium sulphate assay show that at least 8 different bands with myrosinase activity can be observed (Fig. 1B). Some of these bands are complexes consisting of several different polypeptides.
The myrosinase activity at different stages and in different cultivars of *Brassica napus* have been reported by Bones (1990). The two resynthesized lines of *Brassica napus* have a high expression of myrosinases, and 4-6 active myrosinase complexes can be detected by the barium sulphate assay (Tab.1). They also have a high

seed content of glucosinolates. The expression of myrosinases in the double haploids which represents one line with high and one with low glucosinolate content, were considerably lower and 5 active myrosinase complexes could be detected in the line 79. The offspring after crossings between double haploids and the resynthesized lines indicate that the offspring can be divided into two groups with markedly different expression patterns and expression levels of myrosinase (Tab.1).

TABLE 1. Total and specific myrosinase activity, polypeptide classes detected by immunoblots and isoelectric points of active myrosinase complexes detected by the barium sulphate assay after isoelectric focusing in polyacrylamide gels of samples prepared from permanent leaves of *Brassica napus* plants at early flowering stage. The female parent is the line mentioned first in the sexual crossings. ND: not detected, - : not determined.

Lines	**Tot. act.** nmol min^{-1}	**Spec. act** nmol $min^{-1}mg^{-1}$	**Immunoblot** SDS-PAGE	**Bariumsulphate** IEF	**Glucosinolate** µmol g^{-1}
cv. Paroll	16.2	2.8	77	4.9/5.3/5.6	36
Resynthesized oilseed rape					
2305	80.0	6.7	77/68	(4.8)/4.9/5.3/5.6	111
7406	100.8	15.0	77/68	4.8/4.9/5.0/5.3/5.6	168
Double haploids					
91	2.2	0.9	ND	ND	15
79	22.2	4.0	77/68	4.8/4.9/5.0/5.3/5.6	152
Sexual crosses					
91 x 2305	87.0	10.9	77/68	(4.7)/4.8/4.9/5.0/5.3/5.6	29
91 x 7406	9.1	1.3	ND	4.9/(5.0)	25
91 x 79	102.0	12.1	77/68	4.7/4.8/4.9/5.0/5.3/5.6	21
79 x 2305	6.1	0.8	ND	5.0	-
79 x 7406	63.1	9.5	77/68	(4.8)/4.9/5.0	-

Multiple forms of myrosinases in some species of Brassicaceae have been reported earlier (Bones and Slupphaug 1989, Lenman et al. 1990, Bones et al. 1991, 1994). The different forms of myrosinases can be divided into polypeptide classes coded by 2-3 subgroups of the myrosinase gene family. These subgroups most likely contain between 3 and 10 members each. By examination of active myrosinase complexes after isoelectric focusing or native-polyacrylamide gel electrophoresis, both subunits and complexes with multiple polypeptide subunits could be observed. Polyacrylamide electrophoresis of samples which have been reduced and denatured reduced the pattern of bands and basically displayed the products from the subgroups of the gene family.

Thangstad et al. (1993) reported the full-length genomic sequence of two myrosinase genes belonging to the *Myr1* and *Myr2* myrosinase gene families in *Brassica napus*. Based on sequencing of cDNA from different parts and developmental stages it was suggested that these two gene families show different expression patterns. Support for a differential expression of genes in the *Myr1* and *Myr2* families have also been presented by Xue et al. (1991, 1993). Based on the present sequence data (Xue et al. 1992; Chadchawan et al. 1993; Thangstad et al. 1993) and unpublished work, it seems likely that there exists 2-3 myrosinase gene subfamilies and that the subunits observed after SDS-PAGE reflect the gene products of these gene families. It has earlier been shown that the members of the different gene families are differentially expressed (Xue et al. 1993, Bones et al. 1994). The differential expression of myrosinase isoenzymes should be considered when they are used as markers in e.g. breeding programs.

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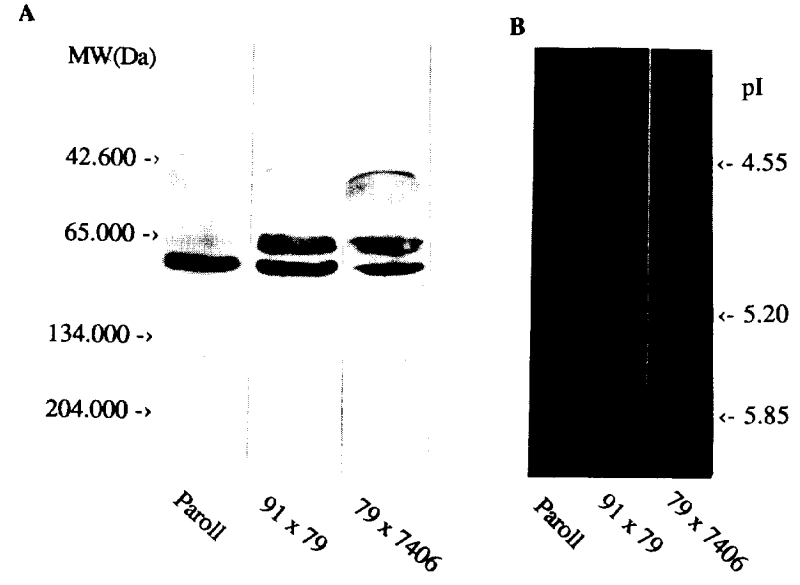

FIGURE 1. (A) Immunoblots after SDS-PAGE and (B) bariumsulphate assay with extracts from lines indicated. The primary antibody used in the immunoblot was a mouse anti-myrosinase monoclonal antibody.

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