Variation for myrosinase activity in some crop *Brassica* species

A. K. Atwal, Manoj Kumar, Pratibha Chauhan, S. S. Banga

Department of Plant Breeding, Genetics and Biotechnology,
Punjab Agricultural University, Ludhiana 141 004, India Email: surin11@rediffmail.com

Abstract

Glucosinolates are the class of sulphur containing secondary metabolites present in all cruciferous plants. These release a variety of compounds (isothiocyanates, thiocyanates and nitriles etc.) following hydrolysis by enzyme myrosinase. Some of these metabolites are defensive against insects and other attractants. The nature of breakdown products is regulated by an associated epithiospecifier protein (ESP). Studies in *Arabidopsis* have shown ESP/myrosinase system can be modified to increase its defense responses. In this communication, we report variation for myrosinase enzyme activity in various *Brassica* species. Inheritance of myrosinase activity in *B. juncea* was also investigated. 128 diverse *Brassica* accessions were assayed for myrosinase activity and glucosinolate content at various development stages. Significant genotypic variation was observed, myrosinase activity was highest in leaves, followed by developing seeds and least in the mature seeds. *B. napus* leaves had the maximum mean value 1.29±0.59 n mole glucose released mg^{-1} min^{-1}, followed by *B. juncea* (0.63 ± 0.05), *B. nigra* (0.52 ± 0.09) and *B. rapa* (0.30 ± 0.03). *B. napus cv.* ACN 50 (2.07) had the highest myrosinase activity and ACN 40 (0.68) the least. In *B. juncea*, it ranged from 0.08 (KH 2099) to 1.50 (ALM 129). Genotypes with appreciably low myrosinase activity were KH 2099 (0.09) of *B. juncea*, VKS 11/29 (0.08) of *B. rapa* and FRG 2 (0.17) of *B. nigra*. *B. rapa* showed strong positive correlation among myrosinase activity recorded at all the development stages. For *B. nigra*, it was reverse. In *B. juncea* and *B. napus*, variable results were indicated. Myrosinase activity in green tissues was negatively correlated with total glucosinolates content. It had a negative association with but 3-ethyl glucosinolate (gluconapin) and a positive correlation with 2-0H-but-3-ethyl glucosinolates (progoitrin). Two to three bands of myrosinase isoenzymes were detected after ultrascanning, when crude extract of myrosinase was subjected of PAGE. Genetic studies in *B. juncea*, indicated the involvement of 3-4 genes, acting in additive fashion, in myrosinase heredity.

Key words: *Brassica*, Myrosinase, Glucosinolates, Variation

Introduction

The myrosinase-glucosinolate system has been a subject of many chemical investigations (Heqnauer 1986). Specific β-thioglucosidases known as myrosinases are responsible for degradation of glucosinolates (β-thioglucoside- N-hydroxysulfatases). The resultant hydrolysis products are more biocidal than the intact glucosinolates, being toxic to both generalist insect herbivores (Li et al., 2000) as well as to crucifer specialist insects (Li et al., 2000). Several myrosinase isoenzymes have been reported in seeds, seedlings and vegetative tissue of oilseed rape (Lenman, 1993). Most studies to date have been focused on variation in glucosinolates without much emphasis on the levels of myrosinase activity in various vegetative and reproductive structures at different development stages. The present studies aimed to screen different crucifer accessions for the variation in myrosinase activity and total glucosinolate levels at varied plant growth stages (at pre- as well as post-anthesis) in different plant parts.

Materials and Methods

Four crop *Brassica* species namely, *B. juncea, B. napus, B. rapa* and *B. nigra* were evaluated for myrosinase activity and glucosinolate content in various crop growth stages over two years. Different plant tissues namely young leaves, pod wall and seeds were collected at varied plant growth stages (pre-anthesis, termination of flowering on main shoot and at maturity). Standard agronomic practices were followed throughout the growing season. Myrosinase activity was measured as described earlier (Li et al., 2002). Total glucosinolates content in mature seeds was measured by the method of Kumar et al. (2004).

Results and Discussion

Analysis of variance revealed significant differences for mean square values of myrosinase activity among the genotypes in different *Brassica* species at almost all the stages of crop growth. In leaves, overall mean values for myrosinase activity were generally lower (Table 1) in diploids (*Brassica rapa* and *Brassica nigra*) as compared to the digenomics (*Brassica napus* and *Brassica juncea*). Higher leaf myrosinase activity of *Brassica juncea* and *Brassica napus* was in consonance with a past report (Xue et al., 1993). It ranged from 0.68 (ACN-40) to 2.07 (ACN-50), 0.08 (KH-2099) to 1.50 (ALM-129), 0.08 (VKS-11/6) to 0.53 (Sunshine) and 0.13 (FRG-2) to 1.37 (N-17) in *B. napus, B. juncea, B. rapa* and *B. nigra* respectively. These ranges are in agreement with those of Li et al. (2002) who reported myrosinase activity ranging from 0.22–0.63 n mol mg^{-1} min^{-1} in *B. juncea* cotyledons. In *B. napus*, leaf myrosinase activity was positively correlated with that of green tissues (developing seeds and developing pod wall), no such correlation was recorded at the other developmental stages. In *B. juncea* and *B. nigra* myrosinase activity of developing seeds and developing pod wall was strongly correlated to that of developing pod wall and in mature seeds (Table 2). *B. rapa* showed strong positive correlation of myrosinase activity at all the developmental stages.

Table 1. Variation of mean myrosinase activity (nmol mg^{-1} min^{-1}) at different developmental stages in crop *Brassica* species

Species	Samples analysed	Myrosinase activity (nmol mg^{-1} min^{-1}) (Mean ± SE)				
		Levels	Developing seeds	Mature seeds	Developing pod wall	Mature pod wall
Brassica napus	45	1.29±0.59	1.12±0.05	0.67±0.03	0.67±0.03	0.27±0.01
Brassica juncea	49	0.63±0.05	0.90±0.09	0.25±0.01	0.25±0.01	0.11±0.01
Brassica rapa	20	0.30±0.03	0.94±0.06	0.29±0.02	0.29±0.02	0.15±0.01
Brassica nigra	14	0.52±0.09	1.02±0.06	0.21±0.01	0.21±0.01	0.09±0.01

Table 2 : Correlation for myrosinase activity (nmol mg-1 min-1) at various developmental stages in *Brassica* species

Developmental stages	Leaves	Developing seed	Developing pod wall	Mature seed	Mature pod wall
Leaves		0.453**	0.344**	0.093	-0.106
		-0.179	-0.173	-0.290	-0.022
		0.745**	0.894**	0.818**	0.730**
		-0.380	-0.288	-0.355	-0.648**
Developing seed			0.836**	-0.036	-0.044
			0.949**	0.866**	0.807**
			0.997**	0.615**	0.585**
			0.824**	0.707**	0.446**
Developing pod wall				-0.088	-0.017
				0.908**	0.835**
				0.667**	0.817**
				0.929**	0.508
Mature seed					0.218
					0.861**
					0.927**
					0.557*
Mature pod wall					

* Significant at P=0.05
** Significant at P=0.01
B. napus *B. juncea* *B. rapa* *B. nigra*

All the crop *Brassica* species investigated showed negative correlation between myrosinase activity and glucosinolates content especially in green tissues (leaves, developing pod wall). Across all the species, a negative and significant correlation between myrosinase activity and but-3-enyl was indicated. In contrast, a positive correlation was recorded with 2-OH-but-3 enyl.

References

Hegnauer R. (1986). Photochemistry and plant taxonomy-An essay on the chemotaxonomy of higher plants. Photochemistry **25**, 1519-1535.

Li Q., Eigenbrode S.D., Strigam G.R., Thiagarajah M.R. (2000). Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica* juncea with varying glucosinolate concentrations and myrosinase activities. J Chem Ecol **26**, 2401-2419.

Li Q., Brown J., Eigenbrode S.D. (2002) Variation in the cross progenies of two *Brassica juncea* lines with relatively low and high myrosinases activities. Can J Plant Sci **82**, 579-582.

Lenman M., Falk A., Xue J and Rask L. (1993) Characterization of a *Brassica napus* myrosinase pusedogene: myrosinases are members of the BGA family of β-glycosidases. Plant Mol Biol **21**, 463-474.

Kumar S., Yadav S.K., Chauhan J.S., Singh A.K. (2004) Total glucosinolates estimation by complex formation between glucosinolates and tetrachloropalladate (II) using ELISA reader. J Fd Sci Technol **41**, 63-65.

Xue J., Philigren V., Rask L. (1993) Temporal, cell specific and tissue preferential expression of myrosinase genes during embryo and seedling development in *Sinapis alba*. Planta **191**, 95-101.