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RESEARCH EXPERIENCE

Designation and affiliation	Duration	Project
Postdoctoral Fellow Interactions Lab TIFR- National Centre for Biological Sciences (NCBS), Bangalore, India.	23/4/2019 to 31/01/2020	Understanding fate of glucosinolates across three trophic levels (plant-herbivore-parasitoid) for crop protection.
Ph.D. Scholar Plant Physiology and Biochemistry Lab, Plant Sciences Department, CSIR-Indian Institute of Integrative Medicine, Jammu, India.	14/02/2012 to 29/11/2018	Biochemical and molecular characterization of Glucosinolate-Myrosinase system in <i>Lepidium latifolium</i> .

EDUCATION

Degree	Board/University	Date of result declaration	Percentage or CGPA
Ph.D	CSIR-Indian Institute of Integrative Medicine, Jammu, India.	20/06/2019	CGPA 8.08
MSc. (Biotechnology)	University of Jammu, India.	01/08/2011	71.87%
BSc. (Chemistry, Zoology, Biotechnology)	University of Jammu, India.	26/07/2009	71.03%
Higher Secondary School Examination (12th)	The Jammu and Kashmir School Board of School Education	16/05/2006	74.16%
Secondary School Examination (10th)	The Jammu and Kashmir School Board of School Education	11/06/2004	88.80%

Publications with citations

1. **Bhat, R.**, Kaur, T., Khajuria, M., Vyas, R., & Vyas, D. (2015). Purification and Characterization of a Novel Redox-Regulated Isoform of Myrosinase (β -Thioglucoside Glucohydrolase) from *Lepidium latifolium* L. *Journal of agricultural and food chemistry*, 63(47), 10218-10226. (Cited by 7)
2. Kaur, T., **Bhat, R.**, Khajuria, M., Vyas, R., Kumari, A., Nadda, G., ... & Vyas, D. (2016). Dynamics of glucosinolate-myrosinase system during *Plutella xylostella* interaction to a novel host *Lepidium latifolium* L. *Plant Science*, 250, 1-9. (Cited by 2)

3. Bhat, H. A., Kaur, T., **Bhat, R.**, & Vyas, D. (2016). Physiological and biochemical plasticity of *Lepidium latifolium* as 'sleeper weed' in Western Himalayas. *Physiologia plantarum*, 156(3), 278-293. (Cited by 10)
4. Kaur, T., **Bhat, R.**, & Vyas, D. (2016). Effect of contrasting climates on antioxidant and bioactive constituents in five medicinal herbs in Western Himalayas. *Journal of Mountain Science*, 13(3), 484-492. (Cited by 4)
5. Kaur, T., Bhat, H. A., **Bhat, R.**, Kumar, A., Bindu, K., Koul, S., & Vyas, D. (2015). Physio-chemical and antioxidant profiling of *Salvia sclarea* L. at different climates in north-western Himalayas. *Acta physiologiae plantarum*, 37(7), 132. (Cited by 9)
6. Koul, S., Kaur, T., Bhat, R., Bindu, K., Kumar, A., Kitchlu, S., & Vyas, D. (2017). Morpho-chemical characteristics of *Salvia sclarea* L. at two different locations in Jammu and Kashmir. *Research Paper*. 4 (1), 19-26. (Cited by 1)

Review with citations

1. **Bhat, R.**, & Vyas, D. (2019). Myrosinase: insights on structural, catalytic, regulatory, and environmental interactions. *Critical reviews in biotechnology*, 39(4), 508-523. (Cited by 5)

Book chapter

- 1 **Bhat, R.**, Khajuria, M., Mansotra DK. A systematic review on global environmental risks associated with pesticide application in agriculture. In: Contaminants in Agriculture and Environment: Health Risks and Remediation. 2019; 96-110.

Awards

1. Joint CSIR-UGC award for Junior Research Fellowship (JRF)/Eligibility for Lectureship NET (2011, 50th rank) in the subject of Life sciences.

Fellowships

1. From 4/2019 to 1/2020: NCBS Bridging Postdoctoral Fellowship
2. From 02/2014 to 02/2017: CSIR-Senior Research Fellowship
3. From 02/2012 to 02/2014: CSIR-Junior Research Fellowship
4. JNU-CEEB stipend (Jawaharlal Nehru University Combined Entrance Exam) for MSc. Biotechnology, India.

Poster presentation

1. Partial purification and characterization of myrosinase from leaves of *Arabidopsis thaliana* (101st Indian Science Congress, University of Jammu, Jammu, 3-7 February 2014).

Oral presentation

1. Role of sinigrin hydrolysis products on *Pieris brassicae* interactions with pepperweed. (107st Indian Science Congress, University of Agricultural Sciences, Bangalore, 3-7 February 2020).

Hands-on Workshop attended

1. Metabolomics workshop jointly organized by NIPGR and AIRF, JNU, New Delhi, India 18th -20th December 2016.

Manuscripts communicated as first author

1. Sinigrin hydrolysis products play an important role in insect interactions during different developmental stages in *Lepidium latifolium*.
2. Effect of temperature and insect herbivory on the regulation of glucosinolate-myrosinase system.

Supervision

Postgraduate dissertations (6 month) 3

Undergraduate dissertations (2 month) 1

Other information

1. Reviewer of journal “World Journal of Biochemistry and Molecular Biology” published by The American Association for Science and Technology (AASCIT).
2. Appointed as evaluator for State Level Camp (Level-II), Jammu & Kashmir of Vidyarthi Vigyan Manthan – India’s Largest Science Talent Search for New India Using Digital Devices (VVM) 2018-19 organized by VIBHA, DST, NCERT and MHRD.
3. Participated in International Conference on Functional & Interaction Proteomics: Application in Food & Health organized by Proteomic Society of India. 14-17th December 2016.
4. Participated in Flower Show 2018 organized by CSIR-IIIM, Jammu on 9 March 2018.
5. Participated in National Seminar cum exhibition on Kisan Mela organized by CSIR-IIIM, Jammu on 13 March 2016.

Research and scientific skills

- Chromatographic techniques- HPLC and affinity chromatography.
- Purification and biochemical characterization of enzymes
- SDS- PAGE, native PAGE and western blotting
- Isolation of DNA, RNA and plasmid.
- PCR and RT-PCR
- Cloning and expression in bacterial system
- *Agrobacterium* mediated transformation
- Transient expression in plant
- Bioinformatic analysis- BLAST, Clustal, phylogenetic tree generation, homology-modelling, active site analysis etc.
- Choice and no-choice insect assay.

REVIEW ARTICLE



Myrosinase: insights on structural, catalytic, regulatory, and environmental interactions

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ABSTRACT

Glucosinolate–myrosinase is a substrate-enzyme defense mechanism present in Brassica crops. This binary system provides the plant with an efficient system against herbivores and pathogens. For humans, it is well known for its anti-carcinogenic, anti-inflammatory, immunomodulatory, anti-bacterial, cardio-protective, and central nervous system protective activities. Glucosinolate and myrosinase are spatially present in different cells that upon tissue disruption come together and result in the formation of a variety of hydrolysis products with diverse physicochemical and biological properties. The myrosinase-catalyzed reaction starts with cleavage of the thioglucosidic linkage resulting in release of a D-glucose and an unstable thiohydroximate-O-sulfate. The outcome of this thiohydroximate-O-sulfate has been shown to depend on the structure of the glucosinolate side chain, the presence of supplementary proteins known as specifier proteins and/or on the physicochemical condition. Myrosinase was first reported in mustard seed during 1939 as a protein responsible for release of essential oil. Until this date, myrosinases have been characterized from more than 20 species of Brassica, cabbage aphid, and many bacteria residing in the human intestine. All the plant myrosinases are reported to be activated by ascorbic acid while aphid and bacterial myrosinases are found to be either neutral or inhibited. Myrosinase catalyzes hydrolysis of the S-glycosyl bond, O-β glycosyl bond, and O-glycosyl bond. This review summarizes information on myrosinase, an essential component of this binary system, including its structural and molecular properties, mechanism of action, and its regulation and will be beneficial for the research going on the understanding and betterment of the glucosinolate–myrosinase system from an ecological and nutraceutical perspective.

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Glucosinolates; glycoside hydrolase; catalytic mechanism; insect interactions; nutraceutical application

Introduction

The *Brassicaceae* has been the focus of intense research as it includes both commercially important oilseed and vegetable crops. These crops are known for the distinct presence of glucosinolates, an important class of sulfur-containing secondary metabolites. Glucosinolates are a diverse class sharing a common core structure consisting of a β-thioglucose linked by a sulfur atom to a (Z)-N-hydroximosulfate ester, and a variable side chain derived from amino acids [1]. *In situ*, glucosinolates are inactive, their hydrolysis is enzymatically driven by spatially separated endogenous enzymes called myrosinases (β-thioglucoside glucohydrolases) [EC 3.2.1.147] that always coexist in plants containing glucosinolates [2,3]. Upon plant damage mainly by herbivory, glucosinolates are decomposed by myrosinase into a number of physiologically active products,

viz., isothiocyanates, thiocyanates, simple nitriles, and epithionitriles. The outcome of this hydrolysis is dependent on the chemical nature of the side chain of intact glucosinolate, the presence of specifier proteins and cellular factors like pH and the presence of metal ions. Myrosinase belongs to a large family of β-glucosidases and is the only enzymes that catalyze the cleavage of the S-glycosidic bond (present in all glucosinolate) using ascorbate as a cofactor and a reaction mechanism that retains the anomeric configuration at the cleavage. The hydrolysis products of glucosinolates are the major regulators of the plant–insect interactions in the family *Brassicaceae*. These assist in combating the attack of generalist insect herbivores and also acts as a stimulatory cue for feeding and oviposition of specialists [4,5]. This complex is also known for its antifungal, nematicidal, allelopathic, and herbicidal properties, and is even

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 Supplemental data for this article can be accessed [here](#).

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Purification and Characterization of a Novel Redox-Regulated Isoform of Myrosinase (β -Thioglucoside Glucohydrolase) from *Lepidium latifolium* L.

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S Supporting Information

ABSTRACT: Myrosinase (ExPASy entry EC 3.2.1.147) is involved in the hydrolysis of glucosinolates to isothiocyanates, nitriles, and thiocyanates that are responsible for various ecological and health benefits. Myrosinase was purified from the leaves of *Lepidium latifolium*, a high-altitude plant, to homogeneity in a three-step purification process. Purified enzyme exists as dimer in native form (~160 kDa) with a subunit size of ~70 kDa. The enzyme exhibited maximum activity at pH 6.0 and 50 °C. With sinigrin as substrate, the enzyme showed K_m and V_{max} values of $171 \pm 23 \mu M$ and $0.302 \mu mol min^{-1} mg^{-1}$, respectively. The enzyme was found to be redox-regulated, with an increase in V_{max} and K_{cat} in the presence of GSH. Reduced forms of the enzyme were found to be more active. This thiol-regulated kinetic behavior of myrosinase signifies enzyme's strategy to fine-tune its activity in different redox environments, thus regulating its biological effects.

KEYWORDS: myrosinase, enzyme purification, enzyme kinetics, redox regulation, glutathione

INTRODUCTION

Glucosinolates are nitrogen- and sulfur-containing plant secondary metabolites present in the family Brassicaceae, of which more than 120 different glucosinolates have been identified.¹ Because of their occurrence in important crops such as cabbage, broccoli, oilseed rape (canola), and the model plant *Arabidopsis*, they are a well-studied class of plant secondary metabolites. Glucosinolates by themselves have little biological activity, but upon plant damage, they are hydrolyzed by myrosinases (β -thioglucosidase glucohydrolase, ExPASy entry EC 3.2.1.147) to form a variety of hydrolysis products, including isothiocyanates, nitriles, epithionitriles, and thiocyanates.² These hydrolysis products are responsible for "mustard bomb": the typical taste and smell of cruciferous vegetables. Glucosinolates and their degradation products, mainly isothiocyanates, have been found to give protection against cancer, cardiovascular and central nervous system diseases, diabetic nephropathy and neuropathy, skin integrity, and *Helicobacter pylori* infection.³ Apart from their health benefits, these degradation products are well-known for their ecological importance in plant–insect and plant–microbe interactions.^{4,5}

Glucosinolate hydrolysis is avoided in situ because glucosinolates and myrosinase are present in different tissues or cellular compartments. Myrosinases are members of the family 1 O-glycoside hydrolase superfamily⁶ with different degrees of glycosylation. They have been reported to be composed of two identical subunits ranging from 60 to 75 kDa with apparent molecular masses of 135–150 kDa.⁷ However, different studies have shown that they can form high-molecular-weight complexes with myrosinase-binding proteins.^{7–9} 3D structural analysis of various myrosinases on the basis of the crystal structure of *Sinapis alba* myrosinase has suggested that the protein folds into a $(\beta/\alpha)_8$ -barrel structure.^{7,10} The active

site has a hydrophobic pocket for binding the variable but generally hydrophobic side chain of glucosinolates and includes specific amino acids that participate in catalysis or interact with the ascorbate cofactor or the glucose or sulfate moieties of glucosinolates. To date several myrosinases from various Brassicaceae members have been purified, including *Brassica napus*,^{11,12} *Lepidium sativum*,¹³ *Sinapis alba*,^{14–16} *Raphanus sativus*,^{17,18} *Armoracia rusticana*,¹⁹ *Crambe abyssinica*,²⁰ and *Brassica oleracea*.²¹ Their characterization has led to a deeper understanding of the glucosinolate–myrosinase system in plants, including activation of myrosinase by ascorbic acid and their specificities with various glucosinolates.

Lepidium latifolium, also known as perennial pepperweed, is an ecologically important plant²² that has attracted the attention of ecologists after been recognized as noxious weed along the western coast of North America.²³ However, the Western Himalayan ecotype of this plant is used as phytofood,²⁴ and several stress-responsive genes have recently been isolated from this ecotype.^{25,26} It has also been shown to be a rich source of sinigrin²⁴ and possesses a very efficient glutathione-mediated redox mechanism.²⁷ The role of myrosinase in thiol-based signaling in *Brassica napus* guard cells²⁸ and allyl isothiocyanate in *Arabidopsis* stomatal closure²⁹ have been recently deciphered. The present study was therefore envisaged to purify and characterize myrosinase from this Himalayan ecotype of *L. latifolium*. We hypothesize a responsive glucosinolate–myrosinase system in this plant owing to its high glucosinolate content.

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Dynamics of glucosinolate-myrosinase system during *Plutella xylostella* interaction to a novel host *Lepidium latifolium* L.

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ABSTRACT

Plutella xylostella L. is a notorious pest of cruciferous crops causing worldwide losses of \$4–5 billion per year. Developing classical biological control to this pest include an introduction of host plants that act as natural enemies showing deviation from the preference-performance regimen in the evolutionary ecology of plant-insect interactions. The present study was designed to understand the role of glucosinolate-myrosinase system during *P. xylostella* interactions with a novel host. Adult moth preference and larval performance study were conducted on a novel host *Lepidium latifolium* L. (LL) that has high sinigrin content and was compared with its laboratory host *Arabidopsis thaliana* (AT). The glucosinolate-myrosinase system was studied in a time course experiment during larval feeding in choice and no-choice experiments. Adult moths visit and prefers LL over AT for oviposition. Conversely, LL leaves were not preferred and proved detrimental for *P. xylostella* larvae. Aliphatic and indolic glucosinolates were found to decrease significantly ($p \leq 0.05$) in AT during initial 12 h of *P. xylostella* challenge, whereas, they were not affected in LL. Also, MYB transcription factor expression and myrosinase activity in LL do not suggest a typical host response to a specialist insect. This preference-performance mismatch of *P. xylostella* on LL mediated by glucosinolate pattern suggests that this novel plant could be utilized in *P. xylostella* management.

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1. Introduction


Plutella xylostella L., diamondback moth (Lepidoptera: Plutellidae), is an important pest of cruciferous crops world over. The high reproductive capacity, dispersal ability, and wide range of host plants allow *P. xylostella* to proliferate wherever cruciferous crops are grown [1]. Because of its ability to rapidly evolve resistance to many synthetic insecticides, *P. xylostella* is considered extremely difficult to control [2], resulting in the worldwide cost of losses of \$4–5 billion per year [3]. Losses occur directly through consumption and contamination of the harvested crop or indirectly through expensive control measures. Female moths have shown strong preferences for cultivated Brassicaceae [4] and certain cultivars within species [5]. Therefore, knowledge of its host recognition


mechanism could lead to the development of effective resistance against this pest in commercially important crops.



Adult moths of *P. xylostella* utilize an integration of chemical and morphological cues, including glucosinolates, cardenolides, plant volatiles, waxes, as well as host plants nutritional quality, leaf morphology, and leaf color, for host plant recognition [1,6]. Of these, the role of glucosinolates and their hydrolysis products for the *P. xylostella* oviposition has been studied extensively [7–11]. There are varying reports on the use of various glucosinolates as an attractant for *P. xylostella* oviposition. Non-volatile indolic and volatile aliphatic glucosinolates play important roles as host recognition cues [9]. Earlier, sinigrin and glucobrassicin or their metabolites were reported to have a stimulatory effect on oviposition [12–14]. It is suggested that sinigrin is spontaneously degraded, and allyl isothiocyanate thus produced gets adsorbed onto the wax of the leaves that would be released slowly over time. Other isothiocyanates, iberin and sulforaphane, which are the hydrolysis products of aliphatic glucosinolates were also found to stimulate *P. xylostella* oviposition [11].

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Effect of contrasting climates on antioxidant and bioactive constituents in five medicinal herbs in Western Himalayas

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Abstract: To understand the effect of climate change on constitutive antioxidant and biochemical metabolites in Western Himalayas, five medicinal herbs were selected and grown at two altitudes in Jammu (305 m) and Srinagar (1730 m) with subtropical and temperate climates, respectively. Significant variations were observed in phenols and flavonoids in *Hypericum perforatum* L., *Matricaria chamomilla* L., *Thymus vulgaris* L., *Cynara cardunculus* L. and *Echinacea purpurea* L. growing at two locations. High altitude temperate site show variable (up to 13 fold) increase in their content. Proteins (1.3 - 1.8 times), sugars (2.8 - 4.1 times) and free amino acid (1.04 - 1.22 times) were also higher at Srinagar (1730 m). Within these plants, *H. perforatum* and *M. chamomilla* have shown higher accumulation of phenols, xanthophylls and proline even at subtropical environment in Jammu (305 m) suggesting potential for increasing their geographical area. The results demonstrate that changing environmental conditions significantly affect the bioactive constituents, which accumulate as a defence strategy by these temperate plants. Their medicinal significance during climate change scenario has also been discussed.

Keywords: Medicinal herbs; Flavonoids; Phenols; Glutathione; Western Himalayas; Climate change

Introduction

Plants contain an enormous variety of chemical compounds known as secondary metabolites that are used for specific odours, tastes and colours. Most of the medicinal properties of plants have been attributed to these compounds and have been utilized by the human race from time immemorial. However, these compounds help plants respond to environmental stimuli in a rapid, reversible and ecologically meaningful manner and thus, plays crucial role in existence of plants in any environment (Metlen et al. 2009). In other words, the environmental factors play an important role in regulating the metabolic content of these bioactive molecules, and dynamic response of these compounds is one of the factors defining plant's adaptation strategy (Silvertown 1998; Tuteja and Sopory 2008). Being sessile, one of the most challenging tasks for plants is to combat the unfavorable conditions that can alter or reduce qualitative and quantitative yield. These molecules such as phenols, flavonoids, anthocyanin, carotenoids, non-protein amino acids together with low molecular weight molecules such as glutathione and ascorbate help the plant to combat stress by acting as scavengers for reactive oxygen species (ROS) or by regulating its cellular redox

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Physiological and biochemical plasticity of *Lepidium latifolium* as ‘sleeper weed’ in Western Himalayas

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To understand the spread of native populations of *Lepidium latifolium* growing in different altitudes in Ladakh region of Western Himalayas, photosynthetic and fluorescence characteristics were evaluated in relation to their micro-environment. Three sites representing sparsely populated (SPS), moderately populated (MPS) and densely populated site (DPS) were selected. Results showed that the DPS had higher photosynthetic accumulation than MPS and SPS. The higher transpiration rate at DPS despite lower vapor pressure deficit and higher relative humidity suggest the regulation of its leaf temperature by evaporative cooling. Intrinsic soil parameters such as water holding capacity and nutrient availability also play crucial role in higher biomass here. The quantum efficiency of PSII photochemistry (F_v/F_m , non-photochemical quenching (NPQ), Φ_{PSII}) and light curve at various PPFDs suggests better light harvesting potential and light compensation point at DPS than the other two sites. Concomitantly, plants at SPS had significantly higher lipid peroxidation, suggesting a stressful environment, and higher induction of antioxidative enzymes. Metabolic content of reduced glutathione also suggests an efficient mechanism in DPS and MPS than SPS. High light intensities at MPS are managed by specialized contrive of carotenoid pigments and *PsbS* gene product. Large pool of violaxanthin and lutein plays an important role in this response. It is suggested that *L. latifolium* is present as ‘sleeper weed’ that has inherent biochemical plasticity involving multiple processes in Western Himalayas. Its potential spread is linked to site-specific micro-environment, whereby, it prefers flat valley bottoms with alluvial fills having high water availability, and has little or no altitudinal effect.

[†]Both authors have contributed equally to this work.

Abbreviations – APX, ascorbate peroxidase; Ax, antheraxanthin; Car, carotenoid; CAT, catalase; Chl, total chlorophyll; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; DEPS, de-epoxidation state; DPS, densely populated site; EDTA-Na, ethylenediaminetetraacetic acid disodium salt; ETR, electron transport rate of PSII; GR, glutathione reductase; g_s , stomatal conductance; GSH, reduced glutathione; GSSG, glutathione disulphide; Lut, Lutein; MPS, moderately populated site; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitro blue tetrazolium; NPQ, non-photochemical quenching; Nx, neoxanthin; PPFD, photosynthetic photon flux density; PSII, photosystem II; RH, relative humidity; ROS, reactive oxygen species; SOD, superoxide dismutase; SPS, sparsely populated site; TBA, thiobarbituric acid; VDE, violaxanthin de-epoxidase; VpdL, vapor pressure deficit; Vx, violaxanthin; WUE_i, intrinsic water use efficiency; Xc, total xanthophylls; Xz, zeaxanthin; β -Caro, β -carotene; Φ_i , quantum yield at a particular value of *i*; Φ_{PSII} , efficiency photosystem II.