Curriculum Vitae

Dr. Aparupa Bose Mazumdar Ghosh, M.Sc., Ph.D.

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Previous Positions:

- Post-Doctoral Research Associate at Kusuma School of Biological Sciences, IIT-Delhi (October 2017 to January 2018)

Education:

- Ph.D. as DST-INSPIRE Fellow, in Life Science & Biotechnology from CSIR-Indian Institute of Chemical Biology (CSIR-IICB), Kolkata and degree awarded by Jadavpur University in 2017
- M.Sc. in Plant Physiology, Agriculture from University of Calcutta (Ballygunge Science College) in 2009 (First Class First and Gold Medalist)
- B.Sc. in Botany Honours from University of Calcutta in 2007 (First rank holder in College Department)
- Higher Secondary Examination (12^{th} Board) from Carmel High School in 2003 under West Bengal Council of Higher Secondary Education
- Madhaymik Pariksha (10^{th} Board) from Carmel High School) in 2001 under West Bengal Board of Secondary Education

Additional Qualification/Certifications:

- Certificate Course on "**General Course on Intellectual Property"** from **World Intellectual Property Organization (WIPO), Geneva, Switzerland**; passed with 80%
- Certificate Course on "**Intellectual Property Rights and Information Technology in the Internet Age"** from **Indian Law Institute (ILI, New Delhi);** passed with **A+** grade

Honours, Awards & Fellowships:

- Awarded DST-INSPIRE Fellowship (NET equivalent) for Doctoral studies (Ph.D.) by Department of Science & Technology (DST); Ministry of Science & Technology, Government of India
- Awarded Gold Medal in Post-Graduation (M.Sc.) for securing 1st class 1st position in University of Calcutta Department

Research Experience & Interests: Life Science & Biotechnology field including both wet lab and dry lab studies. Hands-on experience in plant tissue culture techniques, molecular, transcriptomic, proteomic and bioinformatics–based studies. Interested in developing a career combining both research and teaching in the field of Biosciences

Research Publications:

1. Sequencing, *de novo* assembly, functional annotation and analysis of *Phyllanthus amarus* leaf transcriptome using the Illumina platform. **Aparupa Bose Mazumdar**, Sharmila Chattopadhyay. 2016. ***Frontiers in Plant Science*** 6:1199. doi: 10.3389/fpls.2015.01199

2. Integrated transcriptomic and proteomic analysis of *Arabidopsis thaliana* exposed to glutathione unravels its role in plant defense. Ragini Sinha, Deepak Kumar, Riddhi Datta, Saptarshi Hazra, Dipto Bhattacharyya, **Aparupa Bose Mazumdar**, Ria Mukhopadhyay, Asma Sultana, Sharmila Chattopadhyay. 2015. ***Plant Cell, Tissue and Organ Culture*** 120(3): 975-988. doi: 10.1007/s11240-014-0651-9

3. Establishment of cDNA Library and EST Analysis from Leaves of *Phyllanthus amarus*. **Aparupa Bose Mazumdar Ghosh** and Sharmila Chattopadhyay*. ***International Journal of Biochemistry Research & Review*** 2014. 4(1):1-15. doi:10.9734/IJBCRR/2014/5262

4. Multistep involvement of glutathione with salicylic acid and ethylene to combat environmental stress. Srijani Ghanta, Riddhi Datta, Dipto Bhattacharyya, Ragini Sinha, Deepak Kumar, Saptarshi Hazra, **Aparupa Bose Mazumdar**, Sharmila Chattopadhyay. ***Journal of Plant Physiology*** 2014; 171(11): 940–950. doi:10.1016/j.jplph.2014.03.002

5. Proteomic profiling of γ-ECS overexpressed transgenic *Nicotiana* in response to drought stress. Deepak Kumar, Riddhi Datta, Ragini Sinha, **Aparupa Ghosh**, Sharmila Chattopadhyay. ***Plant signaling & behavior*** 2014. 20(9): e29246. doi:10.4161/psb.29246

6. Leaf proteome profiling of transgenic mint infected with *Alternaria alternata*. Ragini Sinha, Dipto Bhattacharyya, **Aparupa Bose Majumdar**, Riddhi Datta, Saptarshi Hazra, Sharmila Chattopadhyay. ***Journal of Proteomics*** 2013. 93(20):117–132. doi:10.1016/j.jprot.2013.01.02

NCBI GenBank Submissions:

- SRA data submission: **Bose Mazumdar, A** and Chattopadhyay, S. Bioproject: *De novo* sequencing and analysis of *Phyllanthus amarus* leaf transcriptome under Accession PRJNA248079 and ID 248079, May 2014
- **Bose Mazumdar, A.**, Bhattacharyya, D., Chattopadhyay, S. EST analysis from cDNA library of *Phyllanthus amarus* under the accession numbers JK492908 to JK492964, September 2011

Project during Post-Doctoral Research Associateship:

"Study of novel membrane active peptides from marine organisms and their applications in human theragnostics" (Funded by DBT, Ministry of Science &Technology, Government of India

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Ph.D thesis title:

"Molecular study and in-depth transcriptome analysis of *Phyllanthus amarus* leaves identifying lignans and other secondary metabolites biosynthetic pathway gene/s"

Dissertation & Projects during M.Sc.

- Seminar and project presentation on "Biofuel: Prospect in India"
- Dissertation title submitted for M.Sc: "Effect of Fluoride on seed germination and seedling growth of green gram (*Vigna radiata* L.Wilczek)

Professional Activities

- **Professional training:** Attended a Winter School workshop at the Presidency College (now Presidency University), Kolkata on *"Plant Chromosome Techniques and Biometry"* in 2006
- **Professional Memberships:** Life Member of "The Society of Biological Chemists (India)"; Indian Institute of Science (IISc), Bangalore

Presentations at Conferences/Symposiums/Seminars

2017:

- ***38th Annual Meeting of the Plant Tissue Culture Association (India) and a National symposium on the theme, 'Plant Biotechnology: Current Perspectives on Medicinal and Crop Plants'*** held at **CSIR-Indian Institute of Chemical Biology (CSIR-IICB), Kolkata, March 3rd-5th:** Poster presentation entitled "Leaf transcriptome study of hepatoprotective herb *Phyllanthus amarus* gaining a deeper insight on its secondary metabolites" by Aparupa Bose Mazumdar Ghosh and Sharmila Chattopadhyay

2014:

- Invited and participated in ***National Education Summit*** in Gandhinagar, Gujarat on 10 & 11th January
- ***International Symposium- Trends in Plant Science Research*** held at **University of Calcutta, Kolkata, February 15-16:** Poster presentation entitled "Molecular analysis

of a potential medicinal herb- *Phyllanthus amarus*" by Aparupa Bose Mazumdar (Ghosh), Mehar D Kalim and Sharmila Chattopadhyay

- ***2nd International Meet on Advanced Studies on Cell Signaling Network (CeSiN*** **Kolkata, 13th to 15th December:** Poster presentation entitled "Molecular analysis of hepatoprotective herb *Phyllanthus amarus* to identify lignan biosynthetic pathway gene/s" by Aparupa Bose Mazumdar, Mehar D Kalim, and Sharmila Chattopadhyay

2012:

- ***81st Annual Meeting of the Society of Biological Chemists (India) & Symposium on Chemistry & Biology: Two Weapons Against Diseases*** **held at Science City, Kolkata, November 8-11:** Poster presentation entitled "Molecular analysis of a hepatoprotective plant- *Phyllanthus amarus*" by Aparupa Bose Mazumdar and Sharmila Chattopadhyay

2011:

- ***18th West Bengal State Science & Technology Congress*** **held at Ramkrishna Mission Residential College, Narendrapur, Kolkata (28th February– 1st March):** Oral presentation entitled "Transgenic Tobacco Overexpressing γ- ECS Exhibits Disease Resistance" by Srijani Ghanta, Aparupa Bose Mazumdar, Dipto Bhattacharyya, Ragini Sinha and Sharmila Chattopadhyay

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Social Work:

Awarded the Certificate of Social Service by the Apostolic Carmel Educational Society for creating awareness and assisting in raising funds for the eradication of illiteracy, irrespective of race, religion, caste or creed

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Extracurricular Activities:

- Painting (Final Year completed from Bangiya Sangeet Parishad, Ankan Kala Bibhag)
- Singing (trained in Classical from ITC Sangeet Research Academy, Kolkata under Late Pandit A.T.Kanan)

- Dancing (trained in Bharatnatyam from Kalamandalam, Kolkata under Thankumuni Kutty

Achievements:

- Awarded the first prize in Rabindra Sangeet competiton in 1998 conducted by Aabriti Sansad (affiliated to S & R Council) held at Bhilai, Chhattisgarh
- Awarded the second prize in Adhunik song competition in the same above mentioned year and place
- Awarded the second prize in Nazrul geeti song competition in the same above mentioned year and place

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frontiers in Plant Science

ORIGINAL RESEARCH
published: 28 January 2016
doi: 10.3389/fpls.2015.01199

Sequencing, *De novo* Assembly, Functional Annotation and Analysis of *Phyllanthus amarus* Leaf Transcriptome Using the Illumina Platform

*Aparupa Bose Mazumdar and Sharmila Chattopadhyay **

Organic and Medicinal Chemistry Division, Plant Biology Laboratory, Council for Scientific and Industrial Research-Indian Institute of Chemical Biology, Kolkata, India

Phyllanthus amarus Schum. and Thonn., a widely distributed annual medicinal herb has a long history of use in the traditional system of medicine for over 2000 years. However, the lack of genomic data for *P. amarus*, a non-model organism hinders research at the molecular level. In the present study, high-throughput sequencing technology has been employed to enhance better understanding of this herb and provide comprehensive genomic information for future work. Here *P. amarus* leaf transcriptome was sequenced using the Illumina Miseq platform. We assembled 85,927 non-redundant (nr) "unitranscript" sequences with an average length of 1548 bp, from 18,060,997 raw reads. Sequence similarity analyses and annotation of these unitranscripts were performed against databases like green plants nr protein database, Gene Ontology (GO), Clusters of Orthologous Groups (COG), PlnTFDB, KEGG databases. As a result, 69,394 GO terms, 583 enzyme codes (EC), 134 KEGG maps, and 59 Transcription Factor (TF) families were generated. Functional and comparative analyses of assembled unitranscripts were also performed with the most closely related species like *Populus trichocarpa* and *Ricinus communis* using TRAPID. KEGG analysis showed that a number of assembled unitranscripts were involved in secondary metabolites, mainly phenylpropanoid, flavonoid, terpenoids, alkaloids, and lignan biosynthetic pathways that have significant medicinal attributes. Further, Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values of the identified secondary metabolite pathway genes were determined and Reverse Transcription PCR (RT-PCR) of a few of these genes were performed to validate the *de novo* assembled leaf transcriptome dataset. In addition 65,273 simple sequence repeats (SSRs) were also identified. To the best of our knowledge, this is the first transcriptomic dataset of *P. amarus* till date. Our study provides the largest genetic resource that will lead to drug development and pave the way in deciphering various secondary metabolite biosynthetic pathways in *P. amarus*, especially those conferring the medicinal attributes of this potent herb.

Keywords: *Phyllanthus amarus*, next-generation sequencing (NGS), Illumina Miseq, leaf transcriptome, *de novo* assembly, functional annotation, secondary metabolism

Edited by:
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Specialty section:
This article was submitted to Plant Metabolism and Chemodiversity, a section of the journal Frontiers in Plant Science

Received: *13 August 2015*
Accepted: *14 December 2015*
Published: *28 January 2016*

Citation:
Bose Mazumdar A and Chattopadhyay S (2016) Sequencing, De novo Assembly, Functional Annotation and Analysis of Phyllanthus amarus Leaf Transcriptome Using the Illumina Platform. Front. Plant Sci. 6:1199. doi: 10.3389/fpls.2015.01199

Plant Cell Tiss Organ Cult (2015) 120:975–988
DOI 10.1007/s11240-014-0651-9

ORIGINAL PAPER

Integrated transcriptomic and proteomic analysis of *Arabidopsis thaliana* exposed to glutathione unravels its role in plant defense

Ragini Sinha · Deepak Kumar · Riddhi Datta · Saptarshi Hazra · Dipto Bhattacharyya · Aparupa Bose Mazumdar · Ria Mukhopadhyay · Asma Sultana · Sharmila Chattopadhyay

Received: 1 April 2014 / Accepted: 25 October 2014 / Published online: 6 November 2014

Abstract The role of glutathione (GSH) in plant defense has long been known in addition to its substantial role in stress tolerance and antioxidant signalling. In this study, molecular analysis of GSH fed *Arabidopsis thaliana*, exhibiting enhanced GSH content and stress tolerance potential, was performed to explore the intricate position of GSH in the plant defense signaling network. Microarray data revealed the differential regulation of 653 transcripts of which 379 were upregulated and 274 were downregulated by 2-fold or more ($p < 0.05$). Gene enrichment and KEGG database analysis identified glucosinolate (GLS), a plant defense compound, and tryptophan biosynthetic pathways as specifically enriched by GSH. Interestingly, upregulation of genes related to biosynthesis was also observed under enhanced GSH condition. Functional annotation noted upregulation of biotic stress related and ethylene (ET)-related genes like 1-*aminocyclopropane carboxylate synthase* 2 at transcript level. These data were supported by the up-accumulation of ACC oxidase at proteomics level signifying the interplay between GSH and ET in defense signaling pathway. Differential expression of salicylic acid (SA)-mediated signaling genes direct the involvement of GSH with SA. Our proteomic analysis also identified the upregulation of stress and defense related proteins. The effect of GSH on GLS biosynthetic pathways as observed here might be an important information linking GSH to GLS mediated defense. Together, this investigation reveals the association of GSH with tryptophan, lignin and GLS in addition to SA and ET, in plant defense.

Keywords GSH · *Arabidopsis thaliana* · Microarray · Proteomics

Abbreviations

ABA	Abscisic acid
ACC 1	Aminocyclopropane carboxylic acid
ADC2	*Arginine decarboxylase* 2
AIG1	*AVRRPT2-induced gene* 1
APX1	*Ascorbate peroxidase* 1
ASA1	*Anthranilate synthase alpha subunit* 1
*CYP*79B2	*Cytochrome P450* 79*B2*
DAVID	Database for Annotation, Visualization and Integrated Discovery
ERD5	*Proline dehydrogenase*
ET	Ethylene
GLS	Glucosinolate
GPX	Glutathione peroxidase
Grxs	Glutaredoxins
GSH	Glutathione
GSSG	Oxidised glutathione
GST	Glutathione-S-transferase
*GSTU*3	*Glutathione-S-transferase TAU* 3
HPLC	High performance liquid chromatography
HSP	*Heat shock protein*
JA	Jasmonate
KEGG	Kyoto Encyclopedia of Genes and Genomes

Ragini Sinha and Deepak Kumar have contributed equally to this article.

Electronic supplementary material The online version of this article (doi:10.1007/s11240-014-0651-9) contains supplementary material, which is available to authorized users.

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International Journal of Biochemistry Research & Review
4(1): 1-15, 2014

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Establishment of cDNA Library and EST Analysis from Leaves of *Phyllanthus amarus*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study and finally prepared the corrected version of the manuscript. Author ABMG performed the experiment and statistical analysis, literature searches, wrote the protocol, and the first draft of the manuscript. Both authors read and approved the final manuscript.

Research Article

Received 12th June 2013
Accepted 1st September 2013
Published 9th October 2013

ABSTRACT

DNA sequencing of randomly chosen clones from a cDNA library allows thousands of different transcripts to be identified. However, since the likelihood of observing a given transcript is proportional to the expression level of that transcript in the tissue from which the library is derived, often transcripts are represented by several EST sequences. An expressed sequence tags (EST) analysis was undertaken to identify the genes present in the leaves of *Phyllanthus amarus,* which is a small tropical, glabrous herb with several health benefits. Phyllanthin and hypophyllanthin, major bioactive components, present in highest amounts in the leaves, are of significant therapeutic importance like hepatoprotective, antioxidant, antiviral, hypoglycemic, etc. Taken together, sequencing of cDNA clones generated high-quality ESTs (Accession number: JK492908 to JK492964) with high similarities with genes from *Ricinus communis, Onchocerca volvulus*, *Eucalyptus globules, Gossypium hirsutum, Nicotiana tabacum, Solanum* spp. and many more. A BLASTN analysis along with BLASTX analysis of all the unique sequences was performed and was grouped according to the reported activities. Results represented here is the first reference collection of ESTs from this commercially important medicinal herb. This study indicated that the leaf transcriptome contains series of interesting sequences like ALBINO3, ribulose-1, 5 bisphosphate carboxylase/ oxygenase (RUBISCO), chloroplast

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photosystem II chlorophyll A/B-binding protein, stress-responsive proteins like methionine sulfoxide reductase type, etc.

Keywords: cDNA library; expressed sequence tags (EST); Phyllanthus amarus.

ABBREVIATIONS

EST: Expressed Sequence Tag; NCBI: National Centre for Biotechnology Information BLAST: Basic Local Alignment Search Tool.

1. INTRODUCTION

The plant genus *Phyllanthus* (Euphorbiaceae) is widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into 10 or 11 subgenera. [1,2]. *Phyllanthus amarus* Schum & Thonn. popularly known as bhuiamlaki, is found in different parts of India.

Genus: *Phyllanthus*
Species: *amarus*

This important species of the genus *Phyllanthus* has been assigned common names in different languages in India as bhuiamla and sadahazurmani in Bengali, bhuiavala in Bombay, bhonyaanmali in Gujrati, bhuianvalah in Hindi, etc. [3]. In Spain this plant is best known by the common name chanca piedra, which means stonebreaker [4].

P. amarus has a long history of use as an herbal edible source for the treatment of a broad spectrum of diseases in Brazil, India and other countries [1,3]. Works done by various group of researchers globally have shown the huge potential of this herb in treating multi-faceted diseases like jaundice, hepatitis B, HIV, Herpex Simplex virus, cancer, diabetes, inflammation, oxidative stress, etc. [5-9]. The whole plant is used in gonorrhea, menorrhagia and other genital affections. It is useful in gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds. It also has antimutagenic activities [10], antinociceptive activities [11] and antilipidemic potentials [12,13]. The organic compounds of medicinal interest present in this herb include lignans – phyllanthin and hypophyllanthin [14,15], geraniin and 5 flavanoids -quercetin, astralgin, quercetrin, isoquercetin and rutin [16,4]. It also contains minor compounds like hydrolysable tannins like phyllanthusiin D [17], amariin [16], amarulone [18], amarinic acid and alkaloids like ent-norsecurinine, sobubbialine, epibubbialine [17]; diarylbutane, nyrphyllin, and a neolignan, phyllnirurin besides lactones, steroids, terpenoids, and so forth. The plant is bitter, astringent, cooling, diuretic, febrifuge and antiseptic.

Over the past years, construction of cDNA library and its analysis is considered to be an indispensable tool for functional genomic analysis as it provides much more detailed information on the genomic mechanisms underlying diverse processes of the organism [19]. Thus, cDNA library is a powerful and useful tool in the area of biotechnology. It is helpful in expressing eukaryotic genes in prokaryotes, which in turn helps in the transcription process of prokaryotes; facilitate the study of the repertoire of mRNAs expressed in different cells or tissues, and study of alternative splicing in different cells or tissues; helps in discovery of novel genes and cloning of full-length cDNA molecules for *in vitro* study of gene function.

Journal of Plant Physiology 171 (2014) 940–950

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Functional Biotechnology

Multistep involvement of glutathione with salicylic acid and ethylene to combat environmental stress

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ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form 6 March 2014
Accepted 6 March 2014
Available online 14 March 2014

Keywords:
Defense signaling network
Ethylene
Glutathione
Salicylic acid
Stress

ABSTRACT

The role of glutathione (GSH) in plant defense is an established fact. However, the association of GSH with other established signaling molecules within the defense signaling network remains to be evaluated. Previously we have shown that GSH is involved in defense signaling network likely through NPR1-dependent salicylic acid (SA)-mediated pathway. In this study, to gain further insight, we developed chloroplast-targeted *gamma-glutamylcysteine synthetase* (*γ-ECS*) overexpressed transgenic *Nicotiana tabacum* (*NtGp* line) and constructed a forward subtracted cDNA (suppression subtractive hybridization (SSH)) library using *NtGp* line as a tester. Interestingly, in addition to SA-related transcripts like *pathogenesis-related protein* 1a (*PR*1a) and *SAR*8.2 m/2l, 1-*aminocyclopropane*-1-*carboxylate oxidase* (*ACC oxidase*), a key enzyme of ethylene (ET) biosynthesis, was identified in the SSH library. Besides, transcription factors like *WRKY transcription factor* 3 (*WRKY*3), *WRKY*1 and *ethylene responsive factor* 4 (*ERF*4), associated with SA and ET respectively, were also identified thus suggesting an interplay of GSH with ET and SA. Furthermore, proteomic profiling of *NtGp* line, performed by employing two-dimensional gel electrophoresis (2-DE), corroborated with the transcriptomic profile and several defense-related proteins like serine/threonine protein kinase, and heat shock 70 protein (HSP70) were identified with increased accumulation. Fascinatingly, induction of 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) was also noted thus demonstrating the active involvement of GSH with ET. Protein gel blot analysis confirmed the enhanced accumulation of ACC oxidase in *NtGp* line. Together, our data revealed that GSH is involved in the synergistic multiple steps crosstalk through ET as well as SA to combat environmental stress.

Abbreviations: 2-DE, two-dimensional gel electrophoresis; γ-ECS, gamma-glutamylcysteine synthetase; ACC, 1-aminocyclopropane-1-carboxylate; ACC, oxidase1-aminocyclopropane-1-carboxylate oxidase; ACCsynthase, 1-aminocyclopropane-1-carboxylate synthase; BZI-2, bZIP transcription factor BZI-2; dpi, days post inoculation; ERF4, ethylene responsive factor 4; EST, expressed sequence tag; ET, ethylene; GO, gene ontology; GSH, glutathione; GSSG, oxidized glutathione; HPLC, high performance liquid chromatography; HSP70, heat shock 70 protein; IEF, isoelectric focusing; JA, jasmonic acid; MALDI TOF, matrix assisted laser desorption/ionization time of flight; MS/MS, tandem mass spectrometry; NCBI, National Centre for Biotechnology Information; PCA, principal component analysis; PR1a, pathogenesis-related protein 1a; PR4, Pathogenesis-related protein 4; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; SA, salicylic acid; SAR, systemic acquired resistance; SSH, suppression subtractive hybridization; WRKY, WRKY transcription factor.

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http://dx.doi.org/10.1016/j.jplph.2014.03.002
0176-1617/

RESEARCH PAPER

Plant Signaling & Behavior 9, e29246; May; © 2014 Landes Bioscience

Proteomic profiling of γ-ECS overexpressed transgenic *Nicotiana* in response to drought stress

Deepak Kumar, Riddhi Datta, Ragini Sinha, Aparupa Ghosh, and Sharmila Chattopadhyay*

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Keywords: Drought stress, GSH, mass spectrometry, transgenic *Nicotiana*

The contribution of Glutathione (GSH) in drought stress tolerance is an established fact. However, the proteins which are directly or indirectly related to the increased level of GSH in response to drought stress are yet to be known. To explore this, here, transgenic tobacco plants (*NtGp*11) overexpressing gamma-glutamylcysteine synthetase (γ-*ECS*) was tested for tolerance against drought stress. *NtGp*11 conferred tolerance to drought stress by increased germination rate, water retention, water recovery, chlorophyll, and proline content compared with wild-type plants. Semi-quantitative RT-PCR analysis revealed that the transcript levels of stress-responsive genes were higher in *NtGp*11 compared with wild-type in response to drought stress. Two-dimensional gel electrophoresis (2-DE) coupled with MALDI TOF-TOF MS/MS analysis has been used to identify 43 differentially expressed proteins in response to drought in wild-type and *NtGp*11 plants. The results demonstrated the up-accumulation of 58.1% of proteins among which 36%, 24%, and 20% of them were related to stress and defense, carbon metabolism and energy metabolism categories, respectively. Taken together, our results demonstrated that GSH plays an important role in combating drought stress in plants by inducing stress related genes and proteins like HSP70, chalcone synthase, glutathione peroxidase, thioredoxin peroxidase, ACC oxidase, and heme oxygenase I.

Introduction

Environmental stress is the major limiting factor in plant productivity. Considering agricultural perspectives, drought stress is one of the most significant factors responsible for substantial and unpredictable losses in crop production. Drought, salinity, and osmotic stresses cause adverse effects on the growth and photosynthesis, oxidative damage, hormonal changes, and the accumulation of numerous stress-related proteins. These changes are usually the result of tissue dehydration. Tissue dehydration occurs when there is an imbalance between root water uptake and leaf transpiration.[1] Dehydrated plants generally start closing their stomata; however, under some environmental situations or in specific plant genotypes, modification of root water uptake capacity plays a more important role compared with stomatal closure in avoiding stress-induced growth reduction.[2,3] Again, guard cell signaling is of critical importance because it is a key denominator within the plant water budget in drought stress responses.[4]

Drought stress induces the overproduction of reactive oxygen species (ROS) which are highly reactive and toxic, which must be minimized to protect the cell from oxidative damage. The cell organelles, particularly chloroplast and mitochondria are the major sites of ROS production in plants where excessive rate of electron flow takes place. Plant cells are well equipped to efficiently scavenge ROS and its reaction products by the coordinated and concerted action of antioxidant machinery constituted by vital enzymatic and non-enzymatic antioxidant components.[5,6] Prolonged drought stress results in ROS production in plant cell which overwhelm the scavenging action of the antioxidant system resulting in extensive cellular damage and death.[7] Recently, it is also reported that drought is responsible for the development of oxidative stress in plant cell which induces various associated genes.[8]

Cellular redox homeostasis is essential for plant growth, development as well as for the resistance to biotic and abiotic stresses.[9] Glutathione is a key water-soluble antioxidant and plays a central part in ROS scavenging through the GSH-ascorbate cycle and as an electron donor to glutathione peroxidase (GPx). It is the storage form and the long-distance transport form of reduced sulfur, is involved in the detoxification of heavy metals and xenobiotics and in the regulation of the cell cycle. Previous studies have shown that GSH acts as a signaling molecule and mitigates biotic stress through non-expressor of PR genes 1 (NPR1)-dependent/independent salicylic acid (SA)-mediated pathway.[10-13] In our recent investigation it was also noted that GSH may act through multistep signaling pathways to mitigate environmental stresses.[14] The protective role of GSH against low temperature stress was also demonstrated by chemical or genetic manipulation of its level.[15] Besides an increase in the size of the glutathione pool, a high GSH/GSSG ratio is also necessary for

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Submitted: 03/28/2014; Revised: 05/14/2014; Accepted: 05/15/2014; Published Online: 05/20/2014

Citation: Kumar D, Datta R, Sinha R, Ghosh A, Chattopadhyay S. Proteomic profiling of γ-ECS overexpressed transgenic Nicotiana in response to drought stress. Plant Signaling & Behavior 2014; 9:e29246; PMID: 24844531; http://dx.doi.org/10.4161/psb.29246

JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

Available online at www.sciencedirect.com

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www.elsevier.com/locate/jprot

Leaf proteome profiling of transgenic mint infected with *Alternaria alternata*☆

*Ragini Sinha, Dipto Bhattacharyya, Aparupa Bose Majumdar, Riddhi Datta, Saptarshi Hazra, Sharmila Chattopadhyay**

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ARTICLE INFO

Article history:
Received 21 October 2012
Accepted 20 January 2013

Keywords:
Mint
Transgenic
Environmental stress
Differential proteomics

ABSTRACT

The genus *Mentha* has been widely used in food, flavor, culinary, cosmetic and pharmaceutical industries. Substantial damage to this crop happened regularly due to environmental stresses like metal toxicity and pathogen attack. Here, an approach has been taken to raise transgenic mint over-expressing γ-glutamyl-cysteine synthetase (γ-ECS), the rate-limiting enzyme of GSH biosynthesis, resulted enhanced GSH content and its *in planta* expression confers significant tolerance towards abiotic/biotic stresses viz. metal toxicity — Cd, Zn as well as against infection of *Alternaria alternata* and *Rhizoctonia solani*. A differential proteomic analysis through 2-DE and MALDI TOF–TOF MSMS was performed to focus on the altered abundance of functionally important protein species in control and infected transgenic mint. Results showed a significant variation in the protein profile of the infected transgenic plant as compared to the wild/control transgenic counterpart. In addition to protein species related to stress and defense, redox regulation, transcription factors and energy & metabolism, protein species related to signaling and gene regulation as well as cell division also showed differential accumulation in infected transgenic. Hence, proteomics can be used as a tool to decipher the mechanism of action of GSH in providing tolerance against a necrotrophic fungus, *A. alternata* in transgenic mint.

This article is part of a Special Issue entitled: Translational Plant Proteomics.

1. Introduction

Genus *Mentha* belongs to Labiatae, which is a very large family having annual and perennial herbs of economic importance. This family is well known for their use in food, flavor, culinary, cosmetic and pharmaceutical industries. *Mentha* is an important crop having a significant impact on the global and Indian economy. *Mentha arvensis* is cultivated in a large area in the Indo-Gangetic plains in the states of Punjab, Haryana, Uttarakhand, Uttar Pradesh and Bihar, with a maximum area in Uttar Pradesh [1]. In India, mint is cultivated on approximately 160,000 ha of land with annual production of 16,000 t of oil. Today, India is the major global producer and supplier of mint oil and its derivatives in the world.

A major constraint to *Mentha* production is the disease caused by fungal pathogens such as *Puccinia menthae*, *Verticillium dahliae*, *Verticillium albo-atrum*, *Phoma strasseri*, *Erysiphe cichoracearum*, *Alternaria alternata* and *Rhizoctonia solani* [2–4]. Stolon rot caused by *Rhizoctonia* spp. has been the most serious disease in North Indian plains, likewise the leaf blight caused by *Alternaria* spp. causes a reduction in the essential oil yield from 55 to 75%, and the disease is sometimes associated with a dual infection with

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http://dx.doi.org/10.1016/j.jprot.2013.01.020