APARNA SINGH

POSTDOCTORAL FELLOW

UNIVERSITY OF NEW BRUNSWICK

CANADA

PROFILE

Molecular Biologist with 5+ years of international research experience and in-depth background in molecular biology, cellular biology, genetic engineering, gene discovery, multiomics, plant natural products biosynthesis.

CONTACT

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EXPERIENCE

MITACS ACCELERATE INDUSTRIAL POSTDOCTORAL FELLOW

Department of Chemistry, University of New Brunswick, Canada

JUNE 2021-PRESENT

Biochemical characterization of Perrotettinene biosynthesis pathway in *Radula complanata.*

Key roles

Managed and executed research project. Conference presentations, writing manuscripts, grants and project reports. Developed and optimized multiple molecular biology protocols and bioassays. Trained and mentored undergraduate students. Responsible for hands-on training of laboratory protocols and instruments, including PCR, Agarose gel electrophoresis, SDS-PAGE and western blotting, qPCR and HPLC.

MITACS ACCELERATE INDUSTRIAL POSTDOCTORAL FELLOW

Department of Biological Sciences, University of Lethbridge, Canada

JULY 2019-MARCH 2021

Functional characterization of Cannabidiolic acid synthase (CBDAS) gene variants obtained from in-house Cannabis cultivars to determine THC/CBD ratio.

Advanced Teaching Assistant - Molecular Biology

Key roles

Functional analysis of CBDAS variants extracted from genomic data of more than 25 in-house cannabis cultivars. Analyzing SNPs in CBDAS variants and their effect on CBD content Laboratory management: assisted in setting up a molecular biology lab and played a lead role in purchasing instruments/ reagents, writing SOPs, and developing lab protocols. Worked as an Advanced Teaching Assistant and taught Molecular Biology course and lab course to undergraduate students.

POSTDOCTORAL ASSOCIATE

Department of Biological Sciences at the University of Calgary, Canada

JULY 2018-JUNE 2019

Discovering the role of heterodimeric *O*-methyltransferases (OMTs) in noscapine biosynthesis in *Papaver somniferum.*

TECHNICAL SKILLS

Molecular Biology- RNA and DNA extraction, quantitative Real Time PCR, Gateway cloning, MULTIcoli cloning, Restriction digestion and ligation cloning, protein expression and purification from bacterial and plant system, western blotting, SDS-PAGE, plant tissue culture, geldoc system, spectrophotometer, nanodrop, genetic transformation using *Agrobacterium tumefaciens*, transient expression in *N. benthamiana*.

Bioinformatics- Transcriptome data study, BLAST search, Clustal W, Serial cloner, Expasy, candidate genes identification, identification of top expressed genes, KEGG, MEGA, Snapgene, Addgene, Serial cloner.

Neurobiology- Cell culture of differentiated and non-differentiated neuronal cells, nuclear and cytosolic protein extraction, protein assay, single stranded DNA extraction, LDH assay.

Microbiology- Total coliform determination, *E. coli* determination, microbial characterization of water and soil samples, isolation of plant growth promoting bacteria (P.G.P.R) from rhizosphere. Isolation and Biochemical analysis (enzymatic characterization) of heterodimers OMT2:OMT3 and OMT2:6OMT

Key roles

Conducted integrated transcriptomics and metabolomics analysis. Prepared presentations and lab reports. Assisted lab coworkers in research experiments, data collection and analysis. Participated in lab group discussions. Published an article in a scholarly journal. Provided a biweekly research update.

RESEARCHER

Neuronal and Cellular Biology laboratory, University du Quebec at Trois-Rivieres, Canada

JANUARY 2014- AUGUST 2014

Determining anti-inflammatory and anti-apoptotic effects of natural polyphenols on neuronal cells in context to Parkinson's disease.

Key roles

Neuronal cell growth and maintenance. Developed and maintained databases, pulling relevant data. Collected and analyzed data to contribute to manuscripts and publications. Trained undergraduate students, summer interns and volunteers.

ECOTECHNOLOGIST

Ecomen Laboratories, Lucknow, India

July 2012-DECEMBER 2012

Analysis of water, soil and air samples collected from Industrial sites

Key roles

Worked successfully with diverse group of coworkers to accomplish goals and address issues related to our products and services. Worked closely with team members to deliver project requirements, develop solutions and meet deadlines. Prioritized and organized tasks to efficiently accomplish service goals. Demonstrated leadership by making improvements to work processes and helping to train others. Provided excellent service and attention to customers when face-to-face or through phone conversations.

identification of bacteria and fungi from air, water and soil. Chemical and microbiological analysis of wastewater, drinking water and soil samples collected from industrial sites.

Analytical Chemistry- Alkaloid extraction, TLC with different coloration tests (Dragondorff, FeCl3, potassium permanganate), HPLC (Waters, Shimadzu with PDA detector, Agilent) and LC-MS/MS (Waters HPLC coupled to Micromass Quattro LC), enzyme assays.

TEACHING EXPERIENCE

Advanced Teaching Assistant
Molecular Biology.
Department of Biological Sciences, University of Lethbridge, Canada

INTERNSHIPS

"Food Preservation and Management"
Institute of Entrepreneurship and Development, India
(December 2011 - February 2012)

"Microbial Techniques – Learning to Handle Microbes"
Institute of Entrepreneurship and Development, India
(June 2009 - July 2009)

EDUCATION

PH.D. (2014-2018)

Cellular and Molecular Biology
University du Quebec at Trois-Rivieres, Quebec, Canada

THESIS TITLE

"STUDY OF PRECURSOR GENES INVOLVED IN BIOSYNTHESIS PATHWAY OF AMARYLLIDACEAE ALKALOIDS USING INTEGRATED TRANSCRIPTOMICS AND METABOLOMICS APPROACH"

RESEARCH EXPERIENCE

Functional characterization of a candidate gene involved in Amaryllidaceae Alkaloids (AAs) biosynthesis and discovering other precursor genes of the pathway in *Narcissus pseudonarcisssus* 'King Alfred' using integrated transcriptomics and metabolomics. Developed a deep understanding of AAs metabolism by comparative transcriptome and metabolome study. For this, metabolic profiling (TLC, HPLC, and LC-MS/MS) of different tissue extracts was performed and transcriptome database was established of *Narcissus pseudonarcisssus* 'King Alfred' through HI-seq Illumina sequencing to identify potential genes responsible for AAs formation.

MASTERS (2008-2010)

M.Sc. in Biotechnology
Babasaheb Bhimrao Ambedkar University, India

DISSERTATION

"MOLECULAR ANALYSIS OF TRANSGENIC *NICOTIANA TABACUM* & GENETIC TRANSFORMATION OF *WITHANIA SOMNIFERA* WITH *SGT L4* GENE FOR OVEREXPRESSION USING *AGROBACTERIUM TUMEFACIENS*"

BACHELORS (2005-2008)

B.Sc. in Biotechnology
Chhatrapati Shahuji Maharaj University, Kanpur, India

PUBLICATIONS

Google scholar link (*https://scholar.google.com/citations?hl=en&user=TIL_zyAAAAAJ*)

- Laurence Tousignant[1], Aracely Maribel Diaz Garza[1], Bharat Bhusan Majhi[1], **Aparna Singh[1],** Isabel Desgagne-Penix[1,2] **(2021)** "Transcriptome analysis of *Leucojum aestivum* and identification of genes involved in norbelladine formation" **(Accepted-Planta)**
- **Singh A.**, Bilichak A., and Kovalchuk I. **(2020)** "The genetics of Cannabis – genomic variations of key synthases and their effect on cannabinoids content" **(Published- Genome)**
- **Aparna Singh.**, Ivette Menendez-Perdomo, Peter J Facchini, **(2019)** 'Benzylisoquinoline alkaloid biosynthesis in opium poppy – an update' **(Published-Phytochemistry Reviews)**
- **Singh A.**, Marie-Ange massicotte, Ariane Garand, Laurence Tousignant, Vincent Ouellette, Gervais Bérubé and Desgagné-Penix I. **(2018)** Cloning and characterization of norbelladine synthase catalyzing the first committed reaction in Amaryllidaceae alkaloid biosynthesis **(Published – BMC Plant Biology)**
- **Singh A.**, and Desgagné-Penix I. **(2017)** Transcriptome and metabolome profiling of *Narcissus pseudonarcissus* 'King Alfred' reveal components of Amaryllidaceae alkaloid metabolism. **(Published- Scientific Reports)**
- **Singh A.**, and Desgagné-Penix I. **(2015)** Chapter 3 Biosynthesis of Amaryllidaceae alkaloids: A biochemical outlook. **(Published - *Alkaloids: Biosynthesis, Biological Roles and Health benefits*, pp. 53-76. Nova Science Publishers, Editor - Eduardo Sobarzo-Sanchez)**
- **Singh A.**, and Desgagné-Penix I**. (2014)** Biosynthesis of the Amaryllidaceae alkaloids. **(Published-Plant Science Today)**

AWARDS, FELLOWSHIPS AND MEMBERSHIP

- June 2021-Present – Received ***MITACS Accelerate Industrial Postdoctoral Fellowship*** at University of New Brunswick
- July 2019-January 2021-Received ***MITACS Accelerate Industrial Postdoctoral Fellowship*** at University of Lethbridge
- July 2018-June 2019- Received ***NSERC Postdoctoral Fellowship*** at University of Calgary
- June 2016 – Awarded ***Canadian Association of Plant Biology (CAPB) Travel Award*** at CAPB Meeting
- June 2016- Received ***Centre SEVE Travel Scholarship*** from Centre SEVE
- September 2014-May 2018 Received ***NSERC PhD Fellowship*** at University du Quebec Trois-Rivieres
- Membership of the ***Canadian Society of Plant Biologists***

CONFERENCE PRESENTATIONS

- University du Quebec at Trois-Rivieres, 2018
  "Update on study of precursor genes involved in Amaryllidaceae alkaloid metabolism"
- Canadian Society of Plant Biologist Meeting at McGill University, 2017
  "Norbelladine Synthase – A novel enzyme involved in Amaryllidaceae alkaloid metabolism in

Narcissus pseudonarcissus cv. 'King Alfred"

- Centre SEVE Annual Meeting in Bromont, 2016
  "Understanding galanthamine metabolism in *Narcissus pseudonarcissus 'King Alfred'* by comparative transcriptome and targeted metabolism"
- Canadian Association of Plant Biology Meeting, Queen's University, Kingston, 2016
  "Transcriptome and targeted metabolome profile of *Narcissus pseudonarcissus 'King Alfred'* reveals component of galanthamine metabolism"
- 23rd University of Quebec Science Poster Contest, Trois-Rivières, 2016
  "Study of biosynthetic genes of precursors of Amaryllidaceae alkaloids"
- Journee Scientific sur la defense et la metabolism de vegetaux, 2016
  "Discovering unknown genes of Amaryllidaceae alkaloids biosynthesis pathway"
- Groupe de Recherche en Biologie Végétale (GRBV), 2015
  "Novel genes involved in Amaryllidaceae alkaloids biosynthesis"
- 84th Acfas Congress, University of Quebec in Montreal, 2016
  "Study of biosynthetic genes of precursors of Amaryllidaceae alkaloids"
- 83rd Congress of Acfas, University of Quebec in Rimouski, 2014
  "Characterization of the expression of the genes involved in the biosynthesis of precursors of Amaryllidaceae alkaloids."

ATTENDED CONFERENCES AND WORKSHOP (Selected)

- Canadian Society of Plant Biology Conference 2021
- Cyber security online course
  (University of Lethbridge)
- New approaches in LC-MS lipidomics
  (Dr. Dajana Vuckovic, Concordia university)
- Structural Biology of PsOMT2/6OMT
  (Ken Ng, University of Calgary)
- Visualization of cell signalling and metabolism with genetically engineered fluorescent indicators
  (Dr. Robert Campbell, Faculty of Science, University of Alberta)
- The Purification of Arabidopsis thaliana Shewanella - like Protein Phosphatase 1 and Elucidation of its Protein Interactome
  (University of Calgary)
- The role of effector proteins in the evolution of fungi and their interaction with plants
  (Richard Belanger, Chair Professor, Laval University)
- Next generation technologies for tomorrow's crops: getting to the roots of global food security
  (Leon Kochian, Centre SEVE)
- The middle lamella –the glue that holds cells together
  (Anja Geitmann, Mcgill University)

Transcriptome analysis of *Leucojum aestivum* and identification of genes involved in norbelladine formation.

Laurence Tousignant[1], Aracely Maribel Diaz Garza[1], Bharat Bhusan Majhi[1], Sarah-Eve Gélinas[1], Aparna Singh[1], Isabel Desgagne-Penix[1,2*]

[1]Department of Chemistry, Biochemistry and Physics, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada

[2]Plant Biology Research Group, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada

* Correspondence: Isabel.Desgagne-Penix@uqtr.edu , Telephone : 1-819-376-5011

Abstract

Main conclusion

Transcriptome analysis of *Leucojum aestivum* led to the identification of 50 key genes associated with Amaryllidaceae alkaloid biosynthesis including norbelladine synthase which localized in the cytosol and catalyzed norbelladine formation.

Abstract

The Amaryllidaceae alkaloids (AAs) are a large group of plant specialized metabolites, which are known for their biological activities. Although the general chemical reactions in the AA biosynthetic pathway have been proposed, the genes and enzymes of the pathway remain largely unstudied. All AAs are synthesized from a common precursor, norbelladine, by the condensation of tyramine and 3,4-dihydroxybenzaldehyde. The enzyme norbelladine synthase (NBS) which catalyzes the condensation reaction has been characterized at a molecular level from one species, and the subcellular localizations have not been explored; hence, the intracellular compartments wherein the AAs are biosynthesized remain unknown. In this study, a first comprehensive transcriptomic analysis of summer snowflake (*Leucojum aestivum*) was done to identify key genes associated with AA biosynthesis. Fifty orthologous genes were identified and deposited into GenBank. In addition, we identified and further characterized NBS from the transcriptome of *L. aestivum* and previously reported *Narcissus papyraceus*. Phylogenetic analysis showed that *La*NBS, *Np*NBS1 and *Np*NBS2 shared high amino acid identity. The heterologous expression of *La*NBS produced a recombinant protein with NBS activity. Bioinformatic prediction programs and C-terminal GFP tagging in transiently transformed *Nicotiana benthamiana* showed that *La*NBS, *Np*NBS1 and *Np*NBS2 were likely localized to the cytosol which suggests that the AA biosynthesis start in the cytosol. This study provides an Amaryllidaceae transcriptome that will be very helpful to identify genes for characterization studies in AA metabolism *in planta* or using heterologous systems. In addition, our study will facilitate the bioengineering of AA biosynthetic pathway in plants or in microorganisms.

NRC Research Press

MINIREVIEW

The genetics of *Cannabis*—genomic variations of key synthases and their effect on cannabinoid content[1]

Aparna Singh, Andriy Bilichak, and Igor Kovalchuk

Abstract: Despite being a controversial crop, *Cannabis sativa* L. has a long history of cultivation throughout the world. Following recent legalization in Canada, *Cannabis* is emerging as an important plant for both medicinal and recreational purposes. Recent progress in genome sequencing of both cannabis and hemp varieties allow for systematic analysis of genes coding for enzymes involved in the cannabinoid biosynthesis pathway. Single-nucleotide polymorphisms in the coding regions of cannabinoid synthases play an important role in determining plant chemotype. Deep understanding of how these variants affect enzyme activity and accumulation of cannabinoids will allow breeding of novel cultivars with desirable cannabinoid profiles. Here we present a short overview of the major cannabinoid synthases and present the data on the analysis of their genetic variants and their effect on cannabinoid content using several in-house sequenced *Cannabis* cultivars.

Key words: *Cannabis sativa* L., hemp, marijuana, *THCAS*, *CBDAS*.

Résumé : En dépit des controverses l'entourant, le *Cannabis sativa* L. est cultivé depuis longtemps partout dans le monde. Suite à de récents changements législatifs au Canada, cette culture devient importante tant pour ses usages médicinaux que récréatifs. Des avancées récentes en matière de séquençage de variétés tant du cannabis que du chanvre rendent possible une analyse systématique des gènes codant pour des enzymes impliquées dans les voies de synthèse des cannabinoïdes. Des polymorphismes mononucléotidiques dans les régions codantes des cannabinoïdes synthases jouent un rôle important dans la détermination du chimiotype. Une compréhension approfondie des effets de ces variants sur les activités de ces enzymes et sur l'accumulation de cannabinoïdes permettra de sélectionner de nouveaux cultivars dotés de profils souhaités de cannabinoïdes. Dans ce travail, les auteurs présentent une vue d'ensemble des principales cannabinoïdes synthases ainsi que des données sur l'analyse de leurs variants génétiques et de leurs effets sur la teneur en cannabinoïdes à l'aide de plusieurs cultivars de *Cannabis* séquencés à l'interne. [Traduit par la Rédaction]

Mots-clés : *Cannabis sativa* L., chanvre, marijuana, *THCAS*, *CBDAS*.

Introduction

Cannabis sativa L. (including marijuana and hemp) is a herbaceous plant belonging to the family Cannabaceae (Vavilov and Freier 1951; Brizicky 1966). Being one of the major sources of medicine, oil, and fiber, it has been extensively cultivated in many countries (Camp 1936; Godwin 1967; Quimby et al. 1973; Schultes et al. 1974; Kriese et al. 2004; Laverty et al. 2019). Since ancient times, the *Cannabis* plant has been valued for its medicinal properties and use for treating pain, nausea, depression, glaucoma, asthma, and insomnia (Mechoulam et al. 1976; Duke and Wain 1981). Although the therapeutic properties of cannabinoids have been extensively studied, the role of phytocannabinoids within plants is poorly understood. *Cannabis* is diploid and its karyotype consists of nine autosomes and a pair of sex chromosomes ($2n = 18 + XX$ for female or XY for male) (Flemming et al. 2007; Divashuk et al. 2014; Vyskot and Hobza 2015). The haploid genome size of female and male plants is approximately 818 and 843 Mb, respectively (Sakamoto et al. 1998).

The medicinal properties of *Cannabis* are a result of the presence of terpenophenolic compounds known as cannabinoids. They can modulate the human endocannabinoid system and are useful for various physiopathological processes (Izzo et al. 2009). They are named as cannabinoids owing to their typical exhibition of a C21 terpenophenolic structure (Hillig 2004; Brenneisen 2007;

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[1]This Minireview is part of a collection on Genome Biology jointly published by *Genome* and *Biochemistry and Cell Biology*.

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https://doi.org/10.1007/s11101-019-09644-w

Benzylisoquinoline alkaloid biosynthesis in opium poppy: an update

Aparna Singh · Ivette M. Menéndez-Perdomo · Peter J. Facchini

Received: 13 April 2019 / Accepted: 27 August 2019 / Published online: 27 November 2019

Abstract For nearly eight millennia, opium poppy (*Papaver somniferum*) has been bred and cultivated for therapeutic purposes. The medicinal properties of the plant are conferred by specialized metabolites known as benzylisoquinoline alkaloids (BIAs), comprising the narcotic analgesics morphine and codeine, the antimicrobial agent sanguinarine, and the potential anticancer drug noscapine. In addition, naturally occurring thebaine is used for the semi-synthesis of widely prescribed pain-relievers (e.g., oxycodone and hydrocodone), valuable drugs used in the treatment of opioid addiction (i.e., naltrexone), or antidotes for opioid overdose (i.e., naloxone). The complex stereochemistry of many opiates hinders their chemical synthesis and opium poppy remains the sole commercial source of these important pharmaceuticals. For decades, opium poppy has served as a model plant for research aimed at a comprehensive understanding of BIA metabolism. Recent progress in functional genomics has enabled the discovery of a nearly complete collection of BIA biosynthetic genes, many of which are clustered in the opium poppy genome. Advances in synthetic biology have facilitated the successful reconstitution of several BIA biosynthetic pathways in heterologous hosts such as *Saccharomyces cerevisiae* and *Escherichia coli*, although the initially low production levels suggest that commercial scale-up will present additional challenges. This review provides an update of key molecular and biochemical aspects of BIA metabolism in opium poppy, including recent biosynthetic gene discoveries, genomic organization, novel BIA transporters, metabolic regulation, and major efforts in the engineering of pathways in plants and microbes.

Keywords Alkaloid biosynthetic pathways · Gene discovery · Metabolic engineering · *Papaver somniferum* · Plant specialized metabolism · Opiate transport · Regulation of alkaloid metabolism

Abbreviations

2-ODD	2-Oxoglutarate-dependent dioxygenase
4-HPAA	4-Hydroxyphenylacetaldehyde
4′OMT	3′-Hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase
6OMT	Norcoclaurine 6-*O*-methyltransferase
7OMT	Reticuline 7-*O*-methyltransferase
AKR	Aldo-keto reductase
AT	Acetyltransferase
AT1	1,13-Dihydroxy-*N*-methylcanadine 13-*O*-acetyltransferase

Aparna Singh and Ivette M. Menéndez-Perdomo contributed equally.

A. Singh · I. M. Menéndez-Perdomo · P. J. Facchini (✉)
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Singh et al. BMC Plant Biology (2018) 18:338
https://doi.org/10.1186/s12870-018-1570-4

BMC Plant Biology

RESEARCH ARTICLE Open Access

Cloning and characterization of norbelladine synthase catalyzing the first committed reaction in Amaryllidaceae alkaloid biosynthesis

Aparna Singh[1], Marie-Ange Massicotte[1], Ariane Garand[1], Laurence Tousignant[1], Vincent Ouellette[1], Gervais Bérubé[1] and Isabel Desgagné-Penix[1,2*]

Abstract

Background: Amaryllidaceae alkaloids (AAs) are a large group of plant-specialized metabolites displaying an array of biological and pharmacological properties. Previous investigations on AA biosynthesis have revealed that all AAs share a common precursor, norbelladine, presumably synthesized by an enzyme catalyzing a Mannich reaction involving the condensation of tyramine and 3,4-dihydroxybenzaldehyde. Similar reactions have been reported. Specifically, norcoclaurine synthase (NCS) which catalyzes the condensation of dopamine and 4-hydroxyphenylacetaldehyde as the first step in benzylisoquinoline alkaloid biosynthesis.

Results: With the availability of wild daffodil (*Narcissus pseudonarcissus*) database, a transcriptome-mining search was performed for *NCS* orthologs. A candidate gene sequence was identified and named *norbelladine synthase* (*NBS*). *NpNBS* encodes for a small protein of 19 kDa with an anticipated pI of 5.5. Phylogenetic analysis showed that *Np*NBS belongs to a unique clade of PR10/Bet v1 proteins and shared 41% amino acid identity to opium poppy NCS1. Expression of *NpNBS* cDNA in *Escherichia coli* produced a recombinant enzyme able to condense tyramine and 3,4-DHBA into norbelladine as determined by high-resolution tandem mass spectrometry.

Conclusions: Here, we describe a novel enzyme catalyzing the first committed step of AA biosynthesis, which will facilitate the establishment of metabolic engineering and synthetic biology platforms for the production of AAs.

Keywords: Amaryllidaceae alkaloid, Pathogenesis related protein 10, Alkaloid biosynthesis, *Narcissus pseudonarcissus*, Norcoclaurine synthase, Norbelladine

Background

The Amaryllidaceae alkaloids (AAs) are a group of naturally synthesized molecules with more than 600 renowned complex structures [1]. They are pharmacologically active compounds that are classified under three different groups of C-C phenol coupling namely *para-para', ortho-para'* and *para-ortho'* [2]. An outsized variety of pharmacologically active AAs have been identified with the bioactive properties including the acetylcholine esterase inhibitor galanthamine, anti-tumor activity of lycorine and the cytotoxic haemanthamine [3–5]. AAs are obtained chiefly from the extracts of plants from *Galanthus, Leucojum* and *Narcissus* species, as their complicated structures do not enable cost-effective high-yield organic synthesis [6]. Though AAs display a large range of pharmaceutical applications, only galanthamine is accessible in markets as an Alzheimer's treatment drug because of its ability to stabilize behavioral symptoms in the course of six months treatment in comparison to chemically synthesized acetylcholinesterase inhibiting drugs, donepezil and rivastigmine [7].

Previous investigations on the biosynthesis of AAs *in planta* have revealed that all AAs are made from the common metabolic intermediate, norbelladine (Fig. 1)

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SCIENTIFIC REPORTS

OPEN

Received: 11 July 2017
Accepted: 10 October 2017
Published online: 11 December 2017

Transcriptome and metabolome profiling of *Narcissus pseudonarcissus* 'King Alfred' reveal components of Amaryllidaceae alkaloid metabolism

Aparna Singh[1] & Isabel Desgagné-Penix[1,2]

Amaryllidaceae alkaloids (AAs) represent a diverse class of plant specialized metabolites and many display potent pharmacological activities. The AA metabolic pathway is poorly understood and resources are minimal. To enable AA pathway elucidation and novel biosynthetic enzymes discovery, we generated comprehensive metabolomic and corresponding transcriptomic datasets from different tissues of *Narcissus pseudonarcissus* 'King Alfred'. In this study, we performed untargeted UPLC-QTOF-MS metabolite analysis from different tissues, which generated exhaustive list of compounds, including several AAs, most predominant and diverse in bulbs. RNA sequencing of *N. pseudonarcissus* 'King Alfred' bulbs yielded 195,347 transcripts, after assembly. Top expressed genes belong to process like metabolism, survival, and defense including alkaloid biosynthetic genes. The transcriptome contained complete sequences for all proposed genes encoding AA-biosynthetic enzymes such as tyrosine decarboxylase (*TYDC1* and *TYDC2*), phenylalanine ammonia-lyase (*PAL1* and *PAL2*) and phenolic acids hydroxylases (*C4H* and *C3H*) to name a few. Furthermore, transcriptome data were validated using RT-qPCR analysis and expression study in different tissues of *N. pseudonarcissus* 'King Alfred' was performed. Here, we present the first comprehensive metabolome and transcriptome study from *N. pseudonarcissus* 'King Alfred' providing invaluable resources for metabolic engineering and biotechnological applications.

Plants produce a plethora of chemicals important for growth, development, and defense through the primary and specialized (*aka* secondary) metabolic pathways. The specialized metabolites, also named phytochemicals or natural products, are specific and restricted to some taxonomic groups and play pivotal roles in plant defense, protection, and survival[1].

The medicinal properties of the Amaryllidaceae are owed to the presence of specialized metabolites, the Amaryllidaceae alkaloids (AAs), which are specific to this plant family. Several AAs display pharmaceutical activities such as the anti-cancer narciclasine, the anti-parasitic haemanthamine, the anti-viral lycorine and the anti-acetylcholine esterase galanthamine[2–9]. Despite AAs being a huge therapeutic reserve, galanthamine is the only one used medically to treat the symptoms of Alzheimer's disease through its reversible acetylcholine esterase inhibition and its nicotinic receptor binding activity[10,11]. Currently, the knowledge of AA metabolism and regulation is limited and only a few genes encoding biosynthetic enzymes have been identified[12–14]. A better understanding of AA biosynthesis will allow for the development of new cultivars or biotechnologies to help produce these valued phytochemicals. Additionally, over 600 AAs have been identified[15], adding to the diversity of AA chemical structures and also demonstrating the complexity of their biosynthetic pathway.

Previous studies using radiolabeled precursors led to the biochemical elucidation of the initial steps in AA biosynthesis[16–18]. Despite the vast structural diversity, all AAs are derived from a common central intermediate

[1]Department of Chemistry, Biochemistry and Physics, Université du Québec à Trois-Rivières, 3351 boul. des Forges, Trois-Rivières, QC, G9A 5H7, Canada. [2]Plant Biology Research Group, Université du Québec à Trois-Rivières, 3351 boul. des Forges, Trois-Rivières, QC, G9A 5H7, Canada. Correspondence and requests for materials should be addressed to I.D.-P. (email: Isabel.Desgagne-Penix@uqtr.ca)