Shashank S Saini, PhD

Specialized metabolism Biotechnologist

SUMMARY I am an accomplished researcher skilled in successfully executing scientific projects. 5+ years of experience in the biosynthesis of specialized metabolites and their applications. My aspiration is to lead a research team that employs techniques like bioinformatics, biochemical, mass spectrometry, molecular biology, and other synergistic approaches to unravel biological questions like spatio-temporal formation of pharmacologically active plant specialized metabolites and regulatory pathways. I am eager to decipher out metabolic pathways of specialized plant metabolites that hold great potential in agriculture and pharmaceutical industries, as well as their sustainable production in SMART cells as metabolite factory. Overall, my aim is to leverage state-of-the-art techniques such as metabolomics, transcriptomics, network pharmacology, and functional genomics to advance our knowledge of specialized metabolism and contribute to the scientific community's understanding and sustainable commercialization of novel products.

KEY SKILLS

- Specialized Metabolism
- Plant tissue culture (SMART-CELLS)
- Metabolomics (GC-MS & HPLC-DAD/MS)
- Transcriptomics (De Novo & Ref Seq)
- Cloning & functional characterization
- Protein Purification
- Enzyme Kinetics
- Gene Silencing
- E-Sensor Development
- Linux & Python for NGS data analysis
- R language (towards intermediate)
- Data interpretation
- Communication (verbal and written)

VALUES

- Integrity
- Sustainability

HOBBIES

- Trekking
- Yoga
- Reading

LEARNING

• Molecular Docking

PROFESSIONAL & RESEARCH EXPERIENCE

Oct2020-June2023: Newe Yaar Research Center, ARO Volcani, Israel As an ARO postdoctoral fellow at Volcani Israel with Prof. Efraim Lewinsohn, I carried out my research in deciphering "Novel amphetamine and terpenoids class of bioactive specialized metabolite by LC-MS and GC-MS. Further, understanding their biosynthesis at molecular level in Catha edulis. As a part of this initiative, I performed integrative transcriptomics and metabolomics analysis to map out major genes involved in biosynthesis of these novel active metabolites. Furthermore, with the help of molecular biology tools, responsible genes were cloned, heterologously expressed and functionally characterized to have a better understanding of mechanism underlying in the biosynthesis of these metabolites in khat. In order to obtain their sustainable production viz molecular farming for the future usage, the functionally characterized genes are expressed and pathway is engineered using binary vector system in yeast (Saccharomyces cerevisiae). Along with above major project I also worked on various projects going in lab including "Ecological importance of galling aphids in Pistacia palestina" and in "Terpene metabolism genes of Pinus halepensis (Aleppo pine)".

Jan2018-Oct 2020: Indian Institute of Technology Roorkee, IITR, India
Contributed as a Research Associate in a project funded by ICAR-NASF
(National Agricultural Scientific Fund) in the research group of Associate Prof.
Debabrata Sircar. I have discovered distinctive fruit volatiles (VOCs) that accumulate differentially during the ripening and stress condition in apple and papaya using the GC-MS approach. My research laid the groundwork for ongoing studies in my mentor's research groups, in which those distinctive volatiles were detected by an electronic nose sensor for non-invasive post-harvest nutritional quality and shelf-life analysis of papaya and apple. Along with conducting research, I also mentored 4 undergraduates, 3 graduate, and 5 doctoral students. I have also worked on various metabolomics projects with collaborative lab on anticancer effect of Indian Honey and piperin alkaloid from Piperum nigrum.

ACADEMIC QUALIFICATIONS

PhD (2017), Indian Institute of Technology Roorkee

- Thesis title: Biosynthesis of benzoate-derived biphenyl phytoalexins in cell cultures of *Pyrus pyrifolia*.
- **Summary**: My doctoral research was focused on understanding the molecular changes in scab susceptible and resistant cultivars of *Pyrus spp*, prior and after scab infection/elicitor treatment. I have developed callus and suspension culture from scab susceptible and resistant cultivars of *Pyrus* for systematic understanding of the biosynthesis of biphenyl phytoalexin produced upon infection with *Venturia inequalis* a scab causing fungus which make them resistant to scab. While working on this project I have identified and characterized three new enzymes of biphenyl biosynthesis in pear, manuscripts with these findings have been published in reputed journal.
- **Supervisor**: Dr. Debabrata Sircar, Associate Professor, Department of Biosciences and Bioengineering, IIT Roorkee, Roorkee, India

MSc (2009), Ch. Charan Singh University, Meerut

- Biotechnology
- Thesis title: Assessment of adulteration and contaminations in haldi (*Curcuma longa*) and other herbal extracts. Indian Herbs Pvt Ltd, Saharanpur

BSc (2007), Ch. Charan Singh University, Meerut

• Honors in Biotechnology

FELLOWSHIPS / AWARDS

- 2021: Awarded **ARO-Indo Israel** postdoctoral fellowship to pursue research at Newe Yaar Research Center, ARO-Volcani Israel for two years.
- 2018: Qualified **National Eligibility test** in Agricultural Biotechnology conducted by ICAR-ASRB.
- 2017: Awarded post-doctoral position at IIT Roorkee under highly prestigious ICAR-NASF project.
- 2013: Awarded **MHRD scholarship** for pursuing a full time PhD research at **IIT Roorkee**.
- 2013: Qualified Graduate Aptitude Test in Engineering (GATE-2013).

WORKSHOPS/TRAINING

- Attending workshop on 'Proteomics (Molecular Docking)' from Dr Omics lab [09/10/2023-ongoing], Delhi, India (https://dromicslabs.com)
- Attended workshop and training program on 'Next-Generation Sequencing Data Analysis' from August 14, 2022- August 25, 2022 (40 academic hours). Tel Hai College, Upper Galilee, Israel. (Grade attained: 90)

AREAS OF RESEARCH EXPERTISE

General: Phytochemistry, Plant pathogen interaction, Specialized metabolism, Functional genomics, Tissue culture, Bioinformatics & Molecular Biology.

Specific:

<u>Transcriptomic data analysis</u> - Currently I am working in this area to decipher out integrative metabolic pathway & gene responsible for the biosynthesis of pharmacologically and bioactive plant specialized metabolites in plants. [See: Saini et al, *Plant Metabopepole* – 26-27 (2022)].

<u>Metabolomics</u> - A main area of my research expertise is the plant metabolomics and metabolite profiling (of primary and secondary metabolites) using HPLC, GC-MS, and LC-MS. [See: Sarkate and Saini et al, *Scientific Reports* - 17844 (2018); Teotia, Saini et al, *J. Chromatographic Science* - 54 (2016); Kumar M, Saini et al, *Journal of Food Composition and Analysis* - 96 (2021); Kushwaha K, Saini et al, *European Food Research and Technology* – 1977 (2021)].

<u>Metabolic pathway elucidation and engineering</u> - Another main area of my research expertise is elucidation of biochemical pathways "in QUESTION" at enzymatic and molecular level. [See: Saini et al, *J. plant Physiol* – 215 (2017) 154-62; Saini et al, *Physiologia Plantarum* – 165 (2018); Sarkate, Saini et al, *J. plant Physiol* – 221 (2018) 66-73].

<u>Molecular Biology and Heterologous Protein expression</u>- Third area of my research expertise is cloning and functional characterization of crucial plant gene involved in specialized metabolism and their *Agrobacterium* mediated gene transfer to study their localization. **See**: Sarkate, **Saini** et al, *Planta* - 248 (2019) 1-15, Teotia D, Gaid MM, **Saini SS** et al, *The Plant journal* (2019)].

Major Analytical Expertise: NGS-Transcriptome data analysis using Python & R-language, GC-MS, LC-DAD/MS, HPTLC, Real time gene expression analysis, Cloning, Functional characterization, SDS PAGE, Confocal microscopy, Agrobacterium mediated gene transformation, SMART CELLS, Molecular pharming.

SKILLS & TECHNIQUES (Detailed)

Cell and Tissue Culture: Callus, cell culture and hairy root development, elicitor treatment to plant culture system, development and maintenance of fungal culture (*V. inaequalis, S.cervisae*).

Metabolomics: Metabolite extraction (non-volatile as well as volatile metabolites using SPME, head space GC-MS), HPLC, HPTLC, GC-MS/MS, LC-MS/MS based metabolomics of plant metabolites. Novel Metabolite annotation, pathway map creation, metabolite network analysis, comparative metabolomics; targeted and non-targeted metabolomics analyses.

Transcriptomics: Both DE novo and Ref Seq based transcriptome data analysis from high throughput NGS sequencing data to check the spatially and temporally expressed novel genes for specialized metabolites and their network analysis.

Molecular biology Techniques: DNA and RNA extraction, cDNA synthesis, codon optimization according to the host of heterologous expression, primer designing, vector designing, PCR, qPCR, real time PCR, Gene silencing (VIGS).

Recombinant Protein expression in Bacteria, Fungi and Plants: Cloning and heterologous expression of novel biosynthetic gene in *E. coli* and *Yeast*. Furthermore, recombinant proteins purification and confirmation via SDS-PAGE and/or blotting, *Agrobacterium* mediated transformation of explants for colocalization studies and to engineer hairy roots or suspension culture and to sustainably produce active metabolites of purpose.

Protein/Enzyme Study: Recombinant protein purification from heterologusly expressed genes in *E. coli* (BL21) or *S. cervisae* (YHR072) by affinity chromatography and further purification of protein by membrane columns to analyze, functionally characterize enzymes and to study enzyme kinetics.

Microscopic Techniques: Light microscopy, fluorescent microscopy, confocal microscopy (specialization in sub cellular localization of organelle specific proteins).

Statistical and Bioinformatics: Python and R language tools for NGS data and statistical analysis like DEG, GO ontology and pathway enrichment analysis with ANOVA, PCA, PLSDA and metabolite profile analysis using Metaboanalyst5.0. I have knowledge of Hyper 32 for Enzyme kinetics study, Geneious, Chromas and

other online tools required in functional genomics study.

PUBLICATIONS

Research articles

- 1. Das N, Ray N, Patil AR, Saini SS, Waghmode B, Ghosh C, Patil SB, Patil SB, Mote SB, Saini S, Saraswat BL, Sircar D and Roy P. Inhibitory effect of selected Indian honey on colon cancer cell growth by inducing apoptosis and targeting the β-catenin/Wnt pathway, 2022. *Food and Function* (IF: 6.31)
- 2. Kumar M, Agrawal PK, Roy P, Sircar D. Nutritional and metabolomics characterization of the coconut water at different nut developmental stages. *Journal of Food Composition and Analysis*, 2021. (IF: 4.52)
- 3. Kushwaha K, Saini SS, Waghmode B, Gaid B, Agrawal PK, Roy P, Sircar D. Volatile components in papaya fruits are the non-invasive biomarkers to monitor the ripening stage and the nutritional value. *European Food Research and Technology*, 2021. (IF: 3.49)
- **4.** Banerjee S, Katiyar P, Kumar V, **Saini SS**, Varshney R, Krishnan V, Sircar D, Roy P. Black pepper and piperine induce anticancer effects on leukemia cell line. *Toxicology Research*, 2021. (**IF: 2.68**)
- **5.** Banerjee S, Katiyar P, Kumar L, Kumar V, **Saini SS**, Krishnan V, Sircar D, Roy P. Black pepper prevents anemia of inflammation by inhibiting hepcidin over-expression through BMP6-SMAD1/IL6-STAT3 signaling pathway. *Free Radical Biology and Medicine*, 2021. (**IF: 8.10**)
- **6.** Saini SS, Gaid MM and Sircar D. Benzoate-CoA ligase contributes to the biosynthesis of biphenyl phytoalexins in elicitor-treated pear cell cultures. *Plant cell Reports*, 2020. (**IF: 4.96**)
- 7. Teotia D, Gaid MM, Saini SS, Verma A, Yennamalli RM, Khare SP, Ambatipudi K, Mir JI, Beuerle T, Hänsch, R, Roy P, Agrawal PK, Beerhues L and Sircar D. Cinnamate:CoA-ligase is involved in benzoate-derived biphenyl phytoalexin biosynthesis in Malus × domestica 'Golden Delicious' cell cultures. *The Plant Journal*, 2019. (IF: 7.09)
- **8.** Saini SS, Teotia D, Gaid MM and Sircar D. A New Enzymatic Activity from Elicitor treated Pear Cell Cultures Converting trans-cinnamic acid to Benzaldehyde. *Physiologia Plantarum*, 2018. (**IF: 5.08**)
- **9.** Sarkate A, **Saini SS**, Gaid MM, Teotia D, Mir JI, Agrawal PK, Beerhues L and Sircar D. Molecular Cloning and Functional Analysis of a Biphenyl Phytoalexin-specific O-methyltransferase from Apple Cell Suspension Cultures. *Planta*, 2018. (**IF: 4.54**)
- **10.** Sarkate A*, **Saini SS***, Teotia D, Gaid MM, Mir JI, Roy P, Agrawal PK and Sircar D. Comparative metabolomics of scab-resistant and susceptible apple cell cultures in response to scab fungus elicitor treatment. **Nature Scientific Reports**, 2018. (**IF: 4.99**) (#Joint first Author)
- **11.** Sarkate A, **Saini SS**, Kumar P, Sharma AK and Sircar D. Salicylaldehyde synthase Activity from *Venturia inaequalis* Elicitor-treated Cell Culture of Apple. *Journal of Plant Physiology*, 2018. (**IF:3.68**)
- **12. Saini S S**, Teotia D, Gaid MM, Thakur A, Beerhues L and Sircar D. Benzaldeyde dehydrogenase Driven Phytoalexin Biosynthesis in Elicitor treated Pyrus pyrifolia Cell Cultures . *Journal of Plant Physiology*, 2017. (**IF:3.68**)
- **13.** Teotia D, **Saini SS**, Gaid MM, Beuerle T, Beerhues L and Sircar D. Development and Validation of a New HPLC Method for the Determination of Biphenyl and Dibenzofuran Phytoalexins in Rosaceae. *Journal of Chromatographic Science*, 2016. (**IF:1.61**)

- 1. Juneja K, Saini SS and Sircar D. Production of life-saving drugs from Himalayan herbs Ch:13 Sustainable Utilization of Natural Resources, CRC Press Taylor and Francis group, 2017.
- 2. Tomar V, Saini SS, Juneja K, Agrawal PK and Sircar D. Transgenic Technologies and Their Potential Applications in Horticultural Crop Improvement. Advances in Plant Transgenics: Methods and Applications, 2019.

Conference/Presentation

- 1. Saini SS, Bar E, Khankin V, Shotland Y, Rikanatai RD and Lewinsohn E. Transcriptomics and metabolomics analysis of the terpenoid pathway in leaves and roots of Khat (*Catha edulis* Forsk.). Israel Society of Plant Sciences (**ISPS2023**). Weizmann Institute of Science (Israel), Feb 6, 2023.
- **2.** Rikanatai RD, Bar E, **Saini SS**, Shotland Y, Dudareva N, Inbar M and Lewinsohn E. Comparative metabolic patterns in galls induced by different aphid-species on *Pistacia palaestina*. Israel Society of Plant Sciences (**ISPS2023**). Weizmann Institute of Science (Israel), Feb 6, 2023.
- **3. Saini SS**, Rikanatai RD, Bar E, Khankin V, Shotland Y and Lewinsohn E. Interaction of metabolome & transcriptome reveals genes involved in biosynthesis of pharmacoactive terpenoids in khat (*catha edulis* F.). **Plant MetaboPepole**. MIGAL Galilie Research Institute, Agmon Haula (Israel), April 26-27, 2022.
- **4.** Sircar D, Tomar V, **Saini SS.** SMART-Nose: A non-invasive electronic sensor for qualitative and quantitative analyses of plant natural products from Medicinal plants. Page No.: 34; **23rd National convention of society of pharmacognosy and international conferences on new age opportunities and challenges for quality, safety and GMPs in herbal drug development, CSIR-National Botanical Research Institute, Lucknow (India), February 22-23, 2019.**
- 5. Sircar D and Saini SS. E-MEDI-SMART: A non-invasive electronic sensor for qualitative and quantitative analyses of plant natural products from Medicinal plants. Page No.:39 (IL08); 6th Biennial International Conference on New Developments in Drug Discovery from Natural Products and Traditional Medicines (DDNPTM 2018), National Institute of Pharmaceutical Education and Research, Punjab (India), November 15-17, 2018.
- **6.** Sircar D, **Saini SS**. Functional genomics analyses of inducible defense responses to *Venturia inaequalis* infection in scab resistant apple genotype. **4**th **International conference and Exhibition on Pharmacognosy, Phytochemistry and Natural Products**. Page No:14, Sao Paulo, Brazil, August 29-31, 2016.
- 7. Sircar D, Saini SS. Development of Plant natural product –based topical hemostatic agents for rapid wound sealing and healing. XV Annual convention of ISVPT. January 2016.
- **8.** Sircar D, **Saini SS**. Metabolomics and functional genomics approach to decipher disease resistance mechanism in apple against scab disease. **ICMS- NEHU**; IL-II-4. November 2015.
- **9. Saini SS**, Sircar D. Metabolomics approach for novel drug discovery against metabolic disorder from in vitro plant of *Swertia chirata*. **ICMS- NEHU**, Shillong, India. (2015-16); PP-62, November 2015.
- **10. Saini SS**, Sircar D and Thakur A. Secondary metabolite contents and antioxidant capacities of *Pyrus pyrifolia* (Asian pear) callus. **ICMS- RTBTR**, IIT Roorkee, India (2014-15); PP- 27, December 2015.

REFEREES

Dr. Debabrata Sircar

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Prof. Efraim Lewinsohn

Scientist Rank A⁺
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Newe Ya'ar Research Center, Agricultural Research Organization
Volcani, Ramat Yishai 30095, Israel

DECLARATION

I hereby declare that all the information's furnished by me are true to the best of my knowledge.

Place and Date

India

23-01-2024 Shashank Sagar Saini