AMIT KUMAR KUSHWAHA, Ph. D.

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Education	
Doctoral	Worked on a thesis entitled 'Molecular cloning and characterization of
(2014)	tropinone reductase from Ashwagandha (Withania somnifera Dunal) at
	CSIR-Central Institute of Medicinal and Aromatic Plants
	(www.cimap.res.in), Lucknow. Degree awarded by Jawaharlal Nehru
	University, New Delhi (www.jnu.ac.in).
Post-	In Agricultural Biotechnology (M. Sc. Ag.) from Orissa University of
graduation	Agriculture and Technology (www.ouat.ac.in), Bhubaneswar, India with
(2008)	Gold Medal. Worked on a dissertation entitled 'Standardization of protocol
	for genetic transformation of Brinjal (Solanum melongena L).
Graduation	In Agriculture (B. Sc. Ag. Hons.) From Chhatrapati Shahu Ji Maharaj
(2005)	University (www.kanpuruniversity.org), Kanpur, India. Participated in
, ,	RAWE (Rural Agriculture Work experience Programme) and led a team of
	20 fellow students in agriculture extension work in neighboring villages of
	Lucknow.

Professional Experience

DBT-Junior Research Fellow	Department of Metabolic and Structural Biology, CSIR-
(Sep. 2009 to Sep. 2011)	Central Research Institute of Medicinal and Aromatic Plants,
	Lucknow, INDIA
DBT-Senior Research Fellow	Department of Metabolic and Structural Biology, CSIR-
(Sep. 2011 to Sep. 2014)	Central Research Institute of Medicinal and Aromatic Plants,
	Lucknow, INDIA
Kreitman Post-Doctoral Fellow	French Associates Institute of Agriculture and Biotechnology
(Oct. 2014 to March 2015)	of Drylands, Jacob Blaustein Institutes for Desert Research,
	Ben Gurion University of Negev, ISRAEL
Research Associate III	National Institute of Plant Genome Research, New Delhi,
(April 2015 to May 2016)	INDIA
National Postdoctoral Fellow	Department of Biological Sciences,
(April 2017 to April 2019)	Indian Institute of Science Education and Research Bhopal,
	INDIA
CSIR-Senior Research	Department of Biological Sciences,
Associate	Indian Institute of Science Education and Research Bhopal,
(July 2019 to date)	INDIA

Skill Set

DNA	Cloning and sub-cloning, Genomic and Plasmid DNA isolation, PCR, Restriction analysis, Genomic library preparation, and Genome walking.
RNA	Total RNA and mRNA isolation, cDNA library preparation, RACE, qRT-PCR (semi-quantitative, SYBR-green and TaqMan chemistry)
Protein	Recombinant Protein expression and purification, PAGE, Western blotting, Enzyme assays and kinetics, Y2H assay, BiFC assay
Transgenic	Agrobacterium-mediated transformation and subsequent analysis Yeast transformation and subsequent analysis Bacteria transformation and subsequent analysis
Bioinformatics	Online database mining, transcriptome analysis, sequence alignment, and manipulation, <i>in silico</i> promoter analysis like PLACE, PlantCARE, Softberry, Protein 3D structure Prediction, and various tools at EXPASY, Phylogenetic analysis by MEGA5
Chemistry	Extraction of natural products, column chromatography, TLC and GC based separation

Honors, Fellowships, and Awards

- Awarded merit scholarship by Uttar Pradesh Krishi Mandi Parishad, UP during graduation.
- ➤ Qualified All India Biotechnology Entrance Examination 2006 and scholarship awarded by the Department of Biotechnology, India during post-graduation.
- ➤ Awarded University Gold Medal in Agricultural Biotechnology in 2008.
- ➤ Qualified Biotechnology Eligibility Test (BET-2008) and Junior Research Fellowship awarded by Department of Biotechnology, India in 2009.
- Qualified Indian Council of Agricultural Research National Eligibility Test (ASRB-NET for lectureship), 2010 in Basic Plant Sciences.
- > Senior Research Fellowship awarded by Department of Biotechnology, India, in 2011.
- ➤ Kreitman Postdoctoral Fellowship, 2014 by Ben Gurion University of Negev, Israel.
- ➤ CSIR-RA, 2015-16 (a postdoctoral fellowship) by Council of Scientific and Industrial Research, India.
- ➤ N-PDF, 2017 (a postdoctoral research grant) by the Science and Engineering Research Board, DST, India.
- ➤ CSIR-SRA, 2019 by Council of Scientific and Industrial Research, India.

Publications

- A. K. Kushwaha, N. S. Sangwan, S. Tripathi, R. S. Sangwan (2013). Molecular cloning and catalytic characterization of a recombinant tropine biosynthetic tropinone reductase from *Withania coagulans* leaf. **Gene** 516, 238–247. ISSN: 0378-1119. Impact Factor 2.984. NAAS Id G002, Rating 8.50. DOI 10.1016/j.gene.2012.11.091.
- A. K. Kushwaha, N. S. Sangwan, P. K. Trivedi, A. S. Negi, L. N. Mishra, R. S. Sangwan (2013). Tropine forming tropinone reductase gene from *Withania somnifera*

- (Ashwagandha): Biochemical characteristics of the recombinant enzyme and novel physiological overtones of tissue-wide gene expression patterns. **PLoS One** 8(9): e74777. eISSN: 1932-6203. Impact Factor 2.76. NAAS Id P004, Rating 8.77. DOI:10.1371/journal.pone.0074777.
- N. S. Sangwan, L. N. Mishra, S. Tripathi, A. K. Kushwaha (2014). Omics of Secondary Metabolic Pathways in *Withania somnifera* Dunal (ashwagandha). In OMICS Applications in Crop Science, Debmalya Barh (ed). CRC Press, pages 381-404; ISBN 9781466585256.
- N. S. Sangwan, P. Tiwari, S. K. Mishra, R. K. Yadav, S. Tripathi, A. K. Kushwaha, Rajender Singh Sangwan (2015). Plant Metabolomics: An Overview of Technology Platforms for Applications in Metabolism. In PlantOmics: The Omics of Plant Science, Debmalya Barh et al. (eds). Springer India, pages 257-298. ISBN 978-81-322-2171-5.
- KP Vaishak, P. Yadukrishnan, S. Bakshi, A. K. Kushwaha, H. Ramachandran, N. Job, D. Babu, S. Datta (2019). The B-box Bridge between Light and Hormones in Plants. *Journal of Photochemistry & Photobiology, B:* Biology. 191, 164-174. ISSN 1011-1344. Impact Factor 4.067. NAAS Id J403, Rating 9.17. doi.org/10.1016/j.jphotobiol.2018.12.021.
- J. S. Jadaun, A. K. Kushwaha, L. K. Narnoliya, S. M. Trivedi, N. S. Sangwan, R. S. Sangwan (2020). WRKY1-Mediated Transcriptional Modulation of *Withania somnifera* Tryptophan Decarboxylase Demonstrates its Regulatory Role in Fruit-Specific Tryptamine Generation for Production of Withanamides. ISSN 0721-7714. *Plant Cell Reports*. Impact Factor 3.825. NAAS Id P092, Rating 8.99. https://doi.org/10.1007/s00299-020-02574-4.
- P. Yadukrishnan, D. Singh, N. Ravindran, A. K. Kushwaha, N. Job, P. V. A. Rahul, Yadav, H. Ramachandran, L. Bhagavatula, S. Datta (2020) Hormones and light-regulated seedling development. In D.K. Gupta & F.J. Corpas (Eds.), Hormones and Plant Response. (*In press*): Springer.

Manuscripts in Communication/Preparation

- A. K. Kushwaha, N. S. Sangwan, R. S. Sangwan. Molecular cloning and functional characterization of tropinone reductase II gene from *Withannia somnifera*.
- ➤ A. K. Kushwaha, N. S. Sangwan, R. S. Sangwan. Tropinone reductase: New features of an old enzyme.
- ➤ A. K. Kushwaha, N. S. Sangwan, R. S. Sangwan. Tropanes take aerial route: Changing concepts of tropane biosynthesis.
- ➤ F. Sabir, **A. K. Kushwaha**, R. K. Yadav, R. S. Sangwan, N. S. Sangwan. Phenological metabolic and molecular dynamics of bioactive withanolide biosynthesis in reproductive organs of Indian Ginseng: *Withania somnifera*.

Conference Proceedings

- ➤ A. K. Kushwaha# and R. S. Sangwan (2011). Cloning, Heterologous Expression and Catalytic Kinetics of a Recombinant Tropinone Reductase from Ashwagandha (*Withania somnifera*). 80th Annual Meeting of the Society of Biological Chemists (India) at CIMAP, Lucknow, India.
- ➤ A. K. Kushwaha#, N. S. Sangwan, S. Tripathi, R. S. Sangwan (2013). Molecular cloning of a functional tropinone reductase from leaves of *Withania coagulans*. Symposium on Recent Advances in Biochemistry and Biotechnology: Applications in Health, Environment and Agriculture at the University of Lucknow, Lucknow, India.

- ➤ A. K. Kushwaha#, N. S. Sangwan, P. K. Trivedi, A. S. Negi, L. N. Mishra, R. S. Sangwan (2013). Leaves of Solanaceae Plants Possess Full Metabolic Competence to Synthesize Tropane Alkaloids: Evidence from *Withania*. 82nd Annual Meeting of the Society of Biological Chemists (India) and International conference on genomes: mechanism and function at the University of Hyderabad, Hyderabad, India.
- ➤ J. S. Jadaun#, N. S. Sangwan, A. K. Kushwaha and R. S. Sangwan (2015). Identification, isolation and cloning of tryptophan decarboxylase gene from *Withania somnifera* L. Dunal. International conference on medicinal plants: resource for affordable new generation healthcare' (ICOMP-2015). CSIR-CIMAP, Lucknow, India.

#Work presented by

Participation in Conferences, Workshops and Training, Certificate course

- ➤ 2018, Springer-Nature Author workshop on 'How to write and publish scientific articles and manuscripts', at IISER Bhopal, India.
- ➤ 2018, ICGEB Workshop on 'Plant responses to light and stress: emerging issues in climate change', at ICGEB New Delhi, India
- ➤ 2018, General Course on Intellectual Property (DL-101), WIPO Academy, World Intellectual Property Organization, Switzerland.
- ➤ 2013, 82nd Annual Meeting of Society of Biological Chemists (India) and International conference on genomes: mechanisms and function, at CUH, Hyderabad, INDIA.
- ➤ 2013, Recent advances in Biochemistry and Biotechnology: Applications in health, environment and agriculture, at University of Lucknow, INDIA.
- ➤ 2011, 80th Annual meeting of Society of Biological Chemists (India) and conference on 'Metabolic pathway modulations-Applications in Health and Agriculture', Lucknow, INDIA.
- ➤ 2011, Ocimum: Ancient heritage to modern enigma, Lucknow, INDIA.
- ➤ 2010, AROMED: International symposium on current status and opportunities in medicinal and aromatic plants, Lucknow, INDIA.
- > 2006, International Symposium on Computational Biology and Bioinformatics, Bhubaneswar, INDIA.

Gen Bank Submission (Accession number)

KC310706, KF360055, AGY46257.1, AGB56644.1

Professional Association

- Life Member of the Society of Biological Chemists, India. Membership No. 3056
- Life Member of the Indian Science Congress Association, India. Membership No. L22254
- Reviewer for the journal 'Scientific Reports' of NPG.

Research Experience

Post-doctoral work at IISER Bhopal, India (April 2017 to date)

Title: 'An investigation into the role of B-box in leaf senescence in plants' and 'Study of the role of BBX proteins in root development and low phosphate tolerance' Mentor: Dr. Sourav Datta

I am currently engaged in this project that will identify the putative B-box candidate genes that might play a role in age-triggered and/or stress-induced senescence. The identified candidate genes would be characterized for the role and mechanism of induction/regulation of the senescence process in leaves. I have identified three candidates out of the 32 members of the BBX family of proteins as regulators of age-triggered senescence. In addition to BBX proteins, some other molecular players of light signaling have also been identified to regulate the senescence. The characterization of their molecular mechanism is currently underway. In continuation of studies to understand the role of BBX proteins in plant growth and development, I am also working on to identify their role in root development and phosphate starvation response. We have identified a few members of the BBX protein family to be involved in root development and low phosphate adaptation. Currently, the work is in progress to characterize their involvement.

Post-doctoral work at NIPGR, New Delhi, India (April 2015 to May 2016)

Title: Study of the role of Mitogen-Activated Protein Kinase (MAPK) during rice root development

Supervisor: Dr. Alok Krishna Sinha

As a postdoc, I had cloned several PIN genes involved in Auxin transport from rice. I also worked on protein-protein interaction studies using in-gel kinase assays, yeast two-hybrid assays, and bimolecular fluorescence complementation assays.

Post-doctoral work at BIDR, BGU, Israel (October 2014 to March 2015)

Title: Investigation of 'Sulphite Network' to identify novel molecular players and their role in sulphite metabolism and homeostasis in plants.

Supervisor: Prof. Moshe Sagi

As a postdoc, I was involved in the screening of Arabidopsis mutants for sulfotransferases thought to be involved in sulphur metabolism. I also worked on the generation of over-expression lines for the selected genes.

Summary of Ph. D. work at CSIR-CIMAP, India (2009-2014)

Title of work: 'Molecular cloning and characterization of tropinone reductase from Ashwagandha (Withania somnifera Dunal).'

Supervisor: Dr. Rajender S. Sangwan

During my Ph. D. work, partial fragments of two tropinone reductases (TRs) were identified, isolated, and cloned from *Withania somnifera* and *W. coagulans* following a degenerate primed PCR approach. These fragments were converted into full-length cDNAs using RACE PCRs. These full-length cDNAs were sequenced and sequences were characterized by computational approaches. For biochemical characterization of TR genes, ORFs of the TR cDNAs were subcloned into a protein expression vector and recombinant TR proteins were over-expressed in *E. coli*. Recombinant proteins were purified till homogeneity and purified proteins were characterized biochemically for their physico-kinetic properties. This work reported for the first

time isolation of any TR gene from an aerial part. This was the very first report on any gene/enzyme from medicinally valuable plant *W. coagulans* as well (Gene, 2013).

Expression patterns of these genes were also examined at the tissue level and data were correlated to the tissue-specific metabolite profile with respect to the TR genes. Analysis of the gene expression patterns and correlated metabolite profile revealed novel physiological attributes of the pathway in plants, further confirmed by radio-labeling experiments. This led to the first report on the occurrence of tropane biosynthesis in aerial parts of any plant (PLoS One, 2013)

In addition to the work assigned for Ph. D., I was also associated with fellow scholars dealing with the projects related to other pathways of secondary metabolism of *Withania*, especially withanamide biosynthesis.

Summary of M. Sc. Dissertation at OUAT, Bhubaneswar (July 2006- Oct 2008)

Title of work: 'Standardization of Protocol for Genetic Transformation of Brinjal (Solanum melongena L)'

Supervisor: Dr. D. Mahapatra

This project was focused on developing a protocol for *Agrobacterium*-mediated transformation of Brinjal/eggplant *cv* Utkal Anushri. To accomplish the goals, first, a protocol was standardized to develop multiple shoots via callus induction. Different hormonal combinations were tried. Then, parameters were optimized for the *Agrobacterium*-mediated transformation of callus.

Personal details

Date of birth : February 06, 1985

Gender : Male Marital status : Unmarried

Father's Name : Mr. R. L. Kushwaha Permanent Address : 218, Village Amanabad

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References

Dr. R. S. Sangwan

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