Systems analysis of plant metabolism under UV-B radiation by integrating metabolomics and $^{13}CO_2$ labeling

A Thesis submitted by

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Under the supervision of

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Dedicated

to

My beloved

Parents

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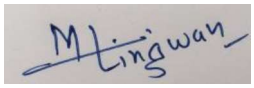
Declaration by the Research Scholar

I hereby declare that the entire work embodied in this Thesis is the result of investigations carried out by me in the **School of Basic Sciences,** Indian Institute of Technology Mandi, under the supervision of **Dr Shyam Kumar Masakapalli,** and that it has not been submitted elsewhere for any degree or diploma. In keeping with the general practice, due acknowledgements have been made wherever the work described is based on finding of other investigators.



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Declaration by the Research Advisor

I hereby certify that the entire work in this Thesis has been carried out by **Maneesh Lingwan,** under my supervision in the **School of Basic Sciences,** Indian Institute of Technology Mandi, and that no part of it has been submitted elsewhere for any Degree or Diploma.

Date: 31/08/2021

Signature:

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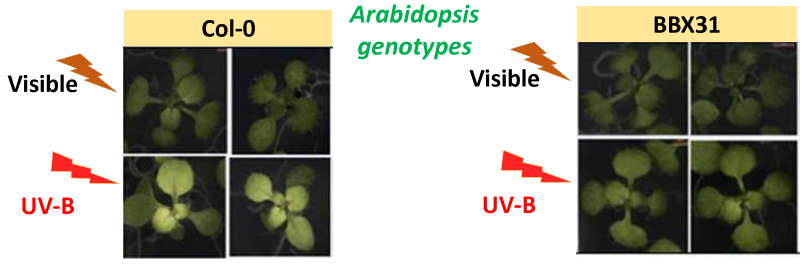
Maneesh Lingwan

Graphical Abstract

Thesis Goal

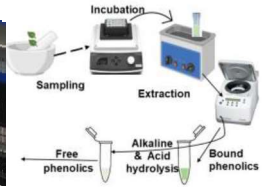
Metabolic Analysis of plant systems under UV-B radiation
Metabolic reprogramming and survival strategy?

Plant Growth Phenotypes analysed



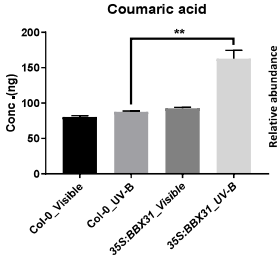
- Optimised robust metabolic analytics workflow
- Novel $^{13}CO_2$ feeding prototype developed

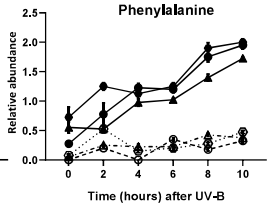
Optimise workflow (GC-MS based)
- Metabolite profiling (Monophenols focused)
- Metabolomics

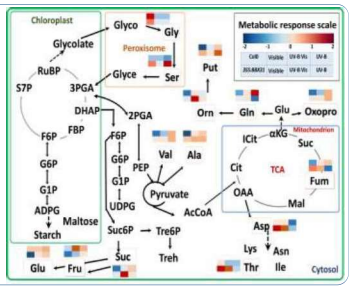


Parallel $^{13}CO_2$ feeding chambers set up

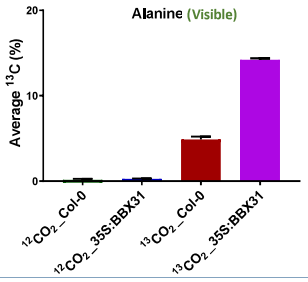
- Metabolomics of Col-0 and BBX31 showed effects on key pathways

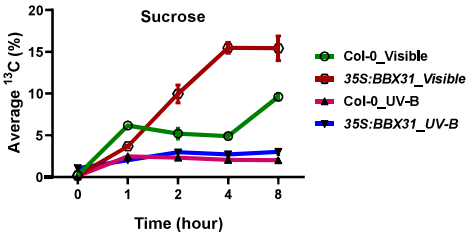






- $^{13}CO_2$ labeling shed light on the dynamic metabolism from source to sink in the genotypes





Major Outcomes of the Thesis

- UV-B mediated system-wide metabolic reprogramming deciphered
- Shikimate, Fatty acid, Phenolics and other pathways affected
- Phenolics levels elevated under UV-B (BBX31 more profound – photoprotection and physiological roles)
- BBX31 overexpressor – high carbon fixation , biomass and metabolism under normal light (13C analysis - novel insights)
- UV-B as a trigger for targeted enrichment of Phenolics proposed
- Robust analytical methods of GC-MS & $^{13}CO_2$ feeding prototype can be adopted widely

Abstract

Deciphering the response of plants to biotic and abiotic stress is of immense scientific interest owing to their role in agriculture and environment. Under optimal and varying environmental parameters, how plants metabolise CO_2 and regulate the metabolic flow from source to sink will allow identifying and developing efficient crops with desirable traits (Allen *et. al.,* 2016). Among the abiotic parameters, UV-B radiation (290 nm to 315 nm), an indispensable element of the sun's spectrum influences growth and development of plants. Plants perceive light signals through photoreceptors and regulate their physiological, developmental and metabolic processes via a family of B-box (BBX) proteins (Song et al., 2020). Among these, BBX31, a transcription factor is reported to play a significant positive regulatory role under UV-B radiation (Yadav *et. al.,* 2019). The metabolic phenotypes of plant systems with altered B-box protein expression under the visible and UV-B radiation are not well-defined. The main goal of this thesis is to decipher the precise metabolic phenotypes of *Arabidopsis thaliana* Col-0 and BBX31 mutant (*bbx31)* and overexpressor (*35S:BBX31)* lines under the influence of visible and UV-B radiation by integrating robust metabolic systems biology approaches of metabolite profiling, metabolomics and $^{13}CO_2$ tracer-based pathway mapping.

First, robust methodologies of metabolite profiling and metabolomics using Gas Chromatography-Mass Spectrometry (GC-MS) data analysis were optimized. This allowed comprehensive analysis of sugars, organic acids, amino acids and secondary metabolites. Much focus is laid on developing a robust methodology for the analysis of free, glycoside and ester bound phenolics, whose levels are known to be influenced under UV-B. Second, the Arabidopsis Col-0 seedlings subjected to UV-B radiation showed negative effects on the morpho-physiological parameters with diminished growth. Also, their metabolic analysis provided detailed insights into the central and secondary metabolic pathways under UV-B. Third, the metabolomics and pathway analysis of Arabidopsis Col-0, *bbx31* mutant and *35S:BBX31* overexpressor provided novel insights into the UV-B mediated metabolism. The morphological and metabolic phenotypes of *35S:BBX31* overexpressor lines are distinct owing to their reprogrammed metabolism under visible as well as UV-B. BBX31 markedly influenced the levels of photosynthetic compounds, central and secondary metabolites. Mainly, the metabolic pathways involving GS/GOGAT, fatty acid, shikimate, phenolics and flavonoids are regulated, eventually conferring photoprotection under UV-B. Finally, with the aim to define the precise metabolic phenotypes, $^{13}CO_2$ labeling of Col-0 and *35S:BBX31* overexpressor lines was undertaken in a novel designed and built prototype of controlled

feeding chambers. The prototype includes mass flow controllers for achieving optimal composition of gases, step change for controlled $^{13}CO_2$ feeding at desired ppm levels, parallel growth chambers with controlled environmental parameters. *BBX31 overexpression* line in comparison to Col-0 showed enhanced rates of incorporation of ^{13}C in the mass isotopomers of sugars, soluble amino acids and proteinogenic amino acids under visible light as well as UV-B respectively. The ^{13}C analysis highlighted distinct metabolic phenotypes of Col-0 and BBX31, eventually shedding light qualitatively on the activities of various metabolic pathways and source to sink CO_2 mapping.

Overall, the robust methodologies of metabolic analysis facilitated defining the metabolic phenotypes of Arabidopsis genotypes (Col-0, *bbx31*, *35S:BBX31*) under the influence of visible and UV-B. The outcomes provided novel insights into our understanding of the BBX31 mediated metabolic rewiring that eventually confer UV-B tolerance in plants. Broadly, the optimised metabolic analysis workflow including plant phenolics and the prototype of $^{13}CO_2$ parallel feeding set up can readily be adopted to investigate other plant systems under biotic and abiotic stress of interest for agricultural applications.

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