Correlation of Phytochrome B with Auxin signaling in Plants

By

SONY KUMARI

LIFE11201204004

National Institute of Science Education and Research,

Bhubaneswar

A thesis submitted to the
Board of Studies in Life Sciences
In partial fulfillment of the requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Of

HOMI BHABHA NATIONAL INSTITUTE

December, 2019

Homi Bhabha National Institute

Recommendations of the Viva Voce Committee

As members of the Viva Voce Committee, we certify that we have read the dissertation prepared by **Sony Kumari** entitled **"Correlation of Phytochrome B with Auxin signaling in Plants"** and recommend that it may be accepted as fulfilling the thesis requirement for the award of Degree of Doctor of Philosophy.

Chairman - Name & Signature with date **Dr. Chandan Goswami**	13/12/19
Guide / Convener - Name & Signature with date **Dr. Kishore CS Panigrahi**	13/12/19
Co-guide - Name & Signature with date (if any)	
Examiner - Name & Signature with date **Prof. Utpal Nath, IISC Bangalore**	13/12/19
Member 1- Name & Signature with date **Dr. Praful Singru**	13/12/19
Member 2- Name & Signature with date **Dr. Pratap Sahoo**	13/12/2019
Member 3- Name & Signature with date **Prof. A. B. Das, Utkal University**	13.12.2019

Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to HBNI.

I/We hereby certify that I/we have read this thesis prepared under my/our direction and recommend that it may be accepted as fulfilling the thesis requirement.

Date: 13.12.19

Place: NISER, Bhubaneswar

Signature
Co-guide (if any)

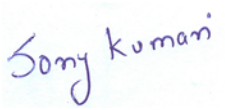
Signature
Guide

[1] This page is to be included only for final submission after successful completion of viva voce.

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at Homi Bhabha National Institute (HBNI) and is deposited in the Library to be made available to borrowers under rules of the HBNI.

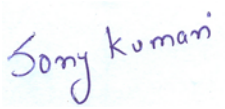
Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the Competent Authority of HBNI when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.



Sony Kumari
NISER, Bhubaneswar

DECLARATION

I, hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree / diploma at this or any other Institution / University.



Sony Kumari
NISER, Bhubaneswar

List of Publications arising from the thesis

Journal

1. *Uncovering the molecular signature underlying the light intensity-dependent root development in *Arabidopsis thaliana*. **Sony Kumari**, Sandeep Yadav, Debadutta Patra, Sharmila Singh, Ananda K Sarkar and Kishore C S Panigrahi. *BMC Genomics. 2019, 20(1):596.*
2. *An understanding towards light dependent auxin dynamics in protoplast system using a luciferase-based auxin sensor. **Sony Kumari** and Kishore Panigrahi. *Pantnagar Journal of Research. 2019, Vol 17(1) Jan-Apr*.
3. *Light and auxin signaling cross-talk programme root development in plants. **Sony Kumari** and Kishore Panigrahi. *Journal of biosciences. 2019, Mar 1;44(1):26.*

Book Chapters

*Influence of Light-Hormone Interaction on Seedling development in *Arabidopsis thaliana*. **Sony Kumari** and Kishore Panigrahi. *Cambridge Scholars Publishing. Proceedings of International Conference on Plant Development Biology, 2018.*

*Root growth and development influenced by abiotic stress due to climate change. **Sony Kumari,** Debadutta Patra, Nidhi Mishra and Kishore C S Panigrahi. *Springer Nature 2019.* **Communicated**

Other publications

Alkaline phosphatase activity of a phosphate solubilizing *Alcaligenes faecalis*, isolated from Mangrove soil. Bikash Chandra Behera, Himendra Yadav, Santosh Kumar Singh, Bijay Kumar Sethi, Rashmi Ranjan Mishra, **Sony Kumari**, Hrudayanath Thatoi. *Biotechnology Research and Innovation. 2017, 1.1: 101-111*.

Conference proceedings

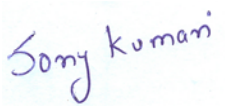
Heavy Metal Absorption Efficiency of two Species of Mosses (*Physcomitrella patens* and *Funaria hygrometrica*) Studied in Mercury Treated Culture under Laboratory Condition.

Abanti Pradhan, **Sony Kumari**, Saktisradha Dash, Durga Prasad Biswal, Aditya Kishore Dash, Kishore C. S. Panigrahi. *IOP Conference Series: Materials Science and Engineering*. *2017, Vol. 225. No. 1. IOP Publishing.*

***Pertaining to thesis**

Conference Presentations:

1. Interaction of Phytochromes and auxin signaling in root pattering. **Sony Kumari** and Kishore Panigrahi. *National Conference on Bioprospecting in Life Sciences Research for Human Welfare & 41st Annual Conference Orissa Botanical Society*. Sambalpur University. Odisha, India.
2. Correlating the role of red-light signaling pathway with root architecture in *Arabidopsis thaliana*. **Sony Kumari** and K.C. Panigrahi. *International Symposium on Insight to Plant Biology in the Modern Era*. Bose Institute, Kolkata, India.
3. Phytochrome and sugar signaling cross-talk to modulate root architecture in *Arabidopsis thaliana*. **Sony Kumari**, Debadutta Patra, Durga Prasad Biswal, K.C. Panigrahi. *International Conference on Plant Development Biology and 3rd National Arabidopsis Meeting*. National Institute of Science Education and Research, Odisha, India.
4. Interaction of phytochrome and sugar signaling affecting root development in *Arabidopsis thaliana*. **Sony Kumari**, Debadutta Patra, Durga Prasad Biswal, K.C. Panigrahi. *International Symposium on Plant Photobiology*. Matsue, Japan.
5. Light and sugar signaling cross-talk regulates root architecture in *Arabidopsis thaliana*. **Sony Kumari**, Debadutta Patra, Durga Prasad Biswal, K.C. Panigrahi. *National Conference on Biotechnological Interventions for Environmental stress Management in Plants and Microbes & 42nd Annual Conference of Orissa Botanical Society*. Utkal University, Odisha, India.
6. Involvement of carbon nanoparticles in light dependent root patterning of *Arabidopsis thaliana*. **Sony Kumari**, Nidhi Mishra, Debadutta Patra, Kishore Panigrahi. *National Conference on "Plants for Sustainable Development and Clean Environment" and 43rdAnnual Conference of Orissa Botanical Society (OBS)*. Centurion University, Odisha, India.



Sony Kumari
NISER, Bhubaneswar

Dedicated to my family and friends…

ACKNOWLEDGEMENT

First of all, I would like to thank my PhD. supervisor Dr. Kishore Panigrahi for his valuable ideas and support during my PhD. He has not only helped in improving my research abilities but also enriched my writing and communication skills. He always pushed me to think first, analyse and then execute any scientific problem. This attitude helped me a lot to work directional and independently.

I would also like to thank my thesis monitoring committee members: Dr. Chandan Goswami, Dr. Praful Singru, Dr. Pratap Sahoo and Prof. Anath Bandhu Das for their valuable suggestions and motivation to fulfill all the objectives. I would also like to thank DAAD exchange program, Prof. Klaus Palme, Dr. Cristina Dal Bosco and Dr. Alexander Dovzhenko, University of Freiburg, for giving me opportunity to work at an international platform. They gifted luciferase construct for the protoplast transformation. I am also thankful to Dr. Ananda Sarkar, Sandeep and NIPGR facility for helping me at the crucial stage of my PhD period. I also like to thank Dr. N. Dey, Ahamed and the plant growth facility at ILS, Bhubaneswar which was a life saver during the shifting of NISER from transit to main campus at Jatni. I would also like to thank Mr. Subhayan from Chemistry dept. NISER for supporting me with some essential stuffs.

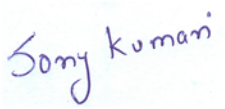
I sincerely thank NISER, SBS staffs and central facility for their co-operation and support. My gratitude also goes to greenhouse members Dr. Balaji, Mr. Ashok, Mr. Surya Narayan and greenhouse facility.

I also thank Dr. Madhusmita Panigrahy for guiding me with her valuable suggestions and my senior Dr. Abhishek Kumar for helping in starting my work at lab. My heartfelt thanks goes to my lab members specially Ms. Maitree Pradhan (taught me the first technique at lab), Mr. Debadutta (my lab support system and lab partner), Mr. Durga and Mr. Kishan for their timely and

unconditional support in my professional and personal life. Durga helped me to improve my work strategies and never thought of day or time while helping me. I would like to say special thanks to Debadutta as he was always there to help me without thinking about clock and we both were student and mentor of each other. I also appreciate the valuable inputs of other lab members: Mr. Aaram, Ms. Subhashree, Dr. Priyanka, Ms. Khushboo, Dr. Aditya, Ms. Anamika, Ms. Soumya, Mr. Braj, Ms. Nibedita, Mr. Linkan, Mr. Jyoti, Mr. Mukundan, Mr. Aman, Ms. Anisa, Ms. Alena, Mr. Swarnendra, Dr. Bedabrata, Mr.Sarat and other juniors at lab. I am also grateful to all the seniors from other labs, Dr. Anoop, Dr. Usha, Dr. Charles, Dr. Uday (taught me first technique at NISER, IOP Campus), Dr. Ashutosh, Dr. Ankit, Dr. Biswaranjan, Dr. Sushree, Dr. Sanjima, Dr. Manoj, Dr. Mitali, Dr. Gargi, Dr. Biswajit and juniors: Late Malloji Vinay, Ms. Himani, Ms. Bushra and Ms. Somdatta. I also appreciate the wonderful contribution of my batchmates cum friends Dr. Shrikant (biggest support), Dr. Subhransu, Dr. Rakesh and Mr. Yakub who always stood beside me during the struggling days. Dr. Subhransu and Mr. Yakub always motivated me to work hard. Sincere thanks to Dr. Shrikant who helped not only in PhD work but also taught me to keep patience and finish work properly. He was always there to help me in professional and personal life. I would like to give special thanks to Mr. Sushant Kumar Singh (my genuine critic) from VECC Kolkata, who doesn’t belong to my subject, still boosted me and supported with directional strategies. I also thank my hotel buddies Dr. Anima (my master chef), Dr. Rita, Dr. Srijani and friends Dr. Avanendra, Dr. Samir and Ms. Nidhi (my little girl) for their lovely company and moral support. I also extend my thanks to my local guardian and friends Ms. Arati (sister) and Mr. Gautam, with whom I started my journey at NISER and they made my stay homely and wonderful. I especially thank Shrikant, Durga, Debadutta and Nidhi for proof reading my thesis.

My PhD work would not have been successful without the love, blessings and support of my parents, brothers, sister-in-law (sisters) and my three lovely kids. I give my whole hearted thanks to them for their guidance and making me reach to this position. I am grateful to them for making me so strong to handle the hurdles of PhD and complete all the goals. I would also like to thank my grandparents and family for their faith in me.

I also acknowledge HBNI, DAE for the financial support and Director, NISER for allowing me to carry out my work at this prestigious institute.



Sony Kumari
NISER, Bhubaneswar

CONTENTS

Abstract

Correlation of Phytochrome B with Auxin signaling in Plants

Light as an external factor plays important role in the growth and development of plants. On the other hand, phytohormones are the principal internal regulators controlling various plant responses. They have been investigated to govern several key processes in plants, beginning with seed germination till their maturity. Phytochrome B (PHYB) is the major red-light photoreceptor and performs various functions in plant development. Auxin is the principal phytohormone which controls many critical plant activities. Roots are essential organ which assist in water and mineral intake, provide support and interact with microbes in soil to help in presenting disease resistance. PHYB and auxin signaling have been documented to crosstalk and control several developmental aspects of plants. However, limited information is available about the PHYB and auxin interaction in root growth control. Along with this, the intermediate components playing role in this interaction are also not well studied. The key objective of this current thesis work is to explore the cross-talk of PHYB with auxin signaling in root architecture and protoplast system. In the present work, it has been investigated that different light intensity affect the root architecture and also alter the expression of genes engaged in phytohormone, light and circadian pathways. It has also been documented that the carbon nanoparticle and sugar mediated alteration in root morphology and in expression of phytohormone associated genes are light quality and PHYB dependent. Auxin dynamics has been shown to be similar in *phyB* mutant and wild-type protoplast. Hyposensitive red-light signaling mutants with altered root phenotype have been generated which can possibly provide information about the molecular players in the auxin and PHYB cross-talk in the root development. This current thesis work provides critical information about the involvement of red

light and PHYB in root morphology as well as possible candidates involved in the interaction of PHYB and auxin signaling.

Summary of thesis

Phytochrome B (PHYB) is the principal photoreceptor which perceives red light and regulates several features of root architecture. It has been shown to control primary root elongation, lateral root development, root hairs, gravitropism etc. It senses the shade condition or reduction in the ratio of red/far-red light and alters the root morphology depending upon the quantity of light. Different intensity of light has been shown to affect the root morphology but the components engaged in this phenomenon are not completely known. In the present work, the candidate genes which are altered in the roots under different light intensity have been investigated. The genes playing role in circadian, phytohormone and light regulated pathways were found to be significantly altered.

Carbon nanoparticles (CNPs) are the nanomaterial which are known to be very beneficial in the field of agriculture and affect the root development. The involvement of red light or PHYB in CNP mediated root growth has not been studied till yet. In the present work, CNP has been shown to alter primary, lateral and adventitious roots, root waving and root coiling phenomenon in *phyB-9* mutant seedlings under different light conditions. They also altered the phytohormone associated genes in the root under white and red light.

PHYB controls the root development by interacting directly or indirectly with various internal factors of plants such as sugar and phytohormones. Sugar and phytohormones are the major internal elements which determine the root morphology and physiology. Sucrose and glucose are the main sugar types which are present in the plant and are involved in root development. Auxin is the key phytohormone which participates in root growth. However, the molecular players present in the PHYB-sugar signaling crosstalk and PHYB -auxin signaling crosstalk are not well documented. In the current study the root architectural changes have been documented in presence

of different light and sugar types. The auxin related genes in the root under these experimental conditions were also analysed. Sucrose, glucose, maltose, mannitol and sorbitol have been observed to influence the primary root length, root waving and root coiling processes in *phyB-9* mutant seedlings under different light conditions. They also altered the expression of auxin associated genes in the root under white and red light.

Auxin and PHYB interactions have been studied in intact plants. However, their interaction has not been well explored in the protoplast system. In the current work, it has been attempted to study auxin dynamics in the *phyB-9* protoplast with a novel chemiluminescence ratiometric auxin sensor. It was observed that auxin dynamics is similar in the *phyB-9* and wild-type protoplasts with red and white light treatment. In contrast, the expression of auxin signaling genes was different in the *phyB-9* and wild-type protoplasts.

In the present work, several hyposensitive PHYB mutant lines (under red light) were generated which have shown alteration in PHYB nuclear complex formation and root morphology. These mutants possibly have defect in red light and auxin related pathways. This thesis work delivers important clues about the involvement of red light and PHYB in root growth and the potential elements associated with the PHYB-auxin crosstalk.

LIST OF ABBREVIATIONS

ABA	Abscisic Acid
ABI5	ABA Insensitive 5
At	*Arabidopsis thaliana*
ARR6	*Arabidopsis* type a Response Regulator 6
ABCB	ATP-Binding Cassette subfamily B
AFB	Auxin F-box
ANN1	Annexin
ATHB4	*Arabidopsis thaliana* Homeobox-leucine zipper protein-4
AUX/IAA	Auxin/Indole-3-Acetic Acid
AUX1/LAX	Aux1/Like Aux1
AXR2	Auxin Resistant 2
ARFs	Auxin Response Factors
AUXREs	Auxin Responsive Elements
bHLH	Basic Helix-Loop-Helix
BP	Biological Process
B	Blue
BZR1	Brassinazole-Resistant 1
BR	Brassinosteroid
CNP	Carbon Nanoparticle
CC	Cellular component
CTAB	Cetyltrimethylammonium Bromide

CAB	Chlorophyll A/B Binding
CCA1	Circadian Clock-Associated 1
Col-0	Columbia
CO	Constans
COI1	Coronatine Insensitive 1
COL3	Constans Like 3
COP1	Constitutive Photomorphogenic 1
CIP1	COP1 Interacting Protein 1
CSN6A	COP9 Signalosome complex subunit 6A
CRYs	Cryptochromes
CYP79B2	Cytochrome p450 monooxygenases
CK	Cytokinin
DEGs	Differentially Expressed Genes
DR5	Drought Resistant 5
EPR1	Early Phytochrome Response 1
ER	Endoplasmic Reticulum
EMS	Ethyl Methane Sulfonate
ET	Ethylene
EIN2	Ethylene Insensitive protein 2
FC	Fold Change
FDR	False Discovery Rate
FR	Far-red
FHY1	Far-red elongated Hypocotyl 1

FHL	FHY1-Like
FT	Flowering locus T
fCNTs	Functionalized Carbon Nanotubes
GO	Gene Ontology
GA	Gibberellic Acid
GLU	Glucose
GFP	Green Fluorescent Protein
GH3	Gretchen Hagen 3
gin2	*glucose insensitive 2*
GUS	β-glucuronidase
HFR1	Long Hypocotyl in Far-red1
HGI	Horizontal Growth Index
HIRs	High Irradiance Responses
HXK1	Hexokinase 1
HY5	Elongated Hypocotyl 5
HYH	HY5 Homologue
IAOX	Indole-3-Acetaldoxime
IAM	Indole-3-Acetamide
IAA	Indole-3-Acetic acid
IAN	Indole-3-Acetonitrile
IBA	Indole-3-Buytric acid
IPYA	Indole-3-Pyruvic acid
JA	Jasmonic Acid

JAZ	Jasmonate Zim domain
KMD3	Kiss Me Deadly 3
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAF1	Long After Far-red light 1
Ler	Landsberg erecta
LFRs	Low Fluence Responses
LB	Luria-Bertani broth
LD	Long Day
MAL	Maltose
MAN	Mannitol
MED	Mediator
MF	Molecular Function
MWCNTs	Multi-Walled Carbon Nanotubes
MS	Murashige and Skoog
NMs	Nanomaterials
NITs	Nitrilases
NPH3	Non-Phototropic Hypocotyl 3
OD	Optical Density
PAT	Polar Auxin Transport
PER	Peroxidase
PIN	Pin-formed
PGP	P-glycoproteins
PHOTs	Phototropins

PHYs	Phytochromes
PIFs	Phytochrome Interacting Factors
PIL5	PIF3-Like 5
PAR1	Phytochrome Rapidly Regulated 1
psi2	*phytochrome signaling 2*
PEG	Polyethylene Glycol
PVP	Polyvinylpyrrolidone
PRR9	Pseudo Response Regulator 9
POLYUBIQ	Polyubiquitin
R	Red
ROS	Reactive Oxygen Species
RAG1	Response to Auxins and Gibberellins 1
RIN	RNA Integrity Number
RTH1	Root Hairless 1
RHS	Root Hair Specific
RTCS	Rootless concerning Crown and Seminal roots
SA	Salicylic Acid
SDG	Set Domain Group
SM	Sensor Module
SAR	Shade Avoidance Response
SWCNTs	Single-Walled non-functionalized Carbon Nanotubes
SLCYP1	*Solanum lycopersicum* cyclophilin
SRL1	Solitary root 1

SAUR36	Small Auxin Upregulated RNA 36
SOR	Sorbitol
SKP1	S-phase Kinase-associated Protein-1
SL	Strigolactone
SUC	Sucrose
SUR2	Superoot 2
SPA1	Suppressor of PHYA 1
TOR	Target Of Rapamycin
TOC1	Timing of Cab expression 1
TIR1	Transport Inhibitor Response 1
TRA	Tryptamine
TAA1	Tryptophan Aminotransferase 1
TAR1	Tryptophan Aminotransferase-Related
UVR-8	UVB-Resistance 8
VLFRS	Very-Low Fluence Responses
WD	Wave Density
W	White
wei	*weak ethylene insensitive*

LIST OF FIGURES

Chapter 3

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Chapter 1

1. Introduction

1.1. *Arabidopsis thaliana* and root development

1.1.1. *Arabidopsis thaliana* a classical model plant

Arabidopsis thaliana (*At*) is a facultative long-day dicot plant. It belongs to Brassicaceae family and is widely spread in the American, Asian and European regions. It has a shorter life-cycle of ~2-3 months and produces large number of seeds at a time. It is smaller in size and needs lesser space to grow. The genome size of *At* is approximately 120 mega bases that are distributed in five pair of chromosomes. Its genome has been fully sequenced and annotated, due to which gene manipulation has become easier (1,2). Large number of transgenic and mutant lines can be generated in a short duration of time. Although there are about 1000 ecotypes of *At*, Landsberg *erecta* (*Ler*) and Columbia (Col-0) are the most commonly used.

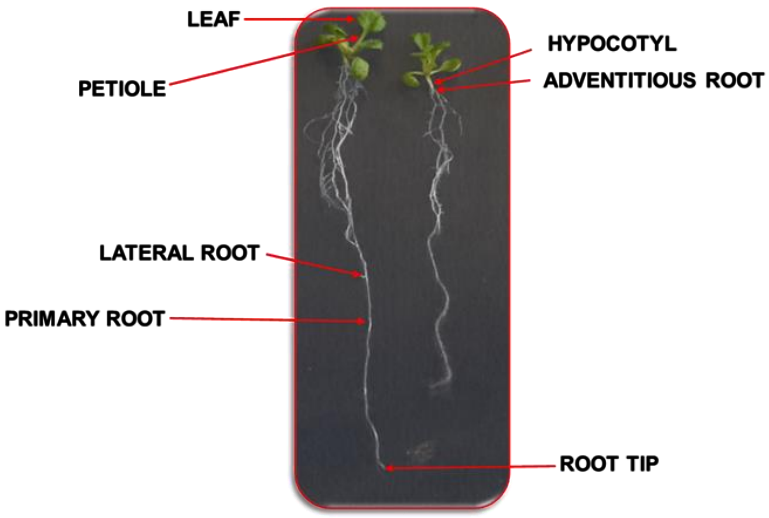


Fig.1.1. Structure of ten-day old *Arabidopsis* seedling

A young seedling has various distinct parts such as hypocotyl, leaves, primary, lateral and adventitious roots and root hairs (Fig.1.1).

1.1.2. Root morphology and architecture

Roots are the essential organ of plants as they provide support and anchorage to the plants. They also help in absorption of water and different minerals from the soil. The *At* seedlings can be grown on media plate, their root system is very simple and can be analysed with ease. Primary root is the main root arising from the base of the hypocotyl. The tip of primary root invades through the pores of the soil, where root cap covers the root tip and provide protection.

Root is divided into three different zones: meristematic zone, elongation zone and zone of cell differentiation. Meristematic zone is present behind root cap and it consists of dividing cells, here cells keep on dividing continuously. Above meristematic zone elongation zone is present, where cell elongation occurs that leads to lengthening of roots. Cell differentiation zone is present above zone of elongation, where cells get matured and differentiated. Primary root tissue has three different of cell layer from outside to inside: epidermis, cortex and endodermis. The outer layer is epidermis which helps in absorption of water and minerals and it possess root hairs which enhance the rate of absorption process. The cortex cells are involved in conducting water and minerals to vascular tissues and other parts of plants. The inner most layer is endodermis; it regulates the passage of water and minerals from cortex to vascular tissues. The vascular tissues are present inside the endodermis and surrounded by another layer known as pericycle (3,4). Xylem and phloem are the vascular tissues present in the root, xylem is involved in water and mineral transport while phloem helps in transportation of food material such as carbohydrates (3).

Along with primary root, *At* has secondary, tertiary and adventitious roots (Fig.1.2) (5). The primary roots are first to develop from the embryo. The secondary or lateral roots originate from primary root in a post-embryonical manner. They initiate from pericycle cell layer which is present adjacent to the xylem pole and further tertiary roots emerge from secondary roots. Adventitious roots also originate post-embryonically and develop from the root and shoot junction. In root apical meristem, the epidermal cells which are in the vicinity of two cortex cells, produce root hair cells. In differentiation zone, the root hair cells form root hairs (6). The primary, lateral, adventitious roots and root hairs all together help in anchorage to soil as well as in absorption of water and minerals from the soil.

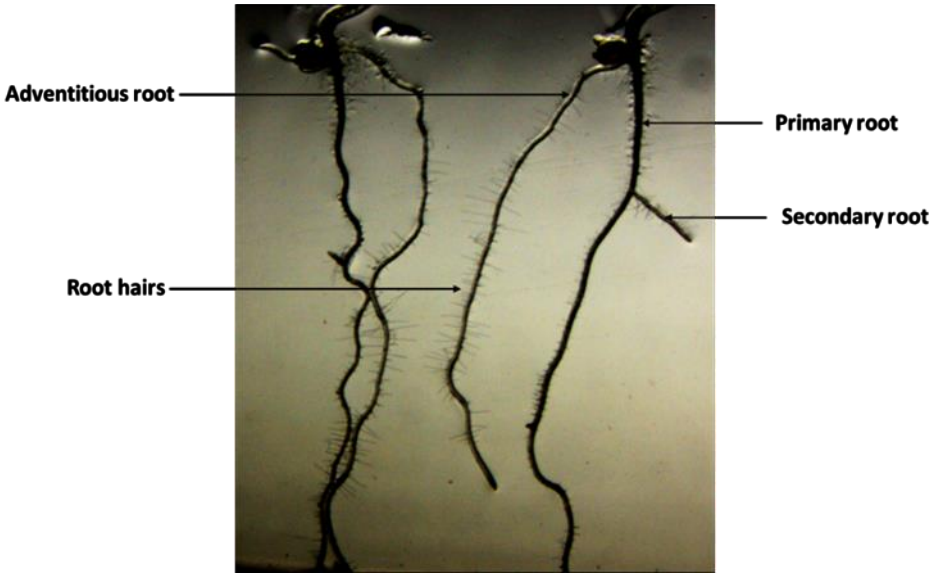


Fig.1.2. Basic root architecture of *Arabidopsis thaliana*

Roots do not grow straight and while growing they form different patterns such as root coils, root waves, skewing etc (Fig.1.3) (7). The movement of roots is influenced by different external factors like gravity, humidity, light, pH, nutrient quality and some of the internal regulators such

as phytohormones and nutrients (8). Gravitropism, thigmotropism and circumnutation might be potential factors causing coiling and wave formation in roots (7, 9, 10). These root patterns could possibly help roots in penetrating through different textures of soil and facilitate plant growth.

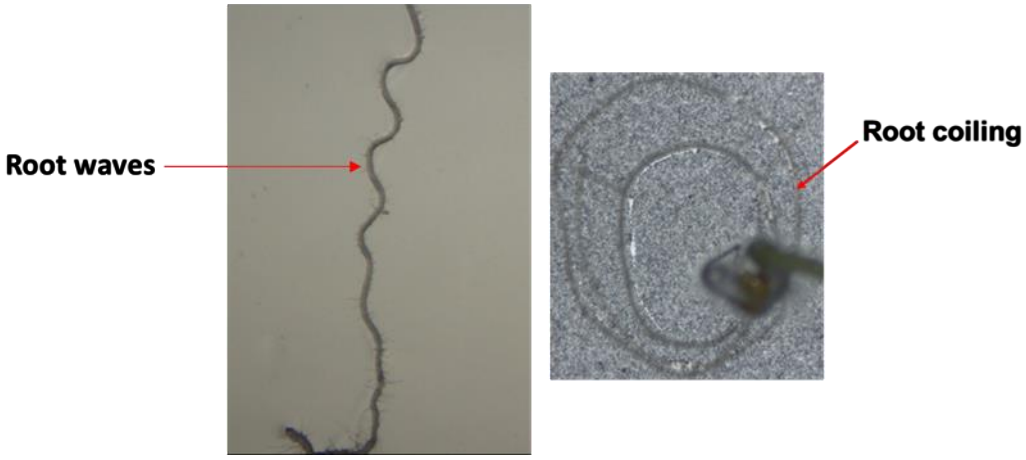


Fig.1.3. Root waving and coil formation in *Arabidopsis thaliana*

1.1.3. Factors affecting the development and growth of roots

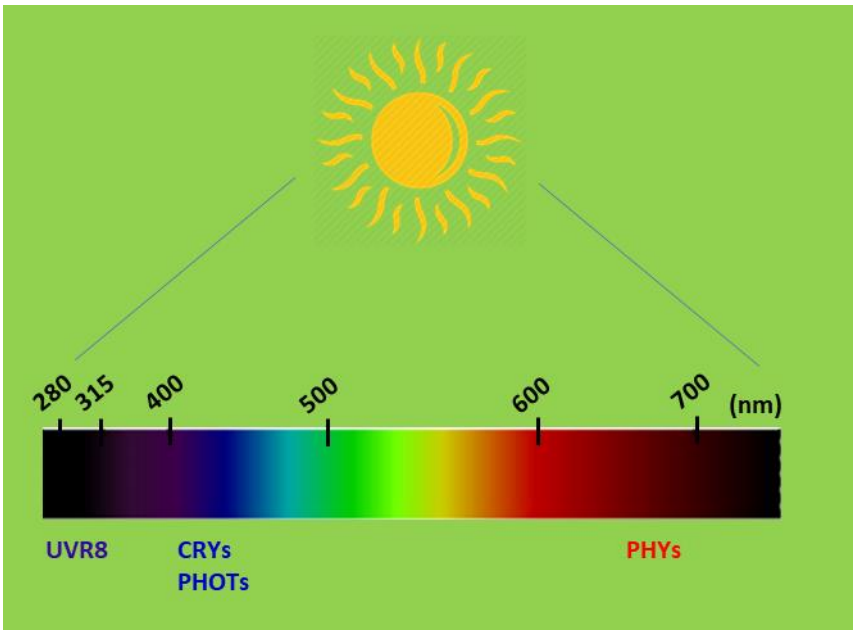
Plants are sessile in nature and their growth is dependent upon various biotic and abiotic factors. The biotic and abiotic components such as microbes, fungi, mycorrhizae, micronutrients, macronutrients, hormones, salt, temperature and light influence the root development in plants (11). These factors individually or collectively affect the health of plants.

The living components affecting plant growth and development are known as the biotic factors. The major biotic factors are bacteria, fungi, insects, earthworm, mycorrhizae etc. These factors have beneficial as well as harmful effects on plant health and productivity. Abiotic factors are the non-living components such as light, phytohormones, nutrients, gases, temperature and humidity which influencing the plant growth. Among these, light is the most vital abiotic factors involved

in regulating root development and phytohormones are considered as the most important internal factors playing vital roles in root growth (12).

1.2. Photoreceptors in *Arabidopsis*

Light is a major source of energy which regulates most of the plant developmental processes beginning from seed germination till senescence. The light quality, quantity, duration and direction have been shown to modulate the development of plants. It plays important role in photomorphogenesis, shade avoidance response (SAR), flowering and phototropism. SAR is a phenomenon in which plants compete for light under lower ratio of red:far-red and that leads to elongation of hypocotyl, lower chlorophyll, smaller leaves, elongated petiole and early flowering (13).



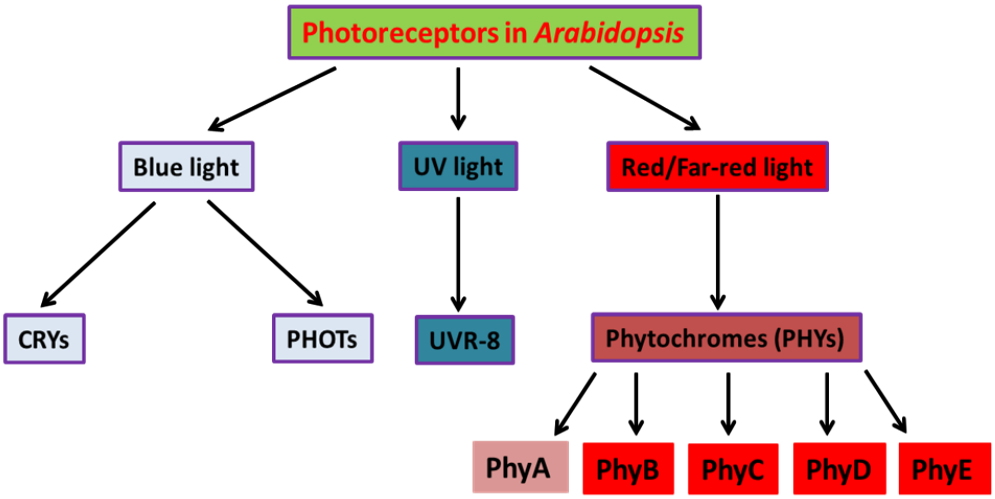


Fig.1.4. Photoreceptors in *Arabidopsis thaliana*

Plants perceive different qualities and quantities of light via various photoreceptors. They possess photoreceptors such as: UVB-resistance 8 (UVR-8), Phototropins (PHOTs), Cryptochromes (CRYs) and Phytochromes (PHYs) which function in different range of wavelengths (Fig.1.4) (14). The photoreceptors are present throughout the plant body with majority of them located in shoot region; however, they are not absent in roots (15).

1.2.1. Phytochromes

PHYs sense the red (R) and far-red (FR) light wavelength. In *At*, PHY gene family consists of five members: PHYA-E. They absorb light in the array of 600-750 nm. PHYA perceives FR light and mediates the FR-dependent light responses whereas the other four PHYs are mainly involved in R light photo perception. PHYs also sense the shade condition under low light. PHYs are dimeric in nature and each of the monomer consists of two components: apoprotein and phytochromobilin. Apoprotein is covalently linked to light absorbing chromophore, phytochromobilin. They exist in two interchangeable forms: Pr and Pfr. Pr and Pfr are the R and FR light absorbing forms respectively. Synthesis of PHY occurs in the cytoplasm in the Pr form,

upon light irradiation Pr changes to Pfr form and enters inside the nucleus (16, 17, 18, 19, 20). It has been reported that PHYB translocates to the nucleus by itself, however, PHYA is carried to the nucleus with the help of proteins for example, FAR-RED ELONGATED HYPOCOTYL1 (FHY1) and FHY1-LIKE (FHL) (19, 20).

Inside the nucleus, in the presence of light, Pfr form interacts with the PHYTOCHROME INTERACTING FACTORS (PIFs), phosphorylates them and that results in the degradation of PIFs. However, in the dark condition PIFs accumulate and interact with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), form complex and further PHYs are degraded in ubiquitin-mediated manner (16) (Fig.1.5). PIFs are the transcription factors with basic helix-loop-helix (bHLH) domain. In *At*, eight type of PIFs are present: PIF1-PIF8 (18, 21). They have been shown usually to act antagonistic to PHYs in most of the plant processes. On the contrary, PIF2 is an exception which acts along with the PHYB and promotes de-etiolation under R, FR and blue (B) light (21). De-etiolation responses are characterized by shorter hypocotyl, more amount of chlorophyll, apical hook opening, etc. PIFs have major roles in hormone signaling, circadian clock regulation, temperature signaling, seed germination, etc (21). COP1 is an E3-ubiquitin ligase and in dark represses the photomorphogenesis. Photomorphogenesis is defined as light dependent morphological responses in plants such as short hypocotyl, chlorophyll accumulation etc (22). COP1 and PIFs are the positive regulators of skotomorphogenesis. Skotomorphogenesis is the dark adaptation in plants, where major part of energy and resources are allocated to hypocotyl elongation at the cost of root and cotyledon growth (23). Light signal transduced by PHYs regulates COP1 and PIFs and further controls different downstream transcription factors for example, ELONGATED HYPOCOTYL 5 (HY5), HY5 HOMOLOGUE (HYH), LONG

AFTER FAR-RED LIGHT 1 (LAF1), LONG HYPOCOTYL IN FAR-RED 1 (HFR1) and stimulate photomorphogenesis (16, 22).

It has been reported that PHYA shares high sequence similarity with PHYC whereas PHYB shows resemblance to PHYD and PHYE. PHYA is a photolabile protein having half-life of ~30 minutes while PHYB-PHYE are photostable (16). As PHYA is a photolabile PHY, it is present in ample amount in plants grown under dark. On the other hand, PHYB-PHYE are found in plants grown under light condition and among these, PHYB is the most abundant one. PHYs mediate three different modes of action; very-low fluence responses (VLFRs), low fluence responses (LFRs) and high irradiance responses (HIRs). VLFRs are induced by low light intensities of B, R and FR. Pr to Pfr photoconversion is mediated by LFRs. Prolonged exposure of high light intensity induces HIRs (24). PHYs are involved in several plant processes for example, seed development, de-etiolation of seedlings, hypocotyl and root growth, circadian rhythm control, shade avoidance, stomatal opening, flowering, etc (25). They are the positive regulators of photomorphogenesis. The Seed germination is controlled by PHYA, PHYE and PHYB, PHYA regulates seed germination under R and FR light whereas PHYB controls R light mediated seed germination. PHYA and PHYE promote seed germination under FR while PHYB promotes the same under R light. (25,26).

De-etiolation events are predominantly controlled by PHYA and PHYB. Along with PHYs, photoreceptors such as CRYs also participate in the regulation of de-etiolation. PHYA mainly regulates this phenomenon under FR and B light, however, PHYB is involved in de-etiolation under W and R light. Other PHYs don’t play significant role in de-etiolation (27). PHYs negatively regulate hypocotyl elongation and they control phototropism in hypocotyl. They have

differential role in root development and root gravitropism (28). PHYs such as PHYA, PHYB, PHYD and PHYE have been shown to participate in circadian clock regulation (29).

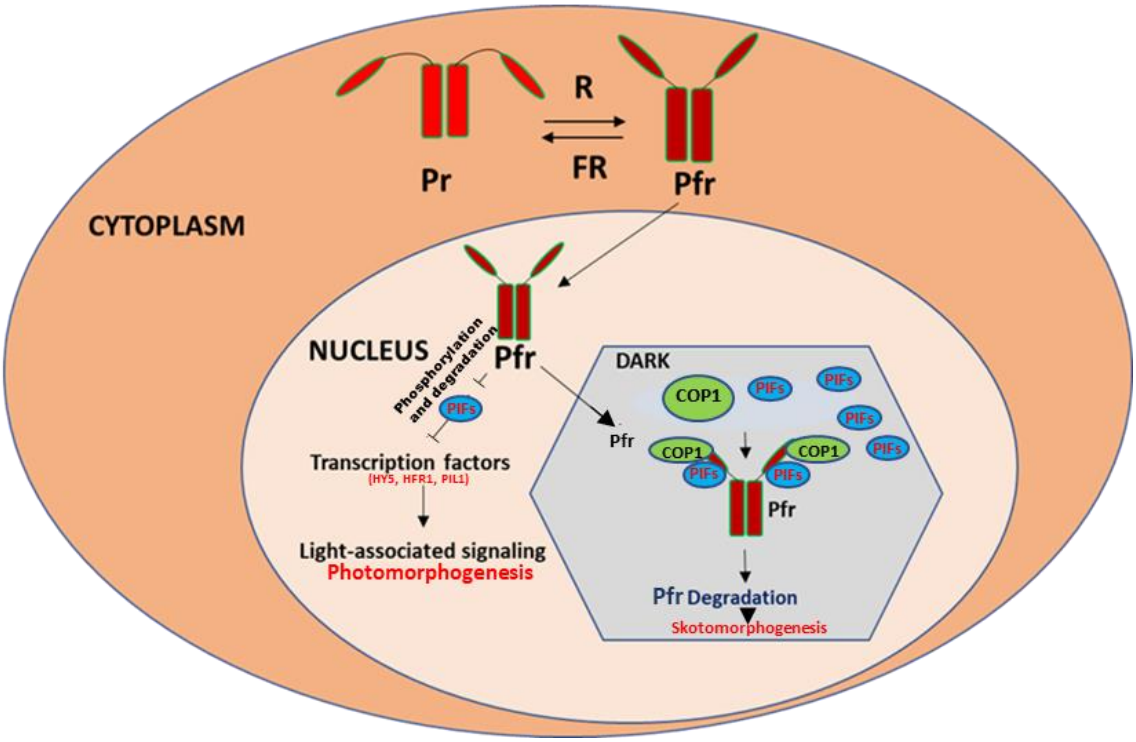


Fig.1.5. PHYB photoconversion and signaling in the plants. On red light irradiation, in cytoplasm Pr changes to Pfr form and then Pfr enters inside the nucleus. Inside nucleus, in presence of light, Pfr phosphorylates and degrades PIFs and promotes photomorphogenesis. Under dark condition, COP1 and PIF degrade PHYB leading to skotomorphogenesis.

1.2.2. Cryptochromes

CRYs are B light photoreceptors, consisting of three members namely CRY1, CRY2 and CRY3. They absorb the light in the range of ~400-500 nm (30). They are unique kind of photoreceptors found in both plants and animals. In plants, they mainly regulate seedling de-etiolation, root growth, flowering time, cotyledon expansion, circadian clock control, development of

chloroplast, stomatal opening and tropism. CRY1 and CRY2 are the principal B light photoreceptors controlling B light-mediated responses. CRY3 has been represented as a unique member of CRY category as it is involved in DNA repair mechanism (31).

CRY1 is photostable and acts as a major B light photoreceptor. However, CRY2 is photolabile and specifically acts under low strength of B light (30). CRY1 and CRY2 interact with different light regulated downstream genes specifically, *HY5*, *HYH*, *COP1* and *SUPPRESSOR OF PHYA 1* (*SPA1*) and govern de-etiolation process (31,32). *HY5* and *HYH* are the photomorphogenic components which positively regulate light-mediated responses. They are important players in light signaling pathways. They are also reported to act as integrators of light and hormone signaling. *SPA1* is mainly involved in skotomorphogenesis and regulates etiolated development of plants. CRY1 present in cytoplasm positively regulates root elongation whereas nuclear CRY1 acts inversely with respect to cytoplasmic CRY1 (33). It has also been reported that CRY1 promotes root elongation, however, CRY2 has been stated to inhibit root growth (34). CRY2 positively regulates photoperiodic flowering and functions opposite to PHYB. However, CRY1 has non-significant role in this event (35).

1.2.3. Phototropins

PHOTs are another category of B light photoreceptor and consist of two members: PHOT1 and PHOT2. Similar to CRYs, they also perceive light in the range of ~400-500 nm. They are involved in root gravitropism, root development, hypocotyl phototropism *etc*. High fluence B light mediated responses are controlled by both PHOT1 and PHOT2, however, PHOT1 has also been reported to regulate low fluence responses (36). Along with NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3), it controls the B light dependent root phototropism (37). NPH3 is known as B light signal transducer. They stimulate root geotropism via managing localisation of

PIN2 and auxin efflux rate (38). PHOT1 has been shown to suppress lateral root number and growth and this is due to reduction in the growth of lateral root epidermal cell (39). PHOT1 and PHOT2 regulate the hypocotyl phototropism via stimulating cytosolic Ca^{2+} (40).

1.2.4. UVB-resistance 8

UVR-8 is a unique kind of photoreceptor which provides resistance to plant from UV-B light (280-320 nm). Usually higher intensity of UV-B light is harmful for plants but it also regulates various plant processes. However, at lower intensity it helps in photomorphogenesis. It regulates hypocotyl elongation, root growth, flowering, stomatal opening control, etc (41). UV-B light promotes photomorphogenesis and hence inhibits hypocotyl elongation. Inhibition of hypocotyl is mediated by interaction of UVR-8 with SPA1 and suppression of COP1 (41). UV-B light negatively regulates the primary root growth, lateral root density and also suppresses the lateral root emergence (42). In this case, the impaired root growth is possibly caused by the alteration in the mechanism of auxin transportation. It also stimulates flowering time via changing the expression level of flowering time genes such as *FLOWERING LOCUS T* (*FT*) and *CONSTANS* (*CO*) (43). CO is a B-box protein and has been shown to accelerate the flowering. *FT* is a mobile signal acting downstream to *CO* and activates flowering by stimulating vegetative to reproductive phase transition (44).

1.2.5. Phytochrome B and its functions

PHYB is a homodimer protein which exists in two interconvertible forms: Pr is the cis form, which absorbs light at 660 nm and Pfr form is the trans form which absorbs light at 730 nm. PHYB protein comprises of three domains present in the sequence of PLD-GAF-PHY. PLD is the Per/Arnt/Sim-like domain which assists in signal sensing. GAF is known as cGMP

phosphodiesterase/adenyl cyclase/*Fhl1* domain. PHY is PAS-related domain which is specific to phytochromes and is important for the Pr-Pfr conversion (45).

Pr has been suggested to be the inactive form whereas Pfr has been considered as the biologically active form performing most of the light-associated functions (16). PHYB has a predominant role in promoting seed germination at higher temperature (22-28ºC). It triggers de-etiolation, stimulation of photomorphogenesis, inhibition of hypocotyl elongation and petiole length under R light (25). It also enhances cotyledon expansion and chlorophyll content but delays flowering. PHYB plays a major role in SAR and inhibits SAR under high R:FR ratio (46). It has been reported to be involved in controlling stomatal index under high irradiance of W and R light (47). Under high R:FR light condition, it acts in redundant manner along with PHYD and PHYE in delaying flowering time. It also takes part in controlling root morphology. It influences root elongation, gravitropism, lateral root growth and root hair development. The root localized PHYB has been investigated to regulate root development (48). It positively regulates root elongation under R light. PHYA has also been known to act with PHYB in this phenomenon. It has been reported that PHYB promotes formation of lateral root and root gravitropism under specific light and temperature (28). Elongation of root hairs is inhibited by PHYB under R light (49).

1.3. Phytohormones in plants

Plant possesses various signaling factors such as phytohormones which belong to a major group of plant growth regulators. Phytohormones are of different types; Auxin, Cytokinin (CK), Ethylene (ET), Gibberellic acid (GA), Abscisic acid (ABA), Strigolactone (SL), Jasmonic acid (JA), Brassinosteroid (BR) and Salicylic acid (SA) (50, 51, 52). These phytohormones play major roles in development of plants at each step of its life cycle.

1.3.1. Auxin

Auxin is the first discovered and the widely studied phytohormone in plants. Indole-3 acetic acid (IAA) is the naturally occurring auxin. It participates in various aspects of plant development. It helps in hypocotyl growth, vascular patterning, development of primary roots, lateral root branching, regulates movement of hypocotyl and root (53, 54). It controls several processes individually or interacts with other phytohormones to perform additional functions in plants. It enhances hypocotyl elongation and promotes apical dominance (55). The predominant growth of main shoot over lateral shoot branches is known as apical dominance. It also promotes shoot vascular differentiation. The factors involved in auxin influx and efflux play important role in vascular patterning (56). In contrast to shoot growth, IAA inhibits primary root elongation and promotes lateral branching. PIN-FORMED (PINs) proteins are the auxin efflux carrier, PIN3 dependent auxin transport stimulates hypocotyl gravitropic responses and also promotes root gravitropism (57).

1.3.2. Gibberellin

GA belongs to a terpenoid group. It is present in plants as well as in many fungi. It influences seed germination, flowering, elongation of stem, root phenotype, etc. It promotes the seed germination and also helps in maintaining uniformity in flowering (58). GA promotes skotomorphogenesis and hence positively regulates stem elongation in dark. It controls the stem elongation by decreasing HY5 stability and promoting PIFs. Hence, GA-mediated stem elongation in dark depends upon HY5 and PIF stability (59, 60). *RESPONSE TO AUXINS AND GIBBERELLINS 1* (*RAG1*) gene acts as a connecting component between auxin and gibberellin signaling. It is a *SMALL AUXIN UPREGULATED RNA 36* (*SAUR36*) type gene and reported to

promote elongation of hypocotyl in dark. However, under light hypocotyl growth is repressed due to lesser expression of *RAG1* (61).

1.3.3. Cytokinin

CK is known to regulate apical dominance, phyllotaxis, leaf senescence and root growth (62). It functions along with the auxin in controlling root-shoot ratio, apical dominance, phyllotaxis and root architecture. It acts antagonistic to auxin in apical dominance and hence suppresses the growth of main shoot (55). It promotes root hair formation, however, inhibits the growth of primary root and initiation of lateral roots. It plays crucial role in phyllotaxis and also reduces the rate of leaf senescence (63, 64). CK mediates reduction in leaf and flower senescence and counter balance the effects of ET.

1.3.4. Ethylene

ET is a gaseous plant regulator and is known for its major contribution in fruit ripening. It also plays vital role in hypocotyl growth, abscission control, leaf development and regulation of root phenotype (65). It interacts with other phytohormone signaling pathways to control plant growth and development (66). It promotes fruit ripening, softening and accelerates its maturity. It also positively regulates hypocotyl elongation which depends upon light fluence and quality (67, 68). ET promotes abscission of fruits, flowers and leaves. The leaf growth and development are dependent on ET concentration as well as plant species. It suppresses root growth and it occurs due to inhibited root cell elongation (65). ET-dependent root inhibition is because of alteration in auxin synthesis and its transport (69).

1.3.5. Abscisic acid

ABA is mainly recognized as stress hormone; it regulates the plant physiology under stress condition and provides resistance to the abiotic stress (70). It is involved in seed germination,

dormancy, hypocotyl growth and root morphology. It protects plants from biotic and abiotic stress and usually functions in adverse condition. It promotes de-etiolation phenomenon and suppresses the hypocotyl growth by HY5 accumulation. *ABA INSENSITIVE 5* (*ABI5*) also plays a vital role in this growth control (71). *ABI5* codes for basic leucine zipper transcription factor and takes part in ABA signaling during seed development. It suppresses primary and lateral root growth and the root growth is dependent upon ABA concentration (72).

1.3.6. Strigolactone

SL is a recently investigated phytohormone which belongs to carotenoid-derived compounds. It has been reported to participate in seed germination of parasitic plants, plant-fungi symbiosis, shoot and root development. It stimulates the germination of seeds in parasitic plants and symbiosis of plant-mycorrhizal fungi (73). It inhibits shoot branching and stimulates elongation of primary and adventitious root but reduces the formation of lateral roots (74, 75). It has also been shown to interact with genes downstream in light signaling and controls the hypocotyl growth (76).

1.3.7. Jasmonic acid

JA is an organic compound and mainly involved in plant defense and abiotic stress tolerance. It regulates root growth and hypocotyl elongation (77). Generally, it delays or suppresses the plant growth and promotes the defense related responses under stress conditions. In this way, it enhances the rate of plant survivability during pathogen infection as well as during herbivore attack (78). It provides tolerance to plants against various stress factors such as cold, salt, drought, heat etc. which involve JA signaling genes such as *Jasmonate ZIM domain* (*JAZ*) and *MYC2* (79). JAZ acts as transcriptional repressor in JA signaling. In presence of Jasmonoyl-L-isoleucine (JA-Ile), a class of JAs, JAZ is degraded by CORONATINE INSENSITIVE 1 (COI1) in ubiquitin

mediated pathway. CORONATINE INSENSITIVE 1 (COI1) is an E3-ubiquitin ligase (80). On the other hand, MYC2 is a transcriptional activator which stimulates JA responsive genes and JA signaling (81).

JA has been shown to suppress primary and lateral root growth. Oxylipin (a kind of JA) results in root waving and also causes failure in apical dominance. In dark, JA inhibits hypocotyl growth in *MYC2* dependent manner (82).

1.3.8. Salicylic acid

Similar to JA and ABA, SA also plays vital role in plant defense and under abiotic stress condition. It also regulates other physiological and morphological features of plant development. It controls photosynthesis, seed germination, thermogenesis and development of flower. It has also been documented that it promotes root and hypocotyl growth (83). SA interacts with other phytohormones, which further control the biotic and abiotic stress in plants.

1.3.9. Brassinosteroids

BRs belong to the group of polyhydroxylated steroid hormones and control many plant developmental aspects such as photomorphogenesis, cell division, root growth etc (84). *BRASSINAZOLE-RESISTANT 1* (*BZR1*) acts as a positive regulator of BR signaling and promotes skotomorphogenesis. It restrains photomorphogenesis by repressing transcription of GATA2 (85). GATA2 is a transcription factor involved in light signaling. *BZR1* promotes hypocotyl growth via interaction with *PIF4* and *COP1* (86, 87). BR also controls root growth by regulating the cell elongation and its organization. It regulates root growth in concentration dependent manner (84). Root growth is promoted at lower concentration of BR while at higher concentration root growth is inhibited. Similarly, it promotes lateral root formation depending upon its

concentration. This root growth control is due to suppression of auxin signaling at higher concentration whereas enhancement of auxin transport at lower concentration (88).

1.3.10. Auxin synthesis, transport and signaling

Auxin synthesis, transport and signaling are the three major activities which regulate the plant development (Fig.1.6). These processes are stringently controlled to maintain the optimal intracellular auxin concentration in every cell and organ.

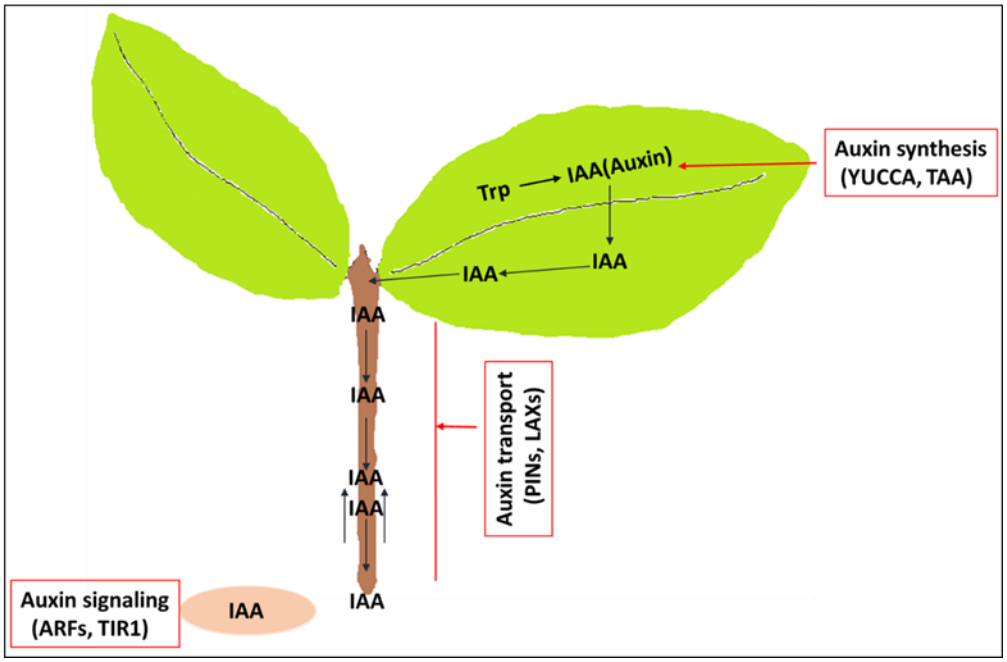


Fig.1.6. Auxin synthesis, transport and signaling in *Arabidopsis thaliana*. IAA synthesized in young tissues such as leaves via Tryptophan dependent pathways. IAA is transported basipetally in stem and in root is transported basipetally as well as acropetally with the help of various carrier proteins. Auxin signaling leads to several auxin responses which involves ARFs and AUX/IAA.

The auxin regulated functions in plants are dependent upon the auxin synthesis, signaling response and their transport mechanism. Alteration in any of these phenomena affects the plant

phenotypes. There are few reports available which have mentioned about the mutants of auxin in *At* (Fig.1.7) (89).

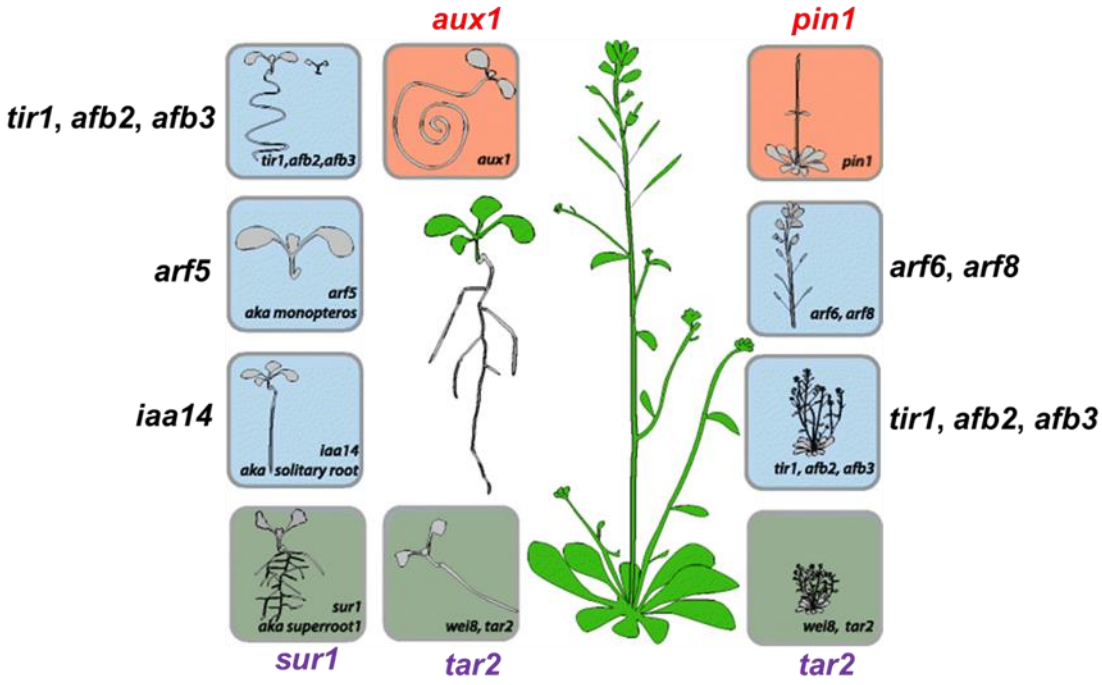


Fig.1.7. Mutants of *Arabidopsis thaliana* defective in auxin biosynthesis, signaling and transport. Auxin biosynthetic mutants are shown in grey boxes, signaling mutants are shown in light blue boxes and transport mutants are shown in light brown boxes (*Paque. S and Weijers. D. 2016*).

1.3.10.1. Auxin biosynthesis

Auxin is synthesized mainly in young meristematic tissues for example, young leaves, shoot and root apex via tryptophan dependent or independent pathways. The major pathway for auxin biosynthesis is tryptophan-dependent. The mechanism and the genes involved in tryptophan independent pathway are still not clearly known (90). Hence, only tryptophan-dependent pathways have been mentioned here. It has been shown that in peroxisome, on beta oxidation of Indole-3-buytric acid (IBA), IAA is produced. Various pathways are known in tryptophan-

dependent auxin synthesis such as Indole-3-acetamide (IAM), Tryptamine (TRA), Indole-3-acetaldoxime (IAOx) and Indole-3-pyruvic acid (IpyA) pathways (Fig.1.8) (91, 92).

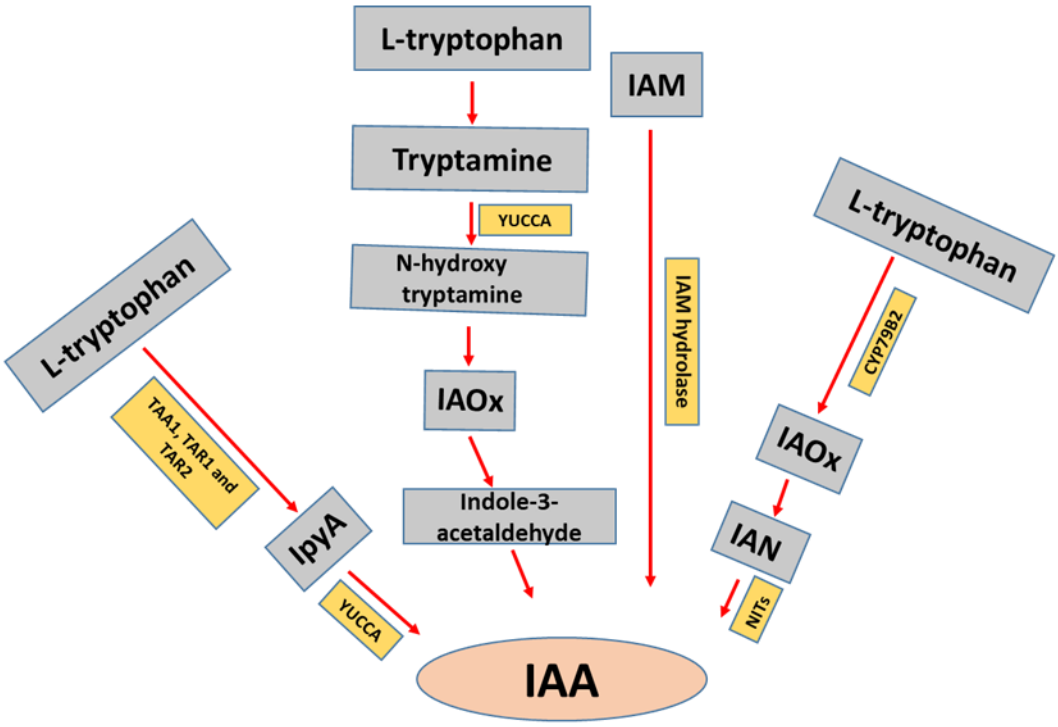


Fig.1.8. Auxin biosynthetic IAM, IpyA, IAOx and TRA pathways in plants.

In IAM pathway, IAM produces IAA with the help of IAM hydrolases. IpyA pathway has been considered as one of the principal auxin biosynthetic pathways in plants. This pathway involves Tryptophan Aminotransferase of Arabidopsis 1 (TAA1), Tryptophan Aminotransferase-Related 1 (TAR1) and TAR2 enzymes which catalyze the conversion of L-Tryptophan to IpyA. IpyA is further converted to IAA via YUCCA enzyme. YUCCA belongs to flavin monooxygenase enzyme family. In IAOx pathway, Cytochrome P450 Monooxygenases (CYP79B2) converts L-Tryptophan to IAOx, which is further translated into Indole-3-Acetonitrile (IAN). Next, nitrilases (NITs) catalyze the conversion of IAN to IAA. Similarly, TRA pathway starts with L-Tryptophan,

which is converted to TRA and then to N-hydroxy tryptamine by YUCCA. After that IAOx is synthesized from N-hydroxy tryptamine and IAOx is further converted into Indole-3-Acetaldehyde. In the final step, IAA is produced from Indole-3-Acetaldehyde. The mutants such as *superroot 1* (*sur1*) and *weak ethylene insensitive 8 tar2* (*wei8 tar2*) are altered in auxin biosynthesis. *sur1* mutant produces high amount of IAA and shows a greater number of lateral roots as compared to wild type (93). In *wei8 tar2* mutant, *TAA1* and *TAR2* genes are defective and this double mutant shows decreased production of IAA (94).

1.3.10.2. Auxin transport

After synthesis, IAA is transported by two ways: non-polar and polar transport pathways. In non-polar pathway, IAA is loaded in phloem tissues after synthesis and then transported to different organs. It occurs at very fast rate and is a passive way of transport, however, here directionality of transport is not clear. It has been suggested to be the principal way of transport in roots.

On the other hand, polar auxin transport (PAT) is regulated but slower than non-polar transport. It is energy consuming process and requires various carrier proteins. It involves acropetal as well basipetal movement. In shoot, PAT is primarily basipetal while in roots, it follows both acropetal as well as basipetal movement. Basipetal transport is defined as the movement of IAA from the base of the organ to the apex however, in acropetal transport IAA is transported from apex to basal part of the organ. In PAT, IAA moves from one cell to another and involves inflow and outflow of IAA. IAA transport in the cell can be through passive diffusion or active transport depending upon the pH of cell environment (95). AUX1/LIKE AUX1 (AUX1/LAX) and PINs are the key influx and efflux carriers of auxin transport respectively (96, 97, 98). IAA directly enters inside the cell via passive transport, but when pH is low in the cell wall, IAA gets

protonated and then transported inside the cell. AUX1/LAX has been known to participate in the process of auxin influx in the cell. In case of high pH inside the cell, auxin needs PINs to get out of the cell or for its efflux (Fig.1.9). In *At*, there are eight types of PIN proteins; PIN1 to PIN8. PINs are asymmetrically distributed throughout the cells; their polarity as well as directionality of auxin flow are correlated. PIN1, PIN3, PIN2, PIN7 and PIN4 proteins are confined to the plasma membrane, however, PIN5, PIN6 and PIN8 are possibly be involved in controlling auxin distribution between endoplasmic reticulum (ER) and cytosol (99).

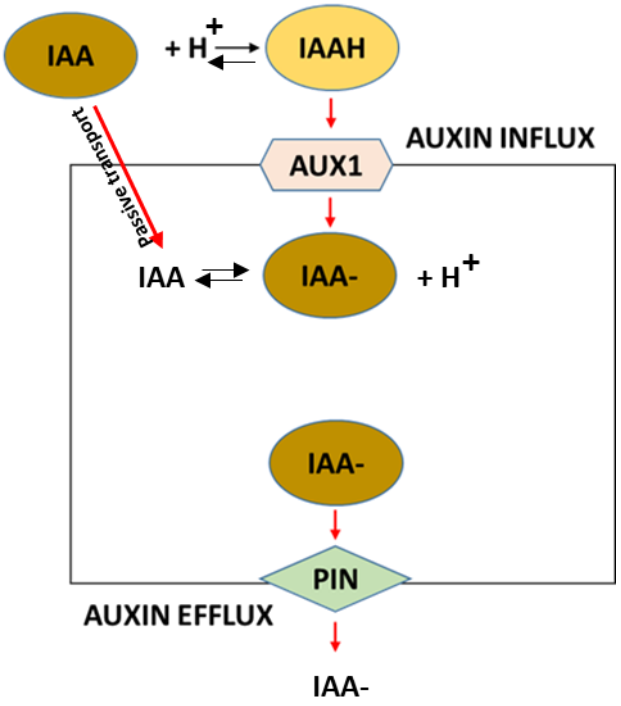


Fig.1.9. Auxin transport in plants. IAA is transported to the cell in two ways one via passive transport in the form of IAA and in another case under low pH, IAA gets protonated and carried inside the cell with help of auxin influx carrier such as AUX1. Inside the cell, where pH is alkaline IAA gets dissociated to form IAA- and further transported outside the cell with the assistance of auxin efflux proteins, PINs.

Along with PINs, ATP-binding cassette subfamily B (ABCB)-type proteins also participate in auxin efflux (100). AUX1/LAX and ABCB are mainly involved in maintaining IAA level in the cells. On the other hand, PINs are meant for the directionality of auxin transport. The auxin concentration and transport regulate the hypocotyl phototropism and root gravitropism (101).

The mutants such as *auxin-resistant 1* (*aux1*) and *pin1* are reported to be malfunctioned in auxin transport. *aux1* mutant is resistant to auxin, ET, has defective gravitropism and altered in auxin influx pathway (102). *pin1* mutant shows defective auxin influx and is characterized by naked inflorescence (103).

1.3.10.3. Auxin signaling

Auxin mediated functions in plants are dependent upon auxin signaling. Auxin signaling is facilitated by auxin response factors (ARFs) and transcription repressors; AUX/IAA. In *At*, AUX/IAA and ARF consist of 29 and 23 members respectively. ARFs have been reported to act as either repressor or activator (104). When auxin concentration is low in the cell, AUX/IAA binds to ARFs and prevents ARF binding to the promoter region of auxin responsive elements (AuxREs) present in the early auxin responsive genes. This leads to transcriptional repression of of auxin responsive genes. However, if amount of auxin is high in the cell, it binds to transport inhibitor response1 (TIR1) receptor of SCF^{TIR1} complex and degrades the AUX/IAA via ubiquitin-proteasome mediated pathway. This makes ARFs free to bind to AuxRE which activates auxin responsive genes (Fig.1.10). SCF^{TIR1} is a ligase which consists of S-phase kinase-associated protein-1 (SKP1), Cullin and F-box protein, TIR1; TIR1 codes for auxin receptor (105). In this way auxin level in the cell regulates the status of ARFs, AUX/IAA, transcription of auxin regulated genes and hence auxin responses. The auxin signaling and response further control several features of plant growth. Mutants such as *auxin F-box* (*tir1 afb2 afb3*), *arf*5,

solitary root-1(*slr-1*/*iaa14*) and *arf6 arf8* have been reported to be altered in auxin signaling. *tir1 afb2 afb3* mutant is less responsive to auxin in controlling primary and lateral roots (106). *arf5* mutant is altered in auxin response and has defect in vasculature and body axis patterning in embryo (107). *slr-1*/*iaa14* mutant shows altered sensitivity to auxin and characterized by lack of lateral roots, reduction in root hairs and defective shoot and root gravitropism (108). In *arf6 arf8* mutant, auxin regulated gene expression is altered and it shows defect in floral bud opening and floral organ elongation (109).

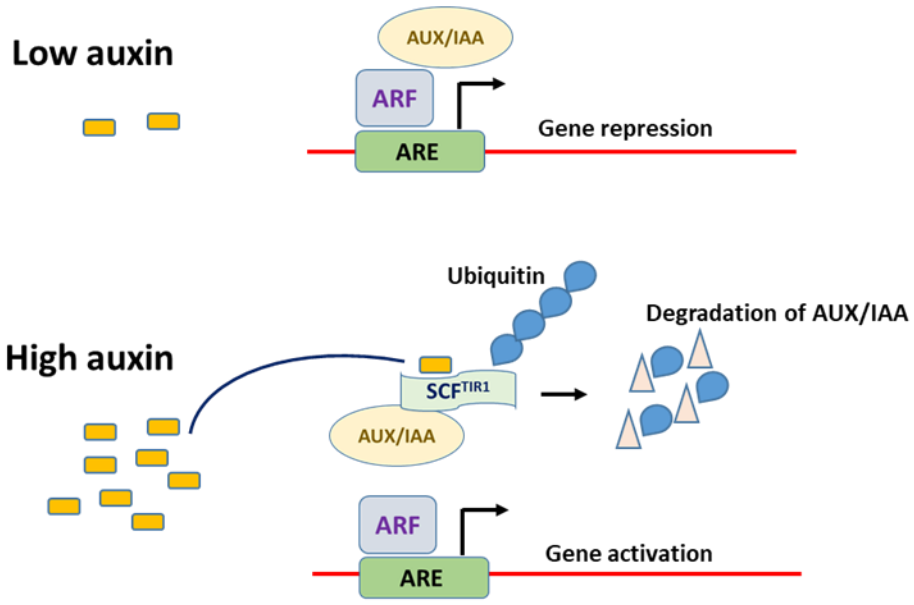


Fig.1.10. Auxin signaling in plants. In the cell when auxin is at low level then AUX/IAA binds to ARF, prevents its binding to ARE and the auxin responsive genes are repressed. In presence of high auxin in the cell, auxin binds to TIR1 and degrades AUX/IAA, then ARF binds to ARE leading to gene activation.

1.4. Interaction of red light and auxin signaling in plants

It has been studied that R light interacts with auxin to regulate different plant processes. PHYB and its downstream genes cross-talk with auxin and control the components involved in auxin synthesis, transport or signaling (110, 111). Pfr form of PHYB has been shown to suppress the auxin synthesis by negatively regulating *TAA1* and enhancing the expression of *SUR2*. *TAA1* and *SUR2* (cytochrome P450 monooxygenase) are activator and inhibitor of IAA biosynthesis respectively. *SUR2* regulates the level of IAA and indole glucosinolate inside the cell. In the shade (low R:FR) condition, IAA concentration has been shown to be enhanced. PHYB regulates the transcription of *GRETCHEN HAGEN 3* (*GH3*), an early auxin responsive gene. *GH3* is known to code for enzymes which mediate IAA conjugation to amino acids. Depending on the IAA concentration, IAA conjugation maintains the storage or degradation of IAA in the cell. PHYB regulates the growth of hypocotyl and root by altering the auxin transport and distribution (112, 113). PHYB controls the abundance of both PINs and P-glycoproteins (PGP) and hence regulates root and hypocotyl development. PGP belongs to the ABCB transporter protein family (110). PHYB negatively regulates the transcript level of *PIN3* gene (114). Low R:FR condition alters the expression of several auxin related genes. Under low R:FR light condition, bHLH factors such as *PHYTOCHROME RAPIDLY REGULATED 1* (*PAR1*) and *PAR2* downregulate *SAURs* genes; *SAUR15* and *SAUR68*. *SAURs* belong to the early auxin responsive genes (115). Like *PARs*, *ARABIDOPSIS THALIANA HOMEOBOX-LEUCINE ZIPPER PROTEIN-4* (*ATHB4*) has also been shown to downregulate *SAUR15* and *SAUR68* genes. *HFR1* reduces the *IAA29* transcript level under low R:FR light condition. *HY5* and *HYH* are known to be major integrators of light and auxin signaling. They also promote *AUXIN RESISTANT 2* (*AXR2*) and *SLR1* genes involved in negative regulation of auxin signaling (116). At high temperature, PIF4 mediated hypocotyl

elongation is due to the upregulation of auxin biosynthetic genes such as *TAA1* and *CYP79B3* (117). These reports stated that R light interacts with auxin and regulates different mechanism at its synthesis, transport and signaling. However, the molecular mechanism behind this regulation needs to be studied in detail. The current thesis work elucidates some of the facts regarding correlation of PHYB and auxin signaling in root growth as well as in protoplast system.

CHAPTER 2

MATERIALS AND METHODS

Chapter 2

2. Materials and methods

2.1. Materials:

2.1.1. Soil types:

At was grown in a mixture of three types of soil. Soil mix consist of soil rite, red and garden soil. Soil rite was purchased from Bhubaneswar, India (Keltech Energies Limited, Bangalore), red and garden soil were obtained from NISER Jatni campus, India.

2.1.2. Bacterial strains and constructs:

DH5α *Escherichia coli*, GV3101 *Agrobacterium tumefaciens* strains and luciferase auxin sensor.

2.1.3. Vectors:

pDONR 201 (donor vector) and pB2GW7 (expression vector).

2.1.4. Enzymes:

Taq polymerase (NEB, M0273L), Phusion polymerase (NEB, M0530S), DNaseI (NEB, M0303S), ApaI (NEB, R0114S), EcoRI-HF (NEB, R3101S), AflII (NEB, R0520S), BamHI-HF (NEB, R3136S), Cellulase R-10 (Duchefa, C8001.0010) and Macerozyme R-10 (Duchefa, M8002.0010).

2.1.5. Kits:

PureLink® HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit (Invitrogen, K2100-27), BP-LR cloning kit (Invitrogen, 12535-027), iScript™ cDNA synthesis kit (Biorad, 1708891), RevertAid H Minus First Strand cDNA Synthesis Kit (Thermofisher Scientific, K1631), Maxima reverse transcriptase kit (Thermofisher Scientific, EP0741), TURBO DNA free kit (Thermofisher Scientific, AM1907), Miniprep kit (Agilent, 400761), SsoFast EvaGreen Supermix (Biorad, 172-5201), iTaq Universal SYBR GREEN Supermix (Biorad, 172-5121) and RNeasy Mini Kit (Qiagen, 74104).

2.1.6. Antibiotics and miscellaneous:

Ampicillin sodium salt (Sigma, A0166), Kanamycin monosulfate (MP Biomedicals, 194531), Streptomycin dihydrochloride pentahydrate (Sigma, 85555) and Rifamycin (MP Biomedicals, 195490). Graphite nanopowder (Type 1, carbon nanoparticle, SRL, 7782-42-5), Ethyl methane sulphonate (Sigma, M0880), Silwett L-77 and BASTA (Phosphinothricin, Goldbio, P-165-250).

2.1.7. Sugars:

Sucrose (Suc, MP Biomedicals, 194018,), D(+) Maltose (Mal, Himedia, PCT0613), D(+) Glucose (Glu, Sigma, G8270), D-Mannitol (Man, Himedia, PCT0604) and D(-) Sorbitol (Sor, Himedia, PCT0606)

2.1.8. Seed lines:

Columbia wild-type (Col-0), PHYB overexpressor (35S:PhyB:GFP) and phytochrome mutants (*phyB-9*, *phyB-5* and *phyA-211*) seed lines were used. 35S:PhyB:GFP and *phyB-5* mutant belonged to Ler ecotype. *phyB-9* and *phyA211* seed lines belonged to Col-0 background.

2.1.9. Media:

Half-strength Murashige and Skoog **(**MS) media, SCA media, Protoplast isolation media, Protoplast transformation media, YEBS media, Luria-Bertani broth (LB) and LB agar.

2.1.9.1. Media for growing plants:

SCA, half-strength MS and hard agar half-strength MS. These media were autoclaved at 120 ºC and 15psi pressure before use.

Composition of SCA media (1L), pH~5.8:

B5 salt (Duchefa Biochemie, M0302), B5 vitamins (Duchefa Biochemie, G0415), $MgSO_4$.7H2O (Duchefa Biochemie, M0513), sucrose and Gelrite (Duchefa Biochemie, G1101).

Table.1. Composition of SCA media (1L)

Chemicals	Amount required
B5 salt (Gamborg's B-5 Basal Salt Mixture)	3.052 gm
B5 vitamins	10 ml
$MgSO_4.7H_2O$	983 mg
Sucrose	15 gm
Gelrite	3 gm

Composition of half-strength MS (1L), pH~5.8:

MS media without sucrose and agar (Himedia, PT021), sucrose and plant tissue culture grade agar powder (Himedia, PCT0901).

Table.2. Composition of half-strength MS (1L)

Chemicals	Amount required
MS	2.2 gm
Sucrose	10 gm
Agar powder	8 gm

Composition of hard agar half-strength MS (1L), pH~5.8:

MS media without sucrose and agar, sucrose and plant tissue culture grade agar powder.

Table.3. Composition of hard agar half-strength MS (1L)

Chemicals	Amount required
MS	2.2 gm
Sucrose	10 gm
Agar powder	15 gm

2.1.9.2. Media, chemicals and enzyme mixtures for protoplast isolation and transformation:

MMC, MSC, MMM, TM, PCA-0, Polyethylene glycol ($PEG_{1500,}$ Roche, 10783641001) and IAA (Sigma, I3750). 40% PEG_{1500} (in 0.413g of Ca $(NO_3)_2.4H_2O$ (Sigma, C2786) and 1.275g of Mannitol) was used. 57 mM stock of IAA (in ethanol) was prepared and diluted in PCA-M media. 10% cellulase (3 gm of cellulase + 27 ml of MMC) and 10% macerozyme (3 gm of macerozyme + 27 ml of MMC) were used.

All media and chemicals were filter sterilized and maintained ~ 600 osmolarity.

Composition of MMC (1L), pH~5.8:

2-(N-morpholino) ethanesulfonic acid (MES**,** Duchefa Biochemie, M1503), $CaCl_2.2H_2O$ (Duchefa Biochemie, C0504) and Mannitol.

Table.4. Composition of MMC (1L)

Chemicals	Amount required
MES	1952 mg
$CaCl_2.2H_2O$	1470 mg
Mannitol	86 gm

Composition of MSC (1L), pH~5.8:

MES, $CaCl_2.2H_2O$ and Sucrose.

Table.5. Composition of MSC (1L)

Chemicals	Amount required
MES	1952 mg
$CaCl_2.2H_2O$	2940 mg
Sucrose	145 gm

Composition of MMM (1L), pH~5.8:

MES**,** $MgCl_2.6H_2O$ **(**Duchefa Biochemie, M0533), $MgSO_4.7H_2O$ and Mannitol.

Table.6. Composition of MMM (1L)

Chemicals	Amount required
MES	1952 mg
$MgCl_2.6H_2O$	2040 mg
$MgSO_4.7H_2O$	2500 mg
Mannitol	86 gm

Composition of TM (0.2 L), pH~5.8:

$MgCl_2.6H_2O$**,** MES and Mannitol.

Table.7. Composition of TM (0.2 L)

Chemicals	Amount required
$MgCl_2.6H_2O$	610 mg
MES	200 mg
Mannitol	17.5 gm

Composition of PCA-M (1 L), pH~5.8:

B5 salts, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and Mannitol.

Table.8. Composition of PCA-M (1 L)

Chemicals	Amount required
B5 salts	3052 mg
$MgSO_4.7H_2O$	250 mg
$CaCl_2.2H_2O$	300 mg
Mannitol	93 gm

Composition of PCA-0 (1 L) made in coconut water, pH~5.8:

B5 salts, PC-Vitamins, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, MES, L-Glutamine (Duchefa Biochemie, G0708), Sucrose, Glucose and 1-Naphthaleneacetic acid (NAA, Duchefa Biochemie, N0903).

Table.9. Composition of PCA-0 (1 L)

Chemicals	Amount required
B5 salts	3052 mg
PC-Vitamins	10 ml
$MgSO_4.7H_2O$	500 mg
$CaCl_2.2H_2O$	300 mg
MES	976 mg
L-Glutamine	50 mg
Sucrose	20 gm
D-Glucose monohydrate	80 gm
NAA	1 mg

Composition of PC vitamins (1L), pH=5.8:

$CaCl_2.2H_2O$, Myo-Inositol (Merck, I5125), Pyridoxin-HCl (Merck, P5669), Thiamin-HCl (Merck, T4625), Biotin (Merck, B4639), Nicotinic acid (Merck, N4126) and Ca-pantothenate (Merck, C8731).

Table.10. Composition of PC vitamins (1L) .

Chemicals	Amount required
$CaCl_2.2H_2O$	20 gm
Myo-Inositol	20 gm
Pyridoxin-HCl	200 mg
Thiamin-HCl	100 mg
Biotin (Vit.H)	2 mg
Nicotinic acid	200 mg
Ca-pantothenate	200 gm

2.1.9.3. Media for growing and transforming bacteria

E.coli was grown in LB broth and on LB agar. *A. tumefacien* was grown in LB broth (Himedia, M1245) and LB agar (Himedia, M1151). For transformation of plants, *A. tumefacien* was grown in YEBS media.

Composition of YEBS media (1L), pH=7.0:

Yeast Extract (MP Biomedicals, 3065-532), Beef extract (Himedia, RM002), Sucrose, Bacto peptone (Fisher, 26315) and $MgSO_4.7H_2O$.

Table.11. Composition of YEBS media (1L)

Chemicals	Amount required
Yeast extract	1 gm
Beef extract	5 gm
Sucrose	50 gm
Bacto peptone	0.5 gm
$MgSO_4.7H_2O$	0.5 gm

2.1.10. Instruments:

White light growth cabinet (Percival, CU36L6), LED growth cabinet (Percival, E-30LEDL3), Shaker incubator (Eppendorf, New Brunswick Innova 44), Laminar airflow work bench (Thermo Scientific, 1300 Series A_2), Centrifuge (Eppendorf 5810R), TissueLyser II (Qiagen, 85300), Mini centrifuge (Eppendorf 5424R), Dry Bath (Labnet International Inc., Accu Block Digital Dry Bath), Motorless Magnetic stirrer (Spinit Tarson, 4050), Electrophoresis tank (Biorad, Mini electrophoresis horizontal), PCR (Biorad, C_{1000} Touch Thermal Cycler), Real time PCR (Biorad, C_{1000} Touch Thermal Cycler, CFX384 Real-Time System), Spectrophotometer (NanoDrop 2000, ThemoFisher Scientific), Vortex mixer (Labnet International Inc., S0200), Bioanalyzer (Agilent Bioanalyzer 2100 system), Gel doc (Biorad, Molecular Image ChemiDoc XRS+), Spectrophotometer (Eppendorf, Kinetic Biospectrum), Scanner (HP Scanjet G4010 Flatbed), pH meter (Merck, Mettler Toledo SevenCompact S210), Water Bath (Grant, JB Nova JBN18), Thermomixer (Eppendorf, Themomixer C), Mithras LB940 luminescence reader with integrated injectors (Bertold Technologies) and Stereozoom microscope (Nikon, SMZ745T).

2.1.11. Chemicals:

2.1.11.1. Plant DNA extraction:

Cetyltrimethylammonium bromide (CTAB) buffer, Isopropanol (Fisher, 13825), Phenol: Chloroform: Isoamyl alcohol (25:24:1) mix (SRL, 69031), 70% ethanol (Merck, 1,00983,0511) and T.E buffer (pH=8.0). CTAB (G Biosciences, DG094), NaCl (Himedia, MB023), EDTA (MP Biomedicals, 194822), Tris (Himedia, MB029), Polyvinylpyrrolidone (PVP, Himedia, RM854) and 2-Mercaptoethanol (Sigma, M6250).

Composition of T.E buffer: Tris-Cl (1M) and EDTA (0.5M)

Table.12. Composition of CTAB extraction buffer (10 mL):

Chemicals	Amount required
10% CTAB	3.0 ml
5M NaCl	2.8 ml
0.5M EDTA (pH 8.0)	0.4 ml
1M Tris-HCl (pH 8.0)	1.0 ml
PVP (M. W=40 KDa)	0.3 gm
2-Mercaptoethanol	0.02 ml

2.1.11.2. Plant RNA extraction:

For manual RNA purification: TRIZOL reagent (Invitrogen, 15596018), chloroform (MP Biomedicals, 193814), Isopropanol and 70% ethanol.

2.1.11.3. Gel electrophoresis:

50x TAE buffer, agarose (MP Biomedical, 100267) and ethidium bromide (G Biosciences, RC-049).

Composition of 50x TAE buffer:

Tris, Disodium EDTA and Glacial acetic acid (Fisher, 21055).

2.1.11.4. Plasmid isolation:

Miniprep:

Solution I, Solution II, Solution III, Phenol: Chloroform: Isoamyl Alcohol, 70% ethanol and TE buffer (pH=8.0) with RNase A (20µg/ml). Glucose, Tris-Cl, EDTA, NaOH (Himedia, MB095), SDS (Himedia, MB010), Potassium acetate (Fisher, 26495) and Glacial acetic acid (Fisher, 21055).

Table.13. Composition of Solution I, II and III:

Solution I (Stored at Room Temperature, RT)	Solution II (Stored at RT)	Solution III (Stored at 4˚C)
50 mM Glucose	0.2 N NaOH	5 M potassium acetate
25mM Tris Cl (pH=8)	1% SDS	Glacial acetate
10 mM EDTA (pH=8)		

For maxiprep:

Invitrogen kit was used.

2.1.11.5. Competent cell preparation and transformation:

$MgCl_2$-$CaCl_2$ solution (80 mM $MgCl_2$ and 20 mM $CaCl_2$) and $CaCl_2$. $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$ were used.

2.1.11.6. Luciferase imaging:

Renilla substrate consists of coelenterazine (Sigma, 3230-50UG) in methanol and further diluted in PBS as working solution. Firefly substrate stock contains D-Luciferin (Biosynth, L-8200), Acetyl Co-A trilithium salt (Applichem, A3753), ATP (Sigma-Aldrich Chemie GmbH, A2383), Tricine (Sigma-Aldrich, T0377), DTT (Duchefa Biochemie, D1309), NaOH (Duchefa Biochemie, S0523), $MgSO_4$ (Duchefa Biochemie, M0513), $MgCO_3$ (Sigma-Aldrich, M7179) and EDTA.

2.1.12. Primers:

Table.14. Primer list for genes

Genes	Forward Primer sequences (5'-3')	Reverse Primer sequences (5'-3')
PAR2	TCTCCTCCGTCTCCATCCTCCG	CATCTTCATCTTCTACGTCGGTCTCGC
ARR6	CCAATACCTTGGTTTGGATGTTGAGGA	GAGTTCATATCCAGTCATACCGGGC
HY5	ACGGCGAGTGCCGGAGTTTGGA	CCGGTGTCCTCCCTCGCTTC
ARF18	GGCGGAGCATGAAACAGATGAGG	CGAATGAAACTCTTGCTTAGTTGGTCC
ARF2	CGTGAACAGGGAAGACCATTCCAG	CAATTCCCTGCTTGTGAACCTTTGTGC
ARF4	TGACTGGAGTCTGTGACTTGGACCC	GTGTGGGAGAGAAACCGAGGGATC
KMD1	AGCTTCCTCCGATTCCTGGTCAAACC	ACCGAATCATGGGCCTGCCACG
PIF4	GCCAAAACCCGGTACAAAACCAGATC	GAGTCGCGGCCTGCATGTGTG
IAA7	AACTTTGGTGGAGGAGCAGCCGG	CCTGGTAGCTTTTGTACATCTTGAGGT
EPR1	ATCCTCGGAAATCGCCTGTTCCATATAC	GGTACACGAATTAGGCGAAGAGCATC
COL3	TGTTTCGACGAGAACGATAGATGTACC	GGTGATAACTGCACAGCTGGCGTC
SAUR9	GCTACTTCAACGACGTGCCAAAAGGT	AGCCACGAGATTGGGACCACATAGC

SAUR26	ATGGCTTTGGTGAGAAGTCTC TTTAGC	CTTCTTCTGGCTCTCGCCGACG TA
LAX2	ATGGAGAACGGTGAGAAAGC AGCTG	ACCAAGCATCATAAGCAGAGC CACC
COL9	ATGGTGTACTGTCGATCCGAT GCAG	TTGCATCTCTCGCAGACAAGTG TCCG
CIP1	GCACGGGTGGAATCAGCAGAG G	GGTGCTTTCCGCCCGCTTGATC
CSN6A	CTCAGCTCAATCCTCCTGCTTC AATC	CTCTGGACACCGATCACACATC C
CSN6B	TCACGAATTGTACCAGCAACA TGAACG	TCCTACCACCTCTTCGGCTGTG
CCA1	GGGAAGAGGGAAGTCAGAAT AACAGG	GTCTGAGGTCCTTGCTCATTAC CG
TOC1	TAGGTCCACCAACCCACAGA	TCAGCACCAAGACCACCATC
PRR9	CAGGTGAGCCAAAGACACCAA CC	CGATATTCTCCTGGTTGCTGCT CG
POLYUBIQ	GGCCTTGTATAATCCCTGATG AA	AGAAGTTCGACTTGTCATTAGA AAGAAA
TIR1	CATTGTTGAGTCTTGGTCCATG TT	GTGGCTGGTCCTCGATTTGA
EIN2	GCGTTGTGACTGGTAAACACT TGGCT	GAGCAGAATTGCTGAGAACTCC GCC
PIN1	TCGCGTTGCCCATAACTCTT	CCCTGTGTTTTGGTAATATCTCT TCA
YUCCA2	GGAACCTTACATAAATGCATC CACTA	CGATTTGGCAAGTTTGGATTG
UBQ	TGGACCGCCTTATCCAACG	CTTGAGGAGGTTGCAAAGGA
IAA2	CGACGCTCCTGCTCTAGACT	AAAACCCCGAAGTTTCGTCT

IAA5	TCCGCTCTGCAAATTCTGTTCGG	CCCAAGGAACATCTCCAGCAAG
GH3.5	AGCCCTAACGAGACCATCCT	AAGCCATGGATGGTATGAGC
LUC	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGAAAGTTTATGATCCA	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACAAGTCTTCTTCTGAGATTAA

2.1.13. Plant growth conditions:

For light intensity experiment, white (W) light :150, 112, 75 and 38 µmol $m^{-2}s^{-1}$ were used. For other set of experiments: W (100 µmol $m^{-2}s^{-1}$), R (50 µmol $m^{-2}s^{-1}$), FR (45 µmol $m^{-2}s^{-1}$) and B (30 µmol $m^{-2}s^{-1}$) light were utilized. The duration of light was long day (LD, 16 hr light/8 hr dark). For R light screening, continuous red light was used. They were grown at 22-24ºC and 70% relative humidity was maintained.

2.2 Methods:

2.2.1. Soil preparation

Soil rite, red and garden soil (5:4:1) were mixed and autoclaved. The autoclaved soil was used for growing the plants.

2.2.2. Media preparation

SCA and MS media were prepared and sterilized at 120ºC for 15 mins. LB broth, LB agar and YEBS media were also sterilized at 120ºC for 15 mins and subsequently antibiotic was added after filter sterilization (except Rifampicin). All media and solution for protoplast isolation and transformation were prepared and filtered sterilized.

2.2.3. Seed sterilization

Seeds were initially washed with 70% ethanol and incubated for 5 mins followed by centrifugation for 2 mins at 5000 rpm. Residual ethanol was thrown and then 100% ethanol was added to seeds and kept for 5 mins. Next centrifugation was done at 5000 rpm for 2 mins and the remaining ethanol was discarded. Then, final centrifugation was executed for 1 min at maximum speed. Rest alcohol was decanted and seeds were kept for drying under sterile condition. The seeds were dried for ~ 3 hours and checked to ensure complete evaporation of ethanol. The dried seeds were further sown on media plates.

2.2.4. DNA isolation

2.2.4.1. Plasmid isolation

Bacterial culture was inoculated in 4 ml LB broth and kept at 37˚C for 15 hrs. Afterward, centrifuged for 30 secs at 4˚C and at maximum speed. Supernatant was thrown and pellet was dried. Then pellet was mixed in 200 µl of chilled Solution I (Sol^n I). After mixing, 400 µl of Sol^n II was added to the resuspended pellet and kept for 5 mins at RT. Further, the solutions were mixed gently by inverting and then 300 µl of ice-cold Sol^n III was added. Again, they were mixed gently and kept for 5 mins at 4˚C. Then centrifuged for 10 mins at 4˚C with maximum speed. After that, supernatant was mixed with Phenol: Chloroform: Isoamyl Alcohol solution and incubated at RT for 15 mins. Again, centrifugation was done at 13,200 rpm for 2 mins at RT and supernatant was collected. Supernatant was mixed with ice-cold isopropanol and kept on ice for 20 mins, followed by centrifugation at maximum speed for 20 mins at 4˚C. Supernatant was thrown, pellet was cleaned with 70% ethanol and then pellet was dried. At the end, the pellet was mixed in 30 µl T.E buffer and quantified using nanodrop.

2.2.4.2. DNA isolation from plants

The plant tissues were homogenized with tissuelyser in liquid nitrogen. Homogenized tissue was mixed with pre-heated CTAB buffer and kept for 30 mins at 65˚C on heating block. Then, resuspended in Phenol: Chloroform: Isoamyl Alcohol solution. After that, centrifugation was done for 2 mins at 4˚C and at maximum speed. After centrifugation, the upper phase was collected, mixed with isopropanol and kept at RT for 10 mins. After incubation, centrifugation was carried out for 10 mins at 4˚C and at maximum speed. Then, supernatant was thrown and pellet was further cleaned twice using 70% ethanol. The pellet was mixed in TE buffer and quantified using nanodrop.

2.2.5. RNA isolation:

For Root samples:

The tissue samples were harvested and ground in tissuelyser with liquid nitrogen. The ground sample was suspended in 1 ml TRIZOL solution and kept at RT for 5 mins. After that, incubated with 200 µl of chloroform for 3 mins at RT, followed by centrifugation at 4˚C for 15 mins and at maximum speed. Aqueous part was collected and incubated with isopropanol and incubated for 5 mins at RT. Solution mix was centrifuged at 4˚C for 10 mins at maximum speed. Then, supernatant was decanted and pellet was cleaned twice with 75% ethanol. Further, pellet was suspended in RNase free water. RNA quality was checked by gel electrophoresis and bioanalyzer. Quantification was done using nanodrop.

For protoplast:

RNA isolation was done with RNeasy mini kit.

2.2.6. DNaseI digestion and precipitation:

For microarray experiments, after RNA isolation, DNase digestion was done with TURBO DNA free kit. After digestion, RNA was mixed with formamide. Then, denaturation was performed at 70˚C for 2 mins and then RNA integrity number (RIN) was calculated and analysed.

For other experiments, isolated RNA was incubated with DNaseI and its respective buffer. The mix was incubated at 37˚C for 10 mins, then 0.5 µl of 1 M EDTA was added and kept for 10 mins at 75˚C. After DNase I digestion, precipitation was performed. 2/3rd volume and 1/10th volume of pre-chilled isopropanol and 3M sodium acetate were added to the mix respectively and incubated for 10 mins at RT. Then, centrifuged at 4˚C for 5 mins and at 13,000 rpm, after that supernatant was thrown and the pellet was cleaned with 70% ethanol twice. Lastly, pellet was suspended in miliQ water.

2.2.7. cDNA preparation:

The cDNA synthesis was performed using Thermofisher and Biorad kits.

2.2.8. *E.coli* and *Agrobacterium* competent cell preparation and transformation

2.2.8.1. *E.coli* competent cell preparation:

Colonies were inoculated in LB broth and incubation was done at 37˚C and at 220 rpm. They were grown till optical density (OD) reached 0.4 at 600 nm wavelength. The culture was incubated on ice for 10 mins and then centrifugation was performed at 4˚C for 10 mins and at 4500 rpm. Extra media was decanted; then pellet was dried and mixed with chilled solution of $MgCl_2$-$CaCl_2$ with the help of vortex. After that centrifuged for 10 mins at 4500 rpm and at 4˚C, then supernatant was decanted and pellet was resuspended in 0.1 M $CaCl_2$ (ice cold) solution. Finally, they were kept in aliquots at -80˚C.

2.2.8.2. *E. coli* transformation:

50 µl competent cell was incubated with 1 µl of plasmid for 30 mins on ice. After incubation, the mix was given heat shock (42˚C) for 40 secs. Next it was incubated for 5 mins on ice. After that, LB media was added and kept at 37˚C for 1 hr. Then centrifugation was performed for 1 min at 6000 rpm, extra media was discarded and pellet was mixed in the remaining media. The suspended pellet was uniformly spread on media plate and kept overnight at 37˚C.

2.2.8.3. *Agrobacterium* competent cell preparation:

Single colony of bacterial culture was inoculated in LB broth and incubation was done overnight at 28˚C and 220 rpm. Overnight grown bacterial inoculum was added to 100 ml of LB media with rifamycin and further grown till OD reached 0.5. When OD reached 0.5, the culture was incubated for 25 mins on ice and centrifuged at 4˚C for 15 mins, and at 5000 rpm. Extra media was thrown and the pellet was washed thrice with 40 ml of 10% glycerol. After that 1 ml of 10% glycerol was added to pellet. Then they were kept in aliquots at -80˚C.

2.2.8.4. *Agrobacterium* transformation:

50 µl competent cell was mixed with 1 µl of plasmid on ice and then shifted to electroporation cuvette (pre-chilled). Then cuvette was kept in electroporation system and electroporation shock was provided for three times. After that, 1 ml of LB broth was added and incubated overnight at 28˚C and 220 rpm. After incubation, centrifugation was done at RT for 1 min at 6000 rpm. The supernatant was removed. Pellet was mixed in residual media, spread over LB agar plate with selection antibiotic and incubated for 2-3 days at 28˚C till the colonies appeared.

2.2.9. Plant transformation and mutant screening

The transformed *Agrobacterium* colony was inoculated in YEBS media (with streptomycin and rifamycin) and incubated at 220 rpm and 28˚C. The OD_{600} nm was measured at different time interval till OD reached to 0.8. Small amount of culture (feeder culture) was kept for next floral dip. When OD reached 0.8, Silwett L-77 was added to the culture and then the floral parts of plant (at bolting stage) were dipped properly in the culture. After dipping, plants were kept in dark for 12 hrs in slanted state and covered with polybag. After 12 hrs, polybag was unwrapped and the plants were transferred to growth rooms. For efficient transformation, floral dip was performed thrice following the above-mentioned steps at an interval of 3 days using feeder culture. After that transformant seeds were harvested and further sown on soil and three times treated with BASTA. The healthy seedlings isolated after BASTA treatment were further screened by PCR.

2.2.10. Protoplast isolation and transformation

The sterilized seeds were sown on SCA media and grown vertically for ~3 weeks. After that in sterile condition, the shoot was separated from roots and chopped. The chopped shoot was transferred to MMC media. They were incubated for 1 hr in MMC media, after that more MMC media was added with cellulase and macerozyme enzymes and incubated overnight at 22ºC in dark. After incubation, digested tissues were slowly agitated by pipetting and then filtered. The filtered solution mix was centrifuged at 100g for 15 mins. Then supernatant was removed and pellet was suspended in MSC media. MMM media was overlaid on suspended protoplast (pellet) and centrifuged. After that intact protoplast was collected from the layer present between MSC and MMM media. Further, protoplasts were suspended in TM media and counted using hemocytometer. The resuspended protoplasts were centrifuged, supernatant was discarded and then fresh TM media was added to it. For protoplast transformation, the plasmid with luciferase

auxin sensor (after maxiprep) was diluted with TM. The protoplasts were incubated with diluted plasmid for 5 mins. Then, PEG was added and kept for 8 mins followed by addition of PCA-0 media. The mixture was kept in dark for 18-24 hrs at 25ºC.

2.2.11. Luciferase imaging

Transformed protoplasts were mixed with different concentration of auxin in white flat bottom plate and incubated for different time intervals under variable light quality. The measurement was performed with luminescence reader. Firstly, firefly substrate was added and measurement was done, after that renilla substrate was added and the signal was measured. Ratiometric luciferase (Firefly/Renilla) signal was analysed with Microwin 2000 software.

2.2.12. Root analysis

Plates containing seedlings were scanned using HP Scanjet Scanner. Root length was calculated using R and ImageJ programs. Root wave density (WD), straightness and horizontal growth index (HGI) were analysed and quantified with R program. Counting of root coils, lateral and adventitious root number was performed under stereo zoom microscope.

2.2.13. PCR and Colony PCR

PCR was done using Taq polymerase and Phusion polymerase enzymes. The standard protocol as mentioned by NEB was followed.

2.2.14. Real time PCR

cDNA samples were mixed with SYBR green and primer mix (forward+reverse) and total volume (10µl) was subjected to real time PCR. Data was analysed with Bio-Rad CFX Manager 3.81. Fold change (FC) of 1.2 with p-value <0.05 was considered. POLYUBIQ or UBI gene was used as internal control for normalization.

Table.15. Conditions for real time PCR:

Steps	Temp (ºC)	Time (sec)
Enzyme Activation	95	30
Denaturation	95	10
Annealing/Extension	60	30
Melt Curve	65-90 (0.5 increment)	0.05 /step

2.2.15. Gateway cloning

2.2.15.1. BP cloning

Amplified product was mixed with pDONR201, BP Clonase mix and T.E buffer, total volume of 10µl was incubated overnight at 25ºC. After incubation, Proteinase K was added and kept for 10 mins at 37ºC. This BP mix was further used for transformation of *E.coli*. The colonies were examined by PCR and restriction digestion.

2.2.15.2. LR cloning

The positive colonies after BP reaction were subjected to miniprep. The plasmid DNA (entry clone) was isolated and used for LR reaction. Entry clone was mixed with destination vector (pB2GW7), LR Clonase and T.E buffer and incubated at 25ºC for 14 hrs. After that mixed with Proteinase K and kept for 10 mins at 37ºC. LR mix was used for transformation in *E.coli*. The colonies were confirmed via colony PCR and digestion with specific restriction enzymes.

2.2.16. Microarray

After analyzing the quality and quantity of RNA, microarray was performed (Affymetrix GeneChip® WT PLUS Reagent Kit Manual Target Preparation for GeneChip® Whole Transcript (WT) Expression Arrays) .

2.2.17. EMS mutagenesis

0.2% EMS solution was prepared with water and added to seeds. The seeds were suspended properly and kept overnight on rotating mixer. Then, extra EMS solution was discarded and seeds were cleaned with water. After that seeds were incubated with water for 1 hr. All the steps were performed in fume hood. The mutagenized seeds were further sown on soil.

2.2.18. Data analysis and Image processing

Adobe illustrator was used for image processing. Statistical analysis and plotting of graphs have been done using OriginPro. Image J and R programs were used for image analysis. One-way ANOVA analysis with Tukey and Bonferroni tests were performed for multiple mean comparison. Probability threshold of 0.05 was considered.

RESULTS

CHAPTER 3

Exploring the molecular components in light fluence dependent root growth in *Arabidopsis thaliana*

Chapter 3.

3. Exploring the molecular components in light fluence dependent root growth in *Arabidopsis thaliana*

3.1. Background:

The light quality and quantity both are involved in regulating plant developmental processes. Light signal is primarily perceived at shoot through various photoreceptors and controls the plant growth, however, roots growing in dark condition also encounter small amount of light. Generally, the light enters inside the soil through the cracks and reaches to the roots. Though, it has also been investigated that light irradiated on shoot, passes through the shoot to the roots and modulates their growth (118). This light signal further activates the photoreceptors present in the roots. Light irradiation of roots causes generation of reactive oxygen species (ROS). ROS at its minimal level results in enhanced root growth but at higher amount it has detrimental effects. When roots are directly exposed to light, *phyB* mutant has shown shorter primary root in comparison to wild-type. However, when roots are subjected to darkness, reduction in primary root length has not been noticed in *phyB* mutant (119).

It has also been reported that, ROS accumulation in the roots of *phyB* mutant is more as compared wild-type when they are irradiated with light. This shows that light signal mediated by PHYB localized in the shoot region may reduce the accumulation of ROS in the roots on light exposure. ABA signaling components present in the shoot have also been reported to participate in this process. In shoot ABA biosynthetic and signaling mediators are stimulated by shoot localized PHY signals, that results in enhanced root growth. ABA stimulates *ABI5* expression in root region which further triggers the expression of *PEROXIDASE 1* (*PER1*). It has been reported that, *ABI5* and *PER1* expression are lesser in *phyB* mutant as compared to wild-type. *PER1* encodes peroxidase

enzyme which helps in detoxification of ROS. Excess ROS inhibits the root growth however, peroxidase detoxifies the ROS in root, retains its optimal level and hence promotes the root growth. Based on these information, it can be concluded that PHYB-mediated enhanced root growth depends on the expression of the *ABI5*, *PER1* genes and the amount of ROS in roots (120). It has also been shown that *HY5*, a downstream light signaling component is stimulated at shoot region, which moves to roots and controls the root morphology (121). MEDIATOR (MED) is a complex, playing important roles in plant development and is engaged in sugar-auxin crosstalk in roots (122). It has been investigated that, the *med18* mutant shows shorter root length, long and dense root hairs along with a reduction in the lateral root number. This alteration in root morphology is because of defect in auxin response and distribution in the root. In *med18* mutant, cell death occurs in root meristem regardless of light exposure to the root (123). Hence, it can be proposed that some signals are triggered at the shoot which translocate and control the root architecture.

The effects of light on root growth has been studied, however, very few reports are available which suggest the impacts of light quantity at molecular level in root development. In tobacco, it has been observed that plants exhibit altered root growth when grown under variable W light intensity and durations. It has been shown that, plants continuously growing under 60 µmol m^{-2} s^{-1} light has lesser fresh root biomass in comparison to the plants growing in presence of 300 µmol m^{-2} s^{-1} light, after 14 or 18 days of light irradiation. Although, after 18 days, the plants grown under 60 µmol m^{-2} s^{-1} for the first 14 days and then under 300 µmol m^{-2} s^{-1} for next 4 days, exhibited intermediate fresh root weight when compared with plants grown for 18 days continuously in presence of 60 and 300 µmol m^{-2} s^{-1} light. This signifies that the light intensity and duration have additive effects on the root growth (124). *Solanum lycopersicum* cyclophilin (*SlCyp1*) is a phloem specific mobile signal, which is translocated to root from the shoot region and regulates root

development in tomato plant. It is a peptidyl-prolyl cis/trans isomerase and has a crucial role in the plant development (125). As light intensity increases, the translocation of SlCyp1 is also enhanced which further promotes root growth. This report has shown that root growth is dependent upon light intensity (126). *Pinus sylvestris L*. (Scots pine), a gymnosperm displayed variability in root development when grown under different FR and R light intensities (1, 10, 25 and 100 µmol m^{-2} s^{-1}). FR light had more significant effects as compared to R light irradiation. The seedlings displayed shorter roots in presence of lower intensities of FR (1 and 10 µmol m^{-2} s^{-1}) when compared to complete darkness. However, the root growth was maximum under higher intensity of FR in comparison to other light conditions (127). This indicates that both optimal light quality and quantity are required for the proper development of roots.

A recent article has reported that direct or indirect light irradiation on *At* roots changes their gene expression. In this report, the seedlings were grown under two different setups. In one setup, roots along with shoots were completely irradiated with W light and the second setup included Dark-Root (D-Root) system where shoots were exposed but roots were masked. D-Root is a device where seedlings are vertically grown on square plates and the plates are further kept in a black methacrylate box where the roots of the seedlings remain in dark. To avoid light reaching to the roots, a black methacrylate comb is fitted on media plate which separates the shoot and root parts allowing root to grow in complete darkness. Under the above-mentioned setups, the expression *PHYA*, *PHYB*, *UVR8*, *CRYs* and *PHOT*s genes were not altered in roots under these two experimental set ups. However, when they were examined in specific regions of roots, they showed differentially expression (128). Since, these reports have shown that the light intensity influences the root growth, the molecular components involved these phenomena are still not clear and need further investigation.

In this chapter, the light intensity-dependent root growth and gene expression were investigated. In the current work, the seedlings were grown under 38, 75, 112 and 150 µmol m^{-2} s^{-1} W light intensities. Under the above-mentioned intensities, root morphology was studied in Col-0 (wild-type), *phy* A and *phyB* mutants. Change in transcriptomics of Col-0 root under different light intensities was further examined through microarray.

3.2. Results:

3.2.1. Phytochrome mutants displayed variable root growth in the presence of different white light intensities

The seeds of Col-0, *phyA-211* and *phyB-9* were sterilized, sown on half MS media and kept for cold stratification. After 72 hrs of stratification, the plates were kept vertically under different W light intensities (38, 75, 112 and 150 µmol m^{-2} s^{-1}).

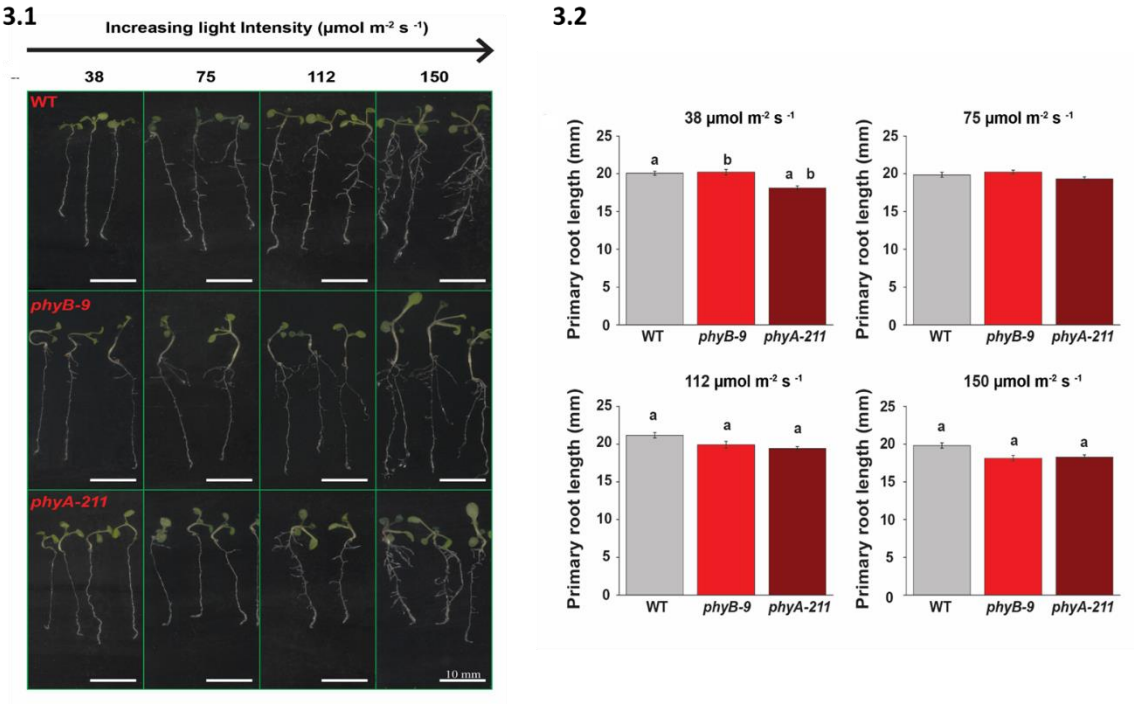


Fig.3.1. Phenotypic differences in root morphology of six-day old seedlings. Fig.3.2. Primary root elongation in Col-0, *phyB-9* and *phyA-211* under 38, 75, 112 and 150 µmol m^{-2} s^{-1} light.

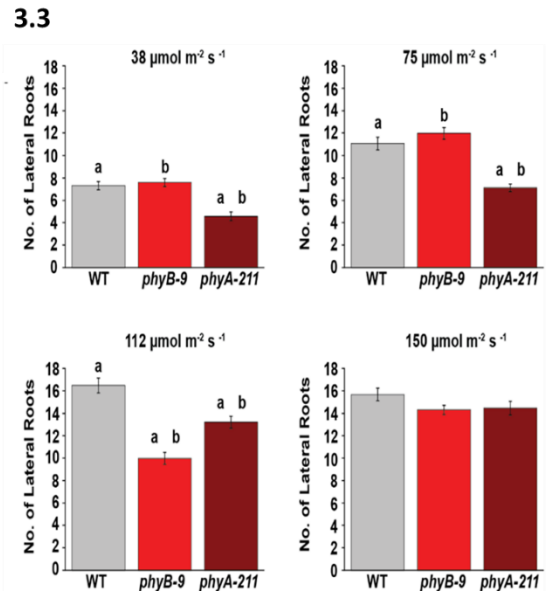
Error bars represent SEM. Same letters signify statistical significance. One-way ANOVA with Tukey and Bonferroni tests was performed with p≤0.05. Five technical replicates were used and each replicate comprised of 20 seedlings. Scale bar=10 mm.

The root development was analysed in six-day old Col-0, *phyA-211* and *phyB-9* seedlings and found that they showed differences in their root morphology under above said light intensities (Fig.3.1). Under lowest light intensity (38 μmol m^{-2} s^{-1}), the primary root in *phyA-211* was shorter than *phyB-9* and Col-0 seedlings. However, under higher intensities of light (150 μmol m^{-2} s^{-1} and 112 μmol m^{-2} s^{-1}), both *phy* mutants displayed slightly shorter root length as compared to Col-0 (Fig.3.2).

The adventitious and lateral root number (emerged lateral roots) were also altered under different light intensities. When light intensity increased from 38 to 150 μmol m^{-2} s^{-1}, the lateral root number in *phyB-9* and Col-0 increased two times but three times in *phyA-211* seedlings. (Fig.3.3). Surprisingly, *phyB-9* displayed lesser number of lateral roots under 112 μmol m^{-2} s^{-1} in comparison to 75 and 150 μmol m^{-2} light intensities (Fig.3.3). Under lower light intensities (38 and 75 μmol m^{-2} s^{-1}), the number of lateral roots was lesser in *phyA-211* as compared to WT and *phyB-9* mutant. On the other hand, in presence of 112 μmol m^{-2} s^{-1} light, the lateral root number was least in *phyB-9* seedlings. Under highest light intensity of 150 μmol m^{-2} s^{-1}, all the genotypes displayed similar number of lateral roots (Fig.3.3).

The number of adventitious roots was observed to increase with increase in light intensity of 38-75 μmol m^{-2} s^{-1} (Fig.3.4). However, it was saturated further after 112 and 150 μmol m^{-2} s^{-1} light intensity. Under all light intensities, *phyA-211* mutant had lesser number of adventitious roots as compared to WT and *phyB-9* seedlings. The increment in the adventitious root number

with increasing light intensity was maximum in *phyA-211* as compared to WT and *phyB-9* seedlings (Fig.3.4).



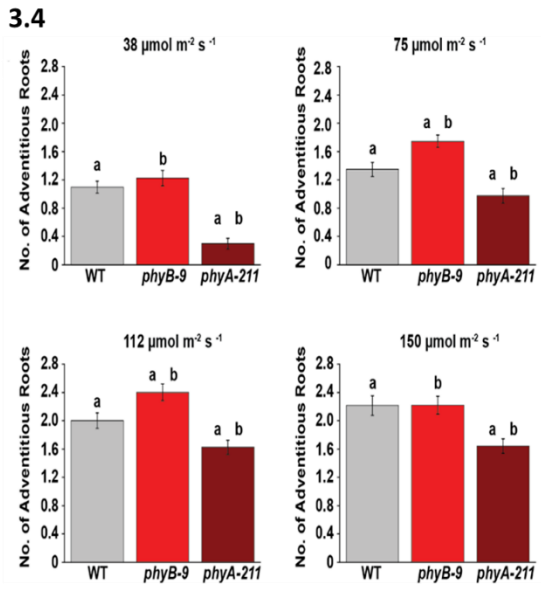


Fig.3.3. Change in lateral root number. Fig.3.4. Adventitious root number altered among all the genotypes under different W light intensities of 38, 75, 112 and 150 µmol m^{-2} s^{-1}. Error bars represent SEM. Same letters signify statistical significance. One-way ANOVA with Tukey and Bonferroni tests was performed with $p \leq 0.05$. Five technical replicates were used and each replicate comprised of 20 seedlings.

3.2.2. Documentation of differentially expressed genes in Col-0 root under different white light intensities

Analysis of root growth showed that different W light intensity altered various aspects of root growth in Col-0 and *phy* mutant seedlings. Furthermore, microarray was done with the root of 5-day old Col-0 seedlings grown on media plates under all the four W light intensities mentioned before in previous section. First of all, RNA isolation was done from the root samples and then the

quality and quantity of RNA were checked. After that, microarray was carried out with two biological and three technical replicates. The details of differentially expressed genes (DEGs) from microarray data was described by using gene level differential expression analysis. DEGs were selected from microarray data considering FC=1.2 and False Discovery Rate (FDR) < 0.05. DEGs were further categorized in six different comparative light intensities:150 versus (vs) 112, 150 vs 75, 150 vs 38, 112 vs 75, 112 vs 38 and 75 vs 38 μmol m^{-2} s^{-1}. Upregulated and downregulated DEGs have been shown in the table 16 and have also been represented in the form of graphs (Fig.3.5). The maximum and minimum number of DEGs belonged to 150 vs 38 and 150 vs 112 light respectively.

Table.16. Total number of DEGs identified under six comparative white light intensities:

Comparative light intensity (μmol m^{-2} s^{-1})	**150 vs 112**	**150 vs 75**	**150 vs 38**	**112 vs 75**	**112 vs 38**	**75 vs 38**
Total number of genes	1014	1424	1789	1163	1518	1047
Genes upregulated	492	717	823	569	700	463
Genes downregulated	522	707	966	594	818	584

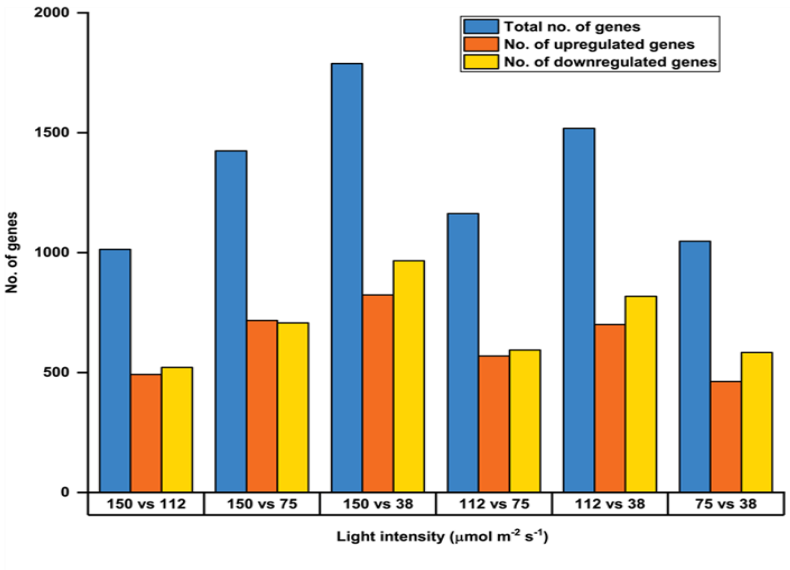


Fig.3.5. Total number of upregulated and downregulated DEGs obtained from microarray analysis of root sample of 5-day old Col-0 seedling under 150 vs 112, 150 vs 75, 150 vs 38, 112 vs 75, 112 vs 38 and 75 vs 38 µmol m^{-2} s^{-1} light condition.

Venn diagram was presented to provide information about common and distinct DEGs from the complete set of genes under different comparative light conditions: 150 vs 112, 150 vs 75 and 150 vs 38 ; 150 vs 112, 112 vs 75 and 112 vs 38 ; 150 vs 75, 112 vs 75 and 75 vs 38 and 150 vs 38, 112 vs 38 and 75 vs 38 µmol m^{-2} s^{-1} (Fig.3.6).

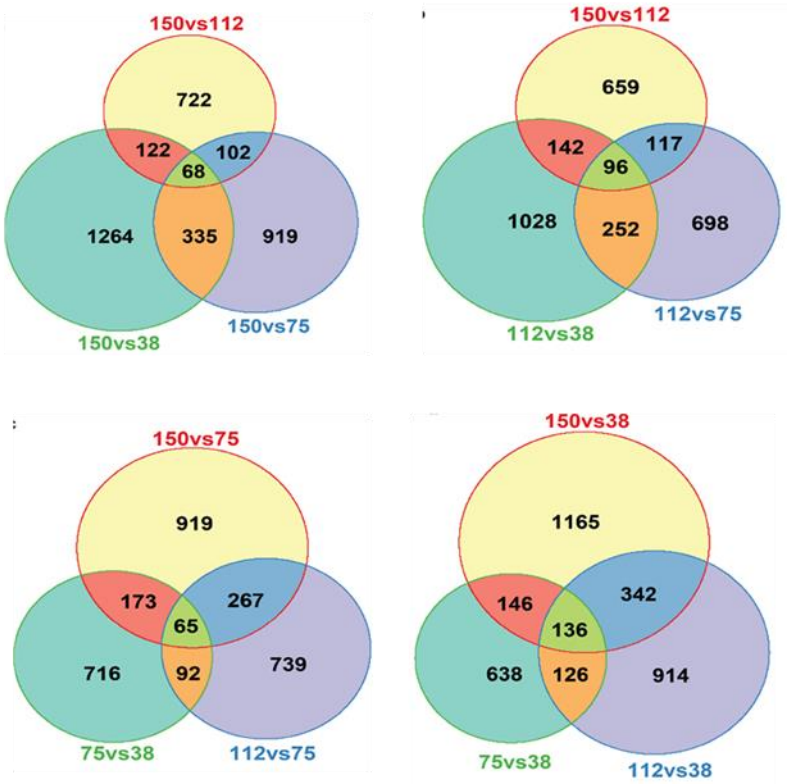


Fig.3.6. Venn diagrams of common and distinct DEGs under variable comparative light intensities of 150 vs 112, 150 vs 75 and 150 vs 38; 150 vs 112, 112 vs 75 and 112 vs 38; 150 vs 75, 112 vs 75 and 75 vs 38 and 150 vs 38, 112 vs 38 and 75 vs 38 µmol m^{-2} s^{-1}. The DEGs in light green coloured circles are the common among three comparative light intensities. The DEGs shown in dark pitch, dark blue and orange coloured circles are overlapping between two comparative light intensities. The DEGs presented in light blue, purple and yellow coloured circles are unique for each comparative light condition.

After analyzing the DEGs, it was investigated that 68 DEGs were found to be common under 112, 75 and 38 µmol m^{-2} s^{-1} light as compared to 150 µmol m^{-2} s^{-1}. In 150 vs 112, 112 vs 75 and 75 vs 38 µmol m^{-2} s^{-1} comparative light condition, 96 common DEGs were found. Relative to 75 µmol m^{-2} s^{-1} light, 65 common DEGs were observed under other light intensities. In case of 150 vs 38, 112 vs 38, 75 vs 38 µmol m^{-2} s^{-1} light condition, 136 overlapping DEGs were found.

At5g22555, *At2g39445*, *At2g44130* and *CYP81F2* genes were common under all the six comparative light intensities.

At5g22555 is known as a hypothetical membrane protein. *At2g39445* codes for phosphatidylinositol *n*-acetylglucosaminyltransferase. *At2g44130* codes for KISS ME DEADLY 3 (KMD3), a F-box protein. *KMD3* is stimulated by a root-knot specific nematode, *Meloidogyne incognita* (129). CYP81F2 is a membrane specific protein which is involved in the synthesis of indole glucosinolate and provides resistance against aphids, fungus, *etc* (130).

3.2.3. Gene ontology enrichment analysis of DEGs from microarray

DEGs obtained from microarray were subjected to Gene ontology (GO) enrichment analysis. It provided information about over-represented DEGs in presence of various light intensities. It helped in understanding the impacts of light intensity on various biological, molecular and cellular processes in the root system.

In general, GO analysis presents information about gene products and their functions based on the existing literature and databases. In this analysis, the genes are classified in three categories or domains: molecular function (MF), cellular component (CC) and biological process (BP). MF category involves function or activity of gene products at molecular level and CC describes the location of gene functioning. BP category explains about the end results of gene function and the associated key pathways. These categories comprise of many distinct and broad terms which are based on the available database information.

GO Ontology Database annotation version and PANTHER Over-representation test were utilised for the analysis (131, 132). Fisher's exact with FDR multiple test correction type was used to categorize the DEGs. In the current work, the over-represented DEGs were classified in different GO categories and terms and summarized in table 17.

Table 17. Most highly affected GO categories and terms under six comparative light intensities:

Comparative light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	GO Category	GO terms	GO Accession
150 vs 112 light	CC	Plasma membrane	GO:0005886
		Cell periphery	GO:0071944
150 vs 75 light	CC	Cell	GO:0005623
		Cytoplasm	GO:0005737
		Membrane	GO:0016020
	MF	Catalytic activity	GO:0003824
150 vs 38 light	BP	Cellular process	GO:0009987
		Metabolic process	GO:0008152
		Cellular metabolic process	GO:0044237
		Organic substance metabolic process	GO:0071704
		Biological process	GO:0008150
	CC	Cell	GO:0005623
		Cytoplasm	GO:0005737
		Membrane	GO:0016020
		Integral component of membrane	GO:0016021

		Intrinsic component of membrane Cell periphery	GO:0031224 GO:0071944
	MF	Ion binding Heterocyclic compound binding Organic cyclic compound binding Molecular function Catalytic activity	GO:0043167 GO:1901363 GO:0097159 GO:0003674 GO:0003824
112 vs 75 light	CC	Cytoplasm Cell	GO:0005737 GO:0005623
	MF	Catalytic activity	GO:0003824
112 vs 38 light	BP	Cellular process	GO:0009987
	CC	Membrane Cytoplasm Cell Intracellular	GO:0016020 GO:0005737 GO:0005623 GO:0005622

DEGs in 150 vs 112 μmol m^{-2} s^{-1} comparative light intensity were enriched under CC category and classified in plasma membrane and cell periphery GO terms. Under 150 vs 75 μmol m^{-2} s^{-1} comparative light intensity, DEGs were grouped in MF and CC domains. In MF category, catalytic activity was the most over-represented term and under CC category the most enriched terms were

cell, cytoplasm and membrane. Under 150 vs 38 μmol m^{-2} s^{-1} light, maximum number of DEGs were present and categorized in BP, MF and CC. In case of this comparative light intensity, cellular, metabolic, cellular metabolic, organic substance metabolic and biological process were the highly represented terms found under BP category. Under CC category, the over-represented terms were cell, cell periphery, membrane, cytoplasm, intrinsic and integral component of membrane. However, the most enriched terms under MF category were heterocyclic compound binding, ion binding, catalytic activity and organic cyclic compound binding.

Similarly, in case of 112 vs 75 μmol m^{-2} s^{-1} comparative light intensity, DEGs were present in all categories, however, the over-represented terms were found only in MF and CC categories. Here in CC category, cell and cytoplasm were the most enriched terms and in MF category, catalytic activity was the highly represented term. DEGs under 112 vs 38 μmol m^{-2} s^{-1} light were categorized in BP and CC class. Cellular process was the most represented term under BP category. On the other hand, CC category comprised of the most enriched terms: cell, membrane, cytoplasm and intracellular. Under 75 vs 38 μmol m^{-2} s^{-1} comparative light, DEGs were minimally enriched and classified in CC and BP categories. The only over-represented term under CC category was respiratory chain.

3.2.4. Kyoto Encyclopedia of Genes and Genomes pathway analysis for investigating gene function

Kyoto Encyclopedia of Genes and Genomes **(**KEGG) color pathway analysis was performed to explore the specific functions of DEGs found under different comparative light intensities.

KEGG analysis gives detailed information about the function and utility of genes in biological, cellular and molecular processes. KEGG analysis works on the basis of available database comprising of information about genome sequencing, bioinformatic details *etc* (133).

KEGG analysis characterized only 184 genes out of 1014 DEGs in case of 150 vs 112 μmol m^{-2} s^{-1} light. Under 150 vs 75 μmol m^{-2} s^{-1} comparative light, KEGG found 290 DEGs out of 1424. Total number of DEGs obtained from microarray data was 1789 under 150 vs 38 μmol m^{-2} s^{-1} light, however, only 420 genes were characterized by KEGG. Under 112 vs 75 μmol m^{-2} s^{-1} light, out of 1163 DEGs, KEGG identified only 249 genes. In case of 112 vs 38 μmol m^{-2} s^{-1} comparative condition, KEGG characterized 314 genes, out of 1518 DEGs. From microarray analysis, 1047 DEGs were obtained under 75 vs 38 μmol m^{-2} s^{-1} comparative light, however, KEGG detected only 172 DEGs. The details of KEGG analysis has been shown in the table 18. KEGG analysis has shown that maximum number of DEGs belonged to metabolic and secondary metabolite biosynthetic pathways under all comparative light intensities.

Table.18. Categorization of DEGs based on KEGG analysis:

Comparative light intensity (μmol m^{-2} s^{-1})	**Total no. of DEGs obtained from microarray**	**No. of genes categorized by KEGG**	**Major pathways**	**No. of genes under each pathway**
150 vs 112 light	1014	184	Metabolic pathway Secondary metabolite biosynthesis Plant hormone signal transduction Protein processing in ER Carbon metabolism MAPK signaling	71 40 19 15 11 11
150 vs 75 light	1424	290	Metabolic pathway Secondary metabolite synthesis Carbon metabolism Amino acid biosynthesis	143 82 24 19

			Glycolysis	15
			Plant-pathogen interaction	13
			RNA transport	13
			Glutathione metabolism	13
			Starch and sucrose metabolism	13
			Purine metabolism	12
			Oxidative phosphorylation	12
			Plant hormone signal transduction	10
			Amino sugar and nucleotide sugar metabolism	10
150 vs 38 light	1789	420	Metabolic pathway	79
			Secondary metabolite biosynthesis	114
			Ribosome	51
			Carbon metabolism	27
			Amino acid biosynthesis	25
			Oxidative phosphorylation	21
			Phenylpropanoid biosynthesis pathway	20
			Plant-pathogen interaction	17
			RNA transport	17
			Spliceosome	17
			Starch and sucrose metabolism	17
			Glutathione metabolism	16
			Plant hormone signal transduction	15
			Glycine, serine and threonine metabolism	13
			Endocytosis	13
			Glycolysis	13
			Amino sugar and nucleotide sugar metabolism	12
			Purine metabolism	12

			Cysteine and methionine metabolism	11
			Photosynthesis	10
			Ubiquitin mediated proteolysis	10
			2-oxocarboxylic acid metabolism	10
			Protein processing in ER	10
112 vs 75 light	1163	249	Metabolic pathway	122
			Biosynthesis of secondary metabolites	63
			Carbon metabolism	18
			Starch and sucrose metabolism	15
			Plant hormone signal transduction	14
			Amino acid biosynthesis	12
			Protein processing in the ER	11
			Phenylpropanoid biosynthesis pathway	10
112 vs 38 light	1518	314	Metabolic pathway	131
			Secondary metabolite biosynthesis	59
			Ribosome	30
			Plant hormone signal transduction	27
			Carbon metabolism	22
			Oxidative phosphorylation	18
			MAPK signaling	13
			Protein processing in ER	13
			Purine metabolism	12
			Spliceosome	12
			Glyoxylate and dicarboxylate metabolism	11
			Amino acid biosynthesis pathway	10
75 vs 38 light	1047	172	Metabolic pathway	64
			Biosynthesis of secondary metabolite	37
			Plant-pathogen interaction	10
			Plant hormone signaling	10

3.2.5. Quantitative PCR validation of DEGs obtained from microarray involved in phytohormone, light and clock-controlled pathways

The DEGs obtained from microarray data were further validated by qRT-PCR. Here, the genes were selected on the basis of their expression levels (from microarray data) and possible roles in root growth. Light, phytohormone and circadian clock have been shown to be the important factors influencing root development (12, 134). Root growth and gravitropism have been reported to be regulated by PHYs (135). Phytohormones control lateral root formation in dose dependent manner and also regulate root hair initiation and growth (136, 137). Lateral root emergence has been shown to be controlled by circadian clock (134). In *Eucalyptus*, it has been reported that exogenous auxin and higher intensity of light promote adventitious root growth (138).

Hence, the genes participating in the auxin responsive and transport pathway: *ARF2*, *ARF4*, *ARF18*, *IAA7*, *LIKE AUX 2* (*LAX2*), *SAUR9* and *SAUR26*; cytokinin signaling genes: *KMD1* and *TYPE A RESPONSE REGULATOR 6* (*ARR6*) and ABA signaling gene: *COP1 INTERACTING PROTEIN 1* (*CIP1*) were selected to explain the impact of different W light intensity on phytohormone associated pathways. Some light signaling genes such as *HY5, PIF4, CONSTANS LIKE 3* (*COL3*), *COL9, PAR2, EARLY PHYTOCHROME RESPONSE 1* (*EPR1), COP9 SIGNALOSOME COMPLEX SUBUNIT 6A* (*CSN6A*) and *CSN6B* were also selected to evaluate the impact of light intensity on downstream light signaling gene expression in roots. To investigate the correlation of clock and light intensity, clock regulated genes for example, *TIMING OF CAB EXPRESSION 1* (*TOC1*), *PSEUDO RESPONSE REGULATOR 9* (*PRR9*) and *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) were selected. These genes were evaluated for all the comparative light intensities.

Under 112 µmol m^{-2} s^{-1} in comparison to 150 µmol m^{-2} s^{-1} light, *COL3* was upregulated however, *PIF4* and *EPR1* genes were downregulated. On the other hand, *ARF2* and *ARF4* were upregulated and *TOC1* was downregulated (Fig.3.7).

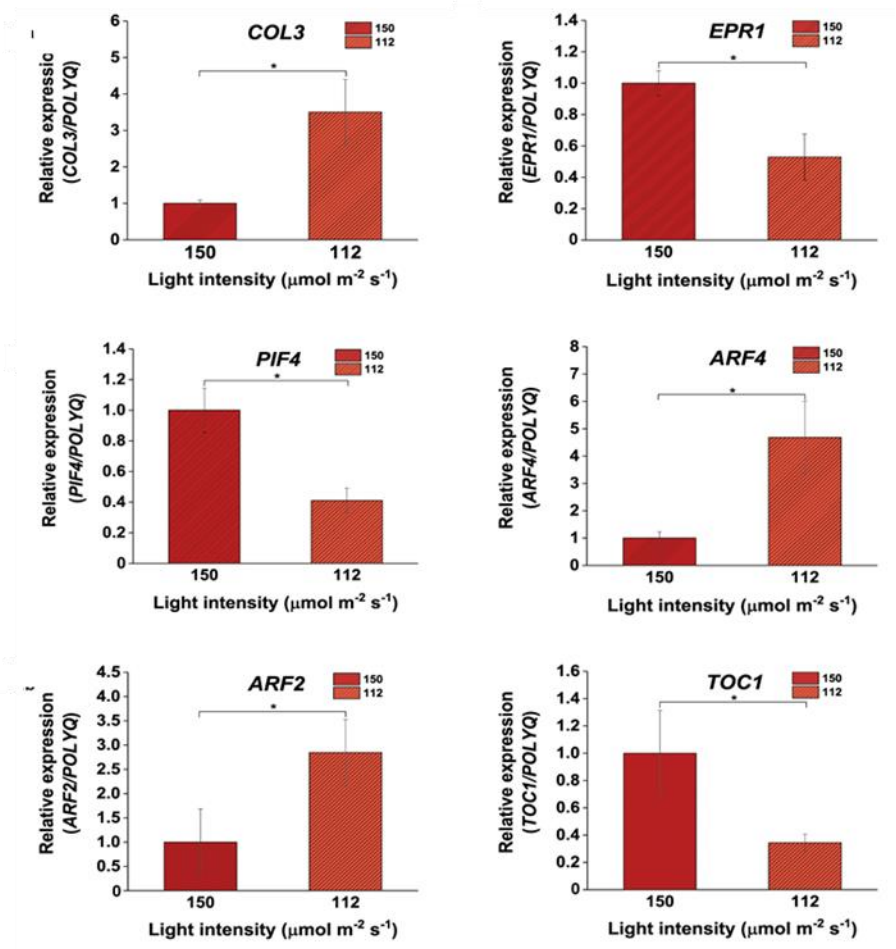


Fig.3.7. Change in expression level of *COL3, EPR1, PIF4, ARF4, ARF2* and *TOC1* genes under 150 vs 112 µmol m^{-2} s^{-1} light condition in the root sample of 5-day old Col-0 seedlings. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here *= p<0.05. qRT-PCR was performed at least in triplicates.

When 75 µmol m^{-2} s^{-1} light was compared to 150 µmol m^{-2} s^{-1} intensity, *PIF4*, *EPR1* and *CSN6B* genes were downregulated but *COL3* was upregulated. On the other hand, *ARF18*, *CIP1* and *KMD1* genes were upregulated however, *LAX2* was downregulated. *TOC1* was also downregulated under this comparative light (Fig.3.8)

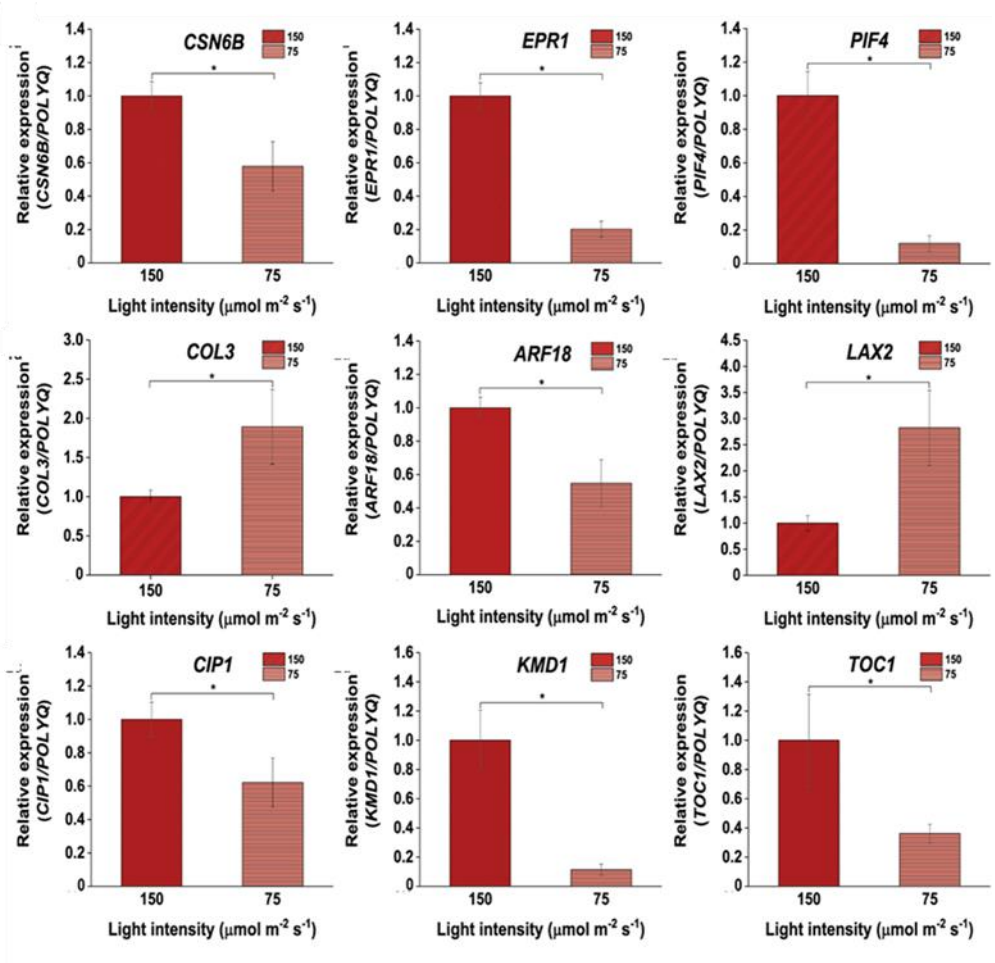


Fig.3.8. Expression of *CSN6B, EPR1, PIF4, COL3, ARF18, LAX2, CIP1, KMD1* and *TOC1* genes under 150 vs 75 µmol m^{-2} s^{-1} light condition was altered in the root sample of 5-day old Col-0 seedlings. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here *= p<0.05. qRT-PCR was performed at least in triplicates.

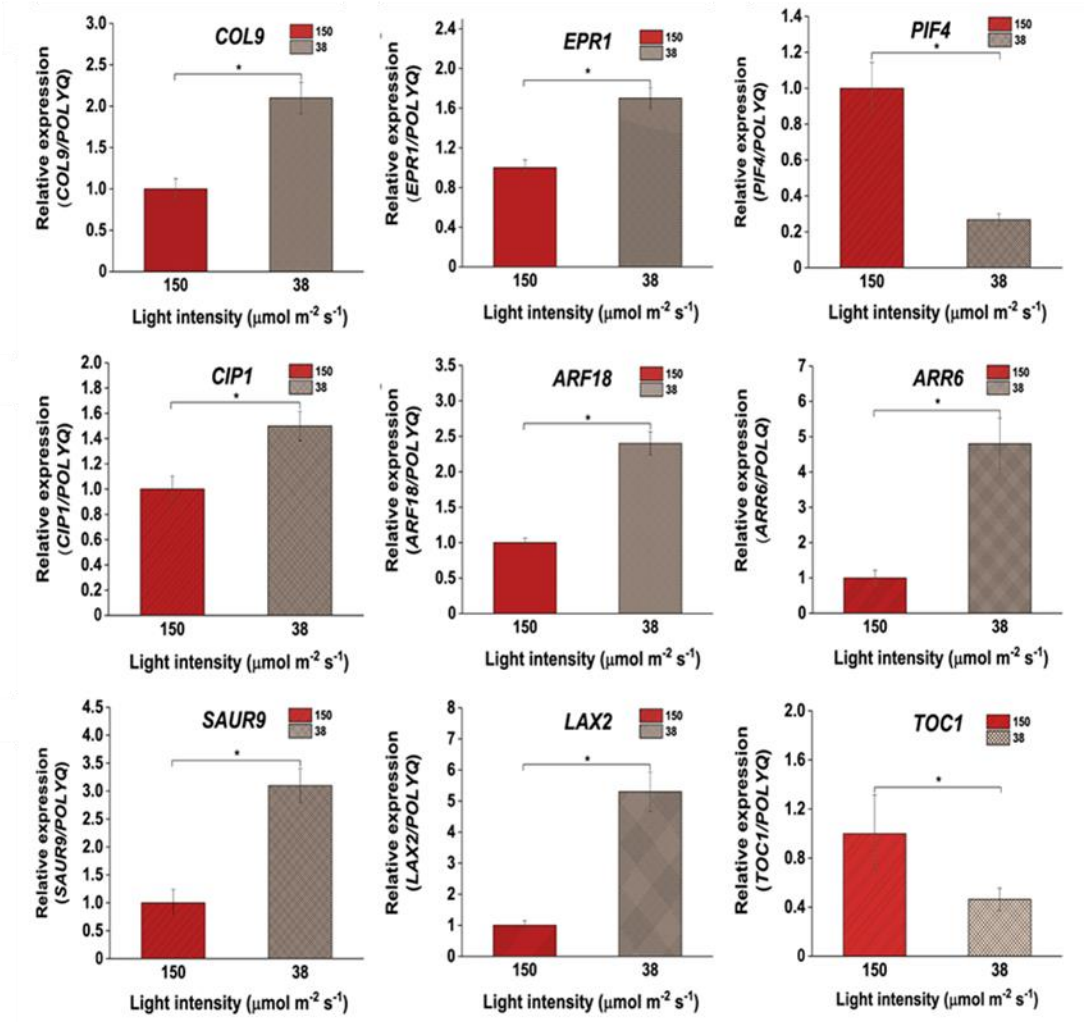


Fig.3.9. Expression of *COL9, PIF4, EPR1, ARF18, SAUR9, LAX2, CIP1, ARR6* and *TOC1* genes was differentially regulated in root sample of 5-day old Col-0 seedlings under 150 vs 38 µmol m^{-2} s^{-1} comparative light. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here *= $p<0.05$. qRT-PCR was performed at least in triplicates.

Under 38 µmol m^{-2} s^{-1} light in comparison to 150 µmol m^{-2} s^{-1}, *EPR1* and *COL9* gene were upregulated while *PIF4* has been shown to be downregulated. Under this comparative light condition, *ARF18*, *SAUR9*, *LAX2*, *ARR6* and *CIP1* genes were upregulated however, *TOC1* gene was downregulated (Fig.3.9).

Under 75 µmol m^{-2} s^{-1} as compared to 112 µmol m^{-2} s^{-1} light, *COL3* and *EPR1* genes were downregulated. In this case, hormone and clock-controlled genes didn't show significant alteration in their expression (Fig.3.10).

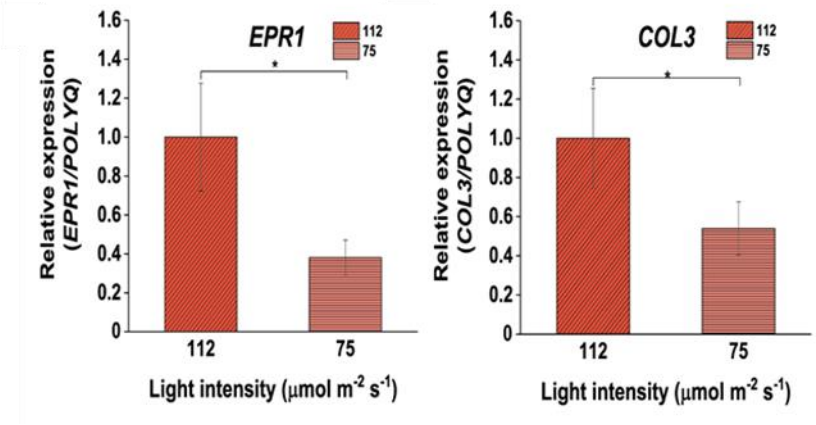


Fig.3.10. Light signaling genes; *EPR1* and *COL3* were downregulated in root sample of 5-day old Col-0 seedlings under 112 vs 75 µmol m^{-2} s^{-1} comparative light. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here *= p<0.05. qRT-PCR was performed at least in triplicates.

Under 38 µmol m^{-2} s^{-1} light in comparison to 112 µmol m^{-2} s^{-1} light, *EPR1* gene was upregulated, however, *COL3* was observed to be downregulated. Furthermore, *ARR6*, *SAUR9* and *SAUR26* genes were upregulated but *ARF2* was downregulated. The *CCA1* gene was upregulated under this slight condition (Fig.3.11).

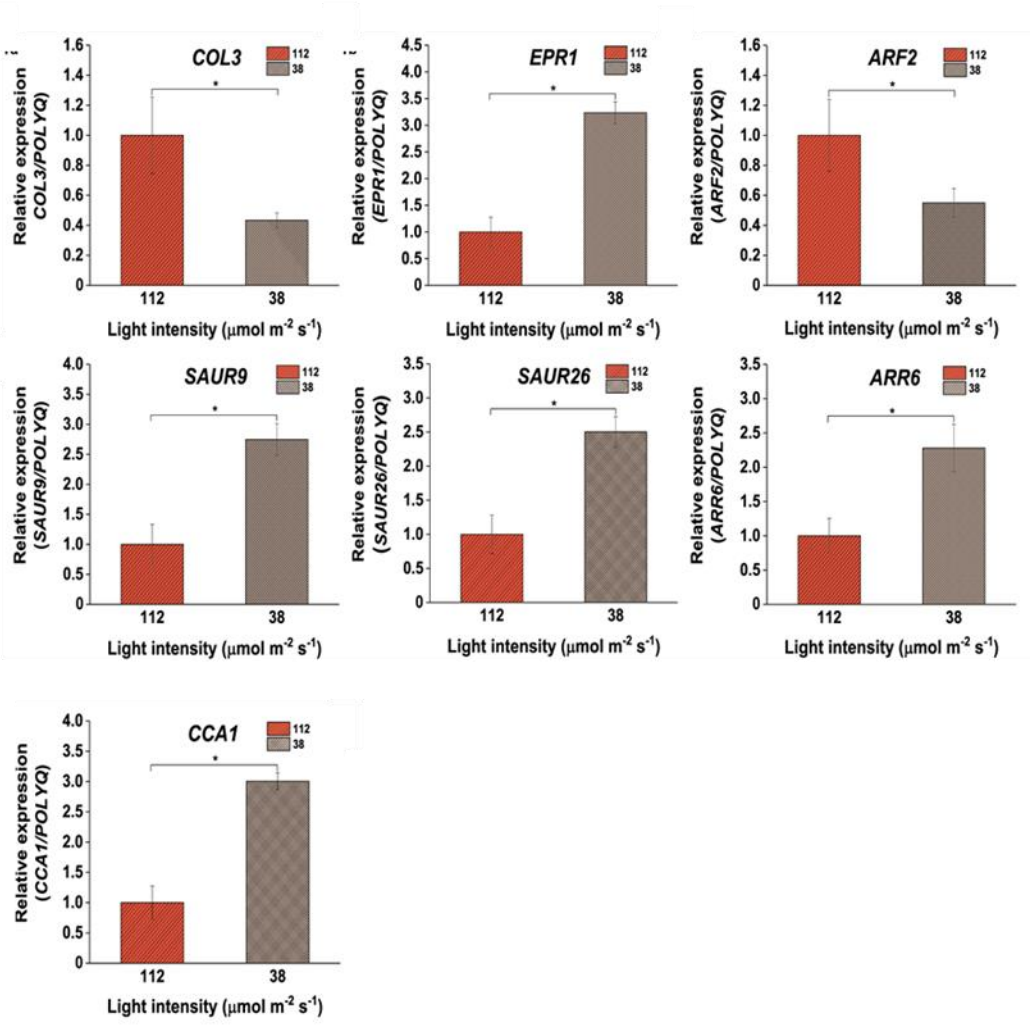


Fig.3.11. *COL3, EPR1, ARF2, SAUR9, SAUR26, ARR6* and *CCA1* genes were altered in root sample of 5-day old Col-0 seedlings under 112 vs 38 µmol m^{-2} s^{-1} light intensity. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here *= p<0.05. qRT-PCR was performed at least in triplicates.

HY5, *COL9*, *CSN6A*, *CSN6B* and *EPR1* genes were upregulated under 38 µmol m^{-2} s^{-1} light when compared with 75 µmol m^{-2} s^{-1} light intensity. The expression of *ARF18*, *LAX2*, *KMD1* and *CIP1* genes was also enhanced. Similarly, *CCA1* gene was also upregulated under this light condition

(Fig.3.12). The difference in expression of *PRR9* gene has been observed to be insignificant under all the six comparative light conditions.

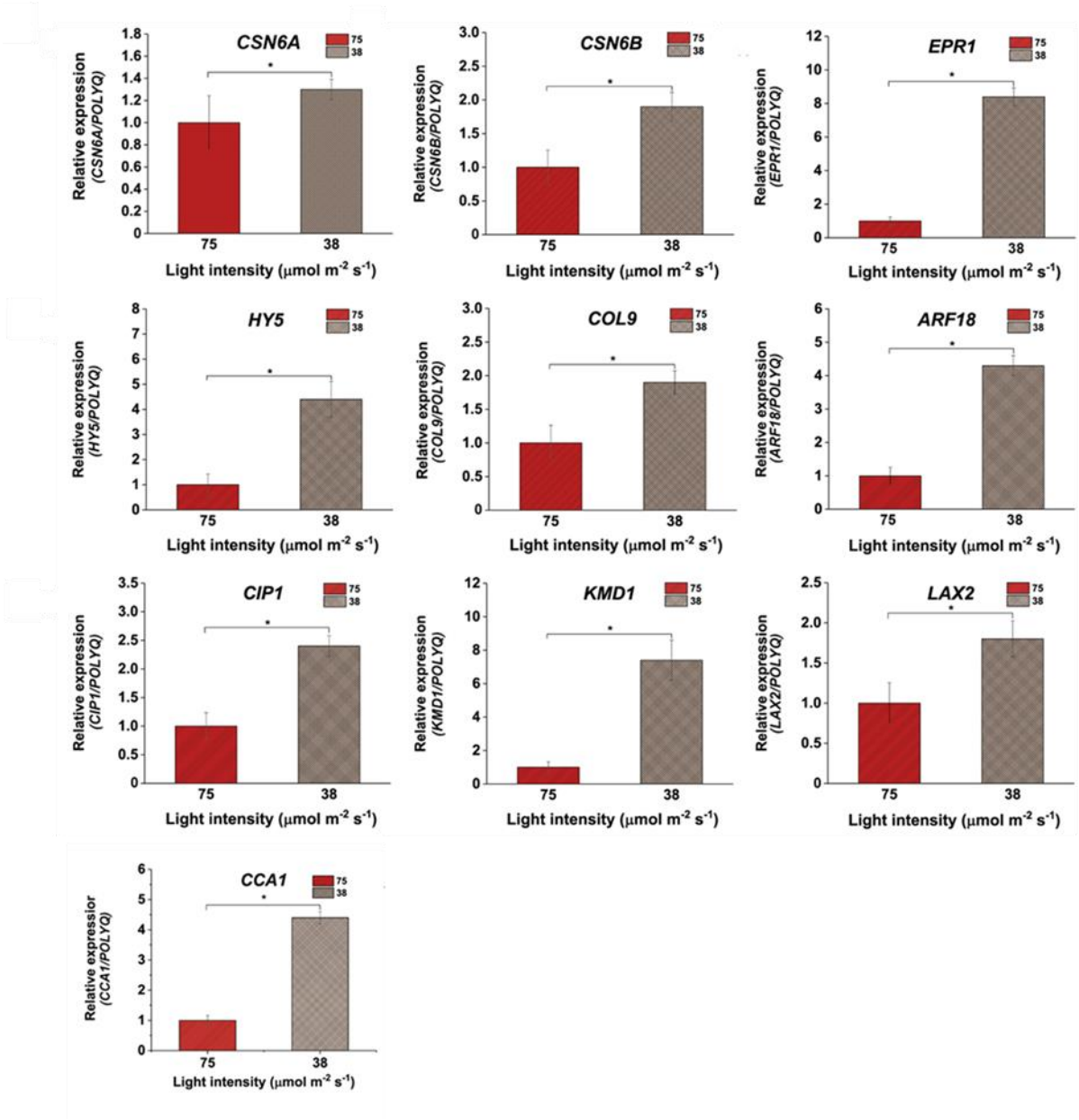


Fig.3.12. Light, phytohormone and clock-ontrolled genes were upregulated in root sample of 5-day old Col-0 seedling under 38 µmol m^{-2} s^{-1} light when compared with 75 µmol m^{-2} s^{-1} light. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with

Tukey and Bonferroni tests was performed, here *= p<0.05. qRT-PCR was performed at least in triplicates.

3.3. Discussion:

The light intensity as well as quality both play crucial role in root development. The survivability rate and health of plants depend upon the root growth. It has been shown that root growth varies in presence or absence of light. Root growth pattern is different if only shoot is irradiated or if roots are directly exposed to light (120). It has reported that, plants display shorter roots and low root to shoot biomass ratio when they are grown under lower intensities of light (119, 124). The total root length, surface area of root, lateral root number and cluster root formation have been shown to increase with increase in light intensity in white lupin (*Lupinus albus* L.). The cluster roots are the parts of primary lateral roots having bottle brush like rootlet cluster along with more than ten rootlets/cm (139). Different photoreceptors present in plants have been investigated to modulate the morphology and physiology of roots. PHYs, CRYs, PHOTs and UVR-8 are directly or indirectly regulate the root growth (25, 34, 39, 42, 140, 141). They are involved in the control of root elongation, lateral root growth, adventitious root growth and root gravitropism. Among PHYs, PHYA and PHYB play predominant role in controlling primary and lateral root growth (12). PHYs perceive R and FR light but can also sense the variation in the ratio of R:FR light. It has been reported that when roots are in direct contact of light then *phyB* mutant displays shorter primary root in comparison to wild-type (119). In the current work, similar result was found at higher light intensities. The present work shows that both *phyA* and *phyB* mutants possess shorter primary root at higher light intensities while at lower light intensity only *phyA* mutant has shorter primary root as compared to Col-0.

Previous reports have shown that in tobacco, root biomass increases with increase in light intensity (60-300 µmol m^{-2} s^{-1} light) (124). The current work has utilized light in the range 38-150 µmol m^{-2} s^{-1} intensity and it was examined that in Col-0, the lateral and adventitious root number enhanced with increase in light intensity. This could be similar to the case of increase in root biomass of tobacco with light intensity as here the lateral and adventitious roots (contributing to the whole root biomass) increased with increasing intensity of light. However, *phy* mutants showed variable root parameters under different light intensity. This suggests that PHYs along with light intensity both are involved in determining the root growth.

It was investigated that when only shoot is irradiated or both shoot and root are irradiated with light, the expression of *PHYA, PHYB, CRYs, PHOTs* in roots was similar in both the cases. (128). The present work has found that under highest (150 µmol m^{-2} s^{-1} light) versus lowest (38 µmol m^{-2} s^{-1} light) light intensity, the expression of light signaling genes such as *PIF4*, *COL9*, *EPR1*, hormone associated genes: *CIP1*, *ARF18, SAUR9, LAX2, ARR6* and clock regulated gene *TOC1* was altered in Col-0 roots when shoot and root both are irradiated. In future, some of the key regulators of light signaling such as other *PIFs*, *FHY1, COP1 etc*. can also be analysed in the root under different light intesnities. Next, other phytohormone associated genes can also been analysed in root under variable light intesnities. The present work can be extended by exploring the gene profiling in the roots of *phyA* and *phyB* mutants under light intensity tested. This will facilitate in explaining the mechanism of light intensity affecting the root growth in *phy* mutants. The current study would be beneficial to understand the mechanisms behind the root growth in the vegetation areas where light intensity varies very frequently. In this way, plants with robust root system to adapt to any adverse light condition can be developed in future.

CHAPTER 4

Light dependent effects of carbon nanoparticles on root architecture in *Arabidopsis thaliana*

Chapter 4.

4. Light dependent effects of carbon nanoparticles on root architecture in *Arabidopsis thaliana*

4.1. Background:

Roots are important part of plants and they help in uptake of water, nutrients and anchorage to soil, leading to healthy growth of plants. It has been reported that light and phytohormones both affect root development. PHYB promotes the lateral root emergence and its growth (113). Auxin increases the lateral and adventitious root growth; however, lateral root is inhibited by CK (53). In presence of light, ET has been shown to reduce root growth, but enhances root hair size and number (142). Few other environmental/abiotic factors such as nanomaterials (NMs) have also been investigated to influence plant growth. In small quantity they don't have any effect, however, after release of excess NMs they alter the growth and development of plants and animals (143). NMs are used in various fields such as agriculture, genetic engineering, drug delivery systems etc. They have also been shown to influence root morphology and its architecture.

There are few reports available which state that light and hormone cross-talk to regulate root growth. COP1 has also been shown to regulate the PIN dependent auxin transport (144). PHY mutants for example *phyA, phyB, phyAphyB* and downstream light signaling mutants such as *hy1-7* have been shown to be resistant to CK and exhibit defects in root growth and root hair development (145). ABA interacts with light signaling to modulate root development as *hy-5* has been reported to be insensitive to ABA in the development of primary and lateral roots (146).

NMs are the colloidal system having 10-1000 nm size. They are of different types, for example, quantum dots, semiconductor, nanopolymer, carbonaceous etc. and they affect the growth and development of plants (147). They also influence root growth, the impacts vary upon type, dose,

size and form of NMs and also depend on type of plant species. They have positive impact at lower dose, however, in excess or after prolonged exposure, they exhibit negative effects on root development. Carbon nanomaterials (CNMs) belong to carbonaceous NM category and have been reported to influence root growth in various ways. CNMs have been classified on the basis of their shape, size and structure in different groups such as carbon dots, carbon nanoparticles, carbon nanotubes, graphene, fullerenes etc (148).

Single-walled non-functionalized carbon nanotubes (CNTs) and functionalized carbon nanotubes (fCNTs) have been reported to exhibit variable effects on root development in different plant varieties. The root growth was more severely affected by CNTs as compared to fCNTs. Root elongation was drastically decreased after 24 and 48 hrs of CNTs exposure in tomato, however, the impact of CNTs was reverse in onion plants. In case of cucumber, root growth was more after 24 hrs of CNTs treatment. On the other hand, in lettuce, fCNTs caused reduction in root growth after 48 hrs of treatment. It was observed that the effect of both type of CNTs on root elongation was insignificant after 24 hrs and 48 hrs in lettuce and cucumber respectively. Furthermore, in cabbage and carrot there was no significant impact of both CNTs after 24 and 48 hrs of treatment (149). This article suggested that, the impacts of NTs on root development are determined by their type, exposure time and also depend on plant variety. Single-walled carbon nanotubes (SWCNTs) in maize, have been reported to promote seminal root growth while suppress root hair growth, however, primary root elongation was unaffected. It was also observed that genes playing vital roles in root development were altered by SWCNTs. *SLR1* is a key regulator of cellular pattering in seminal roots and it was upregulated after 36 hrs whereas downregulated after 72 hrs of SWCNT treatment. *ROOTLESS CONCERNING CROWN AND SEMINAL ROOT* (*RTCS*) gene participates in embryonic seminal root initiation. The expression of *RTCS* genes was enhanced with an increase

in exposure time. In contrast, SWCNTs downregulated *ROOT HAIRLESS 1* (*RTH1*) and *RTH3* genes involved in root hair growth control (150). In *At*, COOH-multiwalled carbon nanotubes (COOH-MWCNTs) was shown to decrease the number of root hairs. However, SA supplemented with COOH-MWCNTs enhanced root hair number. CNTs have also been shown to downregulate the genes playing role in root hair growth, emergence and differentiation. Genes such as *AT4G25790*, *CYP708A2*, *AT3G49960*, *AT4G26010*, *AT1G34510*, *AT5G05500*, *ROOT HAIR SPECIFIC 12* (*RHS12*), *RHS13*, *RHS15* and *RHS19* were downregulated by COOH-MWCNTs (151). In rice, root length and biomass have been investigated to be increased by MWCNTs, Fe-Co-filled CNTs (FeCo-CNTs) and Fe-filled CNTs (Fe-CNTs) at lower concentrations. However, they suppressed the root growth higher dose. They have also been shown to reduce the IAA, ABA, GA, CK, BR and JA content of roots (152). Graphene (another class of CNMs) has been reported to alter root growth in tomato, cabbage, lettuce and red spinach. After 20 days of exposure, root elongation and biomass both were reduced in tomato, cabbage and spinach. Primary root and root hair development were also altered by graphene in all tested plants except lettuce. Root growth was affected the most in tomato by graphene treatment. It has enhanced ROS generation and cell death in roots. It also altered the morphology of cells nearby root elongation region in tomato and cabbage. In red spinach and tomato roots, it triggered detachment of epidermis and in case of cabbage, root surface area was enhanced by graphene treatment (153). In cucumber, increasing concentration of carbon nanoparticle (CNP) has been shown to reduce root elongation (154). It has been reported to enhance primary root length under different monochromatic light in *At* (155).

Thus, it was aimed to study the light quality dependent effects of CNP on root morphology and the hormonal signaling pathways in roots. In this chapter, several features of roots such as, primary, lateral, adventitious root development, root hair formation, root coiling and waving were

analysed. The phytohormone signaling genes involved in root growth control were also investigated.

4.2. Results:

4.2.1. CNP altered primary root elongation, lateral, adventitious root number and root hair growth in Col-0 and *phyB-9* mutant depending upon the light quality

In a recent report, the development of *At* has been studied in dose dependent manner using CNP conc. in the range of 10-500 µg/ml. It was shown that the increase in conc. of CNP make the wild-type plants flower early and the flowering time was least at 500 µg/ml CNP conc. In this study, the primary root was also analysed at 500 µg/ml CNP under different light conditions and it was investigated that CNP enhanced the primary root length (155). With this information, in the current work it has been attempted to understand the involvement of PHYB in root development in presence of CNP under different light conditions. The CNP type used was graphite nano powder and the conc. of CNP was 500 µg/ml. The seeds of Col-0 and *phyB-9* after sterilization were sown on hard agar half-MS with and without CNP (+CNPs and -CNPs) in square plates. After cold stratification for 48 hrs and W light germination induction, the plates were kept vertically for 48 hrs under W, R, FR and B (LD) light. The plates were tilted at 45˚ after 48 hrs, then 5 days after tilting the seedlings were imaged (156).

The primary root elongation was analysed using ImageJ program. It was observed that, in -CNP, *phyB-9* has shorter primary root as compared to Col-0, under W, R and B light (Fig.4.1a, 4.1b and 4.1d). It is similar to the result shown earlier in chapter 3 for W light intensity of 112 and 150 µmol m^{-2} s^{-1}. However, in +CNP only under R light, the primary root was significantly shorter in *phyB-9* as compared to Col-0 (Fig.4.1b).

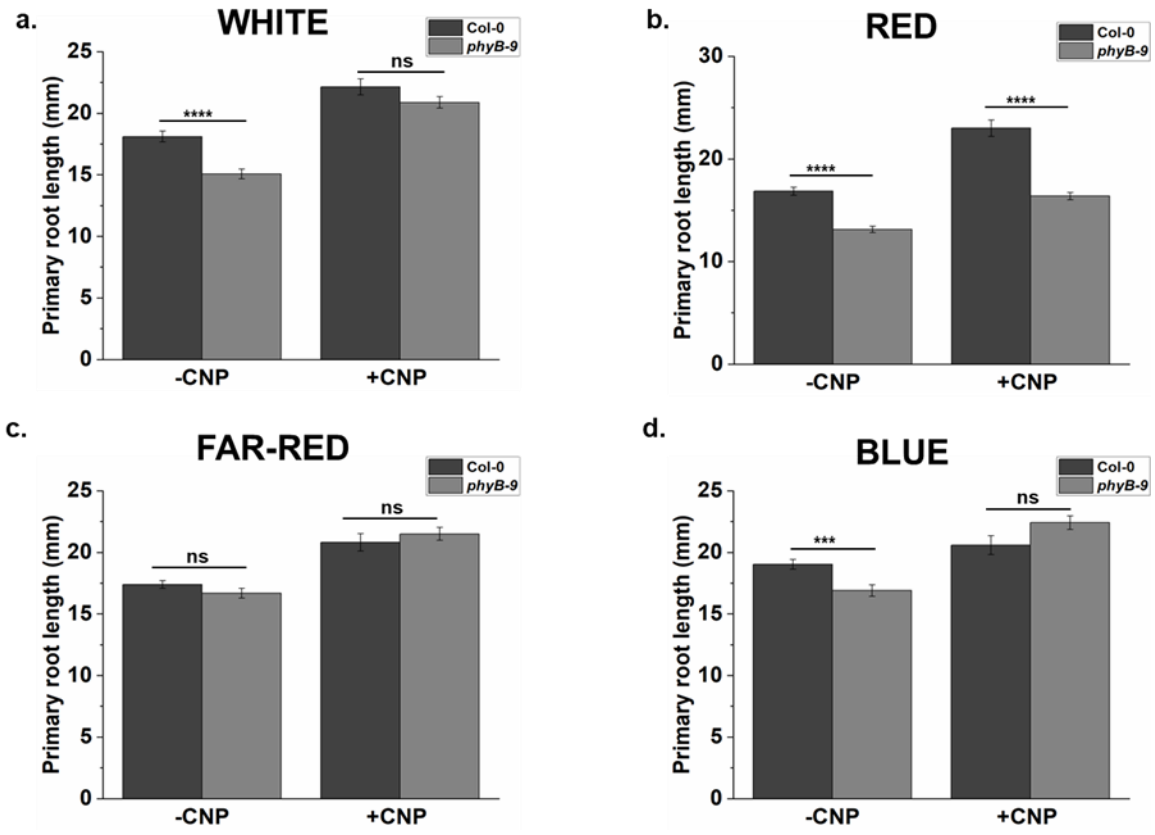


Fig.4.1. Effect of CNP (500 µg/ml) on primary root elongation in 5-day old *phyB-9* mutant seedlings as compared to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

The quantification of lateral, adventitious root and analysis of root hairs were performed under stereo zoom microscope. Quantification data of lateral roots showed that, in -CNP, the number of lateral roots was more in *phyB-9* as compared to Col-0 only under B light (Fig.4.2d). However, in presence of CNP, *phyB-9* had more lateral roots than Col-0 under W and R light (Fig.4.2a and 4.2b).

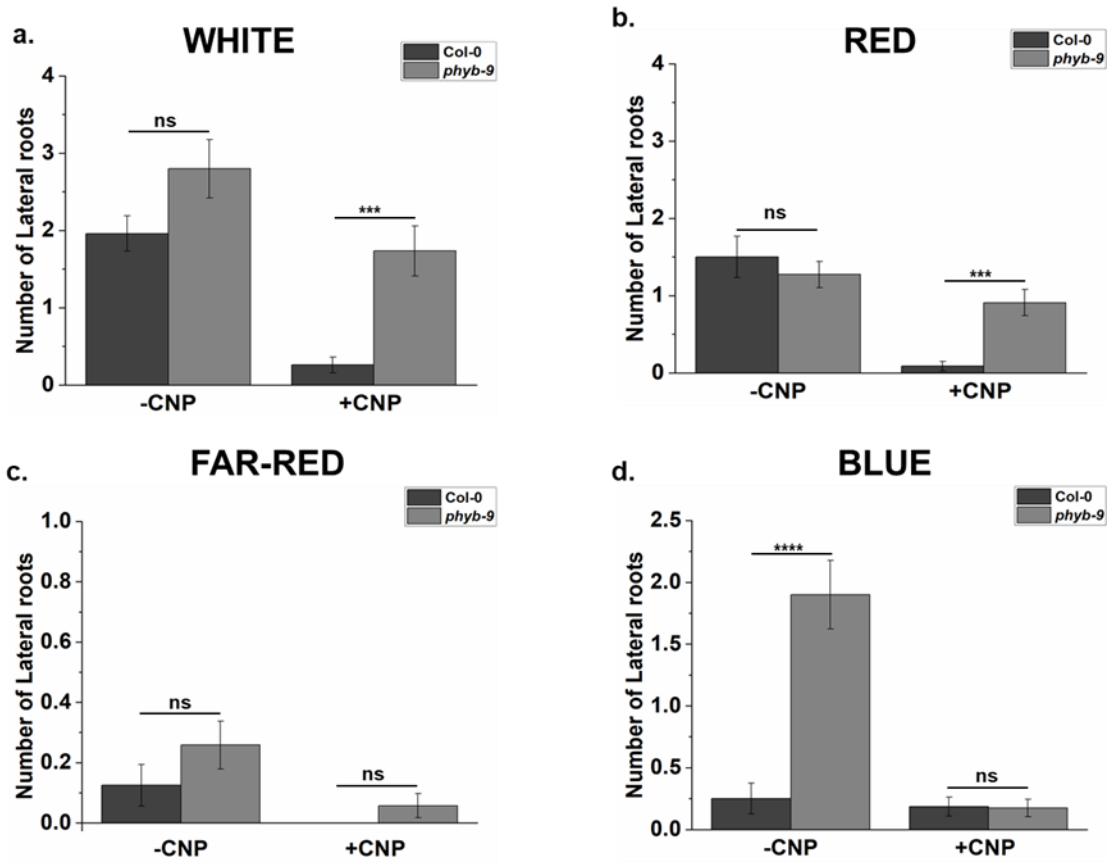


Fig.4.2. CNP (500 µg/ml) influencing lateral root number in 5-day old *phyB-9* mutant seedlings as compared to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

When adventitious root quantification was performed, it was observed that, in -CNP no significant variation was found between the seed lines under all the light qualities. However, in presence of CNP, only under W light, *phyB-9* displayed more adventitious roots than Col-0 (Fig.4.3a).

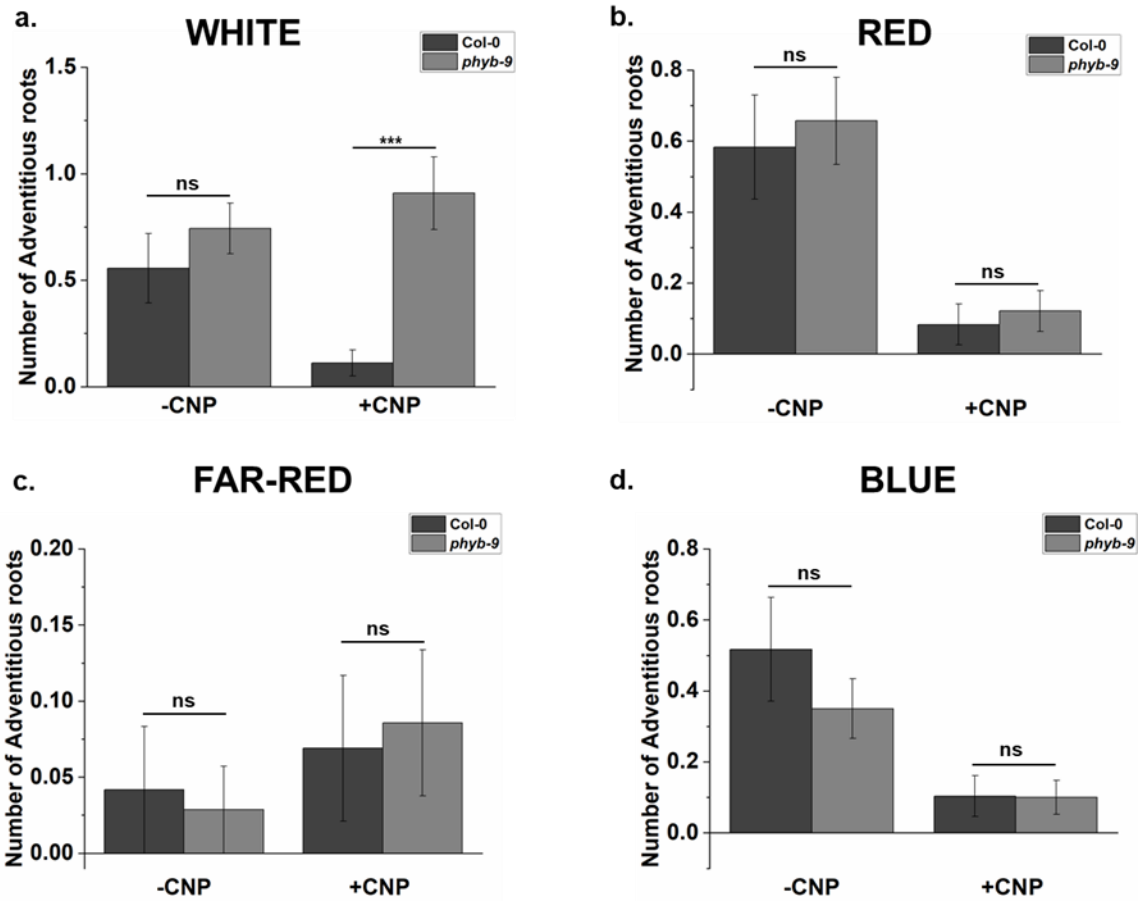


Fig.4.3. Variation in number of adventitious roots of 5-day old *phyB-9* mutant seedlings as compared to Col-0 in presence of CNP (500 µg/ml) under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

The root hair growth was also examined in these two seed lines after 7-days of light treatment. Then it was observed that root hair growth was less in +CNP in both the seed lines, irrespective of light quality. The thickness of root tip region was also lesser in +CNP as compared to -CNP, under all light qualities (Fig.4.4).

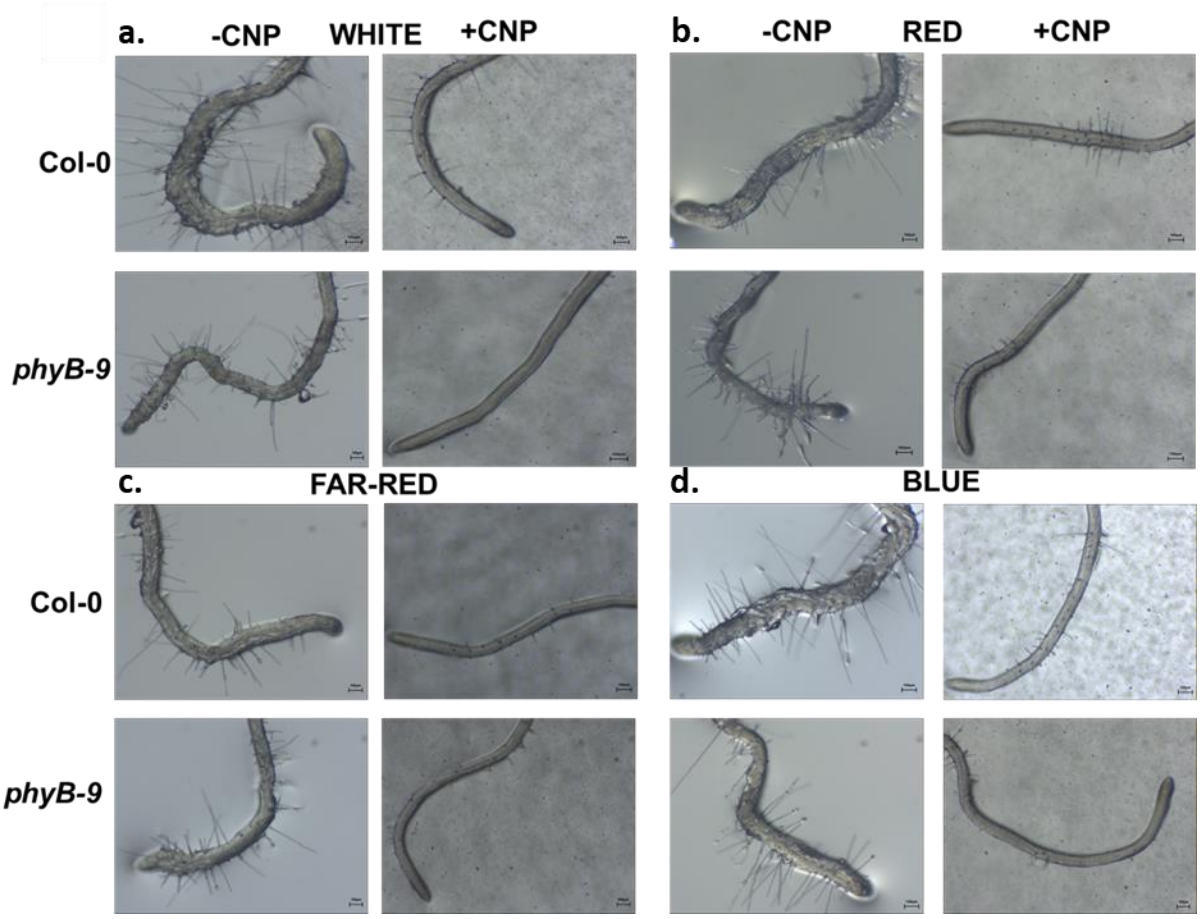


Fig.4.4. Reduction in root hairs and thickness of root by CNP (500 µg/ml) in 5-day old *phyB-9* mutant and Col-0 seedlings under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Scale bar=100µm.

4.2.2. Root wave density and related features varied by CNP depending upon light quality

The primary root elongation, lateral and adventitious roots were investigated to be altered by CNP under different light conditions. Furthermore, root waving and related parameters were analysed in Col-0 and *phyB-9* seedlings. Root waving is an important characteristic of root architecture as it conveys information about how efficiently root tip can penetrate the soil by bending, overcome the mechanical stress and grow inside soil.

Here, the seeds were sown and grown similarly as mentioned in previous section. After 7 days of light irradiation, seedlings were imaged to measure WD, root straightness and HGI and these parameters were analysed by using R program. WD is calculated as the number of waves formed by the root per mm of root length. To understand the details of the root waving, root straightness and HGI were determined. The root straightness (S) characterizes the straightness of a root and is described as the ratio of length of a chord connecting the initial point of root and apex of a primary root (Lc) to the total length of the root (L) (157).

$$S = \frac{Lc}{L}$$

Root growth in plants usually deviates from the vertical axis, and the rate of root growth in the lateral and vertical direction are different. HGI is the parameter to describe the root growth in lateral direction. HGI is defined as the ratio of distance covered by root tip in horizontal direction from the vertical (Lx) and total root length (L) (157).

$$HGI = \frac{Lx}{L}$$

HGI is positive if the root tip deviates towards the right and negative if it deviates in the opposite direction.

Under W, R, FR and B light, in -CNP there was no difference observed in WD of Col-0 and *phyB-9* seedlings (Fig.4.5). However, in +CNP, under W, R and B light, Col-0 had more root WD as compared to *phyB-9* seedlings (Fig.4.5a, 4.5b and 4.5d).

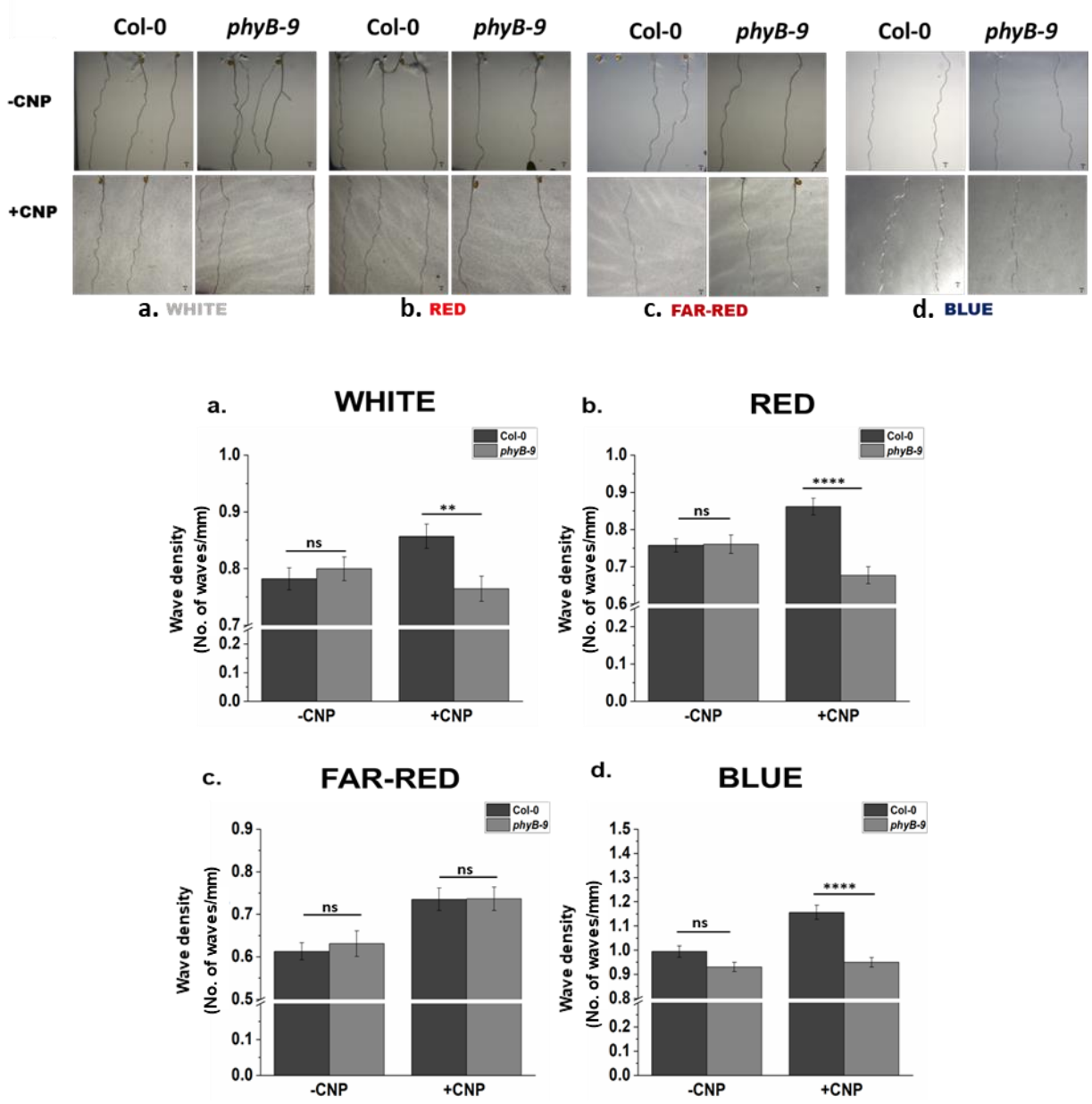


Fig.4.5. Root wave density altered by CNP (500 µg/ml) in 5-day old *phyB-9* mutant seedlings in comparison to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

After that, root straightness was analysed and it was observed that under W and B light, in -CNP *phyB-9* displayed more root straightness as compared to Col-0 seedlings (Fig.4.6a and 4.6d). In +CNP, only under R light Col-0 had more root straightness in comparison to *phyB-9* seedlings (Fig.4.6b).

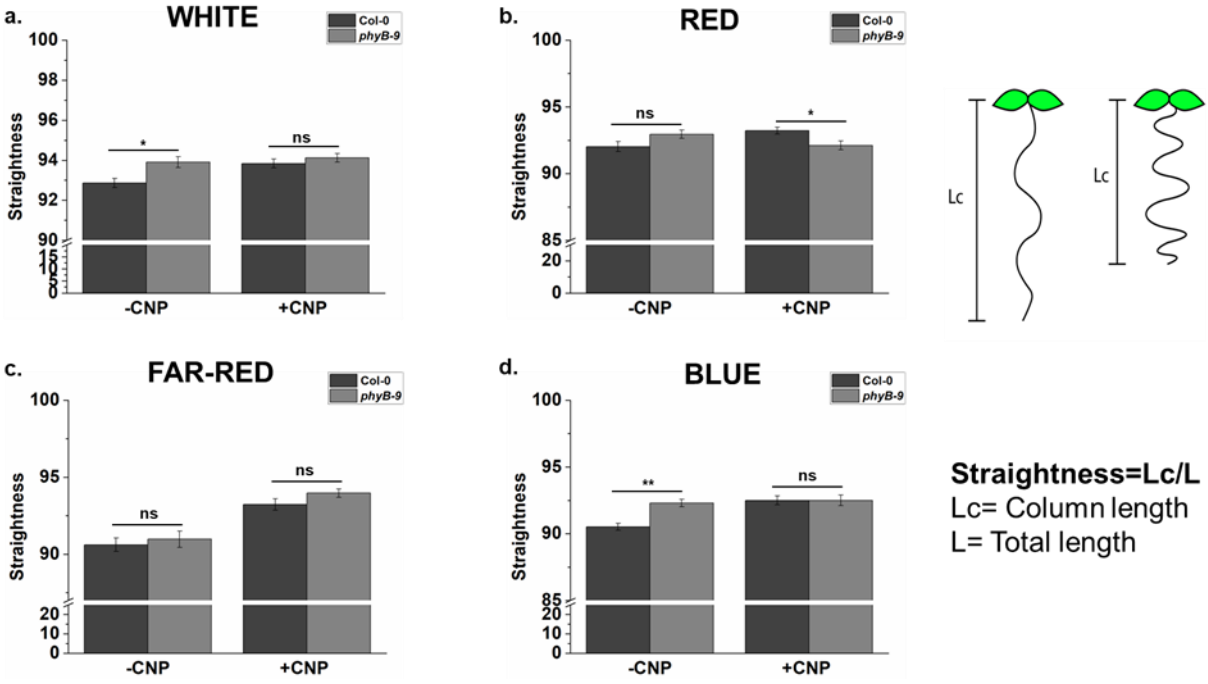


Fig.4.6. Effect of CNP (500 µg/ml) on root straightness in 5-day old *phyB-9* mutant seedlings as compared to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

In presence of CNP, root straightness varied only under R light, then HGI of root in *phyB-9* mutant and Col-0 seedlings was investigated under different light conditions. Under R and B light, in -CNP, Col-0 had more HGI than *phyB-9* seedlings (Fig.4.7b and 4.7d). On the other hand, in +CNP

under W, R and B light, Col-0 displayed more HGI as compared to *phyB-9* seedlings (Fig.4.7a, 4.7b and 4.7d).

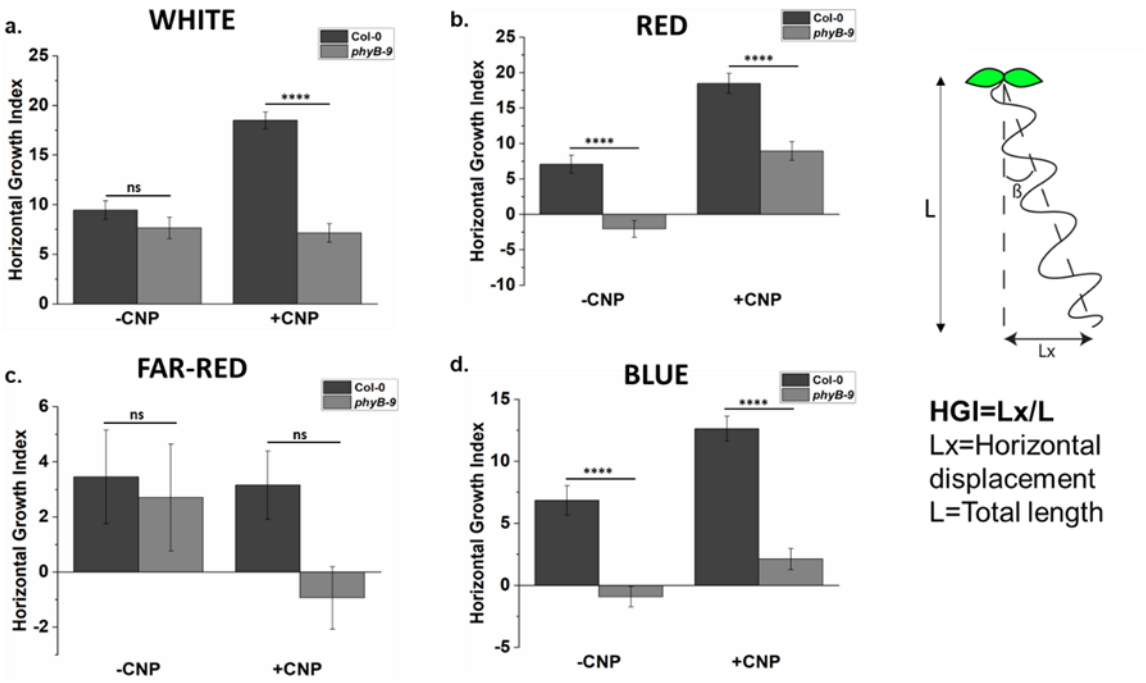


Fig.4.7. Horizontal growth index of root altered by CNP (500 µg/ml) in 5-day old *phyB-9* mutant seedlings as compared to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}), (c) far-red (45 µmol m^{-2} s^{-1}) and (d) blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

4.2.3. Root coiling frequency enhanced by CNPs under different light conditions

It was analysed that CNP-mediated root waving varied upon light quality in *phyB-9* mutant as compared to Col-0 seedlings. Then, root coiling phenomenon was investigated in the seedlings. Root coiling conveys information about graviresponse and thigmotropic response of roots (10). Sterilized seeds were sown on half MS medium in -CNP and + CNP and grown horizontally. After 7 days of light treatment, root coiling was analysed under microscope.

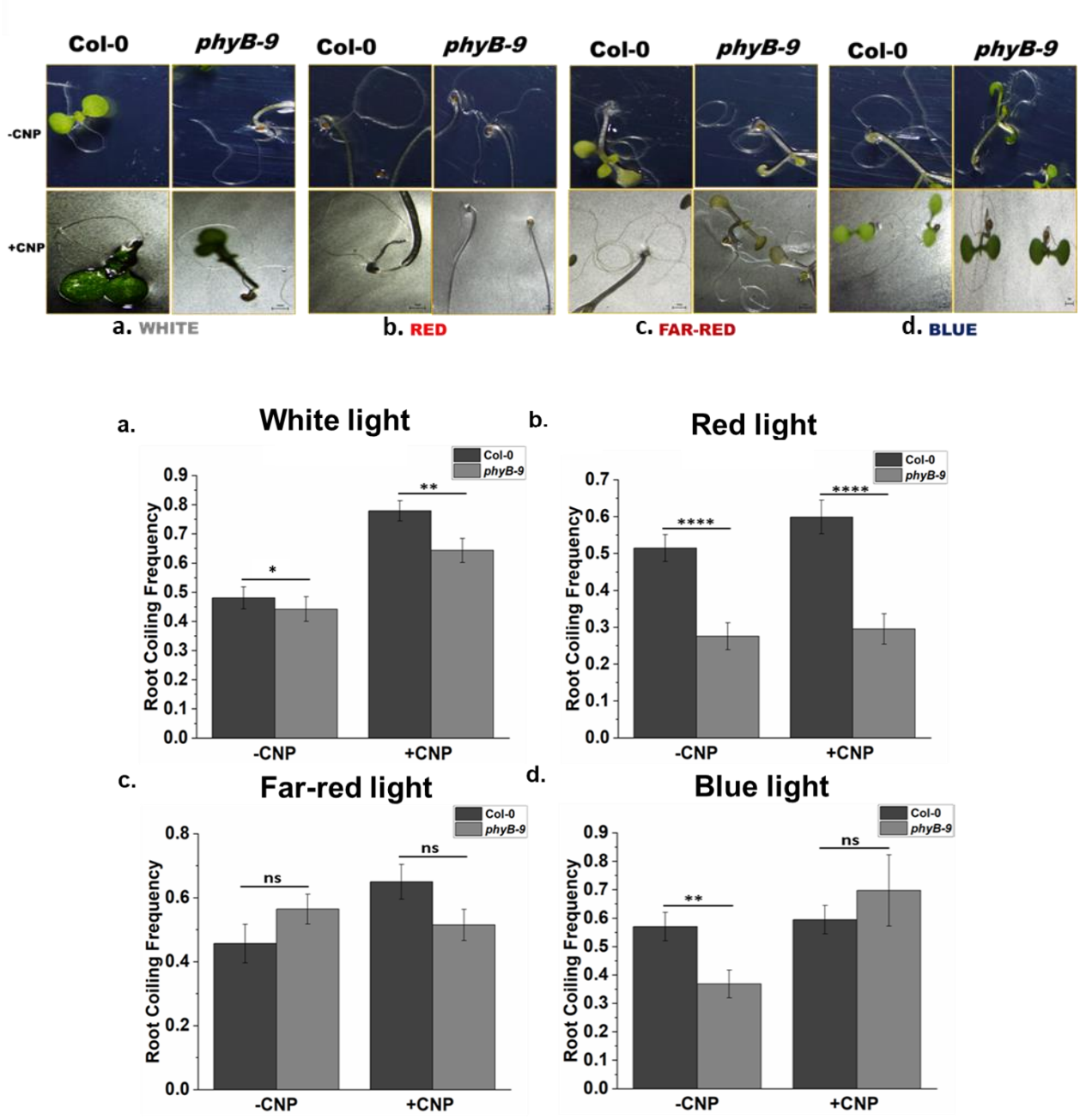


Fig.4.8. CNP (500 µg/ml) influencing root coiling in 5-day old *phyB-9* mutant seedlings as compared to Col-0 (a) under white (100 µmol m^{-2} s^{-1}), (b) under red (50 µmol m^{-2} s^{-1}), (c) under far-red (45 µmol m^{-2} s^{-1}) and (d) under blue (30 µmol m^{-2} s^{-1}) light. Experiment was performed five times and every time 20 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

It was found that, Col-0 formed mostly complete coils, however, *phyB-9* formed mostly irregular and partial coils. Root coiling frequency has been calculated as the ratio of number of seedlings forming coils to the total number of seedlings. Under W, R and B light, in -CNP, Col-0 exhibited more root coiling frequency as compared to *phyB-9* seedlings (Fig.4.8b and 4.8d). In +CNP, *phyB-9* showed lesser root coiling in comparison to Col-0 under W and R light (Fig.4.8a and 4.8b).

4.2.4. Phytohormone signaling genes in root are differentially regulated by CNPs in presence of white and red light

It was investigated in the above-mentioned experiments that CNP alters different aspects of root morphology of *phyB-9* mutant and Col-0 seedlings. Then the impact of CNP on the expression of phytohormone signaling genes was analysed in the roots of *phyB-9* mutant and Col-0 seedlings.

Genes such as *ARR6*, *ETHYLENE INSENSITIVE PROTEIN 2* (*EIN2*) and *TIR1* involved in CK, ET and auxin signaling were analysed under W and R light. In case of -CNP, *ARR6*, *EIN2* and *TIR1* genes were downregulated in *phyB-9* in comparison to Col-0 under W and R light. However, in +CNP, *EIN2* was upregulated under R light and *TIR1* was upregulated both under W and R light in *phyB-9* as compared to Col-0 (Fig.4.9).

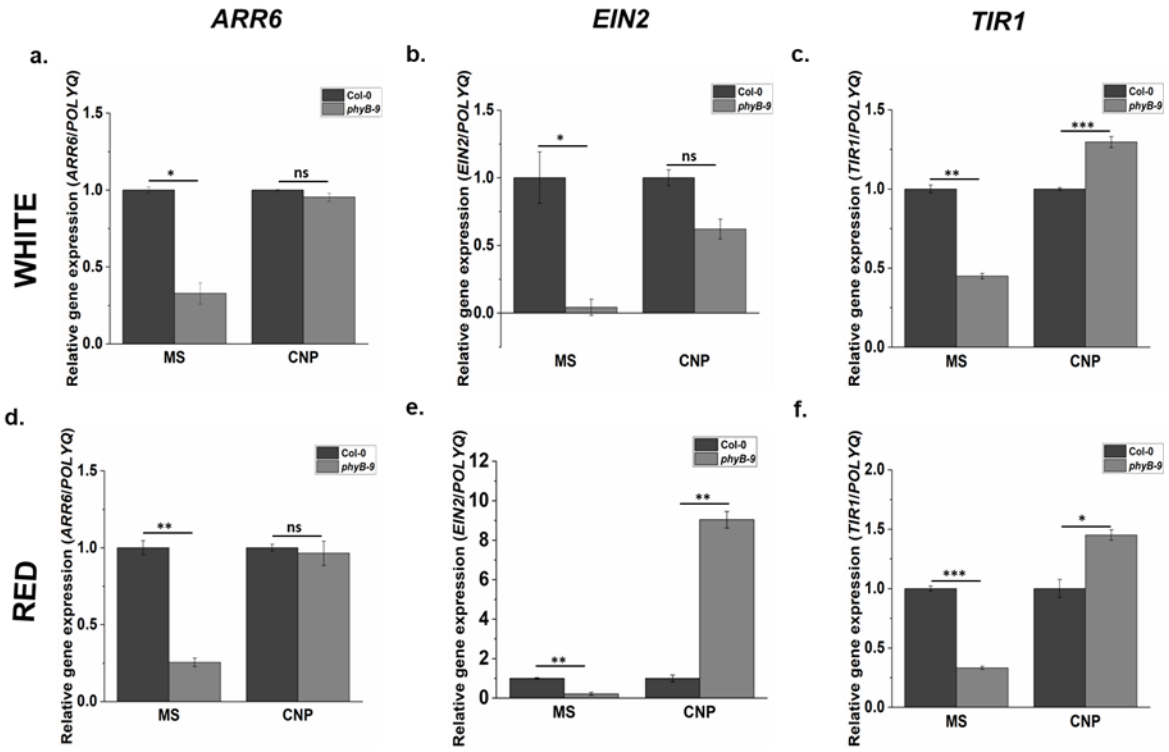


Fig.4.9. Alteration in the expression of (a) *ARR6,* (b) *EIN2* and (c) *TIR1* genes under white (100 µmol m^{-2} s^{-1}); (d) *ARR6,* (e) *EIN2* and (f) *TIR1* genes under red (50 µmol m^{-2} s^{-1}) in *phyB-9* seedlings as compared to Col-0 by CNP (500 µg/ml). Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$. qRT-PCR was performed at least in triplicates.

4.3. Discussion:

Nanotechnology is a booming area nowadays and widely used in agriculture. CNMs have beneficial as well as detrimental effects on plant development (158). The type, size, concentration and exposure time of CNMs determine their effects on plants. They have been reported to increase nutrient uptake efficiency, quality of fruit, seed germination, root growth and other several aspects of plant growth (159). Hence, this decreases the dependency of plants on pesticides. They have

also been shown to alter various features of root morphology and growth. Most of the previous reports have shown the impacts of CNTs on root growth in plants, such as MWCNT enhances the root length in ryegrass and soybean, oxidized-MWCNT increases the root length in mustard and wheat seedling and uncoated-CNTs increase the root elongation in onion and cucumber (160). Carbon nano-dots enhances the root growth in wheat and CNPs such as fullerol have also been reported to enhance the length of roots in wheat (160, 161). Several other reports are available explaining about similar aspects.

CNTs alter the root growth via modulating the expression of genes engaged in the root development such as *SLR1*, *RTCS*, *RTHs* etc (150). Kumar *et al* has shown that CNP (graphite powder) at the concentration of 500 µg/ml enhances the primary root length in Col-0 of *At* (155). In the present work, same concentration of CNP (graphite powder) was utilized and similar results were observed in case of Col-0 seedlings. However, the main interest of this work was to explore the involvement of PHYB in CNP-mediated root growth. PHYB extensively participates in the regulation of root morphology and physiology (12). Various root architectural features have been studied in the present work such primary root length, quantification of lateral and adventitious roots, root wave formation and root coiling in presence of CNPs. It has been reported earlier that *phyB* mutant exhibits shorter primary root in comparison to wild-type (119). In the current work, in absence of CNP *phyB* mutant showed shorter primary root than Col-0 except under FR light. However, in presence of CNP the primary root length of *phyB* mutant and Col-0 become similar except under R light. PHYB has also been shown to alter lateral root development (113, 162). In the present work, lateral and adventitious root quantification data showed that CNP reduces the lateral root number more severely in Col-0 as compared to *phyB-9* mutant, specifically under W and R light. It was observed in the current work that the lateral root number is lesser in Col-0 as

compared to *phyB-9* mutant under W and R light in presence of CNP. On the other hand, present study showed that adventitious root number varied only under white light in presence of CNP between Col-0 and *phyB-9* mutant and the adventitious root number was more in *phyB-9* mutant. In *At*, it has been reported that COOH-MWCNTs suppress the root hair number by negatively regulating the genes playing role in root hair emergence, differentiation and growth (151). Present work has shown that root hair growth was similarly reduced by CNP in Col-0 and *phyB-9* mutant. The genes involved in root hair growth can also be analysed in this case to understand the mechanism behind CNP dependent reduction in root hairs. It was also described in the current study that root WD and HGI are lesser in *phyB-9* mutant as compared to Col-0 in presence of CNP under W, R and B light. The root gravitropism in presence of CNP has also been studied by investigating root coiling in the present work. In the present work, in presence of CNP the root coiling was lesser in *phyB-9* mutant as compared to Col-0 under W and R light. However, under R light, irrespective of CNP root coiling was more in Col-0 as compared to *phyB-9* mutant.

In rice, MWCNT, FeCo-CNTs and Fe-CNTs have been reported to decrease IAA, CK, GA, ABA, JA and BR amount in roots (152). The present study has shown that CNP alters the expression of *EIN2* and *TIR1* genes in *phyB-9* mutant under W and R light. Hence, it can be concluded that PHYB is possibly involved in CNP dependent alteration in root architecture and it occurs due to change in expression of *EIN2* and *TIR1* genes in root. Phytohormone quantification can also be performed in *phyB* mutant in presence of CNP under different light quality. Further experiments can be performed in future to understand the involvement of other photoreceptors in CNP-mediated root growth. Temperature is one of the major factors which controls root elongation (163, 164). The PHYB has been shown to act as temperature sensor (165). Hence, it would be interesting to perform CNP mediated root growth studies under different temperature and light intensity in

phy and *pif* mutant lines. Other phytohormone associated genes involved in root growth can also be analysed in presence of CNP to investigate the mechanism behind the root growth alteration by CNP in detail.

CHAPTER 5

Effect of sugar and light on root architecture and expression of auxin related genes

Chapter 5.

5. Effect of sugar and light on root architecture and expression of auxin related genes

5.1. Background:

Light has been mentioned earlier to be one of the major abiotic factors involved in plant growth. On the other hand, photosynthates or carbon sources (sugar) also contribute to the plant growth pattern depending upon the day length as well as light (166, 167). Phytohormones are also known to act as an essential plant regulator. The role of light and phytohormones in plants have been mentioned in introduction section. Plant synthesizes sugar by photosynthesis and sugar has been shown to regulate hypocotyl elongation, cotyledon opening, flowering time, root growth, etc (168). It has been reported that sugar in higher amount suppresses hypocotyl length in dark and cotyledon opening in presence of light. Suc at low concentration enhances flowering time, however, at higher concentration shows reverse action. Target of rapamycin (TOR) is a kinase which regulates plant growth and development by coordinating carbon source and energy content of the cell. Glu promotes root meristem action and also induces plant TOR kinase (169). It is also involved in transition from juvenile to adult stage via regulating expression of *miRNA156* (167). It has been stated that light and phytohormone interacts to regulate plant development (in introduction part). Similar to this cross-talk, sugar-phytohormone interaction and light-sugar interaction have also been reported to modulate plant growth. The sugar and phytohormone crosstalk have been investigated to synchronize plant growth. This interaction is involved in seedling development, root growth, tuberization etc (170). In embryonic and seedling development, Glu and ABA have been reported act in synergistic manner (171). In early seedling development, exogenous ET causes normal growth of seedlings and compensates the inhibitory effects of Glu and Suc. ABA has been shown to maintain the Suc and Glu sensitivity during seedling development (172). Suc

regulates the tuberization in potato by controlling the level of GA and ABA (173). Sugar and phytohormone also interact to regulate the root development. Auxin signaling has been shown to control Glu-mediated alteration in elongation of primary root, root hair growth, lateral roots and root deviation. Root deviation is defined as the movement of root tip in right or left direction from the main axis of the root (gravity vector). Glu decreases the root meristem via inhibiting accumulation of PIN1 and hence the level of auxin (174). Glu and CK reduce primary root length at higher concentration, however, at lower concentration, Glu promotes root length but CK inhibits the same (175). It has also been shown that light and sugar interact in controlling several aspects of plant growth. The overexpression of PHYB increases hypocotyl growth in presence of Suc under FR light. (176). Hexokinase 1 (HXK1) acts as an integrator of high light intensity and exogenous Glu in plant development. It has been shown that HXK1 mutant, *glucose insensitive 2* (*gin2*) displays alteration in hypocotyl elongation and root growth under low light intensity (177). It has been demonstrated that Suc mediated hypocotyl elongation is dependent on PIFs. Suc has also been shown to enhance the level of PIF5 protein (178). Excess concentration (3%) of Glu promotes leaf area under high intensity of light. At higher intensity of light, sugar acts as photo protectant and hence photosynthetic efficiency is enhanced at higher intensity in the presence of excess amount of sugar (166). HY5 controls the genes playing role in Suc metabolism and transport. It further controls the expression of *FT* gene that consequently regulates induction of flowering (179). Sugar has been reported to stimulate PIF-dependent auxin concentration and also promotes auxin transport and signaling (180). It has also been investigated that light-mediated root growth is dependent upon the sugar transport via phloem (181). Although, light has been shown to interact with sugar, cross-talk of PHYB and sugar in root patterning is still not clearly known. In this chapter, the correlation of different sugar such as disaccharides: Suc and Mal,

monosaccharides: Glu and sugar alcohols: Man and Sor with PHYB in regulating root architecture has been explored.

5.2. Results:

5.2.1. Primary root length decreases in *phyB-9* in different sugars under white, red and far-red light

Different types of sugar have been shown to alter auxin dynamics and root patterning. However, whether sugar dependent root growth is light quality dependent has not been well studied. Sterilised seeds of Col-0 and *phyB-9* were sown on half-MS hard agar medium containing sugars such as Suc, Glu, Mal, Man, Sor (1%) along with -sugar (-S) on square plates.

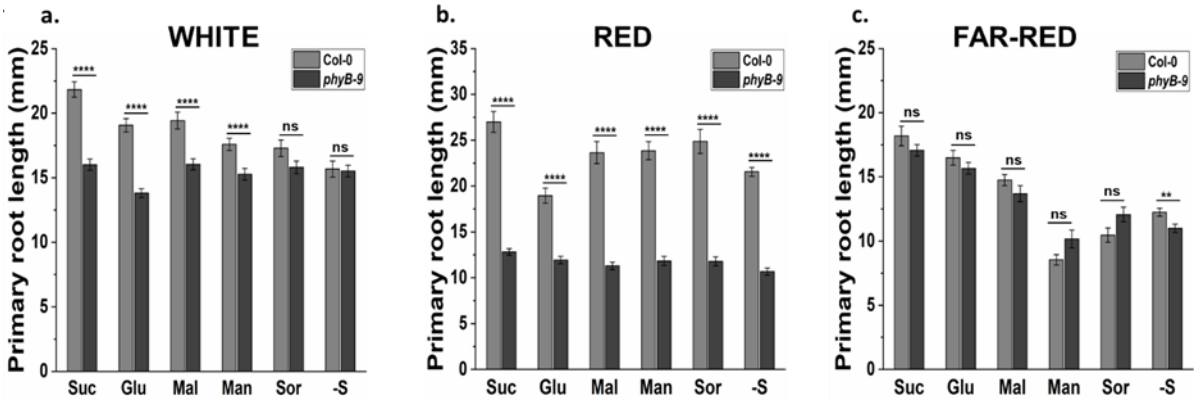


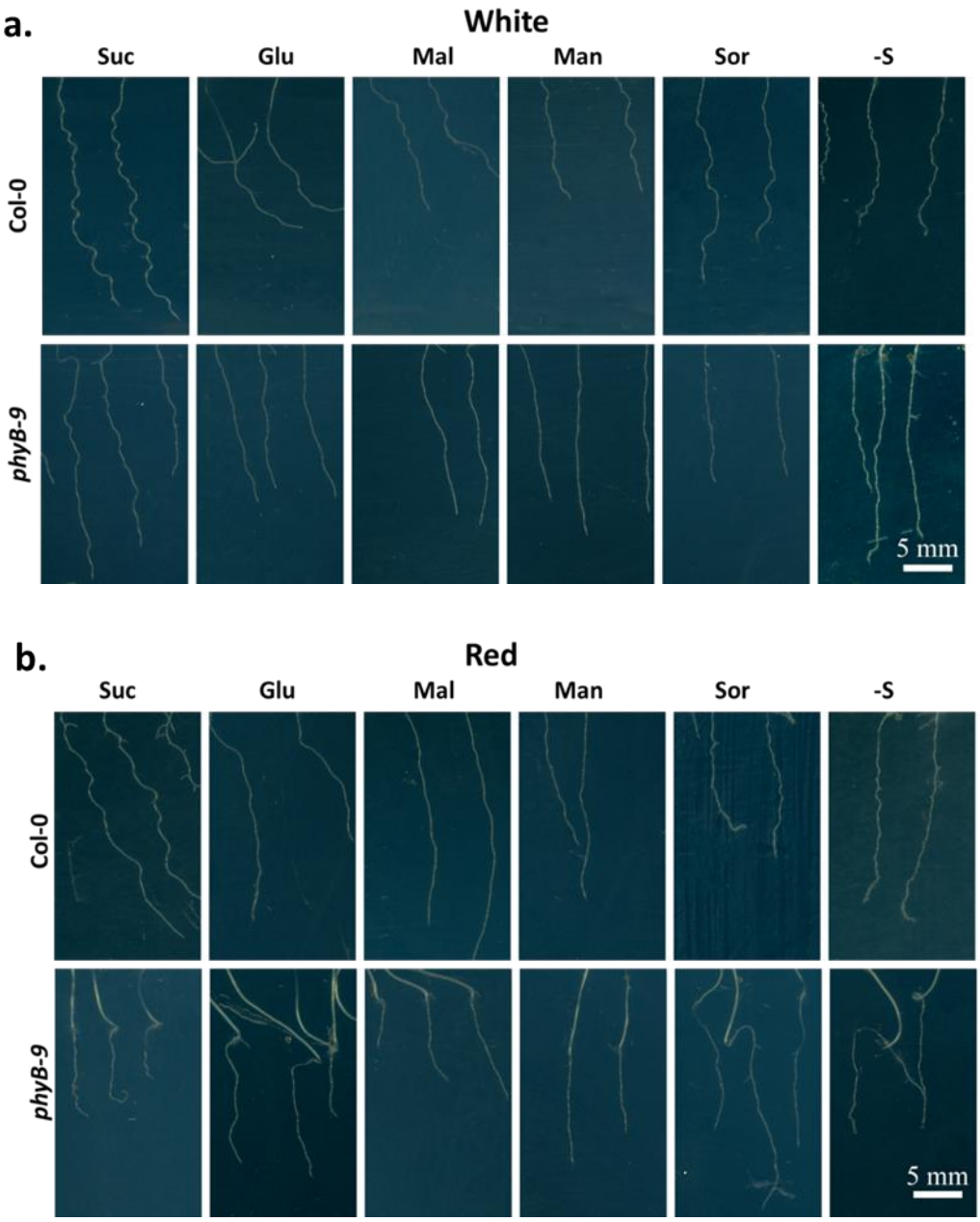
Fig.5.1. Reduction in primary root elongation in presence of sucrose, glucose, maltose, mannitol and sorbitol under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}) and (c) far-red (45 µmol m^{-2} s^{-1}) light in 5-day old *phyB-9* mutant seedlings as compared to Col-0. Experiment was performed five times and every time 15 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

Cold stratification was done for 72 hrs and then kept for germination induction under W light. Further, the plates were kept vertically under different light conditions: W, R and FR. After 48 hrs of transferring in light, the plates were tilted at 45˚. The primary root growth was evaluated 5 days after tilting the plates. The root length measurement was performed with R program.

It was observed that under W light, *phyB-9* had lesser primary root length as compared to Col-0 in the presence of Suc, Glu, Mal and Man (Fig.5.1a). Under R light, primary root length was shorter in *phyB-9* in comparison to Col-0 in presence of all type of sugars as well as in the absence of sugar (Fig.5.1b). On the other hand, under FR light, *phyB-9* had lesser primary root length as compared to Col-0 only in zero sugar condition (Fig.5.1c).

5.2.2. Change in root wave density, straightness and horizontal growth index by different sugar analogues under variable light quality

Sugar type along with light quality showed differential primary root elongation. Further, WD was analysed in the presence of above-mentioned sugar type and light conditions. The seedlings were grown as discussed in previous section. WD was analysed with R program. It was found that, under W light, *phyB-9* had more WD as compared to Col-0 in the presence of Suc, however, in -S, WD was lesser in *phyB-9* seedlings (Fig.5.2a). Under R light, *phyB-9* seedlings had lesser WD than Col-0 in presence of Man only (Fig.5.2b). In case of FR light, WD was lesser in *phyB-9*, in presence of Man as well in absence of sugar (Fig.5.2c).


a.
White
Suc
Glu
Mal
Man
Sor
-S
Col-0
phyB-9
5 mm
b.
Red
Suc
Glu
Mal
Man
Sor
-S
Col-0
phyB-9
5 mm

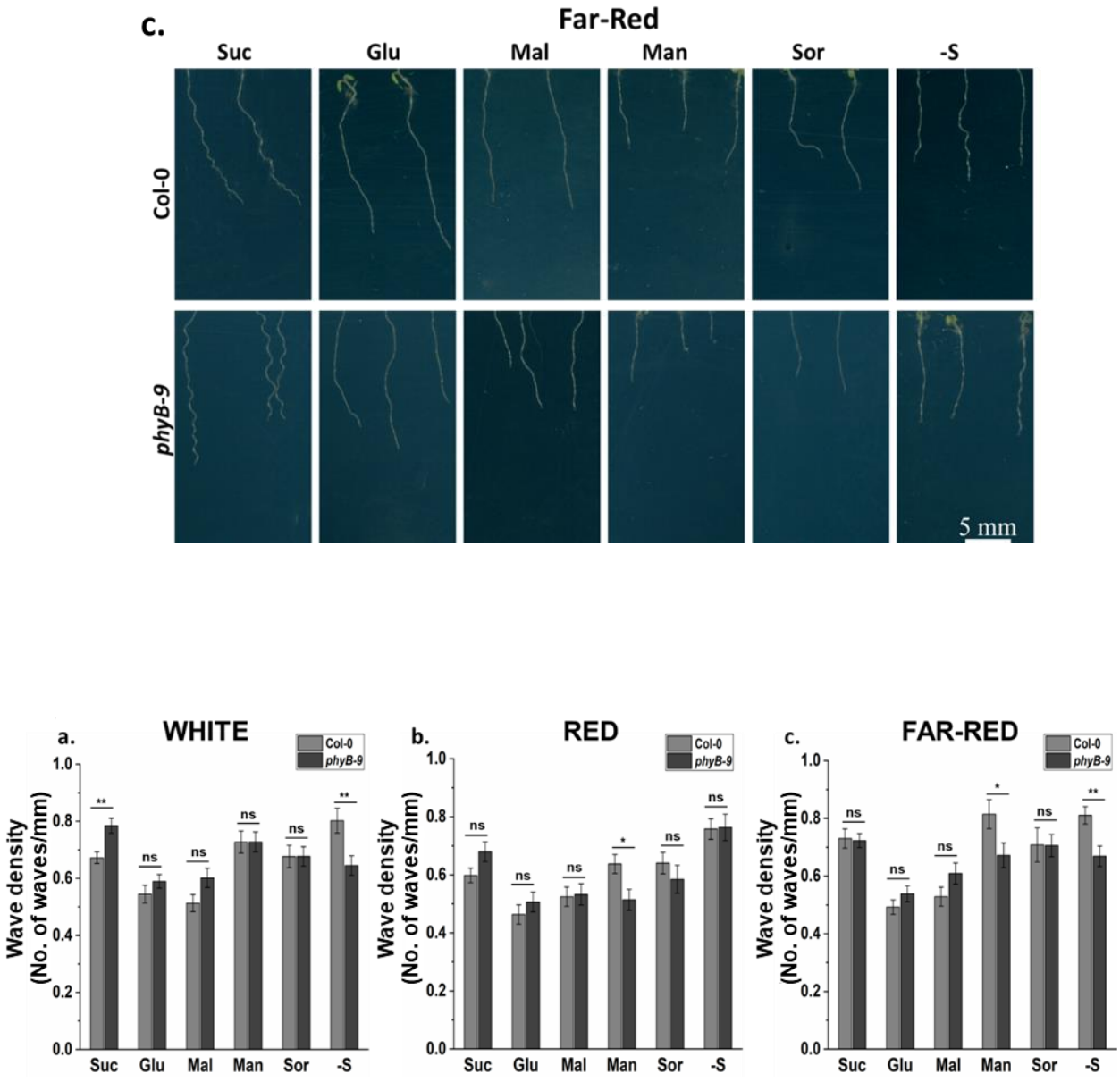


Fig.5.2. Variation in root wave density by different type of sugars under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}) and (c) far-red (45 µmol m^{-2} s^{-1}) light in 5-day old *phyB-9* mutant seedlings as compared to Col-0. Experiment was performed five times and every time 15 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

Although, there was not much significant difference observed in WD, straightness and HGI of roots were analysed further. These parameters were also calculated by R program.

It was noticed that, under W light, root straightness was more in *phyB-9* mutant seedlings as compared to Col-0 in presence of Man and in zero sugar (Fig.5.3a). However, under R light, Col-0 showed slightly more root straightness as compared to *phyB-9* seedlings in presence of Sor (Fig.5.3b). Under FR light, it was observed that root straightness was more in *phyB-9* mutant as compared to Col-0 in presence of Suc, Glu and also in absence of sugar (Fig.5.3c).

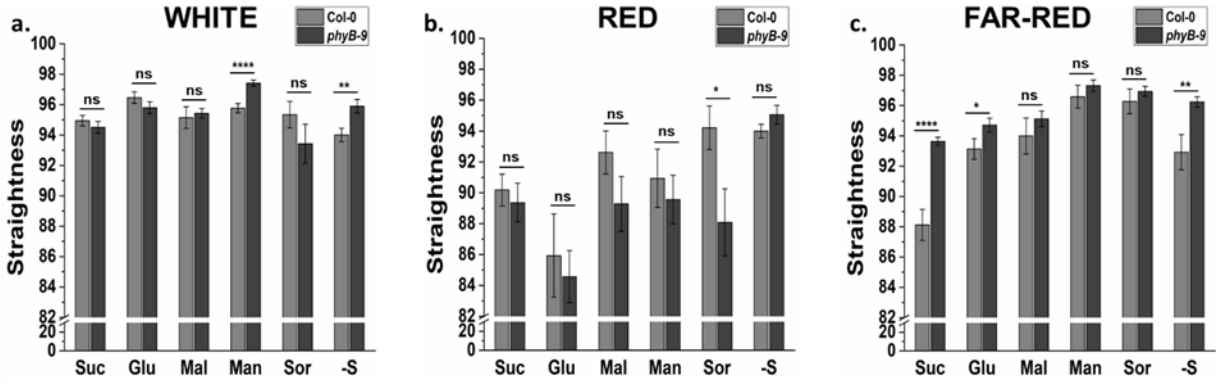


Fig.5.3. Alteration in root straightness by different sugars under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}) and (c) far-red (45 µmol m^{-2} s^{-1}) light in 5-day old *phyB-9* mutant seedlings as compared to Col-0. Experiment was performed five times and every time 15 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

Further HGI was calculated and it was observed that under W light, HGI was lesser in *phyB-9* mutant seedlings as compared to Col-0 in the presence of Suc, Glu, Mal and Man (Fig.5.4a). When HGI was analysed under R light, *phyB-9* seedlings showed lesser HGI as compared to Col-0 in the

presence of Glu, however, it had more HGI as compared to Col-0 in the presence of Man and Sor (Fig.5.4b). Under FR light, HGI was lesser in *phyB-9* in comparison with Col-0, in presence of all type of sugars (Fig.5.4c).

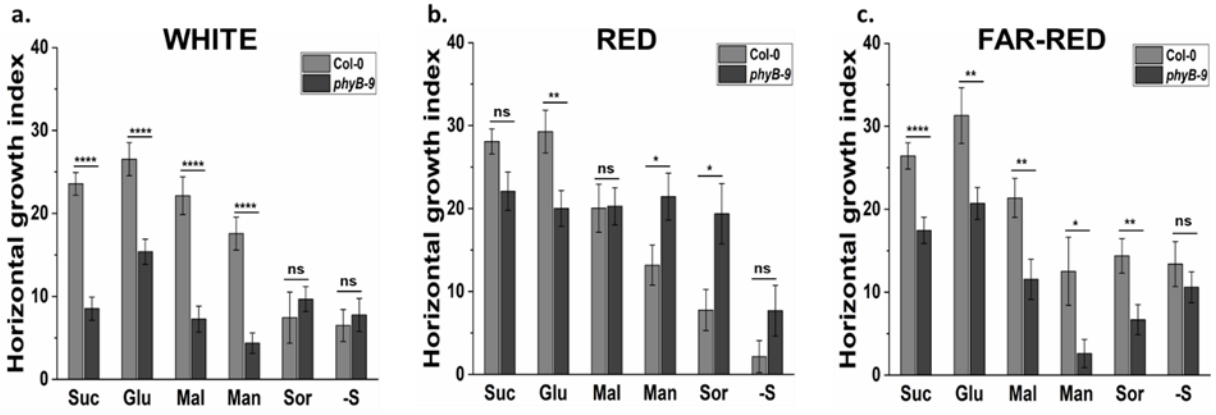
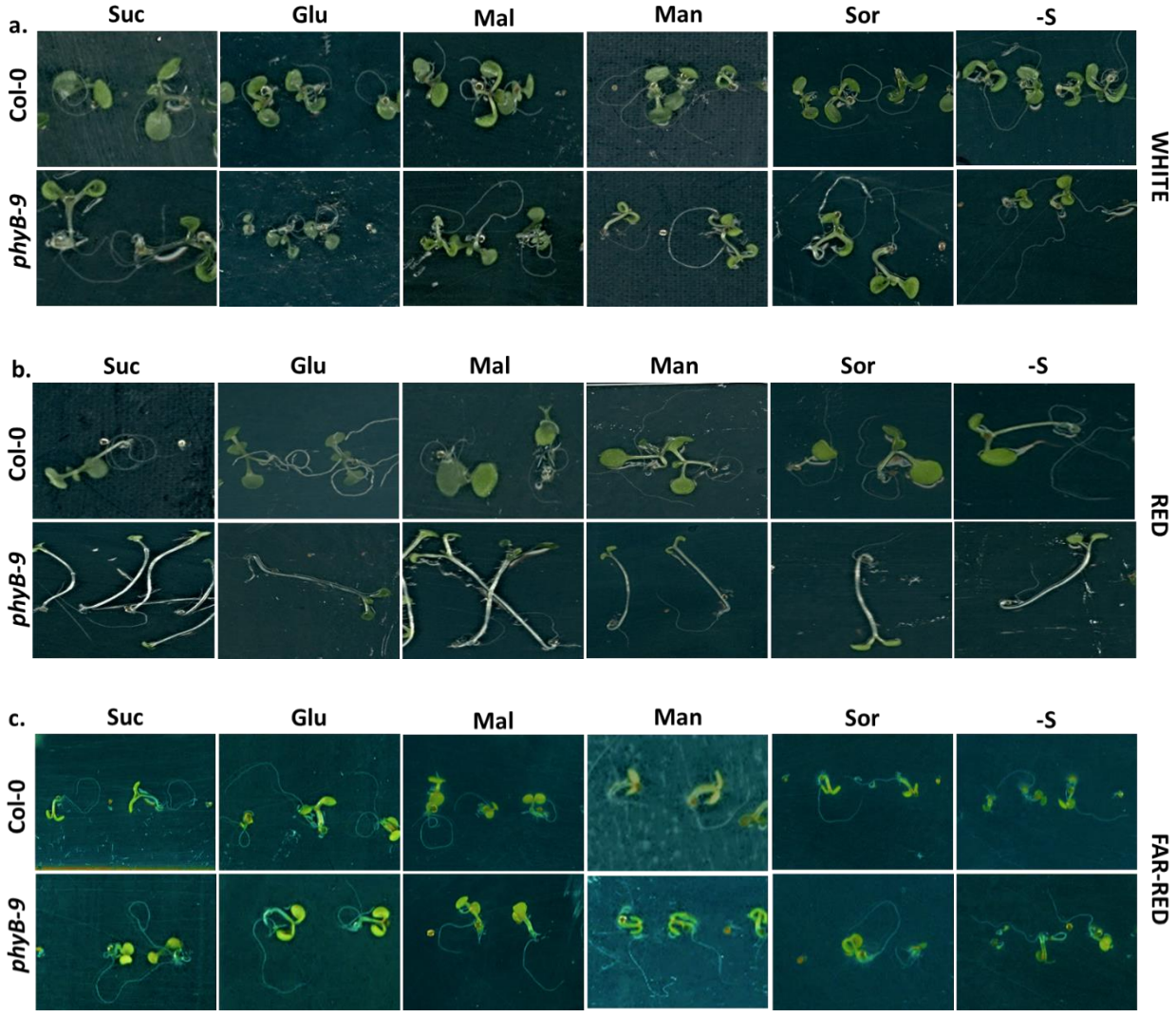


Fig.5.4. Different type of sugar altered the horizontal growth index of *phyB-9* roots as compared to Col-0 under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}) and (c) far-red (45 µmol m^{-2} s^{-1}) light in 5-day old *phyB-9* mutant seedlings as compared to Col-0. Experiment was performed five times and every time 15 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

5.2.3. Root coiling is differentially regulated by sugar type under different light conditions

For root coiling the seeds were sown on normal agar half MS medium in square plates. The plates were kept horizontally under respective light quality after stratification and germination induction. Root coiling frequency were analysed after 7 days of light treatment. Under W and R, Col-0 formed mostly complete coils while *phyB-9* formed mainly partial coils in presence of all sugar tested. However, under FR, coil formation was lesser in both the seedling types as compared to W and R light. It was observed that, under W light, root coiling frequency was lesser in *phyB-9* mutant as

compared to Col-0 seedlings in presence of all types of sugar (Fig.5.5a). Similarly, under R light in presence of all sugars as well in absence of sugar, root coiling was lesser in *phyB-9* as compared to Col-0 seedlings (Fig.5.5b). On the other hand, under FR light, *phyB-9* showed significantly lesser root coiling in comparison with Col-0 seedlings only in presence of Mal and Man (Fig.5.5c). The most significant difference in root coiling between *phyB-9* and Col-0 seedlings was observed under R light.



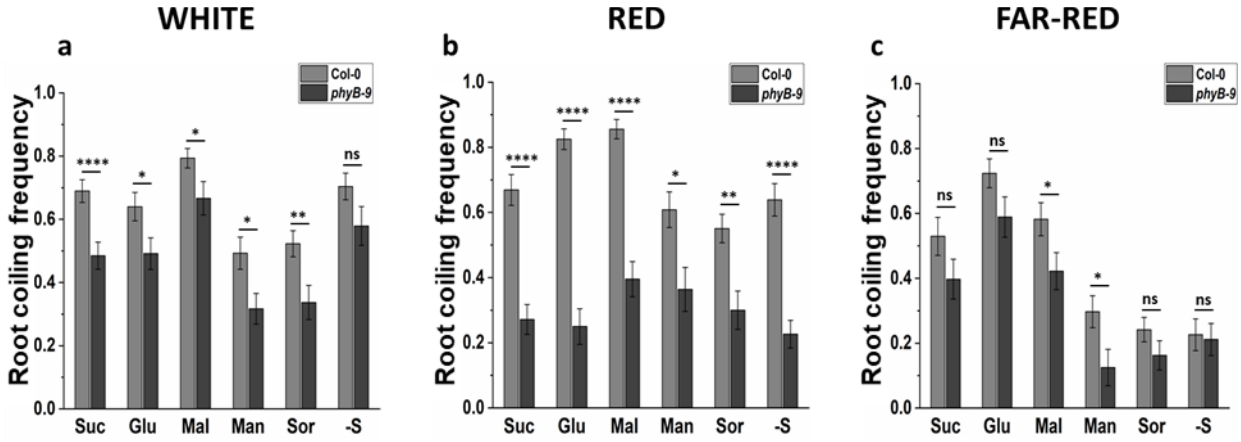


Fig.5.5. Root coiling frequency in presence of sucrose, glucose, maltose, mannitol and sorbitol under (a) white (100 µmol m^{-2} s^{-1}), (b) red (50 µmol m^{-2} s^{-1}) and (c) far-red (45 µmol m^{-2} s^{-1}) light in 5-day old *phyB-9* mutant seedlings as compared to Col-0. Experiment was performed five times and every time 15 seedlings were considered. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

5.2.4. Genes involved in auxin associated pathways are altered by sucrose, glucose and sorbitol under white and red light

Suc, Mal, Glu, Man and Sor showed variable root morphology under W, R and FR light. Further some auxin related genes were investigated by qRT-PCR in presence of Suc, Glu, Sor and -S, under W and R light. The analysis showed that, under W light, *PIN1* was upregulated in presence of Sor and -S in *phyB-9* as compared to Col-0. *TIR1* was upregulated in Glu, Sor and -S in *phyB-9* as compared to Col-0, however, it was downregulated in presence of Suc. On the other hand, *YUCCA2* was upregulated in presence of Suc, Glu and -S in *phyB-9* as compared to Col-0 under W light (Fig. 5.6a, 5.6b and 5.6c). Under R light, *PIN1* was downregulated in presence of Sor in *phyB-9* as compared to Col-0. *TIR1* was downregulated in presence of Suc and Sor, however, in zero sugar, it was upregulated in *phyB-9* in comparison with Col-0. *YUCCA2* was upregulated in

presence of Suc, while it was downregulated in presence of Glu and Sor in *phyB-9* (Fig. 5.6d, 5.6e and 5.6f).

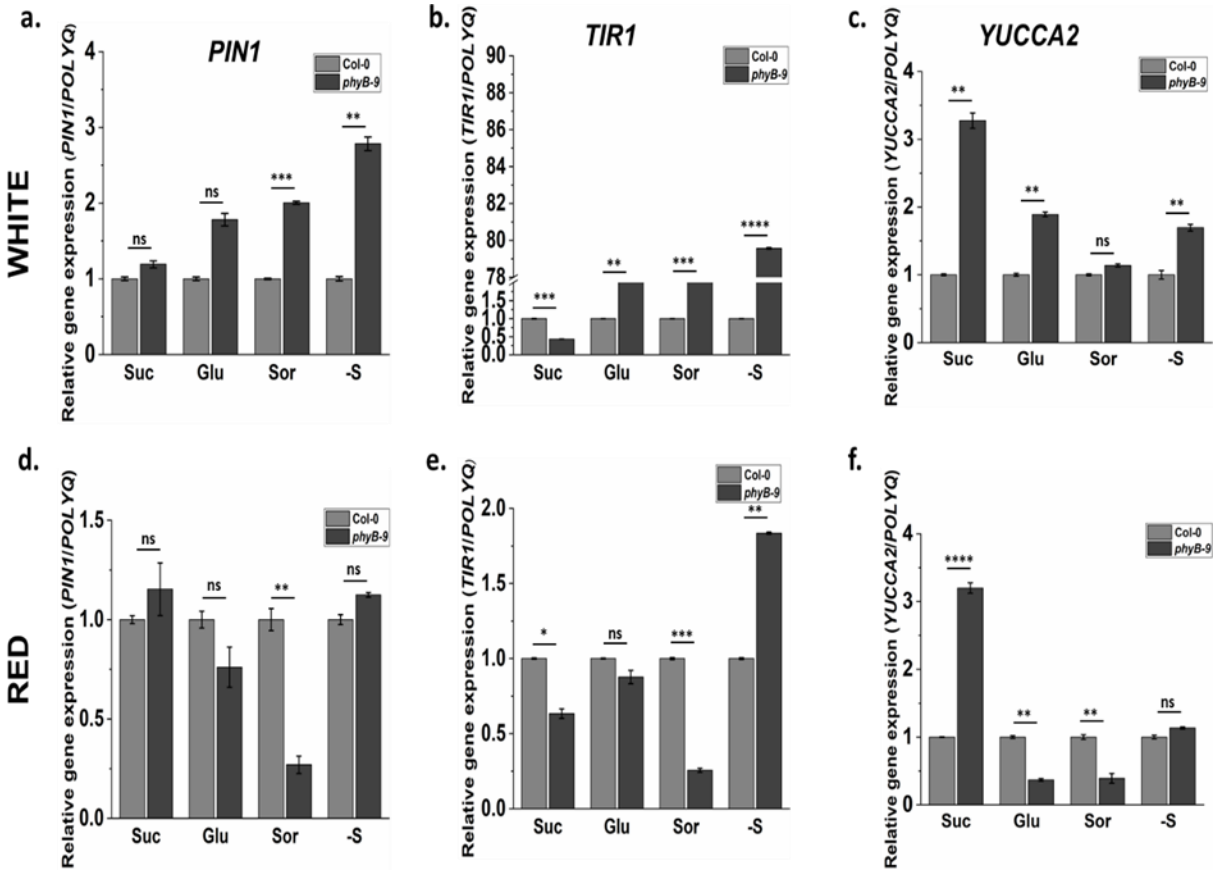


Fig.5.6. Differentially regulation of (a) *PIN1*, (b) *TIR1* and (c) *YUCCA2* genes under white (100 µmol m−2 s−1); (d) *PIN1*, (e) *TIR1* and (f) *YUCCA2* genes under red (50 µmol m−2 s−1) light in presence of sucrose, glucose, sorbitol and no sugar in *phyB-9* seedlings as compared to Col-0. Fold change of 1.2 was considered for gene expression analysis. *POLYQ* gene was used for normalizing the transcript level. Error bar represents SEM. One-way ANOVA with Tukey and Bonferroni tests was performed, here ns = non-significant, * = p < 0.05, ** = p < 0.01 and *** = p < 0.001. qRT-PCR was performed at least in triplicates.

5.3. Discussion:

Light as an ultimate source of energy regulates several physiological and developmental phenomena in plants. It follows different mechanisms to control the root growth (12). Similarly sugar and phytohormones are integral components of plants involved in the development of roots (174, 175). It has been investigated that light controls the root growth by controlling sugar and phytohormone pathways. Suc and Glu are the principal photosynthates present in the plants and have been shown to regulate different aspects of root morphology (182, 183). In maize, Suc and Glu accumulation promoted the lateral root growth from seminal roots (183). Glu has been reported to enhance primary root elongation, number of lateral roots and root deviation. It influences the root growth by altering auxin signaling and transport (174, 184). On the other hand, IAA, CK and BR are key phytohormones which participate in root growth. The roles of phytohormones have been clearly mentioned in the introduction section. PHYs also participate in the root development as explained in the previous sections. *Kircher et al*, 2012 has shown that for light regulated root elongation, the transport of sugar through phloem to root tips is required. They have also documented that PHYs and CRYs possibly be involved in this phenomenon (181). Recently, it has also been shown that annexin (*ANN1* and *ANN2*) genes are involved in post-phloem transport of sugar in the regulation of primary root development. Annexins are the membrane binding proteins which play diverse functions in plants (185). Glu induced alteration in root architecture has been reported to be light intensity dependent. However, Glu stimulate root deviation irrespective of light exposure (184).

However, the cross-talk of PHYB with sugar signaling in root growth is not well studied. It has also not been entirely explored that whether sugar and phytohormone regulated root morphology is light quality dependent or not. In the present work, it has been attempted to solve these questions.

The current study has shown that along with Suc and Glu, other sugar analogues such as Mal, Man, Sor also have significant role in controlling root architecture. Previous reports have shown that *phyB* mutant has shorter primary root as compared to wild-type under W light (120). In the present work, similar results were observed as the primary root length of *phyB-9* mutant was lesser than Col-0 seedlings under W in presence of Suc, Glu, Mal and Man but insignificant in presence of Sor. Under R light, it has been shown here that *phyB-9* mutant has shorter primary root as compared to Col-0 seedlings in presence of all sugar types. However, under FR in presence of all sugar quality, the primary root length has been shown to be insignificant between *phyB-9* mutant and Col-0 seedlings. The present work has also shown that the root WD and straightness don't vary too much between *phyB-9* mutant and Col-0 seedlings under all light conditions and in presence of different sugar analogues tested. However, HGI has been observed to be different in case of *phyB-9* mutant and Col-0 seedlings under W, R and FR light and in presence of all types of sugars. The current work has also documented that *phyB-9* mutant shows lesser root coiling as compared to Col-0 seedlings in presence of all sugar types, but significant difference was observed under W and R light only. This indicates that the root architectural features are dependent upon sugar quality as well as light condition. In the present study, expression of auxin related genes such as *PIN1, TIR1* and *YUCCA2* have also been reported to be altered in presence of W and R light and different sugar quality. It was also observed that, PHYB may play significant role in these processes by regulating the auxin related genes.

In further studies, other phytohormone regulated genes having principal roles in root growth can be investigated with similar experimental conditions in *phyB-9* mutant. Additional features of root morphology for example, root deviation, lateral and adventitious root growth and root hair study can also be performed. In the present work, FR light has been shown to generate minimal variation

in root morphology of *phyB-9* and Col-0, hence, PHYA mutants and other double mutants of PHYs can be utilized for the future study. This will help to understand the cross talk of PHYA and sugar signaling in the root growth. Similar experiments can also be done under different fluences of light in presence of various sugar analogues. Sucrose has been reported to interact with *PIFs* and *HY5* in different physiological processes of plants (178, 179). Hence, the root morphological studies can be performed in *pif* and *hy5* mutants in presence of different sugars and light quality. With these information, the mechanism behind PHY signaling and sugar cross-talk in root development can be established.

CHAPTER 6

A luciferase-based ratiometric auxin quantification to understand auxin and phytochrome crosstalk

Chapter.6.

6. A luciferase-based ratiometric auxin quantification to understand auxin and phytochrome crosstalk

6.1. Background:

Light and phytohormones are the most important components which influence plants at different stages of their lifecycle. Auxin has been shown to act as an crucial factor in controlling plant development (186). PHYs have also been reported to regulate various plant processes. Hence, the study regarding auxin and PHY interaction in plant development needs to be performed in detail. Previously, for phytohormone quantification in plants, techniques such as isotope labelling and GC-MS were utilized (187, 188). These techniques involve tissue disruption and hence, it is difficult to study the dynamics in intact plant.

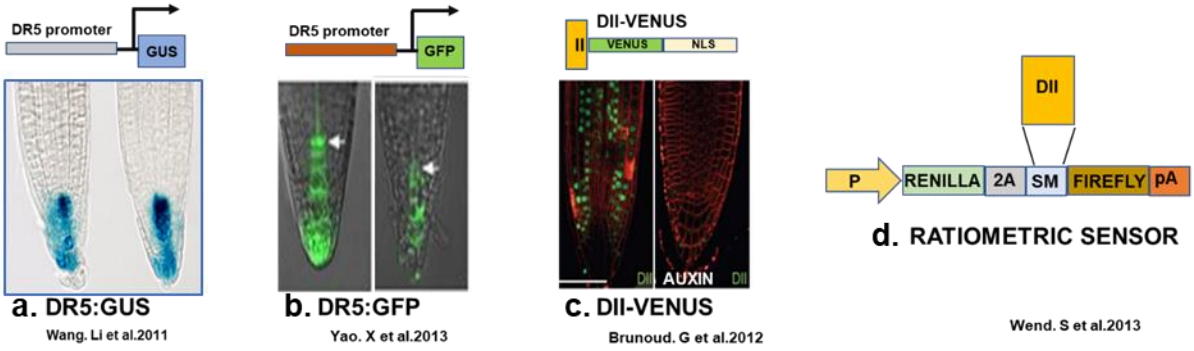


Fig.6.1. Various biological tools for auxin accumulation. (a) The expression of GUS has been shown to more in *arf2-101* mutant root (right) as compared to WT (left) (b) GFP signal has been reported to be higher in WT (left) than *sdg2-3* mutant (right) (c) The signal of DII VENUS when auxin is not present (left) and it vanishes in the presence of auxin (right). (d) Ratiometric sensor involves sensor module (SM) consisting of domain II (DII)/degradation sequence of AUX/IAA which senses the presence of auxin, two luciferases; renilla and firefly for luminescence quantification and 2A peptide for stoichiometrical co-expression of both the luciferases.

However, new techniques /tools have been developed, which are non-invasive in nature and thus are helpful to study auxin dynamics in single cell or intact plants. These tools are DR5:GUS, DR5:GFP, DII-VENUS, etc (Fig.6.1) (189, 190, 191).

DROUGHT RESISTANT 5 (DR5) is an auxin-based synthetic promoter. DR5:GUS and DR5:GFP consist of β-glucuronidase (GUS) and GFP reporters respectively (192). They help in monitoring auxin homeostasis and signaling. However, they don't reflect exact status of auxin accumulation in the cell. In Fig.6.1a, it has been shown that when *DR5:GUS* was expressed in *arf2-101* mutant then GUS expression was observed to be more in *arf2-101* mutant as compared to WT. This indicates that *ARF2* downregulates the *DR5:GUS* expression (189). It was investigated that when *DR5:GFP* was expressed in *set domain group 2* (*sdg2-3*) mutant, GFP signal was more in WT as compared to *sdg2-3* mutant (Fig.6.1b) (190). SET DOMAIN GROUP 2 is a member of H3K4 methyltransferases class regulating histone H3-K4 methylation (193). DII-VENUS is a fluorescence-based tool. It involves the auxin dependent degradation of AUX/IAA (domain II) during auxin signaling. DII-VENUS consists of a segment belonging to domain II (DII) of AUX/IAA protein. When auxin-TIR1 complex binds to the DII of AUX/IAA protein as a result AUX/IAA is degraded (105). In DII-VENUS, DII segment is fused with VENUS sequence, an improved and stable type of GFP. Here, the GFP signal goes down with increasing conc. of auxin (Fig.6.1c). It has provided time-resolved information about auxin distribution in organogenesis at shoot apex as well as in gravitropic responses of root (191). A recently developed chemiluminescence-based ratiometric sensor (pMIR auxin sensor) has been shown to give better and more accurate information about auxin dynamics. It consists of different sequences: Renilla -2A-Sensor Module-Firefly (Fig.6.1d). Renilla and firefly are the luciferases, 2A is a protein sequence helping in stoichiometric co-expression and sensor module (SM) is the sequence from

DII of AUX/IAA (194). This sensor works as such: when auxin is present in very less amount or no auxin is there inside the cell, both the luciferases are expressed and the ratio of firefly to renilla luminescence will be around one. However, when auxin is present in excess, it binds to TIR1 and then SM which further degrades SM along with firefly luciferase. Then, the luminescence of firefly will decrease but renilla luminescence will remain constant, as a result the ratio of firefly to renilla luminescence will decrease and will be less than one (Fig.6.2) (194). This ratiometric luminescence signal provides the exact and accurate information about the distribution of auxin in specific cell and time. This sensor is highly sensitive and cancel out the effects of cellular surrounding or environment.

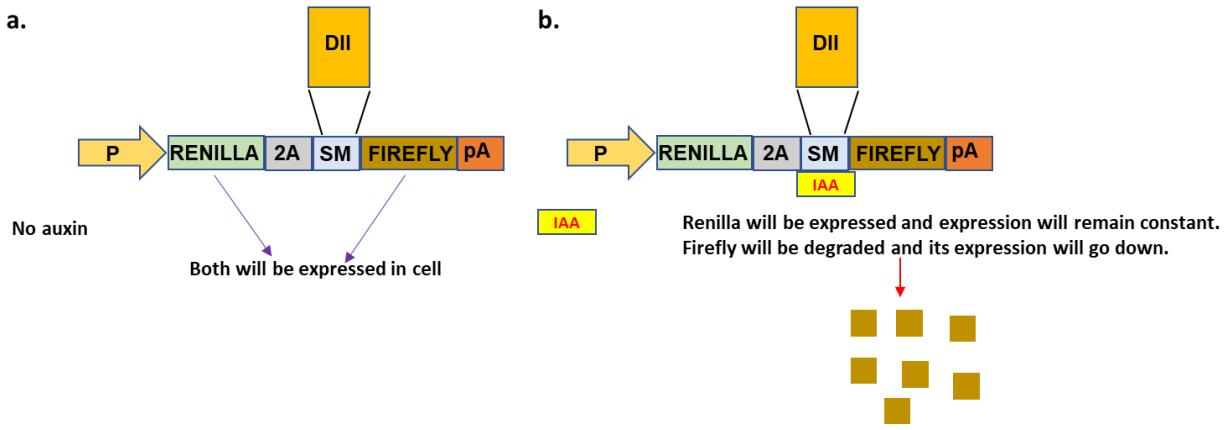


Fig.6.2. Working mechanism of luciferase auxin sensor. (a) In absence of auxin, both the luciferases are fully expressed (b) When auxin is present, it goes and binds to TIR1 and SM, this leads to the degradation of firefly. In this way, signal from renilla remains constant but the firefly luminescence decays with increasing concentration of auxin.

Auxin and PHYs have been shown to cross-talk in controlling plant growth and development (12). There are several reports available which demonstrate about the PHY dependent auxin homeostasis in plants. However, this interaction has not been well documented in protoplast

system or at single cell level. In this chapter, the light dependent auxin dynamics was investigated in protoplasts using luciferase auxin sensor.

6.2. Results:

6.2.1. Auxin dynamics is similar in Col-0 and *phyB-9* protoplast under white light

In a previous article, this ratiometric luciferase auxin sensor was transformed in Col-0 leaf protoplast of *At* and treated with various concentrations of auxin for different duration (5 mins, 15 mins and 45 mins) under W light. Then auxin dynamics was observed to be similar under these experimental conditions (194). In the current work, the sterilized seeds of Col-0 and *phyB-9* mutant were sown on SCA media and kept for 48 hrs of cold stratification. After that the plates were kept vertically and grown under W light. The protoplast was isolated from shoot part of three-week old seedling. Then they were transformed by PEG with auxin sensor and kept in dark for ~20 hrs at 22ºC.

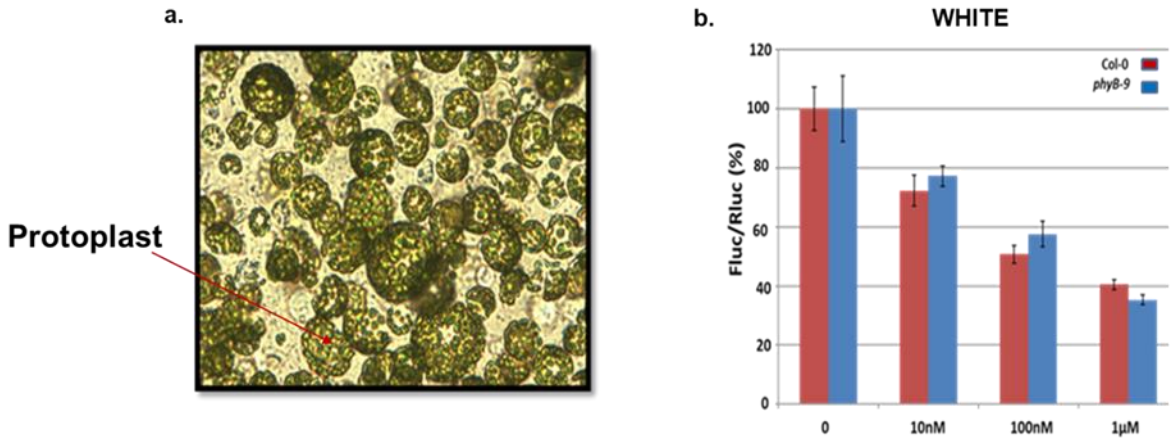


Fig.6.3. Auxin dynamics in protoplasts (a) Protoplast isolated from Col-0 shoot. (b) Ratiometric quantification in the transformed Col-0 and *phyB-9* protoplasts after 30 mins of IAA treatment under white light. Error bar represents SD. One-way ANOVA with Tukey and Bonferroni tests was performed with $p \leq 0.05$. Three technical replicates were used for the experiment.

The transformed protoplasts were subjected to IAA treatment of different strength (10 nM, 100 nM and 1 µM) as well as control, PCA-M. The treatment was done for 30 mins under W light. The ratio of Firefly/Renilla luminescence was observed to be similar between Col-0 and *phyB-9* protoplasts under all auxin treatment. This showed that auxin dynamics follow similar pattern in both the seed lines under this experimental condition (Fig.6.3).

6.2.2. Effect of pre-irradiation of different light quality on auxin dynamics in protoplast system

When it was observed that auxin treatment under W light had no effect on auxin dynamics, then few other experiments were performed under different light qualities. In one of the experiments, Col-0 protoplast was transformed with auxin sensor and then, transformed protoplasts were irradiated for 2.30 hrs by W, R and FR light. After light irradiation, 45 mins of auxin (1nM, 10 nM, 100 nM and 1 µM) treatment was done under above-mentioned light conditions. Then, it was observed that the auxin dynamics was similar in case of all light conditions (Fig.6.4a). Further similar experiment was performed with *phyB-9* protoplast only under R light to understand the involvement of PHYB in the regulation of auxin dynamics in protoplast. The transformed protoplasts of Col-0 and *phyB-9* were irradiated with R light for 2.30 hrs and auxin treatment (1nM, 10 nM, 100 nM and 1 µM) for 45 mins was done in the presence of R light. The auxin dynamics was again found to be similar in Col-0 and *phyB-9* under this condition (Fig.6.4b). The next experiment was performed similarly with a change of 15 mins R light irradiation instead of 2.30 hrs. After 45 mins of auxin treatment under R light, luminescence was analysed and it was observed that Firefly/Renilla luciferase ratio and hence the auxin dynamics was similar in both the protoplasts (Fig.6.4c).

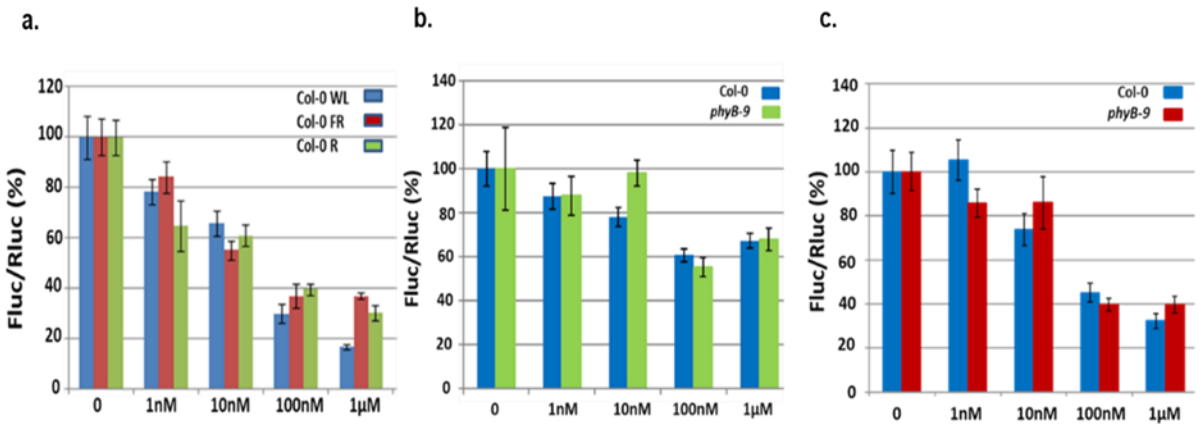


Fig.6.4. Auxin dynamics analysis under different light qualities. (a) Luciferase-based auxin dynamics in transformed Col-0 protoplast after pre-irradiation of white, red and far-red light for 2.30 hrs and 45 mins of IAA treatment under above-mentioned light. (b) Auxin dynamics in transformed Col-0 and *phyB-9* protoplasts, pretreated with red light for 2.30 hrs mins and 45 mins of IAA treatment under red light. (c) Auxin dynamics after 15 mins of red-light treatment followed by 45 mins of IAA treatment under red light in transformed Col-0 and *phyB-9* protoplasts. Error bar represents SD. One-way ANOVA with Tukey and Bonferroni tests was performed with $p \leq 0.05$. Three technical replicates were used for the experiment.

6.2.3. Influence of prolonged light irradiation on auxin dynamics and auxin signaling genes in protoplast

Additional experiments were performed to understand the influence of prolonged light treatment on protoplast system. In earlier experiments, transformation of protoplast was performed in dark, however, two different sets of experiments were further done. In one set, Col-0 and *phyB-9* protoplasts were transformed in dark for 18 hrs and in second set, transformation was performed under R light. The transformed protoplasts from both first and second set were treated with different IAA concentrations (10 nM, 100 nM and 1 µM) for 3 hrs in dark and R light respectively. After that in these two experimental setups, the auxin dynamics was observed to be identical in

both the protoplasts and under both the setups. (Fig.6.5a and 6.5b). Further, the expression profile of auxin signaling genes was investigated in Col-0 and *phyB-9* protoplasts.

Both the protoplasts were transformed in dark, then 4 hrs of red-light irradiation followed by 5 µM auxin treatment for 45 mins were performed. Then the expression of *IAA2*, *IAA5* and *GH3.5* genes were analysed. These genes are involved in auxin signaling and reveal information about auxin response (195, 196).

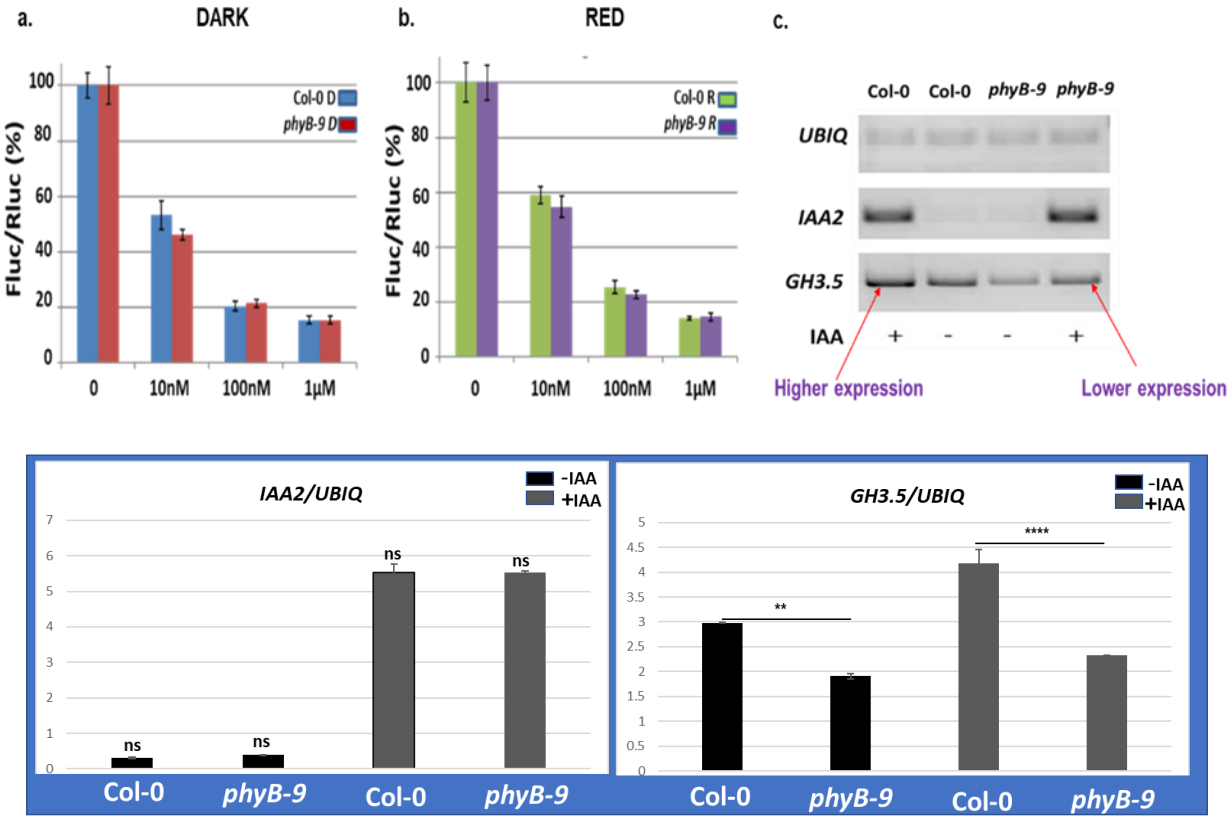


Fig.6.5. Auxin dynamics (a) in Col-0 and *phyB-9* protoplasts after 18 hrs of dark incubated transformation and 3 hrs of IAA treatment under dark (b) in Col-0 and *phyB-9* protoplasts after 18 hrs of red light incubated transformation and 3 hrs of IAA treatment under red light (c) PCR analysis of auxin signaling genes in transformed Col-0 and *phyB-9* protoplasts after 4 hrs of red light irradiation followed by 5 µM auxin treatment for 45 mins under red light. Densiometric analysis of PCR for the differential expression of *IAA2* and *GH3.5* genes before and after IAA treatment. Error bar represents SD. One-way ANOVA with Tukey and

Bonferroni tests was performed with p≤0.05. Three technical replicates were used for the experiment.

It was observed that *IAA5* expression was negligible in any of non-treated or treated protoplasts of Col-0 and *phyB-9*. *IAA2* expression was very low in both the non-treated protoplasts, however in treated protoplasts *IAA2* had higher but similar expression level in both the protoplast types. When *GH3.5* expression was analysed, it was found that, in non-treated as well as treated protoplasts, *GH3.5* expression was lesser in *phyB-9* as compared to Col-0. However, the difference in expression of *GH3.5* was more significant after IAA treatment (Fig.6.5c).

6.2.4. Generation of plant specific auxin sensor construct and transformed wild-type plants with new auxin sensor

When no clear difference in auxin dynamics was observed in the Col-0 and *phyB-9* protoplasts, then new plant specific construct with the luciferase auxin sensor sequence was prepared. The above-mentioned sensor construct is a transient expressing construct and specific to protoplast. This construct cannot be used to transform the plants to make stable transgenic lines as it lacks T-DNA sequence and will not integrate in the genome. The new generated auxin sensor is required for analysing time-resolved auxin quantification in intact plants under different light conditions.

The Renilla-2A-Sensor Module-Firefly sequence was amplified from the existing auxin sensor. The sequence was cloned in pDONR201 construct by BP cloning and then transformed in *E.coli*. After screening of positive clones by colony PCR, further it was cloned in pB2GW7 construct by LR cloning and again transformed in *E.coli*. Then, the positive clones were screened by PCR and then the construct was transformed in *Agrobacterium*. After that, Col-0 plants were transformed by floral dip method with *Agrobacterium* containing new auxin sensor. The transgenic

Col-0 plants were screened by BASTA selection and confirmed with PCR analysis (Fig.6.6). They were further grown till the homozygous generations.

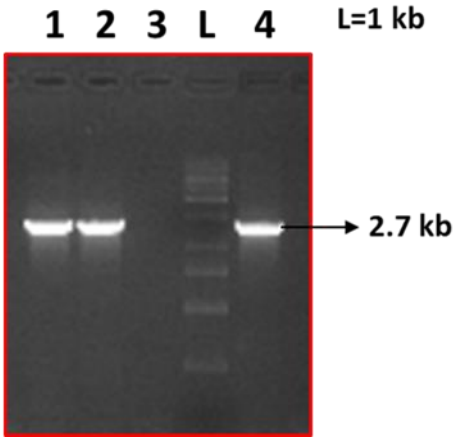


Fig.6.6. PCR analysis for the positive homozygous Col-0 transgenic plants after transformation with new luciferase auxin sensor.

6.3. Discussion:

Several reports are available regarding the roles of light and auxin in plants. Light affects the accumulation, transport and signaling of auxin. They cross-talk with each other and further modulate the plant morphology and physiology (110). Photoreceptors have been reported to interact with auxin to control various plant processes. Under deep shade, PHYA protein accumulates in higher amount which further represses auxin responsive genes and protects AUX/IAA from degradation. In this way, PHYA negatively acts in the SAR process (197). PIF4 has been shown to enhance hypocotyl length under high temperature. It stimulates IAA synthesis by positively regulating the genes involved in IAA biosynthetic pathways (198). Most of the reports about light and auxin crosstalk has been studied in intact plants. However, the auxin dynamics and signaling in response to light in protoplast system have not been well explored.

In a previous report, a chemiluminescence based ratiometric auxin sensor has been generated to study auxin dynamics more accurately in protoplast system. They have shown that the auxin dynamics changes in similar manner after 5, 15 and 45 mins of IAA treatment in the protoplast of Col-0 leaves (194). In the present work, this ratiometric auxin sensor has been utilized to understand how PHYB interacts with auxin or affects the auxin dynamics in protoplasts. It was observed in the present study that auxin dynamics decreases in a similar mode in the Col-0 and *phyB-9* mutant protoplasts after different time of IAA treatment and light exposure (W and R light). This suggests that auxin dynamics is probably same in Col-0 and *phyB-9* mutant protoplasts which is different from the case of intact plant. There is another possibility that the difference in auxin dynamics between Col-0 and *phyB-9* mutant protoplasts is too low to be detected by the auxin sensor tool under the experimental conditions tested. Further in the current work, auxin signaling gene expression was also analysed in the protoplasts. It was found that on IAA treatment, *IAA2* expression was similar Col-0 and *phyB-9* mutant protoplasts, however, the expression of *GH3.5* was more in Col-0 protoplast as compared to *phyB-9* mutant. It has been reported that in intact plant, PHYB negatively regulates auxin signaling and promotes shoot branching. Auxin signaling genes such as *IAA2*, *IAA3, IAA5, IAA6, IAA19, IAA29*, *GH3.5* and *SAUR9* were observed to be upregulated in shoot and mature stem part of *phyB-9* mutant on NAA treatment (199). The expression of *GH3.5* gene been shown to be totally opposite in case of *phyB-9* mutant intact plant and protoplast, hence it can be proposed that the auxin signaling may function differently in intact plant and protoplast system. This study will be helpful to understand the involvement of PHYB or red light signaling in the control of auxin dynamics in protoplast system.

New set of experiments can be further done under different duration of W, R, FR and B light condition. This will give information about light quality dependent auxin dynamics in protoplasts.

It will also provide the details about different photoreceptors involved in regulating auxin dynamics at single cell level. Different intensities of light quality can also be utilised, which will help in understanding the light intensity-based auxin dynamics in protoplast system. In the present work, transgenic plants with the luciferase auxin sensor has been generated which can be utilized further to study the spatio-temporal auxin dynamics in intact plants under different light conditions.

CHAPTER 7

Forward genetic screening of mutagenized phytochrome B over-expressing line to isolate seedlings altered in root patterning

Chapter.7.

7. Forward genetic screening of mutagenized phytochrome B over-expressing line to isolate seedlings altered in root patterning

7.1. Background:

Phytochrome B is a principal red-light photoreceptor and involved in light signaling pathway. It perceives the light and transduces the signal to a cascade of downstream genes such as *HY5*, *HYH*, *PIFs*, etc. They regulate R light specific responses in plants, such as photomorphogenesis (200). When the above-said factors are altered or mutated at any point, it leads to variation in plant phenotype and responses. Mutants of *At* have been generated by various ways such as T-DNA insertion, site-directed mutagenesis, ethyl methane sulfonate (EMS) mutagenesis, radiation-based mutagenesis, etc (201). In forward genetic approaches, T-DNA insertion and EMS mutagenesis are considered as the best choices. In this approach, mutants are screened on the basis of phenotypic differences and no previous assumptions are required. It helps in investigating novel components involved in specific phenomenon or signaling pathways. It is also beneficial in exploring the function of particular genes. EMS mutagenesis is frequently used technique for mutagenesis as it is a much easier process to generate mutants as compared to T-DNA insertion method. It facilitates the generation of huge number of mutants and is less time consuming. It helps in understanding the role of each and every amino acid in protein functioning (202). EMS is an alkylating agent which creates point mutation. However, few reports state that it also creates base-pair insertions or deletions. Ethyl group of EMS reacts with guanine (one of the nucleobases) present in DNA and forms O^6-ethylguanine. O^6-ethylguanine is an abnormal type of guanine, which further transmits during DNA replication. During replication, DNA polymerase add thymine in place of cytosine against O^6-ethylguanine. Hence, this causes random mutation,

creating AT to GC and GC to AT transition point mutation. In 99% cases, it induces GC to AT or AT to GC mutations, however, it can also create stop codon and missense mutations (203). Various light signaling mutants such as *hy3, srl1* etc have been generated by EMS mutagenesis. *hy3* is a PHYB mutant and it has been generated by EMS mutagenesis after screening under W light for longer hypocotyl in comparison to wild-type. This mutant displayed elongated hypocotyl, petiole and long root hairs. It also exhibits early flowering and low chlorophyll content (49). *srl1* mutants were created by mutagenesis of PHYB over-expressor (PHYBox) lines. They are hypersensitive and possess shorter hypocotyl as compared to PHYBox and wild-type under continuous R (cR) light but not under cFR light. They are monogenic, recessive and extragenic R-light specific mutants. They also have larger cotyledon area and shorter petiole length as compared to wild-type. In this mutant, *CHLOROPHYLL A/B BINDING* (*CAB*) expression was shown to be upregulated by two-fold on short pulse of higher fluence of R light irradiation. Hypersensitivity of *srl1* mutant under R light has been investigated to be specifically dependent on PHYB (204). *phytochrome signaling 2* (*psi2*) mutant is also an EMS mutant type. It is hypersensitive to low fluence of R and FR light and possess shorter hypocotyl under these light conditions. R light (~ >45 µmol m^{-2} sec^{-1}) induced hypersensitive response-like necrotic lesions in this mutant. PHYA and PHYB have been shown to be involved in this induction of necrotic lesions (205). N-terminal 651–amino acid present in the domain of PHYB (N651) participates in light perception and transduces light signal to the downstream genes. This domain has various important subdomains: cGMP phosphodiesterase/adenyl cyclase/FhlA, N-terminal extension, PHY and Per/Arnt/Sim-like. To understand their role, N651 expressing lines were mutagenized with EMS solution. The mutants generated were hyposensitive and this response was because of reduced PHYB activity and not its expression (206). In this chapter, hyposensitive mutants of PHYB with altered root architecture

have been generated by EMS mutagenesis to investigate the molecular players involved in red light and auxin signaling cross-talk.

7.2. Results:

7.2.1. Generation of hyposensitive EMS mutants of 35S:PHYB::GFP lines to red light

Light has been shown to alter the rood morphology and PHYB acts as one of the most important photoreceptors affecting the root growth. To investigate the components in the red light or PHYB signaling pathways involved in controlling root growth, mutagenesis of 35S::PHYB:GFP (in *Ler* background) seeds was performed. 35S::PHYB:GFP (approximately 3,00,000 F0) seeds in two batches were EMS mutagenized. EMS solution (0.2% volume/volume in water) was added to the seeds and then incubated overnight. Further, the mutagenized seeds were rinsed several times with water and grown on soil. Out of ~ 3,00,000 seeds, some seeds were lost during washing and some seeds didn't germinate. On soil, some of seedlings became lethal at younger stage, out of the seedlings which reached to the maturity few were sterile, so the seeds were harvested from the rest of the fertile and viable seedlings. The seeds (F1) were pooled and collected, then they were used for screening. For screening purpose, mutagenized seeds were sterilized and sown on square plates containing half-strength MS medium. After cold stratification of 3 days, plates were kept vertically under cR light.

Overexpression of PHYB results in shorter hypocotyl under R light and in present work the mutants showing longer hypocotyl were screened under R light (22). These screened mutants possibly will have mutation in genes involved in PHYB-mediated shorter hypocotyl. Seven-day old seedlings having longer hypocotyl (hyposensitive to R light) as compared to 35S::PHYB:GFP seedlings were screened and bulked on soil. The seeds (F2) were harvested and then the hyposensitive mutants (under R light) were screened till homozygous generations (Fig.7.1a).

Generally, on R light irradiation, PHYB translocate to nucleus and form nuclear bodies. The nuclear bodies are also known as nuclear speckles. The nuclear speckle formation varies upon the quality, fluence and duration of light. The size, shape and number of nuclear speckles are dependent on the light condition (207). In the present study, the homozygous hyposensitive mutants of 35S::PHYB:GFP seeds were further screened for variation in nuclear body formation with GFP signal by microscopy. The 5-day old seedlings were examined under fluorescent microscope and the signal was observed in the hypocotyl part of seedlings. It was found that nuclear complex formation was absent/altered with diffused GFP expression in some of the mutants, however, in some mutants the GFP expression was absent (Fig.7.1b).

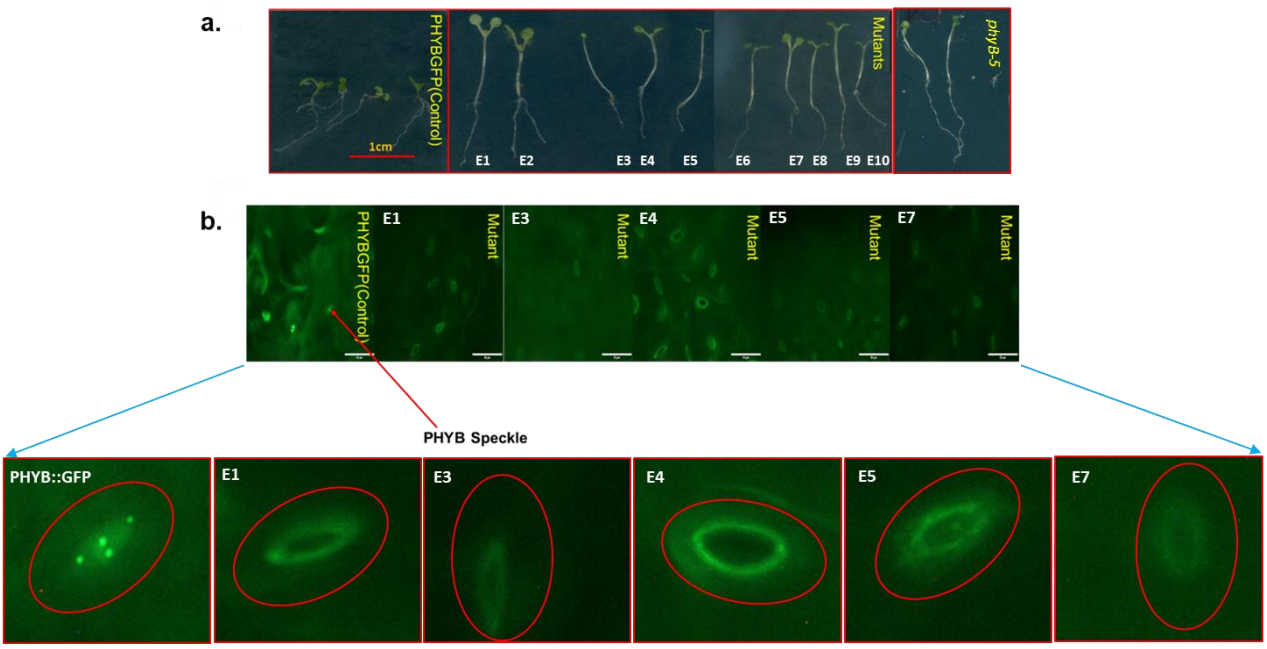


Fig.7.1. 35S::PHYB:GFP EMS mutant lines. (a) Red light screened hyposensitive mutant seedlings of 35S::PHYB:GFP along with 35S::PHYB:GFP (control) and *phyB-5* mutant lines. (b) Alteration in PHYB nuclear speckle formation in hypocotyl of mutant lines of 35S::PHYB:GFP. E= EMS mutants. Scale bar = 1cm in (a) and 50µm in (b).

The mutants showing no GFP expression were eliminated and further study was carried out with the mutants showing absence/alteration in nuclear body formation. This finding indicated that, these hyposensitive mutants had possible alteration in PHYB translocation or defect in nuclear body formation.

7.2.2. Screening of EMS mutants of 35S::PHYB:GFP for alteration in root morphology

The hyposensitive mutant seedlings with absence/altered nuclear speckle formation were further screened for alteration in root morphology under W, FR, R and B light (LD). Then the hyposensitive mutants defective in root morphology specific to R light were selected. The screening was performed after 7 and 15 days of R light treatment.

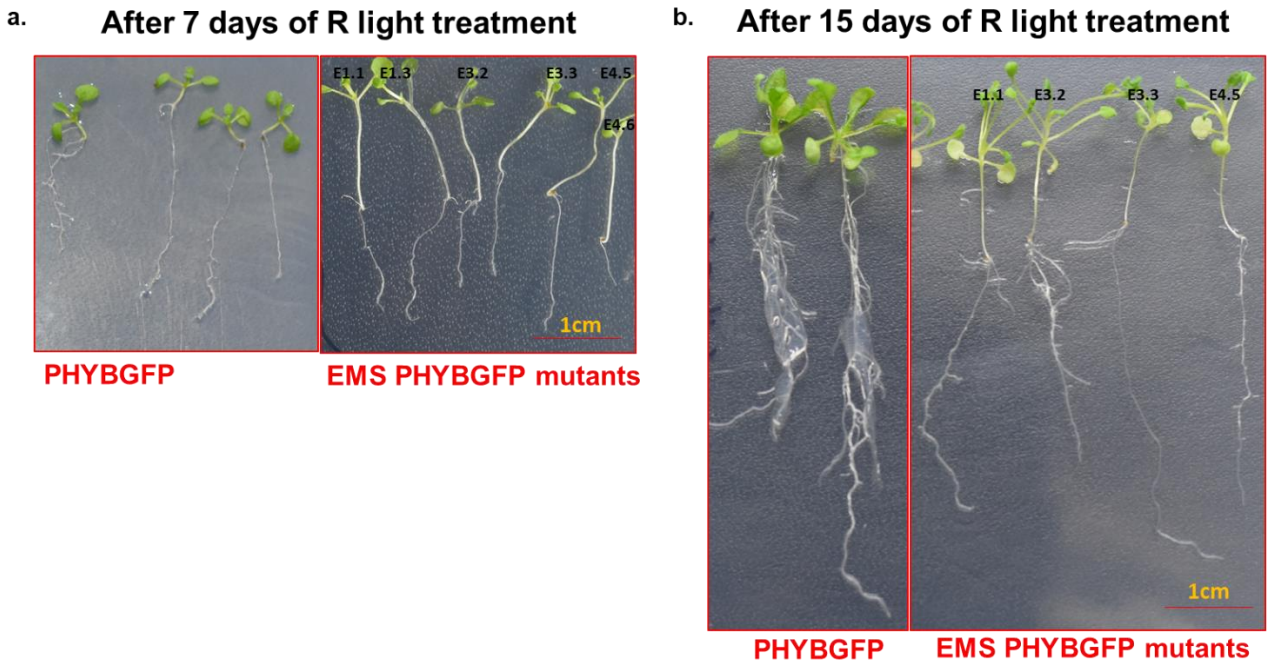
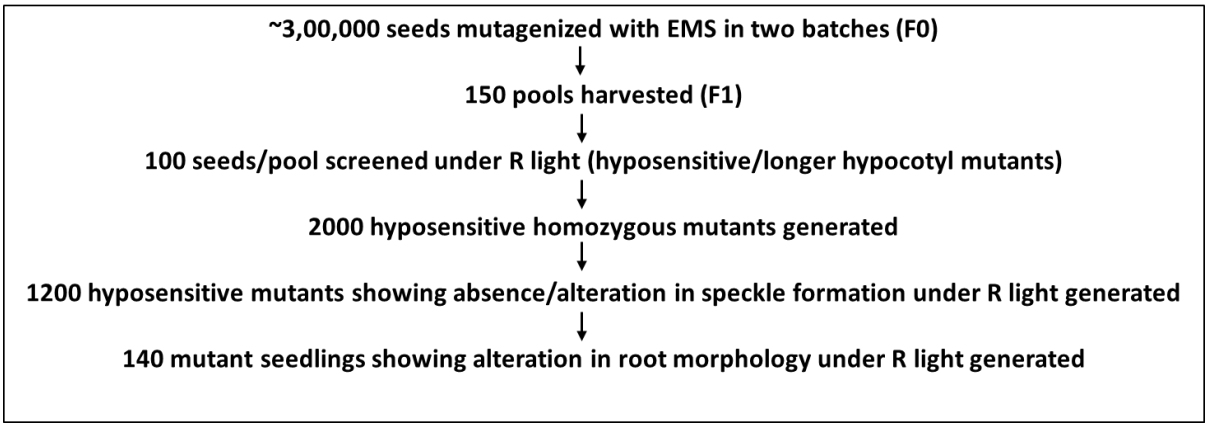


Fig.7.2. Hyposensitive mutants of 35S::PHYB:GFP screened for defects in root patterning (a) after 7 days and (b) after 15 days of R light treatment. E= EMS mutants. Scale bar=1cm.

The mutants defective in root phenotype were further grown. The qualitative analysis indicated that the mutants showed variation in root morphology such as, shorter or longer primary root, alteration in lateral or adventitious root number etc (Fig.7.2).

The plan of EMS mutagenesis, screening and generation of 35S::PHYB:GFP mutants have been summarised in table 19.

Table.19. Flow chart of mutagenesis, screening and generation of 35S::PHYB:GFP mutants



7.2.3. Morphological differences in adult mutant plants

The EMS 35S::PHYB:GFP mutants showing alteration in root morphology were further investigated at adult stage for the defects in their developmental pattern under W light (LD). The mutant plants were grown on soil and analysed after ~3 months. It was observed that, mutants showed variable growth pattern such as stunted growth, altered flowering, long petiole, pale colour leaves, etc (Fig.7.3). This indicated that the mutations generated in 35S::PHYB:GFP lines also affected the plant development at adult stages.

Fig.7.3. Three weeks-old 35S::PHYB:GFP mutants with variable growth defects under white light.

7.3. Discussion:

PHYB and the downstream light signaling genes such as *HY5*, *HYH*, *PIFs*, etc. interact with auxin to control different plant processes (115, 117, 208). There are few reports available which state that R light and auxin interact to modulate the root growth in plants (12). However, the molecular players engaged in the common pathways of auxin and R light signaling are not well documented and need further studies. *hy3* mutant having alteration in gene coding for PHYB has elongated root hairs (49). *hy5* mutant has also shown defect in lateral root growth and it occurs because of modulation in auxin and CK signaling (208). PHYB, PHYE and PHYA have been shown to stimulate the production of lateral roots while PHYD suppresses lateral root production. Among these PHYs, PHYB has the predominant role in lateral root growth. PHYB-mediated lateral root growth is mainly due to change in shoot-root auxin distribution (113). The above-mentioned information are very helpful to explore the involvement of R light in root development, still the

mechanism behind the role of light-auxin crosstalk in the development of root is not well established.

In the present work, it has been attempted to investigate the candidate genes playing role in PHYB and auxin crosstalk in root development. Hence, the EMS mutants of PHYB were generated in this current work. The mutants hyposensitive to R light and had altered root morphology were generated. Further, hyposensitive mutants with different root architecture and altered nuclear complex formation were screened. These mutant lines generated in the current work also displayed developmental defects at adult stage. These hyposensitive mutants of PHYB with altered root architecture and nuclear complex formation can possibly have defect in red light signaling and auxin associated pathways. These mutants can be further subjected to next generation sequencing to know the exact position of mutation. This study can help in providing information about some novel candidate genes involved in PHYB and auxin cross-talk to regulate root development. It will also help in generating plants having improved root growth with drought resistance, pathogen resistance and water logging resistance properties.

CHAPTER 8

SUMMARY AND CONCLUSION

Chapter 8

8. Summary and conclusion

8.1. Exploring the molecular components in light fluence dependent root growth in *Arabidopsis thaliana*

The impact of variable W light intensities (150, 112, 75 and 38 μmol m^{-2} s^{-1}) on root growth of Col-0, *phyA* and *phyB* mutants was investigated. In all four light intensities, *phyA-211* displayed shorter primary root. However, under higher light intensities (150 and 112 μmol m^{-2} s^{-1}) length of primary root was lesser in *phyA* and *phyB* mutants as compared to Col-0. Under constant light intensity, *phyB-9* exhibited more adventitious roots as compared to Col-0 and *phyA-211*. In case of low light intensities (38 and 75 μmol m^{-2} s^{-1}) lateral root number was lesser in *phyA-211* but under 112 μmol m^{-2} s^{-1} light, lateral root number was lesser in *phyB-9* mutant as compared to *phyA-211* and Col-0. However, in presence of 150 μmol m^{-2} s^{-1} light, lateral root number was similar in all the genotypes.

Highest (150 μmol m^{-2} s^{-1}) and lowest (38 μmol m^{-2} s^{-1}) light intensity significantly influenced the expression of genes present in roots since, highest number of DEGs were observed under this comparative light condition. The KEGG colour pathway analysis had revealed that maximum number of DEGs belonged to metabolic, carbon metabolic, biosynthesis of secondary metabolites and plant hormone signaling pathways. Different W light intensity also changed the expression profiling of hormone associated, light signaling and clock regulated genes present in the roots. Genes such as *COL3*, *CIP1*, *EPR1*, *PIF4* and *TOC1* etc were altered under all four light intensities. Alteration of these genes indicated about their potential involvement in the light intensity-based root development. Hence, it suggests that light intensity changes the root morphology by regulating the genes playing roles in hormone associated, light signaling and clock regulated pathways (209).

8.2. Light dependent effects of carbon nanoparticles on root architecture in *Arabidopsis thaliana*

The impact of CNP on root morphology was investigated under W, R, FR and B light. Here, it was explored here, that in +CNP *phyB-9* displayed shorter primary root as compared to Col-0 under R light only. Under W and R light, lateral root number was more in *phyB-9* in +CNP. Adventitious root number was also significantly higher in *phyB-9* in +CNP and only under W light. CNP reduced growth root hairs in both the seed lines irrespective of light quality. Root WD was lesser in case of *phyB-9* as compared to Col-0, in +CNP under W, R and B light conditions. CNP-mediated root straightness was also decreased in *phyB-9* as compared to Col-0 under R light. Similarly, under W, R and B light, HGI in *phyB-9* was lesser with respect to Col-0 in +CNP. *EIN2* and *TIR1* were differentially regulated by CNP, under both W and R light in case of *phyB-9*. It showed that CNP-mediated alteration in root morphology may depend on PHYB. However, decrease in root hair growth by CNP might be independent of light quality and PHYB. It also indicated that, CNP under different light qualities altered the root growth by modulating the expression of phytohormone signaling genes.

8.3. Effect of sugar and light on root architecture and expression of auxin related genes

In the current work, root morphology was studied in presence of different sugars under W, R and FR light. It was investigated that *phyB-9* mutant had shorter primary root in presence of Suc, Glu, Mal and Man under W and R light, however, the difference in primary root length was more significant under R light. Under FR light, insignificant difference in primary root length was observed in presence of all the sugar types. Root WD was observed to contrasting under W light in presence of Suc and zero sugar, as in presence of Suc WD was more in *phyB-9* as compared to Col-0 but it was opposite in zero sugar. On the other hand, under R and FR the difference in root

WD was not significant in presence of any sugar except Man and in Man *phyB-9* had shown lesser WD as compared to Col-0. Root straightness varied depending upon sugar type as well as light quality in *phyB-9* and Col-0. HGI of roots was observed to be lesser in *phyB-9* as compared to Col-0 in presence of Suc, Glu, Mal and Man under W light. Under R and FR, alteration in HGI was observed to be determined by sugar type as well as quality of light in *phyB-9* and Col-0 seedlings. Root coiling was observed to be significantly lesser in *phyB-9* as compared to Col-0 under W and R light in presence of all sugars. However, under FR light, difference in root coiling was insignificant in *phyB-9* and Col-0 seedlings except in presence of Mal and Man. The expression profile of *PIN1*, *TIR1* and *YUCCA2* was variable in *phyB-9* in comparison to Col-0 under W and R light in presence of Suc, Glu and Sor. This showed that, different type of sugar altered the root architecture in various ways depending upon the light quality and PHYB. The light and sugar dependent alteration in root development possibly occur due to change in auxin associated gene expression.

8.4. A luciferase-based ratiometric auxin quantification to understand auxin and phytochrome crosstalk

The interaction of auxin and PHYB was analysed in protoplast system. It was observed that, after pre-irradiation of light for different durations and incubation under different light, auxin dynamics was similar in Col-0 and *phyB-9* protoplasts. Auxin dynamics was observed to be similar in both the protoplasts, however, it was investigated that auxin signaling genes such as *GH3.5* was differentially regulated in *phyB-9* and Col-0 protoplasts. Although, in protoplast system auxin dynamics was investigated to be independent of light quality and duration, the auxin signaling gene expression varied between *phyB-9* and Col-0 protoplasts. To understand the utility and efficiency of the luciferase auxin sensor in plant system, new plant specific auxin sensor was

generated and transformed in Col-0 plants. These transgenic Col-0 plants were generated for the detailed and accurate study of light dependent auxin accumulation in different regions of intact plants at different time point.

8.5. Forward genetic screening of mutagenized phytochrome B over-expressing line to isolate seedlings altered in root patterning

EMS mutagenesis of 35S:PHYB::GFP seeds was performed and mutant lines with longer hypocotyl under R light were generated. They also showed alteration in PHYB nuclear complex formation. They were further screened for variable root morphology in comparison to 35S:PHYB::GFP seedlings. The mutations also caused developmental defects at adult stages. These mutants possibly have mutation at transcriptional or translational level of PHYB or its other downstream genes. These mutants were defective in R light signaling as well as in auxin homeostasis. Hence, these red-light mutants may provide some unique candidates which are involved in R light and auxin cross-talk in root development.

Bibliography

References:

1. Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M. *Arabidopsis thaliana*: A model plant for genome analysis. Science. 1998.

2. Koornneef M, Meinke D. The development of *Arabidopsis* as a model plant. Plant J. 2010.

3. Hopkins WG, Huner NPA. Introduction to Plant Physiology 4th Edition. John Wiley & Sons. 2008.

4. Hodge A, Berta G, Doussan C, Merchan F, Crespi M. Plant root growth, architecture and function. Plant and Soil. 2009.

5. Smith S, de Smet I. Root system architecture: Insights from *Arabidopsis* and cereal crops. Philos Trans R Soc B Biol Sci. 2012.

6. Petricka JJ, Winter CM, Benfey PN. Control of *Arabidopsis* root development. Annu Rev Plant Biol. 2012.

7. Migliaccio F, Fortunati A, Tassone P. *Arabidopsis* root growth movements and their symmetry: Progress and problems arising from recent work. Plant Signaling and Behavior. 2009.

8. Lynch J, Marschner P, Rengel Z. Effect of Internal and External Factors on Root Growth and Development. In: Marschner's Mineral Nutrition of Higher Plants: Third Edition. 2011.

9. Simmons C, Söll D, Migliaccio F. Circumnutation and gravitropism cause root waving in *Arabidopsis thaliana* . J Exp Bot. 2007.

10. Scherer GFE, Pietrzyk P. Gravity-dependent differentiation and root coils in *Arabidopsis* thaliana wild type and phospholipase-A-I knockdown mutant grown on the International Space Station. Plant Biol. 2014.

11. Fortier J, Truax B, Gagnon D, Lambert F. Abiotic and biotic factors controlling fine root biomass, carbon and nutrients in closed-canopy hybrid poplar stands on post-agricultural land. Sci Rep. 2019.

12. Kumari S, Panigrahi KCS. Light and auxin signaling cross-talk programme root development in plants. J Biosci. 2019;44(1).

13. Casal JJ. Shade Avoidance. Arab B. 2012;10:e0157.

14. Briggs WR. Photoreceptors in Plant Photomorphogenesis to Date. Five Phytochromes, Two Cryptochromes, One Phototropin, and One Superchrome. Plant Physiol. 2001;125(1):85–8.

15. van Gelderen K, Kang C, Pierik R. Light signaling, root development and plasticity. Plant Physiol. 2018.

16. Li J, Li G, Wang H, Wang Deng X. Phytochrome Signaling Mechanisms. Arab B. 2011.

17. Chen M, Chory J. Phytochrome signaling mechanisms and the control of plant development. Trends in Cell Biology. 2011.

18. Castillon A, Shen H, Huq E. Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks. Trends in Plant Science. 2007.

19. Hiltbrunner A, Tscheuschler A, Viczián A, Kunkel T, Kircher S, Schäfer E. FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor.

Plant Cell Physiol. 2006.

20. Klose C, Viczián A, Kircher S, Schäfer E, Nagy F. Molecular mechanisms for mediating light-dependent nucleo/cytoplasmic partitioning of phytochrome photoreceptors. New Phytologist. 2015.

21. Pham VN, Kathare PK, Huq E. Phytochromes and phytochrome interacting factors. Plant Physiol. 2018.

22. Nemhauser J, Chory J. Photomorphogenesis. Arab B. 2002.

23. Josse EM, Halliday KJ. Skotomorphogenesis: The Dark Side of Light Signalling. Current Biology. 2008.

24. Casal JJ, Sánchez RA, Botto JF. Modes of action of phytochromes. Journal of Experimental Botany. 1998.

25. Franklin KA, Quail PH. Phytochrome functions in *Arabidopsis* development. J Exp Bot. 2010;61(1):11–24.

26. Reed JW, Nagatani A, Elich TD, Fagan M, Chory J. Phytochrome A and Phytochrome B Have Overlapping but Distinct Functions in *Arabidopsis* Development. Plant Physiol. 1994.

27. Kong S-G, Okajima K. Diverse photoreceptors and light responses in plants. J Plant Res. 2016.

28. Correll MJ, Kiss JZ. The roles of phytochromes in elongation and gravitropism of roots. Plant Cell Physiol. 2005;46(2):317–23.

29. Bognár LK, Hall A, Ádám É, Thain SC, Nagy F, Millar AJ. The circadian clock controls

the expression pattern of the circadian input photoreceptor, phytochrome B. Proc Natl Acad Sci U S A. 1999.

30. Lin C, Todo T. The cryptochromes. Genome Biology. 2005.

31. Liu H, Liu B, Zhao C, Pepper M, Lin C. The action mechanisms of plant cryptochromes. Trends in plant science. 2011 Dec 1;16(12):684-91.

32. Yu X, Liu H, Klejnot J, Lin C. The Cryptochrome Blue Light Receptors. Arab B. 2010;8:e0135.

33. Wu G, Spalding EP. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of *Arabidopsis* seedlings. Proc Natl Acad Sci. 2007;104(47):18813–8.

34. Canamero RC, Bakrim N, Bouly JP, Garay A, Dudkin EE, Habricot Y, et al. Cryptochrome photoreceptors cry1 and cry2 antagonistically regulate primary root elongation in *Arabidopsis thaliana*. Planta. 2006;224(5):995–1003.

35. El-Din El-Assal S, Alonso-Blanco C, Peeters AJM, Wagemaker C, Weller JL, Koornneef M. The role of cryptochrome 2 in flowering in *Arabidopsis*. Plant Physiol. 2003;133(4):1504–16.

36. Christie JM. Phototropin Blue-Light Receptors. Annu Rev Plant Biol. 2007.

37. Zhang KX, Xu HH, Yuan TT, Zhang L, Lu YT. Blue-light-induced PIN3 polarization for root negative phototropic response in *Arabidopsis*. Plant J. 2013.

38. Wan Y, Jasik J, Wang L, Hao H, Volkmann D, Menzel D, et al. The Signal Transducer NPH3 Integrates the Phototropin1 Photosensor with PIN2-Based Polar Auxin Transport in

Arabidopsis Root Phototropism. Plant Cell. 2012.

39. Moni A, Lee AY, Briggs WR, Han IS. The blue light receptor Phototropin 1 suppresses lateral root growth by controlling cell elongation. Plant Biol. 2015;17(1):34–40.

40. Zhao X, Wang Y-L, Qiao X-R, Wang J, Wang L-D, Xu C-S, et al. Phototropins Function in High-Intensity Blue Light-Induced Hypocotyl Phototropism in *Arabidopsis* by Altering Cytosolic Calcium. Plant Physiol. 2013;162(3):1539–51.

41. Jenkins GI. The UV-B Photoreceptor UVR8: From Structure to Physiology. Plant Cell. 2014.

42. Fasano R, Gonzalez N, Tosco A, Dal Piaz F, Docimo T, Serrano R, Grillo S, Leone A, Inzé D. Role of *Arabidopsis* UV RESISTANCE LOCUS 8 in plant growth reduction under osmotic stress and low levels of UV-B. Molecular plant. 2014 May 1;7(5):773-91.

43. Arongaus AB, Chen S, Pireyre M, Glöckner N, Galvão VC, Albert A, et al. *Arabidopsis* RUP2 represses UVR8-mediated flowering in noninductive photoperiods. Genes Dev. 2018.

44. Kim SY, Yu X, Michaels SD. Regulation of CONSTANS and FLOWERING LOCUS T Expression in Response to Changing Light Quality. Plant Physiol. 2008.

45. Sharrock RA. The phytochrome red/far-red photoreceptor superfamily. Genome Biology. 2008.

46. Franklin KA, Whitelam GC. Phytochromes and shade-avoidance responses in plants. Annals of Botany. 2005.

47. Wang FF, Lian HL, Kang CY, Yang HQ. Phytochrome B is involved in mediating red

light-induced stomatal opening in *Arabidopsis thaliana*. Mol Plant. 2010.

48. Costigan SE, Warnasooriya SN, Humphries BA, Montgomery BL. Root-Localized Phytochrome Chromophore Synthesis Is Required for Photoregulation of Root Elongation and Impacts Root Sensitivity to Jasmonic Acid in *Arabidopsis*. Plant Physiol. 2011;157(3):1138–50.

49. Reed JW, Nagpal P, Poole DS, Furuya M, Chory J. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell. 1993.

50. Taiz L, Zeiger E. Plant physiology. California: The. 2002.

51. Wani SH, Kumar V, Shriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop Journal. 2016.

52. Marzec M. Strigolactones and Gibberellins: A New Couple in the Phytohormone World? Trends in Plant Science. 2017.

53. Aloni R, Aloni E, Langhans M, Ullrich CI. Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot. 2006;97(5):883–93.

54. Chae K, Isaacs CG, Reeves PH, Maloney GS, Muday GK, Nagpal P, et al. *Arabidopsis* SMALL AUXIN UP RNA63 promotes hypocotyl and stamen filament elongation. Plant J. 2012;71(4):684–97.

55. Moore TC, Moore TC. Roles of Auxins and Cytokinins in Apical Dominance. In: Research Experiences in Plant Physiology. 1974.

56. Fàbregas N, Formosa-Jordan P, Confraria A, Siligato R, Alonso JM, Swarup R, et al. Auxin Influx Carriers Control Vascular Patterning and Xylem Differentiation in *Arabidopsis thaliana*. PLoS Genet. 2015.

57. Rakusová H, Gallego-Bartolomé J, Vanstraelen M, Robert HS, Alabadí D, Blázquez MA, et al. Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in *Arabidopsis thaliana*. Plant J. 2011.

58. Hedden P, Sponsel V. A Century of Gibberellin Research. Journal of Plant Growth Regulation. 2015.

59. Alabadi D. Gibberellins Repress Photomorphogenesis in Darkness. Plant Physiol. 2004;134(3):1050–7.

60. Alabadí D, Gallego-Bartolomé J, Orlando L, García-Cárcel L, Rubio V, Martínez C, et al. Gibberellins modulate light signaling pathways to prevent *Arabidopsis* seedling de-etiolation in darkness. Plant J. 2008;53(2):324–35.

61. Stamm P, Kumar PP. Auxin and gibberellin responsive *Arabidopsis* SMALL AUXIN UP RNA36 regulates hypocotyl elongation in the light. Plant Cell Rep. 2013;32(6):759–69.

62. Werner T, Motyka V, Strnad M, Schmulling T. Regulation of plant growth by cytokinin. Proc Natl Acad Sci. 2001.

63. Pua E, Davey MR. Plant Developmental Biology - Biotechnological Perspectives. Vol. 2, Plant Developmental Biology - Biotechnological Perspectives. 2010.

64. Kieber JJ, Schaller GE. Cytokinin signaling in plant development. Development. 2018.

65. Schaller GE. Ethylene and the regulation of plant development. BMC Biology. 2012.

66. Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. Front Plant Sci. 2017.

67. Smalle J, Haegman M, Kurepa J, Van Montagu M, Straeten DVD. Ethylene can stimulate *Arabidopsis* hypocotyl elongation in the light. Proc Natl Acad Sci. 1997.

68. Yu Y, Wang J, Zhang Z, Quan R, Zhang H, Deng XW, et al. Ethylene Promotes Hypocotyl Growth and HY5 Degradation by Enhancing the Movement of COP1 to the Nucleus in the Light. PLoS Genet. 2013;9(12).

69. Ruzicka K, Ljung K, Vanneste S, Podhorska R, Beeckman T, Friml J, et al. Ethylene Regulates Root Growth through Effects on Auxin Biosynthesis and Transport-Dependent Auxin Distribution. Plant Cell. 2007.

70. Lim CW, Baek W, Jung J, Kim JH, Lee SC. Function of ABA in stomatal defense against biotic and drought stresses. International Journal of Molecular Sciences. 2015.

71. Chen H, Zhang J, Neff MM, Hong S-W, Zhang H, Deng X-W, et al. Integration of light and abscisic acid signaling during seed germination and early seedling development. Proc Natl Acad Sci. 2008.

72. Sharp RE, Lenoble ME. ABA, ethylene and the control of shoot and root growth under water stress. J Exp Bot. 2002.

73. Mishra S, Upadhyay S, Shukla RK. The role of strigolactones and their potential cross-talk under hostile ecological conditions in plants. Frontiers in Physiology. 2017.

74. Sun H, Tao J, Gu P, Xu G, Zhang Y. The role of strigolactones in root development. Plant

Signaling and Behavior. 2016.

75. Pandey A, Sharma M, Pandey GK. Corrigendum: Emerging Roles of Strigolactones in Plant Responses to Stress and Development. Front Plant Sci. 2016.

76. Jia KP, Luo Q, He SB, Lu XD, Yang HQ. Strigolactone-regulated hypocotyl elongation is dependent on cryptochrome and phytochrome signaling pathways in arabidopsis. Mol Plant. 2014;7(3):528–40.

77. Robson F, Okamoto H, Patrick E, Harris S-R, Wasternack C, Brearley C, et al. Jasmonate and Phytochrome A Signaling in *Arabidopsis* Wound and Shade Responses Are Integrated through JAZ1 Stability . Plant Cell. 2010.

78. Wang L, Wu J. The Essential Role of Jasmonic Acid in Plant-Herbivore Interactions - Using the Wild Tobacco *Nicotiana* attenuata as a Model. Journal of Genetics and Genomics. 2013.

79. Ahmad P, Rasool S, Gul A, Sheikh SA, Akram NA, Ashraf M, et al. Jasmonates: Multifunctional Roles in Stress Tolerance. Front Plant Sci. 2016.

80. Pauwels L, Goossens A. The JAZ proteins: A crucial interface in the jasmonate signaling cascade. Plant Cell. 2011.

81. Liu Y, Du M, Deng L, Shen J, Fang M, Chen Q, et al. Myc2 regulates the termination of jasmonate signaling via an autoregulatory negative feedback loop. Plant Cell. 2019.

82. Chen J, Sonobe K, Ogawa N, Masuda S, Nagatani A, Kobayashi Y, et al. Inhibition of arabidopsis hypocotyl elongation by jasmonates is enhanced under red light in phytochrome B dependent manner. J Plant Res. 2013.

83. Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence: Its role in plant growth and development. Journal of Experimental Botany. 2011.

84. Wei Z, Li J. Brassinosteroids Regulate Root Growth, Development, and Symbiosis. Mol Plant. 2016;9(1):86–100.

85. Luo XM, Lin WH, Zhu S, Zhu JY, Sun Y, Fan XY, et al. Integration of Light- and Brassinosteroid-Signaling Pathways by a GATA Transcription Factor in Arabidopsis. Dev Cell. 2010.

86. Oh E, Zhu JY, Wang ZY. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol. 2012;14(8):802–9.

87. Kim B, Jeong YJ, Corvalán C, Fujioka S, Cho S, Park T, et al. Darkness and gulliver2/phyB mutation decrease the abundance of phosphorylated BZR1 to activate brassinosteroid signaling in *Arabidopsis*. Plant J. 2014.

88. Mussig C. Brassinosteroids Promote Root Growth in Arabidopsis. Plant Physiol. 2003.

89. Paque S, Weijers D. Q&A: Auxin: The plant molecule that influences almost anything. BMC Biol. 2016.

90. Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawaide H, Natsume M, et al. The main auxin biosynthesis pathway in *Arabidopsis*. Proc Natl Acad Sci. 2011.

91. Zhao Y. Auxin Biosynthesis and Its Role in Plant Development. Annu Rev Plant Biol. 2010;

92. Mano Y, Nemoto K. The pathway of auxin biosynthesis in plants. Journal of Experimental Botany. 2012.

93. Barlier I, Kowalczyk M, Marchant A, Ljung K, Bhalerao R, Bennett M, Sandberg G, Bellini C. The SUR2 gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. Proceedings of the National Academy of Sciences. 2000 Dec 19;97(26):14819-24.

94. Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, H. and McSteen, P., 2011. The main auxin biosynthesis pathway in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, *108*(45), pp.18512-18517.

95. Polar Auxin Transport and Asymmetric Auxin Distribution. Arab B. 2007.

96. Swarup R, Péret B. AUX/LAX family of auxin influx carriers—an overview. Front Plant Sci. 2012.

97. Forestan C, Varotto S. The role of PIN auxin efflux carriers in polar auxin transport and accumulation and their effect on shaping maize development. Mol Plant. 2012.

98. Adamowski M, Friml J. PIN-Dependent Auxin Transport: Action, Regulation, and Evolution. Plant Cell Online. 2015.

99. Křeček P, Skůpa P, Libus J, Naramoto S, Tejos R, Friml J, Zažímalová E. The PIN-FORMED (PIN) protein family of auxin transporters. Genome biology. 2009 Dec;10(12):249.

100. Cho M, Cho HT. The function of ABCB transporters in auxin transport. Plant Signal Behav. 2013.

101. Zhang KX, Xu HH, Gong W, Jin Y, Shi YY, Yuan TT, et al. Proper PIN1 distribution is

needed for root negative phototropism in *Arabidopsis*. PLoS One. 2014.

102. Pickett FB, Wilson AK, Estelle M. The aux1 mutation of *Arabidopsis* confers both auxin and ethylene resistance. Plant physiology. 1990 Nov 1;94(3):1462-6.

103. Guenot B, Bayer E, Kierzkowski D, Smith RS, Mandel T, Žádníková P, Benková E, Kuhlemeier C. Pin1-independent leaf initiation in *Arabidopsis*. Plant Physiology. 2012 Aug 1;159(4):1501-10.

104. Quint M, Gray WM. Auxin signaling. Current Opinion in Plant Biology. 2006.

105. Lavy M, Estelle M. Mechanisms of auxin signaling. Development. 2016;143(18):3226–9.

106. Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, et al. Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell. 2005.

107. Konishi M, Donner TJ, Scarpella E, Yanagisawa S. MONOPTEROS directly activates the auxin-inducible promoter of the Dof5.8 transcription factor gene in *Arabidopsis thaliana* leaf provascular cells. J Exp Bot. 2015.

108. Fukaki H, Tameda S, Masuda H, Tasaka M. Lateral root formation is blocked by a gain-of-function mutation in the solitary-root/IAA14 gene of Arabidopsis. Plant J. 2002.

109. Tabata R, Ikezaki M, Fujibe T, Aida M, Tian CE, Ueno Y, et al. *Arabidopsis* AUXIN RESPONSE FACTOR6 and 8 regulate jasmonic acid biosynthesis and floral organ development via repression of class 1 KNOX genes. Plant Cell Physiol. 2010.

110. Halliday KJ, Martínez-García JF, Josse EM. Integration of light and auxin signaling. Cold Spring Harb Perspect Biol. 2009;1(6):1–11.

111. Iglesias MJ, Sellaro R, Zurbriggen MD, Casal JJ. Multiple links between shade avoidance

and auxin networks. Journal of Experimental Botany. 2018.

112. Jensen PJ, Hangarter RP, Estelle M. Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown *Arabidopsis*. Plant Physiol. 1998.

113. Salisbury FJ, Hall A, Grierson CS, Halliday KJ. Phytochrome coordinates *Arabidopsis* shoot and root development. Plant J. 2007;50(3):429–38.

114. Devlin PF, Yanovsky MJ, Kay SA. A Genomic Analysis of the Shade Avoidance Response in *Arabidopsis*. Plant Physiol. 2003.

115. Roig-Villanova I, Bou-Torrent J, Galstyan A, Carretero-Paulet L, Portolés S, Rodríguez-Concepción M, et al. Interaction of shade avoidance and auxin responses: A role for two novel atypical bHLH proteins. EMBO J. 2007;26(22):4756–67.

116. Sibout R, Sukumar P, Hettiarachchi C, Holm M, Muday GK, Hardtke CS. Opposite root growth phenotypes of hy5 versus hy5 hyh mutants correlate with increased constitutive auxin signaling. PLoS Genet. 2006.

117. Franklin KA, Lee SH, Patel D, Kumar SV, Spartz AK, Gu C, et al. Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. Proc Natl Acad Sci U S A. 2011.

118. Lee HJ, Ha JH, Kim SG, Choi HK, Kim ZH, Han YJ, et al. Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots. Sci Signal. 2016.

119. Reed JW, Elumalai RP, Chory J. Suppressors of an *Arabidopsis thaliana* phyB mutation identify genes that control light signaling and hypocotyl elongation. Genetics. 1998.

120. Ha JH, Kim JH, Kim SG, Sim HJ, Lee G, Halitschke R, et al. c phytochrome B modulates

reactive oxygen species homeostasis in roots via abscisic acid signaling in *Arabidopsis*. Plant J. 2018.

121. Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X. Shoot-to-Root Mobile Transcription Factor HY5 Coordinates Plant Carbon and Nitrogen Acquisition. Curr Biol. 2016;26(5):640–6.

122. Raya-González J, López-Bucio JS, Prado-Rodríguez JC, Ruiz-Herrera LF, Guevara-García ÁA, López-Bucio J. The MEDIATOR genes MED12 and MED13 control *Arabidopsis* root system configuration influencing sugar and auxin responses. Plant Mol Biol. 2017.

123. Raya-González J, Oropeza-Aburto A, López-Bucio JS, Guevara-García ÁA, de Veylder L, López-Bucio J, et al. MEDIATOR18 influences *Arabidopsis* root architecture, represses auxin signaling and is a critical factor for cell viability in root meristems. Plant J. 2018.

124. Nagel KA, Schurr U, Walter A. Dynamics of root growth stimulation in *Nicotiana tabacum* in increasing light intensity. Plant, Cell Environ. 2006;29(10):1936–45.

125. Spiegelman Z, Omer S, Mansfeld BN, Wolf S. Function of Cyclophilin1 as a long-distance signal molecule in the phloem of tomato plants. J Exp Bot. 2017.

126. Spiegelman Z, Ham BK, Zhang Z, Toal TW, Brady SM, Zheng Y, et al. A tomato phloem-mobile protein regulates the shoot-to-root ratio by mediating the auxin response in distant organs. Plant J. 2015;83(5):853–63.

127. Razzak A, Ranade SS, Strand Å, García-Gil MR. Differential response of Scots pine

seedlings to variable intensity and ratio of red and far-red light. Plant Cell Environ. 2017;40(8):1332–40.

128. Qu Y, Liu S, Bao W, Xue X, Ma Z, Yokawa K, et al. Expression of root genes in *Arabidopsis* seedlings grown by standard and improved growing methods. Int J Mol Sci. 2017;18(5):1–13.

129. Curtis RHC, Pankaj, Powers SJ, Napier J, Matthes MC. The *Arabidopsis* F-box/Kelch-Repeat Protein At2g44130 Is Upregulated in Giant Cells and Promotes Nematode Susceptibility . Mol Plant-Microbe Interact. 2012.

130. Bednarek P, Piślewska-Bednarek M, Svatoš A, Schneider B, Doubský J, Mansurova M, et al. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science (80-). 2009;323(5910):101–6.

131. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, et al. PANTHER version 11: Expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. 2017;45(D1):D183–9.

132. Gene Ontology Consortium. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res. 2004.

133. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic acids research. 1999 Jan 1;27(1):29-34.

134. Voß U, Wilson MH, Kenobi K, Gould PD, Robertson FC, Peer WA, et al. The circadian clock rephases during lateral root organ initiation in *Arabidopsis thaliana*. Nat Commun. 2015;6.

135. Kiss JZ. Phytochromes A and B Mediate Red-Light-Induced Positive Phototropism in Roots. Plant Physiol. 2003;131(3):1411–7.

136. Moriwaki T, Miyazawa Y, Kobayashi A, Uchida M, Watanabe C, Fujii N, et al. Hormonal Regulation of Lateral Root Development in *Arabidopsis* Modulated by MIZ1 and Requirement of GNOM Activity for MIZ1 Function. Plant Physiol. 2011.

137. Zhang S, Huang L, Yan A, Liu Y, Liu B, Yu C, et al. Multiple phytohormones promote root hair elongation by regulating a similar set of genes in the root epidermis in *Arabidopsis*. J Exp Bot. 2016.

138. Fogaça CM, Fett-Neto AG. Role of auxin and its modulators in the adventitious rooting of Eucalyptus species differing in recalcitrance. Plant Growth Regul. 2005;45(1):1–10.

139. Cheng L, Tang X, Vance CP, White PJ, Zhang F, Shen J. Interactions between light intensity and phosphorus nutrition affect the phosphate-mining capacity of white lupin (*Lupinus albus* L.). J Exp Bot. 2014.

140. Wan J, Zhang P, Wang R, Sun L, Wang W, Zhou H, et al. UV-B radiation induces root bending through the flavonoid-mediated auxin pathway in *Arabidopsis*. Front Plant Sci. 2018.

141. Warnasooriya SN, Montgomery BL. Spatial-specific regulation of root development by phytochromes in *Arabidopsis thaliana*. Plant Signal Behav. 2011.

142. Feng Y, Xu P, Li B, Li P, Wen X, An F, et al. Ethylene promotes root hair growth through coordinated EIN3/EIL1 and RHD6/RSL1 activity in *Arabidopsis* . Proc Natl Acad Sci. 2017.

143. Tripathi DK, Shweta, Singh S, Singh S, Pandey R, Singh VP, et al. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. Plant Physiology and Biochemistry. 2017.

144. Sassi M, Lu Y, Zhang Y, Wang J, Dhonukshe P, Blilou I, et al. COP1 mediates the coordination of root and shoot growth by light through modulation of PIN1- and PIN2-dependent auxin transport in *Arabidopsis*. Development. 2012;139(18):3402–12.

145. Dobisova T, Hrdinova V, Cuesta C, Michlickova S, Urbankova I, Hejatkova R, et al. Light Controls Cytokinin Signaling via Transcriptional Regulation of Constitutively Active Sensor Histidine Kinase CKI1. Plant Physiol. 2017;174(1):387–404.

146. Chen H, Xiong L. Role of HY5 in abscisic acid response in seeds and seedlings. Plant Signal Behav. 2008.

147. Aslani F, Bagheri S, Muhd Julkapli N, Juraimi AS, Hashemi FSG, Baghdadi A. Effects of engineered nanomaterials on plants growth: An overview. Sci World J. 2014;2014.

148. Sharon M, Sharon M. Carbon Nanoforms and Applications. In: Introduction to the nanoworld of Carbon. 2010.

149. Luo M, Nations S, Dai L, Cañas JE, Vadan R, Lee EH, et al. Effects of Functionalized and Nonfunctionalized Single-Walled Carbon Nanotubes on Root Elongation of Select Crop Species. Environ Toxicol Chem. 2008;27(9):1922.

150. Yan S, Zhao L, Li H, Zhang Q, Tan J, Huang M, et al. Single-walled carbon nanotubes selectively influence maize root tissue development accompanied by the change in the related gene expression. J Hazard Mater. 2013.

151. García-Sánchez S, Bernales I, Cristobal S. Early response to nanoparticles in the *Arabidopsis* transcriptome compromises plant defence and root-hair development through salicylic acid signalling. BMC Genomics. 2015;16(1).

152. Hao Y, Yu F, Lv R, Ma C, Zhang Z, Rui Y, et al. Carbon Nanotubes Filled with Different Ferromagnetic Alloys Affect the Growth and Development of Rice Seedlings by Changing the C:N Ratio and Plant Hormones Concentrations. PLoS One. 2016;11(6):e0157264.

153. Begum P, Ikhtiari R, Fugetsu B. Graphene phytotoxicity in the seedling stage of cabbage, tomato, red spinach, and lettuce. Carbon N Y. 2011.

154. Mushtaq YK. Effect of nanoscale Fe3O4, TiO2 and carbon particles on cucumber seed germination. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng. 2011;46(14):1732–5.

155. Kumar A, Singh A, Panigrahy M, Sahoo PK, Panigrahi KCS. Carbon nanoparticles influence photomorphogenesis and flowering time in *Arabidopsis thaliana*. Plant Cell Rep. 2018.

156. Buer CS. Ethylene Modulates Root-Wave Responses in *Arabidopsis*. Plant Physiol. 2003.

157. Grabov A, Ashley MK, Rigas S, Hatzopoulos P, Dolan L, Vicente-Agullo F. Morphometric analysis of root shape. New Phytol. 2005.

158. Verma SK, Das AK, Gantait S, Kumar V, Gurel E. Applications of carbon nanomaterials in the plant system: A perspective view on the pros and cons. Science of the Total Environment. 2019.

159. Mukherjee A, Majumdar S, Servin AD, Pagano L, Dhankher OP, White JC. Carbon nanomaterials in agriculture: A critical review. Frontiers in Plant Science. 2016.

160. Tripathi S, Sarkar S. Influence of water soluble carbon dots on the growth of wheat plant. Appl Nanosci. 2015.

161. Panova GG, Ktitorova IN, Skobeleva O V., Sinjavina NG, Charykov NA, Semenov KN. Impact of polyhydroxy fullerene (fullerol or fullerenol) on growth and biophysical characteristics of barley seedlings in favourable and stressful conditions. Plant Growth Regul. 2016.

162. van Gelderen K, Kang C, Paalman R, Keuskamp D, Hayes S, Pierik R. Far-red light detection in the shoot regulates lateral root development through the HY5 transcription factor. Plant Cell. 2018.

163. Gladish DK, Rost TL. The effects of temperature on primary root growth dynamics and lateral root distribution in garden pea (*Pisum sativum* L., cv. “Alaska”). Environ Exp Bot. 1993.

164. Lahti M, Aphalo PJ, Finér L, Ryyppö A, Lehto T, Mannerkoski H. Effects of soil temperature on shoot and root growth and nutrient uptake of 5-year-old Norway spruce seedlings. Tree Physiol. 2005.

165. Legris M, Klose C, Burgie ES, Rojas CC, Neme M, Hiltbrunner A, et al. Phytochrome B integrates light and temperature signals in *Arabidopsis*. Science (80-). 2016.

166. Eckstein A, Zieba P, Gabryś H. Sugar and Light Effects on the Condition of the Photosynthetic Apparatus of *Arabidopsis thaliana* Cultured in vitro. J Plant Growth

Regul. 2012.

167. Lastdrager J, Hanson J, Smeekens S. Sugar signals and the control of plant growth and development. J Exp Bot. 2014.

168. Ohto M -a. Effects of Sugar on Vegetative Development and Floral Transition in *Arabidopsis*. Plant Physiol. 2001.

169. Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen J. Glucose-TOR signalling reprograms the transcriptome and activates meristems. Nature. 2013.

170. Hartig K, Beck E. Crosstalk between auxin, cytokinins, and sugars in the plant cell cycle. Plant Biology. 2006.

171. Dekkers BJW, Schuurmans JAMJ, Smeekens SCM. Interaction between sugar and abscisic acid signalling during early seedling development in *Arabidopsis*. Plant Mol Biol. 2008.

172. Matsoukas IG. Interplay between sugar and hormone signaling pathways modulate floral signal transduction. Frontiers in Genetics. 2014.

173. Gibson SI. Sugar and phytohormone response pathways: Navigating a signalling network. In: Journal of Experimental Botany. 2004.

174. Mishra BS, Singh M, Aggrawal P, Laxmi A. Glucose and auxin signaling interaction in controlling *Arabidopsis thaliana* seedlings root growth and development. PLoS One. 2009.

175. Kushwah S, Laxmi A. The interaction between glucose and cytokinin signaling in controlling *Arabidopsis thaliana* seedling root growth and development. Plant Signal

Behav. 2017.

176. Short TW. Overexpression of *Arabidopsis* Phytochrome B Inhibits Phytochrome A Function in the Presence of Sucrose. Plant Physiol. 2002.

177. Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, et al. Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science (80-). 2003.

178. Stewart JL, Maloof JN, Nemhauser JL. PIF genes mediate the effect of sucrose on seedling growth dynamics. PLoS One. 2011.

179. Matsoukas IG. Crosstalk between photoreceptor and sugar signaling modulates floral signal transduction. Frontiers in Physiology. 2017.

180. Sairanen I, Novak O, Pencik A, Ikeda Y, Jones B, Sandberg G, et al. Soluble Carbohydrates Regulate Auxin Biosynthesis via PIF Proteins in *Arabidopsis*. Plant Cell. 2012.

181. Kircher S, Schopfer P. Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in *Arabidopsis*. Proc Natl Acad Sci U S A. 2012.

182. Gupta A, Singh M, Laxmi A. Interaction between glucose and brassinosteroid during the regulation of lateral root development in *Arabidopsis*. Plant Physiol. 2015.

183. Ogawa A, Kawashima C, Yamauchi A. Sugar accumulation along the seminal root axis, as affected by osmotic stress in maize: A possible physiological basis for plastic lateral root development. Plant Prod Sci. 2005.

184. Singh M, Gupta A, Laxmi A. Glucose control of root growth direction in *Arabidopsis*

thaliana. J Exp Bot. 2014.

185. Wang J, Song J, Clark G, Roux SJ. ANN1 and ANN2 function in post-phloem sugar transport in root tips to affect primary root growth. Plant Physiol. 2018.

186. Vanneste S, Friml J. Auxin: A Trigger for Change in Plant Development. Cell. 2009.

187. Petersson S V., Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, et al. An Auxin Gradient and Maximum in the *Arabidopsis* Root Apex Shown by High-Resolution Cell-Specific Analysis of IAA Distribution and Synthesis. Plant Cell. 2009.

188. Porfírio S, Sonon R, Gomes da Silva MDR, Peixe A, Cabrita MJ, Azadi P. Quantification of free auxins in semi-hardwood plant cuttings and microshoots by dispersive liquid-liquid microextraction/microwave derivatization and GC/MS analysis. Anal Methods. 2016.

189. Wang L, Hua D, He J, Duan Y, Chen Z, Hong X, et al. Auxin response Factor2 (ARF2) and its regulated homeodomain gene HB33 mediate abscisic acid response in *Arabidopsis*. PLoS Genet. 2011.

190. Yao X, Feng H, Yu Y, Dong A, Shen WH. SDG2-Mediated H3K4 Methylation Is Required for Proper *Arabidopsis* Root Growth and Development. PLoS One. 2013.

191. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, et al. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. Nature. 2012.

192. Chen Y, Yordanov YS, Ma C, Strauss S, Busov VB. DR5 as a reporter system to study auxin response in *Populus*. Plant Cell Rep. 2013.

193. Guo L, Yu Y, Law JA, Zhang X. Set domain group2 is the major histone H3 lysine 4 trimethyltransferase in *Arabidopsis*. Proc Natl Acad Sci U S A. 2010.

194. Wend S, Dal Bosco C, Kämpf MM, Ren F, Palme K, Weber W, et al. A quantitative ratiometric sensor for time-resolved analysis of auxin dynamics. Sci Rep. 2013.

195. Zhang Z, Li Q, Li Z, Staswick PE, Wang M, Zhu Y, et al. Dual regulation role of GH3.5 in salicylic acid and auxin signaling during *Arabidopsis-Pseudomonas syringae* interaction. Plant Physiol. 2007.

196. Taniguchi M, Nakamura M, Tasaka M, Morita MT. Identification of gravitropic response indicator genes in *Arabidopsis* inflorescence stems. Plant Signal Behav. 2014.

197. Yang C, Xie F, Jiang Y, Li Z, Huang X, Li L. Phytochrome A Negatively Regulates the Shade Avoidance Response by Increasing Auxin/Indole Acidic Acid Protein Stability. Dev Cell. 2018.

198. Sun J, Qi L, Li Y, Chu J, Li C. Pif4-mediated activation of yucca8 expression integrates temperature into the auxin pathway in regulating arabidopsis hypocotyl growth. PLoS Genet. 2012.

199. Reddy SK, Finlayson SA. Phytochrome B promotes branching in *Arabidopsis* by suppressing auxin signaling. Plant Physiol. 2014.

200. Luo Q, Lian H-L, He S-B, Li L, Jia K-P, Yang H-Q. COP1 and phyB Physically Interact with PIL1 to Regulate Its Stability and Photomorphogenic Development in *Arabidopsis*. Plant Cell. 2014.

201. Sikora P, Chawade A, Larsson M, Olsson J, Olsson O. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. International Journal of Plant Genomics.

2011.

202. Kim Y, Schumaker KS, Zhu J-K. EMS Mutagenesis of *Arabidopsis* In: Arabidopsis Protocols.

203. Sega GA. A review of the genetic effects of ethyl methanesulfonate. Mutation Research/Reviews in Genetic Toxicology. 1984.

204. Huq E, Kang Y, Halliday KJ, Qin M, Quail PH. SRL1: A new locus specific to the phyB-signaling pathway in *Arabidopsis*. Plant J. 2000.

205. Genoud T, Millar AJ, Nishizawa N, Kay SA, Schafer E, Nagatani A, et al. An *Arabidopsis* Mutant Hypersensitive to Red and Far-Red Light Signals. Plant Cell. 2007.

206. Oka Y, Matsushita T, Mochizuki N, Quail PH, Nagatani A. Mutant screen distinguishes between residues necessary for light-signal perception and signal transfer by phytochrome B. PLoS Genet. 2008.

207. Chen M, Schwab R, Chory J. Characterization of the requirements for localization of phytochrome B to nuclear bodies. Proc Natl Acad Sci U S A. 2003.

208. Cluis CP, Mouchel CF, Hardtke CS. The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. Plant J. 2004.

209. Kumari S, Yadav S, Patra D, Singh S, Sarkar AK, Panigrahi KC. Uncovering the molecular signature underlying the light intensity-dependent root development in Arabidopsis thaliana. BMC genomics. 2019 Dec;20(1):596.

Proceedings of the International Conference on Plant Developmental Biology

Edited by

Madhusmita Panigrahy
and Kishore CS Panigrahi

Cambridge
Scholars
Publishing

TABLE OF CONTENTS

REVIEW PAPER 1

INFLUENCE OF LIGHT-HORMONE INTERACTION ON SEEDLING DEVELOPMENT IN *ARABIDOPSIS THALIANA*

SONY KUMARI, KISHORE CS PANIGRAHI*
NATIONAL INSTITUTE OF SCIENCE EDUCATION AND RESEARCH (NISER), HOMI BHABHA NATIONAL INSTITUTE (HBNI), SCHOOL OF BIOLOGICAL SCIENCES, P.O. BHIMPUR-PADANPUR, VIA JATNI, DIST. KHURDA 752050, ODISHA, INDIA

Keywords: Phytohormones, photoreceptors, root, hypocotyl, light

Key Message: The current review will provide elaborate information regarding the crosstalk of light and phytohormones regulating the root and shoot development.

Abstract

Root and hypocotyl development is influenced by various extrinsic and intrinsic factors. Extrinsically, the quality and quality of light impacts plant development in a variety of ways, while phytohormones act as intrinsic factors regulating the development. Besides, each phytohormone affects the development of root and hypocotyl differently. In the current review, the interactive role of light and phytohormones in root and hypocotyl development has been summarized. Phytohormones play different roles in root and hypocotyl development based on the availability, intensity, and wavelength type of light. Further, light and phytohormones affect the root development and hypocotyl development differently.

* Corresponding Author E-mail: kpanigrahi@niser.ac.in. Tel: 91-06742494226, 91-9439863130

J Biosci (2019) 44:26
DOI: 10.1007/s12038-018-9838-2

Indian Academy of Sciences

Review

Light and auxin signaling cross-talk programme root development in plants

SONY KUMARI and KISHORE C S PANIGRAHI*
School of Biological Sciences, National Institute of Science Education and Research (NISER), HBNI, P.O. Bhimpur-Padanpur, Via Jatni, Dist. Khurda, Odisha 752 050, India

*Corresponding author (Email, panigrahi@niser.ac.in)

MS received 15 February 2018; accepted 26 November 2018; published online 7 February 2019

Root development in plants is affected by light and phytohormones. Different ranges of light wavelength influence root patterning in a particular manner. Red and white light promote overall root development, whereas blue light has both positive as well as negative role in these processes. Light-mediated root development primarily occurs through modulation of synthesis, signaling and transport of the phytohormone auxin. Auxin has been shown to play a critical role in root development. It is being well-understood that components of light and auxin signaling cross-talk with each other. However, the signaling network that can modulate the root development is an intense area of research. Currently, limited information is available about the interaction of these two signaling pathways. This review not only summarizes the current findings on how different quality and quantity of light affect various aspects of root development but also present the role of auxin in these developmental aspects starting from lower to higher plants.

Keywords. Cryptochrome; phytochrome; PIN; primary root

Abbreviations: ARF7, auxin response factor 7; AXR2, auxin resistant 2; BFA, brefeldin A; bZIP, basic leucine zipper; bHLH, basic helix loop helix; COP1, constitutive photomorphogenic 1; CRYs, cryptochromes; CRYox, cryptochrome overexpression; DFL1, dwarf in light 1; FR, far-red; GLK, golden 2-like; HYH, homolog of HY5; HY5, elongated hypocotyl 5; IAA, indole-3 acetic acid; IAA14, indole-3-acetic acid inducible 14; IPA, indole-3 pyruvic acid; NPH3, nonphototropic hypocotyl 3; OsRAA1, Oryza sativa root architecture associated 1; PAT, polar auxin transport; PGPs, phosophoglycoproteins; PHOTs, phototropins; PHYs, phytochromes; PID, protein kinase PINOID; PIF3, phytochrome interacting factor 3; PKS1, phytochrome kinase substrate 1; PP2A, protein phosphatase 2A; P. patens, Physcomitrella patens; PpGH3L1, Physcomitrella patens GH3-like protein 1; PpIAA1, Physcomitrella patens indole-3-acetic acid 1; PpRSL1, Physcomitrella patens root hair defective six-like 1; R, red; RPT2, root phototropism 2; SLR, solitary root; SPA1, suppressor of PHYA 1; TCN1, taichung native 1; TNG67, tainung 67; UVR8, UV-B resistance 8

1. Introduction

Plant development comprises of shoot and root development, which depend on various factors such as light, water, nutrients, temperature, hormones, pathogens, etc. (Lahti et al. 2005; Giuliani et al. 2005; Simonetta et al. 2007). Light and phytohormones act as major external and internal factors respectively and they cross-talk with each other to control different aspects of plant growth in a coordinated manner (Kurepin et al. 2012). This cross-talk plays a crucial role in cotyledon development, seedling etiolation, hypocotyl elongation, root development, etc., throughout the plant's life (Nakazawa et al. 2001; Takase et al. 2004; Sibout et al. 2006). However, the mechanisms behind this cross-talk have not been studied in depth and remains obscure.

The current review is focused to understand how lightauxin interaction regulates root development in plants. Root is an essential organ in plant and it helps in anchorage to soil, absorption of water, oxygen, nutrients and minerals, and it acts as storage organ for water and carbohydrates. Generally, roots growing beneath the soil are negatively phototropic and thus never experience high light but still get influenced by light quality and quantity (Correll and Kiss 2005; Lee et al. 2016). Root development is affected by various extrinsic as well as intrinsic factors; for example, light as an extrinsic factor modulates root architecture and development, and similarly phytohormones act as intrinsic factor (Fu and Harberd 2003). Plants perceive light via various photoreceptors such as phytochromes (PHYs), cryptochromes (CRYs), phototropins (PHOTs), UV-B

An understanding towards light dependent auxin dynamics in protoplast system using a luciferase-based auxin sensor

SONY KUMARI and KISHORE C S PANIGRAHI

School of Biological Sciences, National Institute of Science Education and Research (NISER), HBNI, P.O. Bhimpur-Padanpur, Via Jatni, Dist. Khurda 752050, Odisha

ABSTRACT : Photoreceptors in plants are involved in sensing different light quality and quantity influencing plant growth and development. Auxin is one of the principal phytohormones regulating plant survival and growth. Light and auxin cross-talk has been studied in various aspects of plant development such as root growth, hypocotyl elongation, seed germination etc. Auxin dynamics was previously studied by using different auxin sensors such as DR5: GFP, DR5:GUS, DII-VENUS and luciferase-based sensor. In the current work,luciferase-based auxin sensor has been utilized in protoplast system of wild-type and phytochrome mutant to study light and auxin cross-talk at single cell level. Luciferase-based auxin sensor consisted of two type luciferases namely, firefly and renilla alongwith auxin degron sequence. This study revealed that light dependent auxin dynamics was similar in wild-type and phytochrome mutant with different light treatment for variable time duration in protoplast system. The expression of various auxin related genes was observed to be differentially regulated in both the seed lines.

Key words: *:* IAA, Light, Luciferase, Phytochrome, Protoplast

Light and phytohormones are the two important factors involved in healthy growth of plants. Plants perceive light via photo receptors and the quality, quantity, direction and duration of light perceived determine their proper growth. Plants possess different types photoreceptors for example, phytochromes (PhyA, PhyB, PhyC, PhyD and PhyE) are for far-red and red light perception, cryptochromes (CRYs) and phototropins (PHOTs) are the blue light photoreceptors and UV-B Resistance 8 (UVR 8) is a UV-B photoreceptor (Kiss *et al.,* 2003, Tilbrook *et al.,* 2013). Different types of phytohormones are present in the plants such as, Auxin, Cytokinin, Gibberellins, Ethylene, Abscisic acid, Strigolactone, Salicylic acid and Jasmonic acid, Brassinosteroids affecting plant development (Davies, 2010). Auxin is one of the important phytohormones which control different aspects of plant development. Indole-3 acetic (IAA) acid is the naturally occurring auxin. Auxin has been shown to be involved in hypocotyl elongation, primary and lateral root growth, root gravitropism, organogenesis, flowering, etc. (Jensen *et al.,* 1998; Overvoorde *et al.,* 2010;Kumari and Panigrahi, 2019;Krizek, 2011). Hence, it is very important to study the auxin response, distribution, dynamics, transport and signaling in plants. Few years back, the quantification of phytohormones was done by using techniques such as GC-MS, isotope labelling etc. (Porfirio *et al*. 2016; Petersson *et al.,* 2009). Recent scientific advancements have led to the development of new biological tools for studying the complete status of auxin in plants without plant tissue disruption. The biological tools are DR5:GFP, DR5:GUS, DII-VENUS, chemiluminescent ratiometric sensor etc. (DeMason and Polowick, 2009; Cui *et al.,* 2016; Brunoud *et al.,* 2012;Wend *et al.,* 2013). DR5 is a synthetic auxin responsive promoter, analysis with DR5:GFP and DR5:GUS are done on the basis of green fluorescent protein (GFP) signal and β-glucuronidase (GUS) staining respectively. DII-VENUS and chemiluminescent sensor are GFP and luciferase-based sensors respectively and they work on the principal ofauxin dependent degradation of AUX/IAA. DR5:GFP, DR5:GUS and DII-VENUS have been used to study the auxin dynamics in whole plant however, DII-VENUS has given detailed and precise spatio-temporal information regarding auxin distribution and response in root gravitropism and lateral organogenesis at shoot tip (Brunoud *et al.,* 2012). Chemiluminescent ratiometric sensor (pMIR auxin sensor) has been shown to be one of the best tools to accurately study spatio-temporal auxin dynamics in a single plant cell. This tool consisted of Renilla luciferase-2A-Sensor Module-Firefly luciferase. Renilla luciferase has been linked with 2A (for stoichiometric co-expression) followed by sensor module, which consisted of auxin-dependent degradation

Kumari et al. BMC Genomics (2019) 20:596
https://doi.org/10.1186/s12864-019-5933-5

BMC Genomics

RESEARCH ARTICLE Open Access

Uncovering the molecular signature underlying the light intensity-dependent root development in *Arabidopsis thaliana*

Sony Kumari[1], Sandeep Yadav[2†], Debadutta Patra[1†], Sharmila Singh[2], Ananda K. Sarkar[2] and Kishore C. S. Panigrahi[1*]

Abstract

Background: Root morphology is known to be affected by light quality, quantity and direction. Light signal is perceived at the shoot, translocated to roots through vasculature and further modulates the root development. Photoreceptors are differentially expressed in both shoot and root cells. The light irradiation to the root affects shoot morphology as well as whole plant development. The current work aims to understand the white light intensity dependent changes in root patterning and correlate that with the global gene expression profile.

Results: Different fluence of white light (WL) regulate overall root development via modulating the expression of a specific set of genes. Phytochrome A deficient *Arabidopsis thaliana* (*phyA-211*) showed shorter primary root compared to phytochrome B deficient (*phyB-9*) and wild type (WT) seedlings at a lower light intensity. However, at higher intensity, both mutants showed shorter primary root in comparison to WT. The lateral root number was observed to be lowest in *phyA-211* at intensities of 38 and 75 μmol m^{-2} s^{-1}. The number of adventitious roots was significantly lower in *phyA-211* as compared to WT and *phyB-9* under all light intensities tested. With the root phenotypic data, microarray was performed for four different intensities of WL light in WT. Here, we identified ~ 5243 differentially expressed genes (DEGs) under all light intensities. Gene ontology-based analysis indicated that different intensities of WL predominantly affect a subset of genes having catalytic activity and localized to the cytoplasm and membrane. Furthermore, when root is irradiated with different intensities of WL, several key genes involved in hormone, light signaling and clock-regulated pathways are differentially expressed.

Conclusion: Using genome wide microarray-based approach, we have identified candidate genes in *Arabidopsis* root that responded to the changes in light intensities. Alteration in expression of genes such as *PIF4*, *COL9*, *EPR1*, *CIP1*, *ARF18*, *ARR6*, *SAUR9*, *TOC1* etc. which are involved in light, hormone and clock pathway was validated by qRT-PCR. This indicates their potential role in light intensity mediated root development.

Keywords: Root, Light signaling, Intensity, Gene expression, Auxin, Hormone

Background

Light is an essential parameter for the optimal growth and survival of plants. The quality, quantity, direction and duration of light are important factors required for various aspects of plant development [1]. Root development comprises of different aspects such as primary root elongation, lateral root elongation, lateral root branching, root geotropism, root hair formation etc. Root patterning beneath the soil plays a crucial role in penetration, anchorage and gravitropism leading to absorption of water and nutrient. To perceive light, plants have evolved with many canonical photoreceptors such as phytochromes (PHYs), cryptochromes (CRYs), phototropins (PHOTs) and UVB-resistance 8 (UVR8) [2, 3]. Light regulates the patterning of shoot as well as root system [4]. It has been shown to regulate all the aforesaid aspects of root development at different stages of

* Correspondence: panigrahi@niser.ac.in
†Sandeep Yadav and Debadutta Patra contributed equally to this work.
[1]School of Biological Sciences, National Institute of Science Education and Research (NISER), Homi Bhabha National Institute (HBNI), P.O. Bhimpur-Padanpur, Via Jatni, Dist. Khurda, Odisha 752050, India
Full list of author information is available at the end of the article