



Root growth in Orchid *Dendrobium* cv. Sonia requires shade avoidance response of phytochromes along with regulation of auxin pathway genes

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Abstract

Orchid *Dendrobium* Sonia has wide demand in floral market throughout the world due to its vibrant coloured flowers, flowering recurrence and dense inflorescence. Effects of different monochromatic lights (red, far-red, blue, white) on different developmental and growth responses such as seed germination, shoot and root growth, chlorophyll and carotenoid accumulation were studied in *Dendrobium* Sonia. Asymbiotic seed germination was highest under blue or white light (80%, $p < 0.001$) and least under far-red (55%, $p < 0.001$) suggesting that it could either be controlled by an exceptional novel function of Cryptochrome 1 or the blue wavelengths perceived by PHYA or PHYB in *Dendrobium* Sonia. All analyses were done in 6-months-old plants till 1 year of age. Shoot length increased significantly in white or red light (3.7-fold, 3.5-fold respectively) while was least under far-red (decreased by 1.6-fold) indicating the major regulatory role of PHYB for shoot growth. Root growth in red light in *Dendrobium* Sonia was more similar to hypocotyl adventitious root (HAR) formation considering the *PHYB* transcript expression. We suggest that the root growth (HAR) under red light in *Dendrobium* Sonia is partly attributed to *PHYB*, negatively regulated with *HY5* and positively associated with auxin biosynthesis and accumulation gene *BABY BOOM* (*BBM2*) and efflux carriers such as *LIKE AUXIN RESISTANT 2* and *3* (*LAX2*, *LAX3*). Highest chlorophyll content under far-red and blue might be a hyper-response of shade avoidance response under far-red light in *Dendrobium* Sonia. Taxonomic tree analysis finds *Dendrobium* Sonia closer to *Phalaenopsis* and *Dendrobium catenatum*. The three phytochromes and one cryptochrome sequences were similar to PHYA, PHYB, PHYC and CRY1 respectively. CRY1 was retrieved with one more isoform CRY1_X2, with all these sequences more similar to those of *Oryza sativa*. Further study can clarify the indicating reason of a probable gene loss which is evident from the absence of any sequence similar to CRY2 in root RNA isolates of *Dendrobium* Sonia.

Keywords Orchid · *Dendrobium* · Sonia · Root · Phytochrome B · Auxin · Red light · Monochromatic light

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Introduction

Orchidaceae family is considered as one of the highly evolved groups of flowering plants. Among the 2700 genus of the family, the *Dendrobium* orchids are well known for their aesthetic value of long-lived, beautiful flowers, diverse and distinct floral morphology, and it is the second largest genus in Orchidaceae, with more than 1500 species (Lopez & Runkle, 2005). *Dendrobium* is also widely used in Southeast Asian countries as a medicinal herb i.e., Fengdou (Zha et al., 2009). Generally, members of Orchidaceae including *Dendrobium* exhibit a long juvenile phase before they flower, which ranges from a few months to 2–3 years depending on the species (Chase, 2005; Chase et al., 2015). Orchids are among few of the flowering plants that have successfully occupied an epiphytic or lithophytic niche (Zhang et al., 2017). Orchid development including the floral induction and flowering is affected by factors such as light wavelengths and temperature (Lopez & Runkle, 2005). Most tropical *Dendrobium* species grow luxuriantly at vegetative stage at temperature between 24 and 30 °C and are highly tolerant to high intensity of light as compared to other genera of orchids. One of the important *Dendrobium* cultivars namely *Dendrobium* cv. Sonia (hereafter *Dendrobium* Sonia) has wide demand in floral market throughout the world due to its vibrant dark purple-pink flowers, flowering recurrence and dense inflorescence, mostly used as cut flowers or potted plants (Martin et al., 2006; Ching et al., 2012). It is an important orchid hybrid (*Dendrobium* Cesar x *Dendrobium* Tomie Drake) closest to *Dendrobium* and *Phalaenopsis* (Hu et al., 1998; Suwanagul et al., 2007; Wahba et al., 2014). It is generally propagated through seeds, however micropropagation of protocorm-like bodies (PLB) is the widely accepted technique for the production of plant material (Julkiflee et al., 2014). For commercial production of *Dendrobium* cut-flowers, optimized growth can be obtained under controlled conditions in polyhouse or greenhouse equipped with shade net preferably black in color (Zhang et al., 2014). However, it is difficult to supply the flowers round the year due to limited knowledge of growth and flowering physiology in *Dendrobium* Sonia. There is no clear-cut recommendation available for proper growth of plants of *Dendrobium* Sonia because of lack of thorough studies. Only in few genera like *Phalaenopsis* and *Cattleya* some success has been achieved in controlled flowering (Dueck et al., 2016). Growth physiology and yield of functional components in artificial and natural conditions vary (Tian et al., 2022), subject to different environmental factors and diseases (Dehghi et al., 2016) leading to varied plant growth, flower quality and yield (Billore et al., 2017; Shin et al., 2008). *Phalaenopsis*

plants grown under red shade nets show early induction of flowering and more number of flowers than those grown under blue or black shade nets. However, plants grown under blue shade nets display larger leaves and leaf biomass as well as larger roots (Leite et al., 2008). Hence, growth physiology and the effect of different wavelengths of light is important for utilizing the plant resources while understanding the basis of photomorphogenic and flowering processes. As compared to other crops or flowering plants, the role of different monochromatic lights in the growth and development of orchids has been insufficiently studied.

Photoreceptors mediate various physiological and developmental processes in plants including seed germination, seedling development, stem length inhibition (Paik et al., 2019), root system architecture (Kumari et al., 2019), regulation of flowering time, sensing the day length and shade avoidance response (Panigrahy et al., 2020) etc. Light not only affects the growth and development, but also meristematic cell activity in orchids. The blue (B) light photoreceptors cryptochromes (CRYs) and red (R) light-sensing phytochromes (PHYs) play major regulatory roles in several plant developmental responses including seed germination, de-etiolation etc. (Heschel et al., 2008; Yang et al., 2017). In tomato, CRY1a controls seed germination by regulating the hormonal balance and degrading the hydrolase enzymes (de Oliveira et al., 2023). In *Cymbidium* R induces callus formation, however, PLB formation decreases in R (Le et al., 2004). Similar results have been observed in *Phalaenopsis*, where R promotes callus proliferation in comparison to B (Le et al., 2004). In addition to understanding the growth and physiological responses to different wavelengths of light, it is important to have information about photoreceptor genes in *Dendrobium* Sonia genome. Different environmental factors including the fertiliser applied for growth, temperature, relative humidity etc. may influence the observed growth and developmental responses in *Dendrobium* Sonia due to interaction with downstream components of different photoreceptors (Sudeep et al., 2018). Photosynthetic orchids such as *Phalaenopsis* and *Dendrobium catenatum* have total 8–9 copies of photoreceptor genes including the PHYs, CRYs, phototropins (PHOTs) and UV Resistance Locus 8 (UVR8). However, mycoheterotrophic orchids such as *Platanthera* encounters loss of CRY, PHOT2 and PHYB, consistent with their living habitat in low-light environment (Li et al., 2022).

Plant roots can be categorized into embryonic roots, primary, lateral and adventitious roots (Tian et al., 2022). The aerial roots of the closest relative *D. catenatum* are exposed to air, face severe water and nutrient scarcity, added with different environmental stresses (Priya et al., 2023) including drought and heat to fit to the epiphytic and lithophytic life style of the plant. These epiphytic

roots bear photosynthetic characteristics with chlorophyll when young and have outstanding ability to absorb water with very slow water loss rate (Tian et al., 2022). Root initiation, elongation and architecture are well known to respond to auxin hormone concentration in roots (Kumari et al., 2019), which in turn is modulated by PHYs (Raya-González et al., 2014; Salisbury et al., 2007). However, involvement of auxin or PHYs in root system architecture in epiphytic roots have hardly been studied (Oliveira et al., 2019).

This study demonstrates growth pattern of *Dendrobium* Sonia in different wavelengths of light. To gain sequence information on R and B light photoreceptors, PHY and CRY sequences were studied in root-isolated RNA samples from *Dendrobium* Sonia. It not only provided the sequence information, but the sequence similarity, homology and domain structures could also be presented, which may shed light on light perception in *Dendrobium* Sonia to elicit the epiphytic and lithophytic habit. The auxin pathway genes and *PHY* transcript levels in the root RNA isolates of *Dendrobium* Sonia were analysed. The results of our study demonstrated the processes of the anomalous characteristics of epiphytic root of *Dendrobium* Sonia with special emphasis on interaction between the photoreceptor PHYs, CRYs and auxin expression and accumulation.

Materials and methods

Seed germination

Seed capsule of the orchid *Dendrobium* Sonia was surface sterilized and cut to release the seeds, which were then spread uniformly on sterile Murashige and Skoog (MS) medium plates (Himedia, Catalogue# PT100-1L) under dim green light. The plates were then kept in different irradiations of monochromatic light including B ($\lambda_{\max}$ = 450 nm, 50 $\mu\text{mol}/\text{m}^2/\text{s}$), R ($\lambda_{\max}$ = 660 nm, 50 $\mu\text{mol}/\text{m}^2/\text{s}$), far-red (FR) ($\lambda_{\max}$ = 730 nm, 30 $\mu\text{mol}/\text{m}^2/\text{s}$), white light (WL) (100 $\mu\text{mol}/\text{m}^2/\text{s}$) in 16/8 hour light/dark cycle in plant growth chambers (Percival Scientific, USA) or in constant dark (D) conditions. Monochromatic lights were obtained from LED lights equipped in the growth chambers and WL was obtained from fluorescent cool white light tubes (Philips 17-watt F17T8/TL741/ALTO) fitted in the growth chambers (Biswal and Panigrahi, 2022). After 30 days, the seeds were assayed for germination under stereo-zoom microscope (Nikon SMZ745T) and percentage of germinations were calculated from observation of 200 seeds in 3 biological replicates. Data represented is the mean of three biological replicates.

Plant growth condition and phenotypic analysis

Plants of *Dendrobium* Sonia in juvenile stage (6 months) of ~1' in height were grown in plant growth chambers under B, R, FR and WL in 16/8 hour light/dark cycle at 25 °C and under constant D conditions as described previously. The nutrient solution was prepared by dissolving 1 g/L of Polyfeed Fertilizer (IFFCO, India) (N₂, P₂O₅, K₂O 19% w/v). The plants were sprayed with water regularly and with standard nutrient solution (Biswasa et al., 2021) once every week. The nutrient solution was prepared by dissolving 1 g/L of Polyfeed Fertilizer (IFFCO, India) (N₂, P₂O₅, K₂O 19% w/v). Plants were regularly photographed every month and different characters such as plant height and number of roots were recorded. Data for the plant height and number of roots are the mean of 30 plants (10 plants in each replicate measured 3 times). Significant data points are marked with * at $P \leq 0.05$. Test of significance was done using one-way ANOVA with the Turkey's multiple comparisons in Prism version 7.0 software.

Chlorophyll and carotenoid content estimation

For chlorophyll estimation, 3rd leaf was sampled from ~10 months old plants grown under different light conditions. Nearly 100 mg of leaf tissue was crushed with liquid nitrogen, incubated in 2 ml of 100% DMF (Dimethyl formamide) and incubated in dark at 4 °C for 48 h. The pigments were extracted after centrifugation at 10,000 RPM and 2 ml of extract was used for estimation. Chlorophyll a (Chl a) and Chl b and total chlorophyll was estimated according to Porra et al. (1989), Moran et al. (1980) by noting the absorbance at 663.8 nm, 646.8 nm. Carotenoid estimated was done according to Wellbrun et al. (1994).

RNA isolation and gene expression

One year old *Dendrobium* Sonia plants (pre-flowering stage) were kept under various monochromatic light conditions (R, FR, B, WL) for 5 days in long day (LD) conditions. The root was then cut out and immediately frozen in liquid nitrogen. The frozen roots were ground into fine powder using RNAase-treated mortar and pestle. The powder was then used for RNA extraction using Qiagen Plant RNeasy mini kit (Catalogue #74104). The cDNA was prepared from 1 μg of RNA of each sample using Bio-Rad iScript™ Reverse Transcription Super-mix, (Catalogue # 1708840). Quantitative RT-PCR (qRT-PCR) was performed using Bio-rad CFX384 Touch™ Real time detection system. Reaction mixture of 10 μl for amplification contained 5 μl of Universal SYBR® Green super mix, 500 nM each forward and reverse primer, 25–50 ng RNA and 3 μl nuclease free water). PCR was carried out in hard-shell 384-well PCR plates (Bio-Rad,

Catalogue #: HSP3805) as per the programme: (i) 95 °C: 3 min, (ii) 35 cycles of 95 °C: 30 s, 60 °C: 30 s, 72 °C: 30 s and (iii) 72 °C: 5 min. For each sample, data was collected in triplicates. Ct values were normalized to *ACTIN* for various light conditions and then subtracted from individual Ct values of different samples to calculate ΔC_t . Relative values were obtained from $2^{-\Delta C_t}$ (Pfaffl et al., 2001). Turkey's comparison test was conducted using One-way ANOVA in Graphpad-Prism to calculate significance differences.

The primer sequences used in this study are present in Suppl Table 1. All the sequences taken for qRT were from the *Dendrobium Sonia* and were highly conserved as compared to *D. catenatum* and are obtained from de novo RNA sequencing data from root isolates of *Dendrobium Sonia* taking corresponding *D. catenatum* sequences as source sequences (Suppl Table 2) with variable percentages.

De novo transcriptome analysis of *Dendrobium Sonia*

De novo transcriptome analysis from root RNA isolates was carried out using illumina Hiseq platform at AgriGenome Labs, Hyderabad, India. The workflow comprised of:—Fastq quality checking and filtering, de novo *transcriptome* assembly, expression estimation and transcriptome annotation. Paired-end library at sequencing platform yielded RIN value 7.0 indicating good quality reads. Sequencing yielded 205,142,240 raw reads with Q30 value 94.55 (error probability ≥ 0.001). Processing of raw reads by removal of adapters, low-quality bases, removal of ribosomal sequences and aligning with silva database (Bowtie2 version 2.2.9) and subsequent work flows using sam-tools (version 0.1.19), sambamba (version 0.6.5), BamUtil (version 1.0.13) tools resulted 137,043,312 high-quality reads with 13,373.87 Mb bases. The high-quality reads were then assembled using Trinity with default options. A total of 432,420 transcripts were assembled after cd-hit-est. The trimmed reads were aligned to the assembled transcriptome (length ≥ 200 bp) using Bowtie2 program with maximum 1-mismatch in the seed region (length=31 bp). Of all filtered reads, about ~93.29% of reads from sample were properly aligned back to the assembled transcriptome resulting 75,231 transcripts with FPKM ≥ 1.0 . The assembled transcripts were compared with TAIR Araport protein database using BLASTX program. The tophit matches with E-value cutoff of 10^{-5} were retained for further annotation. Overall, we found 67,496 of assembled transcripts have at least one hit. The predicted proteins from BLASTX were annotated against TAIR_araport, UniProt, Pathway and KEGG database. The raw data and the processed reads of the de novo transcriptome assembly was submitted at NCBI GEO repository with ID GSE252123. The raw data can be viewed with the link: <https://www.ncbi.nlm.nih.gov/geo/>

[query/acc.cgi?acc=GSE252123](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE252123) and reviewers token number: yrqzoqwmzbivdqf.

Bio-informatic analysis

Sequences of all genes used in this study were selected from *D. catenatum* genome sequence (Zhang et al., 2016). These sequences were used to design primers presented in Supplementary Table 1. These primers were used for gene expression analysis. The corresponding regions were sequenced with an estimation of gene size from *D. catenatum* sequences. The sequences obtained from the root RNA isolates of *Dendrobium Sonia* using the abovementioned primers were compiled to obtain the cDNA sequences. The complementary protein sequences were used for alignment using EXPASY (www.expasy.ch). The homologous locus details in *Arabidopsis thaliana* and *D. catenatum* genomes are presented against each sequence in Supplementary Table 2. Sequence similarity percentage was obtained from BLAST tool of NCBI (www.ncbi.nlm.nih.gov). Phylogenetic analysis was based on Taxonomy common tree built from the information obtained from standard NCBI database Taxonomy common tree analysis (Common Taxonomy Tree). The taxonomic tree was built by added the specific division, order, family, genus and species *Dendrobium* belongs to.

Results

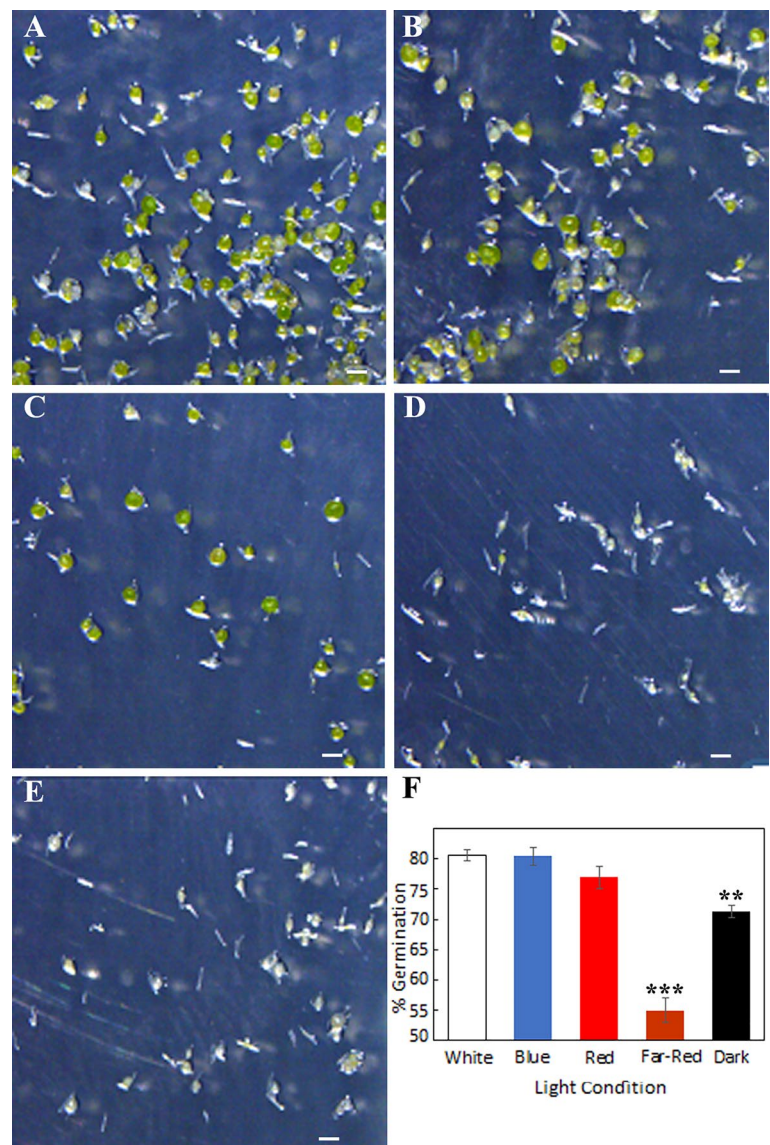
Effect of different monochromatic lights on seed germination

Seed germination was assayed after 1 month under different light conditions including WL, B, R, FR and D (Fig. 1A–F). Germination % was recorded to be highest under WL (i.e., 80.6%) (Fig. 1A) followed by B (Fig. 1B) and subsequently by R (Fig. 1C) and lowest in FR light (i.e., 54.9%) (Fig. 1D). Seeds germinated under FR light were less green as compared to WL, B and R light. Worth noting that 71.3% germination occurred to in darkness (Fig. 1E). However, germination which happened in Ddark may be due to the green light exposure while inoculation (Fig. 1E). However, the dark-germinated seeds were non-viable and were unable to grow further.

Effect of different monochromatic lights on shoot and root growth

Length of either newly emerged plant or active growth of the young plant under different lights including WL, B, R, FR were measured once in every month over a period of 6 months (Fig. 2). WL irradiations produced longest shoot (i.e., 14.5 cm) (Fig. 2B) followed by R (i.e., 14 cm)

Fig. 1 Effect of different light quality on seed Germination in *Dendrobium Sonia*. Seeds of *Dendrobium Sonia* were germinated on MS medium plate and were kept for 30-days under different lights: **A** fluorescence cool white, **B** blue, **C** red, **D** far-red, or in **E** dark. Photographs were taken after 30-days under a stereo-zoom microscope. **F** Germination percentage was assayed on 30th day after sowing (DAS) from observations of at least 100 seeds each with two biological replicates in each light quality. Scale: 100 μ m. Significant data points are marked with ** at $P \leq 0.01$, *** at $P \leq 0.001$. Test of significance was done using one-way ANOVA with the Turkey's multiple comparisons in Prism version 7.0 software



(Fig. 2D) when compared to the plants at the start of the experiment (Fig. 2A). Least growth was observed under FR light followed by that in B light. Under WL condition, shoot length was significantly increased ~4.2-folds of the initial length (Fig. 2F), whereas, it was least (~36%) under B light (Fig. 2C). Significant shoot growth not observed under FR light (Fig. 2E).

Root development was observed for 6 months to understand the effects of different monochromatic light on root growth (Fig. 3). It was found that root numbers were increased to nearly 3-folds under R (Fig. 3) followed by the WL, where it was increased nearly 2-folds (Fig. 3C). Whereas, B and FR lights induced nearly no more root growth even after 6 months (data not shown). These results indicated that while WL and R promoted shoot growth, R light best promoted root growth followed by WL.

Estimation of chlorophyll and carotenoids

Photosynthetic efficiency is represented by photosynthetic pigments such as chlorophyll. Total chlorophyll and carotenoids in the 10-month-old plants were significant and highest in the plant samples grown under FR light followed by WL (Fig. 4) while with comparable amounts of chlorophyll in samples grown under B, R or WL (Fig. 4). Chl a and Chl b pigment contents also followed a similar trend of being highest in samples grown under FR light, with only deviation of lowest Chl a amount was found in the samples grown under R light. These results were striking with the highest amount of chlorophyll and carotenoid content under FR light.

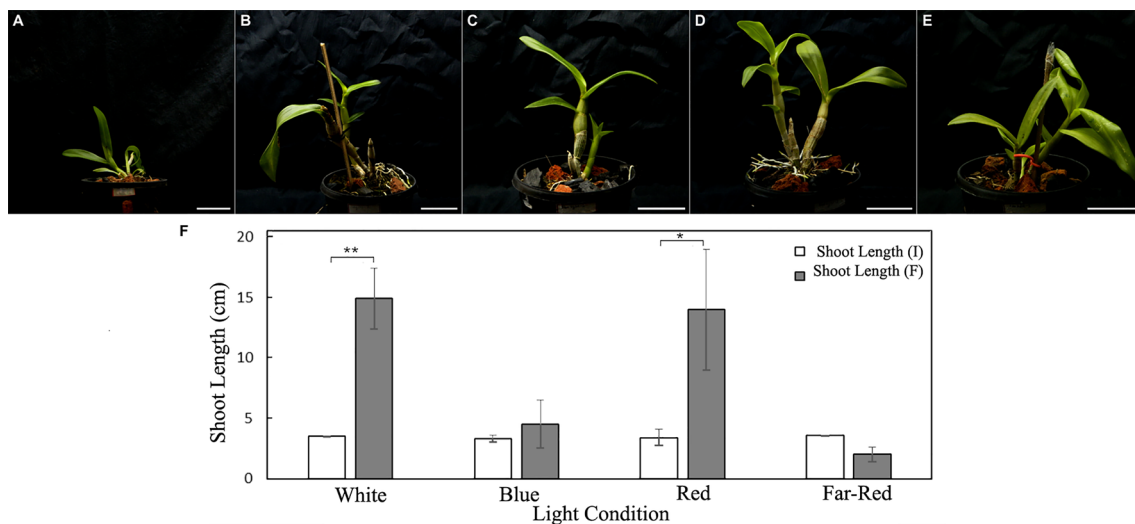


Fig. 2 Effect of different light quality on shoot growth in *Dendrobium Sonia*. Plants of *Dendrobium Sonia* of 6-month-old (juvenile stage) were grown for 6 months under different light conditions. **A** Representative picture of 6-month-old plant of *Dendrobium Sonia* **B–E** 1-year-old plants of *Dendrobium Sonia*. One representative plant picture for each light quality is shown: **A**, **B** fluorescence cool white light; **C** blue light; **D** red light; **E** far-red light. **F** Shoot length was

measured for plants grown under different light sources. Data present the mean of 30 plants grown in 3 replicates of 10 plants each. Significant data points are marked with * at $P \leq 0.05$, ** at $P \leq 0.01$. Test of significance was done using one-way ANOVA with the Turkey's multiple comparisons in Prism version 7.0 software. Scale: 5 cm

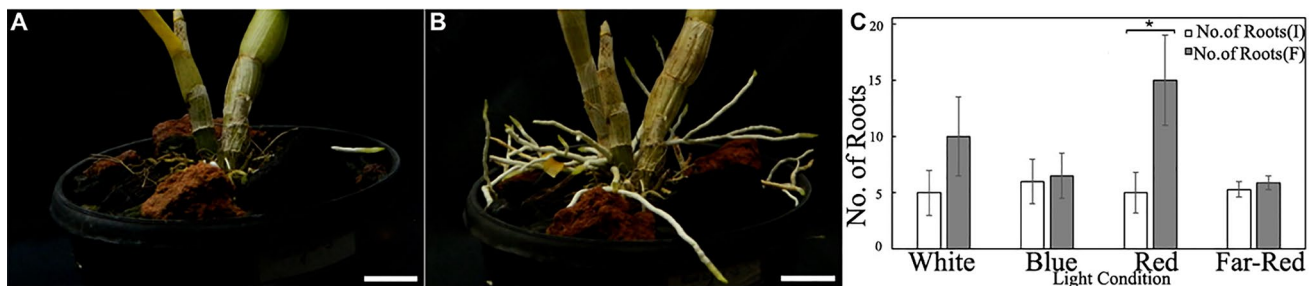


Fig. 3 Effect of different light quality on root growth in *Dendrobium Sonia*. Plants of *Dendrobium Sonia* of 6-month-old (juvenile stage) were grown for 6 months under different monochromatic light qualities. Root growth was visually assayed by counting the number of roots under each light quality and photographs were taken. Representative pictures of the base of the plant: **A** before transferring to

red light, **B** after 6-months of growth under red light. **C** Root number was counted for plants grown under different monochromatic light source. Data present the mean of 30 plants grown in 3 replicates of 10 plants each. Scale: 2 cm. Significant data points are marked with * at $P \leq 0.05$. Test of significance was done using one-way ANOVA with the Turkey's multiple comparisons in Prism version 7.0 software

Structural and sequence analysis of phytochromes in *Dendrobium Sonia*

Dendrobium is classified under Dendrobiinae subtribe in the Malaxideae sub-class (Fig. 5A). The genome of *Dendrobium Sonia* is not sequenced, however is known to be the close relative of *Phalaenopsis* and *D. catenatum* (Fig. 5B). Taxonomically, it was also found closer to the *Phalaenopsis* which belonged to *Vandae*, another tribe in Orchidaceae (Chase et al. 2015). *Dendrobium* was distant to *Poaceae* family subdivided under *Asparagales* class and also to the *Eudicotyledons* which branches further to *Arabidopsis* and is a branch of *Liliopsida* which

is upstream of *Asparagales* (Fig. 5A). A search for PHY and CRY sequences in the de novo transcriptome assembly data of *Dendrobium Sonia* root RNA isolates revealed 3 PHYs with typical N-terminal photosensory module and C-terminal regulatory module (Fig. 5C) Similarly, the CRYs possess the N-terminal PHR domain and the C-terminal CCE domain (Fig. 5C). Analysis of the sequence identities of PHY and CRY of *Dendrobium Sonia* with other plant species revealed that they follow the standard phylogeny of land plant evolution (Fig. 5A). Since both *Dendrobium* and *Oryza sativa* belong to monocotyledonae, the PHYB of *Dendrobium Sonia* is more identical to *Oryza* instead of *Arabidopsis*, which belongs to eudicotyledonae. A

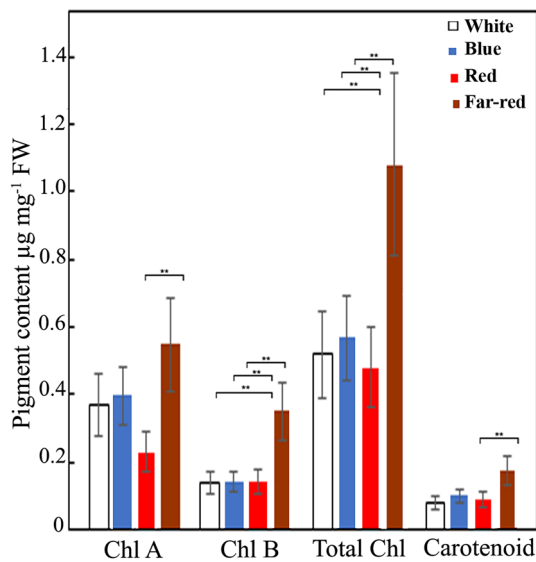


Fig. 4 Effect of different monochromatic light qualities on chlorophyll and carotenoid content in *Dendrobium Sonia*. Chlorophyll and carotenoid content was assayed from 1-year-old plant at the end of the experimental time frame. Data represented are mean of 3 biological replicates in each light quality with 10 plants grown in each instance. Significant data points are marked with ** at $P \leq 0.01$. Test of significance was done using one-way ANOVA with the Tukey's multiple comparisons in Prism version 7.0 software

comparative analysis of the *PHY* and *CRY* sequences in *Dendrobium Sonia* with representative species from two other branches of the Orchidaceae family, i.e. *Apostasia* and *Phalaenopsis*, revealed a high sequence identity following the expected order of phylogeny in plant evolution. For instance, *PHYB* of *Dendrobium Sonia* is 96.2% identical to its sister species *D. catenatum*, while showing 91.3% and 82.2% identity with *Phalaenopsis equestris* and *Apostasia shenzhenica*, respectively (Fig. 5D). The variations in sequences primarily arise from the loops connecting the functional domains. Given that all three *PHY*s share the same constituent functional domains, we conducted a comprehensive analysis of sequence identities for to ensure accurate annotation (Fig. 5E). The results clearly demonstrated a distinct segregation in sequence identity values between *PHY*As, *PHY*Bs and *PHY*Cs across all species.

Overall, our analysis unveiled the existence of conserved *PHY*s and *CRY*s in *Dendrobium Sonia*, strongly suggesting their involvement in photomorphogenic responses within the Orchidaceae family.

Dendrobium Sonia was detected with only one *CRY* (868 amino acids), which is more similar to *CRY1*, whereas no sequence similar to *CRY2* was found in the *Dendrobium Sonia* RNA samples (Fig. 5B). Another isoform of *CRY1* (i.e. *CRY1_X2*) was detected in *Dendrobium Sonia* (Fig. 5B). The protein sequence alignment of *CRY1_X2* (Fig. 5D, Suppl Table 1) showed 95.5% sequence similarity

with the *CRY1_X2* isoform of *D. catenatum*. Sequencing similarities of *PHY*s and *CRY*s of *Dendrobium Sonia* with *O. sativa* provide evidence of closer evolutionary divergence and functional conservation of these genes across families.

Genetic analysis of root specific phenotype under red light

As maximum root growth was observed under R light, transcript expression analysis was selectively done from R light-grown root samples. To understand the genetic control of R light-specific root growth in *Dendrobium Sonia*, some root-specific genes were selected for transcript expression analysis. These genes were *SHORT-ROOT (SHR)*, *LIKE AUXIN RESISTANT 2 (LAX2)* and *LAX3*, *PLETHORA4 (PLT4)* and *NAM/ATAF/CUC transcription factor 1 (NAC1)*. The regulations of these genes from *Arabidopsis* were taken as benchmarks. Additionally, primers for *PHYB* and *LONG HYPOCOTYL 5 (HY5)* were also designed for light signaling pathway. *SHR* transcript levels were highest under R and FR and slightly reduced by 10% under WL (Fig. 6A), however the changes in FC were non-significant. *SHR* expression was not detected under B. Both *LAX2* and *LAX3* showed highest expression under R light. Their expression levels were reduced non-significantly to $\frac{3}{4}$ th of the R levels under FR. Similar to the *SHR* expression pattern, the *LAX2* and *LAX3* expressions were also least under B preceded by those under WL (Fig. 6B, C). *PLT4* or *BBM2* transcript were highly induced under R followed by FR light, and this induction was significant as compared to the transcript levels under WL. *PLT4* showed minimum expression under B light (Fig. 6D). *NAC1* showed contrasting regulation patterns under different light qualities with highest expression under FR light. While the *NAC1* expression under R light was threefold reduced as that of the FR, it was nearly zero under WL and undetected under B light (Fig. 6E). *PHYB* transcript expression in *Dendrobium Sonia* root samples was surprisingly very high under FR and reduced under R (0.2-fold), least under WL and undetectable under B (Fig. 6F). *HY5* transcript levels were detected in all light conditions with higher levels under FR, B and lowest abundance under WL. Transcript abundance of *HY5* under R was half as that in the FR light condition (Fig. 6G).

Discussion

Dendrobium Sonia is the major dominator in the Asian flower market (Pratama et al., 2023). Taxonomically *Dendrobium Sonia* is closest to *D. catenatum* and *Phalaenopsis* (Suwanagul et al., 2007). Our taxonomic tree analysis placed *Dendrobium* closer to *O. sativa* than *A. thaliana*. We found three *PHY*s and one *CRY* sequences which were similar to

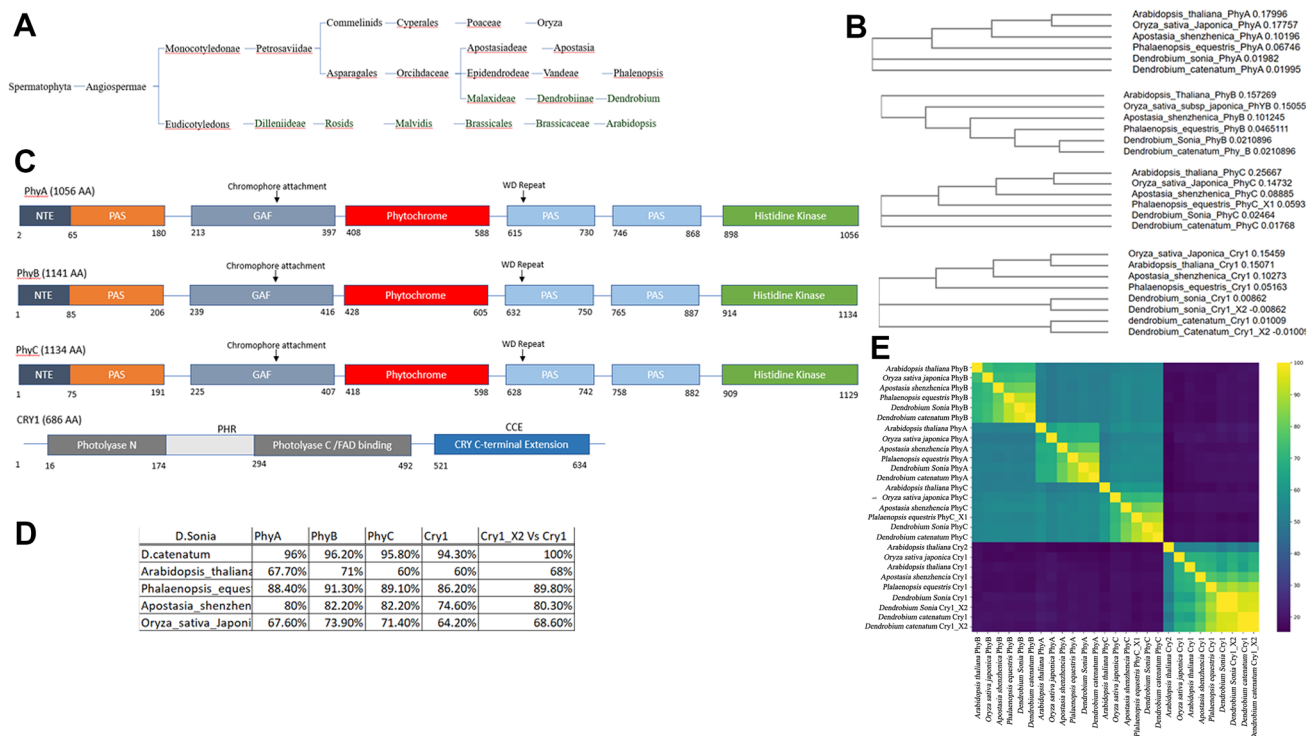


Fig. 5 Taxonomic representation and sequence information of phytochromes and cryptochromes of *Dendrobium* cv. Sonia. **A** Taxonomic position of *Dendrobium* is shown with reference to the model dicot *Arabidopsis*, model monocot *Oryza* and its closest genus such as *Apostasia*, *Vandaeae* and *Phalaenopsis*. **B** Dendrogram representation and **E** Heatmap showing pairwise sequence identity for various phytochromes (PHYA, PHYB and PHYC) and cryptochromes (CRY1 and CRY2) of representative species *Arabidopsis thaliana*, *Oryza sativa* japonica, *Apostasia shenzhenica*, *Phalaenopsis equestris*, *Dendrobium catenatum* and *Dendrobium* Sonia. The percent identity matrix was calculated using the multiple sequence alignment tool Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustal/>) with default values. The heatmap was plotted using an in-house script, where yellow corresponds to high sequence conservation, and blue corresponds to low sequence conservation. **C** Amino acid-wise domain analysis (Figure is not to scale) and **D** percentage protein sequence similarity of *Dendrobium* Sonia with *A. thaliana*, *Oryza sativa*, *D. catenatum*, *P. equestris* and *A. shenzhenica*.

PHYA, PHYB, PHYC and CRY1 respectively. CRY1 was retrieved with one more isoform CRY1_X2, with all these sequences more similar to those of *O. sativa*. The existence of CRY1_X2 isoform in *D. catenatum* indicated that it could be required for specific physiological function of epiphytic orchids like B-light-induced seed germination. However, the exact spectral properties and physiological function of CRY1_X2 requires further study. We could not find any sequence similar to CRY2 in root RNA isolates of *Dendrobium* Sonia, indicating either a gene loss or it may be found in shoot samples. The absence of CRY2 as well as the presence of CRY1_X2 isoform in root RNA isolates prompts questions about its functional compensation or alternative pathways regulating root development in *Dendrobium* Sonia, and requires further investigations. However, due to their extreme habitat, unique epiphytic, lithophytic and aerial habit, some even having photosynthetic properties in the roots, gene loss is more prevalent in many orchid species (Li et al., 2022).

Photoreceptors play important roles in different stages of plant life starting from seed germination, shoot and root

growth, chlorophyll development etc. (Paik et al., 2019; Kumari et al., 2019; Panigrahy et al., 2020). They even mediate the light quality and quantity information to regulate photosynthesis (Kochetova et al., 2022). We observed differential responses of growth and development in *Dendrobium* Sonia for different light qualities. For example, while seed germination was highest under WL and B, shoot growth was highest in WL and R. On the other hand, while root growth was maximum under R and WL, photosynthetic pigment content was highest under FR and B. These observations suggested that different photoreceptors may be specifically attributed to control different responses in *Dendrobium* Sonia, which is very much dissimilar to the generalized and overlapping functions of photoreceptors in plants (Panigrahy, 2004). However, striking growth responses under yellow and green light (which are generally considered to be quite ineffective developmentally) with regards to germination, shoot and root growth have already been reported in many orchids (Billore et al., 2017, Islam et al., 2019). Seed germination is generally mediated by PHYB in a reversible manner and by PHYB irreversibly under low light (Su et al.,

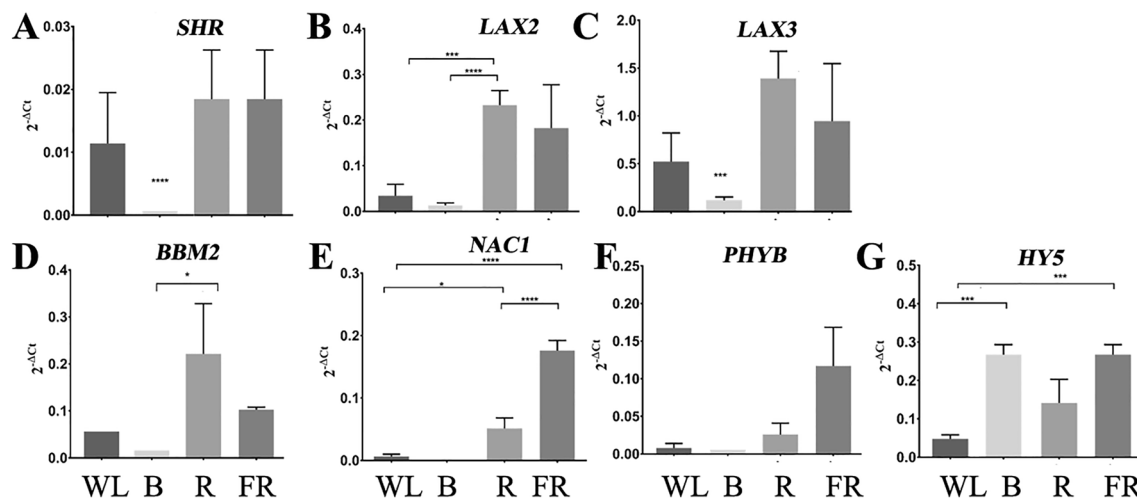


Fig. 6 Transcript expression analysis of genes involved in root growth, auxin pathway and light signalling in *Dendrobium Sonia*. Root samples of 1-year-old plants were used for RNA isolation. Transcript levels of **A** *SHORT-ROOT (SHR)*, **B** *LIKE AUXIN RESISTANT 2 (LAX2)* and **C** *LAX3*, **D** *PLETHORA4 (PLT4)*, **E** *NAM/ATAF/CUC transcription factor 1 (NAC1)*, **F** *PHYTOCHROME B (PHYB)* and **G** *LONG HYPOCOTYL 5 (HY5)* were determined

using qRT-PCR approach. Ct values were normalized to *ACTIN* for various light conditions, subtracted from individual Ct values for different samples to calculate ΔC_t . Relative values were obtained from $2^{-\Delta C_t}$ method. One-way ANOVA using Graphpad-Prism Version 5 along with Turkey's comparison test was done to determine the significant differences. Data represented are mean of 3 biological replicates

2017), and inhibited by the CRYs (Stawska et al., 2019, Jose et al., 2014). Since the FR light was least and B was most effective for seed germination in our experiment, it could be suggested that the seed germination response could either be controlled by a novel function of CRY1 or the B wavelengths may be perceived by PHYA/PHYB in *Dendrobium Sonia*. However, further studies with PHYs and CRY1 expressions in orchid seeds could provide insights of this process. Report of B light promoting germination in *Mormodica* (Wang et al., 2020a, 2020b) supported the similar observation in our study.

SHR is known to control radial root patterning, asymmetric cell division and lateral root formation (Helariutta et al., 2000). Our observation of highest induction of *SHR* transcript and highest root growth under R light indicates relevance to the previous reports. However, *SHR* transcript expression in different light conditions was apparently not correlated with the root numbers in *Dendrobium Sonia*. *SHR* is localized in the stele, and is known to control radial patterning of roots (Helariutta et al., 2000). Despite the similar and comparable expression levels of *SHR* were under R and FR, a higher number of roots was observed under R light than FR. Moreover, though *SHR* expression was least in WL and not detected in B, the number of roots under WL was next to R and that under B was similar to the number of roots formed under FR. These results suggested that *SHR* transcript expression was correlated with root growth only under R, and not under B, FR or WL light conditions in *Dendrobium Sonia*. Shoot and root growth were promoted

by R and WL while being dampened by B light in *Dendrobium Sonia*. Similar reports of positive effects of R light on the growth and development of PLB (Habiba et al., 2014) in *Dendrobium kingianum* and the regeneration of adventitious roots in *Picea abies* (Alallaq et al., 2020) supported our observation. The findings of Lee et al. (2017) that high R ratio and warm white lights promoted vegetative growth in *Phalaenopsis* and *Doritaenopsis* supported our observation of highest shoot growth under R followed by WL (Lee et al., 2017). Probably, other factors and hormone pathways regulate the root and shoot growth in *Dendrobium Sonia*, which has epiphytic roots (Tian et al., 2022). AUX1/LAX auxin influx transporters, i.e. LAX2 and LAX3, play major roles in lateral root emergence and embryonic root cell organization controlled by auxin (Ugartechea-Chirino et al., 2010, 2008). Highest expression of LAX2 and LAX3 genes under R indicated that root formation and emergence is highly promoted under R light and positively regulated by auxin, since LAX2 and LAX3 are known to control lateral root growth (Swarup et al., 2008). Though expression of these two gene transcripts were higher in FR, root initiation was least under this light condition, indicating the involvement of other factors for the growth retardation under FR. As R and WL have been known to promote root (Kumari et al., 2019) and adventitious root growth in an auxin-dependent manner (Alallaq et al., 2020), we suggest the involvement of auxin in this process. Moreover, LAX2 and LAX3 being the auxin influx carriers confirm the involvement of auxin for root growth under R in *Dendrobium Sonia*. A recent report showing

exogenous application of auxin promoting root growth in the closest relative *D. catenatum* strongly supported our inference of involvement of auxin for R light-induced root growth in *Sonia* (Tian et al., 2022). The *PLT4* (also known as *BABY BOOM* or *BBM2*) along with other *PLETHORA* group of proteins regulate root development in a dose-dependent manner (Santuari et al., 2016). These proteins also regulate the cell division, differentiation, radial patterning in root cells (Galinha et al., 2007). In addition, they control auxin biosynthesis and accumulation at the quiescent center (QC) in roots (Santuari et al., 2016). Our observation of the highest expression of *BBM* transcript under R light not only suggest the its involvement in the root growth, but also the requirement of auxin for this process in *Dendrobium Sonia*. However, the *BBM2* transcript abundance in FR and WL does not correlate with their root growth indicating other mechanisms may interplay in promoting root growth under WL and dampening root growth under FR. *NAC1* is known to promote lateral root development transducing auxin signal downstream of the auxin receptor Transport Inhibitor Response 1 (TIR1) (Xie et al., 2000). However, we observed that the transcript expression of *NAC1* was highest under FR light among the 4 light conditions tested. Thus, *NAC1* may not be involved in the process of root formation. *PHYB* is the major R light photoreceptor and also involved in different developmental responses linked to the environment (Priya et al., 2023) such as shade avoidance, gravitropism, biotic stress and temperature sensing (Hernando et al., 2021). But in *Dendrobium officianale* FR light has been shown to play an important role in shade avoidance response through expression of different light and auxin signaling genes including *PHYA*, *CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)*, *SUPPRESSOR OF PHYA-105 (SPA)*, *HY5*, *INDOLE-3-ACETIC ACID (IAA)* and *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA)*. Under FR light, synthesis of IAA through TAA, which is involved in production of etodolac from tryptophan and pyruvate, is activated leading to elongated hypocotyls, which is characteristic of shade avoidance response (Li et al., 2021). The upregulation of *CYP90A1 (Cytochrome P450 family 90 subfamily A polypeptide 1)* under FR-enriched light in *D. officianale* (Li et al., 2021) supported our observation of highest chlorophyll and carotenoid content under FR light in *Dendrobium Sonia*. This phenotype is probably an hyper-response of shade avoidance response (SAR) under FR light in *Dendrobium Sonia*, which was also found in *Phalaenopsis* as higher chlorophyll content under lower light intensity (Lee et al., 2017). FR light has been shown to add to photon capture leading to ~30% of increase in biomass (Zhen et al., 2020a, Zhen et al., 2020b.). Certain gymnosperms show greening of seedling under continuous FR light (Mathews & Tremonte, 2012). It was shown that *PHYA* can upregulate the synthesis of active protochlorophyllide (Pchl) under FR light.

Pchl under FR light. This response varies from species to species in a tissue-specific manner. Moreover, the FR-mediated enhancement of *Pchl* has been suggested to be enhanced by the *PHYA'* and inhibited by *PHYA''* form of *PHYA* (Sineschekov, 2004). Probably *Dendrobium Sonia* and other orchids use the FR or dim or low light to maximize the photon capture and use in photosynthesis (Zhen et al., 2022). Therefore the observed accumulation of chlorophylls in FR light in the orchid *Dendrobium* may represent a unique adaptation to a shaded environment.

HY5 transcription factor is known as one of the master regulators of several light-regulated developmental processes in plants. It binds physically to different *PHYs* to regulate photomorphogenic responses and is degraded by COP1-mediated proteasome degradation. It is also known to regulate root system architecture in darkness in roots (Zhang et al., 2019). Elevated transcript expression of *HY5* in all light conditions in *Dendrobium Sonia* root samples reconfirmed its role in root system architecture. *HY5* also affects auxin accumulation to control lateral root growth (Zhang et al., 2019). *HY5* accumulates to higher levels in roots of plants with their aerial parts illuminated, while its abundance is decreased in roots of etiolated seedlings (Miotto et al., 2021). The higher *HY5* transcript accumulation in *Dendrobium Sonia* roots under FR and B lights in this study indicates a possible newer role of *HY5* in root system architecture of epiphytic, above-ground roots. *HY5* accumulation may negatively affect root growth in epiphytic roots of *Dendrobium Sonia*, as the accumulation of its transcript in R (with highest root growth) was half as that under FR or B, which had least root growth. *HY5* is known to regulate lateral root emergence in *Arabidopsis* under FR light by decreasing PIN-FORMED 3 (PIN3) and LIKE AUX1 3 (LAX3) auxin transporters (van Gelderen et al., 2018). However, the regulation of *BBM2*, *LAX2* and *LAX3* in controlling root growth in a R light-dependent manner is novel and needs further supportive evidence from other species. Adventitious roots (ARs) arise post-embryonically, and are different from the primary and lateral roots. In higher plants, ARs can function as respiratory organs, storage organs, prop roots and even respond to different stress conditions such as nutrient deprivation, wounding and flooding in many plant species (Steffens et al., 2016). Moreover, ARs from hypocotyls (HAR) are another type of ARs, which are induced specifically in darkness and shown to be negatively regulated by *PHYB* under R light (Li et al., 2021). In the present study, *PHYB* transcript expression was decreased in R and WL as compared to FR. This observation indicated that decreased expression of *PHYB* in R could induce the set of genes for HAR formation similar to that in other higher plants. Hence, root growth in R in *Dendrobium Sonia* can be compared to HAR formation at genetic level considering *PHYB* transcript expression under R, as *PHYB* is known to suppress SAR by

its own deactivation (Panigrahy et al., 2020; Wang et al., 2020a, 2020b). Regarding the regulation due to the elevated *PHYB* transcript under FR for the root growth phenotype of *Dendrobium Sonia* in our study, the following indications are followed. It was shown that *PHYB* can sense low R:FR ratio in shaded environment in *D. officinale* to maximize the SAR (Li et al., 2021). Hence, elevated *PHYB* transcript under FR in our study indicated an aggravation of the SAR in *Dendrobium Sonia* root samples. While the study suggests a SAR under FR light in root samples of *Dendrobium Sonia*, additional experiments involving physiological and biochemical analyses could elucidate the underlying mechanisms causing this response.

Moreover, aerial roots of *Dendrobium Sonia* represent negatively gravitropic response being hung or exposed outside and growing in air. Hence probably opposite transcript pattern of *PHYB* response under R and FR light may be required for growth of such epiphytic roots in *Dendrobium Sonia*, and requires further study. Further, the expression pattern of the selected genes in this study might vary across different tissues or developmental stages in a spatio-temporal manner. The above indicated variability in the gene expression patterns in different tissues and developmental stages may provide in-depth insights of overall growth and development of *Dendrobium Sonia*. Considering the molecular complexity involved in light responses, interdisciplinary advancement with bioinformatics and computational biology data analysis and interpretation can be enhanced.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40502-024-00781-9>.

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Data availability All related data are available within the manuscript and its online supplementary files. All data related to De novo transcriptome assembly can be found in the publicly available at NCBI GEO repository ID: GSE252123 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE252123>; Reviewers token number to view the NCBI submission: yrqzoqwmzbivdqf.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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