Vol. 21(6), pp.287-291, June 2022
DOI: 10.5897/AJB2020.17170
Article Number: 8132A0F69285
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

*Full Length Research Paper*

# The role of N-terminal module of PhyB in modulating root and hypocotyl growth length in Arabidopsis

**Njimona Ibrahim[1,2*] and Baluška František[3]**

[1]Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, P. O. Box 6133, Yaounde, Cameroon.
[2]Bamenda University of Science and Technology (BUST), Bamenda, Cameroon.
[3]Institute of Cellular and Molecular Botanics (IZMB), University of Bonn, Bonn, Germany.

Received 20 May, 2020; Accepted 24 September, 2021

**Phytochrome belongs to red/far-red light family of photoreceptors. It exists in two spectral forms named red light absorbing form (Pr), said to be the inactive form and far-red light absorbing form (Pfr) which is the active form. This photoreceptor is structurally divided into two modules- the amino acid (N-) terminal photosensory module and caboxylic acid (C-) terminal His kinase-like catalytic output module. Five different types exist in Arabidopsis (PhyA-E). Roots and hypocotyls elongation in Arabidopsis is regulated by photoreceptors one class of which is phytochrome. The role of phytochrome B (PhyB) in red light responses has been established through studies using PhyB mutant and truncated versions. N-terminal module of PhyB containing 651 amino acids was shown to be biologically active in regulating photomorphogenesis. Meanwhile, the C-terminal module was long assumed to be involved in downstream signal transduction. Recently, this module was suggested to play a role in integrating red and blue light signaling to circadian clock. Here, the study shows that the C-terminal module of PhyB is needed for root growth and strongly modulates the root to hypocotyl ratio at 22°C. At an elevated temperature (34°C), this ratio was altered suggesting a role of this module in temperature signaling during plant growth.**

**Key words:** Phytochrome, red/far-red, Roots, hypocotyls.

## INTRODUCTION

In all living organisms, responses to stimuli are mediated by signal transduction pathways governed by their physiological performance under given established conditions. In both prokaryotes and eukaryotes, one of the key strategies from which originates intracellular signal-transduction is protein phosphorylation. Using ATP as the phosphate donor, protein kinases catalyze auto-phosphorylation (phosphorylation of themselves) or trans-phosphorylation (phosphorylation of other protein substrates) at particular amino acid residues. Until now,

*Corresponding author. E-mail: njimona@yahoo.com.

protein phosphorylation has been identified on His, Ser, Asp, Tyr, Thr, Lys, Arg and Cys residues (Matthews, 1995; Khoury et al., 2011). As regards this substrate specificity, protein kinases have been grouped into three main categories, viz: Tyr kinases, Ser-Thr kinases, and His kinases. In eukaryotic systems, the Tyr and Ser-Thr kinases are dominant. In bacteria, His kinases are dominant and are involved in diverse responses like thermosensing, nutrient sensing, photoreceptor, chemotaxis and osmosensing. Here, while His kinases perceive the signal, signal transduction occurs by means of phosphotransfer to another class of signal transducer proteins known as the response regulators (RRs). Signal transduction of this type is called, “Two Component System” (TCS), since it comprises two kinds of conserved proteins, namely: His kinases and their corresponding RRs. Two types of this mode of phosphotransfer are now well described: the first type is found mainly in bacteria and involves transfer of the phosphoryl group directly from the His kinase to the response regulator and the second type that is more complex and involves a hybrid His kinase where the phosphotransfer occurs from the conserved His to the conserved Asp residue, existing within the same protein. Here, there is mediation of the phosphotransfer to the response regulator by an additional protein, the His-containing phosphotransfer protein, which on its own has a conserved His phosphorylation site. Two component systems encoded by many genes have been identified and properly described in both Prokaryotes and Eukaryotes including plants (Mijakovic et al., 2016; Chang and Stewart, 1998). This kinase activity which is strongly modulated by temperature could possibly be one of the mechanisms which plants use to adjust their internal temperature amidst constant fluctuations of environmental temperature (Josse et al., 2008).

Plants are sisal organisms which have no other option than to optimize their growth in the area in which they find themselves. Plants show both developmental plasticity and an adaptive ability for growth development and modification in response to the constant changing of environmental signals (Franklin and Quail, 2010). Among important environmental signals are light, gravity and temperature, with plant growth development, survival and productivity found to be greatly affected by light and temperature. Major photoreceptor families saddled with signaling and light perception in plants consist of red (R)/far-red (FR) reversible phytochromes, blue (B)/ultraviolet-A (UV-A) responsive cryptochromes, phototropins, and ultraviolet-B (UV-B) absorbing photoreceptors (Yang et al., 2009; Galva˜o and Fankhauser, 2015; Li and Mathews, 2016; Somers and Quail, 1995; Goosey et al., 1997). The integrated signalling governed by these photoreceptors lead to regulation of numerous light- and temperature-dependent growth and developmental responses, comprising skotomorphogenesis, photomorphogenesis and thermomorphogenesis, chloroplast differentiation and development, leaf development, and other processes occurring throughout the life cycle, like flowering and senescence. Light has distinct effects on different tissues during the process of photomorphogenesis such as inhibition in hypocotyl growth, but promotes growth and development in cotyledons and emerging leaves, as well as in roots. Such antagonistic responses in distinct tissues could be maintained by means of distinct pools of photoreceptors regulating growth promotion in roots or cotyledons, as well as distinct photoreceptors inhibiting elongation of hypocotyl (Tóth et al., 2001).

In photomorphogenesis, phytochromes and cryptochromes are the main players which accumulate at different patterns and levels in different tissues owing to developmental signals (Somers and Quail, 1995; Goosey et al., 1997; Tóth et al., 2001; Sharrock and Clack, 2002). Nevertheless, significant overlap is also exhibited by these photoreceptors in their expression patterns, which only partly supports a role for spatially distinct photoreceptors in the control of antagonistic growth responses that are light-dependent in different tissues (Tóth et al., 2001). Therefore, the distinct impacts of light on growth promotion in some tissues alongside inhibiting expansion in others is possible due to distinct signalling cascades downstream of the activated photoreceptors in distinct tissues.

Arabidopsis phytochrome (Phy) photoreceptor family consists of two types: type 1(PhyA) and type 2 (PhyB-E) which are further classified into five members (PhyA-E). The type11 to which PhyB belongs is the most abundant and light stable. These Phys function as molecular light switches. In the dark, Phys appear in their inactive red light-absorbing (Pr) form. After capturing a photon by the covalently bound linear tetrapyrrole chromophore, they are converted to the active far-red light-absorbing conformer (Pfr). Pfr initiates downstream signaling events in the cytosol or in the nucleus. The active Pfr form is converted to Pr by far-red light (Rockwell et al., 2006) or by a thermally driven process named dark reversion in the absence of light (Mancinelli, 1994). The Pfr conformers of Phys are transported into the nuclei, where they form characteristic nuclear bodies (Kircher et al., 2002). Interestingly, Pr and Pfr forms of phytochromes have overlapping absorption spectra which permit them to monitor F/FR ratio of sunlight. Phytochrome B (PhyB) is the major red/far-red light-absorbing phytochrome receptor in light-grown plants. The characteristic domain structure of PhyB and analysis of PhyB mutants displaying altered light sensing or signaling capabilities suggested that the N-terminal domain is required for light absorption, whereas downstream signaling cascades are activated by the C-terminal His kinase like module (Park et al., 2000). However, it has been shown that N-terminal fragments of PhyB containing 651 amino acids of the

photoreceptor fused to bacterial GUS protein (providing dimerization motifs) and nuclear localization signals (NLS) were biologically active in regulating photomorphogenesis (Matsushita et al., 2003; Oka et al., 2004). These reports have also demonstrated that the 651-amino acid, N-terminal fragment retained the function of PhyB in controlling flowering. This could possibly imply that the His kinase-like subdomain, which is located within the C-terminal module of PhyB, is dispensable for downstream signaling mediating photomorphogenesis and flowering. Nonetheless, other reports (Palágyi et al., 2010) provide proof that the carboxyl-terminal module is required to mediate circadian entrainment in white light, indicating a role for this module in integrating red and blue light signaling to the clock (Njimona and Lamparter, 2011). It has previously been suggested that, the C-terminal of bacterial phytochrome Agp1 may have a thermosensing function. Also, the spectral properties of cynobacterial phytochromes Cph1 was shown to be modulated by temperature (Njimona et al., 2014). Here, the research shows that PhyB derivatives containing 651 amino acid residues of the N-terminal module is functional in mediating inhibition of root growth and that this inhibition is altered at an elevated temperature (34°C) in Arabidopsis. This research further provides evidence that the root to hypocotyl ratio is reduced by a factor of 2 in the 651-NLS compared to the wild type, hence the carboxyl-terminal module may be required to mediate root length elongation in the nucleus at this temperature, suggesting a role for this module in integrating light and temperature signaling to root elongation. This further supports the fact that this part of the phytochrome molecule is involved in downstream signal transduction.

## MATERIALS AND METHODS

### Plant materials and growth conditions

The WT *Arabidopsis thaliana* used in this study is Columbia-0 (Col-0). The studies conducted in Germany in the city of Karlsruhe and Bonn showed that both results were the same hence no influence on the environment. The mutants of it B651-NLS, B651-NES, phyB-9 and BFL lines were described previously (Palágyi et al., 2010). All seeds were surface-sterilized with 6% (v/v) bleach solution for about 10 min, rinsed several times with sterile water and then sown on half-strength Murashige and Skoog (1/2 MS) medium containing 0.8% (w/v) agar. Thereafter, the seeds were stratified for 2 days at 4°C in darkness to synchronize germination; the plates were transferred to a growth chamber with continuous white light (about 100 µmol $m^{-2}$ $s^{-1}$) and maintained at 23°C for 5 days. Germinated seeds were then incubated at different temperatures as given in the text for 72 h on vertical Petri dishes. All treatments were done in white light. Thereafter images were taken, hypocotyl and root lengths were measured using Image J. A total of 50 plants each in 5 separate repeats were considered for each mutant's line and wild type. The root and the corresponding hypocotyls of each of the plants were measured. The mean length of the root and hypocotyls were calculated. Error bars are the standard error.

## RESULTS AND DISCUSSION

The hypocotyls growth of seedlings is inhibited by light, mediated by phytochromes and cryptochromes (Poppe et al., 1998). The N-terminal photosensory module of PhyB alone, consisting of 651 amino acids, was reported by many groups to be biologically active in regulating photomorphogenesis. All of these studies were done at ambient temperature. To examine whether the C-terminal His-kinase catalytic output module plays a role in root and hypocotyls elongation at different temperatures under continuous white light, we grow Arabidopsis wild type and four PhyB mutants: B651-NLS carrying a nuclei localization signal at the C-terminal, B651-NES with nuclei exclusion signal at the C-terminal, PhyB deficient mutant (PhyB-9) and PhyB complementation line PhyB-OX (all lines were previously described (Palágyi et al., 2010). The data shows that no significant difference was found between hypocotyls of the lines grown at 23°C except for phyB-9 mutant line with ca 0.53 cm taller (Figure 1A). Interestingly, the wild type and 651-NLS have hypocotyls to root ratio of 1:3 and all other mutant lines showing less (Figure 1B). On a warm day (34°C), the C-terminal His kinase like catalytic output module suppresses both root and hypocotyls elongation (Figure 2A). At this temperature, the hypocotyls to root ratio was found to be 1:5 for the wild type and PhyB-OX line, surprisingly the mutants PhyB-9 and 651NES line has factor of ca 2 less. This strongly shows that the C-terminal module of PhyB modulates hypocotyls to root ratio under these conditions.

In nature, root systems of most terrestrial plants are shielded from light exposure by growing in a dark soil environment. Although only shoots are normally exposed to light in nature, it has been reported that from about 14 different types of photoreceptors expressed in Arabidopsis, most of them are also found in the root where they participate in root growth control (Kiss et al., 2003; Briggs and Lin, 2012). There are also reports that light can only penetrate a few millimeter into the soil due to its high absorbance (Woolley and Stoller, 1978). Nevertheless, human action on the ground and natural disaster such as route construction, farming, sudden changes in temperature, earthquake, heavy rain, wind etc. often happen which allows light to penetrate deeper and come into contact with some roots. Decades ago, transparent Petri dishes were introduced and are currently in use as a simple system for cultivating plants. This type of cultivation allows for easy access to the root system for imaging and analysis, but in the standard set-up it does not prevent light interference with roots. Main functions of roots include the acquisition of water and nutrients from the soil. Since nutrient availability may be

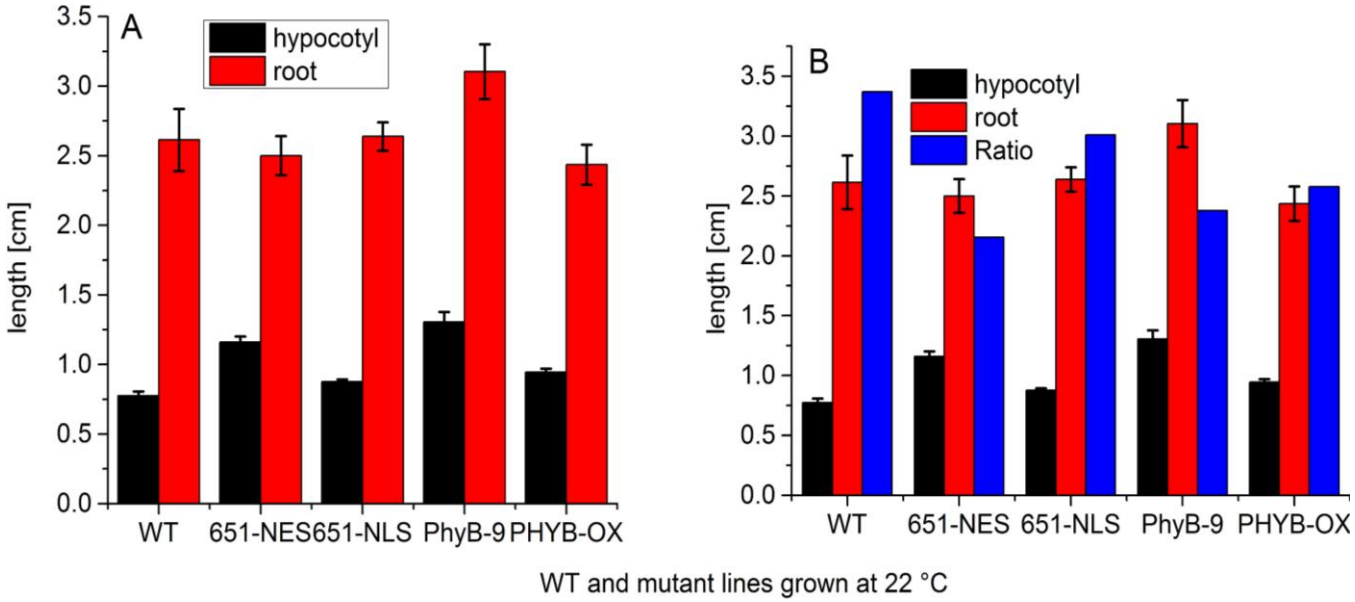


**Figure 1.** Hypocotyls and root lengths of Arabidopsis wild type and mutants grown at 22°C under continuous white light. Mean values ± standard error.

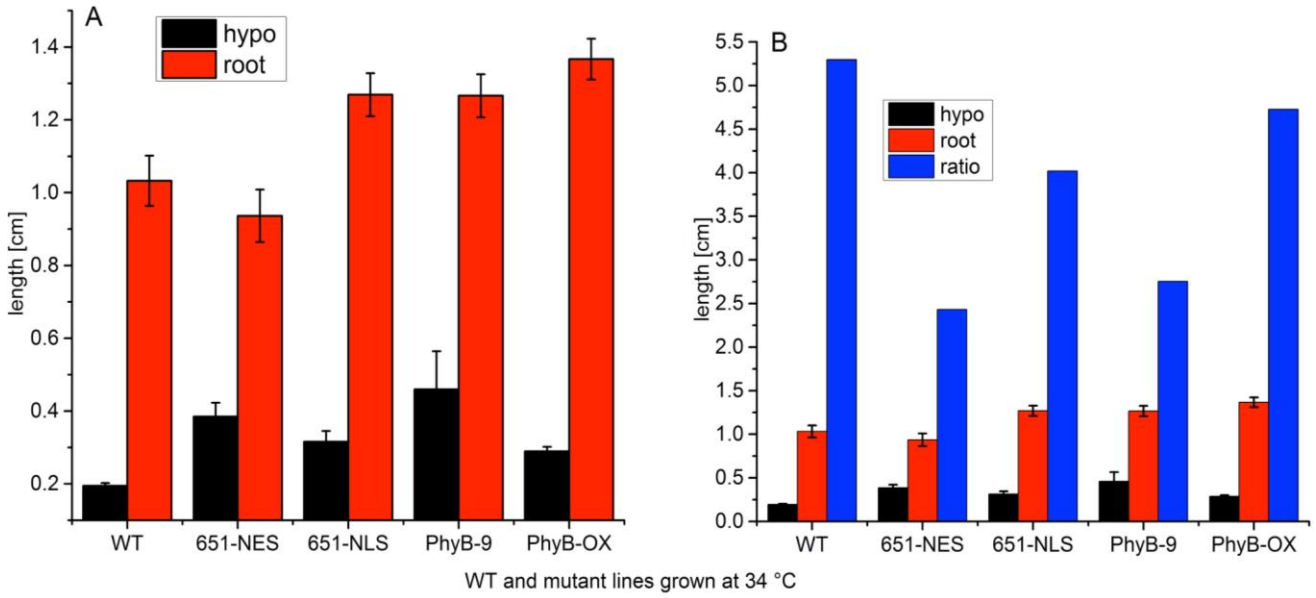


**Figure 2.** Hypocotyls and root lengths of Arabidopsis wild type and mutants grown at 34°C under continuous white light. Mean values ± standard error.

limited, plants form root hairs that increase the total surface of primary and lateral roots, as well as enhance nutrient acquisition (Gilroy and Jones, 2000). In our cultivation system, the whole plant was exposed to white light (ca 100 µmol $m^{-2}$ $s^{-1}$). We cannot exclude the fact that this type of cultivation could alter the ratio of root to hypocotyl length compared to when grown in its natural environment. What is really striking is the difference in root to hypocotyl length ratio between the wild type and the various mutants. The work from Koini et al. (2009) was amongst the first to demonstrate that plant acclimation to high temperature requires PhyB interacting

protein PIF4, suggesting a pivotal role of PhyB in temperature sensing; in line with this, we suggest that this could possibly be due to different strength of interactions at the C-terminal of PhyB with PIF4 or/and other phytochromes interacting factors.

## Conclusion

This study shows that PhyB act as both photo-sensing and thermal-sensing molecular in Arabidopsis.

## Recommendation

Further experiments are however needed to pool-down PIF4 together with C-terminal at this temperature.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

Njimona Ibrahim is thankful to Professor Tilman Lamparter (Karlsruhe Institute of Technology, Germany) for very stimulating discussions on this paper and Professor Laszlo Kozma-Bognar (Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary) for providing the wild type and all the mutant seeds used in this work. This work was partly supported by Programms Migration für Entwicklung, Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) and Bamenda University of Sceince and Technology (BUST).

## REFERENCES

Briggs WR, Lin CT (2012). Photomorphogenesis—from one photoreceptor to 14:40 years of progress. Molecular Plant 5(3):531-532.

Chang C, Stewart RC (1998). The two-component system. Regulation of diverse signaling pathways in prokaryotes and eukaryotes. Plant Physiology 117(3):723-731.

Franklin KA, Quail PH (2010). Phytochrome functions in Arabidopsis development. Journal of Experimental Botany 61(1):11-24. doi:10.1093/jxb/erp304

Galva˜o VC, Fankhauser C (2015). Sensing the light environment in plants: Photoreceptors and early signaling steps. Current Opinion in Neurobiology 34:46-53.

Gilroy S, Jones DL (2000). Through form to function: root hair development and nutrient uptake. Trends in Plant Science 5(2):56-60.

Goosey L, Palecanda L, Sharrock RA (1997). Differential patterns of expression of the Arabidopsis PHYB, PHYD, and PHYE phytochrome genes. Plant Physiology 115(3):959-969

Josse EM, Foreman J, Halliday KJ (2008). Paths through the phytochrome network. Plant, Cell and Environment 31(5):667-678.

Khoury GA, Baliban RC, Floods CA (2011). Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. Scientific Reports 1:90. doi:10.1038/srep00090

Kiss JZ, Correll MJ, Mullen JL, Hangarter RP, Edelmann RE (2003). Root phototropism: how light and gravity interact in shaping plant form. Gravitational and Space Research 16(2):55-60.

Kircher S, Gil P, Kozma-Bognár L, Fejes E, Speth V, Husselstein-Muller T, Nagy F (2002). Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. The Plant Cell 14(7):1541-1555.

Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2009). High Temperature-Mediated Adaptations in Plant Architecture Require the bHLH Transcription Factor PIF4. Current Biology 19(5):408-413.

Li FW, Mathews S (2016). Evolutionary aspects of plant photoreceptors. Journal of Plant Research 129(2):115-122.

Mancinelli AL (1994). The physiology of phytochrome action. In: Kendrick RE, 896 Kronenberg GHM eds. Photomorphogenesis in Plants. Dordrecht: Springer897 Netherlands pp. 211-269.

Matsushita T, Mochizuki N, Nagatani A (2003). Dimers of the N-terminal domain of phytochrome B are functional in the nucleus. Nature 424(6948):571-574.

Matthews HR (1995). Protein kinases and phosphatases that act on histidine, lysine, or arginine residues in eukaryotic proteins: a possible regulator of the mitogen-activated protein kinase cascade. Pharmacology and Therapeutics 67(3):323-350.

Mijakovic I, Grangeasse C, Turgay K (2016). Exploring the diversity of protein modifications: special bacterial phosphorylation systems. FEMS Microbiology Reviews 40(3):398-417.

Njimona I, Lamparter T (2011). Temperature effects on Agrobacterium phyto chrome Agp1. PLoS One 6(10):e2597.

Njimona I, Yang R, Lamparter T (2014) Temperature effects on bacterial phytochrome. PloS ONE 9(10):e109794.

Oka Y, Matsushita T, Mochizuki N, Suzuki T, Tokutomi S (2004). Functional Analysis of a 450 – Amino Acid N-Terminal Fragment of Phytochrome B in Arabidopsis. The Plant Cell 16(8):2104-2116.

Palágyi A, Terecskei K, Adám E, Kevei E, Kircher S, Mérai Z, Kozma-Bognár L (2010). Functional analysis of amino-terminal domains of the photoreceptor phytochrome B. Plant Physiology 153(4):1834-1845.

Park CM, Bhoo SH, Song PS (2000). Inter-domain crosstalk in the phytochrome molecules. Seminars in Cell and Developmental Biology 11(6):449-56.

Poppe C, Sweere U, Drumm-Herrel H, Schäfer E (1998). The blue light receptor cryptochrome 1 can act independently of phytochrome A and B in Arabidopsis thaliana. Plant Journal 16(4):465-471.

Rockwell NC, Su YS, Lagarias JC (2006). Phytochrome Structure and Signaling Mechanisms. Annual Review of Plant Biology 57(1):837-858.

Sharrock RA, Clack T (2002). Patterns of expression and normalized levels of the five Arabidopsis phytochromes. Plant Physiology 130(1):442-456.

Somers DE, Quail PH (1995). Phytochrome-Mediated Light Regulation of PHYA- and PHYB-GUS Transgenes in Arabidopsis thaliana Seedlings. Plant Physiology 107(2):523-534.

Tóth R, Kevei E, Hall A, Millar AJ, Nagy F, Kozma-Bognár L (2001). Circadian clock-regulated expression of phytochrome and cryptochrome genes in Arabidopsis. Plant Physiology 127(4):1607-1616.

Woolley JT, Stoller EW (1978). Light Penetration and Light-induced Seed Germination in Soil. Plant Physiology 61(4):597-600.

Yang S, Jang IC, Henriques R, Chua NH (2009). Far-Red Elongated Hypocotyl 1 and FHY1-like associate with the Arabidopsis transcription factors LAF1 and HFR1 to transmit phytochrome A signals for inhibition of hypocotyl elongation. Plant Cell 21(5):1341-1359.