CHARACTERIZATION OF *Synechocystis* AND PLANT PHYTOCHROMES, AND THE ROLE OF PHOSPHORYLATION IN PHYTOCHROME SIGNAL TRANSDUCTION IN PLANTS

by

Jeong-Il Kim

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Chemistry

Under the Supervision of Professor Pill-Soon Song

Lincoln, Nebraska

December, 2002

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

DISSERTATION TITLE

Characterization of Synechocystis and plant phytochromes, and the role of phosphorylation in phytochrome signal transduction in plants

BY

Jeong-Il Kim

SUPERVISORY COMMITTEE:

	Approved	Date
Signature	Pill-Soon Song	11/26/02
Typed Name	Dr. Pill-Soon Song	
Signature	John J. Stezowski	11/26/02
Typed Name	Dr. John J. Stezowski	
Signature		11/26/02
Typed Name	Dr. Gerard S. Harbison	
Signature	Amit Mitra	11/26/02
Typed Name	Dr. Amit Mitra	
Signature	David Smith	12/06/02
Typed Name	Dr. David L. Smith	
Signature		
Typed Name		

University of Nebraska | GRADUATE COLLEGE

CHARACTERIZATION OF *Synechocystis* AND PLANT PHYTOCHROMES, AND THE ROLE OF PHOSPHORYLATION IN PHYTOCHROME SIGNAL TRANSDUCTION IN PLANTS

Jeong-Il Kim, Ph.D.

University of Nebraska, 2002

Advisor: Pill-Soon Song

Phytochromes are a wavelength-dependent light switch for photomorphogenesis in plants and also in many prokaryotes. To extend the knowledge of phytochromes, two *Synechocystis* phytochromes, Cph1 and Cph2, and a Type II phytochrome, *Arabidopsis* phyB were characterized. *Synechocystis* phytochromes have very similar photochromism to plant phytochromes except for their blue-shifted absorption peaks. However, Cph1 do not show any increase of α–helix content upon light absorption and Cph1 apoproteins exist mainly as monomers, whereas plant phytochromes exist as dimers, suggesting that the mechanisms of photoperception and upstream signaling are different between plant and bacterial phytochromes. In addition, the discovery of Cph2 as a photoconvertible photoreceptor proves that prokaryotes also use multiple phytochromes, similar to plants.

Plant phyB has shown a few different characteristics from phyA, including less efficient photoconversion, faster dark reversion, different proteolytic patterns and no α–helix increase during photoconversion. These results suggest that the chromophore-apoprotein interactions of phyB are different from those of phyA. These results may also help understand the different roles between phyA and phyB in plants. The dimerization

domain assay showed that the PAS-B domain is responsible for phytochrome dimerization, not the PAS-A domain.

The role of phytochrome phosphorylation on Ser598 in the hinge region was proven to be a signal switch controlling the interaction between phytochromes and their signal transducer proteins. Since the phosphorylation of this site prevented the interaction of phyA with its signal transducers such as NDPK2, the transgenic plants with Ser598Ala phyA mutant showed hypersensitivity to light and dwarf phenotype. Of three known serine sites of phytochrome phosphorylation, Ser17 was identified as the site for phytochrome autophosphorylation and kinase activity. The domain study for plant phytochrome kinase activity showed that the histidine kinase related domain (HKRD) was not necessary for its kinase activity. Furthermore, deletion of this HKRD increased the kinase activity of phyA. The possible kinase domain was the PRD domain including the PAS-A region and the hinge region of phytochromes.

ACKNOWLEGMENTS

I would like to express my sincere gratitude to my advisor, Prof. Pill-Soon Song, who has not only guided me with his knowledge and advice, but also supported me wisely with patience throughout my studies at the University of Nebraska-Lincoln. This dissertation would not have been accomplished without his support.

I would like to thank my committee professors, Dr. John J. Stezowski, Dr. David. L. Smith, Dr. Gerard S. Harbison and Dr. Amit Mitra for their help and support. I also thank Dr. Veniamin N. Lapko and Dr. Seong-Hee Bhoo for valuable discussion about phytochrome research. I especially thank Dr. Chung-Mo Park at Seoul National University (previously at Kumho Life and Environmental Science Lab. (KLESL) in Korea) and Dr. Si-Myung Byun at Korea Advanced Institute of Science and Technology (KAIST), who have provided advice, encouragement, and insights into science.

I wish to express my special appreciation to Korea Kumho Petrochemical Company (KKPC) and the president of KKPC, Chan-Koo Park who provided the opportunities to acquire great experience here. I also wish to thank KLESL and its members for their invisible help.

I would like to thank my colleagues, Yu Shen, Joe Spicha, Sara Williams, Jun-Sik Lee, Young-Seong Suh and many other people at chemistry department for valuable discussion and for creating a great environment to accomplish my study.

Finally, I am deeply grateful to my parents and brothers for their love. I would like to thank my wife, Yun-Jeong Han, who has been the best colleague during my study and also provided indispensable support with incessant love, and my daughter Ji-Soo who is one of my best results.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

[θ]	: mean residue molar ellipticity
aa	: amino acid
AOX	: alcohol oxidase
bp	: basepair
CBD	: chitin-binding domain
CCA	: complementary chromatic adaptation
CD	: circular dichroism
Cph1	: cyanobacterial phytochrome apoprotein
DMSO	: Dimethyl sulfoxide
DTT	: dithiothreitol
EDTA	: ethylenediaminetetraacetic acid
FPLC	: fast performance liquid chromatography
FR	: far-red light
FRc	: continuous FR
GFP	: Green Fluorescent Protein
GST	: Glutathione S-transferase
HK	: histidine kinase
HKD	: histidine kinase domain
HPLC	: high performance liquid chromatography
HRD	: histidine kinase-related domain
IPTG	: isopropyl thio-β-galactoside
kb	: kilobasepairs
kDa	: kilodalton(s)
LB	: Luria-Bertani medium
MT	: mutant
NDPK2	: nucleoside di-phosphate kinase-2
NTE	: N-terminal extension
OD_{600}	: optical density at 600 nm

ORF	: open reading frame
PAS	: Per/Arnt/Sim domain
PBS	: Phosphate buffered saline
PCB	: phycocyanobilin
PCR	: polymerase chain reaction
PEB	: phycoerythrobilin
Pfr	: far-red light-absorbing form of phytochrome
phyA	: phytochrome A
phyB	: phytochrome B
PIF3	: phytochrome-interacting factor-3
PIP(s)	: Phytochrome interacting protein(s)
PKA	: protein kinase A
PKS1	: phytochrome kinase substrate-1
PMSF	: phenylmethylsulfonyl fluoride
Pr	: red light-absorbing form of phytochrome
PRD	: PAS-related domain
PVDF	: polyvinylidene difluoride
PΦB	: phytochromobilin
R	: Red light
Rc	: continuous R
SAR	: ratio of the absorbance maxima in the visible region to the absorbance at 280 nm for the Pr form
SDS	: sodium dodecyl sulfate
SDS-PAGE	: sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	: size exclusion chromatography
TBS	: Tris-buffered saline
UV/VIS	: ultraviolet and visible
YPD	: yeast extract peptone dextrose
WT	: wild-type

Chapter 1:

Background

1.1. Introduction to phytochromes

Throughout their life cycle, plants and plant cells continually respond to signals to which they adapt to alter their physiology, morphology, and development. Of the signals, light is an important signal for plant development, influencing nearly all aspects of the life cycle from germination to flowering. This absolute requirement of light for growth is the key feature that distinguishes higher plants from animals. Light is the energy source for photosynthesis and it also acts as an information source to tell plants about its environment. There are three known classes of plant signal-transducing photoreceptors: red/far-red light absorbing phytochromes, blue light absorbing cryptochromes, and phototropin (Kendrick and Kronenberg, 1994; Batschauer, 1998; Fankhauser and Chory, 1997). Of these, the best characterized biochemically and physiologically are phytochromes (Smith, 1995; Song et al., 1996; Kim et al., 2002).

In the 1950's, phytochrome, termed for "plant color", was characterized as the pigment that controls lettuce seed germination in red and far-red light (Borthwick et al., 1952). It is now known as a molecular light switch for "photomorphogenesis", which is the process of plant growth and development in response to light signals (Song et al., 1996; Batschauer, 1998; Neff et al., 2000). The photomorphogenic responses of plants include germination, stem growth, chloroplast development, biosynthesis of chlorophylls and other pigments, flowering and circadian rhythm (Chory et al., 1996; Shinomura,

1996; Khurana et al., 1998; Neff et al., 2000). All aspects of photomorphogenesis does not occur in the absence of a light signal. Therefore, photomorphogenesis can perhaps be considered a phenomenon that constantly modulates a plant's ability to harness light energy most efficiently. Since the mechanism of photomorphogenesis is yet to be deciphered completely, it is important to study the characteristics of the photoreceptors, their action mechanisms, and signal transduction pathways.

1.2. Structure/function of phytochrome

1.2.1. Synthesis of the chromophore and its ligation to phytochrome

Phytochromes are dimeric chromopeptides (monomer sizes of 120~130 kDa) that carry a covalently linked chromophore. The chromophore moiety of phytochromes is phytochromobilin (PΦB) which is covalently linked to the apophytochrome via a thioester linkage to a cysteine residue in the most conserved domain among phytochromes (Lagarias and Rapoport, 1980). The phytochrome chromophore is synthesized in the chloroplast of plants (Terry and Lagarias, 1991). The biosynthesis pathway for PΦB is common to chlorophyll synthesis from 5-aminolevulinic acid (ALA) to protoporphyrin IX. The pathway branches from chlorophyll biosynthesis at the point of conversion of protoporphyrin IX to heme (Muramoto et al., 1999; McDowell and Lagarias, 2001). From the heme, the first committed step in chromophore synthesis is the cleavage of the tetrapyrrole ring of heme (Fig. 1.1). This reaction is catalyzed by a heme oxygenase encoded by the *HY1* gene in *Arabidopsis* (Davis et al., 1999a; Muramoto et al., 1999). Then, 3E-PΦB is synthesized by PΦB synthase and PΦB isomerase (McDowell and Lagarias, 2001; Frankenberg et al., 2001; Terry, 1997).

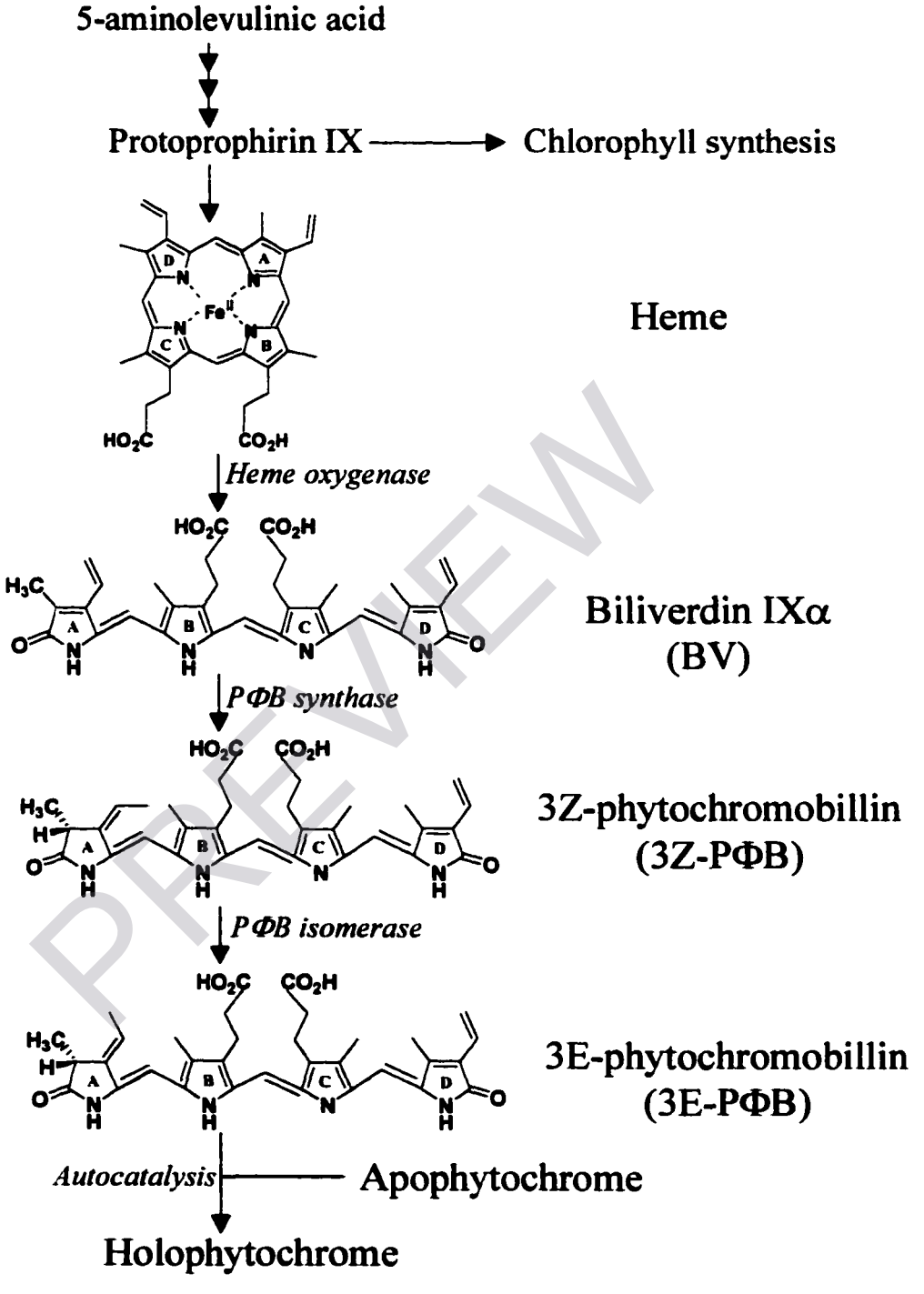

Fig. 1.1. Biosynthesis of PΦB and holophytochrome in plants.

Phytochrome apoprotein binds to the 3E-PΦB in cytoplasm to yield holophytochromes, occurring autocatalytically by using a bilin lyase activity residing within the phytochrome polypeptide (Lagarias and Lagarias, 1989; Terry et al., 1993). This reaction requires the bilin lyase domain (BLD) of the phytochromes, and the minimal BLD is recently shown as less than 200 amino acids long (Wu and Largarias, 2000).

1.2.2. Photochromism of phytochrome

The striking characteristic of phytochromes is its reversible photochromism: the property of changing color on photon absorption and of reverting to the original form on the absorption of another photon (Fig. 1.2). Phytochromes are synthesized in the red light-absorbing form (Pr, λmax = 660nm) which can be phototransformed into the far-red light-absorbing form (Pfr, λmax = 730nm) upon exposure to red light (Fig. 1.2b) (Kendrick and Kronenberg, 1994). The absorption of red light triggers a "Z" to "E" isomerization of the chromophore in the C-15 double bond between the C and D rings of the linear tetrapyrrole (Fig. 1.2a) (Braslavsky et al., 1997), resulting in Pfr form. From the Pr and Pfr absorption spectra (Fig. 1.2b), a difference spectrum can be obtained by subtracting one spectrum from the other (Fig. 1.2c), which represents the photochemical characteristic of the phytochromes. Conformational changes in the protein are accompanied upon this photochromism (Song, 1999; Park et al., 2000). Pfr can be converted to Pr either by a slow non-photoinduced reaction (Dark reversion) or much faster via absorption of far-red light. Two spectral forms of phytochrome display distinct but broad absorption bands (Fig. 2b) that overlap in certain regions, resulting in a photoequilibrium between the two forms after irradiation with monochromatic light.

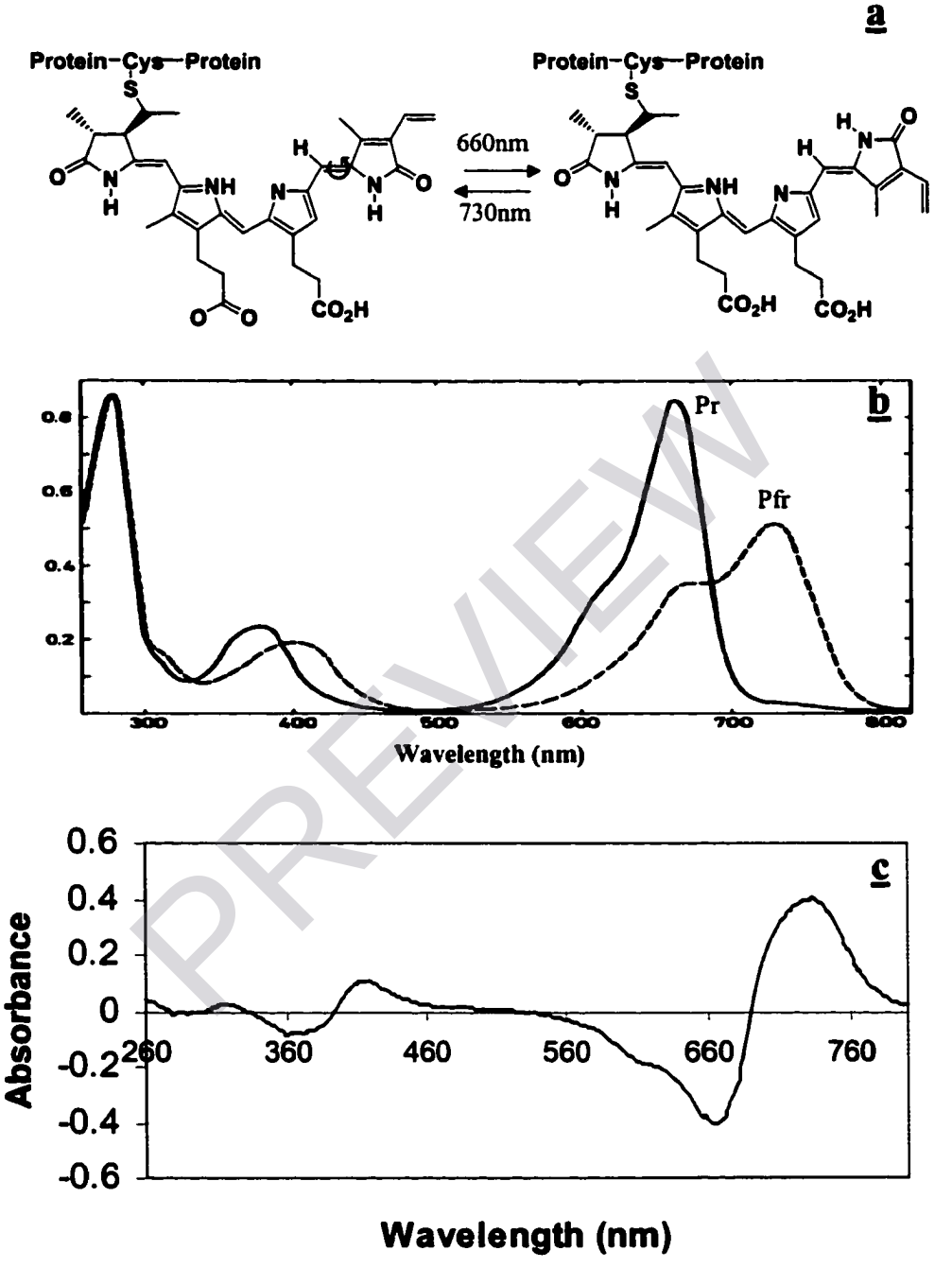

Fig. 1.2. Photochemical property of phytochrome. a. Photoisomerization of PΦB in the Pr-to-Pfr photochromic transformation. b. Absorption spectra of the light interconvertible Pr and Pfr forms of phytochrome. c. Difference spectrum of phytochrome. Pfr spectrum was subtracted from Pr spectrum.

Due to the promotive effect of the red light on most physiological responses, Pfr has been considered the active form and Pr has been considered the inactive form of phytochrome (Khurana et al., 1998).

1.2.3. Phytochrome molecular species

Comparisons of phytochrome sequences from plants indicate that multiple isoforms exist within the same plant. For example, five isoforms (phytochrome A to E) have been isolated from the model plant *Arabidposis thaliana* (Mathews and Sharrock, 1997; Clark et al., 1994; Sharrock and Quail, 1989). These different isoforms often have greater homology to paralogs from other plant species than to other phytochromes within the same species. For example, phyA from *Arabidopsis* is 65-80% identical to phyA sequences from other monocots and dicots but is only 48-52% identical to self-encoded phyB-E sequences (Fig. 1.3). Despite these differences, regions of high amino acid sequence homology exist among all isoforms, suggesting that all phytochromes might have a similar biochemical mode of action (Quail et al., 1995; Clark et al., 1994). However, phenotypic analyses of phytochrome-deficient mutants or transgenic plants overexpressing different phytochromes showed that specific isoforms control distinct facets of photomorphogenesis. For example, phyA regulates seed germination and seedling growth in response to continuous far-red light (FRc). In contrast, phyB regulates seed germination in continuous red light (Rc), plant growth in response to the R/FR ratio and end-of-day FR, and flowering time (Quail et al., 1995; Smith, 1995).

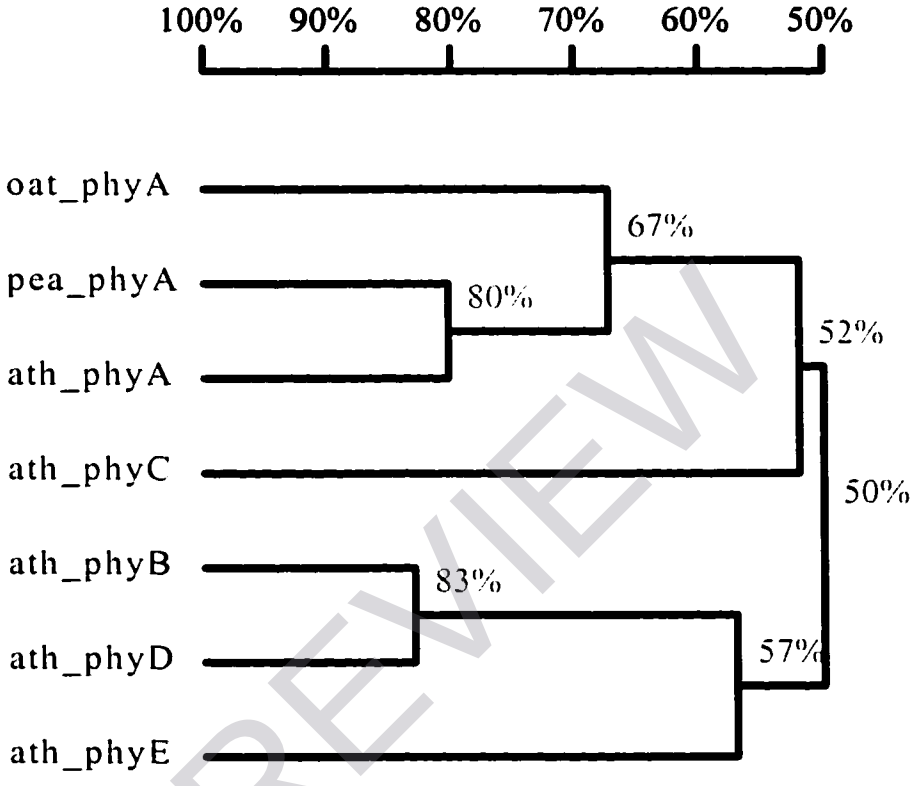

Fig. 1.3. Genetic distance tree of phytochrome protein sequences. Oat_phyA, phytochrome A of *Avena* (oat); pea_phyA, phytochrome A of *Pisum* (pea); ath_phyA to ath_phyE, phytochrome A to E of *Arabidopsis*. This sequence alignment was done by using DNAMAN program (Lynnon BioSoft).