

# Identification of a Light-Harvesting Chlorophyll a/b-Binding Protein Gene in *Gardenia jasminoides*

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**Abstract—Objective:** The major light-harvesting complex of photosystem II, Lhcb, is assembled in its trimeric form, equipped with chlorophyll and xanthophylls, embedded in the thylakoid membrane of green plants, and play critical roles in harvesting light energy for photosynthesis. The objective of this work is to isolate a gene of Lhcb in *Gardenia jasminoides*. **Methods:** A *Gardenia jasminoides* fruit cDNA library was constructed, and the GJLhcb cDNA was isolated from the cDNA library by sequencing method. **Results:** The GJLhcb cDNA is 946bp, contains a predicted 795bp open reading frame that encodes 265 amino acids. A phylogenetic analysis was conducted with previously characterized Lhcb from other plant species. A three-dimensional trimers model of GJLhcb was built. **Conclusion:** The phylogenetic analysis suggested that the GJLhcb has a chloroplast transit peptide, the mature form has 230 amino acids; and contains an N-terminal Thr as a potential phosphorylation site, which is important in regulation of state transitions. The three-dimensional structure of GJLhcb is similar with the structure of Lhcb1 in *Spinacia oleracea*. This is the first Lhcb identified in *Gardenia jasminoides*.

**Keywords—**Light-harvesting chlorophyll a/b-binding protein (LHCB); *Gardenia jasminoides*; photosynthesis component; cDNA library; gene cloning.

## I. INTRODUCTION

Oxygenic photosynthesis is driven by two photosystems (PS II and PS I) that work in series to convert light energy into chemical energy, which involves the collection of solar energy via two types of light-harvesting complexes (LHC-I and LHC-II). LHC-II is the most abundant of these complexes in thylakoid membranes, and consists of pigments which are bound to the light-harvesting chlorophyll a/b-binding proteins (LHCBs or CABs). The Lhcb is normally associated with chlorophyll and xanthophylls and serves as the antenna complex. These antenna complexes absorb sunlight and transfer the excitation energy to the core complexes of PS II in order to drive photosynthetic electron transport. LHC-II consist of minor antenna complexes Lhcb4(CP29), Lhcb5(CP26), Lhcb6(CP24) and Lhcb7 and major antenna complexes that comprise homo- and hetero-trimers of Lhcb1, Lhcb2, and Lhcb3[1]. These proteins are encoded by a large multigene family nuclear

genes [2]. In *Arabidopsis*, there are at least five and three multiple loci encoding Lhcb1 and Lhcb2 respectively, the amino acid sequences of these individual Lhcb1 and Lhcb2 proteins are slightly different and the differences are not conserved in plant species; and three multiple genes for Lhcb4, and one each for Lhcb3, Lhcb5 and Lhcb6. In rice, there are three multiple genes for Lhcb1, and one each for Lhcb2-6 [3].

Expression of Lhcb genes is tightly regulated by developmental process and by multiple environmental signals [4, 5]. Lhcb is frequently reported to be transcriptionally repressed in response to light stress [6]. Since the light quality and intensity can vary during the day, plants need to rapidly adjust the relative absorption cross sections of the two PSs. The ability to dissipate large fractions of their absorbed light energy as heat is a vital photoprotective function of the peripheral light-harvesting pigment-protein complexes in photosystem II of plants [7], and photosynthetic carotenoids, which perform both light-harvesting and photoprotective roles are essential to the photosynthetic process[8]. Through state transition process, it leads to a redistribution of excitation energy between PS I and PS II. In plants, when PS II is preferentially excited compared with PS I, part of the major light-harvesting complex II (LHC II) migrates to PS I to form a PS I-LHC II supercomplex. A stable PS I-LHC II supercomplex is purified from *Arabidopsis* and maize plants [9]. In *Arabidopsis*, state transitions are regulated by the reversible phosphorylation of an N-terminal Thr on Lhcb1 [10, 11]. And state transitions in *Arabidopsis* occur only when both Lhcb1 and Lhcb2 are present, and the functional roles of Lhcb1 and Lhcb2 are different but complementary. *Arabidopsis thaliana* plants lacking only Lhcb2 contain thylakoid protein complexes similar to wild-type plants, where Lhcb2 has been replaced by Lhcb1. In contrast, in the absence of Lhcb1, only a small number of Lhcb2 homotrimers are formed, results in the absence of thylakoid membrane remodeling, which as been suggested to be typical for state transitions [12].

*Gardenia jasminoides* originates in Asia and has been in cultivation for at least a thousand years. The fruit of *G. jasminoides* is used in Asian countries as a natural colorant, and as a traditional herbal medicine. Crocin, crocetin and geniposide are the main secondary metabolites in the fruit, and they all exhibit a wide range of pharmacological

activities [13]. In this paper, we identified and analyzed a light-harvesting chlorophyll a/b-binding protein (GjLhcb) in *G. jasminoides*.

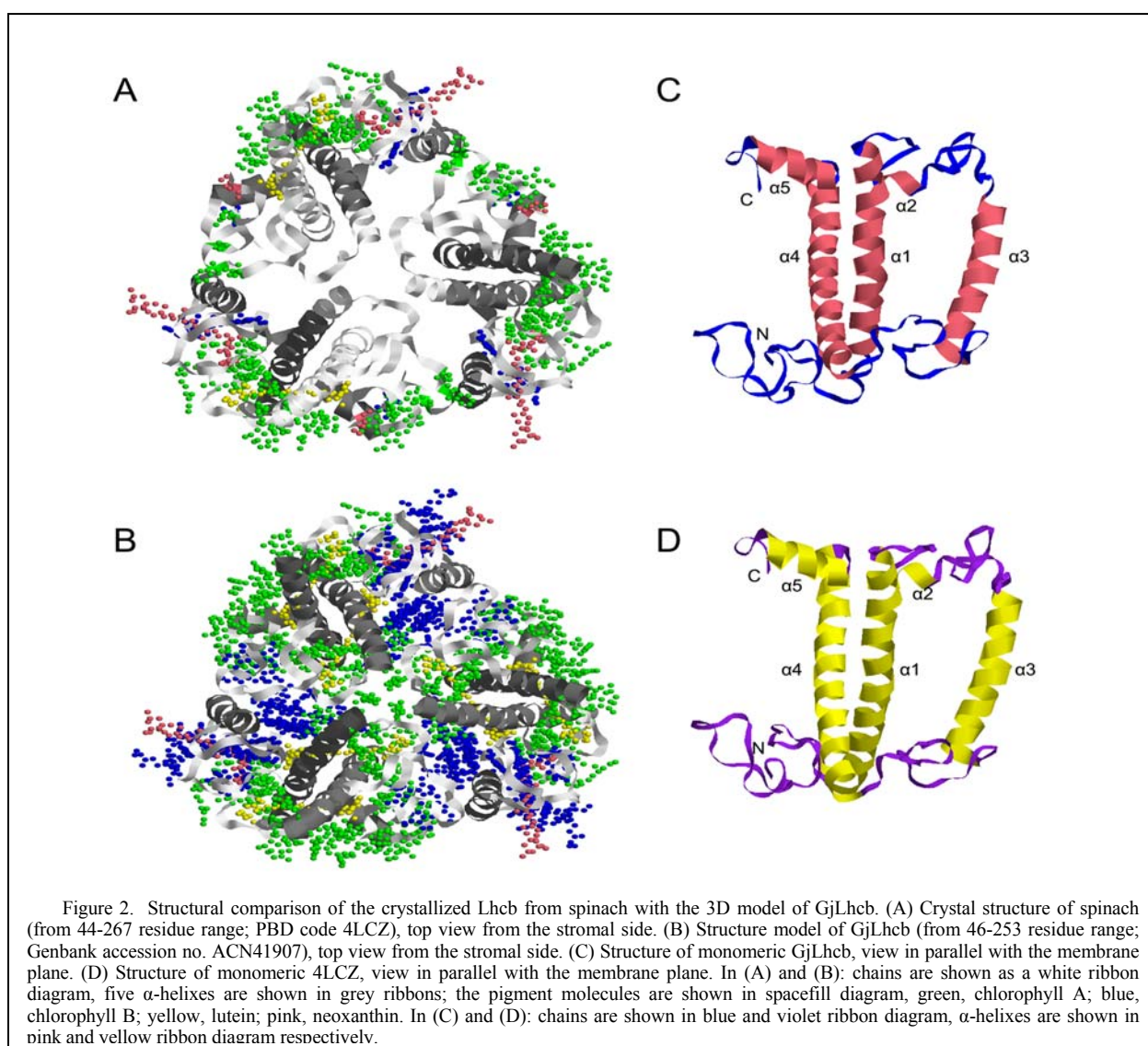
Total RNA was extracted from Gardenia fruit (stage II ), using a modified CTAB (hexadecyl trimethyl ammonium bromide) based extraction protocol [14]. From total RNA, the cDNA library construction and

Figure 1. Alignment of GJLhcb with six Lhcb from other plant and algae. The abbreviation and Genbank accession number are: Gja (*Gardenia jasminoides*, ACN41907), Ptr (*Populus trichocarpa*, XP\_002316737), Ath (*Arabidopsis thaliana*, NP\_174286), Nta (*Nicotiana tabacum*, CB22\_TOBAC), Osa (*Oryza sativa*, CB22\_ORYSJ), Cre (*Chlamydomonas reinhardtii*, AF479777) and Sol (*Spinacia oleracea*, CAJ77390). Star, potential Thr phosphorylation site; underlined, chloroplast transit peptide in Sol; identical residues are shaded; Alpha helices of the spinach Lhcb are marked with H.

conserved  $\alpha$ -helices. The specific differences are in their N-terminal regions, where mostly has chloroplast transit peptide. The GjLhcb is more similar to Lhcb3 of plants (identity range from 85.39% to 93.18%) than to algal counterparts (identity 65.91%), as shown in (Fig. 1). The major PS II antenna in seed plants is composed of the Lhcb1, Lhcb2 and Lhcb3 proteins. Lhcb1 and Lhcb2 are highly similar and usually present in multiple copies, they together account for ~90% of the polypeptide in major PS II antenna [2], whereas Lhcb3 is more divergent, is encoded by a singly copy gene [15]. By alignment GjLhcb with the multiple Lhcb3 isoforms of *A. thaliana*, the GjLhcb is more similar with Lhcb1.3 of *A. thaliana*. The major PSII antenna complex are most abundant in the

thylakoids, it plays different roles in light harvesting and photoprotection, suggest its expression level depend on environmental conditions. It has been shown that the LHC-II protein content is reduced in high light conditions. Multiple copies of Lhcb1 may convenient for an optimal integrated regulation of the LHC- II content. In *Zea mays*, different Lhcb1 isoforms are accumulated at different levels depending on growth conditions [16]. When there is too much light, LHC- II complex regulates distribution of excitation energy between PS II and I through phosphorylation of Lhcb1 at its N-terminus [10, 11]. Sequence alignment suggest that the Thr38 in GjLhcb is a potential phosphorylation site for state transitions regulation. Phosphorylation and dephosphorylation of LHCII are fundamental events occurring in the state transition processes in plants. Transitions are driven by

reversible LHCII phosphorylation by the STN7 kinase and PPH1/TAP38 phosphatase. [17]. In *Arabidopsis*, the phosphorylated light-harvesting complex protein Lhcb1 can bind state-transition phosphatase TAP38/PPH1, the structure of the TAP38/PPH1-phosphopeptide complex is obtained. The first binding segment in Lhcb1 is the five-residue segment (Arg-Lys-pThr-Val-Ala) near the N-terminal peptide of Lhcb1, and the second site in Lhcb1 is the five-residue segment (Lys-Pro-Lys-Gly-Pro) near the first five-residue segment [18]. The correspond two peptide segments in GjLhcb is (Lys-Arg-Thr38-Thr) and (Arg-Pro-Val-Ser) respectively, they are two four-residue segments, the corresponding residue Pro in the first five-residue segment and the Arg in the second five-residue is missing. The potential phosphorylation Thr38 residue in GjLhcb is in the first segment.



The 3D (three dimension) model structure of GjLhcb was predicted using SWISS-PDB software, the X-Ray diffraction at resolutions down to 2.6 Å of spinach Lhcb1.2 (PDB code 4LCZ) trimer was used as template [19]. The amino acid sequence of GjLhcb (from 46-253 residue

range) has 89.29% amino acid identity with Lhcb1.2 (from 44-267 residue range). The quaternary structure was successfully built as trimers (Fig. 2). Each monomer has five  $\alpha$ -helical domains as Lhcb1.2 from spinach; helices 1, 3 and 4 are transmembrane  $\alpha$ -helix, two short amphiphilic

$\alpha$ -helices on the luminal side as 2 and 5; and placing the C-terminus on the luminal side, N-terminus on the stromal side. In the spinach Lhcb1.2, each monomer binds eight chlorophyll A molecules, six chlorophyll B molecules, two Lutein molecules and one Neoxanthin molecular. In GjLhcb model, there are eleven chlorophyll A molecules, three chlorophyll B molecules, two Lutein molecules and three Neoxanthin molecules were predicted bind to the trimer.

In eukaryotes the photosynthetic antenna system is composed of subunits encoded by the light harvesting complex (Lhc) multigene family. These proteins play a key role in photosynthesis and are involved in both light harvesting and photoprotection. The main LHC-II comprises three polypeptide components (Lhcb1, b2 and b3) of ~232 amino acids bound to chlorophyll molecules. The three proteins have similar polypeptide sequences, structure and function, with tendencies to become phosphorylated and to form trimers. In this study, we characterized the GjLhcb protein in *Gardenia jasminoides* by analyzing its sequence, suggesting its a functional protein with conserved phosphorylation site and could constitute LHC- II trimers unit.

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