C. R. Biologies 337 (2014) 302–310

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Molecular biology and genetics/Biologie et génétique moléculaires

Isolation and functional characterization of DNA damage repair protein (DRT) from *Lepidium latifolium* L.

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ARTICLE INFO

Article history:
Received 23 January 2014
Accepted after revision 15 March 2014
Available online 26 April 2014

Keywords:
Cold stress proteins
Arabidopsis
Lepidium
Plastocyanin
Real-time polymerase chain reaction

ABSTRACT

We have isolated and *in silico* characterized a cold regulated plastocyanin encoding gene from *Lepidium latifolium* L designated as *LlaDRT*. Its cDNA sequence (JN214346) consists of a 504 bp ORF, 48 and 205 bp of 5′ and 3′ UTR regions, respectively encoding a protein of 17.07 KDa and pI 4.95. *In silico* and phylogenetic analysis of *LlaDRT* suggested that the protein has features of a typical plastocyanin family member and of a nearest relative of the predominant isoform of *Arabidopsis* (PETE2) plastocyanin. Validation of stress response of *LlaDRT* by qPCR under different abiotic stress regulators viz salicylic acid, jasmonic acid, calcium chloride, ethylene and abscisic acid revealed its possible regulation and crosstalk amongst different pathways.

1. Introduction

Studies involving abiotic stress tolerance in plants have identified that the expression of many genes vary in response to various abiotic stresses *viz.* temperature stress (both low and high), drought, salinity, oxidative stress, etc. [1], which resulted in reduced crop productivity [2,3]. Studies carried out for genome wide mRNA abundance have validated that the expression of around 5–30% of the genes are controlled by various abiotic stresses [4,5]. Plants have attained different strategies (mainly adaptive) at morphological, physiochemical, biochemical and cellular levels, which help them survive environmental extremes. Upregulated genes have always been given more importance, and extensive research on members of DREBs, MYB, bZIP proteins, and zinc finger families represents their importance in response to various environmental stresses. However, only a handful of literature [6] is available on genes, which are downregulated and research on all these genes are still lacking behind for elucidating their possible roles in the regulation of plants responses to stress.

Reports on genes encoding chlorophyll *a*/*b*-binding proteins, plastocyanin, and PSI subunit proteins have revealed their downregulation in response to cold stress [7–9]. It has been established that the plants acquire increased tolerance to photo-inhibition [10,11] particularly, owing to the shifting of photosynthetic carbon metabolism [12]. Plastocyanin, a type-I copper-containing a protein is found in cyanobacteria, algae, and plant chloroplasts. It is considered as one of the most abundant proteins in thylakoids [13,14] and plays a role in both linear and cyclic photosynthetic electron transport [15]. Plastocyanin, synthesized in the cytosol, acquires its mature form and Cu^{2+} co-factor [16] in the thylakoid lumen [17]. Light-stimulated responses are causative

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http://dx.doi.org/10.1016/j.crvi.2014.03.006
1631-0691/

agents for the increase of plastocyanin mRNA levels and have been reported to play an important role in *Arabidopsis*, *Hordeum*, and *Nicotiana* [18,19]. *Arabidopsis, Nicotiana, Oryza* and other higher plants have been observed to possess/express two isoforms of plastocyanin [20–23]. PETE2 (DRT112) protein has been preferentially observed to be more abundant than PETE1 and their independent regulation for performing different roles in copper homeostasis as well as electron transport is known [24,25]. Studies on double mutant generated for *Arabidopsis* plastocyanin genes revealed their failure for growth due to an inhibition in light-driven electron transport [26]. Overexpression of the *Spinacia* plastocyanin promoter in *Nicotiana* has revealed a differential regulation of plastocyanin expression by oxidized and repressed plastoquinone pool [27].

Lepdium latifolium L. ecotype Ladakh shows remarkable ability to withstand harsh environmental stress conditions [28] at different developmental stages, making it an excellent candidate for the exploration of genes related to abiotic stresses. Hence, *Lepidium* can serve as a source of genes and regulatory elements for abiotic stress tolerance through translational genomics approach and may also further aid in providing a deeper insight into the interaction of genes and networks, clarifying the interaction of genes and regulatory networks under adverse conditions [29]. Here, we describe the isolation, full-length cloning and expression pattern of DNA damage repair/toleration protein from *Lepidium latifolium* L. in response to various abiotic stresses.

2. Materials and methods

2.1. Plant material

Seeds of *L. latifolium* L. (Ladakh ecotype) were procured from Leh, India (11,500 ft asl). The collected seeds were germinated and seedlings were grown at $25 \pm 2\,^{\circ}C$, 150 $\mu mol\, m^{-2}\, s^{-1}$ light intensity, 75% relative humidity and a 16:8 h photoperiod [30].

2.2. Rapid amplification of cDNA ends (RACE) and intron mapping

Total RNA was isolated from *L. latifolium* plants (three-week seedlings) by RNeasy mini kit (Qiagen, Germany) using the manufacturer's protocol from unstressed (control) and stressed leaves (4 °C for 24 h) of *L. latifolium*, quantified by spectrophotometry (Biorad, USA) and stored at −80 °C until further use. Based on EST sequence (FG618360), gene specific primers using Primer3 [31] were designed for RACE, which was carried out using GeneRacer™ kit (Invitrogen, USA) following the manufacturer's protocol. The amplified fragments were cloned in pGEM-T Easy vector (Promega, USA) and sequenced by M13 universal primers. The sequenced 5′ and 3′ RACE fragments were aligned together for removal of overlapping sequences and generation of full-length *LlaDRT* from *Lepidium*. Subsequently, extreme forward and reverse primers were designed from the ends of the full-length *DRT* gene sequence. All the primers designed for the study of *LlaDRT* are listed in Table 1.

2.3. Multiple alignments and bioinformatics analysis

The full-length *LlaDRT* (JN214346) cDNA and the deduced amino acid sequence were compared against a non-redundant database of NCBI. Multiple alignments of the retrieved full-length amino acid sequences from the database were generated using ClustalW [32] and a bootstrapped phylogenetic tree [33] was obtained following the neighbour-joining method [34] using MEGA5 software [35]. The conserved motifs in sequences encoded by DRT cDNAs were detected by analysing protein sequences by MEME and BLOCKS software [36]. Conserved domains in DRT encoding sequences were analysed using

Table 1
Description of the oligonucleotides used in this study.

S. No	Name	Sequence (5′–3′)	Length (mer)
1	Primers for 3′ RACE		
	LlaDRT F1	CGAGGTTGCCTTGACTGAGC	20
	LlaDRT F2	GCCACATCAGGGTGCTGGTA	19
	GeneRacer™ 3′ outer	GCTGTCAACGATACGCTACGT AACG	25
	GeneRacer™ 3′ nested	CGCTACGTAACGGCATGACAG TG	23
2	Primers for 5′ RACE		
	LlaDRT R1	GCCATACAACAATCACGGTCCA	22
	LlaDRT R2	CCTCTAATTGAGGCCGAGAC	20
	LlaDRT R3	TACCAGCACCCTGATGTGGC	20
	GeneRacer™ 5′ outer	CGACTGGAGCACGAG GACACT GA	23
	GeneRacer™ 5′ nested	GGACACTGACATGGA CTGAAG GAGTA	26
	GeneRacer™ RNA oligo sequence	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA	44
	Oligo dT primer	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA	60
3	Primers for full-length cloning and intron mapping		
	LlaDRT F	ATGGCCTCAGTAACCTCAGCC	21
	LlaDRT R	TTAGTTAACGGTGAGTTTACCG	22
4	Primers for qPCR		
	26SrRNA F	CACAATGATAGGAAGAGCCGAC	22
	26SrRNA R	CAAGGGAACGGGCTTGGCAGAAT	23
	LlaDRT F1	CGAGGTTGCCTTGACTGAGC	20
	LlaDRT R1	GCCATACAACAATCACGGTCCA	22