CHARACTERIZATION OF ABIOTIC STRESS GENES FROM DIFFERENT SPECIES OF EUCALYPTUS

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Abstract

The stresses causing dehydration damage to the plant cell like cold, drought, and high salinity are the most frequent environmental stresses that influence plant growth, development and restraining productivity in cultivated areas world-wide. Many drought, salinity and cold inducible genes causing tolerance to environmental stresses in many plants include Dehydrin1 (DHN1), Dehydrin2 (DHN2), Dehydrin10 (DHN10), putative phosphate transporter (Ecpt2), choline monoxygenase (CMO) and DREB/CBF1c genes. Gene specific primer pairs were designed for each gene using DNAStar software. These genes were amplified from different species of eucalyptus such as Eucalyptus camaldulensis, E. globulus, E. tereticornis and E. gunni through PCR. Dehydrin2 gene of E. camaldulensis and dehydrin10 gene of E. globulus were cloned using the TA Cloning® Kit with pCR®2.1 vector and sequenced. The Dehydrin genes sequences were submitted to GeneBank: Eucalyptus globulus dehydrin10 gene (Accession No. HG915712) and E. camaldulensis dehydrin 2 gene (Accession No. HG813113). The amino acid sequence of Dehydrin10 from E. globulus showed 97% homology to E. globulus DHN10 (JN052210) and Dehydrin2 from E. camaldulensis presented 94% homology to E. globulus DHN2 (JN052209). These genes can be employed in generating drought resistant crop plants.

Key words: Drought, Cold, Salinity tolerance, Dehydrin gene, Eucalyptus.

Introduction

The genus Eucalyptus belongs to the Myrtaceae family and is the second most important forestry plant that consists of approximately 734 species and 3 hybrids growing in different regions. Eucalyptus is native to Australia and introduced in Asia, Africa, America and South Europe with minimum 12 million of hectares planted (Carbonnier et al., 2004). It is a commercially important woody tree for paper pulp as it is major source of cellulose, timber and in furniture industries.

Several species of the Eucalyptus genus are used for medicinal values in the Chinese folk medicine. The leaves of the Eucalyptus species are traditionally used as anti-inflammatory, antipyretic remedies, analgesic for plants for reproduction and survival (Fernández et al., 2012). The proteins related to Dehydrin present in numerous woody plant species like Eucalyptus, birch, pistachio, Rhododendron, blueberry and peach during autumn, attaining maximum level during winter concurring maximum freezing resistance (Fernandez et al., 2012).

Eucalyptus species are used for a variety of amenity and wood and tolerant for water logging, salinity, cold and drought. The origin and variation of various traits of eucalyptus is large, so collection of the stock is significant when planting. It is grown widely; so much of its silvicultural and pest information is recognized (Potts & Dungey, 2004). In the current study isolation, identification and characterization of stress related genes from four Eucalyptus species: Eucalyptus camaldulensis, E. globulus, E. gunni, E. tereticornis was carried out using different bioinformatics tools.
Materials and Methods

Collection of plant material: The young leaves of four different Eucalyptus species (E. camaldulensis, E. globulus, E. gunni and E. tereticornis) were collected from different areas of Lahore, Pakistan.

DNA extraction and primer designing: Genomic DNA was isolated from young leaf tissues of Eucalyptus species by using CTAB method (hexadecyltrimethyl ammonium bromide) (Doyle & Doyle, 1990; Stewart and JrVia, 1993) with some modifications. One g of leaves were chopped in liquid nitrogen to a fine powder and collected in 2 ml eppendorf. Preheated Extraction buffer (20 mM EDTA, 2%CTAB, 100 mM Tris–HCl, NaCl and 2% β–mercaptoethanol) was added to this eppendorf tube and incubated at 65°C for 30 min. mixed with chloroform–octanol (24: 1 v/v) and centrifuged at 7000 rpm for 10 min. The aqueous layer was separated, mixed with an equal volume of chilled isopropanol and centrifuged, later on pellet was washed with 70% ethanol and dried. Then stored in TE buffer pH 8.0.

DNA concentration was determined by UV–spectrophotometer (at 260 nm). The forward and reverse primers were designed (Table 1) to amplify full length dehydrin 1 gene, dehydrin 2 gene, dehydrin 10 gene, choline monooxygenase gene, CMO 2 (putative phosphate transporter) gene, and DREB/CBF1c genes (Tables 1, 2).

PCR amplification: The PCR reactions were performed in 25 µl volume with reaction mixture containing 2 µl of 20ng µl−1 genomic DNA, 2.5µl 10x PCR buffer, 1 µl MgCl2(50 mM), 2 µl of 2.5mM dNTPs, 0.6 µl each primers(10 mM) and 0.2µl Taq polymerase (Thermo Scientific Gene JET PCR Purification Kit CAT. # 00129583). The PCR amplifications were initiated by 95°C for 5 min followed by 35 cycles consisting of denaturation at 95°C for 30 s; annealing at 58°C for 45s, extension at 72°C for 1:30 min; and a final extension of 7 min at 72°C (Primus 96 Programmable thermal cyclers). The PCR products were visualized on 0.8% agarose gel with molecular DNA marker (Fermentas life science) stained in ethidium bromide. The amplified PCR products were cloned in InsTA clone™ PCR Product Cloning Kit (CAT # 1275240) from Invitrogen Life Technologies.

Cloning of PCR product and sequence analysis: The PCR product was ligated in T/A cloning vector and transferred into E.coli (DH5α strain). The white colonies were used to isolate plasmid and screened for the presence of insert by double restriction (Fig. 3). The positive clones were sequenced (Macrogen Company). The consensus sequence was generated from plus and minus strand sequence by using DNASTAR seqman software. The final sequence was submitted to EMBL database (http://www.ebi.ac.uk/ena). The gene sequences were run on BLAST n search of NCBI Gene Bank and selected sequences were used from results of BLAST n to generate phylogenetic tree.

Results and Discussion

We have characterized Dehydrins gene dehydrin 1, dehydrin 2, and dehydrin 10 gene, putative phosphate transporter gene, DREB/CBF1c and choline monooxygenase gene from different Eucalyptus species such as E. camaldulensis, E. globulus, E. tereticornis and E. gunii. Dehydrin gene is responsible for abiotic stress resistance in plants. It is involved in drought, cold and salinity tolerance. The dehydrin1 gene fragment of approximately 959 bp was amplified through PCR from Eucalyptus camaldulensis, with the designed forward and reverse primers.

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<th>Table 1. Eucalyptus gene specific primers.</th>
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<th>Table 2. Stress Genes of Eucalyptus.</th>
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The gene dehydrin 10 of Eucalyptus globulus was amplified via polymerase chain reaction (Fig. 1), then cloned inside the cloning vector pCR 2.1 and transferred into E.coli DH5α strain. The sequenced gene consists of 549 nucleotides encoding a 98 residues. The protein consisted of two functional domains, with a calculated molecular mass of 10.94 kDa and pI value of 6.45, it belong to KS group of dehydrin. Similarly Fernandez et al. (2012) isolated EuglDH10 (Genbank Accession: JN052210) having an open reading frame of intron less 294 bases, 98 amino acids with calculated pI value of 6.87 and deduced molecular mass of approximately 11 kDa, this Dehydrin belongs to the KS group.

The dehydrin 2 gene of Eucalyptus camaldulensis was amplified through PCR (Fig. 2), then cloned inside into pCR 2.1 cloning vector and transferred in E.coli DH5α strain. The sequenced DNA segment has 1051 bp containing one intron and a coding sequence of 777 nucleotide bases that consists of 259 amino acids, molecular mass of 29.47 kDa and pl value of 5.20, it belong to SK2 group of dehydrin. These results are strongly agreed with the study of Fernandez et al. (2012) isolated EuglDHN2 (JN052209) is 2.130 bp long; A coding sequence consisting of 777 nucleotide bases and is composed of about 259 nucleotide bases. It is about 130 base pair in length, has an intron of about 113 bp in length and a coding sequence consisting of about 774 bases which code for 258 amino acids with a 30kDa molecular mass, and an isoelectric point calculated to be 4.82, it belongs to SK2 group.

Sequencing of dehydrin 2 & dehydrin 10 genes: Recombinant plasmid DNA were prepared from the confirmed positive screened clones of dehydrin genes and sequenced using M13 forward and reverse primers. The sequences of these Dehydrin genes were submitted to GeneBank under accession no. HG915712 and E. camaldulensis dehydrin 2 gene under accession no. HG813113. E. globulus dehydrin 10 gene shows 99% homology with E. globulus dehydrin 10, and E. camaldulensis dehydrin2 gene shows 94% homology with E. globulus dehydrin 2 gene. The amino acid sequence of Dehydrin 2 E. camaldulensis and Dehydrin 10 E. globulus were compared with previously sequenced Dehydrin and phylogenetic relationship among them were determined.
The Dehydrin 10 *Eucalyptus globulus* (Fig. 4) presented 98% homology with *E. globulus* Dehydrin10 (AER27689.1), reported by Fernandez et al., 2011 from Chile region and this outgroup shows 64% homology with cold stress protein *citrus trifoliate* (gb|AAA99963.1|), 64% low temperature inducible SLT166 *Glycine max* (AB070349.1), 58% with LIPS *Oryza sativa* (BAA24979.1), 55% and 54% with Drought induced protein in *Retama raetam* and dehydrin 1 *Corylus avellana,-paradise*. The gene KS-type dehydrin (K-segment containing lysine) EUGLDHN10 is present as a multicopy gene which is alike to the core 15 gene in *Citrus* genome (Porat et al., 2002).

The *Eucalyptus camaldulensis* Dehydrin 2 (Fig. 5) show 94% homology with *E. globulus* Dehydrin 2 reported by Fernandez et al., 2011 from Chile region, and this outgroup show 61% homology with Dehydrin1 plantago major, 62% homology Dehydrin salvia miltiorrhiza and Dehydrin protein capsella burea-pastoris, 63% with dehydrin Populus alba x Populus glandulosa, 61% with Populus maximowiczii and Populus davidiana, 46% with *coffee canephora*. A distinct gene present in the genome of *E. globulus* encodes EuglDHN10 which is also seen in the gene ccDH2DHN in the *C. canephora* genome (Hinniger et al., 2006) and the gene csDHN in *Citrus sinensis* (Porat et al., 2004).

The absence of glycine-rich repeat and acidic nature of EuglDHN2 recommend that it belongs to acidic dehydrin subfamily (Danyluk et al., 1998). It had been reported in wheat that it preferentially associated in the sensitive vascular transition area with plasma membrane where cold-induced dehydration is expected to be most severe. Danyluk et al. (1998) reported that this acidic Dehydrin may play an imported role under dehydrative conditions in preventing destabilization of plasma membrane. Moreover, the hydrophilic nature of EuglDHN1 and EuglDHN2 polypeptides is well suitable to stabilize membranes and replace water during dehydration through polar interactions. *E. globulus* Dehydrin10 (Fig. 6) presents 97% similarity with a Eugl DHN10 (JN052210) and 62% similarity with a Dehydrin10 from the *Solanum commersonii* (ACJ26759).
Fig. 6. The optimized alignment of *E. globulus* Dehydrins with Dehydrins homologues. This amino acid alignment was made using the Clustal W program and then optimized further manually. The shaded block shows identical amino acids. The rectangles with broken lines shows the S segments and the solid dark rectangles demarcate the K segments.

Fig. 7. The optimized alignment of *E. globulus* dehydrin with DHNs homologues. This amino acid alignment was generated using the software and then optimized further manually. The Shaded blocks show identical amino acids.
In the present study, analysis of EuglDHN10 protein showed that the Histidine residue composes 12.2% of the total content of amino acids. Also, the EuglDHN10 residues had a high level of transcript in the tissues of stem. This protein EuglDHN10 might be involved in the long distance transport mediated by the phloem which has been reported in castor bean DHN (Kruger et al., 2002). Solanum sogarandinum and Solanum tuberosum report the same phenomenon which provides support to the supposed role of EuglDHN10 in transport of iron, and might also be involved in the mechanism providing protection against oxidative stress (Rorat et al., 2004) in cold. E. camaldulensis Dehydrin2 shows (Fig. 7) 94% similarity with EuglDHN2 (JN052209) and shows 53 and 51% similarity with two poplar DHNs, Populus alba × Populus tremula var. glandulosa dehydrin (ABHI1546) and Populus maximowiczii dehydrin (ABS12346) respectively.

EuglDHN1 and EuglDHN2 are hydrophilic polypeptides which substitute water and also contribute towards stabilization of membranes by forming polar interactions during the process of dehydration. The K-type DHNs have a physiological role of the iron binding quality of a castor bean (Ricinus communis), DHN (Kruger et al., 2002) along with Citrus unshiu DHN which is rich in His residues (Hara et al., 2005). His residues do not much protein, and they build up only 2% of amino acids (Ueda et al., 2003). The dehydrin 10 Eucalyptus globulus gene consist of 98 amino acids (Fig. 8), has a change in position number 28 and 36, Glu (Q) with Histidine (H) and Met (M) with Thr (T) from E. globulus DHN10 (AER27689).

The dehydrin 2 E. camaldulensis gene consists of 259 amino acids, (Fig. 8) change in position 48 Lys(K) with Met (M), 79 Leu (L) with Phe (F), 90 Val (V) with Ser (S), from 141 to 146 KKKKKK with EHTTVP, from 150 to 157 EKKKKEEEE with YEEHTIFP, at position 177Ala (A) with Glu (E) from dehydrin E. globulus. It had been suggested that Dehydrin are highly evolved proteins, through conformational modifications in their K segment, might stabilize cellular membranes beneath stress conditions (Koag et al., 2003) and selected to sustain maximum configurational flexibility to resist unspecific aggregation and collapse (Mouillon et al., 2006; Koag et al., 2009).

Conclusion and Future prospects

Abiotic stress signaling is an important area with respect to increase in plant productivity under adverse conditions. The products of these genes may be characterized for their stress tolerance levels and potent could be further used for transgenesis of stress tolerance in economically important crops.

References


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