Plant Physiology and Biochemistry 111 (2017) 174–178

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Transcription activation activity of ERD15 protein from *Morus indica*

Bushra Saeed*, Paramjit Khurana

Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India

ARTICLE INFO

Article history:
Received 10 October 2016
Received in revised form
25 November 2016
Accepted 25 November 2016
Available online 27 November 2016

Keywords:
Early responsive to dehydration 15
Mulberry
Transcription activation
Yeast

ABSTRACT

Early Responsive to Dehydration (ERD) genes are described as rapid response mediators of dehydration stress. Recently, ERD15 has emerged as a novel stress induced transcription factor which might be involved in mediating distinct stress responses in plants. In order to determine whether mulberry ERD15 can act as functional transcription factor, yeast-based assays were performed. Mulberry ERD15 was found to drive high level reporter gene expression in yeast which suggests it may function as a transcription factor. However, due to lack of an identifiable DNA binding domain, deletion analysis was carried out to determine the putative region of the protein involved in mediating protein-DNA interaction. Our results indicate that the region between 70 and 100 amino acids is critical in conferring transcription activation activity and might harbor the DNA binding region of ERD15.

1. Introduction

Mulberry is an economically important plant used for traditional medicine, fodder and timber. Mulberry is commercially used for rearing silkworm (*Bombyx mori*) making it indispensable for the sericulture industry (Khurana and Checker, 2011). Owing to large cumulative leaf area and rapid growth rate, water limitation is a major constraint for mulberry cultivation (Guha et al., 2010). Recently several investigations on functional characterization of drought responsive genes have been undertaken in mulberry (Lal et al., 2008; Das et al., 2011; Checker et al., 2012a; Liu et al., 2015; Zhou et al., 2016).

Drought and salinity stress are among the key limiting factors affecting plant yield (Bartels and Sunkar, 2005). Early responsive to dehydration (ERD) genes were identified on the basis of their capacity to be rapidly induced by dehydration (Kiyosue et al., 1994a). Rapid induction of these genes could be suggestive of their critical role in mediating drought stress responses. Early Responsive to Dehydration 15 was initially described as a small hydrophilic protein (Kiyosue et al., 1994b). Recently ERD15 and related proteins have been suggested to belong to a small, ubiquitous gene family specific to plants (Aalto et al., 2012). ERD15 family proteins have a characteristic PABP interacting Motif2 (PAM2), PAM2 Associated Element1 (PAE1) domain followed by an acidic region and a conserved IqQPR sequence in the C-terminal end (Aalto et al., 2012). Overexpression of ERD15 resulted in decreased tolerance to drought and freezing in response to ABA, suggesting that alteration of ERD15 expression affects ABA responses in *Arabidopsis thaliana* (Kariola et al., 2006). Nonetheless, a corresponding increase in tolerance to *Erwinia carotovora* was additionally observed, implicating a probable role of AtERD15 as a negative regulator of ABA responses (Kariola et al., 2006). On the contrary, overexpression of ERD15 from *Solanum pennellii* resulted in tolerance to drought, salt and cold stress as demonstrated by enhanced survival, reduced lipid peroxidation and increased osmolyte production (Ziaf et al., 2011). Furthermore, ERD15 has been found to be a functional transcription factor in *Glycine max* possibly acting at the confluence of endoplasmic reticulum and osmotic stress signaling (Alves et al., 2011b). Thus in the present study, identification of the transactivation potential of mulberry ERD15 was undertaken and the region crucial for driving high level transcription was identified.

2. Materials and methods

2.1. Stress treatments

For analysis of gene expression, mature leaves of *Morus indica* cv. K2 plants maintained in University of Delhi (South Campus) were used. Leaves of similar developmental stage (second and third leaves from the apex) were treated for 4 h. **Mock:** Detached leaves were mock treated in Reverse Osmosis (RO) water at 28 °C, **Abscisic acid (ABA):** placed in 100 μM ABA at 28 °C, **Mannitol (Man):** placed

* Corresponding author.
E-mail addresses: bushra.saeed1@gmail.com (B. Saeed), param@genomeindia.org (P. Khurana).

http://dx.doi.org/10.1016/j.plaphy.2016.11.020
0981-9428/

in 500 mM mannitol solution at 28 °C, **Salt stress (SS):** placed in 250 mM NaCl solution at 28 °C, **Cold stress (CS):** placed in RO water at a temperature of 6 ± 2 °C, **Heat stress (HS):** placed in RO water at a temperature of 40 ± 1 °C.

2.2. RNA isolation and quantitative real time PCR

Total RNA was extracted from mulberry leaves following GITC procedure (Chomczynski and Sacchi, 1987) followed by a DNase treatment using RNeasy plant mini kit (Qiagen) according to manufacturer's instructions. First strand cDNA was synthesized using 2 μg total RNA in a reaction volume of 50 μl using high capacity cDNA archive kit (Applied Biosystems). The primers were designed by using Primer Express 2.0 software (PE Applied Biosystems) using default parameters and analyzed by BLAST tool against nr database (NCBI) and melting curve analysis. Quantitative real time PCR was carried out in Stratagene Mx3005P (Agilent Technologies). The data was normalized using Elongation Factor 1α as an internal control and relative expression values were calculated using ΔΔCT method. The data represents average of two biological replicates with three technical replicates each. Primers used in this study can be found in Supplementary Table 1.

2.3. Isolation and cloning of mulberry ERD15

Partial sequence of MiERD15 was initially identified in the root cDNA library of *Morus indica* cv. K2 (Checker et al., 2012b). The MiERD15 coding sequence was completed with the help of 3′ RACE using SMART™ RACE amplification kit according to manufacturer's instructions (Clontech) using mature leaf cDNA. The identified EST was used as a template for primer designing (Supplementary Table 1). The full length coding sequence obtained was cloned in pDrive vector (Qiagen) and sequenced.

2.4. Transactivation assay in yeast

For evaluating the ability of mulberry ERD15 to act as a functional transcription factor, complete coding sequence of MiERD15 was fused to the GAL4 DNA binding domain of the pGBKT7 vector (Clontech). Successful cloning was confirmed by digestion followed by sequencing. Moreover, in order to determine which region of the protein possesses a functional transactivation domain, different deletion constructs from the N-terminal (MiERD15Δ1-6) were amplified and cloned in pGBKT7 vector. List of primers can be found in Supplementary Table 1. These constructs were transformed in *Saccharomyces cerevisiae* strain AH109 by lithium acetate mediated method according to Yeast Protocols Handbook (Clontech). The transformants were grown on synthetically defined (SD) medium lacking tryptophan (SD −W) for 2–3 days at 30 °C. The transformants were then spotted onto medium lacking tryptophan (SD −W), lacking both tryptophan and histidine (SD −WH) and supplemented with 10 mM 3-aminotriazole (3-AT).

For colony filter lift assay, the transformants were blotted onto Whatman filter paper and lysed by repeated freeze thawing in liquid nitrogen. The filter paper was then incubated in Z buffer containing X-gal as described in Yeast Protocols Handbook (Clontech) and photographed after color development. The β-galactosidase reporter gene activity was quantified by using liquid culture method using o-nitrophenyl β-D-galactopyranoside (ONPG) as substrate (Yeast Protocols Handbook, Clontech). The experiments were repeated at least thrice.

3. Results and discussion

3.1. Identification of stress responsive ERD15 from mulberry

A partial sequence encoding ERD15 identified from root cDNA library was isolated and characterized. The 507 bp open reading frame is comprised of deduced protein of 168 amino acids with a predicted molecular weight of 19.24 kDa. Since *Arabidopsis thaliana* ERD15 transcripts (Kiyosue et al., 1994a) are known to accumulate at high levels under dehydration stress, the expression of MiERD15 was analyzed under different abiotic stress treatments using quantitative real time PCR. The expression of MiERD15 was found to be considerably induced (more than five-fold) under simulated drought stress (Fig. 1A). The expression of MiERD15 was also found to be induced markedly under ABA treatment, salinity and temperature extremes (Fig. 1A). Our data correlates with the previous reports of drought responsiveness of ERD15 from *Arabidopsis thaliana* (Kiyosue et al., 1994a, 1994b), *Glycine max* (Alves et al., 2011b) and *Ipomoea batatas* (Shao et al., 2014). Apart from biotic stress simulations (salicylic acid, wounding and *Erwinia carotovora*), AtERD15 is induced strongly by ABA application and drought suggesting a potential role in ABA dependent drought stress signaling (Kariola et al., 2006). Likewise, ERD15 from *Solanum pennellii* is responsive to ABA, drought, salinity and cold (Ziaf et al., 2011). Also, the expression of ERD15 from *Solanum lycopersicum* (Ziaf et al., 2016) is enhanced under drought, salinity, cold, wounding and hormonal treatments (ABA, SA, GA and ethylene). Thus, the expression of ERD15 from diverse plant species indicates its responsiveness to not only drought but several abiotic and biotic stress conditions.

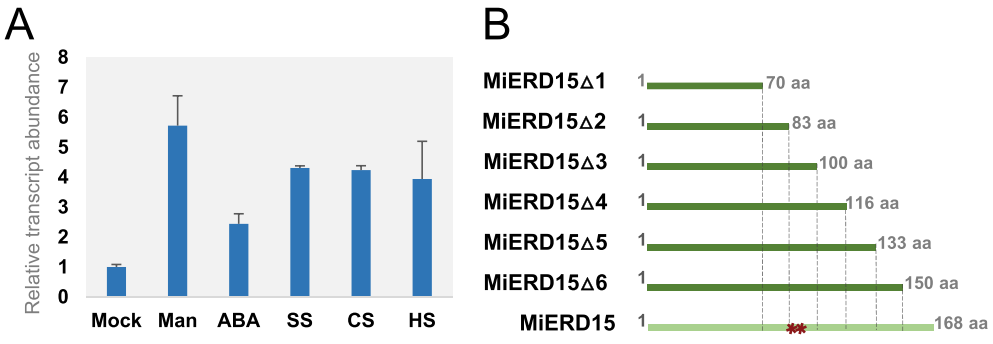

Fig. 1. Deletion analysis of abiotic stress inducible ERD15. A. Gene expression analysis of ERD15 under different environmental stress treatments for 4 h in *Morus indica*. Mock: Mock treated with RO water, ABA: treated with 100 μM ABA, Man: Drought Stress simulated by treatment with 500 mM mannitol, SS: Salt Stress simulated by treatment with 250 mM NaCl, CS: Cold Stress, HS: Heat Stress. Data represents mean ± standard deviation, n = 2 with three technical replicates each. **B**. Schematic representation of deletion constructs analyzed for the mapping of the region crucial for driving high level transcription.