FUNCTIONAL CHARACTERIZATION OF A DEHYDRIN OF CACTUS PEAR (OpsDHN1)

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Introduction. Dehydrins (DHNs) are the most studied group of the LEA proteins. DHN proteins are thermostable, intrinsically disordered, and highly hydrophilic. These proteins are characterized by the presence of one or more K-segments (lysine-rich consensus regions) that form a putative amphipathic α-helix. This structure allows stabilizing proteins in water stress environments. Y- and S-segments frequently appear in DHN sequences (Allagulova et al. 2003).

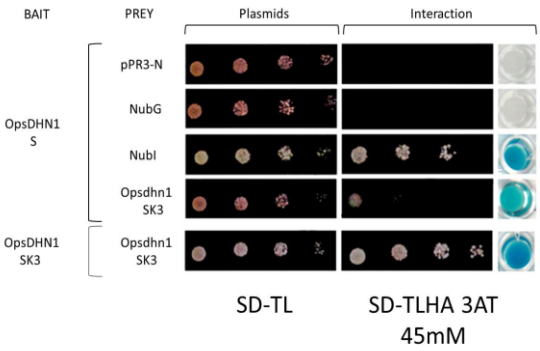
Several transgenic studies have revealed the positive effect of the expression of DHN genes in the tolerance to abiotic stress; however, functional mechanism of the DHNs is less understood. In our research group, we isolated and characterized a gene encoding an SK_3-type acidic DHN (OpsDHN1) of *Opuntia streptacantha*. In this study, it was showed that OpsDHN1 over-expression in *Arabidopsis thaliana* leads to enhance tolerance to freezing stress (Alfaro Ochoa et al. 2012).

In this study, we focus on the characterization of OpsDHN1 dehydrin, mainly on two aspects; the first one is related to the potential interaction of OpsDHN1-OpsDHN1 and the second on the characterization of putative PEST degradation regions of the OpsDHN1. For the characterization protein interaction we used the Split ubiquitin system (DUAL Hunter system) (Hammani et al. 2011), which is a variant of the classic yeast two-hybrid genetic assay. On the other hand, an in silico analysis of OpsDHN1 protein, we detected two putative PEST sequences (proline, glutamic acid, serine, threonine rich regions) in the C-terminal; a characteristic of proteins with a high rate of turnover (Rechsteiner and Rogers 1996).

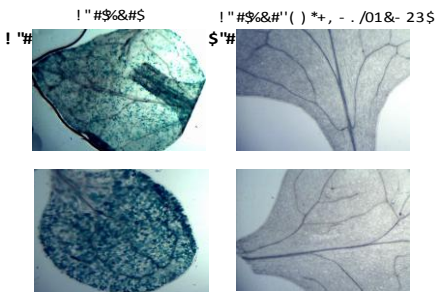
Methods. A short version of OpsDNH1 (1-97 aa) was PCR amplified and cloned into the pDHB1 Bait vector by directional *Sfi*I restriction sites. Bait vector generated and a Prey vector containing the ORF of OpsDHN1 gene were co-transformed into yeast strain NMY51 according to the manufacturer's instructions (Dualhunterbiotech).

GUS::OpsDHN1-PEST fusion was generated by PCR using the ORF of *GUS* gene without stop codon and the last 378 bp of *OpsDNH1* gene that encodes the PEST sequences. The fusion was cloned into pMDC32 plant expression vector under the control of 2x35S promoter. As a positive control, the *GUS* gene without PEST sequences was cloned into pMDC32 vector. The resultant constructs were used for the *Agrobacterium*-mediated transformation of *A. thaliana* plants. GUS staining was done according to Jefferson (1987).

Results. We found that OpsDHN1 dehydrin interacts with itself in the yeast system, but when we analyzed the short version of OpsDHN1 (1-97 aa, without lysine-rich regions), a decrease in the interaction between the short version and the full OpsDHN1 version was observed. This data suggests that the segment excluded of the OpsDHN1 is important for the protein interaction (Fig. 1)**.**



Related to *GUS*::OpsDHN1-PEST fusion, it shows a reduction in GUS activity, where the GUS signal was abated, as compared with the control *GUS* construction, where all cells are blue stained (Fig. 2A, B)



Conclusions. Our data showed that the OpsDHN1 C-terminal is important for the protein interaction between OpsDHN1-OpsDHN1. On the other hand, the translational fusion *GUS*::OpsDHN1-PEST leads to specific degradation to the GUS reporter protein.

References.

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