AtGRDP1 gene encoding a glycine-rich domain protein involved in abiotic stress

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Introduction. Seed maturation and germination involve changes in gene expression, as well as physiological and metabolic events; however, they are not well understood. The phytohormone abscisic acid (ABA) plays a crucial role in these adaptive strategies. Molecules related to ABA recognition and signalling, such as the ABA-insensitive (ABI) transcription factors, ABI3, ABI4, and ABI5, have been identified (Hirayama and Shinozaki, 2007)**.** GRPs are a group of proteins characterised by a high content of glycine (from 40 to 70%) and repetitive sequences of residues that forms a $(Gly\text{-}X)_n$ motifs (Sachetto-Martins *et al*., 2000). A small number of plant glycine-rich proteins (GRPs) have been characterised.
The aim is the characterization of *AtGRDP1* (At2g22660), a novel *A. thaliana* gene encoding a DUF1399-glycine-rich domain protein.

Methods. Identification of the T-DNA insertional mutant line (http://signal.salk.edu; Alonso *et al*., 2003), generation of *A. thaliana* over-expressing lines (Zhang *et al*., 2006), quantitative RT-PCR (qRT-PCR) of the *AtGRDP1* gene (Livak and Schmittgen, 2001), expression analysis of *AtGRDP1* gene under abiotic stress and ABA treatments and estimation of *ABI3* transcript levels in *Atgrdp1*-null mutant and 35S::*AtGRDP1* over-expressing lines.

Results. The At2g22660 cDNA is 2789bp in length, containing an open reading frame of 2460bp encoding a polypeptide of 819 amino acids with a predicted molecular mass of 89.486 kDa. Sequence analyses revealed diverse domains in the At2g22660 protein: a domain of unknown function (DUF1399), a putative RNA binding motif (RNP) and a glycine-rich domain (GRD). Therefore, we named this protein AtGRDP1 (***A****rabidopsis* ***t****haliana* **G**lycine-**R**ich **D**omain **P**rotein **1**). The transcript level of *AtGRDP1* in Arabidopsis (Col-0) under different inductors of abiotic stress was determinate in 15-day-old plantlets grown on 0.5 x MS liquid medium exposed to: A) 150 and 175 mM NaCl, B) 13 and 16 mM LiCl, C) 5 and 6% sorbitol D) 5 and 6% mannitol. Expression was determined by qRT-PCR using SYBR green dye. The effect of glucose and ABA on *AtGRDP1* gene expression was assessed. A gradual increase of the *AtGRDP1* mRNA level by glucose in a dose- and time-dependent manner was observed, beginning at 6h with gene repression achieving the highest levels after 24h of treatment. On the other hand, we tested the expression of the *AtGRDP1* gene under several ABA concentrations (0.1, 3, and 25 µM) at 3, 12, 24, and 48h of treatment. A similar behaviour to that obtained with glucose was observed in ABA treatments. Inhibitory experiments of seed germination were carried out with Col-0, *Atgrdp1*-null mutant and 35S::*AtGRDP1* over-expressing lines on 0.5 x MS medium containing 0, 5, 7, and 9 µM ABA. The *AtGRDP1* gene disruption causes a germination sensitivity phenotype on ABA treatments. The 35S::*AtGRDP1* over-expressing lines stayed green, and the root development was continuous, being viable on a growth medium containing high concentrations of ABA such as 5, 7, and 9 µM, where Col-0 plants are unable to grow. We evaluated *ABI3* gene expression in the Col-0, *Atgrdp1*-null mutant and 35S::*AtGRDP1-6* over-expressing lines. For this, qRT-PCR experiments were carried out in 15-day-old plants subjected to ABA (0, 0.1, 1, and 9 µM) treatments at 12, 24, and 48h. This increased induction of *ABI3* transcript in *Atgrdp1*-null mutant, a key factor in ABA signalling, could be correlated with the ABA sensitive phenotype observed in mutant lines at the germination and seedling stages.

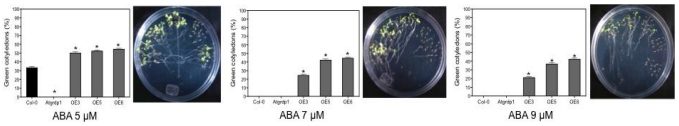

Fig.1 Green cotyledons percentage was evaluated 28 days after seed germination on condition of 5, 7, 9 mM ABA.

Conclusions. This study reveals the presence of a novel family of proteins containing the DUF1399 and glycine-rich domains in *Arabidopsis*. The deregulation of *AtGRDP1* gene levels affects the response to ABA, possibly through regulation of the *ABI3* gene. These results suggest that *AtGRDP1* gene plays a regulatory in ABA signalling, and tolerance to abiotic stress.

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References.

1.-Alonso JM, Stepanova AN, Leisse TJ, (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301, 653-657.
2.- Hirayama, T. and Shinozaki, K. (2007). Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. Trends in Plant Science 12, 343–351.
3.- Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4), 402-408.
4.- Sachetto-Martins G, Franco LO, de Oliveira DE. (2000). Plant glycine-rich proteins: a family or just proteins with a common motif? Biochimica et Biophysica Acta 1492, 1-14.
5.- Zhang X, Henriques R, Lin SS, Niu QW, Chua NH. (2006). Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. Nature Protocols 1(2), 641-646.