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THE AtRGGA RNA BINDING PROTEIN REGULATES RESPONSES TO ENVIRONMENTAL STRESSES THROUGH A WIDE RANGE OF PROTEIN PARTNERS AND RNA TARGETS

PUNZO P.*, MASSIMINO I.*, AURILIA V. **, DEDOW L.***, NURCATO R.*, BAILEY-SERRES J.***, GRILLO S.*, BATELLI G.*

*) CNR-IBBR, National Research Council of Italy, Institute of Biosciences and Bioresources, Via Università 133, 80055 Portici (Italy)
**) CNR-ISAFOM National Research Council of Italy, Institute for Agricultural and Forest Systems in the Mediterranean, Via Patacca 85 80056 Ercolano (Italy)
***) Department of Botany and Plant Sciences and Center for Plant Cell Biology, University of California Riverside, Riverside, CA 92521 (USA)

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Environmental stresses, such as drought and salinity, severely affect plant growth and productivity. RNA regulatory mechanisms are recently emerging as key processes participating in the coordination and modulation of cellular responses and adaptation to environmental stimuli. These mechanisms include transcriptional and post-transcriptional regulation of gene expression, such as synthesis, transport, translation, stability control and decay of transcripts, and require the action of RNA-binding proteins (RBPs). In a previous study, we genetically characterized the Arabidopsis AtRGGA, encoding a glycine-rich RBP, in responses to drought and salt stress. Here, we use a combination of methods to infer the protein function in the RNA regulatory mechanisms through isolation of protein and ribonucleic partners. Using Electrophoresis Mobility Shift Assays (EMSA) and recombinant His-RGGA we show that AtRGGA is capable of binding RNA molecules in vitro, such as the small ribosomal RNAs 5S and 5.8S. The immupurification of RNA-protein complexes in planta confirmed the specificity of AtRGGA-rRNA interaction in vivo and revealed that AtRGGA is able of binding all RNA components of the ribosomes and additional RNAs, including mRNAs. Sequencing of the immunopurified RNAs isolated from stress-treated (NaCl 120mM) transgenic plants (35S::FLAG-AtRGGA), allowed the identification of AtRGGA-targets under salt stress in vivo. Interestingly, several genes involved in abiotic stress responses, such as DIL19, MYB102, DREB2A, DREB2B, HAI1, HAI2 were significantly enriched in the AtRGGAimmunopurified samples after salt treatments. Finally, a yeast two-hybrid assay screening performed with an Arabidopsis cDNA library identified putative AtRGGA protein interactors. Most of the partners such as APUM24, RANBP1, and ZCF125 are mainly involved in RNA processing, transport and ribosome biogenesis. Taken together, the obtained results indicate a role of AtRGGA in post-transcriptional control of gene expression during salt stress through an extended web of interactors and targets.

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