

cGMP SIGNALING IN PLANT-PATHOGEN INTERACTIONS

BELLIN D., HUSSAIN J., BOUNEB M., KLEINFELDER FONTANESI K., PACHAIAPPAN R., VANDELLE E., DELLEDONNE M.

Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona (Italy)

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Cyclic GMP (3', 5'-cyclic guanyl monophosphate) is known as a key second messenger which regulates a wide variety of cellular responses in several living organisms such as bacteria, fungi, and animals. Its presence and role in plants has been long debated but in the last decade a growing number of reports have described both the occurrence and function of cGMP in higher plants. These reports, mainly based on pharmacological approaches, clearly highlighted its involvement in mediating nitric oxide and other hormones plant signaling in several important physiological processes like biotic and a-biotic stress responses, but detailed knowledge about the mechanisms is still very limited. We are interested in clarifying the genetic basis of cGMP signaling in plant especially during plant- pathogen interaction.

One of the main limitations in cGMP studies in plants is due to the difficulties related to measurement of its content in plant tissues. All available detection systems are expensive and allow processing only limited number of samples. In this project therefore we started applying a new Alpha-Screen (Perkin Elmer) FRET based immunoassay for high throughput cGMP measurement which was never applied in plants. Reproducibility of the assay as well as error introduced by different cGMP extraction procedures from plant tissues have been carefully evaluated and quantified in order to establish a protocol for measurement of cGMP content in a high number of plant samples using this system. We are currently employing this assay in our lab in order to analyze the dynamic of changes in cGMP content in plant tissues upon nitric oxide or pathogen treatment.

cGMP content in plants is regulated by the activities of the enzymes guanylate cyclase (GCs), that specifically synthesize cGMP from GTP, and phosphodiesterase (PDE), that hydrolyze cGMP to GMP. Unfortunately the specific enzymes operating this turnover in *Arabidopsis thaliana* have not yet been clearly identified limiting our possibility to alter through a classical genetic approach the cGMP content in plant. As an alternative we expressed the heterologous mammalian genes for a soluble guanylate cyclase and a phosphodiesterase in *Arabidopsis thaliana* plants. Transgenic lines constitutively expressing a functional soluble mammalian guanylate cyclase (alpha and beta sub-units) and presenting a cGMP content 10 to 50 time-fold higher than wild type plants have been obtained. Moreover, transgenic *Arabidopsis thaliana* plants expressing either a constitutive or inducible bovine phosphodiesterase were also obtained. We are currently characterizing these plants both morphologically and in terms of response to biotic and a-biotic stresses with the final aim of better clarifying thorough this approach the role of cGMP signaling in plant-pathogen interaction.