Università degli Studi di Napoli Federico II

PhD in Biotechnology - 38th cycle

Dr. Martina Chiara Criscuolo

Prosystemin: toward a deeper understanding of the molecular role in plant defense and in crop protection

Tutor(s): Rosa Rao (AGR/07), Pasquale Chiaiese (AGR/07), Simona Maria Monti

Department: Dipartimento di Agraria, Via Università, 100 - 80055 - Portici (NA)

Plants developed very efficient immunity system against stress agents based on the recognition of enemy's molecular patterns that are released during plant-parasite interaction known as Pathogen-associated molecular patterns (PAMP), Microbe-associated molecular patterns (MAMP) and Herbivore associated molecular patterns (HAMP). In addition, plants are able to recognize other alarm signals such as phytocytokines and Damage-associated molecular patterns (DAMP) or other endogenous elicitors that trigger plant defense responses (Choi et al., 2016). A well-known phytocytokine is Systemin (Sys), an octadecapeptide found in tomato, which is part of a large precursor of 200 amino acids, called Prosystemin (ProSys). Sys promotes long-distance defense responses by amplifying the jasmonic acid signaling pathway, that leads to the production of defense compounds (McGurl et al., 1992; Ryan, 2000, Gust et al., 2017). Interestingly, ProSys is an Intrinsically Disordered Protein (IDP) and this suggested a more complicated role of this precursor in tomato defenses because IDPs are able to interact with different molecular partners being likely involved into different biological functions (Buonanno et al., 2018). The ProSys molecular network of protein-protein interactions, recently described, includes almost one hundred predicted interactors (Natale et al., 2022). In addition, it was discovered that ProSys, devoid of Sys peptide ($ProSys_{(1-178)}$), have its own biological activity against different stress agents like pathogenic fungi and chewing insects and this activity involves oligogalacturonides production (Molisso et al., 2022). In order to identify the shortest regions of ProSys which conferred the biological activity, the amino acidic sequence of the precursor was analyzed through several bioinformatic tools. This investigation showed the presence of 4 repeats of 33 amino acids which contain further shorter motifs, called repeat motifs (RMs) and preliminary studies showed that these sequences are able to protect tomato plants against an arrays of biotic and abiotic stresses (Rao, unpublished).
The five objective of this project are illustrated in the graphical abstract and here summarized. One objective is to improve the knowledge about the functions of $ProSys_{(1-178)}$ and RMs in plant protection. For this purpose, $Prosys_{(1-178)}$ and RMs will be studied, in comparison to Sys through transcriptomic data, gene expression studies, bioassays with stress agents and secondary metabolites analysis. Furthermore, plant treated with RMs will be tested in greenhouse and open field to evaluate the efficiency of protection provided by RMs treatments. Another objective is to investigate the molecular determinants that define some ProSys-other protein interactions selected among those validated through *in vitro* and *in vivo* experiments (Natale et al 2022). Finally, the activity that will be performed by the company participating to this project, Materias

S.R.L has as object the extension of the RMs patent, active in Italy, to the largest number of countries interested in sustainable crop protection and formulation of new biostimulants.

References

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