DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di progetto di Dottorato

Titolo della ricerca: "Genetic determinants of pectin methylesterase-related immunity in plants: new perspective in plant protection"

Docente guida proposto: Daniela Bellincampi

DESCRIZIONE DELLA RICERCA

Obiettivi della ricerca (max 4000 car.)

The project aims at providing new knowledge and technological solutions to plant infections caused by fungal pathogens. Cell wall (CW) is an essential element of plant monitoring system to perceive stress-derived signals triggering specific resistance responses. Plant cell wall alterations affecting cell wall integrity (CWI) have been demonstrated to impact on disease resistance. Pectin, one of the main components of CW, is methylesterified in the Golgi and secreted in the CW in a high methylesterified form. In this compartment, pectin methylesterases (PMEs) remove methylesters producing polyanionic homogalacturonan, methanol and protons. Evidences indicate that PMEs participate in the remodeling of CW with alteration of CWI and activation of plant immunity.

PMEs are widespread in plants and microorganisms and belong to a large multigene family whose members display different expression profiles and mediate different physiological responses.

PMEs play critical role in the outcome of plant-fungus interaction but much remains to be discovered with regard to their transcriptional and post-translational regulation.

Differently from fungi and bacteria, higher plants PMEs are frequently organized in pre-pro-proteins. Pre domain includes a common signal peptide (SP) for CW export. The mature active part is preceded by an N-terminal extension (PRO region) which shares similarity with the endogenous PME inhibitors. Subtilisins, serine-like proteases (SBTs), cleave the PRO-region, at conserved processing site, for the activation and secretion of the active PME domain. Plant SBTs belong to the large S8A subfamily of 56 members in Arabidopsis. Plant PMEs are also regulated by proteinaceous inhibitors (PMEIs) belonging to a large multigene family. PMEIs have been identified in many dicots and in monocot plants. The contribute PMEs, SBTs and PMEIs genes in plant immunity is still largely unknown and will be investigated in the present project. In addition, the PME-related traits and their underlying genes identified in the project in the Arabidopsis thaliana model plant will represent an excellent tool to identify homologues genes in crop varieties

to produce a durable resistance to pathogens. Among pathogens, *Botrytis cinerea* a necrotroph, responsible for pre-harvest and postharvest disease, is considered the second most important fungal pathogen at global level. Grapevine yield and quality is reduced by *B.cinerea* which causes bunch rot. *Vitis vinifera-B.cinerea* interaction will be studied to unravel the molecular bases of regulation of PME activity during infection. This research will be developed in collaboration with Dr. Claudio Moser Plant Biology and Physiology Unit-Research and Innovation Center -Fondazione Edmund Mach. A multidisciplinary approach including glycome profiling, LC-MS/GC-Mass Spectrometry and confocal microscopy will be exploited. The signal pathways triggering PME activity in Arabidopsis during infection will be identified by using different molecular approaches.

The specific objectives of the project include:

-Identification and characterization of pathogen-induced pectin metylesterification-related genes (PMR) in Arabidopsis and of grape orthologs in grapevine.

- Timing and levels of expression of Arabidopsis and grape *B.cinerea* induced PMR orthologs will be integrated with CW and fungal disease parameters

-Biochemical characterization of PMR proteins from Arabidopsis and grapes

- Signaling: identification of factors mediating transcriptional regulation of PMR during infection

- Identification of PMR secretion routes during *B. cinerea* infection

The results foreseen in this project will have an impact well beyond the species and pathogen here studied: most likely, analogous molecular mechanisms control PME activity during infection with other necrotrophic pathogens and in other crop species.

Stato delle conoscenze e referenze (max 4000 car.)

The intensification of agricultural practices and climatic changes lead to an increased need for crop protection against pathogens. The identification of new genetic traits able to improve the plant resistance to pathogens is necessary to assure yields and food security. Development of resistant cultivars represents a major environmentally friendly solution for both breeders and plant pathologists.

The plant cell wall (CW) is the foremost interface at which interactions between plants and pathogens take place. Fungal pathogens are responsible for the most devastating crop diseases.

Botrytis cinerea, a necrotrophic pathogen the cause of grey mold disease, is considered the second most important fungal plant pathogen at global level (Dean et al., 2012).

The plant susceptibility to necrothrophs and the efficiency of CW degradation is largely affected by CW composition and structure (Malinovski et al.,2014; Lionetti and Metraux, 2014).

Pectin is a main component of CW. Pectins are synthesized and methylesterified in the Golgi. Pectin is demethylesterified in the apoplast by pectin methylesterases (PMEs) which release protons and methanol in the apoplast. The level of pectin esterification affects the CW plasticity and integrity and makes pectin susceptible to the degradation by pectic enzymes. In several plants-microbe interactions the level of pectin esterification correlates with an increased resistance to pathogens (Lionetti et al., 2012;). PMEs belong to a large multigene family which members display different modes of action and are finely regulated during plant growth and development. PMEs are critical during plant-pathogen interactions and influence plant resistance to diseases although the regulation and role of the single PME isoforms need to be clarified (Bellincampi et al., 2014). Genetic, biochemical and molecular approaches have been shown that the reduced expression of specific PMEs in Arabidopsis and wheat resulted in a reduced susceptibility to fungal and bacterial pathogens (Bellincampi et al., 2014; Raiola et al., 2011 Lionetti et al., 2015). In addition to the transcriptional control, PMEs are regulated by endogenous serine-like proteases (SBTs) and protein inhibitors (PMEIs).

In addition to the multiple PME and PMEI isoforms induced in Arabidopsis by different pathogens (Lionetti et al. 2012) ; evidences indicate that specific PME and PMEI isoforms are also induced by *B.cinerea* in *Vitis vinifera* and can be involved in the plant response to the pathogen (Haile et al.,2017).

Biobliography

Bellincampi D et al. (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci 5: 228

Dean, R et al. (2012) The Top 10 fungal pathogens in molecular plant pathology. Mol.Plant Pathol., 13: 414

De Lorenzo G et al. (2018) Extracellular DAMPs in Plants and Mammals: Immunity, Tissue Damage and Repair. Trands Immunol.39:937

Haile ZM et al. (2017) Molecular analysis of the early interaction between the grapevine flower and Botrytis cinerea reveals that prompt activation of specific host pathways leads to fungus quiescence . Plant, Cell and Environment 1-20.

Komarova T et al. (2014) Cell wall methanol as a signal in plant immunity. Front Plant Sci. 5: 101.

Lionetti, V and Metraux, JP (2014) Plant cell wall in pathogenesis, parasitism and symbiosis. Front.Plant Sci., 5: 612.

Lionetti V et al.(2012) Methyl esterification of pectin plays a role during plant-pathogen interactions and affects plant resistance to diseases. J Plant Physiol 169:162330

Lionetti Ve t al. (2015) Cell wall traits as potential resources to improve resistance of durum wheat against Fusarium graminearum. BMC Plant Biology 15:6

Lionetti V et al. (2017) Three pectin methyl esterase inhibitors protect cell wall integrity for immunity to Botrytis. Plant Physiology 173: 1844

Malinovsky, FG et al. (2014) The role of the cell wall in plant immunity. Front.Plant Sci., 5: 178

Lavori pubblicati negli ultimi 5 anni dal Docente Guida (2015-2019)

1. Lionetti V., Raiola A., Cervone F., Bellincampi D. (2014) Transgenic expression of pectin methylesterase inhibitors limits tobamovirus spreading in tobacco and Arabidopsis. Molecular plant pathology 15:265-274

2. Tauzin A., Sulzenbacher G., Lafond M., Desseaux V., Reca I. B., Perrier J., Bellincampi D., Fourquet P., Lévêque C. and Giardina T. (2014) Functional characterization of a vacuolar invertase from *Solanum lycopersicum*: post-translational regulation by N-glycosylation and a proteinaceous inhibitor. Biochimie 101: 39-49

3. Bellincampi D, Cervone F., Lionetti V. (2014) Plant cell wall dynamics and wall related susceptibility in plant-pathogen interactions. Frontiers in Plant Science 5:228 doi: 10.3389/fpls.2014.00228

4. Lionetti V, Raiola A, Cervone F, Bellincampi D. (2014) How do pectin methylesterases and their inhibitors affect the spreading of tobamovirus? Plant Signaling and Behaviour 9:12 e972863 DOI 10.4161/15592316.2014.972863

5. Cook C., Francocci F., Cervone F., Bellincampi D., Bolwell P.G., Ferrari S., Devoto A. (2015) Combination of pretreatment with white rot fungi and modification of primary and secondary cell walls improves saccharification. BioEnergy Research 8:175-186 DOI 10.1007/s12155-014-9512-y

6. Lionetti V., Giancaspro A., Fabri E., Giove S., Reem N., Zabotina O.A., Blanco A., Gadaleta A., Bellincampi D. (2015) Cell wall traits as potential resources to improve resistance of durum wheat against Fusarium graminearum. BMC Plant Biology 15:6 doi:10.1186/s12870-014-0369-1

7. Lionetti V., Alessandro Raiola A., Mattei B. and Bellincampi D. (2015) The grapevine VvPMEI1 gene encodes a novel functional pectin methyl esterase inhibitor associated to grape berry development. Plos one 10(7):e0133810. doi: 10.1371/journal.pone.0133810

8. Reem N. T, Pogorelko G., Lionetti V., Chambers L., Held M.A, Bellincampi D., Zabotina O.A. (2016) Decreased Polysaccharide Feruloylation Compromises Plant Cell Wall Integrity and Increases Susceptibility to Necrotrophic Fungal Pathogens. Front. Plant Sci., 2016 10,7:630 doi. 10.3389/fpls.2016.00630

9. Tundo S., Kalunke R., Janni M., Volpi C., Lionetti V., Bellincampi D., Favaron F., D'Ovidio R (2016) Pyramiding PvPGIP2 and TAXI-III but not PvPGIP2 and PMEI

enhances resistance against *Fusarium garaminearum.* MPMI 29:629-639 doi: 10.1094/MPMI-05-16-0089-R

10. Lionetti V., Fabri E., De Caroli M., Hansen A. R., Willats W.G.T., Piro G., and Bellincampi D. (2017) Three pectin methyl esterase inhibitors protect cell wall integrity for immunity to Botrytis. Plant Physiology 173: 1844–1863 doi:10.1104/pp.16.01185

11. Rigano M.M., Lionetti V., Raiola A., Bellincampi D., Barone A. (2018) Pectic enzymes as potential enhancers of ascorbic acid production through the D-galacturonate pathway in Solanaceae. Plant Science 266: 55-63 DOI:10.1016/j.plantsci.2017.10.013

12. Giancaspro A., Lionetti V., Giove S. L., Zito D., Fabri E, Reem N., Zabotina O.A., De Angelis ., Monaci L., Bellincampi D., Gadaleta A. (2018) Cell wall features transferred from common into durum wheat to improve Fusarium Head Blight resistance. Plant Science 274.121-128

Fondi disponibili per svolgere il programma di ricerca.

2018- Ateneo “Sapienza” Università di Roma- progetti medi prot. RM11816432F244FD-” Pectin integrity regulation in plant immunity: new perspective in plant protection”

2018- Lazio INNOVA -PROGETTI DI GRUPPI DI RICERCA Conoscenza e Cooperazione per un Nuovo Modello di Sviluppo –Prot n. 85-2017-15080 -Tecnologie "green" per una agricoltura sostenibile: protezione da fitopatogeni e fertilizzanti di colture agroalimentari mediante biomolecole ottenute da reflui oleari.”

Collaborazioni con laboratori nazionali ed internazionali

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