DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di progetto di Dottorato

Titolo della ricerca: " Plant immunity mechanisms triggered by PME activity"

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DESCRIZIONE DELLA RICERCA

Obiettivi della ricerca (max 4000 car.)

The project aims at providing new knowledge to improve plant resistance to fungal disease moving towards a sustainable agriculture. Cell wall (CW) pectin forms a matrix composed of three main components called rhamnogalacturonan I, rhamnogalacturonan II, and homogalacturonan (HG), a major pectic polymer consisting of α-1,4-linked galacturonic acids. HG is secreted in a highly methyl-esterified form and selectively de-methyl-esterified by PMEs during plant physiology. Different plant PMEs are activated in plants upon pathogen attacks. PME activity could be involved in the production/activation of different defense alarm as Oligogalacturonides and/or Methanol. Moreover, de-methyl-esterification of HG could strengthen the cell walls, because It can form calcium bonds that promote the formation of supramolecular pectic gel that could contrast pathogen penetration. Despite this knowledge, the role of plant PME activity induced during defense has never been clarified. Using the combination of innovative approaches of molecular biology, glycomics, pathology and genetics of plants we attempt to unravel the role of plant PME activity in CW integrity sensing during plant immunity.

The specific objectives of the project include:

- Unveil the dynamic of the release of PME-related DAMPs during plant-pathogen interaction. Defence activated PME activity could assists the generation of Oligogalacturonides (OGs). OGs, the best characterized damage-associated molecular patterns (DAMPs), activate plant immune responses. PME also generates methanol (MeOH) which function as a systemic pectin-derived intra-plant or inter-plant alarm signal. The project aims to obtain new insight about the contribution of PME activity on the release of DAMPs or danger signals during pathogen attacks. The PME isoforms involved in the production of OGs and MeOH during disease will be identified and how their activity dynamically affects the release of these plant alarms will be defined.

- Effect of defense activated-PME activity on the pectin structure and plant immunity

Evidences indicate that plants modify the pectin structure to protect CW integrity against pathogens. A fine mapping of the alteration of CW composition in Arabidopsis WT and pme mutants will be investigated through glycome profiling. To identify pectin changes associated to PME activity during fungal penetration, the imaging of pectin dynamics will be investigated at the point of fungal penetration by using pectin molecular probes COS488, OG7-13 or monoclonal antibodies 2F4, JIM5 and PAM1 against pectin with different degree of methyl esterification or pectin calcium crosslinks. dyes as propidium iodide (PI) will be used to estimate the fraction of Ca^{2+} bound to HG.

Stato delle conoscenze e referenze (max 4000 car.)

The CW is the foremost interface at which interactions between plants and fungi take place(Lionetti and Metraux, 2014). Fungal pathogens use CW degrading enzymes (CWDEs) to degrade different polysaccharides of plant CWs, gaining access to host tissues and causing extensive maceration. HG, the major component of pectin, is an important target of the degradation. HG, is synthesized in the Golgi where it is methyl esterified by pectin methyl transferases and is secreted in an highly methyl esterified form to the CW. In this compartment, pectin methyl esterases (PMEs, E.C. 3.1.1.11), catalyse the de-methyl esterification of HG releasing free carboxyl ester groups, protons and methanol. PME isoforms may have different modes of action and produce HG with different degree and distribution of methyl esterification. The methyl esterification status of HG plays a critical role during plant–pathogen interactions and affects plant resistance to diseases (Lionetti et al., 2012)**.** In Arabidopsis several PME isoforms show an altered expression in response to *B. cinerea* and other pathogens suggesting that plants temporally coordinate the balance of PMEs during infection to regulate the degree and distribution of methyl esterification (Lionetti et al., 2017). The degradation of HG by pathogens is sensed by plants. HG breakdown fragments (e.g. Oligogalacturonides; OGs), released upon partial degradation of HG by fungal PGs, are the best characterized damage-associated molecular patterns (DAMPs) in plants (Lionetti, 2015). Some evidence indicates that PME activity is required to produce de-methyl esterified OGs with elicitor activity against *B. cinerea*. Since the degree and distribution of methyl esterification of HG varies in plant tissues during plant development and biotic stress, OGs released under pathogen attack may also have a different methyl esterification. PME is responsible for the release of methanol from plants, which may function as a systemic intraplant or interplant alarm signal by alerting adjacent non-infected tissues or neighboring plants. The molecular mechanisms behind methanol release and signalling are still largely unknown.

-Lionetti V,et al.Three Pectin Methylesterase Inhibitors Protect Cell Wall Integrity for Arabidopsis Immunity to Botrytis. Plant Physiol. 2017 Mar;173(3):1844-1863.
-Lionetti V. PECTOPLATE: the simultaneous phenotyping of pectin methylesterases, pectinases, and oligogalacturonides in plants during biotic stresses. Front Plant Sci. 2015 May 13;6:331.
-Lionetti V, Métraux JP. Plant cell wall in pathogenesis, parasitism and symbiosis. Front Plant Sci. 2014 Nov 6;5:612.
-Lionetti V et al.. Methyl esterification of pectin plays a role during plant-pathogen interactions and affects plant resistance to diseases. J Plant Physiol. 2012 Nov 1;169(16):1623-30.

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

1: Pogorelko GV, Juvale PS, Rutter WB, Hütten M, Maier TR, Hewezi T, Paulus J, van der Hoorn RA, Grundler FM, Siddique S, Lionetti V, Zabotina OA, Baum TJ. Re-targeting of a plant defense protease by a cyst nematode effector. Plant J. 2019 Feb 23. doi: 10.1111/tpj.14295. [Epub ahead of print] PubMed PMID: 30801789.

2: Giancaspro A, Lionetti V, Giove SL, Zito D, Fabri E, Reem N, Zabotina OA, De Angelis E, Monaci L, Bellincampi D, Gadaleta A. Cell wall features transferred from common into durum wheat to improve Fusarium Head Blight resistance. Plant Sci. 2018 Sep;274:121-128. doi: 10.1016/j.plantsci.2018.05.016. Epub 2018 May 23. PubMed PMID: 30080595.

3: Rigano MM, Lionetti V, Raiola A, Bellincampi D, Barone A. Pectic enzymes as potential enhancers of ascorbic acid production through the D-galacturonate pathway in Solanaceae. Plant Sci. 2018 Jan;266:55-63. doi: 10.1016/j.plantsci.2017.10.013. Epub 2017 Oct 26. PubMed PMID: 29241567.

4: Stavolone L, Lionetti V. Extracellular Matrix in Plants and Animals: Hooks and Locks for Viruses. Front Microbiol. 2017 Sep 12;8:1760. doi: 10.3389/fmicb.2017.01760. eCollection 2017. Review. PubMed PMID: 28955324; PubMed Central PMCID: PMC5600933.

5: Lionetti V, Fabri E, De Caroli M, Hansen AR, Willats WG, Piro G, Bellincampi D. Three Pectin Methylesterase Inhibitors Protect Cell Wall Integrity for Arabidopsis Immunity to Botrytis. Plant Physiol. 2017 Mar;173(3):1844-1863. doi: 10.1104/pp.16.01185. Epub 2017 Jan 12. PubMed PMID: 28082716; PubMed Central PMCID: PMC5338656.

6: Tundo S, Kalunke R, Janni M, Volpi C, Lionetti V, Bellincampi D, Favaron F, D'Ovidio R. Pyramiding PvPGIP2 and TAXI-III But Not PvPGIP2 and PMEI Enhances Resistance Against Fusarium graminearum. Mol Plant Microbe Interact. 2016 Aug;29(8):629-39. doi: 10.1094/MPMI-05-16-0089-R. Epub 2016 Jul 27. PubMed PMID: 27366923.

7: Reem NT, Pogorelko G, Lionetti V, Chambers L, Held MA, Bellincampi D, Zabotina OA. Decreased Polysaccharide Feruloylation Compromises Plant Cell Wall Integrity and Increases Susceptibility to Necrotrophic Fungal Pathogens. Front Plant Sci. 2016 May 10;7:630. doi: 10.3389/fpls.2016.00630. eCollection 2016. PubMed PMID: 27242834; PubMed Central PMCID: PMC4862258.

8: Lionetti V, Raiola A, Mattei B, Bellincampi D. The Grapevine VvPMEI1 Gene

Encodes a Novel Functional Pectin Methylesterase Inhibitor Associated to Grape Berry Development. PLoS One. 2015 Jul 23;10(7):e0133810. doi: 10.1371/journal.pone.0133810. eCollection 2015. PubMed PMID: 26204516; PubMed Central PMCID: PMC4512722.

9: Lionetti V. PECTOPLATE: the simultaneous phenotyping of pectin methylesterases, pectinases, and oligogalacturonides in plants during biotic stresses. Front Plant Sci. 2015 May 13;6:331. doi: 10.3389/fpls.2015.00331. eCollection 2015. PubMed PMID: 26029230; PubMed Central PMCID: PMC4429564.

10: Lionetti V, Giancaspro A, Fabri E, Giove SL, Reem N, Zabotina OA, Blanco A, Gadaleta A, Bellincampi D. Cell wall traits as potential resources to improve resistance of durum wheat against Fusarium graminearum. BMC Plant Biol. 2015 Jan 19;15:6. doi: 10.1186/s12870-014-0369-1. PubMed PMID: 25597920; PubMed Central PMCID: PMC4298115

11: Lionetti V, Raiola A, Cervone F, Bellincampi D. How do pectin methylesterases and their inhibitors affect the spreading of tobamovirus? Plant Signal Behav. 2014;9(12):e972863. doi: 10.4161/15592316.2014.972863. PubMed PMID: 25482766; PubMed Central PMCID: PMC4623000.

12: Lionetti V, Métraux JP. Plant cell wall in pathogenesis, parasitism and symbiosis. Front Plant Sci. 2014 Nov 6;5:612. doi: 10.3389/fpls.2014.00612. eCollection 2014. PubMed PMID: 25414718; PubMed Central PMCID: PMC4222219.

13: Lionetti V, Cervone F, De Lorenzo G. A lower content of de-methylesterified homogalacturonan improves enzymatic cell separation and isolation of mesophyll protoplasts in Arabidopsis. Phytochemistry. 2015 Apr;112:188-94. doi: 10.1016/j.phytochem.2014.07.025. Epub 2014 Aug 13. PubMed PMID: 25128920.

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.”

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