The role of E-2-hexenal and β-amino butyric acid in plant defense responses

A. Scala

SUMMARY

Plants are sessile and cannot avoid being attacked by an almost infinite number of microorganisms and herbivores. However, plants are masters in using molecular weapons against their attackers. Plant defense responses are the result of a complex signaling network, in which the hormones jasmonic acid (JA) and salicylic acid (SA) play a central role. However, also Green Leaf Volatiles (GLVs) must be taken into account as important players in plant defenses since they are very quickly produced / emitted upon herbivory or pathogen infection by almost every green plant. GLVs have been reported to be important in indirect defenses, but these volatiles are probably one of the fastest weapons exploited, being also able to trigger or prime plant defense responses. In this thesis *E*-2-hexenal is the GLV of choice to study because it has antimicrobial activity, can induce defense related genes, it is a Reactive Electrophile Species (RES), triggers γ-amino butyric acid (GABA) accumulation and inhibits root growth. In addition, we focused our research on GABA, a non-protein amino acid, which is reported to be induced by different stresses and it is tightly connected with *E*-2-hexenal.

In *chapter 2* of this thesis we investigated the effect of a mutation in the *HYDROPEROXIDE LYASE (HPL)* gene on the Arabidopsis-DC3000 (*Pseudomonas syringae* pv. *tomato* strain DC3000) interaction. As a major consequence of this *HPL* mutation we found that the susceptibility to the bacterial pathogen was reduced compared to wt, due to differences in the levels of defense hormones during the infection. Indeed JA levels were lower in the *hpl* mutant at 48 hpi while SA levels were higher at 24 hpi than in the wt, indicating that SA-dependent defenses are activated earlier in *hpl1* than in wt plants. Arabidopsis defenses are regulated by these two major phytohormones that are well-known to be have antagonistic effects. Moreover, we found that a treatment of Arabidopsis plants with *E*-2-hexenal prior to infection, resulted in an increased susceptibility to DC3000, partially mediated by coronatine, (a JA-isoleucine mimic made by DC3000 to

antagonize SA-dependent defenses) and partially via OCTADECANOID-RESPONSIVE ARABIDOPSIS 59 (ORA59)-dependent JA signaling pathway in the plant.
In *chapter 3* we tried to dissect the role of different GLUTAMATE DECARBOXYLASE (GADs) in response to biotic and abiotic stress. In literature it is stated that the major GAD isoforms involved in the GABA biosynthesis are GAD1, GAD2 and GAD4. Overall we found that different combinations of *gad1, gad2 and gad4* mutations had different effects, not only on the plant susceptibility to DC3000 and JA and ABA levels, but also on root system architecture during salt stress. Our data indicate that GABA might interferes with the hormonal crosstalk used to tightly control these stress responses.
In *chapter 4* we characterized an *E-2-hexenal response* mutant, *her2*, which mapped to the gene At5g63620 that encodes a putative GroES-like zinc-binding alcohol dehydrogenase. We confirmed its mitochondrial localization and we found that HER2 shares high similarity with a SSADH-Acetylating enzyme from a thermoacidophilic bacterium, *Metallosphaera sedula.* The overexpression of *HER2* induced a extremely sensitive growth root phenotype in seedlings after the *E*-2-hexenal treatment, thus confirming the role of HER2 in mediating *E*-2-hexenal response. Moreover the *her2* mutant showed higher susceptibility to DC3000, indicating that HER2 has a role in plant defense responses. Interestingly we also found that the *E*-2-hexenal specifically oxidized the redox state of the mitochondria.
In *chapter 5* we developed Arabidopsis lines in which the HPL expression is driven by chemical induction with the aim to have a tool to study GLVs during, for example, the DC3000-Arabidopsis interaction.
Overall our research elucidated new aspects of GLVs, especially *E*-2-hexenal, and its interaction with GABA and opened new interesting scenarios that are worth to follow for further research especially by using the tools we developed in this thesis i.e. *gad* mutants and HPL inducible Arabidopsis lines.