Research interests

Understanding plant – pathogen interaction on the molecular level

Summary

As sessile organisms, plants have to cope with numerous abiotic and biotic stresses in order to survive and successfully reproduce. Phytopathogens such as microbes, fungi, oomycetes, arthropods and parasitic plants pose major biological threats to plants and cause tremendous crop losses worldwide. In agronomy, crop protection against biotic stressors is essential to guarantee sustainable global food production. Thus, it is of great relevance, and one of the major interests of my research team, to understand molecular resistance mechanisms of plant defense against all kinds of phytopathogenic invaders. This knowledge serves as a basis to prevent crop failures.

For plants, it is critical to sense pathogenic attackers at the cell surface. This is accomplished by receptor proteins which are able to recognize “danger” either as endogenous signals (Damage-associated molecular patterns, DAMPs) or as exogenous signals (Microbe-associated molecular patterns (MAMPs). MAMP/DAMP perception switches on intracellular signaling cascades which activate cellular defense programs in infected host plants and ultimately lead to resistance. In our research projects, we focus on molecular patterns, their perception via membrane-bound receptors and the activation of cellular signaling programs in the context of plant—plant interactions. Identified resistance-conferring receptors together with the corresponding molecular pattern of any pathogen, allow for a detailed analysis of the co-evolutionary aspects of plant and pathogen. Moreover, identified resistance mechanisms serve as important basis to advance breeding efforts and to provide biotechnological approaches for sustainable crop protection.

Methods – Summary

To measure “defense”, “resistance” or “susceptibility” of plant—pathogen systems in a quantitative manner we apply multiple established bioassays. We have diverse pathogen growth assays to determine the growth of bacteria, oomycetes, fungi and parasitic plants on host plants. Additionally, we monitor immune activation by measuring stress-related phytohormone ethylene, reactive oxygen species (ROS), MAP-Kinase activation or the induction of defense marker genes in plant samples treated with pathogen-derived elicitors. All of these methods serve as convenient tools for the quantitative measurement of defense responses to pathogens, elicitors or well-defined molecular patterns. For treatments of plant samples, we use crude pathogen extracts, (pre-) purified or synthesized defense-triggers (e.g. chitin, peptides, etc.).

To successfully identify novel resistance-related receptors, we genetically screen crop germplasms, including natural cultivars/accessions, crossings thereof or existing introgression lines. To find new pathogenic elicitors, we prepare extracts of a pathogen (microbe, fungi, parasitic plant, etc.) and purify it using analytical methods (chromatography, mass spectrometry) to isolate and ascertain the defense-triggering elicitor. With receptor—ligand pairs at hand, we apply biochemical approaches to quantitatively assess receptor-ligand binding by e.g. “surface plasmon resonance” (SPR) or microscale thermophoresis (MST). In summary, we have assembled a robust tool-kit for detailed analyses of molecular mechanisms going on during plant-pathogen interactions.

Description of research projects

Molecular mechanisms of susceptible and resistant plant—plant interactions

Plant parasites directly connect to the vasculature of host plants thereby stealing water, nutrients, and carbohydrates consequently leading to tremendously reduced biomass and losses in seed yields of the infected host plants.

The major goals of my research team are the elucidation of molecular mechanisms during the interaction of parasitic plants with host plants. Thereby we are following two major routes and try to address both phenomena, susceptibility and resistance (see overview in Fig. 1). In susceptible interactions, the parasitic plant hooks up certain, yet unknown, plant developmental signaling pathways to abuse it by redirecting and withdrawing nutrients, water and carbohydrates. Up to date nearly nothing is known about how such connection is established while the infection stays unrecognized by the parasitized host.

Complementary, working with host plants that are resistant to an infection by a parasitic plant, we aim to understand this stress-avoidance on molecular level. Does plant resistance against parasitic plants function in a similar manner such as known for plant resistance against microbial pathogens?

In terms of agronomic applications, steps to understand the molecular mechanisms behind both, susceptibility and resistance, together with the successes in recent breeding efforts will provide fundamental knowledge to generate resistant crops.

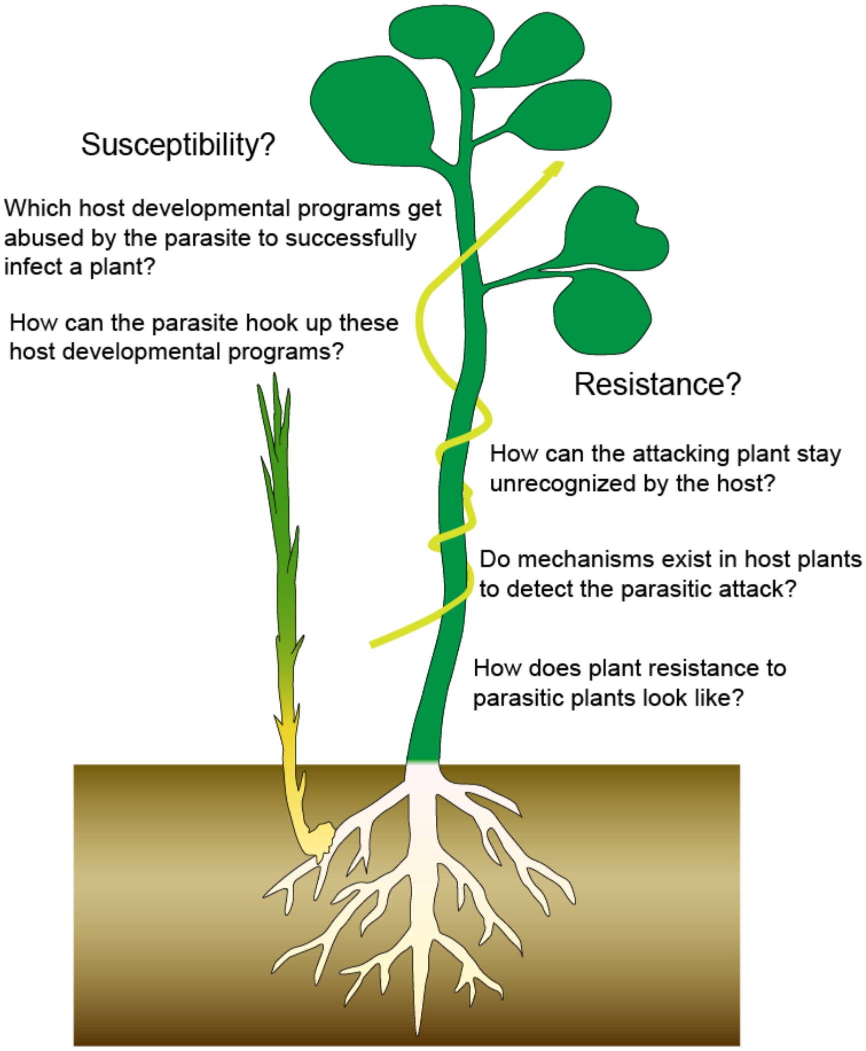

Fig. 1 Overview plant–plant interaction projects; some of the questions addressed by the Albert Team. Picture shows a model host plant infected by both a root parasite (e.g. *Orobanche* or *Striga* spp.) and a shoot parasite (*Cuscuta* spp.)

a) Plant-plant interactions of *Cuscuta* spp. with hosts

At present, we are mainly using *Cuscuta* spp. (Fig. 2) as a model plant parasite to addressing the questions raised above. Living as obligate holoparasites with a very broad host spectrum, plants of the genus *Cuscuta* possess neither roots nor expanded leaves. To survive, *Cuscuta* enwinds the stems of host plants and develops haustoria, establishing connections directly to the host's vascular bundles. This haustorium penetration and accompanying wounding seems to go undetected by most host plants and the parasite succeeds in representing itself as an attractive sink by probably mimicking a part of "host-self". The chances and possibilities of hosts for defence may differ from those known for plant-microbe interactions. Given their significant evolutionary distance from microbes, over time all higher plants have established an innate immune system to defend against microbial pathogens.

By means of pattern recognition receptors (PRRs), plants can sense so-called microbe-associated molecular patterns (MAMPs) and initiate defensive signaling programs that help the plant to restrict pathogen growth.

How can plants detect plants? We are interested in identifying signals and bioactive molecules on the parasite's side that trigger visible or measurable responses in the host plant, for either plant development or for defense. On the host side, we study signaling and responses in the infested plant, focusing on perceptual systems that initiate downstream developmental signaling programs and control cellular responses.


Fig 2. *C. reflexa* on *Coleus blumei*. (susceptible host)

b) A chance exception: Cultivated tomato is resistant to *Cuscuta reflexa*

The relationship between cultivated tomato (*Solanum lycopersicum*) and *Cuscuta reflexa* offers a convenient tool for gaining insights into how secreted parasitic signaling molecules might trigger a host plant reaction. Tomato is resistant to *C. reflexa* and actively responds with a clearly visible hypersensitive-like reaction precisely at the penetration site of *C. reflexa* haustoria (Fig. 3). This strong response leads to tomato cell-wall suberinization and prevents the host from haustoria penetration, and consequently leading to the death of *C. reflexa* (Fig. 3). Such defense reactions are unique to cultivated tomato and do not occur in other plants of the *Solanaceae* family.

In search for the parasite's defense-triggering molecules, extracts of *C. reflexa* stem and haustoria were tested for their capability to trigger typical MAMP responses specific to tomato, such as ethylene production, oxidative burst, or the induction of marker genes.

The ethylene response seemed to work best in tomato but was absent in other related (non-resistant) plants of the Solanaceae (e.g., *S. tuberosum, Nicotiana tabacum,* or *Nicotiana benthamiana*), and so it was further used to screen *Cuscuta*-extracts for defense triggering molecules. Using stepwise purification of crude parasitic extracts by chromatography and monitoring of each purification step for its ability to trigger ethylene production, we purified a small Glycine-Cysteine-rich protein, which is the trigger of the defense responses in cultivated tomato. This protein localizes to the cell walls of *Cuscuta* and can be found in all parts and organs of the plant. Probably, its original function is related to cell wall stability and flexibility, especially for the middle lamella. More detailed analyses are currently in progress.

Fig 3. *C. reflexa* on resistant *S. lycopersicum* ~14 d post infestation

How do such defense-triggering molecules get sensed by tomato? After finding a wild tomato species, *Solanum pennellii*, that is insensitive in the ethylene bio-assay, we screened a collection of ~50 introgression lines (*S. lycopersicum x S. pennellii*) (Eshed & Zamir, 1995) for ethylene response and mapped one essential locus: On chromosome eight we identified a gene encoding a Leucine-rich repeat receptor-like protein (LRR-RLP), which we termed **Cuscuta Receptor 1 (CuRe1)**. After heterologous expression of the *CuRe1* gene in *N. benthamiana* (usually insensitive to *C. reflexa* extracts), this plant started to respond by means of ethylene production when treated with crude *C. reflexa* extracts or with pure Cuscuta factor peptide. This Peptide detection occurred in a highly sensitive and dose-dependent manner. In addition, when infecting transgenic, *CuRe1*-expressing *N. benthamiana* plants with *C. reflexa,* plants were significantly more resistant to parasite invasions demonstrating that CuRe1 contributes to tomato's resistance against *Cuscuta*. The first part of this project was published in Science (Hegenauer et al. 2016). A manuscript for the recently identified parasitic molecular pattern, termed as Cuscuta factor, is currently in preparation.

Future aims in short and long term

In addition to the locus on tomato chromosome 8, we mapped a second resistance-relevant locus on tomato chromosome 12. We identified a gene-cluster whose genes encode for potential resistance receptor genes. These receptor genes were cloned and are currently under *in depth* investigation – also for the ability to sense microbial pathogens. In comparison to CuRe1 which initiates typical responses of a pattern-triggered immunity, the second locus seems to be rather responsible for resistance comparable to effector-triggered immunity. These findings indicate a second layer of resistance present in tomato also described for plant-microbe interactions in the famous zig-zag-model by Jones and Dangl in 2006. Our recent findings as well as ongoing and future research will majorly contribute to create and to substantiate the bigger picture of host plant immunity against parasitic plants and to establish a model similar to the zig-zag model such as described for the interaction of plants with microbes (Jones and Dangl, 2006).

In the long term, we further aim to transfer our knowledge gained from the tomato—*Cuscuta* system to other plant—plant systems as well as to plant—microbe interactions. Understanding these mechanisms of immunity will deliver ground-breaking insights to create crops resistant to parasitic plant and pathogen attacks.

c) Parasitic molecular cues and their effects to susceptible hosts

The recognition of *C. reflexa* as “alien” by CuRe1 and other receptors is an exclusive exception of tomato. Usually, the parasite stays unrecognized by its host and actively manipulates it towards susceptibility: parenchymal cells of the host's vascular bundles start to divide, differentiate and finally connect to the parasitic cells of both Xylem and Phloem. What type of molecular cues initiate and regulate these mechanisms of susceptibility?

In a very recently DFG-funded project (AL 1426/4-1) and related preliminary work, we cloned promoters of host plant genes that are up-regulated in host plants during a *Cuscuta* infection. Using them as promoter::reporter-gene (luciferase) constructs in plant systems, these constructs serve as tools to detect luciferase activity upon treatment with stepwise purified *Cuscuta* extracts and will finally help to identify new Cuscuta factors (CuFs) that manipulate host plant signaling in a manner of virulence factors such as known from microbial invadors. First results indicate the presence of different new CuFs, which will be further characterized and identified.

In a complementary project we aim to identify the detection mechanisms for parasitic CuFs in host plants and want to gain knowledge about the susceptibility-related cellular signaling. We will use *Arabidopsis* receptor mutants for which collections are available in our laboratory. Mutant plants will be grown, infected with *Cuscuta* and further analyzed in regard to altered susceptibility. Subsidiary, we have diverse *Arabidopsis* accessions at hand (1001 Genomes project) that we will check. Relevant genes of obtained phenotypes with disturbed susceptibility will be identified either by classical map-based cloning or by using Genome-wide association studies (GWAS).

Novel components of CuF-perception or related signaling components are further analyzed with well-established methods such as studies on receptor-ligand interaction, localization, protein-protein interaction, etc. Furthermore, we are proving the biological relevance by determining the *Cuscuta* biomass after growth on mutants or transgenic plants. This will help to decipher the molecular and biological role of novel components required to establish a plant-plant interaction.

Future aims in short and long term

Which host plant signaling routes are part of the susceptible plant-plant interaction? Which host plant receptors are involved? Since the parasite is not a microbe but also a plant, it has all the molecular tools and mechanisms to hook up the required host plant developmental programs and related perception mechanisms. In the long term, we aim to identify the required components such as phytohormones, proteinaceous and low-molecular signaling cues together with the related receptors and signaling cascades that may get sequentially activated in a spatial and temporal order during the long-lasting process of the plant-plant connection. Understanding susceptibility and related signaling will open new perspectives and may help to control parasite growth, e.g. by designing susceptibility-antagonizing substances which act as blockers of a parasite invasion. Moreover, knowledge about susceptibility may also help to understand and force symbiotic interactions of plants with microbial or fungal partners.

In the long-term, it will be a further aim to study the parasite's strategy of redirecting and withdrawing nutrients, solutes and carbohydrates when the parasite is properly connected to the host. How does the parasite increase the host's photosynthesis rate, the carbon fixation, nutrient- and water-uptake? What can we learn from this and how could we use such mechanisms to maybe tune crop plants?

The agronomical impact of root parasitic broomrapes

The root holoparasitic plant *Phelipanche ramosa* infecting cultivated tomato (*Solanum lycopersicum*).

Photo by Dr. Mouna Khalloufi

Parasitic plants of the genus *Striga*, *Orobanche* or *Phelipanche* are obligate hemiparasitic plants, which cause severe damage and crop failure to sunflower, maize, rice, tomato, fababeans and other crops.

In frame of an EU-/BMBF-funded project within the PRIMA-program an international collaborative research project has been established together with a company and ten Research Groups from Greece, Tunisia, Jordan, Egypt, Spain and Morocco termed as "ZeroParasitic". In this project, we aim to find "**Innovative Sustainable Solutions for broomrapes: Prevention and Integrated Pest Management approaches to overcome parasitism in Mediterranean cropping systems"** (official project title).

ZeroParasitic is a three-year project, aiming to deliver innovative sustainable solutions to overcome broomrape plant parasitism in key Mediterranean cropping systems. Genetic and molecular approaches will be used at three critical levels to gain new insights on potential regulatory targets of the infection: the broomrapes *per se*, the host plants and their interaction (host-parasite). Research will target two of most important Mediterranean crops such as industrial tomato and faba beans. Surveillance tools utilizing remote and satellite images will be employed for monitoring and large-scale parasitism documentation. Innovation tools will consist of molecular approaches for screening and identification of tolerant/resistant hosts and hormone host-parasite interactions. Deciphering the molecular basis of resistance by utilizing pattern recognition receptors (PRRs) and defense-triggering molecular patterns are aiming to support breeding of resistant host plants. Solutions will be part of an integrated pest management (IPM), targeting several innovations such as prevention, biological/non-chemical approaches, and other IPM strategies. Agronomic practices will be scientifically supported by a validated “Decision Support System” (DSS) across various Mediterranean locations and farming systems. Solutions will be socioeconomically evaluated, implemented and disseminated in a participatory way. Solutions will be highly accessible via an innovative web platform designed to satisfy requirements of a wide range of end-users. The project is expected to start in the beginning of 2019.