Research group: “The functions of lipopolysaccharide in plant-bacteria interactions”

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Picture credit: Astrid Eckert/TUM

How Plants **Fight Back**

The immune system of plants is currently a hotbed of research. How do plants detect disease-causing agents such as bacteria and how do they combat these pathogens? Stefanie Ranf is interested in answers to these questions. She hopes that the findings from her research will help equip plants with natural protection against pathogens and secure our food supplies.

The model plant *Arabidopsis thaliana* (thale cress) is often used for laboratory testing. Its relatively small genome is fully sequenced and can easily be genetically manipulated for research purposes.

Claudia Doyle

Wie Pflanzen sich wehren

Pflanzen müssen sich genau wie Tiere gegen Krankheitserreger verteidigen. In einer Pflanze sind nahezu alle Zellen in der Lage, eine effiziente Abwehrreaktion gegen Krankheitserreger in Gang zu setzen.

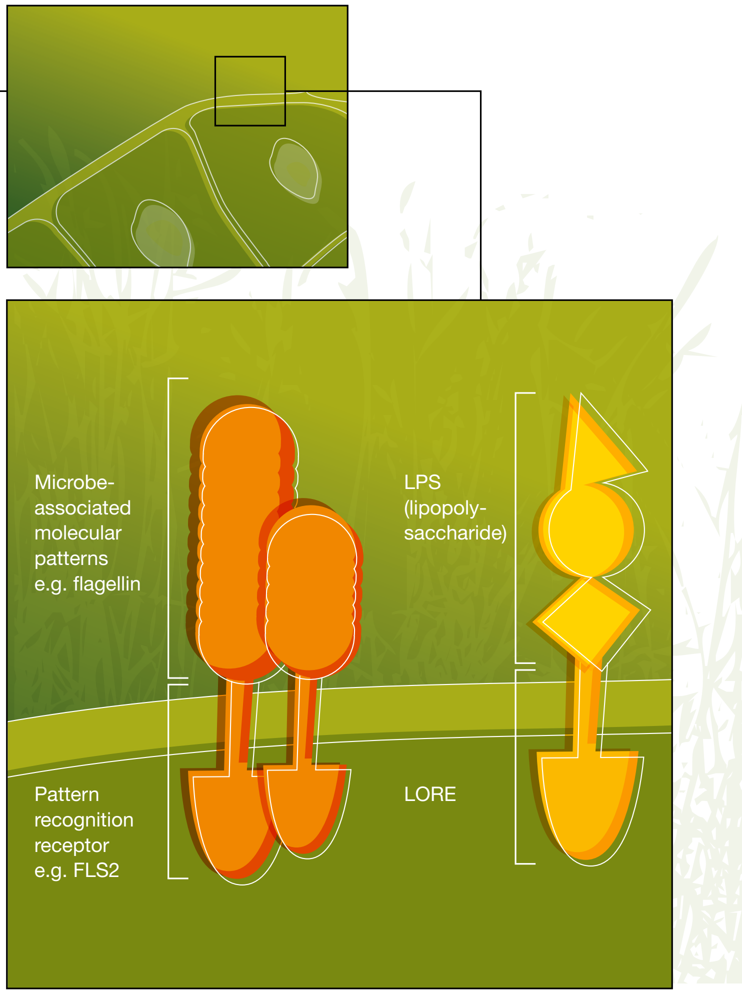
Voraussetzung für eine Immunreaktion ist, dass der Angriff überhaupt bemerkt wird. Meist nutzen Pflanzen dazu Immunrezeptoren auf der Zelloberfläche. Sie funktionieren wie eine Antenne. Die Rezeptormoleküle durchspannen die äußere Zellmembran und schauen an beiden Seiten hinaus. Erkennen sie außerhalb der Zelle verräterische Bakterienmoleküle, so leiten sie die Information ins Zellinnere weiter.

Einen besonders wichtigen Rezeptor namens LORE hat Dr. Stefanie Ranf vor Kurzem entdeckt. LORE erkennt das bakterielle Molekül Lipopolysaccharid, kurz LPS genannt. Jedoch besitzen nur Kreuzblütler wie Kohl, Senf oder Raps den LORE-Rezeptor. Mithilfe von gentechnischen Verfahren ließe sich LORE von Kreuzblütlern auf wichtige Nahrungspflanzen übertragen. Dies hätte keine Auswirkungen auf Geschmack, Aussehen oder Ertrag der Pflanzen. Es würde sie lediglich resistenter machen.

Stefanie Ranf interessiert sich nicht nur dafür, wie Pflanzen ihre Feinde wahrnehmen. Die Biochemikerin will auch herausfinden, mit welchen Strategien die Bakterien sich tarnen und vor dem pflanzlichen Immunsystem verstecken. „Es ist ja nicht so, dass die Bakterien nur darauf warten, von der Pflanze entdeckt und umgebracht zu werden“, sagt die 39-Jährige, die am Wissenschaftszentrum Weihenstephan der TUM eine eigenständige Forschungsgruppe leitet.

Vermutlich verändern die Bakterien auch ihr LPS dermaßen, dass Pflanzen es nicht mehr erkennen können und sie gleichzeitig resistenter gegen die Abwehrreaktionen der Pflanzen sind. Von Bakterien, die Tiere infizieren, ist ein solches Verhalten bereits bekannt.

Ranfs Forschung ist nicht nur wichtig für die Züchtung krankheitsresistenter Kulturpflanzen. Auch die Sicherheit von Lebensmitteln soll davon profitieren. „Es wäre schön zu sehen, dass das, was wir machen, auch einen nachhaltigen Effekt hat. Das wäre die beste Belohnung für unsere Arbeit.“ □



Plants have to defend themselves against attack, so it is vital that they can identify invaders in the first place. Plant cells are thus equipped with various surface receptors to detect bacterial molecules and activate defense mechanisms inside the cells – one of them is LORE: LipoOligosaccharide-specific Reduced Elicitation.

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Around a year ago, Dr. Stefanie Ranf took the stage in an auditorium in the German city of Hamburg. She opened her laptop, picked up the microphone and began to talk about her research into plant immunity. Her audience consisted solely of fellow researchers – all of whom were investigating the immune systems of humans and animals, as well as bacteria and their toxins.

What Ranf had to say astonished her listeners. They began whispering and murmuring how fascinating it was to hear that even plants have an immune system. “I was really very surprised that there was so little awareness around our research up until that point,” recalls Ranf. A group leader at the TUM School of Life Sciences Weihenstephan, she specializes in plant-bacteria interactions.

Of course, just like animals, plants also have to defend themselves against pathogens. Both animals and plants thus possess a natural, innate immune system. Animal cells are additionally equipped with an adaptive immune system, which uses specialized immune cells and antibodies to ward off invaders. Plants, however, lack this type of system.

On the other hand, in plants, almost every cell is able to trigger an efficient immune response to pathogens. “The plant system isn’t weaker; it’s just different,” confirms Ranf. And exploration of plant immunity is now advancing by leaps and bounds.

The 39-year-old biochemist is interested in understanding how plants detect pathogens, how they defend themselves against them, and what processes take place inside the cells as this occurs. Plants with healthy defenses are essential to secure human food supplies. Our ability to harvest sufficient volumes of healthy foodstuffs in the future depends on crop resistance to disease. ▷

Dr. Stefanie Ranf

An inquisitive mind

Stefanie Ranf studied biochemistry at the University of Regensburg. With a degree under her belt, she decided to spend time abroad, joining the University of South Carolina in Columbia (USA) as a visiting researcher investigating MAP kinase signal transduction. "I learned to work independently over there," recalls Ranf. "It would never have crossed my mind to ask my boss what I should do next."

Her interest in plant research also stems from that period. On returning to Germany, she thus sought out a green topic for her doctoral thesis, which she completed at the Leibniz Institute of Plant Biochemistry in Halle/Saale (Germany).

In 2013, Ranf started leading her own research group at the Chair of Phytopathology (Prof. Ralph Hückelhoven) at the TUM School of Life Sciences Weihenstephan. The 39-year-old particularly appreciates the interdisciplinary collaboration between the various faculties at TUM. "Life is really easy here, because people are genuinely keen to achieve things together," she affirms.

Ranf is now trying to foster the same autonomous work ethic in her students that she previously benefitted from herself. "If someone wants to produce lab equipment with a 3D printer or test a new approach to an experiment, I certainly won't stand in their way."

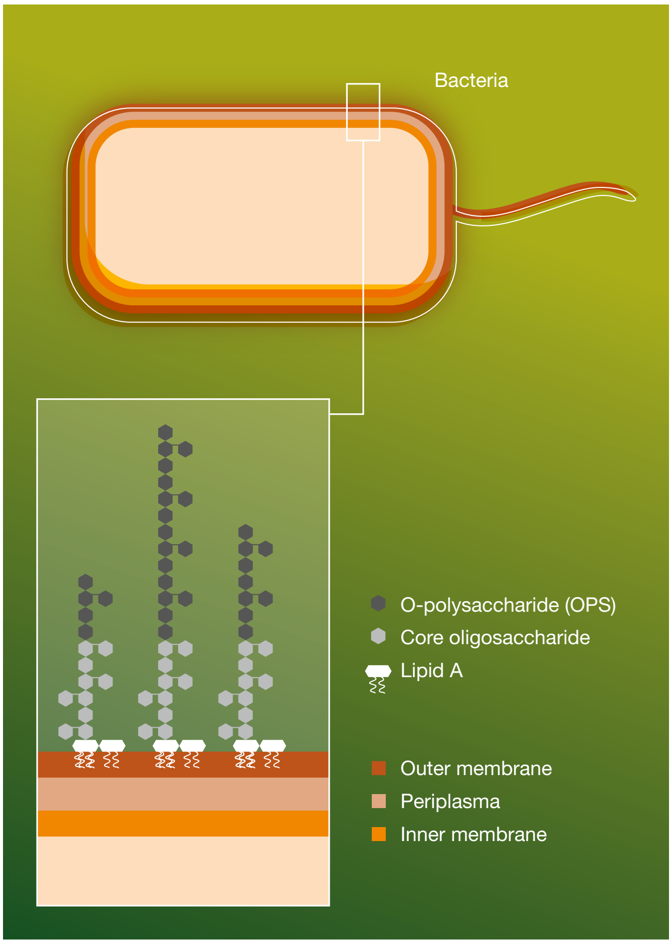
Since 2015, Stefanie Ranf has also been a member of the Collaborative Research Center 924 (SFB924), which investigates molecular mechanisms regulating yield and yield stability in plants. In 2016, she was awarded an Emmy Noether fellowship by the German Research Foundation (DFG). Since then, Ranf has been researching both the plant immune system and pathogenic bacteria with an independent research group.

Picture credits: Astrid Eckert/TUM

"It would be great if we could make a lasting difference through our work." *Stefanie Ranf*

Every plant cell has a natural immunity mechanism
To trigger an immune response, a plant obviously has to first detect an attack. Plants usually use immune receptors for this purpose: molecules that traverse the external cell membrane and protrude from each side. These work like antennas. If they sense telltale bacterial molecules outside the cell, they transmit this information to its interior.
It has been clear since at least the 1970s that bacterial molecules trigger immune responses in plants. The molecules in question are often common to multiple different types of bacteria. A case in point here is flagellin, the protein forming the bacterial flagellum that enables microorganisms to move. Another is lipopolysaccharide, or LPS – the main component of the outer membrane of Gram-negative bacteria. These include the Salmonella and coliform bacteria dangerous to humans, for instance, as well as others pathogenic to plants.
However, the receptors plants use to recognize these molecules went undetected for a very long time. The initial breakthrough came when the flagellin receptor was identified seventeen years ago. But the LPS receptor was to evade discovery until relatively recently.

Initial contradictions
Since LPS occurs in so many types of bacteria, the LPS receptor is an important checkpoint in efforts to improve the plant immune system. Soon, plant researchers all over the world were carrying out tests to determine which plants were equipped with the right antennas to "pick up" LPS. Yet as the findings streamed out of the labs, contradictions emerged. Each working group was investigating different bacteria in different plants, leading to a jumble of individual studies and little meaningful progress.
In part, this was due to the elusive nature of LPS – an extremely complex and variable molecule with challenging chemical properties. Its size and structure vary from one bacterial species to another, and even bacteria of the same species form extremely different LPS variants. Only its basic composition always remains the same.
"Another problem was that no one knew how plants sense LPS," Stefanie Ranf explains. The LPS receptor in animals had already been identified but it has little in common with the plant-specific equivalent. The two systems evolved independently of each other.
And so Ranf took matters into her own hands, testing more than twenty thousand different seedlings of the model plant thale cress *(Arabidopsis thaliana)* to determine whether their immune system was activated by LPS. And since good LPS is hard to source, she asked her colleagues at Germany's Research Center Borstel (Leibniz-Center for Medicine and Biosciences) to show her how to isolate it from bacteria so she could do it herself. ▷



Bacteria are surrounded by multiple membranes. The outer membrane includes LPS molecules (lipopolysaccharide). They don't all look the same, differing significantly in size and structure. However, their basic composition always remains the same, with lipid A – a di-phosphorylated di-glucosamine carrying four to seven fatty acids – embedded in the outer cell membrane. Chains of sugar molecules – some lengthy and branching – protrude from the cell surface.

Graphics: ediundsepp (source: TUM); Picture credit: Astrid Eckert/TUM

Various mutations were created in the genome of these *A. thaliana* plants, occurring at random points. Possibly also in the gene encoding the immune receptor? Stefanie Ranf harvests the seeds from this first plant generation.

Ranf's first few weeks in the lab brought her no further. In the end, though, she was able to identify four plants that displayed no response to contact with LPS. Could it be that their LPS receptor was faulty? She tested this hypothesis by genetically mapping and sequencing the mutation, then comparing it with the reference genome for thale cress. Sure enough, she found that all these plants exhibited a mutation in a gene that coded a receptor whose function was previously unknown. She named this receptor LORE.

Only cruciferous plants recognize LPS

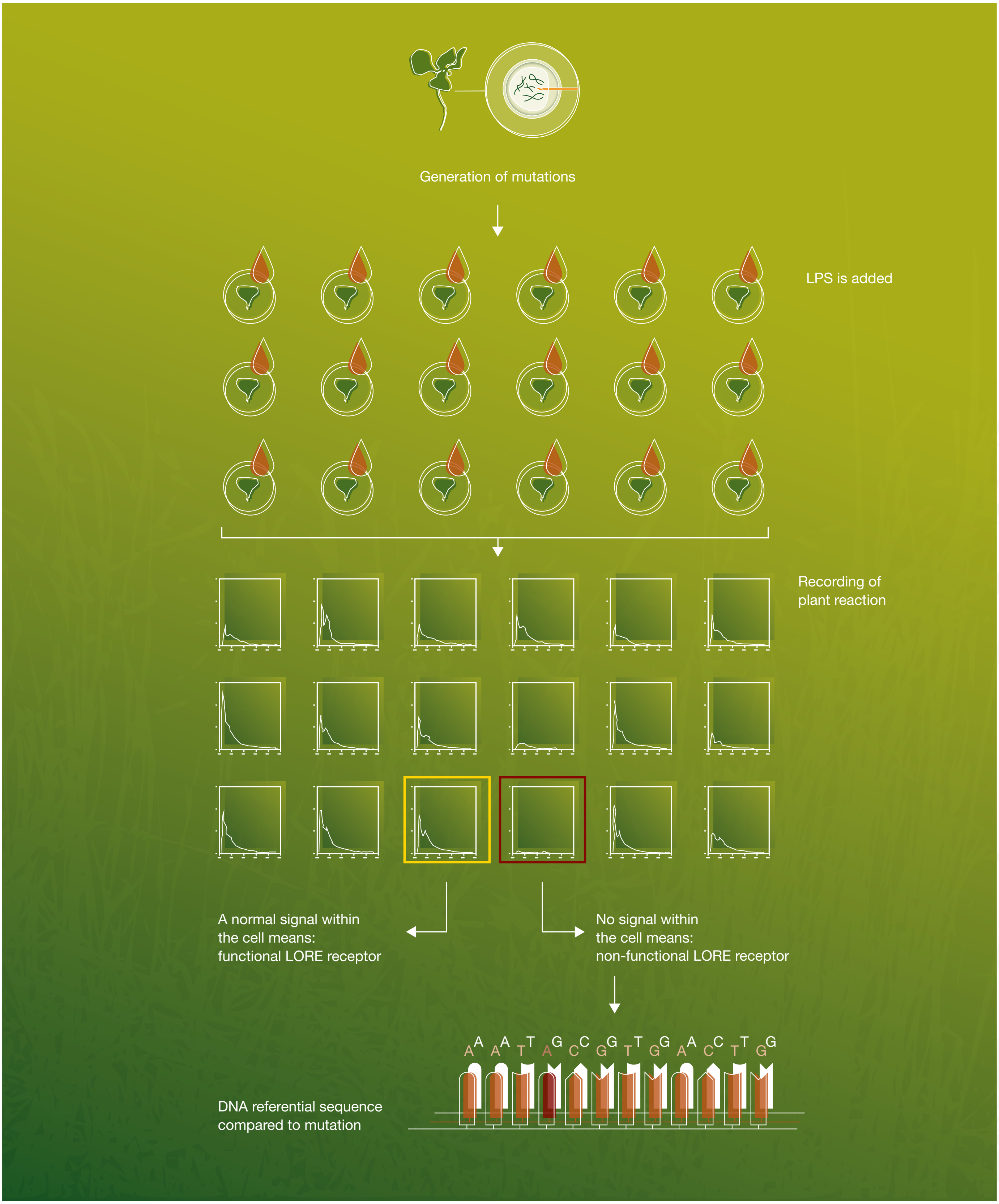
Other cruciferous plants such as cabbage, mustard and rapeseed are also equipped with the LORE receptor on the surface of their cells and are thus able to detect LPS. Or, to be more precise, not LPS in its entirety but the section at the end of the molecule known as lipid A – a sugar molecule with fatty acids of varying lengths.
Interestingly, LORE does not recognize LPS in coliform or Salmonella bacteria. However, it reacts very strongly to LPS in bacteria of the Pseudomonas and Xanthomonas genera. Depending on the species, pseudomonads affect olive trees, cucumbers and tomatoes, among others. Meanwhile, targets for Xanthomonas include citrus fruits, bananas and rice. All of these plants have one thing in common: they lack LORE – the antenna that would enable them to recognize LPS.

LORE could be transferred from cruciferous plants to other important food crops using genetic engineering. The benefits of this type of genetic crop protection are clear. To understand how it works, however, we need to look a little more closely at the plant immune system.

Receptors activate defenses

Plants have hundreds of different receptors on the surface of their cells, which they use to sense the surrounding environment. As soon as they detect a pathogen, their defense mechanism kicks in. They strengthen their cell walls to hold back intruders, switch their metabolism to defense mode and produce oxygen radicals and antimicrobial substances to kill off invaders.
"All plants are equipped with this system – the receptors just switch it on," describes Ranf. Transferring LORE from cruciferous crops to other plants would have no impact on their taste, appearance or yield, she explains. It would simply improve their resistance.
To date, though, the use of green genetic engineering has been viewed with suspicion in Germany. So Ranf is continuing her quest to identify LORE or other LPS receptors in other plants. With clever cross-breeding, these receptors could then be introduced to specific plants to create stronger lines. ▷

The seedlings grow in a sterile medium. When they reach a sufficient size, each seedling is placed in an individual compartment.

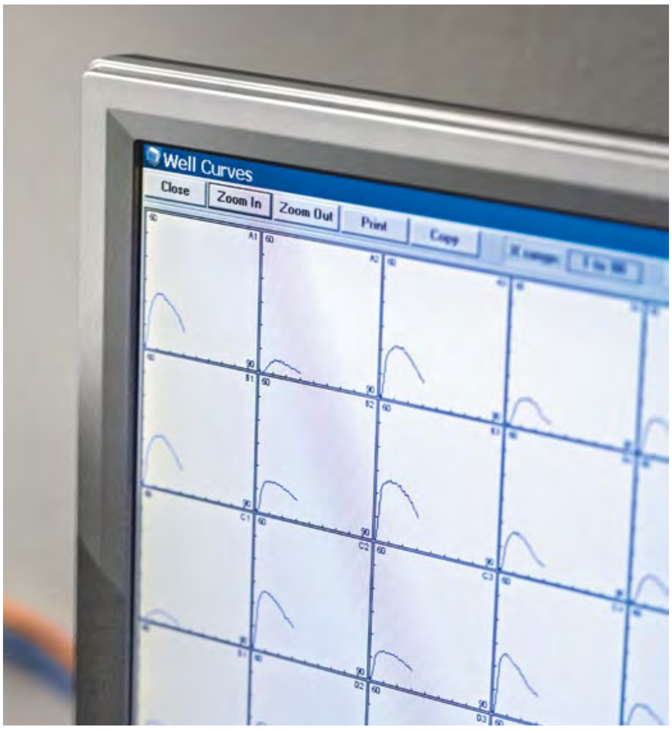


Graphics: ediundsepp (source: TUM); Picture credits: Astrid Eckert/TUM

The seedlings from the mutated plants are germinated in a sterile medium, then LPS is added. If the plants react to LPS, there is a spike in the graph. If this is missing, the plants are not reactive to LPS and are likely missing the relevant receptor to sense it. These LPS insensitive plants all exhibit a mutation in a gene that codes for the LORE receptor.

After 8 to 10 days, the LPS molecule from pseudomonad bacteria is added by doctoral candidate Milena Schäffer to the young plants. Will they identify it and react to it?

The monitor shows which plants are activated by LPS, with a significant upswing in their graphs. If this curve is missing, it means the plant does not react to LPS. There may be a mutation in its LPS receptor.



Stefanie Ranf tests if mature plants with a mutation in the LORE gene are more susceptible to infection with pathogenic bacteria. To this end, cultures of *Pseudomonas syringae* bacteria are grown under sterile conditions.

Picture credits: Astrid Eckert/TUM

In the next step, leaves are infected by simply pressure-infiltrating a solution containing the bacteria into the leaf interior using a needleless syringe.

However, for Stefanie Ranf, the scope extends beyond the way plants recognize their enemies. She is also keen to expose the strategies bacteria use to camouflage themselves and hide from the plant immune system. "It's not as though bacteria just hang around waiting to be discovered and destroyed by the plant," she points out.

Under cover

To remain undetected, some bacterial species simply shed their telltale flagella. Ranf suspects the bacteria also adapt their LPS in such a way that plants no longer recognize it. This change also improves their own resistance to the plant's defense mechanisms. It seems probable that relatively small modifications are sufficient here. Discarding one or two fatty acids from lipid A renders bacteria virtually invisible to the plant's immune system. That is the theory at least.

This type of behavior has already been observed in bacteria infecting humans and animals. The plague's causative agent *Yersinia pestis*, which is transmitted by fleas, deduces from the temperature change that it has successfully made the leap from flea to human. Once there, it swiftly discards two fatty acids from lipid A and is thus hardly recognizable by the human immune system. Salmonella bacteria, which trigger severe diarrheal diseases, also go into stealth mode by slightly modifying their lipid A.

"It's not as though bacteria just hang around waiting to be discovered and destroyed by the plant – they go under cover."

Stefanie Ranf

***A. thaliana* plants harboring** a functional LORE receptor sense the bacterial LPS and initiate immune responses to halt pathogen spread. If the LORE receptor is not functional, bacteria proliferate and cause disease symptoms – yellow and wrinkled leaves (left).

Most likely, bacteria also make use of this type of mechanism when attacking plants. Furthermore, a single bacterial species never occurs alone in a plant, but shares its environment with numerous other microorganisms. "These are all interlinked," explains Ranf, "and our aim is to determine how the communities interact." Some of them are even good for the plant, promoting growth or helping to defend against pathogens. Ranf is aiming to establish a better understanding of this interplay between bacteria and their plant hosts, supported since 2016 by funding from the German Research Foundation (DFG) through the Emmy Noether Programme.

Plants as intermediate hosts

Not only does Ranf's research have important ramifications for breeding disease-resistant crops; it also has the potential to enhance food safety. This is because some bacteria such as coliforms and Salmonella, which mainly target mammals including humans, use plants as intermediate hosts.
Coliform and Salmonella bacteria are known to survive in plants and can produce toxic substances there. It then makes no difference how thoroughly food is washed, as the bacteria and their toxins reside inside the plant tissue. So if plants improve their defenses, humans also stand to gain.
"It would be great if we could make a lasting difference through our work. That would be the best possible reward for our research," concludes Ranf. *Claudia Doyle*

Picture credits: Astrid Eckert/TUM