INTERVIEW WITH DR.CHRISTIAN

POSITION: Deputy Scientific Director
INSTITUTION: INRA

We contacted Dr. Christian Huyghe under the recommendation of Camille Lenoir, another researcher at INRA in Paris. We were convinced that his multidisciplinary experience in sciences, education but also implication in public policies could be of great interest for the Softer Shock project.

WHAT IS YOUR GENERAL OPINION ABOUT OUR PROJECT?

→ Softer Shock is very interesting, as it goes beyond synthetic biology and falls within an agro-ecological approach. The vegetal is made to be considered as a whole ecosystem, in accordance with the most recent vision scientists have towards vegetal. Today, we are witnessing a shift in mentalities, and the whole framework of agricultural development that we used to teach few years ago is changing. Leaves are being less considered as sterile surfaces on which a product against a certain pathogen is applied. Now we know that the leave is in fact covered with a complex ecosystem composed of bacteria, but not only, called phytobiome.

WOULD YOU FIND IT MORE RELEVANT TO MAINTAIN THE PROTEINS AT THE LEAVE SURFACE BY FORMING A BIOFILM IN WHICH THEY WOULD BE EMBEDDED ?

→ With a biofilm, there is a risk to block the plant respiration process. According to me, it would be preferable to keep the bacteria free in their medium, maybe added with an adjuvant to maximize their adhesion. It would be interesting for you to contact an expert in this domain to get more precise information.

WHAT DO YOU THINK OF OUR STRATEGY TO USE ANTIFREEZE PROTEINS FOR THE COLD-SPECIFIC RESPONSE?

→ It is a good idea to act on ice crystal formation to reduce frost damages. However, seeking crystal dissolution could lead to the opposite effect. On the contrary, you should induce ice crystal formation on the surface: as they solidify, ice crystals will release latent heat and warm the leave. The formation of extracellular ice is not always deleterious. Initially, it has a protective role as it induces the water leaking and concentrates the plant dry matter content. This mechanism makes it less sensible to freezing stress. Besides, this technique is currently used during cold seasons: through continuous water sprinkling, the creation of an ice layer around the plant favors its warm up. This could therefore be an interesting approach, despite being counter-intuitive. You should look into ice-nucleation proteins.

WHAT DO YOU THINK OF OUR IDEA TO PRODUCE A WHITE LAYER FOR THE HEAT RESPONSE?

→ This is a very good idea to act on the plant albedo. Some plants naturally possess this ability to modify their surface and make it reflective, even if they do not represent a majority. It is notably the case of some desert plants, and also an alpine sedum-type species. I even heard that an Israeli team based on this principle to develop smart roof coatings. They recorded a 5°C decrease in the surface temperature. The produced compound does not necessarily need to be a protein. According to me, the secretion of a white neutral compound could modify the plant albedo. I am pretty sure you will end up finding one!

DO YOU HAVE ANY IDEA OF WHICH COMPOUND COULD BE USED TO DISPLAY A WHITE COLORATION? WHAT DO YOU THINK OF CASEIN?

→ You will need to do more searches to know precisely what is responsible for giving a white coloration, in the case of trichomes for example, in order to induce it on your plant. Using casein seems risky to me as it is a nutritive element, notably used to make fungi grow, and consequently non-neutral for the plant. It could be interesting to use chitin, a more neutral element. However, this is a highly expensive compound.

IS THERE A RISK OF INHIBITING PHOTOSYNTHESIS BY MODIFYING SURFACE REFLECTIVE PROPERTIES?

→ In your system, albedo induction will be activated in response to high temperatures only (above 37°C). At this temperature, the plant enters a “survival” state and does not grow anymore, or very slowly. Indeed, a mechanism induces stomatal closure and the plant does not capture much light. So in any case, photosynthesis is not really possible in those conditions. However, you have to make sure that the white coloration is reversible as temperatures come back to lower values.

HOW CAN WE CHOOSE OUR MICROORGANISM TO LIMIT THE PLANT IMMUNE RESPONSE AS MUCH AS POSSIBLE?

→ When I read your project description, I feel like you make the hypothesis that the leaf surface is a neutral and sterile environment which will not react to the presence of your microorganisms. This hypothesis is wrong: plant surface is actually covered with a complex ecosystem mostly composed of bacteria. Benefiting from various plant secretions, they are unequally dispersed on its surface and especially present in high concentration around stomata. Among the different bacterial populations present, only few are symbiotic: most are neutral, and some are pathogenic. You should therefore select an organism that is neutral for the plant. Using a fungus could also be of interest: as it has already been done before, you should be able to better control the resulting plant reactions. For example, the fungus *Lecanicillium muscarium* has been used as a biocontrol agent against an insect. Many other examples can be found, as the fungus named *Coniothyrium minitans* and also used in biocontrol. One advantage of fungi it that their slow development rate on the plant allows to limit eventual immune responses. On the other hand, if an insect is present, the fungus can surround and eliminate it very quickly.

WHAT DO YOU THINK OF THE IDEA TO FORM A BIOFILM AT THE PLANT SURFACE?

→ Creating a biofilm on a plant appears complicated to me, precisely because of all bacterial populations already present at the leave surface or at the root level, which could potentially alter its formation.

WHAT WILL BE THE FAITH OF LIVING BACTERIA ON THE PLANT, AND ONCE DEAD IN THE SOIL?

→ It is not likely that bacteria will penetrate inside of the plant: generally, only pathogenic species are capable of entering. They should also remain on the plant itself, there is low risk that they fall on the ground. Once dead, the eventual bacteria present on the soil will be treated as organic debris: after being degraded into simple residues, they will then enter into the pool of organic matter.

WHICH PULVERIZATION TECHNIQUE SHOULD WE USE TO APPLY OUR PRODUCT?

→ Many methods exist today. Pulverizing a biological solution containing microorganisms should be similar in method to classical phytosanitary products. You just have to be careful not to clog water nozzles in case of a biofilm formation.

DO YOU HAVE OTHER REMARKS?

→ Instead of a single plasmid, you could also do a co-culture with two types of modified bacteria, both containing a different plasmid.

→ The biocontrol approach is quite trendy today, and your project can fall within this category. This term gathers all methods aiming at protecting plants by using natural mechanisms (insects, bacteria, fungi…) and represents a very recent research sector. Two different strategies can be used to create biocontrol agents: transformation and screening.

CONCLUSION

This interview with Dr Christian Huyghe was very complete and he quickly grasped our objectives with the Softer Shock project. He was the first to introduce us the notion of "biocontrol", within which our method falls in some ways. It was highly rewarding for us to see that an expert like him was enthusiastic and encouraging towards our ideas! Many thanks to him from the whole iGEM IONIS team.