Chitosan nanoparticles functionalized with double stranded RNA as a novel strategy for plant protection

The main aim of the project is to obtain a novel system of plant protection against the pathogens by strengthening the antifungal and plant immune boosting properties of chitosan. The goal will be achieved by delivering to pathogen and to plant cells chitosan nanoparticles (ChNPs) associated with double strand RNA (dsRNA) designed to trigger RNAi-based processes leading to the pathogen elimination and stronger plant immune response.

The project is based on three hypotheses: 1. Chitosan treatment activates genes and processes of plant acquired resistance. 2. The moderate anti-fungal activities of chitosan will be enforced and more specific by dsRNA molecules linked to ChNPs. The dsRNA-ChNP association will improve dsRNA stability and its delivery to the fungal cells. 3. The final plant-protecting efficiency of dsRNA-ChNP complexes will be the result of synergy of anti-fungal activity of chitosan, chitosan-triggered plant immunity and RNAi-based processes induced by dsRNA.

The four work packages (WPs) include transcriptome analysis of barley (**WP1**) and *Fusarium graminearum* (*Fg*) (**WP2**) treated with chitosan. The aim of both is to identify and to analyze the genes responsible for immune boosting in plant and antifungal against *Fusarium* activity of chitosan. The knowledge of the genes will be used (in **WP4**) to design and to synthesize the dsRNA molecules specifically targeting in association with ChNPs the selected *Fg* and barley genes. The **WP3**, done in Dr. M. Piątkowski team at the Cracow University of Technology, is designated to characterize diverse batches of chitosan, to generate and to characterize ChNPs and, having the results from the WP1 and WP2, to load dsRNA on the selected batches of ChNPs. The properties of dsRNA-ChNPs will be tuned using dsRNA targeting the reporter genes: the *AmCyan* in *Fg* AmCyanPH-1 strain and the *PDS* in barley. Visual screening of the gene's silencing rates (the AmCyan fluorescence in *Fg* and leaf photobleaching in barley) will be used to assess these parameters of dsRNA-ChNPs which are important for efficient delivery of dsRNA and for the dsRNA stability. The selected *Fg* genes downregulated upon chitosan treatment will be used to design dsRNA-ChNPs. We expect that targeting the genes will enhance the native antifungal activity of chitosan and, what more, it will make this activity highly specific against a particular *Fusarium* species or strain. In a similar strategy a selected barley gene(s) important for immune boosting (the reaction observed in plant after chitosan treatment) will be selected for designing and generation of dsRNA-ChNPs with the aim of enhancing this type o response.

The project will provide the knowledge on genes and processes specifically involved in barley immune response and barley acquired resistance activated after chitosan treatment. The knowledge will be used in a subsequent steps to design dsRNA-ChNPs specifically targeting and eliminating the invading organism. The most important and novel results will include: (i) verification of the project hypotheses, (ii) identification and annotation of barley and *Fg* genes regulated in response to chitosan treatment, (ii) detailed procedures of dsRNA loading on ChNPs (iii) detailed physiochemical characteristics of chitosan, ChNPs and dsRNA-ChNPs complexes. It has to be underlined that both components i.e. chitosan, ChNPs and short dsRNA molecules are biodegradable leaving no environmental pollution. What more, the chitosan and chitosan-derived products are biocompatible with a long history of safety use in agriculture including organic agriculture.

The experience in activation of the RNAi-based processes in plants for functional genomics and novel crop characteristics (the PI research) and the experience in chitosan and ChNPs chemistry as well as biomedical applications of chitosan-derived products in Dr. M. Piątkowski team (the main collaborator) are complementary to reach the goals of the project. We expect that the strategy has the potential to be adapted for other plant pathogens in particular those where no other means of protection are known.