Plants are constantly exposed to the infections. Static character of plants that makes impossible for them to relocate easily to more suitable locations and their lack of active, systemic immune system similar to one in humans and animals is a substantial obstacle for plants in facing biotic stress. However, in nature, plant infections occur sporadically and are in most cases are rather exceptions than a rule. It is mostly due to the fact that plants developed various sophisticated defense mechanisms of protection against infection factors and other biotic stress. Lack of such mechanisms would cause deadly infections in plants even at very little exposure to pathogens. The first line of defense constitute the physical barrier such as cuticula, thickened cellular walls, and metabolites toxic to pathogens. This is called constitutional resistance, and it is present all the time, regardless whether the plant is under pathogen attack or not. Additionally, plants possess active defense mechanisms that are being induced by the presence of pathogens. It is due to genetic predispositions of plant that it is equipped in induced immunity which at normal conditions is tuned down and it is turned on during the pathogen attack. Currently two levels of induced immunity are being recognized: (1) immunity induced by pathogen molecules in present in for example pathogen cell walls; it is a basic immunity, also called horizontal, (2) immunity induced by special proteins (effectors) called avirulence factors (avr) produced by pathogens as means of decreasing the basic immunity. The plant, on the other hand, through receptor (protein), which is produced by resistance gen R, recognizes the effector (avr) and induces immunity. This type of resistance used to be called vertical.

Among many different defense mechanisms of horizontal immunity, big hopes are being put on Systemic Acquired Resistance, SAR and Induced Systemic Resistance, ISR. Induced resistance acts against broad spectrum of pathogens, is durable, can be transferred onto the plant’s next generation and can be induced not only by pathogens but also by other elicitors (substances), which have ability to stimulate the plants immunity similarly as it happens during the pathogen attack, mimic plant-pathogen interaction. These substances do not lead to resistance of pathogens against them, as it often happens in the case of classical plant protection products, which are meant to act on the pathogens directly. Natural elicitors are often substances isolated from plants, animals, and other microorganisms such as laminarine, chitin, chitosan and other natural substances that are produced by plants during the induction of resistance, such as salicylic acid (SA), jasmonic acid (JA), ethylene, systemine, which when used externally lead also to induction of resistance in treated plants. However, the highest effectiveness among resistance inducers is expressed by chemical substances such as benzo(1,2,3)thiadiazole (BTH), DL-ß-aminobutyric acid (BABA), oxalic acid, azeleic acid, 2,6-dichloroisonicotinic acid, (INA) and their derivatives.

Among many plant pathogens the most common are fungi, but their occurrence and infections can be effectively limited by the use of various fungicides in form of synthetic plant protection products. In contrary, we do not possess any of such commercially available plant protection compounds acting directly against viruses, and prophylactics very often fail. Viruses are molecular parasites strongly associated with their host thus all chemical compounds that are harmful to virus are also harmful to plants. Because of this so much hope is put in use of induction of systemic resistance coming from the plant, upon external induction, and which can interfere with initial stages of viral infection or replication. What is also important ,viruses due to their specific association to plants place particularly high demands to induction of systemic resistance. Effectiveness of SAR at the level of 80% can be satisfactory in limiting the infections of fungi (to the harmless level), but it will not be satisfactory in combating viral infections. Therefore, the aim of the proposed project is to investigate the possibility of induction of immunity, and assessment of effectiveness of new stimulators of immunity, which will be developed and characterized in the course of the project. We will investigate how those new inducers work on the macroscopic and molecular level, in order to examine the scientific basis of operation of the newly developed SAR inducers in reducing viral infections.

The first and most important task of the proposed research is to develop the most effective SAR elicitors. Two methods for derivatizing plant resistance inducers are being proposed: (1) by introduction of new functional group to the molecule of known inducer in order to reinforce SAR effect, and (2) preparation of new bifunctional immunity inducers through preparation of salts, where the cation and anion come from two different substances and, at least one of them, is SAR inducer, and the second one introduces an additional biological activity. Resistence inducers such as BTH, BABA, GABA, INA, and SA will be subjected to these modifications. So far, beside our preliminary studies in 2014 no scientific reports on the attempts of using bifunctional salts as SAR resistenace inducers, especially against viral infections, was ever reported in the literature. This technique makes it possible to connect in one molecule two different chemicals in order to improve their physicochemical properties and biological activity. The formation of such salts requires development of the conditions for the chemical modification of the starting materials to form cations and anions. Often, such modifications can change substance desirable properties, or the resulting substance does not meet the expected final results. However, obtaining bifunctional salts gives a chance to, for example, combine two immunity-inducing elicitors which act through different signaling routes and thus may lead to synergistic effects on resistance induction.

In ecology of virus spreading by insect vectors plays an important role, which is particularly troublesome in the production of virus-free seed. This is an economically important problem and have not yet been resolved. Therefore, during our research we will also focus on the synthesis of bifunctional salt, which combine the resistance inducers with substances such as insecticides. Such approach can lead to the situation where insecticide ion will cause elimination of aphids. Unfortunately, aphids before they die will still manage to make a few punctures and introduce the virus into the plant but then SAR inducer present in the salt should prevent or limit infection and replication of virus in host cells.

The biological activity of the new salts and modified substances will be initially evaluated in the greenhouse tests (screening tests) using a standarized model of plant-virus e.g. tobacco (Nicotiana tabacum) var. Xanthi nc and Tobacco mosaic virus (TMV), using hypersensitivity effect as indicator (local, necrotic spots). Comparison of the number of necrotic spots on the leaves treated with elicitor and untreated (control) will allow for precise determination of resistance induction, and description of its level. In addition, the phytotoxicity of the substance will be evaluated by spraying of seedlings of various plants. Then, the most promising compounds will be selected for specific biological tests that include: (i) direct effect of salt on viruses - tobacco plants will be inoculated with TMV incubated in inducers; (ii) influence of bifunctional salts on the induction of resistance in various host plant and the various methods of use; (iii) the impact of SAR on the transmission of viruses by insects - vectors contaminated with viruses will be exposed on plants treated with bifunctional elicitors (inducer + insecticide); (iv) the impact of SAR on the transmission of viruses by seeds - tomato plants infected with tomato black ring virus will be treated with inducer every 2 weeks until seed harvest and then the rate of seed transmission from plants treated and untreated will be evaluated, and (v) the impact of the genetic diversity of the virus (various strains and biotypes) on the efficiency of SAR - tomato plants will be treated with resistance inducers and subsequently inoculated with different biotypes of the same virus and after approx. 7 and 14 days the accumulation of the virus will be determined using real-time PCR.

Plants under the influence of biotic and abiotic stress trigger the activity of many genes. Depending on the stressor, in this case, the type of elicitor, signal pathways are activated, mainly those related to salicylic acid (SA), but also jasmonic acid (JA) and ethylene (ET), which are genetic markers indicating the activation of individual signaling pathways. To explain the biological effect and the scientific basis of the actions undertaken by the plant upon the use of new elicitors, experiments using molecular biology techniques will be performed for a high range of host-pathogen systems. For each of the analyzed plant-virus system accumulation of the pathogen in the host cells will be analyzed, and related to different concentrations and different methods of application (watering to the root, spraying, etc.) of resistance inducers. Additionally the level of the phytohormone - salicylic acid in plant tissues will be determined and compared between plants untreated (control) and treated with developed inducers and viruses.

Proposed research on the topic of systemic acquired resistance in the light of its activity against viral infections may provide fundamental knowledge and open possibility of using this phenomenon as an important part of modern plant protection, competitive to common plant protection products, and transgenic plants still not accepted by the majority of consumers.