Stringent response is key player in plant-microbe interaction

Petrova O.E.¹, Parfirova O.I.¹, Sergeeva J.P.², Gorshkov V.Y.¹

¹Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russia;

²Kazan Federal University, Kazan, Russia

E-mail: poe60@mail.ru

Key message. Bacterial SpoT-dependent SR was activated in *Pectobacterium atrosepticum* when pathogen infected potato as well as tobacco plants. Pba induced plastid stringent response in tobacco plants, but not in potato plants. Jasmonic acid defense pathway was activated in tobacco plants, provoking rapid maceration of plant tissues. Salicylic acid defense pathway was induced in potato plants, probably ensuring a more prolonged coexistence of the phytopathogen with its host.

Keywords: stringent response, salicylic acid defense pathway, jasmonic acid defense pathway, ppGpp.

The endosymbiotic hypothesis of the origin of chloroplasts suggests that these organelles arose as a result of the symbiosis of a free-living photosynthetic protobacterium with an ancient eukaryotic cell. In the process of co-evolution of the host and symbiont, a radical reorganization of prokaryotic genome occurred but the chloroplasts retained the main elements of bacterial signaling pathways that are involved in the regulation of the functions of these organelles. One such signaling pathway is a stringent response (SR).

New aspect of stress response study is an analysis of bacterial and plastid SR during plant-microbe interactions. Since an infection is stress situation for host plant as well as a pathogen, stringent response can be induced in both "partners-in-infection". The study of these coincidental in time phenomena, initiated under plant-microbe contact, would be essential to fully understand the evolutionary interplay between plants and symbionts/pathogens.

In this study we have used two model pathosystems: phytopathogenic bacterium *Pectobacterium atrosepticum* SCRI1043 and specific host plant potato *Solanum tuberosum*, co-evolving with pathogen for a long time and *P. atrosepticum* and non-specific host plant tobacco *Nicotiana tabacum*. The expression level of the genes, encoding bacterial and plastid stringent response and plant salicylic and jasmonic acid defense systems, was analyzed by RT PCR.

Bacterial SpoT-dependent SR was activated in pectobacteria when pathogen infected potato as well as tobacco plants. Apparently, phytopathogenic bacterium always perceives the contact with the host plant as a stressful event and responds to it by induction of SR.

P. atrosepticum induced plastid stringent response in tobacco plants. The expression level of *NtRSH2* gene, encoding the synthase of signal molecule ppGpp, increased more than eight-fold reliably at first hour of infection and remained at constant level during 6 hours of experiment. Herewith the expression of hydrolase *NtRSH1* gene did not change during 3 hours and then decreased.

Surprisingly, plastid SR was not activated in potato plants under pectobacterial infection. The expression level of ppGpp synthase *StRSH2* even declined 8-fold by the sixth hour. No expression change of ppGpp hydrolase *StRSH1* gene was revealed in potato plants under bacterial infection, compared to non-infected plants.

It is considered that guanosine tetraphosphate modulates salicylic acid signaling in plants. We have analyzed the expression profiles of genes encoding key genes of salicylic acid signaling defense pathway *PR1*, and antagonistic to it jasmonic acid signaling defense pathway *lox* (lipoxygenase) and *aoc* (allene oxide cyclase) under SR. In tobacco plants the expression of *PR1* gene increased only 4-fold under SR period. In the same time the expression of *lox* and *aoc* gradually increased 360-fold and 27-fold, respectively, during all experimental period. Rapidly progressive necrosis led to death of 100% tobacco plants. A very sharp induction of expression of *PR1* gene was observed in potato plants: the expression level of this gene increased 43-fold under 3 hours of infection and then decreased to control level. Herewith no induction of *lox* and *aoc* gene expression occurred. Symptoms of the disease developed slowly in potato and only 53% of plants died after 25 days post inoculation.

Generally accepted that salicylic acid induces defense responses against biotrophic pathogens, whereas jasmonic acid defense pathway is effective against necrotrophs. Soft rot enterobacteria of genus *Pectobacterium* are classical and well-studied examples of necrotrophic plant pathogenic bacteria. Nevertheless, life style of any phytopathogenic bacteria contains an initial biotrophic stage, in which the bacteria avoid the elicitation of or suppress plant defenses. Obviously, if the salicylic acid defense pathway is induced in potato chloroplasts in the first hours of infection, pathogenic bacteria either die or are forced to remain at the biotrophic stage for a long time. Conversely, the early induction of jasmonic acid defense pathway in tobacco plants under pectobacterial infection could be the cause of the rapid maceration of tobacco tissues and the death of plants.

Thus, the phytopathogen containment mechanism in a framework of asymptomatic infection, associated with the lack of activation of SR and with the trigger of salicylic acid defense pathway in specific plant host potato probably ensures a more prolonged coexistence of the phytopathogen with its host, which can be considered as a more perfect form of plant-microbe interactions developed during coevolution.

Project RFBR 20-34-70043.