dr hab. Grażyna Dąbrowska
Zakład Genetyki
Wydział BiOŚ UMK
tel. 566114576
browsk@umk.pl

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Role of the stringent response in crop plants growth and development

1. Opis projektu doktorskiego

The stringent response was found to takes place in chloroplasts less than 20 years ago. The effectors of the response are guanosine tetraphosphate and guanosine pentaphosphate (ppGpp and pppGpp), unusual nucleotides jointly reffered to as (p)ppGpp or magic spots. These effectors are synthesized by nucleus-encoded RSH proteins that are known to function in chloroplasts. The research work of the last four years has shown that (p)ppGpp inhibit chloroplast transcription (e.g. of rRNA, tRNA, genes encoding proteins involved in translation and photosynthesis), translation and the production of many metabolites. The molecular changes invoked upon the accumulation of (p)ppGpp eventually cause a decrease in the efficiency of photosynthesis and the reduction of plant growth. However, still, the stages of plant development that are being affected with/regulated by those nucleotides are not known (reviewed in Boniecka et al., 2017, Planta 246:817-842).

Thus, the aim of the project is to elucidate the role of the stringent response in plant development, in particular of rapeseed (*Brassica napus* L.), as it is an important crop plant belonging to the same family as the model plant *Arabidopsis thaliana*, and its genome has been sequenced. We assume, based on our research (Boniecka et al., 2019, Ind Crops and Prod 130:478-490), that the stringent response is involed in seed development and maturation. Our hypothesis states that (p)ppGpp during late stages of plant development, by decreasing the efficiency of photosynthesis, bring seeds into a dormant state. Moreover, we belive that the stringent response might be involved in the regulation of other developmental processes, especially of those in which photosythesis is strongly involved, such us rosette development or stem and flower formation. This is an extremely important topic for modern agriculture.

The project includes following tasks:

1. Rapeseed plants growth in a field experiment and plant sample collection at different phenological growth stages listed in the BBCH classification for *Brassica napus* L.: 0 – germination, 1 – leaf development, 3 – stem elongation, 5 – inflorescence emergence, 6 – flowering, 7 – development of fruit, 8 – ripening and 9 – senescence.

2. Analysis of *B. napus RSH* gene expression by means of qPCRs, at all mentioned stages of rapeseed development, and by means of *BnRSH* transcripts immunodetection. The latter analysis will be done both in vegetative and generative tissues of rapeseed plants being at 7–9th growth stages, as the stringent response might be involved in nutrient remobilization and reallocation from vegetative into generative tissues (reviewed in Boniecka et al., 2017, Planta 246:817-842).

Primers and *q*PCR conditions for checking *BnRSH* gene expression were already designed and assesed, respectively (Boniecka et al., manuscript in preparation). The immunodetection of *BnRSH* transcipts in plant tissues will be done using digoxigenine (DIG)-labelled probes, the anti-DIG primary antibody and the fluorochrome-conjugated secondary antibody. The probe hybrydization followed by immunodetection will be performed with thin sections of samples embedded in BMM resin, prepared with the use of vibratome.

3. Measurements of (p)ppGpp content in plant tissues, using liquid chromatography in combination with tandem mass spectrometer (UHPLC-MS/MS) with quadrupole (mass analyzer) and electrospray (ESI; in collaboration with the Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Cracow, Poland; according to Boniecka et al., manuscript in preparation).

4. Analysis of particular chloroplast gene expression (qPCRs) and protein content (western blots), in accordance with the recently established protocols (Boniecka et al., manuscript in preparation).

Because the experiment will be conducted in the field, three years of sample collection followed by the mentioned analysis (3 biological replicates) have to be conducted.

The project is very important both from the scientific as well as the economical point of view. It will help to elucidate the role of the phenomena of the stringent response and to unravel plant developmental stages at which, by the regulation of the activity of the stringent response, plant growth can be stimulated, e.g. with the use of synthetic (p)ppGpp analogues that can inhibit the response (Syal et al., 2017, Antimicrob Agents Chemother 61, e00443-17). Thus, the knowledge of the activity of the stringent repsonse at particular stages of plant development can help to use the regulation of (p)ppGpp content as a tool to stimulate plant development.