

*Vicia faba* subsp. *minor* (faba bean) is one of the earliest plants "domesticated" and utilized by humans. It is also widely used today as a culinary ingredient in many cuisines in the world, a fodder plant for agriculture, while L-DOPA (dopamine precursor), an amino acid derived from it, is effectively used to treat Parkinson's disease. The plant is highly valued for both its highly nutritious composition and its ability to grow under unfavourable conditions (including insufficient hydration, increased salinity and other potentially harmful soil chemicals).

The research performed on the intensely dividing root meristem cells of faba bean, has shown that they have an extremely high ability to survive even in adverse conditions. Interestingly, the mechanisms responsible for its extremely high survival rate have not yet been identified.

It has been shown that in *V. faba* cells blocked in S-phase by hydroxyurea (HU), and then induced to premature chromosome condensation (PCC) by the action of HU and caffeine (CF), a population of PCC-type mitotic chromosomes was observed, which showed strong pulverization and, at the same time, remained their competence for DNA replication. It has also been shown that *V. faba* cells were able to survive PCC induction and fully regenerate within 12 hours after their transfer to water (the whole cell cycle of faba bean 'closes' in 24 hours). In our opinion, this ability results from the evolutionary "preparation" of a plant for highly efficient and possibly fast process of DNA replication, as well as extremely effective mechanisms of DNA repair.

The proposed project aims to investigate the mechanisms responsible for the *V. faba* adaptability and protection against negative environmental impacts. Therefore, it is planned to implement highly precise methods of imaging replication dynamics and the changes observed at the chromatin level.

In the first stage of the study, the impact of replication stress and PCC induction on the behaviour of the progressive replication fork and possible involvement of backup replication start points (replication origins) will be specified. Double immunostaining with replication markers (e.g. CldU, IdU) will be performed, also in combination with the molecular combing protocol previously developed by our team. Mathematical modeling of the DNA replication, and process of restarting of DNA replication during PCC, will also be carried out (in cooperation with our scientific partner in the UK). These studies will allow us to better understanding of replication dynamics and response to its disturbances not only in plants, but also - due to analogies in the organization of the process of DNA replication - in all eukaryotic organisms having a large genome, measured in Gb (including humans).

In the next stage of the project, the chromatin structure will be studied, with particular emphasis on heterochromatin regions (due to the fact that the heterochromatin is the most vulnerable to DNA damage caused by under-replicated sites due to the inhibitory effect of HU). It is planned to determine the localization and quantitative involvement of heterochromatin-related protein HP1 ( $\alpha$ ,  $\beta$  and  $\gamma$ ) in the cells 'before' and 'after' the action of hydroxyurea, as well as during PCC induction. Comparative genomic hybridization (CGH) technique will allow to verify the effect of hydroxyurea and caffeine on the structure of heterochromatin.

It is also planned to investigate whether the elongation of the retention time of the replication fork does not increase the probability of incorrect re-association of core histones to DNA. For this purpose, we will perform a precise chromatin immunoprecipitation (ChIP) for CAF1A proteins and selected components of MCM2-7 complex (e.g. MCM2). CAF1A is an assembly factor that functions in *de novo* assembly of nucleosomes during DNA replication. MCM2-7 proteins are a family of six related proteins required for initiation and elongation of DNA replication.

In the final stage of the research, it is planned to model all the events taking place, among others, in the form of 3D visualization with the use of graphic programs (the project manager is a specialist in this field).

The results will help us to confirm (or deny) the hypothesis - we believe that *V. faba's* adaptive abilities may be due to its ability to slightly re-organize the chromatin structure, especially in the region of facultative heterochromatin.

Knowing which mechanisms are particularly responsible for the survival of plants under stressful conditions can be of great importance to growers, especially when selecting and/or developing new varieties for growth in the regions that are unfavourable to cultivation.

The adaptive capacity of plants that grow in adverse environment, particularly in the context of increasing climate change and pollution of the Earth, our planet, is a factor which may soon become the target of breeding modifications affecting most agricultural crops.