In the face of a significant threat of negative climate changes, the water shortages and osmotic stress will be most probably a critical negative factor influencing plant cell functioning in incoming decades. Adaptation to these adverse conditions will be a key determinant of surviving numerous plant species. It is crucial to know as much as possible about processes of acquiring and maintaining stress tolerance and adaptation.

Surprisingly, despite the massive number of reports on the plant cell response to abiotic stress conditions, the molecular adaptive **mechanisms which allow plant cells live years in continuously adverse conditions and adapting to them are almost unknown** as, in a vast majority, were only predicted from results of experiments on stressed plants.

In the same time, our latest, yet unpublished, results of transcriptomic analysis for adapted BY2 cells showed, that the molecular status of plant cells adapted to ionic- and non-ionic stress condition was surprisingly similar to controls. Those plant cells simply ‘learned to live’ in adverse environments. Our planned experiments will verify the existence of the state of **‘new molecular homeostasis’** and discover how it developed.

Numerous phenotypic observations revealed **that plants living in chronic stress conditions are smaller**. It is postulated that this decrease in size/mass is **a ‘cost’ of life in stress conditions** paid to prevent death. We are very interested in this cost. In our project we plan to study the intensity of energy-consuming processes of protein turnover as a marker of **the molecular stability** of adapted cells as well as the **factor believed to be directly connected with the regulation of cell size**.

In our opinion, the most interesting **question** is**: ●How the coordination between the molecular status – energy management – cell size regulation differ between adapted, stressed and ‘control’ plant cells.** Such knowledge will allow us to find out what is really crucial for the successful adaptation process.

Data on linkage of cell size regulation and energetic costs of protein burden lacks not only for adapted but generally for stressed plants making our proposal highly innovative.

The data obtained in our project will be unique, but will also constitute the basis (will be tested) of the future study at the organismal level e.g. of started **at ID PAS study of the adapted vegetative offspring of poplar**.

We have an unique **experimental system of tobacco** BY2 suspension cells **gradually adapted** to ionic and non-ionic osmotic stress in 2005-2006 y. Those in vitro lines are unique because they have **unified genetic background (they originated from one flask of suspension BY2 culture), fully controlled conditions of growth and the hundreds of cell generations living in continuous harsh stress conditions that are lethal for non-adapted plant cells. This will be our ‘testing ground’ of stress adaptation.**

We will analyze four BY2 lines adapted since 15 years to various osmotic stress conditions (NaCl, KCl, mannitol and sorbitol stress) and controls. We also intend to perform the gradual adaptation of BY2 to increasing stress conditions (from 0 mM to 450 mM mannitol/sorbitol and 190 mM NaCl/KCl) and to gradually reduced concentration of the osmotic agent in adapted lines (from 450 mM mannitol/sorbitol and 190 mM NaCl/KCl to 0 mM). This experiment (necessary to distinguish molecular adjustments caused by chronic and acute stresses) will allow us to answer a fascinating, additional question: ● Whether the return to control conditions is experienced by adapted plants similarly as stress and whether this re-adaptation will be connected with gradual changes in the cell size, subcellular organization and cell’s metabolism.

We plan high-throughput analyzes at the level of the transcriptome, proteome and metabolome but also extensive microscopic analyzes, including 3D analysis of cell size and mitochondria superstructures or ribosome number. We will also carefully analyze oxidative stress and anti-ROS response as well as the energetic status of analyzed adapted to stress conditions, re-adapted to control conditions and control (kept all the time in control conditions) tobacco BY2 cells.

According to our working hypothesis, the ‘molecular stability’ will be rising up in time to reach the ‘state of the new homeostasis (how?/when?)’. In contrast, energy production will be strongly correlated with the protein burden, the oxidative stress level and the cell size in the ‘trade-off’ mode (how?).