

Involvement of vacuolar processing enzyme (VPE) and lipase in the degradation of autophagic bodies in cells of embryo axes of lupin (*Lupinus* spp.) germinating seeds

The main aim of this project is to describe the role of vacuolar processing enzyme (VPE) and lipase in the degradation of autophagic bodies in plant cells. Given that there is an almost total lack of knowledge about vacuolar lytic enzymes involved in the degradation of autophagic bodies in plants, a hypothesis is that VPE and lipase are involved in the degradation of autophagic bodies during autophagy in cells of lupin embryo axes.

Autophagy, which means “self-eating”, plays a crucial role in the degradation of useless or damaged cell components. It is a conserved process that occurs similarly in fungal, animal, and plant cells. Under normal conditions, autophagy occurs at low intensity, but as a result of various stress factors, the process is dramatically intensified. The first visible symptom of macroautophagy (the most common kind of autophagy) in plant cells is the appearance in the cytoplasm of a cup-shaped structure, called the phagophore. The phagophore elongates, surrounding and simultaneously separating the fragment of the cytoplasm together with organelles or other components of the cell that are intended for degradation. The final stage of phagophore differentiation is the complete surrounding of the cargo and its separation inside the autophagosome. This is a vesicle with a double, bilayer lipid-protein membrane containing cargo intended for autophagic degradation. In plants, the autophagosome fuses with the vacuole creating autophagic bodies. Degradation of autophagic bodies occurs rapidly and begins immediately after their appearance in the vacuole, but only a few publications describe the degradation of the autophagic bodies, and this stage of autophagy is often described by generalities, predictions and suggestions. Compared to the initial stages of autophagy, the mechanism and regulation of the degradation of the autophagic bodies are very poorly investigated and understood. However, these are key stages on the path to recycling cellular components in the entire autophagy process.

Previous studies carried out at the Department of Plant Physiology of Adam Mickiewicz University (AMU) show that the lipid content in starved lupin embryo axes is higher than in those fed with sucrose. These results were surprising and difficult to interpret as they are in complete contradiction with literature data which indicated a marked intensification of the decomposition of storage compounds under sugar starvation. At the same time, lipolytic activity was also clearly higher in starved lupin embryo axes. Our other data indicate that autophagy occurs intensively in cells of starved lupin embryo axes. Symptoms of autophagy included an increase in cell vacuolisation and a decrease in phosphatidylcholine levels, one of the metabolic indicators of autophagy. It was also found that in starved embryo axes that were simultaneously fed with asparagine (a central amino acid in lupin seed metabolism), the degradation of autophagic bodies was slowed, resulting in their accumulation in the vacuole. Such an observation was surprising, as the accumulation of autophagy bodies inside the vacuole without the use of autophagy inhibitors had not been previously described. In parallel, lipolytic activity was also significantly reduced by asparagine.

So far, only one vacuolar lytic enzyme (VPE) has been suggested to be involved in the degradation of autophagic bodies in plants. The plant VPE may act similarly to yeast Pep4 by activating cascades of other hydrolases that are responsible for the hydrolysis of various structures inside the vacuole, including autophagic bodies. Nevertheless, to date, there is no evidence for the involvement of VPE in the degradation of autophagic bodies in plants. The best-known and described protein involved in the degradation of the autophagic bodies in yeast is Atg15, a putative lipase. Since autophagy is a conserved process that occurs similarly in fungal, animal, and plant cells, the lipase may also be involved in the degradation of autophagic bodies in plant cells.

Based on our previous results and literature data, we propose a study to describe the role of VPE and lipase in the degradation of autophagic bodies in embryo axes of germinating white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet) seeds cultivated *in vitro* under different carbon and nitrogen nutrition (sugar starvation and sucrose and asparagine nutrition). In this project, will be determined of the effect of selected autophagy inhibitors on the degradation of autophagic bodies. Changes in the expression level of genes coding VPE and lipase will be analyzed. In addition, changes in VPE and lipase protein level will be determined. VPE activity will be analyzed as well. Aim one of the task will be to determine the vacuolar localization lipase in the plant cells.

It will be a significant success to obtain evidence on the involvement of VPE and lipase in the degradation of autophagic bodies in plants. The continuation of the research on autophagy in cells of lupin germinating seeds and publication of new results will contribute to a significant input to the knowledge in this field. The proposed research will clarify one of the last scientifically unexploited aspects of autophagy in plant cells. In addition, the results of the project will be particularly important and valuable as the research will be conducted on plant germ cells. Until now, with the exception of the research conducted in the Department of Plant Physiology at AMU in Poznań, such material has not been used at all in the study of autophagy in plants.