

A question we increasingly need to ask ourselves in today's world is: how can we feed a growing global population while protecting the planet at the same time? According to UN projections, by 2050 the Earth will be home to over 10 billion people. Importantly, most of this growth will occur in developing countries. This means we must find new, more efficient, and sustainable ways of farming to meet humanity's needs. One of the key areas of such research is urban agriculture and controlled-environment farming, such as advanced greenhouses and vertical farms. Technologies like aeroponics and hydroponics (soilless cultivation methods) make it possible to grow plants using minimal area and up to 95% less water than traditional farming. What's more, these systems are far more resilient to climate change and droughts, and can be used even in regions with poor soil quality.

But a rising population will increase more than just the demand for food. Plants are also a valuable source of bioactive compounds, so it's equally important to develop cultivation methods for medicinal plants, which are used to produce pharmaceuticals and dietary supplements. In our project, we aim to develop a methodology that not only increases the yield of harvested plant material but also boosts the concentration of active compounds it contains — all while aligning with the United Nations Sustainable Development Goals. To achieve this, we will use soilless aeroponic systems for cultivation, along with Pulsed Electric Field (PEF) as a stressor for the plants. After carefully adjusting its parameters, PEF triggers a process known as electroporation, which involves an increase in both the number and size of pores in the cell membranes of tissues. Once PEF is no longer applied, these newly formed pores close, and the plant survives the procedure, allowing the process to be repeated several times throughout its growth. According to one hypothesis, electroporation induces oxidative stress in plant tissues, which in turn stimulates the production of antioxidants and other secondary metabolites, including the bioactive compounds used in medicine and pharmacy. Additionally, this type of stimulus causes an increase in plant biomass as a stress response. We have already observed both of these key effects in our previous studies. However, we now want to explore in more detail how PEF influences plant metabolism and physiology. To do this, we will examine changes in gene expression and microscale anatomical changes. To our best knowledge, this will be the first study in the world to investigate plant responses to this type of stressor in such a comprehensive way, while employing methodology which application potential stands out for its simplicity, low cost, and high effectiveness.

For our study, we selected leopard lily – *Iris domestica* (L.) Goldblatt & Mabb. – a widely known medicinal plant listed in the European Pharmacopoeia, which has been used in pharmacy for centuries. Its rhizome is a valuable pharmacopoeial raw material with documented antioxidant properties and high therapeutic potential, particularly in alleviating symptoms of menopause. The plant will be cultivated in the aforementioned aeroponic systems, using a nutrient solution with carefully tailored composition and physicochemical properties. The growth environment (lighting, humidity, temperature etc.) will be strictly controlled to ensure optimal conditions for plant development. While similar systems are already widely used in the food industry to enhance the growth of edible above-ground plant parts (such as lettuce, basil, tomatoes, or cucumbers), their main advantage lies in allowing unrestricted development of underground organs. By applying PEF to the plants periodically during their growth, we aim to maximize the concentration of active compounds in their roots and rhizomes and boost biomass production, all while maintaining plant vitality. Each year of the project, we will use seeds harvested from the previous generation to see whether subsequent generations of *I. domestica* become naturally better adapted to the stressor. This may lead them to develop richer phytochemical profile or grow larger in size, which also is a novel and promising approach in this field.

Expected results of the project include promoting *I. domestica* growth and increasing its phytochemical content, along with elucidating mechanisms of the plant response contributing to these observations. This methodology can easily meet future legal and industrial standards, contributing to the improvement of quality, purity, bioactivity, and production efficiency of plant-based raw materials – both agricultural and pharmacopoeial – on a large scale. We believe it holds the potential to revolutionize the way active compounds are obtained, both in the production of dietary supplements and in the development of new drugs. Moreover, using leopard lily as an easy-to-grow model plant paves the way for transferring these solutions to other medicinal plant species essential to modern phytotherapy, as well as to food crops.