Part A. CV

Personal Information	**Evgenia Koukara**
Birthplace and Date	Pieria, Greece, 15/06/1996
Languages	Fluent English **(**Certificate of Proficiency in English from the University of Michigan), Native Greek
Email	ekoukara@uth.gr

Education

September 2015-July 2019	**Bachelor's degree** from the Department of Biochemistry and Biotechnology, University of Thessaly
October 2019-July 2021	**Master of Science** Advanced experimental and computational biosciences, Department of Biochemistry and Biotechnology, University of Thessaly Master thesis: Genome editing in *L. japonicus* using CRISPR/Cas9 technology
March-April 2020	Docking of AKBA into the binding domain of the Glycocorticoid receptor (GR), supervisor Assis. Pr. Georgios Papadopoulos
July-September 2020	Development of a toehold switch for the detection of West Nile Virus in Greece; Assembly of putative toehold switches and assessment of in vitro West Nile Virus detection sensitivity, supervisor Assis. Pr. Antonios Giakountis

Professional experience (practice)

July-August 2018	Anna Dranidou Diagnostic Lab, Katerini, Greece: worked as a laboratory assistant under the guidance of Dr. Anna Dranidou

Research experience

July 2019-	Laboratory of Plant and Enviromental Biotechnology, University of Thessaly Development of a CRISPR/Cas9 system optimized for use in model legume *L. japonicus*, research leader Pr. Kalliope Papadopoulou
February-June 2019	Undergraduate thesis: Mutagenesis of *L. japonicus* AMY2 using a CRISPR/Cas9 system, supervisor Pr. Kalliope Papadopoulou

Laboratory experience

- Molecular cloning (classical techniques, Gateway® technology, Golden Gate DNA assembly method)
- CRISPR/Cas9 design
- Bacterial plasmid isolation and plant genomic isolation
- PCR, colony PCR
- Creation of competent cells and transformation (chemical and electroporetion)
- Agrobacterium rhizogenes mediated hairy root transformation
- Agroinfiltration in *N. benthamiana* leaves
- Molecular docking

Conferences

1) 66th Panhellenic conference of the Hellenic society for biochemistry and molecular biology

2) 69th Panhellenic conference of the Hellenic society for biochemistry and molecular biology

Main Scientific Interests/Achivements

Since my third year of bachelor studies, I have been interested in the field of plant biotechnology. The elective course in Synthetic biology came to complete the fascination of mine around engineered plants and how their utility can help us elucidate the intricate biology behind them. Later, while I was conducting my bachelor thesis, I learnt about the triterpenoid metabolism and its entanglement in *L. japonicus*- endophyte interactions. I have seen how different triterpene pathways regulate different aspects of nodulation and FsK colonization, which is another symbiotic assosiation of the plant. Even though this interaction between triterpenes and symbioses has been studied in the last few years, the actual mechanisms of action behind each triterpene pathway remain unexplored. To be more precise, the protein partners of each triterpene are still unknown, and this is the case for the majority of triterpenes in all plant species. Consequently, I wanted to combine my two interests, synthetic biology and triterpene-protein interactome, and further explore them during the next three years of my PhD program.

As the main approach to gene studies is functional analysis in mutant plants, I started to learn a lot about CRISPR/Cas9 technology and how I can utilize it in *L. japonicus* to answear many questions around the legume physiology. So, during my research years as a master student, members of the host laboratory and I came up with an **efficient cloning strategy** that would facilitate efficient assembly of any CRISPR/Cas9 binary vector. We decided to employ the Golden Gate DNA assembly method as it is well-known for its effective cloning and assembly of multiple parts in a single step. First, we created a toolkit of CRISPR/Cas9 level 0 modules and level 1 transcriptional units necessary for CRISPR/Cas9-mediated gene editing. After CRISPR target selection, each CRISPR/Cas9 binary vector can be assembled in two cloning reactions. Cloning parameters and transformation conditions have been optimized to ensure identification of positive clone in a few days.

The above CRISPR/Cas9 system was selected to ensure **optimized mutagenesis** in the studied model legume *L. japonicus.* It includes endogenous promoters that ensure optimal expression of Cas9 and sgRNA. Even the T-DNA architecture was taken into consideration to enhance the mutation frequency of each system. Additionally, it employs a double selection scheme for efficient selection of true trangenic tissues during stable transformation. My plan is to employ this system and generate a series of mutant plant lines depleted of certain triterpenes.

I look forward to investigating the phenotype of the generated triterpene-depleted plants, including the external phenotype and the internal transcriptomic and metabolomic changes. I am even more excited to identify putative protein partners of those triterpenes in the two studied symbioses. By combining these two approaches, I am eager to recond exactly how each triterpenoid pathway affects the two studied symbioses and if, the triterpenes have contrary effects, how they are balanced out to allow efficient symbiotic nitrogen fixation and shape FsK colonization pattern.

Like many other members of the plant research community, I also wish to contribute in enhancing the quality of human life but also preserve the biodiversity of ecosystems. By embarking on this journey, the laboraroty of Plant and Environmental Biotechnlogy and I plan to extrapolate the acquired knowldege and techniques to legume crops and enhance crop yield and quality, while also generating means as to preserve our ecosystems.