Escola Tècnica Superior d'Enginyeria Agrària

Universitat de Lleida

IRBLleida Institut de Recerca Biomèdica

Understanding how metabolism can make or break a plant's ability to adapt to extreme conditions: the Tamarix case study in the Negev

A RESEARCH PROJECT PROPOSAL

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ABSTRACT

Research proposals are a necessary step towards successfully carrying out any desired research project. The main goal of this project includes learning what research proposals are, where to send them and how to write them. The proposal described in 4. PROPOSAL is based on the guidelines provided by the European Commission's Horizon 2020 initiative.

The aforementioned proposal is based on the unique properties of the desert plant *Tamarix* found in the Negev (Israel). The research project proposed is mainly focused on the plant's survivability in high salinity, arid environments. If conducted correctly, this project could lead to knowledge on how to transfer the salt resistance mechanisms *Tamarix* possesses to economically important crops and, therefore, help cope with issues related to global climate change such as desertification. An alternative to growing food crops in stressful conditions is dedicating the areas affected by these conditions to biomass production for biofuels.

Overall, writing a bachelor's thesis that revolves around planning a research project along with composing a proposal for it is a critical skill for aspiring researchers to acquire. Projects will remain as ideas unless they are brought into the "real world" by gaining interest and funding through proposals.

RESUM

Les propostes de recerca són un pas necessari per a dur a terme els projectes de recerca en qüestió amb èxit. L'objectiu principal d'aquest projecte inclou aprendre què són les propostes de recerca, a on enviar-les i com redactar-les. La proposta descrita en l'apartat 4. PROPOSAL es basa en els directius proporcionats per la iniciativa d'*Horizon 2020* de la Comissió Europea.

La proposta mencionada prèviament es basa en les propietats úniques de la planta desèrtica *Tamarix* que es troba al Nègueb (Israel). El projecte de recerca que es proposa es centra sobretot en la supervivència de la planta en ambients àrids i amb una salinitat elevada. Si es desenvolupa correctament, aquest projecte podria conduir a la informació necessària per a transferir els mecanismes de resistència a la sal que posseeix *Tamarix* a conreus amb una alta importància econòmica. Així, podria ajudar a fer front als problemes relacionats amb el canvi climàtic global, com pot ser la desertificació. Una alternativa a conrear camps afectats per condicions d'estrès és dedicar aquestes àrees afectades a la producció de biomassa per biocombustibles.

En general, escriure un Treball de Final de Grau que es basa en planificar un projecte de recerca i elaborar una proposta sobre aquest és una habilitat crucial per als investigadors aspirants. Els projectes es romandran com a idees si no es porten al món real a través de l'interès que aquests generen i del finançament obtingut per les propostes.

RESUMEN

Las propuestas de investigación son un paso necesario para llevar a cabo los proyectos de investigación en cuestión con éxito. El objetivo principal de este proyecto incluye aprender qué son las propuestas de investigación, dónde enviarlas y como redactarlas. La propuesta descrita en el apartado 4. PROPOSAL se basa en los directivos proporcionados por la iniciativa de *Horizon 2020* de la Comisión Europea.

La propuesta mencionada previamente se basa en las propiedades únicas de la planta desértica *Tamarix* que se encuentra en el Néguev (Israel). El proyecto de investigación que se propone se centra sobretodo en la supervivencia de la planta en ambientes áridos y con una salinidad elevada. Si se desarrolla correctamente, este proyecto podría conducir a la información necesaria para transferir los mecanismos de resistencia a la sal que posee *Tamarix* a conreos con una alta importancia económica. Así, podría ayudar a hacer frente a los problemas relacionados con el cambio climático global, como puede ser la desertificación. Una alternativa a conrear campos afectados por condiciones de estrés es dedicar estas áreas afectadas a la producción de biomasa para biocombustibles.

En general, escribir un Trabajo de Final de Grado que se basa en planificar un proyecto de investigación y elaborar una propuesta sobre este es una habilidad crucial para los investigadores aspirantes. Los proyectos se permanecerán como ideas si no se llevan al mundo real a través del interés que estos generan y del financiamiento obtenido por las propuestas.

1. INTRODUCTION

Scientific research is an important pillar of modern society, opening the gateway to a sustainable and ideal future. Research provides knowledge that can prove to be beneficial to all communities – not just the scientific community.

In order for a research project to achieve the desired results, financial support is a necessity. A common requirement to access financial support in most cases is a research proposal: a document describing a proposed study created and tailored to investigate a problem (Al-Riyami, 2008). A wide selection of grants is available to researchers around the globe. In this case, the focus will mainly be on those available to researchers within universities and research organizations in Europe.

1.1 Funding

1.1.1 Human Frontiers Science Program (HFSP)

The HFSP is an international program of research support aimed at innovation in life sciences research with international collaboration (Human Frontier Science Program, 2019).

They offer two kinds of research grants: Young Investigators' Grants and Program Grants. Grants are awarded to innovative groups working on projects that would otherwise not receive funding from national programs. They are strictly aimed at postdoctoral level basic research.

1.1.2 Horizon 2020

Horizon 2020 is an initiative from the European Commission, specifically the European Union Research and Innovation Program, designed to act as a financial instrument from 2014 to 2020. It was created as a part of the Innovation Union flagship initiative of the Europe 2020 strategy for smart, sustainable and inclusive growth (Walburn, 2010).

Proposal submissions must be limited to the topics covered by calls released throughout the year. There are, however, calls that provide more freedom while selecting a project topic. These are known as open calls, and they cover a broad spectrum of subtopics.

All calls have strict application periods that must be respected. Some calls require a specific format to be followed. The details on the exact requirements that must be fulfilled for an application to be considered are specified on each call's funding and opportunities page.

European Research Council (ERC)

The ERC provides several financing options such as starting, consolidator and advanced grants (European Commission, 2019a). These grants heavily merit academic achievements and are awarded to those who are most likely to provide promising research.

The ERC's mission is investigator-driven, allowing researchers to determine which direction they deem to be of utmost importance in modern research.

1.2 Motivation

This Bachelor's Thesis revolves around the process of writing a research project proposal. In order to do so, one must know how to write a proposal based on where it is to be sent. The destination is an important element to take into consideration given that it may mean the difference between the proposal being accepted or rejected. Many calls require a specific format that must be carefully followed.

A proposal must include all of the main ideas and aspects of a research project displayed in an attractive, clear and concise manner. Essentially, the goal is to present the project in a way that the desired parties gain interest in it. Only then will it be able to continue through the process.

Taking this into account, there are two main aspects to this thesis: investigating how a proposal should be written in order to move forward with the project and researching the proposal topic itself.

2. OBJECTIVES

The first objective consists in learning how to correctly write a proposal, a useful skill to have when working in a research group. It is important to know which resources are available to make projects viable, such as different funding opportunities, and the processes required to obtain them. This entire process is what turns an idea into a tangible, feasible project.

In order to have a project, a topic must be selected. In this case, the proposed project aims to study the metabolomic and transcriptomic characteristics of the halophytic *Tamarix* located in the Negev (Israel).

Therefore, this project involves learning how to coordinate two different groups from different countries: Israel and Spain. It is quite useful to understand how to collaborate with other teams on the same project. There are many funding opportunities aimed towards interdisciplinary and multi-country projects that facilitate their execution.

The idea of the project is to discover which metabolic pathways and gene expression patterns contribute to *Tamarix*'s coveted properties which enable it to survive in elevated salinity conditions and drought. *Tamarix* potentially possesses survival mechanisms unique to those found in other plants, and these could be easily applied to salt sensitive plants. Discovering this would help explore new possibilities and lead to the goal of creating genetically modified crops able to survive in harsh environments. This achievement would ultimately contribute towards ensuring global food security.

3. METHODOLOGY

Elaborating the previous introduction (1. INTRODUCTION) required exploring the current state of research project proposals in Europe. This involved gathering information from various organizations, such as the European Commission, that offer financial support for certain scientific endeavors.

There must be a specific project planned out to be able to apply for a loan or grant. An idea for a project was developed after consulting with the Systems Biology and Statistical Methods or Biomedical Research group at the University of Lleida - Lleida Biomedical Research Institute (IRB Lleida).

After that, a project proposal is needed in order to display all of the information required for the desired project. Carrying out the aforementioned task involved writing from the perspective of a researcher employed by a public research center, such as the University of Lleida - IRB Lleida.

This proposal (detailed information in 4. PROPOSAL) was written by following the Bachelor's in Biotechnology Thesis Style Guide provided by the *Escola Tècnica Superior d'Enginyeria Agrària* (ETSEA, University of Lleida). The detailed proposal requirements specified by Horizon 2020 were used as a template and were adapted to fit the previously mentioned style guide (European Commission, 2019b).

Specific information had to be present in the proposal, and therefore an extensive amount of research had to be done on the topic. This information was obtained after reading numerous scientific articles on the subject, as well as consulting various researchers.

The budget required determining which elements would be necessary to carry out the project. To do so, the budget was split into six categories: personnel, equipment, consumable material, travel, other and overhead. A list was created containing the indispensable components along with their individual prices.

4. PROPOSAL

4.1 Summary

Call	FET Open
Topic	Crop resistance and survival in challenging environments
Type of Action	Research and innovation actions
Proposal Number	H2020FET-TAMARIX
Proposal Acronym	TAM2020
Proposal Title	Understanding how metabolism can make or break a plant's ability to adapt to extreme conditions: the *Tamarix* case study in the Negev
Duration (months)	36
Key words	salt stress, drought stress, *Tamarix*, abiotic stress

Abstract Climate change has negatively affected plant growth, increasing the occurrence of drought and elevated soil salinity.

Studying defense mechanisms that plants have developed to survive in elevated salinity and drought affected environments is a necessary investment due to the increasing percentage of areas that present these qualities. This leads to lower crop yields, an unacceptable consequence due to the world's ever-increasing population and the need to keep up with its alimentary needs.

Different species of *Tamarix* have adapted to adverse conditions in the Negev desert, surviving in droughts and soils with a salt content found to be too high for most glycophytes. Discovering which metabolic pathways are

more active in *Tamarix* could be the key to characterizing an alternative defense mechanism for these conditions in other plants. Transforming economically important crops with the genes involved in the metabolic pathway would allow them to have a better chance of survival.

4.2 Background and Significance

4.2.1 Salinity

Climate change has a negative impact on food security due to its effect on crop yields. One of the consequences involves an increase in the concentration or accumulation of soluble salts in soil; a process known as salinization (Bockheim and Gennadiyev, 2000). Soil salinization is a threat to agriculture, reducing average glycophytic crop yields by 50-80% (Panta et al., 2014).

Desalination plants remove mineral elements from saline water. These plants produce consumable water from seawater and provide it to the surrounding inhabitants. Israel is a clear example of a country with a water resource problem. This is what lead to the construction of one of the world's largest desalination plants (Sauvet-Goichon, 2007).

Reject brine is a product of the desalination process that is, in some cases, disposed of in unlined pits. Brine has higher salt concentrations than the seawater it comes from. It can seep into the soil and contaminate the groundwater and surrounding soils, elevating their salt concentrations (Mohamed et al., 2005).

4.2.2 Plant adaptations to salinity

Many plants, such as glycophytes, are sensitive to higher concentrations of salts, including economically important crops like *Triticum aestivum* (wheat) or *Zea mays* (maize). Halophytes, by contrast, need a higher concentration of ions than that found in non-saline soils in order to thrive.

Glycophytes and halophytes have evolved to tolerate soil salinity to a low or high degree respectively. These differences are seen in which mechanisms each type of plant has developed. Plants may undergo osmotic, ionic and oxidative stress when exposed to saline soils (AbdElgawad et al., 2016).

When exposed to osmotic stress, glycophytes try to limit the ion influx into the shoot whereas halophytes take up these ions, displaying a high capacity for partitioning (Hasegawa et al., 2002). Glycophytes reduce water uptake into plant roots, an action which must be compensated. In plants such as *Arabidopsis thaliana* and maize, there is a reduction of the root hydraulic permeability in order to minimize water loss (Horie et al., 2012).

There is evidence that proline is involved in adaptation to osmotic stress in plants (Fichman et al., 2015). Proline, glycine betaine and sugars can be used for osmoprotection; they are compatible solutes capable of maintaining cell turgor (Wang and Vinocur, 2003).

4.2.3 Drought

A drought is a natural hazard that consists in a deficiency of precipitation (Şen, 2015). Some plants have developed certain mechanisms to survive in drought or scarce water conditions. Major crops such as wheat, maize and *Oryza sativa* (rice), however, have not developed such mechanisms. Droughts can have an immense effect on food production and threaten global food security.

Climate change affects crops around the globe by increasing temperatures. There is a high likelihood that growing season temperatures will surpass the most extreme seasonal temperatures recorded from 1900 to 2006 for most of the tropics and subtropics by the end of the 21st century (Lobell et al., 2008). Predictions state that these conditions will become even more frequent globally in the future. Therefore, economically important crops, among others, will have lower survival rates if nothing is done.

4.2.4 Plant adaptations to drought

Plants have an array of different mechanisms that ensure their survival in drought conditions. Photosynthesis is reduced through stomatal closure by abscisic acid and metabolic impairment (Reddy et al., 2004). This process exchanges growth for survivability. Drought tolerant plants do not present an increase in abscisic acid and, therefore, do not reduce photosynthesis (Robertson et al., 1994). This also means that their growth is not penalized.

Other plant hormones such as cytokinin, gibberellic acid and ethylene are also involved in drought stress response. Cytokinin has the ability to delay drought related leaf senescence (Robertson et al., 1994). Gibberellic acid represses growth when plants are exposed to drought conditions (Wang et al., 2008), and ethylene promotes leaf senescence (Basu et al., 2016).

4.2.5 *Tamarix*

The genus *Tamarix* comprises of an array of flowering plants originating in arid environments in Eurasia and Africa. One of these environments is the Negev desert located in Israel, for example, where some *Tamarix* species are used as wind breakers in agricultural fields.

The normal annual rainfall in Be'er Sheva, near the Negev, detected by the Israel Meteorological Service from 1981 to 2010 is 195 millimeters (Israel Meteorological Service, 2019). It is also reported that the amount of rainy days with more than 1 millimeter of rain is less than 1 from May to October.

These statistics indicate that *Tamarix* is well adapted to desert climates. *Tamarix* is capable of surviving in periods of water stress with miniscule fluctuations in its physiological processes (Xu et al., 2007). This stability does not jeopardize its growth, making it an attractive study topic.

4.3 Hypotheses and Objectives

4.3.1 Hypotheses

This project is based on two hypotheses (H):

H1: *Tamarix* possesses different mechanisms than those currently known in other plants from present research that allow it to grow and prosper in desert climates. There are also different species belonging to *Tamarix* with different attributes. These attributes may confer characteristics favoring better or worse survival in adverse conditions.

H2: The genes expressed in *Tamarix* that help it survive in adverse conditions can be transferred to other plant species and improve their survivability in these conditions.

4.3.2 Objectives

There are two main goals (G) for this project:

G1: Establish which genes in *Tamarix* are important for drought and salinity resistance.

G2: Contribute to transforming plants to allow them to survive in drought ridden or high salinity environments.

These goals can be accomplished when the objectives (O) have been completed:

O1: Retrieve representative samples from different species of *Tamarix* growing in the Negev (Israel). This will lead to a reliable study of *Tamarix*, and it will contribute to the validation of H1.

O2: Collect omics data from the samples. This information will help determine which metabolites and pathways are unique to resistant species of *Tamarix*. It will also contribute to the validation of H1.

O3: Establish which pathways are relevant to drought and salinity resistance in *Tamarix*. This will help determine which genes are needed to confer resistance. It will also contribute to the validation of H1 and H2.

O4: Create transformed plants that are more resistant to drought and salinity than the wildtype of the same plant. This will confirm whether the established resistance mechanisms in *Tamarix* are effective or not in other plant species. It will also contribute to the validation of H2.

O5: Elaborate articles and attend conferences to disseminate results to the scientific community. This will help other groups gain interest in the project and make future investigation opportunities possible. It will also contribute to H1 and H2.

4.4 Materials and Methods

This project requires the collaboration between two research groups: one located at the IRB from the University of Lleida (UdL) and another at Tel-Aviv University (TAU).

Both research groups have access to all of the equipment needed for the described project.

In order to carry out this project, 3 years are needed. Each task proposed by a work package must be completed by adequate and qualified personnel (

Table 1). The tasks are divided between the two groups IRB and TAU. Each research group must be comprised of at least one principal investigator, two senior investigators and one investigator.

Table 1: Personnel needed for the project by the Lleida Biomedical Research Institute (IRB) and by Tel-Aviv University (TAU).

Group	Initials	Role	Group	Initials	Role
IRB	IPI	Principal Investigator	TAU	TPI	Principal Investigator
IRB	ISI	Senior Investigator	TAU	TSI	Senior Investigator
IRB	IIA	Investigator (predoctoral)	TAU	TIA	Investigator (predoctoral)

4.4.1 Work Packages

Achieving the goals and objectives mentioned in 4.3 Hypotheses and Objectives requires the completion of five work packages (WP) which are briefly described below.

WP1: Gather *Tamarix* biological samples.

- **WP1.1**: Collect samples that represent expression patterns relevant to the environmental conditions in both summer and winter.

WP2: Collect omics data from samples.

- **WP2.1**: Identify compounds present in *Tamarix*.
- **WP2.2**: Perform transcriptomics analyses.

WP3: Perform statistical analyses on data from WP2.

- **WP3.1**: Create tools to transform the data based on the required format for its destination.
- **WP3.2**: Run different statistical tests on metabolomics data.
- **WP3.3**: Interpret results and perform a pathway analysis.
- **WP3.4**: Compare metabolomic and transcriptomic results.

WP4: Test relevant genes in another species.

- **WP4.1**: Select a salt-sensitive species and transform with genes found.
- **WP4.2**: Observe changes relative to salt sensitivity in transformed plant. Quantify changes and determine results through statistical analyses.

WP5: Dissemination of results.

- **WP5.1**: Publish two scientific papers in reputable journals.
- **WP5.2**: Attend three conferences.

WP	1	**Start trimester**	1	**End trimester**	2
Participants	TPI, TIA			**Person-months**	12
Objectives	O1				
Materials	• Experimental plot in the Negev • 3 lines of salt tolerant *Tamarix nilotica*. 3 adjacent trees of each line with salt, 3 adjacent trees of each line without salt. • 1 line of less salt tolerant *Tamarix aphylla* (control). 3 adjacent trees with salt, 3 adjacent trees without salt. • Brine (salty water) • Reclaimed water • Drip system • 1.4 mL tubes • Dry ice • Cryogenic grinder • Ultra-low temperature freezer				

Methods	WP1.1: Have each line divided into 2 groups: with salt and without salt. The first group must be watered with brine, and the second group must be watered with reclaimed water. For each line, collect samples one hour after sunrise from adjacent trees of young branches with tiny leaves. Collect samples in both summer and winter. Freeze samples with dry ice and grind with the cryogenic grinder using 1.4 mL tubes. Store samples at -70ºC.
Deliverables	-

WP	2	**Start trimester**	3	**End trimester**	4
Participants	IPI, IIA			**Person-months**	12
Objectives	O2				
Materials	• GC-MS service • Transcriptomics service				
Methods	WP2.1: Take frozen samples and send to GC-MS service located at the University of Lleida. WP2.2: Send samples to an external genomics service such as BGI.				
Deliverables	-				

WP	3	**Start trimester**	5	**End trimester**	8
Participants	IPI, ISI, IIA			**Person-months**	36
Objectives	O3				
Materials	• Computers compatible with R 3.6.0, Python3				
Methods	WP3.1: Gather data collected from GC-MS and identify compounds present in the samples. Enter all information in an easy to parse database. Write scripts using Python3 to transform the data to fit the input format required by different tools. WP3.2: Write scripts using R to run statistical tests on metabolomics data. WP3.3: Interpret results from statistical tests and determine a pathway for unique metabolites in resistant *Tamarix* lines. Combine different pathways found with KEGG to define a general metabolic pathway. WP3.4: Compare metabolic analysis results with transcriptomics results received from the external genomics service. Determine common results from both analyses.				
Deliverables	*Tamarix* important metabolite database, *Tamarix* metabolic pathway with candidate resistance metabolites				

WP	4	**Start trimester**	9	**End trimester**	12
Participants	TPI, TSI, TIA, IPI, ISI, IIA			**Person-months**	72
Objectives	O4				
Materials	• *Arabidopsis thaliana* plants • *Agrobacterium tumefaciens* • Sucrose • Silwet L-77				
Methods	WP4.1: Research salt-sensitive plant species that are easy to maintain and grow in a lab setting, such as *Arabidopsis thaliana*. Isolate genes that convey salt resistance. Transform the plants following the floral dip method (Zhang et al., 2006). This method consists in dipping the above ground parts of *Arabidopsis thaliana* (flowers) in a solution with sucrose + Silwet L-77 + *Agrobacterium*. WP4.2: Record changes observed in transformed plants and compare to wildtype.				
Deliverables	Transformed *Arabidopsis thaliana* plants (higher resistance to salt vs wildtype)				

WP	5	**Start trimester**	5	**End trimester**	12
Participants	IPI, ISI, IIA, TPI, TSI, TIA			**Person-months**	72
Objectives	O5				
Materials	-				

Methods	WP5.1: Write at least two articles during this time period based on findings. Send them to be reviewed and published by reputable journals. WP5.2: Attend one international conference during the second year (trimesters 5 through 8). Attend two international conferences during the third year (trimesters 9 through 12).
Deliverables	Articles based on research findings

4.5 Expected Results

This project, if carried out successfully, should yield distinct significant results. Each objective leads to a key part of the research project.

O1: There should be enough samples to accurately represent different lines of *Tamarix* with and without high concentrations of salt throughout summer and winter. These samples should be compatible with a posterior transcriptomic analysis.

O2: The main components of each sample should be clearly identified. This information should also suffice for a posterior metabolomic analysis.

O3: The key metabolites and genes related to plant survival in adverse conditions, such as high salinity, should be identified.

O4: *Arabidopsis thaliana* plants should successfully be transformed with salt resistance genes and be able to survive in higher salinity environments.

O5: Results should be communicated to other research groups working on similar projects.

4.6 Project Timetable

The project will take a total of 3 years (36 months) to complete. Each work package should be completed within the expected time frame detailed in

Table 2.

Since the project team is comprised of two groups from different countries, there should be meetings scheduled between them throughout the project. The plan for disseminating results described in 4.8 Dissemination of Study Results includes attending three conferences. This will be exploited to set up meetings between both groups during at least two of the conferences. Meetings with members from both groups will help make the project process as smooth as possible by avoiding misunderstandings, facilitating brainstorming and promoting teamwork.

Table 2: Timetable showing time needed for each work package (WP, described in section 4.4.1) for the entire 36-month project (divided into trimesters). Colored squares (blue) indicate that the work package is active in that timeframe. Grey squares indicate that the work package is inactive during the timeframe.

	Trimester											
Work Package	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
WP1.1	■	■										
WP2.1			■	■								
WP2.2			■	■								
WP3.1					■	■						
WP3.2					■	■						
WP3.3							■	■				
WP3.4								■				
WP4.1									■	■		
WP4.2										■	■	■
WP5.1					■	■	■	■	■	■	■	■
WP5.2					■	■	■	■	■	■	■	■

4.7 Expected Impacts

The main outcome of this project is the discovery of which genes are relevant to salt resistance and the mechanisms that they are involved in. This also includes the creation of *Arabidopsis thaliana* plants that are more resistant to higher concentrations of salt.

The findings from this project can contribute to solving important socioeconomic problems. Creating salt resistant crops is a crucial step towards ensuring global food security. This would, for example, allow areas with limited water sources to grow crops using brine from water desalination.

Another possible impact generated from this project could be the production of biomass for biofuels with *Tamarix* (Eshel et al., 2011). Knowing which resistance related characteristics *Tamarix* may possess and selecting these for biomass production would increase efficiency. This would be a useful way to take advantage of environments with adverse conditions.

4.8 Dissemination of Study Results

Maximizing the brunt of the results will help achieve the desired outcome discussed in 4.7 Expected Impacts. To do so, a plan proportionate to the scale of the prospective project has been elaborated.

During the second year, two members from each group will attend one international (Spain) conference. An example of such is the Soil, Plant and Water Sciences conference in Madrid. The metabolites in quantities unique to *Tamarix* should be identified. A preliminary understanding of the pathways involved in salt resistance should be achieved.

During the third year, two members from each group will attend one national (Czech Republic) and one international (Netherlands) conference. Examples include the World Congress on Plant Biotechnology & Agriculture (Prague) and the European Congress on Biotechnology (Maastricht). The metabolic pathways involved in salt resistance should be defined. *Arabidopsis thaliana* plants should successfully be transformed with related salt resistance genes and should grow properly without being inhibited by higher salt concentrations.

The project members will send their results in an article to high-impact scientific journals such as the Plant Biotechnology Journal and Molecular Plant to be published. The goal is to have at least two published articles describing the results.

4.9 Ethical Considerations

This project proposal does not include any elements that violate current codes of ethics. The genetically modified plants (*Arabidopsis thaliana*) proposed by this project will be kept within the laboratory. This does not go against European Union Law (EUR-Lex, 2001).

4.10 Budget

The prospective budget for this project has been calculated and can be seen in Table 3. It is divided into six categories:

- Personnel
  - Pre-doctoral researcher: 20,000 € / year (3 years IRB, 3 years TAU)
  - Lab technician: 21,000 € / year (3 years TAU)
- Equipment: centrifuge, cryogenic grinder, ultralow temperature freezer, vortex, ceramic mortars, computers
- Consumable material:
  - Plastics and glassware: micropipettes and tips, glass pipettes, Pasteur pipettes, Eppendorf tubes, polypropylene funnels
  - Reagents, solvents and liquids: liquid nitrogen, methanol, ribitol, chloroform, ethanol, tris, LiCl, isopropanol, sodium acetate, agar, EDTA, SDS, phenol, isoamyl-alcohol, pGEM-T Easy, MS medium, casein hydrolysate, proline, mannitol, hygromycin, NAA, BAP, Silwet L-77
  - Gardening: drip system, brine, pliers, *Arabidopsis thaliana,* planters, soil
  - Other: dry ice, gloves, gold particles, *Agrobacterium tumefaciens*, hard drives

- Travel: Costs related to travel to and from conferences, accommodation, diet and inscription.
    - Soil, Plant and Water Sciences conference (Madrid):
        - Transportation: 240 € (IRB), 650 € (TAU)
        - Accommodation and registration: 1,420 € (IRB), 1,420 € (TAU)
        - Food and beverage: 320 € (IRB), 320 € (TAU)
    - World Congress on Plant Biotechnology & Agriculture (Prague):
        - Transportation: 580 € (IRB), 420 € (TAU)
        - Accommodation: 330 € (IRB), 330 € (TAU)
        - Registration: 500 € (IRB), 500 € (TAU)
        - Food and beverage: 300 € (IRB), 300 € (TAU)
    - European Congress on Biotechnology (Maastricht):
        - Transportation: 300 € (IRB), 650 € (TAU)
        - Accommodation and registration: 2,500 € (IRB), 2,500 € (TAU)
        - Food and beverage: 350 € (IRB), 350 € (TAU)
- Other: Costs related to publishing fees and external services (GC-MS, transcriptomic analysis, RT-PCR).
- Overhead: calculated according to Horizon 2020 guidelines (25% of the total direct costs).

Table 3: Finances for both groups, IRB and TAU, needed to execute the project. The requested funding is in k€ (1 k€ = 1,000 €). There are six categories: personnel (Pers.), equipment (Equip.), consumable material (Cons.), travel, other and overhead (Over.).

Group	Pers.	Equip.	Cons.	Travel	Other	Over.	Total
IRB	60	2	1	6.7	3.3	18.3	91.3
TAU	123	12	12	7.3	12.2	41.6	208.1
Total	183	14	13	14	15.5	59.9	**299.4**

5. DISCUSSION

Research project proposals are a necessary step toward successfully obtaining funding for any given project and projecting its importance to other members of the scientific community, allowing it to move forward. Only then can a project take off and get results that could potentially change the world and the lives of its inhabitants for the better.

Writing a research project proposal for a bachelor's thesis is perhaps the most useful way to learn which processes are necessary in order to complete a real-life project.

In this particular case, the requirements for applying to a Horizon 2020 call were considered while writing the proposal. This seemed to be a good option, especially when contemplating the fact that this project involves the collaboration between two distinct research groups. The European Commission encourages and prioritizes interdisciplinary, multi-country partnerships. This project focuses on combining plant science, bioinformatics and genetic engineering with the help of groups from two eligible countries, making it an excellent candidate for Horizon 2020.

The proposal requirements established by the European Commission for Horizon 2020 FET-open research projects are more restrictive and bureaucratic than those established by the Bachelor's in Biotechnology Thesis Style Guide from the University of Lleida. In order to deliver a more complete proposal directed towards the University's evaluation, the Horizon 2020 format was used as a guideline instead of as a strict template.

The proposal itself was able to be written thanks to the knowledge acquired from studying the Bachelor's Degree in Biotechnology offered by the University of Lleida. Further investigation on topics such as statistics, bioinformatics and plant physiology was needed to attain sufficient information on the chosen project. This is what helped the project proposal be as thourough as possible.

In conclusion, writing a research project proposal has incorporated a wide pool of skills. These include bibliographic research, experiment design and communication, among others.

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