DEVELOPMENT OF A NOVEL RHIZOBACTERIAL BIOFERTILIZER TO RELIEVE SALT STRESS IN *Cicer arietinum*

Marc Kessels Solé

Project Proposal | Bachelor's in Biotechnology | May 2022

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INTRODUCTION

In the midst of the current environmental and agricultural landscape, which demands the intensification of production practices, technology with a low environmental impact is needed. Here, the chickpea (*Cicer arietinum*), due to its low yield, arises as an underexploited resource with great potential for improvement [1]. In the Spanish peninsula, its deficiency in production is caused, in part, by the unfavourable distribution of rainfall, the occurrence of biotic attacks, and the susceptibility of cultivars to abiotic stresses. In fact, amongst the latter, salinity has been shown to be most relevant, since chickpea tolerance is poor and more than a quarter of fresh water in the national territory is salinized, as shown in Figure 1 [2].

Fortunately, in recent years, bacteria that promote plant growth (PGPB) through various physiological mechanisms have been isolated, displaying the ability to alleviate this stress [3]. However, these strains are applied through peats or solutions, which provide low protection from the environment. Thus, inoculants must be further formulated into a product that withstands storage and delivery to the field. For this, the immobilization inside an alginate matrix with diverse filling materials, such as perlite, bentonite or starch, has been studied, since these materials confere a higher mechanical resistance [4].

Objectives Taking this into account, the objectives of this proposal are the following: **1)** the isolation and identification of PGPB species found in the rhizosphere of *C. arietinum* in the Spanish peninsula, **2)** the study of the effect of their inoculation on salt stressed plants, **3)** the formulation of the inoculant into an entraped product, with additives to stabilize it and **4)** the trial of the product at a small scale greenhouse cultivar and a large scale field experiment.

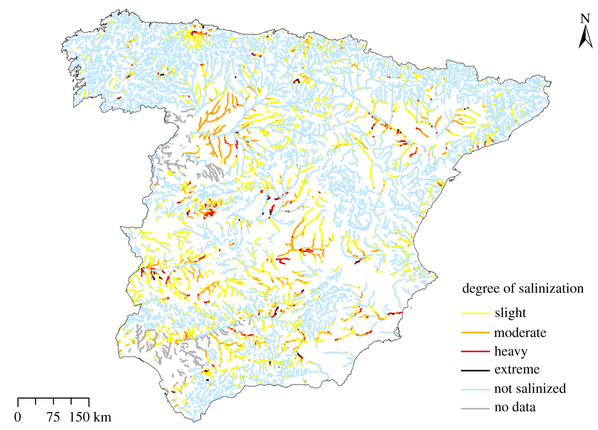

Figure 1: Map evidencing the degree of salinization in Spanish rivers [2].

METHODOLOGY

The methodology put forth to accomplish the specific objectives of this proposal is based on established plant physiology and microbiology protocols, begining with obtaining potential PGPB from a Spanish field, as seen in Figure 2 [5].

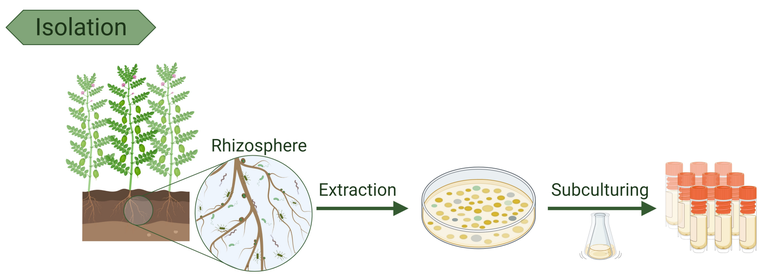

Figure 2: Schematic representation of the method to obtain native bacterial strains.

After isolation, the experimental strategy is approached as a funnel design, where subsequent characterization steps select the strains that yield the best results under salt conditions (Figure 3).

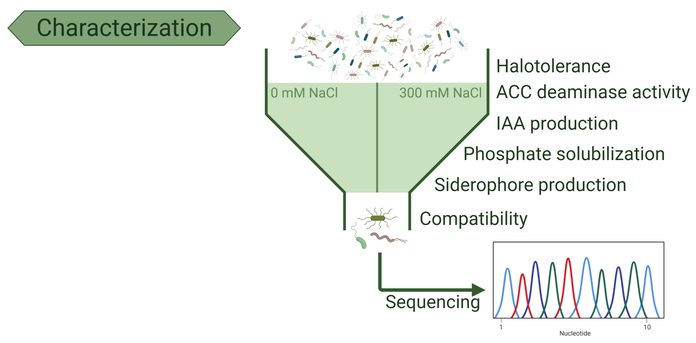

Figure 3: Schematic representation of the method to characterize and identify halotolerant PGPB strains.

Once identified, their effect on chickpea growth under salt stress will be studied through physiological parameters (Figure 4). First, in a pot experiment, the individual strains and all their possible combinations will be analysed, as exemplified in Table 1. Afterwards, the same experiment will be repeated but with the bacteria entraped in alginate along with each individual filling material. However, only the two best performing treatments, in terms of yield and metabolite content, will be used for the second assay.

Table 1: Example list of the treatments that will be tested on chickpea plants.

Treatment
Non-PGPB inoculated (control)
Isolate 1
Isolate 2
Isolate 3
Isolates 1 and 2
Isolates 2 and 3
Isolates 1 and 3
Isolates 1, 2 and 3

From here, a scaling up to a field experiment with the inoculants that display the best results in each step, both in planktonic form and in alginate beads, will be performed.

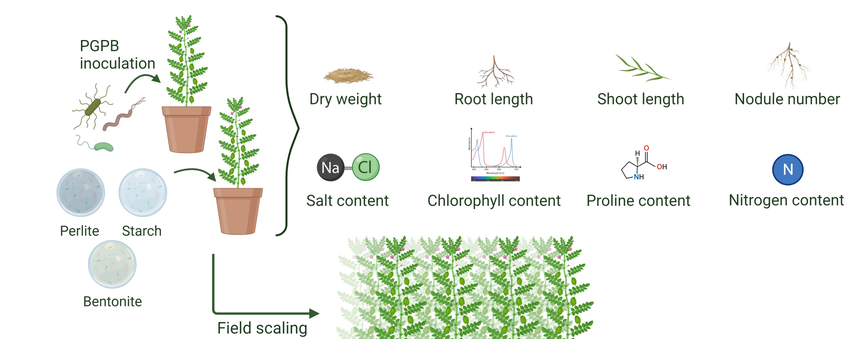

Figure 4: Schematic representation of the experimental design to study the effect of PGPB inoculation on chickpea plants under salt stress.

TIMELINE AND BUDGET

Taking into account the workload of each step in the methodology, a timeline for the project has been devised (Figure 5). Additionally, a preliminary budget has been calculated to estimate its yearly cost, considering the most important aspects of the research and the main supplies needed (Table 2).

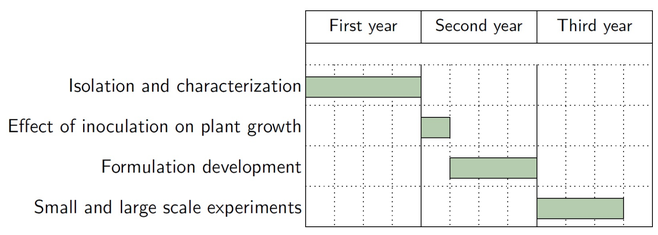

Figure 5: Planned project timeline based on the proposed methodology. The activities to be conducted have been scheduled according to the proposal objectives.

Table 2: Projected yearly and total cost of the proposal. The total cost has been calculated for a project duration of three full years.

Category	Cost (€/year)	Full cost (€)
Personnel	30 000	90 000
Technical services	10 000	30 000
Travel	5 000	15 000
Publication	2 500	7 500
Equipment	3 500	10 500
Supplies	15 000	45 000
Total	66 000	200 000

EXPECTED RESULTS

During the first part of the project, several strains are expected to be isolated from the rhizosphere. Once the best performing ones are identified, based on their physiological responses under salt conditions, their inoculation is expected to promote plant growth. Furthermore, even though salt content may vary, physiological variables, such as dry weight and proline, chlorophyll and nitrogen content, are expected to increase.

After entrapment, the treatments should display greater results than the planktonic inoculants (Figure 5). However, this will be the first study to compare the differences between three different filling materials in the same plant and under the same stress. Thus, even though they all are expected to be superior to alginate entrapment alone, the filler that will yield the best result is unknown.

Lastly, upon validation in a pot experiment, the results in the field trial are expected to replicate those of the smaller scale. However, the disarray of many parameters will likely ocurr.

Disemination plan The obtained results will deserve publication in Q1 scientific journals. Furthermore, they will be presented in national and international scientific congresses and, if the final formulation can be proposed for application, inquiry into the development of a patent will be started before publication. Lastly, since reliable information regarding biofertilizers is not readily available to the public, a divulgative work will be prepared from the expertise acquired in this project.

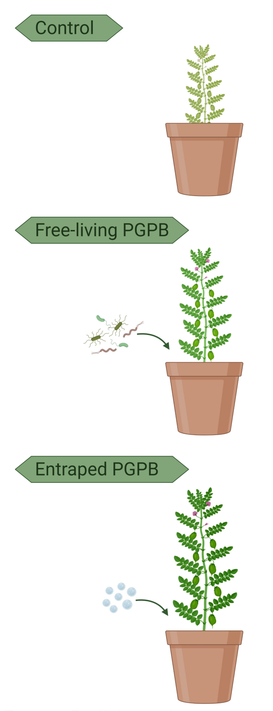

Figure 5: Schematic representation of the general expected results.

CONCLUDING REMARKS

At the end of this project, a novel field-tested biofertilizer based on the entrapment of rhizobacteria with demonstrable improvements in plant growth could be achieved. Still, further steps will have to be taken before its implementation into the market, especially regarding product regulation and scaling up.

To conlude, this work will broaden the current knowledge regarding rhizosphere ecology, through the identified strains adjunct to chickpea cultivation, and biofertilizer application, since no published comparison between filling materials exists, while developing an applicable integrated solution to mitigate salt stress in chickpea cultivars in the Spanish peninsula.

References

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