Experimental strategy for the improvement of the resistance to Common Bacterial Blight (CBB) in common bean (*Phaseolus vulgaris*)

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This PhD research project is aimed at better characterising the resistance to CBB in common bean, in order to facilitate the breeding process. The experimental approach will combine the development of new molecular markers associated with the resistance and a reverse genetic approach that aims at identifying genes involved in the resistance.

Strategia sperimentale per la progettazione ottimale di unità di ultrafiltrazione per il recupero di biopolimeri di interesse alimentare

Questo progetto di tesi di dottorato mira ad aumentare le conoscenze in merito alla resistenza alla malattia CBB in fagiolo con l’obiettivo di facilitare il miglioramento genetico della resistenza. L’approccio sperimentale scelto consisterà sia nello sviluppo di nuovi marcatori molecolari associati alla resistenza che nello studio a livello molecolare della resistenza, tramite un approccio di genetica inversa.

# **1. State-of-the-Art**

Common bean (*Phaseolus vulgaris L*.) is one of the most important grain legume crops worldwide, with over 33 Mha cultivated in 2019 and a production of about 29 million tons (FAOSTAT, 2019). Global common bean production is affected by major biotic stresses: common bacterial blight is one of the most serious diseases of beans, is endemic to most regions where common bean is cultivated and can cause severe yield reduction, even higher than 40% (Singh and Miklas 2015). The bacterial disease is seed-born and caused by *Xanthomonas phaseoli* pv. *phaseoli* and *Xanthomonas citri* pv*. fuscans* (Tugume et al., 2019)*.* Different strategies can be used to manage the disease, these go from cultural practices, such as crop rotation and use of pathogen-free seeds to chemical control, however the preferable method is the use of resistant or tolerant genotypes.

In common bean, the study of CBB resistance has been ongoing for several years and allowed the identification of at least 27 quantitative trait loci (QTLs) for CBB resistance (Chen et al., 2021). At the same time, breeding of common bean for resistance to CBB has been continuously performed over the last 50 years, and cultivars/breeding lines were obtained either from traditional method, using pathogen inoculation and disease screening or by marker-assisted selection (MAS), using three major QTLs linked with the SCAR markers SAP6 on Chr 10, SU91 on Chr 08, and BC420 on Chr 06 (Singh and Miklas, 2015). The marker BC420 has to be avoided in many bean market classes because it is linked with the V locus for seed color (Mutlu et al., 2005), which causes darkened hues, streaks, and spots, making the seeds non-commercially viable.

The breeding programs were successful in generating cultivars showing a certain degree of resistance to CBB, however they lack a complete resistance to the disease (Viteri and Singh, 2014). Furthermore, cultivars with high levels of combined resistance to both less and highly aggressive bacterial strains are lacking as well as cultivars resistant at all plant aerial parts (leaves, flowers and pods) (Singh and Miklas, 2015). Despite the high numbers of QTLs identified, it emerges the necessity to develop new markers to be used for MAS, this is especially true given the variation of resistance observed in cultivars sharing the same markers associated with the resistance (Viteri and Singh 2014). Despite the several years of work, no major resistance genes were molecularly characterised in common beans (Chen et al., 2021). This lack of information is likely due to the difficulties encountered in common bean transformations, which make the functional validation of genes challenging (Hnatuszko-Konka et al., 2014). A possibility in this regard will be the use of a mutagenized population for targeted induced local lesions in genomes (TILLING) for identifying mutants with improved resistance (Fanelli et al., 2021).

# **2. PhD Thesis Objectives and Milestones**

In light of the present state of the art, this PhD project have the following general objectives:

- the selection of the best inoculation method to distinguish between different levels of resistance to CBB;

- the obtainment of new molecular markers to be used for breeding applications;

- the obtainment of mutants of candidate genes in a mutagenized population.

This PhD project will benefit both the private sector, by providing new information for breeders but also the scientific community by advancing the knowledge of the molecular mechanisms involved in the resistance.

Within the overall objective mentioned above, this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Test of inoculation methods and generation of segregating population.**

Different inoculation methods will be tested and the most effective in differentiating the susceptibility of common bean germplasm will be selected (A1.1). The segregating populations for the mapping will be obtained by crossing resistant and susceptible genotypes, this activity has been initiated during the first year of PhD (A1.2).

A2) **Identification of new molecular markers associated with the resistance in mapping populations.**

This activity will consist in the generation of a consensus linkage map to identify molecular markers potentially linked to the resistance (A2.1), the segregating populations (F2) will be evaluated for the resistance and analysed for the recombination of the molecular markers (A2.2). The association mapping will produce new markers associated with QTLs (A2.3).

A3) **Reverse genetic approach for identifying the genes responsible for the resistance.**

A set of candidate genes will be identified either by looking at RNA sequencing experiments in genotypes with different susceptibility or by identifying orthologues of S genes in other species (A3.1). A sequencing approach will be used for identifying mutants of the selected genes in a (M2) chemically mutagenized population (A3.2). The mutants will be tested for the resistance to evaluate the involvement of the selected genes in the process (A3.3).

A4) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Set-up of Population and Inoculation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Inoculation methods |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2) Segregating population |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Molecular markers resistance***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Consensus map |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Recombination and resistance  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 3) Association mapping |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Reverse genetic approach***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Candidate genes identification |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
| 2) Sequencing of population |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3) Resistance analysis in mutants |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

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