

The main goal of this project is to identify the key genes that control flowering in response to different environmental cues in three wild species of Old World lupins: *Lupinus cosentinii* Guss., *L. hispanicus* Boiss. et Reut., and *L. pilosus* Murr. To do this, we will breed accessions with different responses to the photoperiod (length of daylight) and vernalization responsiveness (period of low temperature) to create new genetic populations. These will then be studied to determine which genes are responsible for the observed differences. We will also look at the parts of the genes that control their activity to see if changes affect how they work and the plant's characteristics. This study aims to help us better understand how plants regulate flowering induction and how they adapt to different environments.

The world needs new, more sustainable, high-protein, and nitrogen-fixing crops to reduce the use of artificial fertilizers. **Among possible candidates, lupins also stand out because they are well-suited to dry, sandy soils, which are increasingly in demand due to changing climate conditions.** While lupins are used for various purposes, including food production and soil fertilization, they require genetic improvement to thrive in unstable climates. **One of the main challenges in modern lupin genetics is adjusting their flowering and maturation time to match local climate conditions. This issue is even more important now, when extreme weather events, such as heat waves and droughts, pose significant obstacles to yield, especially for late-flowering plants.** Efforts to optimize flowering time and disease resistance have been limited due to a limited gene pool of already cultivated lupin crops. **Exploiting the genetic potential of wild lupin species adapted to diverse environments could improve existing crops or speed up the domestication of new lupin species better adapted to the climate of tomorrow.**

Based on the preliminary findings from our research, we have **selected three distinct wild lupin species, namely *L. cosentinii*, *L. hispanicus*, and *L. pilosus*, as potential subjects for an in-depth genetic study.** These species exhibit considerable diversity within their respective populations, such as days to flowering, vernalization response, and sensitivity to changes in daylight duration (photoperiod). **Our initial findings indicate that the regulation of flowering induction and vernalization response in these species may be regulated by multiple genes,** with the potential influence of several Quantitative Trait Loci (QTLs). **Consequently, we propose developing recombinant inbred line (RIL) mapping populations to enable the linkage analysis and QTL mapping, facilitating the identification of the major genes responsible for the aforementioned traits.**

We will establish new plant populations **through the crossbreeding of two parent plants with differing traits such as flowering time, vernalization response, and photoperiod sensitivity.** For each of the three plant species, we aim to generate a minimum of two mapping populations to encompass a diverse range of genetic variations. The F₁-F₅ generations will be cultivated in a controlled glasshouse environment under "speed breeding" lamps, which are designed to accelerate growth and maturity for subsequent generations. Starting from the F₂ generation and continuing through at least the F₅ generation, we will utilize the SSD (single-seed-descent) method to maintain and replicate the genetic composition of the mapping populations. Following this, we plan to sequence the genomes of the parental lines within each population using two contrasting methods: PacBio which generates very long sequences, and Illumina NovaSeq to obtain short-read data. The combination of both sequencing approaches is necessary for analysis of complex plant genomes, and will ensure the best possible quality of assembled genomes. The phenotyping of the F₅ populations will be carried out under two distinct photoperiods (8h and 16h), with two treatments (vernalized and non-vernalized), and across three diverse environments, including glasshouse and field conditions in two different locations.

Subsequently, we will analyze all F₅ plants using cost-effective, genome complexity reduction methods: DArT-seq and RAD-seq, and leverage the resulting genetic markers to construct genetic maps. These genetic and physical (genome assembly) maps for each species will be integrated to facilitate the QTL mapping – tracking of the specific regions in the genome, which most likely contain genes related with the studied traits, **leading to the identification of candidate genes associated with the regulation of flowering induction and responsiveness to vernalization within studied species.**