I. Research project objectives

Global climate changes combined with population growth are forcing the world of science to search for a new source of protein in order to support the adaptation of agriculture and reduce crop losses in the near future. ***Lupinus albus*** **L. (white lupin) is one of the most important source of proteins alternative to soybean (*Glycine max* L.).** Its seeds contain high concentrations of essential amino acids (34-45%) and oils (10-13%) in dry matter. Additionally, *L. albus* is a long day plant and in comparison to soybean (short day) it is better adapted to Polish climate, and as a result is an excellent perspective for a new source of protein. Thus, **according to specialist and breeders *L. albus* may become a critical component of traditional and functional food, as well as animal feed and green manure in the next few years**. However, current varieties still require genetic improvement in order to maximise their agronomic potential and to better adopt to short growing season. One of the major breeding limitation of *L. albus* is prolonged time of flowering and maturing.

Our previous studies using world seeds collection, revealed the complexity of flowering control in white lupin, dispersed among numerous Quantitative Trait Loci (QTLs) localized on several chromosomes. Additionally, we found that early flowering in the *L. angustifolius* L., considered as a reference species for the genus, is conferred only by 1,4-kb deletion in the promotor region of a single dominant gene *Flowering Locus T (FT)* homologue. **Therefore, the regulation mechanisms controlling early flowering in *L. albus* and *L. angustifolius*, are quite different, despite their close relationship. As a species which flowering regulation differ substantially from the reference, *L. albus* has been selected as a relevant model for the assay of flowering initiation pathways in this project.**

The main objective of this proposal is to identify known and novel noncoding RNA involved in flowering induction of *L. albus*, and to create a new, comprehensive model of flowering induction, relevant not only for lupins, but also for the entire legume family. To achieve this goal, an integrative analysis of genome, transcriptome and degradome supplemented with RT-qPCR will be performed.

II. Describe the research to be carried out

To collect leaf samples for sRNA-Seq and RT-qPCR, the phenotyping experiments will be performed in controlled temperature and humidity in two photoperiods. One set of plants will be grown at 8h day, and second one at 12h day length. A half of both sets will be vernalized before sowing. The samples of every line will be gathered at the beginning of light phase and an hour before the end of the light phase each week. Differential small RNA sequencing will be performed. Known and novel miRNA will be identified and annotated using available genetic resources and *de novo* miRNA prediction software. The RT-qPCR will be performed with specific primers for candidate miRNAs and targeted genes, revealed using degradome study.

III. Reasons for choosing the research topic

In my previous project, we studied the genetic diversity and population structure within white lupin lines collection, and created a set of hypothetical subpopulations of genetically similar accessions. Significant correlation between the phenotype and distribution of lines in the subpopulations was confirmed, but the standard deviation shows that despite the exact matching with the identified group, the number of days from sowing to flowering are different for each line. **It strongly indicated that there are additional flowering induction mechanisms.** Despite identification of an novel genetic source of early flowering in *L. albus*, which was the main goal of the SONATINA project, **additional regulation within the formed subpopulation was observed. Moreover, genome-wide association study (GWAS) revealed several markers correlated with flowering time and anchored in intergenic regions, matching the hypothetical miRNA clusters. It suggested that additional, equally important regulation relies on miRNA.**

Due to global climate warming, the optimal time frame for sowing of vernalization-dependent lupins is being reduced, therefore thermoneutrality and short vegetative phase should be the most important characteristics in new cultivar selection. Research proposed in this project significantly expands and continues on the previous project on *L. albus* flowering. This study, supplemented with our previous results, **will constitute the first comprehensive analysis of *L. albus* early flowering induction mechanisms, including genetic background, small RNA identification and analysis of transcriptome and degradome sequencing data** to elucidate the role of each layers in white lupin flowering regulation. Obtained results concerning miRNAs and their target genes along with our previous genetic variation results **will allow to create a new, comprehensive model of flowering induction, relevant not only for lupins, but also for the entire legume family.** It could facilitate the understanding of white lupin flowering induction mechanism and allow for the conscious selection of genetic crossing components.