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Grain legumes are valuable source of nutrients for animal feed and human food production. In spite of global sovbean (*Glycine max*) domination, some GMO-free alternatives with an untapped agronomic potential exists. These includes the Old World lupins (OWL) from the genus Lupinus, native to the Mediterranean region and as such well-adapted to European climate. Furthermore, lupins are an unique research object among legumes due to the complex flowering regulation which includes vernalization (a period of low temperature) requirement to promote flowering. Noteworthy, optimal flowering time is one of the key issues affecting yield productivity, especially in the era of changing climate. Old World lupin species so far have been used mainly as an animal feed component and ecological soil nitrogen fertilizer. From approximately twelve species, only three underwent domestication and are used as crops. Some preliminary attempts have been made in order to domesticate the remaining 'wild' OWL, however, none of these have been successful, perhaps due to the lack of knowledge on molecular control of agronomic traits. Importantly, identification of early-flowering, thermoneutral lines was a critical step in domestication of three lupin species, enabling temperature- and photoperiod (the period of daily illumination, received by an organism)-independent sowing. Therefore, understanding of flowering regulation in other OWL species could greatly enhance the future breeding programs involving wild lupins. Interestingly, our previous research have shown that **despite their close** relationship, the detailed mechanism of flowering regulation differs significantly between three lupin crops: Lupinus angustifolius, L. albus and L. luteus, which may imply similar differences among the other OWL species. Indeed, preliminary research highlighted large variation in vernalization responses between particular lines of L. pilosus and L. cosentinii. Nevertheless, neither the molecular mechanism of flowering regulation in wild OWL species, nor the comparative studies of their photoperiod and vernalization response have been performed. The main goal of this project is to decipher the mechanisms of flowering induction in wild species of Old World lupins and to unravel the variety of these processes within studied species.

The experimental part of the project will consist of the following steps:

- 1. The seeds of 11 wild OWL species (over 400 lines) will be sown in greenhouse in order to assay flowering time as well as vernalization responsiveness.
- 2. The DNA from each plant sample will be isolated and sequenced using high-throughput Diversity Array Technology. This genotyping (the process of determining differences of an individual by examining the individual's DNA sequence and comparing it to another individual's sequence) technique will be used to compare each line within species and then to group the similar lines together (early- or late-flowering).
- 3. The results from the previous steps will be used in selection of two different lines (e.g. early and late) for all studied species. These lines will be subjected to RNA and DNA sequencing, which will provide an information about all transcribed genes, their differences, non-transcribed sequences e.g. introns etc. This will facilitate the development of specific primers pairs (short, single-stranded DNA fragments, used for amplification of the sequence between them) for selected genes.
- 4. The final step will involve the analysis of expression level (expression profiling) of selected genes in flowering induction. The plants will be grown in controlled-environment chamber, with two lines for each wild lupin species. Genes which expression level changes significantly during flowering/vernalization will be proposed as regulators of flowering for particular species and subjected to further comparative studies.

Flowering process and its regulation as well as vernalization response are currently among the most discussed issues in the plant molecular biology, which is reflected in articles published in top scientific journals. Understanding the variability of flowering time within wild species of Old World lupins will greatly facilitate the selection of lines that may be domesticated in the future, or could be useful in improvement of the modern cultivars. Finally, this project will generate the first, high quality genomic resources for the studied species, which would allow complex comparative studies of model and non-model legumes.