Genetic and molecular basis of early flowering of yellow lupin (*Lupinus luteus* L.) – summary

Yellow lupin (*Lupinus luteus* L.) is an annual crop that belongs to legume family (*Fabaceae*) and originates from the Mediterranean Basin. Yellow lupin is a facultative long-day plant and its natural populations are late flowering and have high vernalization requirements. It is valued for its high seed protein content and a positive effect on soil structure and fertility, resulting from the ability to symbiotically assimilate atmospheric nitrogen and a deep root system, respectively. Protein from yellow lupin seeds can be successfully used as a substitute for imported soybean meal in the nutrition of farm animals.

Early flowering and thermoneutrality are the key agronomic traits of lupins, enabling their cultivation for seeds in temperate climate. Genetic and molecular regulation of flowering induction was best described for a model plant – thale cress [(*Arabidopsis thaliana* (L.) Heynh.]. There are four main flowering induction pathways: vernalization, photoperiod, gibberellin and autonomous pathway. Output signals from these pathways converge in the regulation of expression of so-called floral integratory genes. *FLOWERING LOCUS T* (*FT*) is the key floral pathways integrator, responding to detection of environmental signals (temperature, photoperiod and light quality), encoding florigen. Expression of *FT* is tightly controlled by transcription factors and epigenetic mechanisms in response to environmental conditions and plant developmental status. Four *FT* homologs were identified in yellow lupin genome: *LlutFTa1a*, *LlutFTa1b*, *LlutFTc1*, and *LlutFTc2*. The main *FT* repressor from vernalization pathway in a model plant, Arabidopsis, is the *FLOWERING LOCUS C* (*FLC*). *FLC* undergoes epigenetic repression in the result of vernalization, which relieves *FT* gene from its repression and allows induction of flowering. However, *FLC* is absent in genomes of many legume species, including yellow lupin.

The aim of this study was to determine the candidate gene, or genes, that may confer early flowering and thermoneutrality in yellow lupin, as well as to determine the sequence polymorphism of these genes, which may underlie observed variance in the number of days to flowering and vernalization responsiveness in germplasm panel. Quantitative trait *loci* (QTL) mapping for the number of days to flowering and mapping of molecular markers anchored in the sequences of 22 genes from the flowering induction regulation pathways was performed. This mapping revealed co-segregation of the marker developed for the *LlutFTc1* gene and the main QTL for thermoneutrality (which is also one of the main QTLs for time to flowering). Markers for the *LlutFTc2* and *LlutFTa1a* genes were localized in the another two QTLs for time to flowering.

A series of insertion-deletion polymorphisms in the promoter regions of *LlutFTa1a* and *LlutFTc1* genes were detected, showing significant correlation with the number of days to flowering and/or response to vernalization in 111 analyzed collection materials. Sequencing also revealed the presence of insertion of *Copia*-like retrotransposon in the third intron of *LlutFTc2* gene which also showed significant correlation with the studied traits.

Results of expression profiling of *FT* homologs using RT-qPCR method suported the hypothesis on their subfunctionalization in yellow lupin. *LlutFTa1a* and *LlutFTc2* genes may regulate flowering induction in response to photoperiod, whereas *LlutFTc1* - in response to both photoperiod and vernalization. In the case of the *LlutFTa1b* gene no significant association was found between identified polymorphisms and time to flowering of yellow lupin.

To conclude, early flowering in yellow lupin is a quantitative trait, determined by at least a few genes. It is associated with the occurrence of thermoneutrality, i.e. the loss of vernalization requirements to induce flowering. *LlutFTc1* is the main gene conferring early flowering and thermoneutrality in yellow lupin. Large deletion (2227 base pairs) in the *LlutFTc1* promoter discovered in early flowering and thermoneutral lines, is expected to cause a loss of binding sites for the transcriptional repressor proteins, such as AGL15 and SUF4, resulting in de-repression of *LlutFTc1*. This de-repression subsequently results in the induction of flowering independent of the presence of inductive photoperiod and fulfilment of vernalization requirement. Also in the case of the *LlutFTa1a* gene, polymorphism within the promoter likely leads to the loss of one of the only two binding sites of the repressor protein (herein TOE2), resulting in reduced repression of *LlutFTa1a* despite the lack of inductive photoperiod. An insertion of a 5269 bp *Copia-like* retrotransposon in the *LlutFTc2* gene may in turn lead to an attenuation of *LlutFTc2* expression in lines carrying this allele (these lines are also one of the latest flowering accessions in analyzed germplasm panel).