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Developing lupin crop into a major and sustainable food and feed source



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Comparative genomics of *Lupinus angustifolius* L. gene-rich regions

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State of the art and aim: Recently, a significant development of molecular genetic resources for the narrow-leafed lupin (Lupinus angustifolius L.) has been observed. These include genetic maps with gene-anchored markers, nuclear DNA libraries, a draft genome sequence aligned to the linkage groups, reference transcriptome assembly and cytogenetic markers tagging specific chromosomes. The aim of this research was to localize and analyze several L. angustifolius generich regions (GRRs) using the resources mentioned above, together with those generated for other legume species. Complex approach involved: DNA fingerprinting, sequencing, genetic mapping and molecular cytogenetics, followed by bioinformatic analyses.

Results and discussion: The L. angustifolius genomic BAC library was screened with several gene-based probes, to track genes involved in symbiotic nitrogen fixation and fatty acid synthesis, as well as with SSR-probes to focus on gene-rich regions in general (Książkiewicz et al. 2013, 2015). BAC clones carrying particular sequences were isolated, fingerprinted and assembled into contigs. BAC-end sequence (BES) annotation allowed us to choose clones for sequencing, targeting particular genome regions. L. angustifolius reference genetic map (Kamphuis et al. 2015) was supplemented with new BES- and BAC-derived markers. Physical and genetic mapping was supported by multi BAC-FISH; new chromosome-specific landmarks were identified. BESs and BACs were aligned to L. angustifolius genome draft assembly, where they tagged several hundred sequences. Orientation of more than half of anchored scaffolds was identified by paired BESs. The in silico detection of coding regions revealed that the average gene density was higher than 10 genes/100 kb. Using genetic linkage distances and consensus band size data, the ratios of physical to genetic distances were calculated. Values obtained for generich regions were below 150 kb/cM. The physical-to-genetic distance ratios supported the results of functional annotation. Comparative analysis of sequenced L. angustifolius GRRs revealed numerous syntenic links to the sequenced legume genomes (Arachis duranensis, A. ipaensis, Cajanus cajan, Cicer arietinum, Glycine max, Lotus japonicus, Medicago truncatula, Phaseolus vulgaris, and Vigna radiata). Some regions of the nuclear genomes retained their quasi-ancestral structures, visualised by identical order and orientation of microsyntenic blocks. The maximum-likelihood and Bayesian phylogenetic inferences provided novel evidences for ancient duplications and triplications in the L. angustifolius genome, which putatively have occurred after the divergence of ancestor lineage to Lupinus, Arachis, and Glycine.

References:

Książkiewicz et al. (2013) BMC Genomics, 14, 79

Kamphuis et al. (2015) Plant Biotechnol J, 13(1), 14-25

Książkiewicz et al. (2015) Plant Mol Biol Rep, 33, 84-101

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Tags: genome; synteny; sequencing; BAC-FISH; molecular marker; narrow-leafed lupin

Genotyping by sequencing of white and narrow-leafed lupins

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State-of-the-art and aim:

Over the last years significant progress in generation of publicly available sequence resources has occurred, including three crop lupin species: white (*Lupinus albus*), yellow (*L. luteus*), and the narrow-leafed (*L. angustifolius*) (Książkiewicz et al. 2015). Flagship achievements encompass: transcriptome assemblies and gene indices (all three species), linkage maps (white and narrow-leafed lupin), and a draft genome assembly (narrow-leafed lupin). These resources, together with DNA-based molecular markers, can facilitate the gene-based prediction of phenotype. Marker assisted selection (MAS) has recently become an integral part of plant breeding programs and has accelerated new cultivar development. However, the most of the marker systems provided for lupins are ineffective due to high cost of scoring and low level linkage with particular traits. Advances in sequencing technologies have driven down sequencing costs and launched increasing interest in genotyping by sequencing (GBS), which generates ready to use markers based on sequence polymorphism.

Results and discussion: The aim of our ongoing research is to generate a technology of monitoring the narrow-leafed and white lupin lines carrying particular QTLs (quantitative trait loci) conferring agricultural traits. It will enable allele-directed selection in breeding programs. This target will be reached by determination of relationships between variability of white and narrow-leafed lupin phenotype quantitative traits, genetic polymorphisms of markers linked to these traits, genetic information contained in genome regions carrying these loci, and transcriptional activity of genes encoded by these sequences. The narrow-leafed lupin accessions from the world germplasm collection, together with recombinant inbred lines (RILs) from two mapping populations, 112 of narrow-leafed lupin and 193 of white one, are currently being profiled by GBS. Polymorphisms of obtained markers will be correlated with agricultural traits by association mapping. Phenotype observations include plant morphology and physiology, content of alkaloid and other non-nutrient and nutrient components, and resistance to biotic and abiotic stresses. Consensus, high density linkage maps containing both newly developed GBS and existing gene-based PCR and AFLP markers (Phan et al. 2007 and Vipin et al. 2013) will be constructed to support the identification of genomic regions in the marker-trait association study. Obtained linkage maps, composed mainly of sequence-based markers, will be also used for synteny survey in comparative mapping approach to sequence legume genomes (Arachis duranensis, Arachis ipaensis, Cajanus cajan, Cicer arietinum, Glycine max, Lotus japonicus, Medicago truncatula, Phaseolus vulgaris, and Vigna radiata).

References:

Phan et al. (2007) DNA Research, 14, 59-70 Vipin et al. (2013) Breeding Science, 63, 292-300 Książkiewicz et al. (2015) Plant Mol Biol Rep, 33, 84-101

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Tags: GBS, white lupin, narrow-leafed lupin, molecular markers, next-generation sequencing