YELLOW LUPIN TRANSCRIPTOME SEQUENCING TOWARDS IDENTIFICATION OF GENES ASSOCIATED WITH RESISTANCE TO *FUSARIUM SP.*

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RNA-SEQ

RNA-seq experiment was performed for **four yellow lupin genotypes (two resistant and two susceptible)**. Each genotype was grown in **two distinct environments: multiannual lupin monoculture** (infected plants) **and control field with crop rotation** (non-infected plants) (Table 1). The experiment was carried out in two biological replicates resulting in the total of **16 lines incorporated in the RNA-Seq analysis.**

Total RNA was extracted from leaves with aid of SV Total RNA Isolation System (Promega). TruSeq RNA Sample Preparation Kit (Illumina) was used to **cDNA libraries construction**. Sixteen RNA-seq libraries were sequenced using the **Illumina NGS platform** (2x75 bp PE, HiSeq 1500). **A minimum of 59 million high quality sequence reads were obtained for each of biological replicates.**

TRANSCRIPTOME ASSEMBLY

The reference transcriptome was *de novo* assembled on the basis of Lord and Perkoz libraries, using CLC Genomics Workbench 8.0.3 software. **We received 55043 contigs of an average length of 1058 bp** (N50 = 1497 bp). Summary of contig measurements and contig length distribution are presented in Table 2 and Figure 1, respectively, while nucleotide distribution is shown on Figure 2.

FUNCTIONAL ANNOTATION

Functional annotation of the transcriptome assembly was conducted using Blast2GO bioinformatic platform. **Terms of Gene Ontology (GO)** were distributed according to **biological processes, molecular function and cellular component** (Figure 3).

DIFFERENTIAL EXPRESSION

Gene expression levels were calculated using CLC Genomics Workbench on the basis of the assembled reference transcriptome and RNA-Seq short reads. **Comparison of type and abundance of transcripts from resistant and susceptible plants revealed differentially expressed genes belonging to a range of functional categories.** Candidate genes potentially involved in resistance to *Fusarium sp.* will be incorporated in **qPCR analyses to confirm differential expression in the wider group of susceptible and resistant lupin genotypes.**

LUPINUS LUTEUS L.

Yellow lupin is one of three lupin crops. Lupins farming gained much attention recently, due to the fact that **lupin seeds are a valuable protein source.** On the other hand a serious threat in yellow lupin cropping are **fungal diseases e.g. fusariose and anthracnose,** which may result in the total destruction of plantation. Up until now, only **one gene *Fus1* underlying resistance to *Fusarium sp.* has been identified** and used in the yellow lupin breeding programs, however its **molecular function has not been yet determined.**

Table 1. RNA-Seq experiment scheme.

Resistant genotypes	Susceptible genotypes
Lord/I	Perkoz /I
Lord/NI	Perkoz/NI
Z-505/3/I	Z-505/16/I
Z-505/3/NI	Z-505/16/NI

* I – infected plants, NI – non-infected plants

Table 2. Contig measurements.

	Length
N50	**1497**
Minimum	298
Maximum	12264
Average	1058
Count	**55043**

This is the first study to investigate the molecular background of resistance to fusariose in yellow lupin on the basis of RNA-Seq results. Transcriptome sequencing data serves as a basis in the reference transciptome de novo assembly and differential gene expression analysis to identify candidate genes involved in the resistance to *Fusarium sp.*

Figure 1. Contig length distribution.

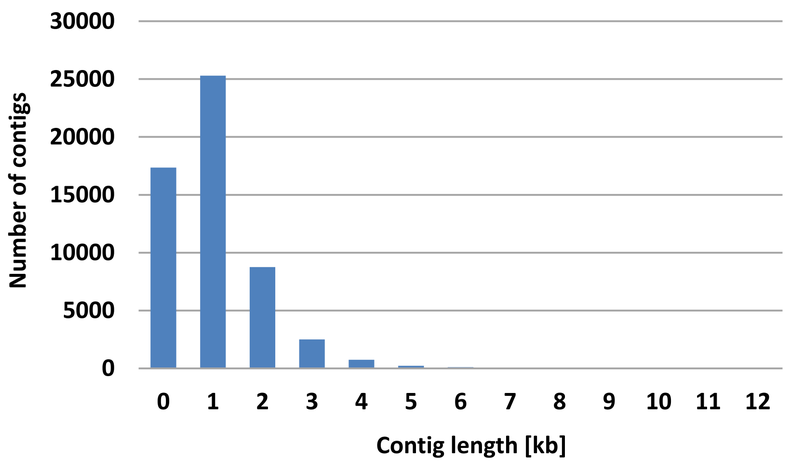

Figure 2. Nucleotide distribution in yellow lupin transcriptome assembly.

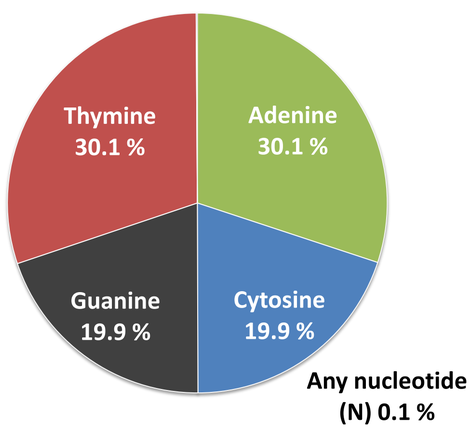

Figure 3. GO terms distribution according to biological processes, molecular function and cellular component (level 4 – top 20)

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