The narrow leafed lupin (*Lupinus angustifolius* L.) is considered as valuable crop, a rich source of plant protein for feed and food. Due to symbiosis with root nodule bacteria lupins have an ability to bind atmospheric nitrogen and transfer it to soil as nitrogen compounds. The most dangerous biological threats of the narrow-leafed lupin are diseases caused by fungi, like anthracnose and Phomopsis stem blight. Anthracnose is caused by pathogenic fungus *Colletotrichum lupini*. This disease was first observed in 1912 on white lupin in Brasil, but in 1939 reached south-east region of the USA, bringing considerable yield losses in the narrow-leafed lupin crops. In 1982 anthracnose was observed on white lupin in France, in 1983 in Ukraine, in 1988 in Russia, and in years 1995-1997 also on the narrow-leafed lupin in Poland, Germany, Austria, Portugal, United Kingdom, and Belarus. Very soon this disease reached all countries, where lupins were cultivated. Anthracnose is actually considered as the most devastating lupin disease in many countries of Europe, North America and South America. The vast majority of the narrow-leafed lupin cultivars and lines are very susceptible to anthracnose. Very resistant are: Astralian Wonga, Tanjil, 83A:476, German Bo7212, Belarusian BGB-6 and Myrtan. Moderately resistant are Kalya, Coromup and Mandelup. Wonga, Tanjil and 83A:476 possess the same dominant resistance gene, called Lanr1. Kalya has other dominant gene, Lanr2, whereas Coromup and Mandelup also carry other gene, AnMan. Molecular markers closely linked to these three genes were developed and the implementation of these markers in breeding programs confirmed the presence of heritable resistance sources. Anthracnose resistance in lines from Denmark and Belarus results from the presence of at least three of four genes, named Rcl1, Rcl2, Rcl3, i Rcl4.

Phomopsis stem blight is caused by pathogenic fungus *Diaporthe toxica*. This disease was first observed at lupins in Germany in 1880. The first occurence of Phomopsis stem blight in Poland was noticed in early 1950. on yellow lupin. In the following years disease symptoms were observed from time to time on lupins cultivated in Europe, Africa, Australia and USA. The presence of *D. toxica* was also confirmed on seeds of the narrow-leafed lupin in Poland. Resitant cultivars are Merrit, Gungurru, Yorrel, Warrah, Belara, Moonah, Quilinock, Myallie, Kalya, Tallerack, Tanjil and Wonga, and very resistant - line 75A:258. Cultivar Merrit is a selection from a cross between cultivar Illyarrie and line from Spain, carrying resistance gene Phr2, whereas line 75A:258 is derived from a cross between cultivar Marri and line from Morocco, having gene Phr1. Cultivars Tanjil and Wonga carry resistance gene PhtjR.

The objective of this proposal is to identify *L. angustifolius* genes involved in recognition of infection and launching of defense responses against two fungi: *C. lupini* and *D. toxica*. The hypothesis is that defense response against *C. lupini* is based on early recognition of fungus activity and efficient downstream transduction of recognition signal. Thus, genes of anthracnose resistance, Lanr1, Lanr2, AnMan, Rcl1, Rcl2, Rcl3 and Rcl4 are involved in detection of specific molecular patterns related to pathogen (effectors) and launching effector-triggered immunity. The response against *D. toxica* appears later, during latent stage of infection, after fungal penetration over the first line of defense, structural barriers at plant surface, and results in slower development of disease symptoms. Genes of Phomopsis stem blight resistance, Phr1, Phr2 and PhtjR are participating in recognition of molecular patterns associated with damage of plant organism and initiation of basal immunity (microbial-associated molecular patterns-triggered immunity).

Assay of fungal isolates of *D. toxica* and *C. lupini* will be performed in the project with the use of methods of microbiology and molecular genetics. Then, evaluation of resistance/susceptibility of *L. angustifolius* lines for anthracnose and Phomopsis stem blight in temperature and humidity favoring growth of fungi will be done. Selection of germplasm for disease resistance survey will be based on our preliminary studies, literature data and this experiment. To supplement these analyses, the polymorphism of DNA markers linked to anthracnose and Phomopsis stem blight resistance in *L. angustifolius* accessions will be evaluated. To answer the question which genes are contributing to pathogen detection and initiation of defense response, the experiment will be set up in which plants will be inoculated with fungi, *D. toxica* i *C. lupini*. Leaves from this experiment will be then analyzed in terms of gene expression, with the reference to control pathogen-free plants. Innovative technology, next generation sequencing of RNA, RNAseq, will be harnessed to identify many - even several thousand - of genes active during experiment. The assembly procedure of such sequences is sophisticated and requires the implementation of appropriate programs. Teams at the Institute of Plant Genetics of the Polish Academy of Sciences have experience in experiment planning, expected number of reads received as well as available bioinformatics methods for *de novo* assembly of short reads in lupin, and have also frequently updated computer pipeline for handling large number of sequence data. The prediction of localization and sequence of genes will be executed with the use of programs enabling detection of genes based on particular sequence motifs as well as based on similarity to other, already recognized genes. Reference sequences from model plant legume, soybean, *Glycine max*, will be used. Biological processes and molecular functions in plant organism of newly described genes will be assigned. The structure of genes itself will be also analyzed, focusing on existing protein domains. Sequence polymorphism of candidate genes in large collection on lines differing in resistance/susceptibility will be assayed, including putative binding sites for protein factors regulating gene activity, transcription start site and any differences in coding sequences resulting in changes of encoded protein sequence and structure. The narrow-leafed lupin genome is sequenced but only partially; it has a form of thousands of short fragments. The majority of these fragments are not assigned to the genetic map of the species. Candidate genes originating from such unlinked sequences will be localized on the map using methods of molecular genetics: marker development based on the gene sequence, and analysis of marker polymorphism in mapping population consisting of lines derived from cross between diverse parental lines. The participation of selected genes in plant-fungus interactions will be analyzed more thoroughly, by evaluation of gene activity at six points of time, representing different stages of disease development. Genes involved in defense reaction development will be subjected to evolutionary relationship inference, with the use of two different methods - Bayesian and maximum likelihood / maximum parsimony. This survey will be done using sequences elucidated from *L. angustifolius* transcriptome and genome sequences of 9 legume species, *Medicago truncatula, Lotus japonicus, Cicer arietinum, G. max, Phaseolus vulgaris, Cajanus cajan, Arachis ipaensis, Arachis duranensis*, and *Vigna radiata*. The comparative analysis of sequences will be done to check if regions carrying these genes, despite complex patterns of evolution, retained similar structure reflected by identical order and orientation of consecutive genes. The results will be shown on linear and circular plots using appropriate software.

To summarize, this project will exploit and apply recently developed genomics tools and resources to address unanswered questions related to plant-pathogen interactions, using *L. angustifolius* as a model. The pioneering approach will be harnessing next generation sequencing technologies, what will enable gene discovery, including detection of rare transcripts, as well as modifications of gene structure and gene sequence polymorphism. It will improve our understanding of the molecular mechanisms

controlling plant immunity. The results will illuminate the relationships between two main tiers of plant resistance to pathogenic fungi and will elucidate the role of downstream components of plant defense. Key findings will be published in high impact journals and presented at international and national conferences. The project will provide the valuable resources for the research community, which will be of great interest for those focusing on molecular genetics and plant physiology. These data will be stored in the open access internet platforms. The results of this project will not be directly applicable in breeding but will contribute to the knowledge about molecular mechanisms involved in defense response and provide breeding community resources to promote state of the art breeding methodology.