Cost STSM Scientific Report

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Because of the major handicap in phytoplasma genomics being the isolation of sufficient phytoplasma DNA, different approaches to isolation of DNA were employed. DNA was isolated from 2 tobacco plants and 2 Catharanthus roseus found in Serbia and infected with stolbur phytoplasma. The DNA from 2 Catharanthus roseus was isolated using the 3% CTAB extraction protocol; from one tobacco DNA was isolated using Dellaporta buffer first and then going on with the DNeasy Plant Mini DNA extraction kit, while for the other tobacco after usage of Dellaporta buffer, 3% CTAB protocol was applied. It is thought that this procedure (Dellaporta) enriches for phytoplasma and mitochondrial DNA while selecting against nuclear and chloroplast DNA. The SDS/potassium acetate precipitation that occurs during this protocol, eliminates most of the plant carbohydrates which can inhibit restriction and other DNA modifying enzymes.

After the extraction, PCR with universal phytoplasma primer pair P1/P7 was performed on these 4 samples and on two samples of Catharanthus roseus infected with AY phytoplasma and extracted with 3% CTAB protocol, also from Serbia. To exclude the possibility of multiple infection and to verify the success of the extraction and the PCR, positive P1/P7 amplicons of 4 Catharanthus roseus were sequenced and obtained sequences were assembled with Pregap4 program from Staden package (the P1/P7 amplicons of both tobaccos were already sequenced). For further experiments, the DNA from two tobacco samples extracted with Dellaporta protocol and infected with stolbur phytoplasma as the enriched DNA and the DNA from two Catharanthus roseus extracted with 3% CTAB protocol and also infected with stolbur phytoplasma as a control, were chosen.

Calculated and designed repeat based oligomers and specific primers were used for isothermal amplification using different conditions and different kits. Results were compared and samples selected for Illumina sequencing, which is still in progress.

A phytoplasma protein database was set up for identification of Illumina derived contigs (draft sequences) that will be used for the selection of possible marker genes. Orthologs of four phytoplasma genomes were calculated, manually curated and assigned to functional groups. The actual annotation of the four phytoplasma genomes was checked by BLAST, Pfam and InterPro software, all three available on the internet. A large number of proteins and structural RNA encoding genes were checked and several genes and corresponding protein sequences were aligned for the design of universal primers but without success.

To summarize-different methods for DNA isolation and enrichment of phytoplasma DNA were successfully performed. The enriched material was generated by usage of calculated oligomers and the sequencing of obtained amplicons is in progress. A database for efficient quantification and for evaluation of the content was built. We will continue with the data analysis, if the sequence data will be available and informative.