Expression of microRNAs and target genes in Scots pine (*Pinus sylvestris* L.) needles in response to methyl jasmonate treatment

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The aim of this study was to investigate the effect of MeJA treatment on microRNA (miRNA) and gene expression in *P. sylvestris* needles and to compare expression levels of miRNAs and their target genes. A combined strategy—high throughput sequencing and computational prediction—was utilized to identify conserved *P. sylvestris* miRNAs from 6 small RNA and 5 transcriptome libraries (Figure 1). The obtained mature miRNA sequences were analyzed and filtered based on known characteristics of plant miRNAs, and compared to other plant miRNAs available in databases. The differences between the groups (with MeJA treatment and control) were analyzed using CLC Genomics Workbench 12.0.3. Expressed data were normalized and the parameters for statistical analysis were set up: normalization method - total, counts reported as - reads per 1000000, total count filter cuttoff – 5.0, estimate tagwise dispersions, Bonferroni and FDR correction. The number of differential expressed (DE) miRNAs and target genes between samples was obtained by fold-change≥1.5 and p≤0.05. GO term enrichment was assessed by use of Blast2GO PRO plugin for CLC Genomics Workbench (Figure1).

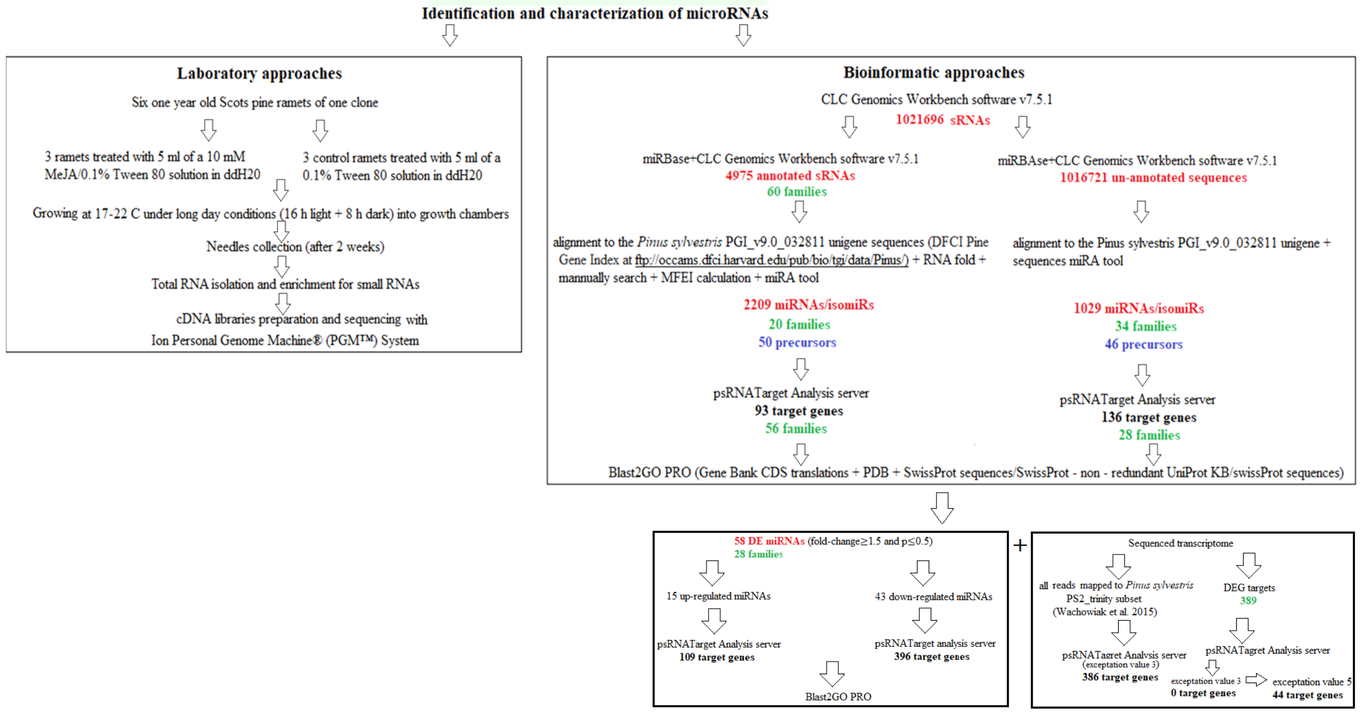

Figure 1. Methods utilised for identification and characterization of conserved and novel miRNAs and DE miRNAs.

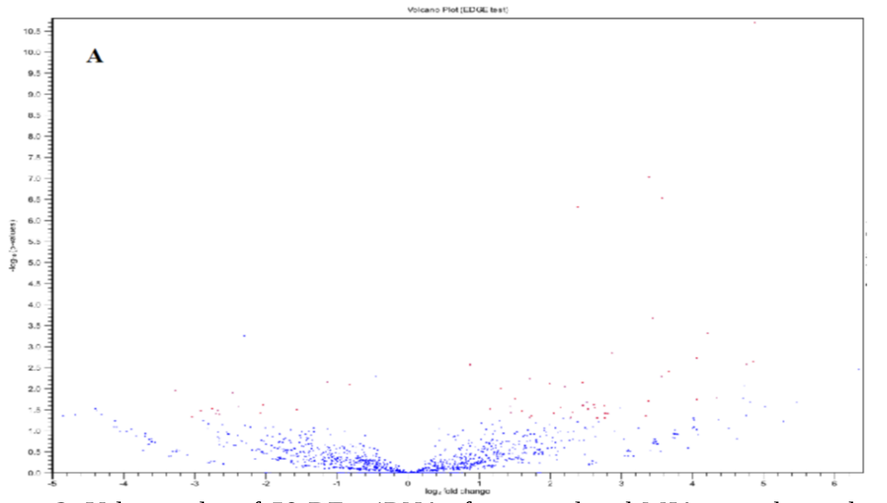

Figure 2. Volcano plot of 58 DE miRNAs for control and MJA treated samples. The X-axis is the fold change (log2 scale) of up and down-regulated miRNAs. The Y-axis represents the p-value (log10) for the statistical test of differences between the samples. The red dots represent the miRNAs that were DE (P value ≤ 0.05, fold change > 1.5 of absolute values). Blue dots represent the miRNAs that are up and downregulated, but without statistical significance.

RESULTS

1,021,696 unique small RNA sequences obtained, 4975 conserved miRNA sequences and 1029 potential novel miRNAs were identified. The most highly expressed conserved miRNA families identified in this study were miR951, miR950 and miR946, which have only been reported in conifer species (*Pinus sylvestris*, *Pinus taeda*, *Pinus densata*, *Picea abies*) and miR396, miR482. 58 miRNAs from 28 families were confirmed to exhibit significantly differential expression (DE) in the control and MeJA treated samples. The Empirical analysis of DGE-test used for Volcano Plot, tool for visualizing DE miRNAs between two compared groups, were produced (Figure 2). 15 miRNAs were up-regulated and 43 were down-regulated. Target genes representing enzymes - glycosylases, hydrolases, oxidoreductases and laccase were the most up-regulated and oxidoreductases, transferring phosphorus-containing groups, transferases, hydrolases were the most downregulated. Figures 3 and 4 show biological process and molecular function GO classifications by all target genes of DE miRNAs.

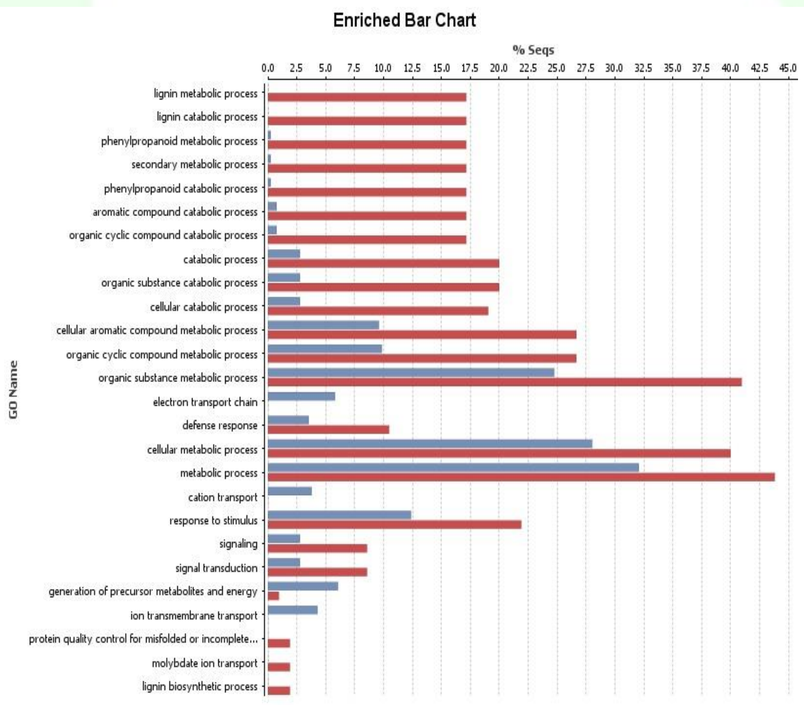

Figure 3. GO term enrichment for biological processes for target genes (targets from psRNATarget tool) for down (red bars) and up-regulated (blue bars) miRNAs (control vs. MJA).

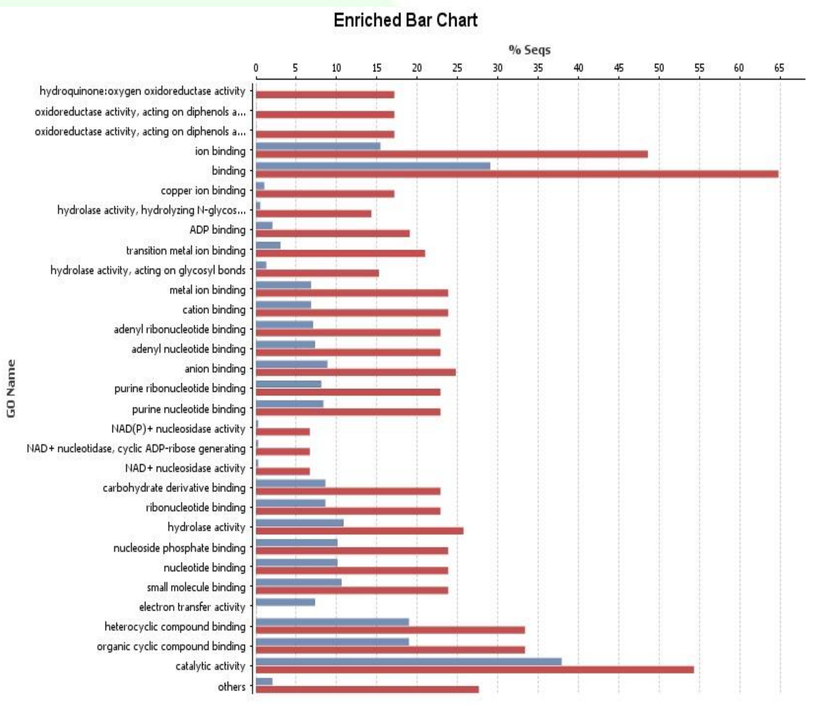

Figure 4. GO term enrichment for molecular functions for target genes (targets from psRNATarget tool) for down (red bars) and up-regulated (blue bars) miRNAs (control vs. MJA).

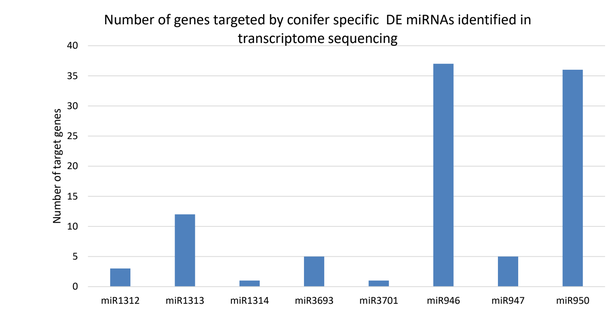

Figure 5. Conifer specific DE miRNAs identified in transcriptome sequencing.

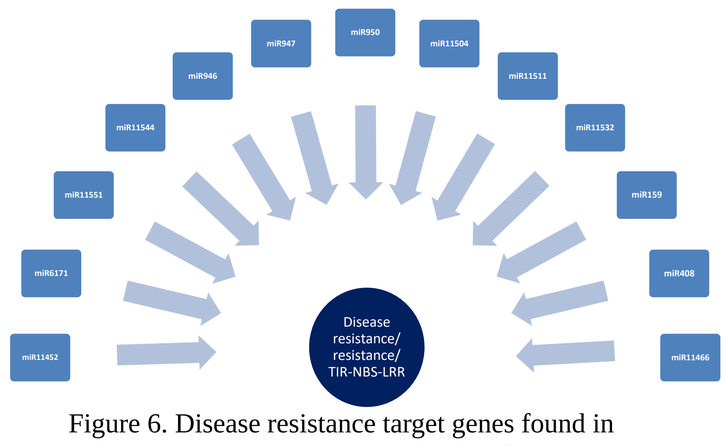

Figure 6. Disease resistance target genes found in *P.sylvestris* transcriptome mapped to reference vs. DE miRNAs.

41 DE miRNA from 8 families were conifer specific conserved miRNA families (Figure 5). The other DE miRNAs were conserved miRNAs, which are also found in angiosperm species (as reported in mirBase). 43 down- regulated miRNAs and 15 up-regulated miRNAs were found, based on normalized expression means. Transcriptome analysis revealed 21 miRNA from 13 DE miRNA families, from which 3 are conifer specific, associated with 46 disease resistance target transcripts (Figure 6). DE of miRNAs and their respective target genes in opposite directions was identified in some cases. (Figure 7).

CONCLUSIONS - The majority of highly conserved plant miRNAs were identified, as well as some conserved miRNAs previously reported to be monocot specific. No conserved dicot-specific miRNAs were identified. A number of potential gymnosperm or conifer specific miRNAs were found, shared among a number of conifer species. Potential target genes were identified, of which the targets of highly conserved miRNAs present in most plant families were transcription factors, while the conserved conifer-specific miRNA targets were involved in disease resistance. Transcriptome analysis revealed DE miRNAs associated with disease resistance.

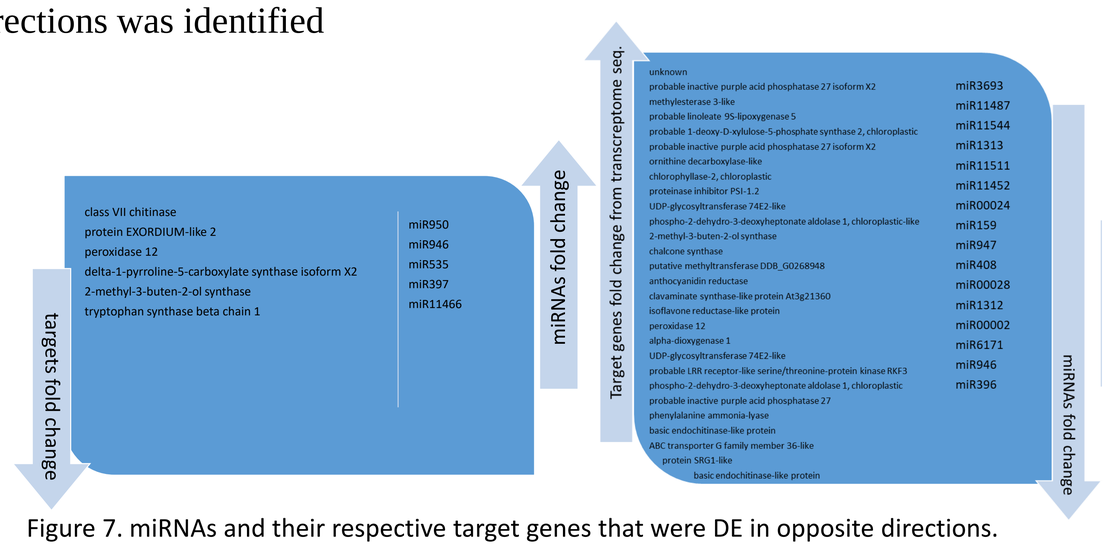

Figure 7. miRNAs and their respective target genes that were DE in opposite directions.