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Exploring miRNA like small RNAs in *Puccinia striiformis* using *in-silico* approach

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The advancement in sequencing techniques has made it possible to study small RNAs like miRNA, siRNA, etc., comprehensively and efficiently. The microRNAs are short (~20-24 nucleotides) non-coding RNAs that negatively regulate the gene expression post-transcriptionally in animals and plants. This negative regulation can be either by degradation of messenger RNA (mRNA) or by inhibition of protein translation. The existence of miRNAs in plants and animals is well studied in plants but in fungi, it is limited. Here, we report the prediction of miRNAs in fungus *Puccinia striiformis tritici* (*Pst*) using EST data available in NCBI. *In silico* approach has been used to predict the miRNAs and their hairpin precursors in this fungal pathogen. A total of 7550 ESTs were available in NCBI for *Pst* which were pre-processed using a perl program named 'ESTtrimmer' and then assembled using CAP3 assembly program and resulted into 6106 assembled ESTs. Of 6106 ESTs, 1215 precursor miRNAs were predicted that resulted into 120 miRNAs of which 71 were unique sequences. We identified targets of 120 predicted miRNAs. For target identification, 6106 initial EST sequences of *Pst* were used. Of these, 3167 ESTs were scanned for sequence complementary sites on hit sequences from BLAST (allowed 4 mismatches). These complementary targets were allowed to fold and their secondary structures were analyzed for efficient folding that result into stable complex with lower MFE (minimal free energy). Of these, 232 potential targets were annotated successfully. The targets identified were mostly from fatty acid metabolism and its biosynthesis, signaling pathways, amino acid metabolism, purine metabolism, basal transcription factors, etc. It is well known that PAMP-triggered immunity (PTI) plays a vital role in the resistance of plants to numerous potential pathogens. Our further study will include experimental validation of these predicted miRNAs to evaluate the effectiveness of our method. Our findings will improve the understanding towards the role of these small RNAs in fungal kingdom and pathogenicity of *Pst*.

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Regeneration and *Agrobacterium* mediated transformation in *Brassica juncea* L Czern & Coss

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Development of an efficient plant regeneration system is a prerequisite for any plant tissue culture approach for crop improvement. The work was undertaken to develop a regeneration system for *B. juncea* genotypes RH 406 and RH 555 using seedling explants. Hypocotyls and cotyledons from 5-day old seedlings were used as explants. Hypocotyl and cotyledon explants excised from *in vitro* grown 5-day old seedlings were cultured on MS medium supplemented with different concentrations and combinations of growth regulators. Highest percent shoot formation was observed on MS medium with 2.5 mg/l BAP (64.85±1.42) from cotyledon explants in genotype RH 406. Highest shoots per explant were obtained on MS medium fortified with 2.5 mg/l BAP in genotype RH 406 and in genotype RH 555; it was obtained on MS medium supplemented with 1.0 mg/l BAP. Eight rooting media were tried for root formation in regenerated shoots. Maximum rooting response was obtained on MS medium supplemented with 0.2 mg/l NAA i.e., 93.44±0.80 and 90.67±1.45 in genotypes RH 406 and RH 555 respectively. The regenerated plants were transferred to a mixture of sand: Soil in 1:1 ratio in pots. Percent survival obtained was higher in genotype RH 406 i.e., 81.8% as compared to RH 555 where it was 67%. Transformation protocol was developed in genotype RH 406 as hypocotyls and cotyledons explants of this genotype showed GUS assay test positive. Cotyledon explants showed 75% while hypocotyl explants showed 80% GUS expression. Hence, regeneration and *Agrobacterium* mediated transformation conditions were developed.

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