To design and functionally analyze an miR targeted reporter gene construct using *In planta* assay

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Abstract

Plants, upon exposure to environmental stress, express sophisticated and co-ordinated responses to reprogram interconnected defense networks and metabolic pathways. These responses are governed by intricate molecular and biochemical signal transduction processes, which act in harmonization to determine tolerance and sensitivity at a holistic level. Modern studies in plant stress biology include identification of key genes that influence stress tolerance and plant growth under the imposing stress and the verification of gene functions using knockout mutants or over-expression lines. Abiotic stresses induce aberrant expression of many miRNAs, suggesting that miRNAs could be a new target for genetically improving plant tolerance to certain stresses. MicroRNAs (miRNAs), 21-24 nts in length, are an extensive class of endogenous small RNA molecules that provide a fascinating option for engineering tolerance to environmental stress by serving as non-protein coding switches of both desirable and undesirable gene expressions. During abiotic stress, miRNAs function by regulating target genes within the stress regulated networks like signalling components and a variety of transcription factors (TFs). It is important to identify the expression domains of the miRs and their targets to understand how the spatio-temporal regulations are co-ordinated under stress. In this project, we aim to design reporter gene construct containing the target site of selected miR. We selected pCambia1302 vector with aim to insert the target site before the ATG of GFP gene such that the coding frame remains intact. Later, the functionality of the designed construct can be checked by expressing the constructs *In planta* using Agroinfiltration.

Objectives

In planta assay for analyzing the miR expression domains by:

- 1 Construction of GFP vector: miRNA target site.
- 2 Agroinfiltration to check the functionality of construct.
- 3 Performing *In planta* assay.

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Introduction

Recently, a new layer of gene regulation has been identified with the discovery of Post Transcriptional Gene Silencing (PTGS). This is mediated by a diverse family of small RNA molecules that can be categorized based on their origin and functions. microRNAs (miRs) constitute an important class of the small RNA family. These include endogenously transcribed 20 to 24 nt non-coding RNA molecules that regulate gene expression in a sequence specific manner.

microRNA biogenesis in plants.

To date, most studies of miRNA-target interactions have focused primarily on the characteristics of the sequence complementarity between the miRNA and putative target sites in the mRNA. Through this project we are trying to make a reporter gene construct containing the target site of miR159b and analyze its functionality by agroinfiltration in *Nicotiana benthamiana* followed by transient assay

Strategy

Materials and Methods

MATERIALS: Binary vector pCambia 1302 was used for inserting our designed oligo and *Agrobacterium* strain LBA4404 was used for transformation. Model plant *Nicotiana benthamiana* was used for Agroinfiltration.

Methodology: 1) Retrieval of miR159 sequences from rice and arabidopsis and 2) predictions of miRNA target genes 3) Designing the Construct containing 21-mer TGGAGCTCCTTCACTCCAAG

1) The sequence for miR159 were fetched from **miRBase: the microRNA database.**

2) A search of miR159 targets in Arabidopsis and Rice was carried out using the program **psRNATarget: A Plant Small RNA Target Analysis Server** that is developed to identify target transcripts of miRNAs.

To **standardize the assay**, available *Agrobacterium* strain in the lab containing a GFP expression vector was used for infiltration in the young tobacco leaves.

Results

Detection of GFP Fluorescence after 96h(3 dpi) and 144h(5dpi) of agroinfiltration showed GFP expression and thereby confirmed non-binding of target(MP in this case) and miR.

Scopes

- Can check and minimize off-target effect and also can be used as a reporter construct library for immediate screening.
- Can be a boon technology for developing miRNA based therapeutics (eg. molecular pharming in plants, to validate kinase as true targets for developing kinase inhibitors)
- Can act as a prognostic, diagnostic or predictive biomarker in a therapeutic intervention.

Conclusions

The novel construct designed for this research project is yet to be validated and so the absolute end results of the cloning experiments with designed construct could not be obtained. The subsequent experiments to cloning were intentionally performed with pre-formed construct in the lab pCambia1302::MP (movement protein) for standardization of the assay. GFP fluorescence detection was detected as miR DOESN'T BIND to MP thus validating the methodology.

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