ABSTRACT

Thesis Title: Functional Investigation of a novel wheat seed-specific miRNA by short tandem target mimic (STTM)

Written by: Abdul Razzak (fypkm7)

Course: Master of Science in Agricultural Biotechnology

Institute: Institute of Genetics and Biotechnology

Primary thesis advisor: Dr. Kis Andras, Ph.D., Research Fellow

Institute of Genetics and Biotechnology

Wheat is a crucial cereal crop that plays a significant role in feeding a significant percentage of the world's population. Scientists have been investigating the genetic factors that influence the growth and development of wheat to find ways to overcome the difficulties associated with crop production. RNAi is a highly effective method for studying gene function and can be accomplished by either post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). Among the different RNAi approaches, microRNA (miRNA) knock-down is a popular strategy, that can be implemented using short tandem target mimic (STTM). Therefore, exploring a novel wheat seed-specific miRNA through the STTM technique presents a promising way of gaining insights into the mechanisms that regulate seed development in wheat.

STTMs have a size of about 100 nucleotides (nt) and consist of two tandemly arranged miRNA binding elements, each of which is designed with a mismatch located at the miRNA cleavage site. The miRNA binding elements are connected by a flexible stem-loop linker that spans between 48-88 nt. The STTM method has been used to silence various miRNA families in Arabidopsis and several model and staple crops such as tomato, rice, wheat, tobacco, Medicago, soybean, poplar, cotton, common bean, and barley. Our main aim was to make a STTM construct to block the function of a novel wheat seed specific miRNA2187 to prove its potential target that is Pol V subunit messenger RNA, predicted bioinformatically. STTM construct was introduced into plant cells through Agrobacterium-mediated wheat transformation.

Seeds of the spring wheat (*Triticum aestivum* L.) genotype ‘Fielder’ were sown at weekly intervals in a peat and sand mix (5:1). Fielder rose in growth chambers (Conviron, Winnipeg, Canada) under 16h/8h light/dark period. 70% humidity with light levels of 800 μmol.m-2s-1

provided by fluorescent tubes and tungsten lighting. Plants are not sprayed with fungicides or insecticides at any stage of growth. The STTM construct has two flanking miRNA sequences with 3 nt bulge at the 10th nucleotide, linked by an 88 nt linker sequence. The construct was made and first introduce into E. coli (strain DH5α) and was finally introduced into *Agrobacterium tumefaciens* strain AGL1. Wheat embryos were extracted at 14 days post-anthesis and were put for cocultivation with Agrobacterium for transformation. Single vector transformation and co-transformation with the GRF4:GIF1 chimera gene were taken into consideration. Callus was induced and went through several selection media. Successfully regenerated plants were put into the LSF medium for root growth. Plants with strong roots were transferred into giffy followed by transfer into the soil.

Transformed plants were put for PCR analysis, and simultaneously hygromycin, GRF4:GIF1 chimera gene, and STTM unit were targeted. Plants either positive with only STTM unit or STTM unit and GRF4:GIF1 were taken for further analysis. Total RNA was extracted from transformed and control lines seeds at 8 and 12dpa. First, several transformed lines were analyzed with semi-quantitative PCR including controls for comparison. Actin and Ubiquitin were used as housekeeping control genes. Promising lines in semi-quantitative PCR were subjected to RTq-PCR analysis. Clearly, it was found that PolV messenger (NRPE1) RNA level was elevated in STTM transformed lines as compared to control line.