THESIS SUMMARY

Thesis Title: Investigating the function of a novel wheat seed-specific miRNA by over expression in transgenic wheat (*triticum aestivum* L.) lines.

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microRNAs (miRNAs) are small noncoding RNAs crucial for regulating gene expression by targeting specific messenger RNA (mRNA) molecules. Within the cytoplasm, small RNAs facilitate PTGS by targeting complementary mRNA molecules, leading to their degradation or translational repression. Conversely, in the nucleus, small RNAs arrange TGS by directing repressive epigenetic modifications, such as DNA cytosine methylation and histone methylation, to homologous genomic regions. In plants, a notable pathway for small RNA-mediated epigenetic modifications is RNA-directed DNA methylation (RdDM). This process involves specialized transcriptional machinery centered around two plant-specific RNA polymerase II (Pol II)-related enzymes: Pol IV and Pol V. Pol IV transcribes a single-stranded RNA which is copied into a double-stranded RNA by (RDR2). The dsRNA is processed by DCL3 into 24 -nucleotide siRNAs. On the other hand, Pol V transcribes a scaffold RNA that base-pairs with AGO4-bound siRNAs. RDM1 links AGO4 and DRM2, which catalyses de novo methylation of DNA.

Identification of microRNAs (miRNAs) serves as the initial step in unraveling their functional roles. Deep sequencing technology provides insight into the expression levels of each miRNA based on read counts. With NGS we found a new wheat specific miRNA during small RNA library preparation from grain, and it was called miRNA tae2187. PsRNATarget predicted that

Pol V is a potential target of this miRNA. To prove it in planta, miRNA overexpression lines were produced.

Seeds of the spring wheat (*Triticum aestivum* L.) *cv.* 'Fielder' were grown under controlled environment in growth chambers (Sanyo) under 16h/8h light/dark period, 70% humidity with light levels of 50 µE $m^{-2}s^{-1}$ provided by fluorescent tubes and tungsten lighting. Plants were not sprayed with fungicides or insecticides at any stage of growth.

The overexpression construct having precursor B sequence was made with the help of restriction digestion and ligation assay. The construct was made and first introduced 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. Co-transformation of pC61K_Pre2187B with a vector having GRF4:GIF1 chimera gene was 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 Jiffy followed by transfer into the soil.

DNA was extracted from regenerated lines and were put for PCR analysis against hygromycin, Kanamycin, GRF4:GIF1 chimera gene and precursor B sequence. Plants positive with hygromycin resistance gene (*hptll*) were taken for further analysis. Total RNA was extracted from the leaf of transformed and WT control lines. First, miRNA expression was checked in leaf with the help of small RNA northern hybridization. Although miRNA expression was seen but later it was found that plants were only transformed with pCubiGRF4:GIF1_NOS vector not with over expression pC61K_Pre2187B vector. The most suited hypothesis of this can be the induction of this miRNA gene which may occur during the callus induction and plant regeneration from tissue culture.