Appendix 1. – Thesis topic application form

THESIS TOPIC*

APPLICATION FORM

Deadline: May 15., November 15.

Filled by the student!

Student Name: MOHAMMAD DANISH Neptun ID: CTE8CK

Course: Agriculture Biotechnology

Level of Education: BA/BSc / ~~MA/MSc*~~ Grade:

Specialisation*: Plant

Student address: danishaliq2@gmail.com e-mail

Name of Host Institute / Department: Institute of Genetics and Biotechnology.

Primary supervisor name and position: Dr. Kis András, Research Fellow

Independent Consultant name, position, workplace: Mohammad Ali, PhD Student

Thesis topic: Investigating the Function of a Novel Wheat Seed-specific miRNA by over-expression in Transgenic wheat (Triticum aestivum L.) Lines

Date: 2024 year 04 month 29 day

Primary supervisor	Independent consultant	Student
	Mohd Ali	M. Danish

MATE
HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Szent István Campus, Gödöllő
Address: H-2100 Gödöllő, Páter Károly utca 1.
Phone.: +36-28/522-000
Homepage: https://godollo.uni-mate.hu

Appendix 4 – Declaration

STUDENT DECLARATION

Signed below, MOHAMMAD DANISH, student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, at the BSc/MSc Course of Agriculture Biotechnology declare that the present Thesis is my own work and I have used the cited and quoted literature in accordance with the relevant legal and ethical rules. I understand that the one-page-summary of my thesis will be uploaded on the website of the Campus/Institute/Course and my Thesis will be available at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes no*

Date: 2024 20 04 month 29 day

M. Danish
Student

SUPERVISOR'S DECLARATION

As primary supervisor of the author of this thesis, I hereby declare that review of the thesis was done thoroughly; student was informed and guided on the method of citing literature sources in the dissertation, attention was drawn on the importance of using literature data in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes (no *)

Approval of thesis for oral defense on Final Examination: approved not approved *

Date: Gödöllő 20 24 04. month 29 day

signature

***Please, underline the correct choice!**

Szent István Campus, Gödöllő
Address: H-2100 Gödöllő, Páter Károly utca 1.
Phone.: +36-28/522-000
Homepage: https://godollo.uni-mate.hu

ABSTRACT OF THESIS

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

Written by: Mohammad Danish

Course: Master of science in Agricultural Biotechnology

Institute: Institute of Genetics and Biotechnology

Primary thesis advisor: Dr. Kis András, PhD, Research Fellow, Institute of Genetics and Biotechnology

Secondary thesis advisor: Mohammad Ali, PhD candidate, Institute of Genetics and Biotechnology

Abstract: microRNAs (miRNAs) are a class of small regulatory RNA molecules which play a pivotal role in the regulation of gene expression in cells. The main function of miRNAs is to inhibit or fine-tune the expression of specific genes by binding to the mRNA target molecules inducing their degradation or translational inhibition. We found a new, uncharacterized, grain specific wheat miRNA during genome wide analysis of small RNAs. This novel miRNA seems to be seed specific since it was absent from small RNA libraries made from leaf. First, the biological presence of this miRNA was validated by small RNA northern blot hybridization using total RNA extracted from 10 and 12 days post anthesis seeds. Bioinformatic analysis revealed NRPE1, a subunit of the plant specific Polymerase V (Pol V), as a potential target. To study the biological function of this miRNA in planta, transgenic wheat lines, over-expressing the target miRNA were produced by *Agrobacterium*-mediated transformation of immature wheat embryos (*Triticum aestivum* L. cv. 'Fielder'). We successfully generated 3 lines containing 11 plants in total. All the plants were positive for the PCR of *hygromycin resistance* (*hptII*) gene. A co-transformation was achieved with a vector containing miRNA precurosr sequence (pC61K_Pre2187) and a helper vector containing GRF4:GRF1 gene pCubiGRF4:GIF1_NOS which helps in regeneration of plants. But unfortuntaely only plants were transformed with pCubiGRF4:GIF1_NOS vector.

Keywords: wheat, miRNA, Polymerase V, Over-expressor lines