MATE
HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

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Appendix 1. – Thesis topic application form

THESIS TOPIC*

APPLICATION FORM

Deadline: May 15., November 15.

Filled by the student!

Student Name: ___Owais Ahmad Kutty_____ Neptun ID: _SJQUHL

Course: _____M.Sc Agricultural Biotechnology_____

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

Specialisation*: _____Plant Biotechnology_____

Student e-mail address:__ owais.kuttay@gmail.com_____

Name of Host Institute / Department: _____Hungarian University of Agriculture and Life Sciences/ Institute of Genetics and Biotechnology/Department of Plant Biotechnology

Primary supervisor name and position:__ Dr. György Szittya (scientific advisor)_____

Independent Consultant name, position, workplace:_ Dr. Péter Gyula (Project leader, senior scientist)

Thesis topic: __ **Investigation of *SlDML2* promoter spatial and temporal expression pattern with reporter constructs in tomato**

Date: _____2024____ year _____04_____ month ___22____ day

Primary supervisor | Independent consultant | Student

Filled by the host course leader/coordinator and the Head of host institute/department!

Application for the thesis topic is accepted/not accepted*

Date: ___2024____year ____04_____ month __22___ date

_____
Course leader/coordinator

Student and thesis topic is admitted/not admitted by the Institute/Deparment*

Date: _____year _____ month _____ day

_____
Head of Institute/Department**

***Please undreline the relevant choice!**

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Appendix 4 – Declaration

STUDENT DECLARATION

Signed below, _____Owais Ahmad Kutty__, student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, at the BSc/MSc Course of __Agricultural 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_____ month _04_____ day__22

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: _____2024 ___ month _____04__ day___22

signature

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

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THESIS

OWAIS AHMAD KUTTY

M.Sc. Agricultural Biotechnology

Gödöllő
2024

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences
Szent István Campus
Agricultural Biotechnology

Investigation of *SlDML2* promoter spatial and temporal expression pattern with reporter constructs in tomato

Primary supervisor: Dr. György Szittya
(Group leader, scientific advisor)

Secondary supervisor: Dr. Péter Gyula
(Project leader, senior scientist)

Author: **Owais Ahmad Kutty**
SJQUHL

Institute: Institute of Genetics and Biotechnology, Department of Plant Biotechnology

Gödöllő

2024

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ABSTRACT OF THESIS

Thesis title: Investigation of *SlDML2* promoter spatial and temporal expression pattern with reporter constructs in tomato

Author name : Owais Ahmad Kutty

Course, level of education: M.Sc Agricultural Biotechnology (Plant Biotechnology)

Host Department/Institute: Institute of Genetics and Biotechnology/Department of Plant Biotechnology

***Primary thesis advisor**:* Dr. György Szittya (Group leader, scientific advisor)

***Secondary thesis supervisor*:** Dr. Péter Gyula (Project leader, senior scientist)

Epigenetics refers to heritable changes in gene expression that occur without modification of the underlying DNA sequence. DNA methylation and demethylation are two of the many mechanisms by which epigenetic changes can occur. These changes can affect gene expression and contribute to plant development. DNA demethylation can occur either passively or actively. Active DNA demethylation is regulated by DNA demethylases. There are four DNA demethylases in tomato (*SlDML1-4*). *SlDML2* controls fruit ripening. In line with this, its expression is induced during the breaker stage of ripening. Altering the *cis*-regulatory elements (promoters) of genes *in vivo* could result in phenotypes different from that of a null mutant. To map the regulatory elements that govern *SlDML2* expression, we created deletion series in the *SlDML2* promoter by multiplex CRISPR/Cas9 genome editing. One of our mutants contained a 2104 bp deletion and a 1 bp insertion in the proximal part of the *SlDML2* promoter which was shown to be differentially methylated during ripening. The mutant had severe developmental defects that could be a result of the deletion in its promoter. We measured the expression of *SlDML2* in the tomato shoots with RT-qPCR and found that the expression level was lower in the mutant. However, this method is not suitable to investigate the spatial and temporal expression in high detail. Therefore, we created two, a mutant and a wild-type *SlDML2* promoter driven GFP gene constructs (pICH86966 D2pro-GFP and pICH86966 A120pro-GFP) to investigate the expressional difference of *SlDML2* in a non-invasive way. The modules were assembled using the Golden Gate assembly method. We have started transformation of these gene constructs into tomato plants. We are expecting to observe a markedly different expression pattern using fluorescence microscopy in case of the two constructs that could help us explain the observed severe developmental phenotype.

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Appendix 6 – Thesis Review Report

DIPLOMADOLGOZAT/SZAKDOLGOZAT BÍRÁLATI LAP
THESIS REVIEW REPORT

A dolgozat készítőjének neve, Neptun kód / Candidate's name, neptun code:

_____Owais Ahmad Kutty, SJQUHL_____

A dolgozat készítőjének szakja, tagozata, képzési helye / Candidate's department, training place:

____ Institute of Genetics and Biotechnology/Department of Plant Biotehnology_____

A dolgozat címe / Title of the thesis:

Investigation of *SlDML2* promoter spatial and temporal expression pattern with reporter constructs in tomato _____

A bíráló neve, beosztása, szervezeti egység / Thesis evaluator's name, title, department:

_____

A diplomadolgozat nem fogadható el/ The thesis cannot be evaluated if:

- súlyos szakmai tévedéseket tartalmaz / it contains major technical errors,
- szegényes a felhasznált forrásmunkák köre, / the amount of sources used is not efficient,
- súlyosan megsérti a tartalmi formai követelményeket / or it severly violates the formal requirements.

Plágium, hivatkozás nélküli jelentős szövegfelhasználás esetén a dolgozat összpontszáma: 0! / In case of plagiarism, when the sources of quotations are not indicated, the total score of the thesis is 0. Kérjük jelölje az értékelésének megfelelő pontszámokat az 1-től 5-ig terjedő pontskálán. Please, evaluate the reviewing aspects below on scale 1 to 5.

I. <u>Témaválasztás / Choice of topic</u>

1. Célkitűzések, logikai ív, koherens gondolatmenet / Objectives, logical and coherent train of thoughts:

1 2 3 4 5

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II. Szakirodalmi feldolgozás / Use of literature

2. Az elméletek, fogalmak, modellek ismerete, alkalmazása / The knowledge and application of theories, concepts and models:

1 2 3 4 5

3. Elemző, értékelő, összehasonlító, kritikai észrevételek / Analytic, evaluative, comparative and critical observations:

1 2 3 4 5

4. Szakirodalmi hivatkozások / Literature references:

1 2 3 4 5

III. Egyéni vizsgálat (amennyiben a dolgozat szakirodalmi áttekintés témájú, az itt szereplő kérdéseket a szakirodalom feldolgozásának színvonala alapján szükséges értékelni) / Individual research (if the thesis is a literature review, these questions should be evaluated according to the quality of reviewing)

5. A kutatási kérdések/hipotézisek / Stating research questions/hypotheses:

1 2 3 4 5

6. Az adatgyűjtés és adatfeldolgozás módszertana/ The method of data collection and processing:

1 2 3 4 5

7. Elemzőkészség / Analytical skills:

1 2 3 4 5

8. Következtetések és javaslatok / Conclusions and suggestions:

1 2 3 4 5

IV. Formai követelmények / Formal requirements

9. A dolgozat stílusa / The style of the thesis:

1 2 3 4 5

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10. A dolgozat struktúrája / The structure of the thesis:

1 2 3 4 5

A DOLGOZAT ÖSSZPONTSZÁMA / TOTAL SCORE OF THESIS: _____

Plágium / Plagiarism: Elfogadható színvonalú forráskezelés / Acceptable use of literature*

Érdemjegy /Final grade:
0-25 pont: elégtelen/insufficient (1)
26-31 pont: elégséges/sufficient (2)
32-38 pont: közepes/satisfactory (3)
39-44 pont: jó/good (4)
45-50 pont: jeles/excellent (5)

Védésre javaslom / I recommend it for final examination*: igen nem

ÉRDEMJEGY / GRADE: _____

Általános összefoglaló vélemény a dolgozatról / General, summarizing opinion about the thesis:

_____
_____
_____
_____
_____
_____
_____
_____
_____

A bíráló szakmai kérdései / Questions of the thesis referee:

_____
_____
_____
_____
_____

Kelt: _____ év _____ hó _____ nap

_____
Bíráló neve és beosztása/Referee name and position
Bíráló munkahelye/Referee place of work