INVESTIGATING THE POTENTIAL INTERACTORS DURING *PLT7*-DRIVEN DNRR

A Thesis

submitted to

Indian Institute of Science Education and Research Pune in partial fulfillment of the requirements for the BS-MS Dual Degree Programme

by

SUBRAMANYA GOND

Indian Institute of Science Education and Research Pune

Dr. Homi Bhabha Road,

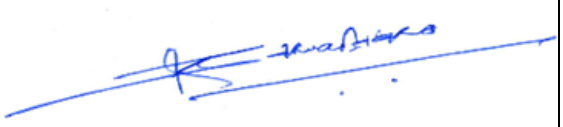
Pashan, Pune 411008, INDIA.

From May 2023 to March, 2024

Supervisor: Dr. KALIKA PRASAD

Certificate

This is to certify that this dissertation entitled **"Investigating the potential interactors during *PLT7*-driven DNRR" t**owards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Subramanya Gond** at Indian Institute of Science Education and Research under the supervision of **Dr. Kalika Prasad**, Department of Biology, during the academic year 2023-2024.



Dr. Kalika Prasad

Associate professor

Biology Division, IISER Pune

Committee:

Name of your Guide: Dr. **Kalika Prasad**, Biology Division, IISER Pune

Name of Your TAC: Dr. **Siddhesh Kamat**, Biology Division, IISER Pune

This thesis is dedicated to my parents and to my beloved ones

Declaration

I hereby declare that the matter embodied in the report entitled "**Investigating the potential interactors during *PLT7*-driven DNRR"** are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education & Research (IISER) Pune, under the supervision of **Dr. Kalika Prasad,** and the same has not been submitted elsewhere for any other degree. Wherever others contribute, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussions.

Subramanya Gond

(20191035)

BS-MS dual degree student

IISER Pune

Table of Contents

List of tables

List of figures

Abstract

Among different regeneration models, *Arabidopsis thaliana* exhibits an intriguing regeneration response in which adventitious roots arise from an excised leaf explant without external hormone supplementation. This regenerative response is known as De novo root regeneration (DNRR). Recent studies have also observed that depending on whether the cut end of the excised leaf is in contact with the substrate, the leaf can either form an adventitious root or a callus to facilitate wound healing. *PLETHORA(PLT)* genes, also known as *AINTEGUMENTA-LIKE (AIL)* genes, are found to play a crucial role in plant regeneration. Overexpression of *PLETHORA7/AIL7(PLT7)* induces DNRR in the leaf even without the cut end being in contact with any surface. Therefore, *PLT7*-driven DNRR might bypass mechano-dependent cell fate. To understand how *PLT7* functions in an excised leaf, it is essential to elucidate the possible interacting co-factors governing its function. So, this project aims to identify novel interacting partners of *PLT7* during DNRR. These interacting partners can help us understand how *PLT7*-driven DNRR is induced and maintained.

Acknowledgments

I want to extend my heartfelt appreciation to Dr. Kalika Prasad for the support and guidance that he provided throughout my thesis. I also like to thank my mentor, Akansha Ganguly, for all her assistance and advice during my thesis.

I want to thank my expert, Dr. Siddhesh Kamat, for all his valuable input. Furthermore, I am grateful to Mabel Mattew, Harish Yadav, Dr. Mohammad Aiyaz, and my batchmates, Amish and Pratham, for their help during my thesis. I also thank Dr. Lieven De Veylder for providing the seeds of *the scl* mutant plants and the plasmid for the *SCL5* translational reporter construct for the experiments. I am thankful to the microscopy facility at IISER Pune for providing all the microscopy facilities needed for my experiments. Last and most importantly, I am grateful to my family and friends who were there for me in my good and bad times.

Contributions

Contributor name	Contribution
S.G, A.G, K. P	Conceptualization Ideas
S.G, A. G	Methodology
S.G, A. G	Software
S. G	Investigation
K. P	Resources
S. G	Writing - original draft
S. G	Writing - review and editing
K. P	Supervision

Author's contributions: Subramanya Gond (S.G), Akansha Ganguly (A.G), Kalika Prasad (K.P).

This contributor syntax is based on the Journal of Cell Science CRediT Taxonomy[1]

1 https://journals.biologists.com/jcs/pages/author-contributions

Chapter 1

INTRODUCTION:

1.1: Regeneration

Since time unknown, humans have been fascinated by the phenomenon of regeneration. This is apparent from ancient Greek mythologies involving entities like Prometheus and Tityus to modern fictional characters such as Wolverine and Deadpool (Papavramidou,2019). Advancements in science and technology have provided new insights into regeneration at the cellular and molecular levels. Regeneration can be classically defined as the process by which a living organism restores, or repairs damaged organs or tissues in response to wound sustained (Ikeuchi et al., 2016). The process of regeneration ranges from repairing minor cellular injuries to forming new multicellular entities from a single cell. The regeneration process is vital for an organism's growth, development, and survival. The extent of regenerative capability may vary from species to species. Some organisms, like salamanders and xenopus, can regrow their amputated organs, while flatworms could manifest a completely functional organism from its severed body (Figure 1) (Fu et al., 2018,). Interestingly, though, the animal regeneration phenomenon is quite restricted compared to the plant kingdom (Poss., 2010).

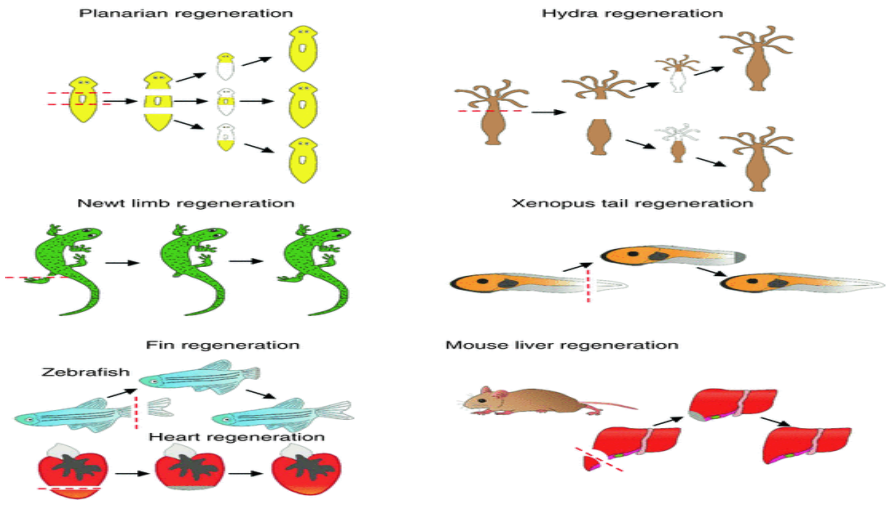


Figure 1: Regeneration in animals (Fu et al., 2018)

1.2. Regeneration in plants

Plants are primarily sessile and yet can grow indeterminately. Despite a lack of cell migration, they display a high degree of developmental plasticity (Alberts et al., 2002). Plasticity refers to plants' ability to adapt and evolve in response to ever-changing environmental conditions. This makes them one of the planet's largest and most diverse organisms. In most organisms, regeneration is limited to specific cell linkage, whereas it's quite comprehensive in plants. This peculiarity can be credited to the plant cells. Plant cells can make all possible cell types; this ability is called totipotency (Price and Smith, 1979). A plant cell can reprogram itself to undergo "differentiation" or "transdifferentiation," leading to de novo organogenesis, to restore or repair a tissue or organ in response to injury or damage (Chatfield et al., 2013; Liu et al., 2014; Kareem et al., 2016).

Furthermore, a single plant cell has the capability to regenerate and develop into a new plant (Shin et al., 2020). This ability of plants has been commercially exploited in plant breeding using tissue culture techniques, especially in food crops like bananas. Similarly, it is also used in the preservation of rare and endangered plant species like opium poppy (*Papaver somniferum*) and mojave wild marjoram (*Lippia junelliana*) (Nessler, 1982; Juliani et al., 2011).

1.3. Types of regeneration in plants:

Among all model organisms, *Arabidopsis thaliana* has been the first choice for studying plant regeneration due to its short life span, ease of cultivation, and well-characterized genome. The availability of established community resources and the genetic and physiological homology with other plants, especially with food crops, makes it optimal.

Two major categories of regeneration responses exhibited by Arabidopsis are those induced by tissue culture and those caused by mechanical injury (Mathew and Prasad, 2021). These are termed as

a) Tissue culture-induced regeneration
b) Mechanical injury-induced regeneration

1.4. Tissue culture-induced regeneration:

It refers to the regeneration of plants from an explant or tissue in in-vitro conditions. There are two types of tissue culture-induced regeneration, i.e., Direct tissue culture-induced regeneration and indirect tissue culture-induced regeneration. In direct tissue culture-induced regeneration, organs develop straightway from explant tissues. In the case of indirect tissue culture-induced regeneration, there is an intermediate step in which cells proliferate to form an undifferentiated mass of cells known as a callus (Figure 2) (Mathew and Prasad, 2021).

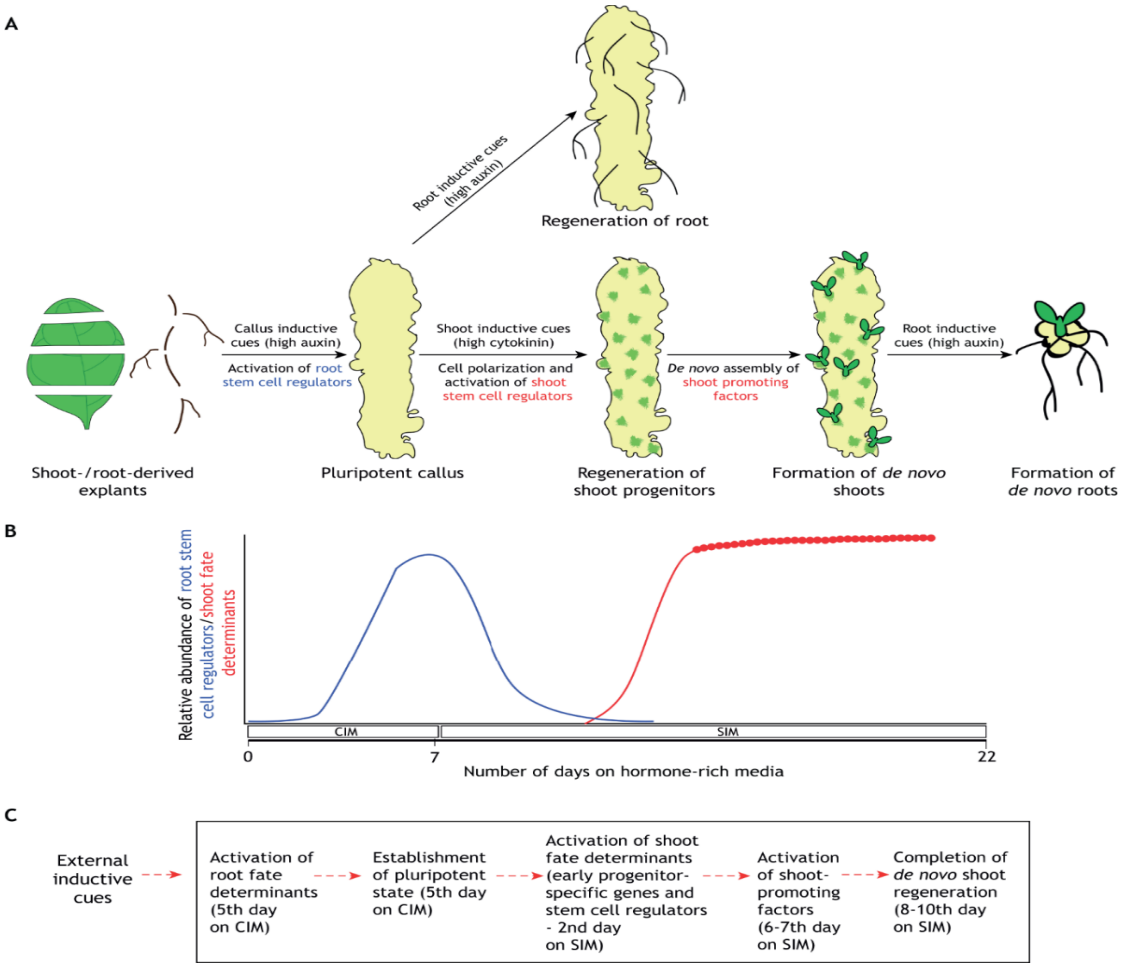


Figure 2: Examples for regeneration in case of callus mediated regeneration (Mathew and Prasad, 2021).

1.5. Mechanical injury-induced regeneration:

Plants are subjected to a wide range of biotic and abiotic stress that can lead to mechanical injuries. Mechanical injury-induced regeneration is observed in two scenarios: firstly, when the injured organ remains connected to the parent plant, and secondly, in a situation where the damaged organ is excised (Figure 3) (Mathew and Prasad, 2021). This regeneration response involves the activation of multiple signaling pathways. These signaling pathways involve different hormonal signals, which catalyze wound-healing responses (Nanda and Melnyk, 2018; Omary et al., 2023; Varapparambath et al., 2022).

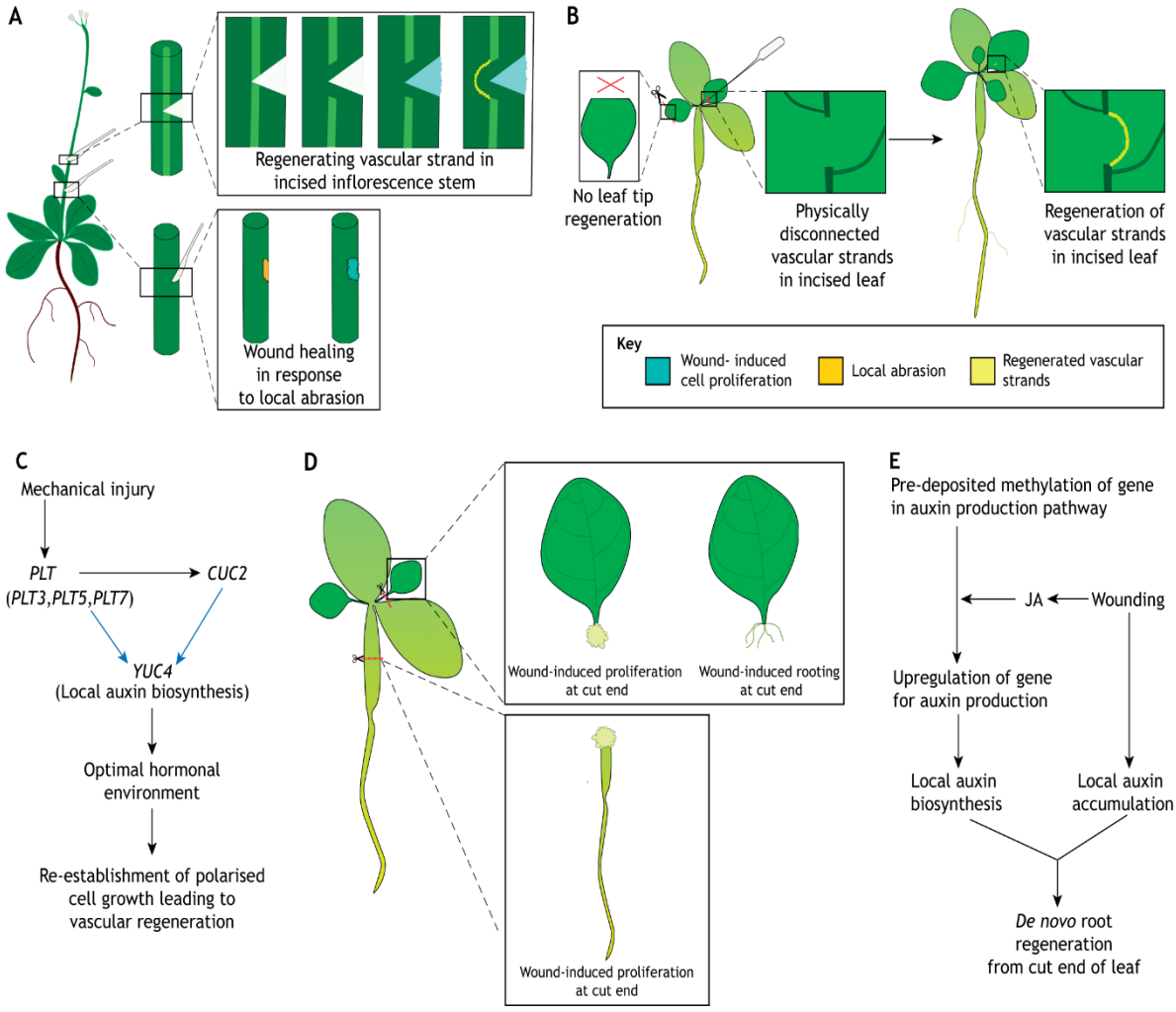


Figure 3: Examples for regeneration in case of mechanical injury **(**Mathew and Prasad, 2021).

1.6. De novo regeneration:

This is observed in plant organs, which are excised or detached from the parent body. This is the second type of mechanical injury-induced regeneration. Studies have shown that when an organ or component is excised from the parent plant, it triggers the formation of a new organ with a unique identity, termed de novo organogenesis (Chen et al., 2014). De novo organogenesis is seen in both vitro and in-vitro situations, for example, de novo shoot regeneration (Shin et al., 2020).

In the case of leaves, they exhibit a fascinating generative response called De novo root regeneration (DNRR). DNRR is a phenomenon wherein adventitious roots emerge from a leaf explant even without external hormone supplementation (Chen et al., 2014). The regenerative ability of a leaf is age-dependent and shows different regenerative responses defending on the rosette from which the leaves have been obtained (Sun et al., 2020).

1.7. Phases in DNRR:

The process of DNRR can be divided into three distinct stages.

a) The initiation of early signaling pathways, which are activated by different stimuli at the cut end. This results in the auxin out flux.

b) Accumulation of auxin in procambium and cambium cells.

c) In the last stage, the cell fate is determined- competent cells transform into founder cells, followed by another cell division to form the root primordium. Next, root apical meristem cells develop, leading to root tip emergence (Xu, 2018).

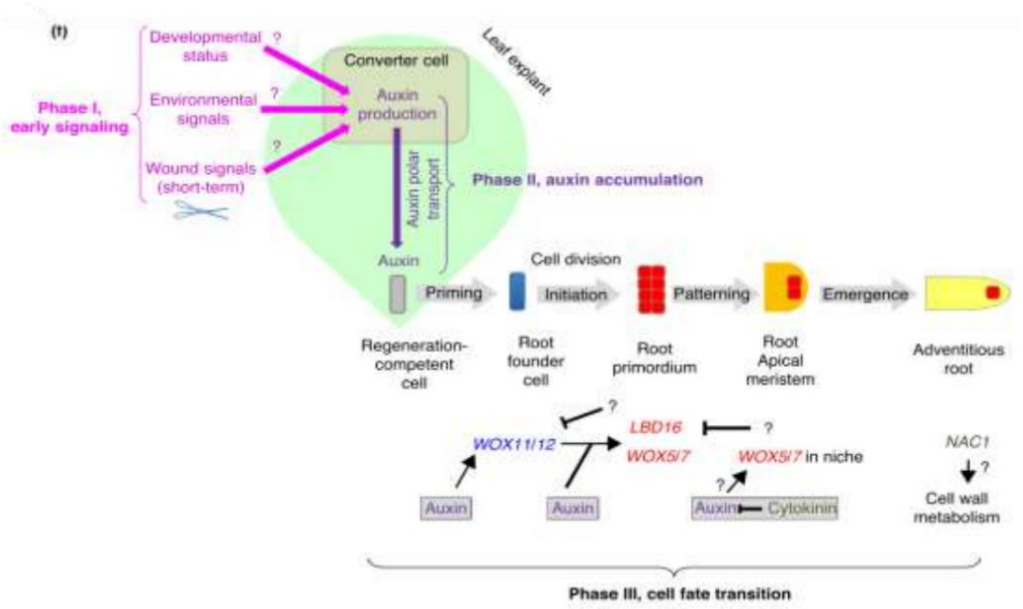


Figure 4: Phases in DNRR (Xu, 2018)

1.8. Touch mediated DNRR:

The regenerative responses in plants vary from organ to organ. The leaves can give rise to both shoot and root depending on the cues (Wan et al., 2023). However, the mechanisms by which the cell fate is defined have been a curious question among many researchers.

Latest findings have shown that the DNRR process works in a contact-dependent manner. Depending on the placement of the injured end of a leaf with respect to the substrate, it can either form an adventurous root or a callus (Figure 5). Recent findings have also found that the phytohormone auxin plays a crucial role in DNRR (Shanmukhan et al., 2021).

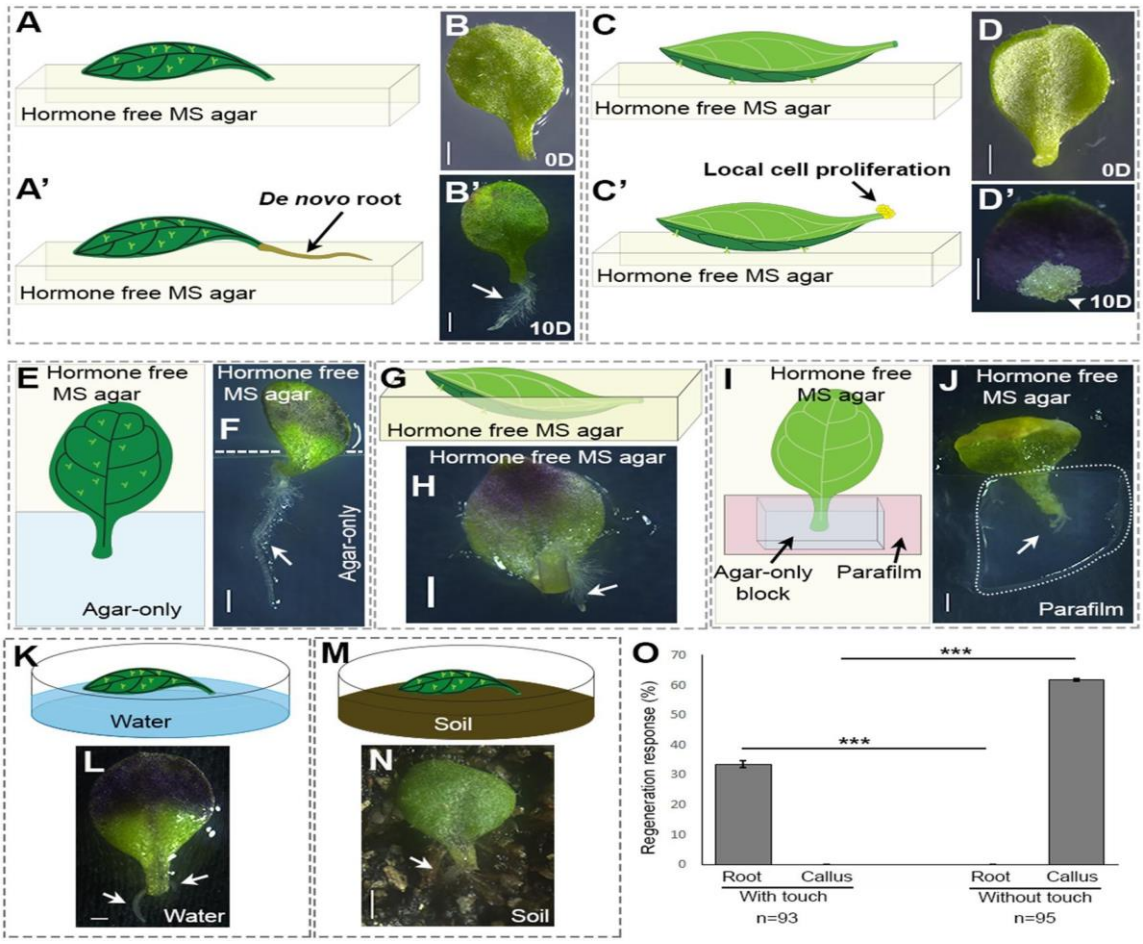


Figure 5: Touch mediated DNRR (Shanmukhan et al., 2021).

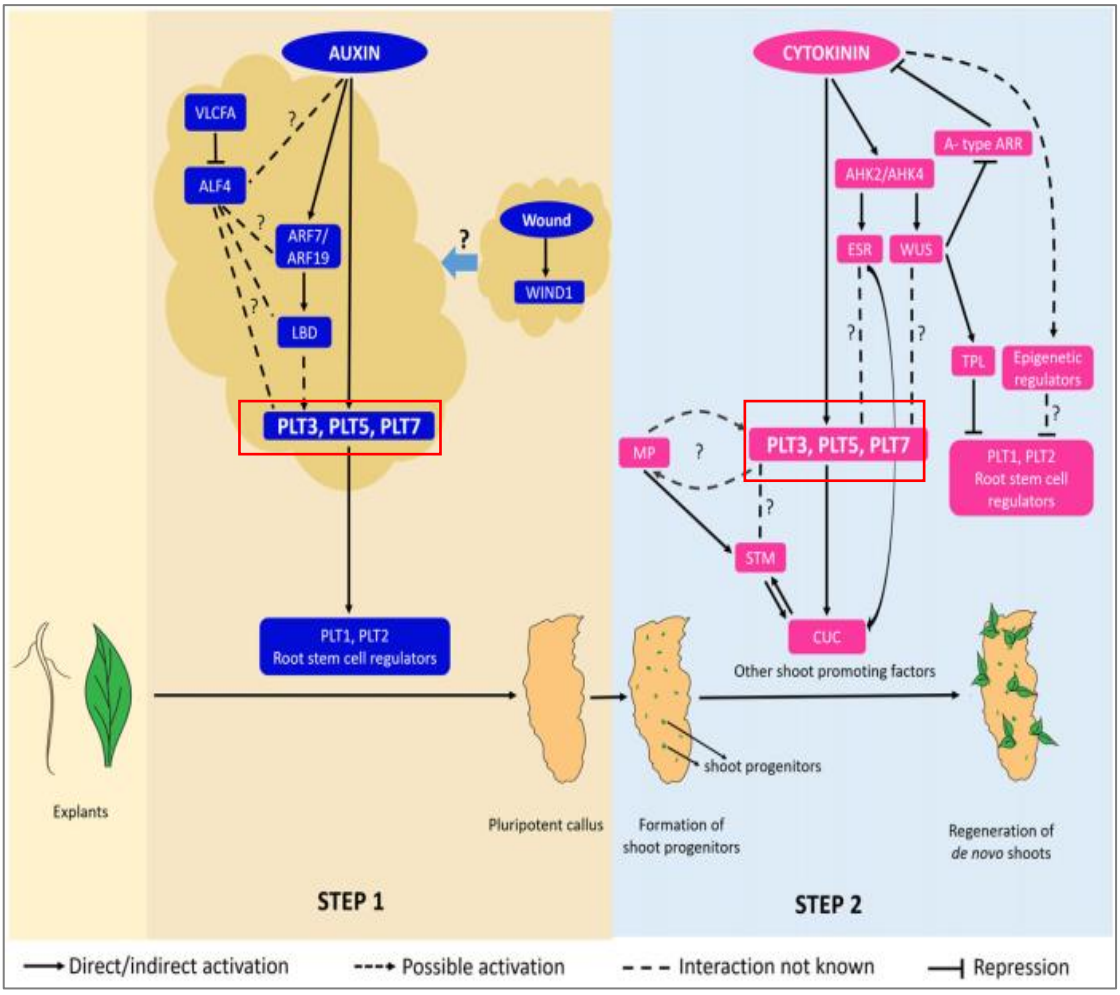
1.9. Molecular basis for regeneration in plants:

The plant regeneration process is governed by a cluster of genes and phytohormones. Phytohormone auxin regulates cell division, differentiation, and organogenesis, crucial processes during regeneration. Auxin is mainly responsible for de novo root regeneration. At the same time, phytohormone cytokinin promotes shoot formation. In tissue culture, a precise balance of auxin and cytokinin supplementation is essential for the specification of de novo regeneration (Ikeuchi et al., 2019). Genes like *WUSCHEL (WUS)* and *CLAVATA (CLV)* are crucial in meristem regeneration (Somssich et al. 2016). *PIN-FORMED 1 (PIN1)* gene regulates auxin polar transport (Braybrook and Harada, 2008). Auxin also activates factors such as *AUXIN RESPONSE FACTOR (ARFs)* and other related genes (Liu et al., 2014; Chen et al., 2016).

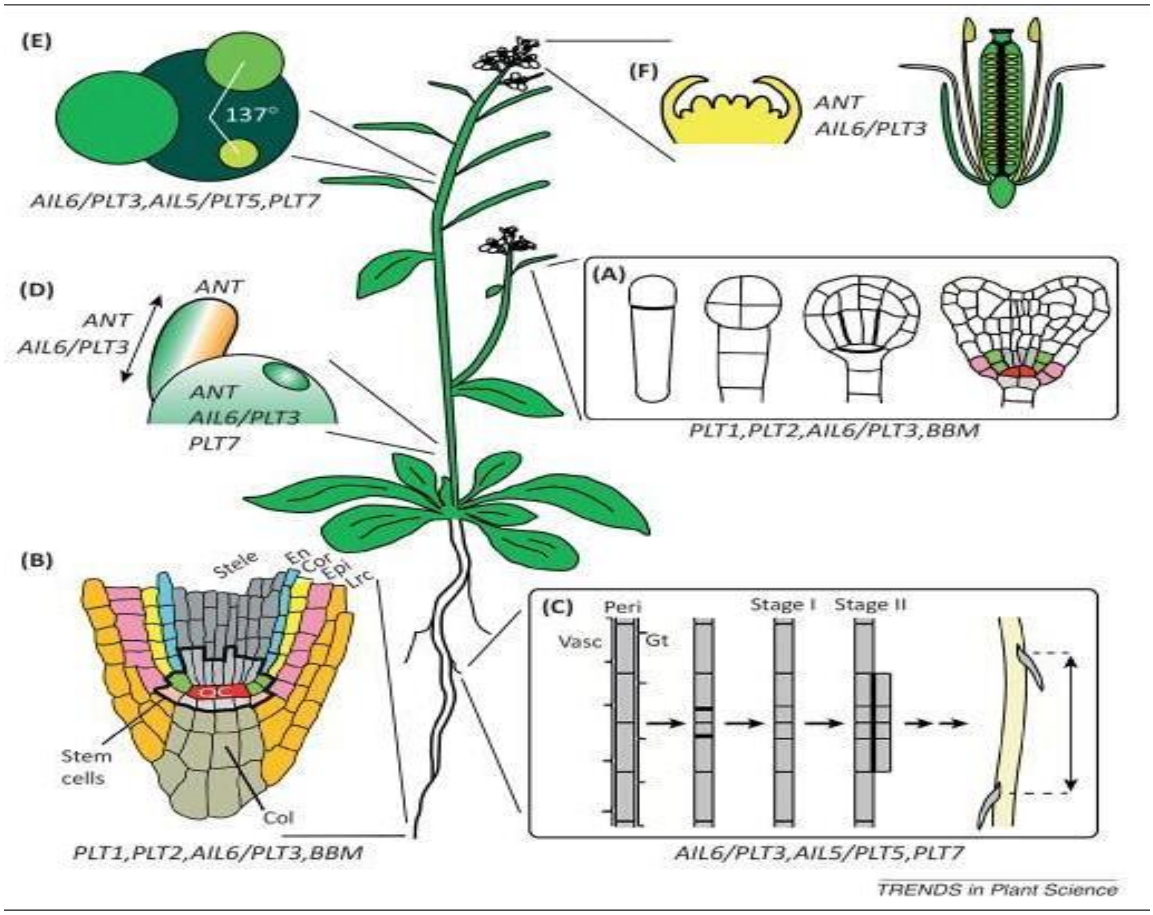
The *PLETHORA (PLT7),* also referred to *as AINTEGUMENTA-LIKE (AIL)* genes, are the family transcription factors belonging to the double *APETALA2/ ETHYLENE RESPONSE FACTOR (AP2/ERF)* domain family and are involved in regulating different plant developmental and regenerative processes (Santuari et al.,2016). In particular, *PLT3, 5*, and *7* govern de novo shoot regeneration through a dual-step mechanism.

a) They control and maintain stem cells, forming shoot progenitors.
b) Additionally, these transcription factors regulate the other factors which promote shoot formation like the *CUP-SHAPED COTYLEDON2 (CUC2)* to conclude the process (Kareem et al., 2015).

These genes are also important in regulating the patterning of de novo organs during lateral root patterning (Du and Scheres 2017).



(a)



(b)

Figure 6 a and b: Role of *PLT* genes in plant growth and development (Horstman *et al.*, 2014; Kareem *et al.*, 2016)

2.1. Motivation for the project:

In the context of touch-mediated DNNR in leaves, auxin accumulation is observed when it is in contact with the surface. The activation of auxin-responsive *INDOLE-3-ACETIC ACID INDUCIBLE 9 (1AA9)* and *MONOPTEROS (MP/ARF5)* was also observed. Notably, the *plt3,plt5,plt7* triple mutants failed to demonstrate DNNR responses. This indicates that PLT genes are indispensable for this process (Shanmukhan et al., 2021). However, the mechanism behind DNRR is still unclear. This raises fundamental questions, such as, how is this initiated in context with touch? What factors govern this? What are the molecular cues responsible for this? What determines the fate of these cells at the molecular level? Answering this question could reveal novel mechanisms behind DNRR and regeneration processes in general. The overexpression of *PLT7* was found to induce DNRR even without the cut end being physically in contact with any substrate (Shanmukhan et al., 2021). So, we hypothesize that *PLT7*-driven DNRR can bypass mechano-dependent cell fate. To

fully comprehend how *PLT7* operates in an excised leaf, it is crucial to identify the co-factors that might interact with it and affect its function. This will enable us to comprehend the mechanism of DNRR induction and maintenance.

2.2. Goals:

Aim: 1) To investigate the Potential cofactors in *PLT7*-driven DNRR:

Objective 1: Study the DNRR responses using the *Scl* mutants of Arabidopsis.

Objective 2: Study the expression pattern of *PLT7* during DNRR.

Chapter 2:

MATERIALS AND METHODS:

2.3. In Silico Analysis:

To investigate the molecular basis of DNRR and identify the potential interacting partners of *PLT7*, a comparative analysis between the mass spectrometry data of callus (Anju S, unpublished data) and the RNA sequencing data of DNRR (Pan, et al 2019) was done. The TRUE/FALSE callout method was used to identify the clusters with upregulated gene expression in *PLT7*-MS genes. A total of 331 common hits were found. A Gene Ontology analysis was performed to identify nuclear-localized genes that may have DNA binding or co-transcription factor functions. To learn more about this analysis, please visit *http://bioinformatics.sdstate.edu/go/* .

TAIR ID	GENE/PROTEIN	DESCRIPTION
AT3G09840	CDC48A	Cell division regulator 48A
AT3G53230	CDC48D	Cell division control protein homolog D
AT2G36530	ENO2/MBP-1	Bi-functional gene module, codes for enolase *ENO2*, and transcriptional repressor *MBP-1* (2nd exon ATG isoform)
AT1G44900	MCM2	DNA replication licensing factor 2
AT1G50600	SCL5	Scarecrow-like protein 5
AT2G16440	MCM4	DNA replication licensing factor 4
AT2G46900	uncharacterised	Transcription factor 25 (Uniprot A0A178VSX5), protein matches *TCF25* family (transcriptional repressors)
AT3G18230	uncharacterised	*PB-1* domain present, Possible *ARF/Aux/IAA* family protein

Table 1: MS Vs RNA seq data

SCARECROW LIKE 5, a GRAS domain protein within *PHYTOCHROME A SIGNAL TRANSDUCTION 1 (PAT1)* subfamily*,* was among the common hits for nucleus proteins (14). *GRAS* domain proteins consist of *GIBBERELLIC ACID INSENSITIVE (GAI)*- an essential regulator in the gibberellin signaling pathway (Achard et al., 2006), *REPRESSOR of GAI -,* a mutant allele of *GAI* (Peng et al., 1997) *and SCARECROW-a* protein involved in root development (Sbabou et al., 2010). These proteins are specific to the plant kingdom and are involved in plant developmental processes. The conserved C-terminus GRAS domain gives these proteins a unique identity. This includes SAW, VHIID, LHR II, LHRI, and PYRE motifs. The variable N-terminus region of grass domain proteins contains IDD and DELLA domains. Recent studies have shown that *SCL5, SCL21*, and *PAT1* act redundantly to regulate root tissue

regeneration by activating the upregulation of the *DNA-BINDING ONE FINGER 3.4 (DOF3.4)* (Bisht et al., 2023, Hofmann 2016, Wang et al., 2020). This study aims to verify whether *SCL5* is an interacting partner of PLT7 during DNRR.

2.4. Genomic DNA extraction:

Leaves were collected and snape-freezed in liquid nitrogen. The extraction of genomic DNA was done using the cetyltrimethylammonium bromide (CTAB) reagent method as per *https://cshprotocols.cshlp.org/content/2009/10/pdb.rec11984.short*. The genomic DNA obtained was later stored at -20 degrees Celsius for future use.

2.5. Genotyping:

Genotyping is a method used to identify homozygous and heterozygous mutants. Here, separating out heterozygous mutants is extremely important for an experiment. In the experiment, a heterozygous mutant can cause redundancy, hence affecting the experimental results. The mutants are created by adding the T-DNA insertions between the genes of interest, making them functionally ineffective.

Three different types of primers are used for genotyping. These are used in 2 combinations

1. LP (left border primer) +RP (Right border primer), this combination of primer is used to amplify the gene of interest.

2. LPB (T-DNA border primer) + RP - this is used to amplify the T-DNA insertion segment.

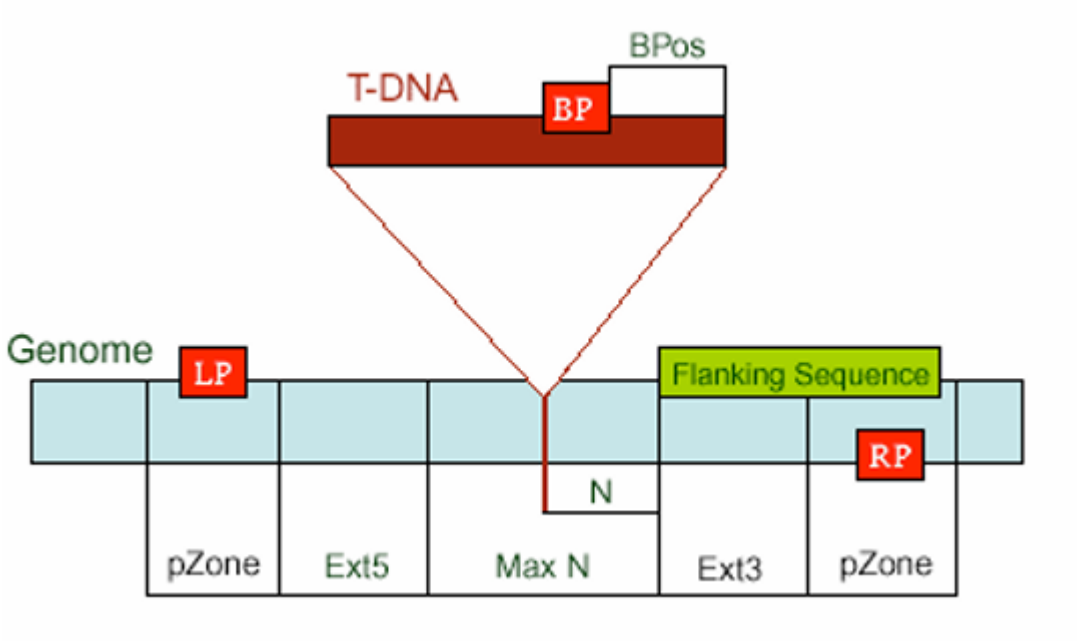


Figure 7: Schematic representation of T-DNA insertion (Batth et.al., 2020)

Here, the heterozygous mutant would show bands for both the gene and the T-DNA insertion in an electrophoresis gel. A homozygous mutant would only have bands for T-DNA, indicating that the T-DNA is inserted in both strands of DNA.

2.6. Plant materials:

All experiments were conducted using a plant model*, Arabidopsis thaliana* - ecotype Columbia (Col-0). Seeds first underwent surface sterilization with 70 percent ethanol. A second wash was done with 20 percent bleach. Later, seven consecutive sterile water washes were followed to wash remove bleach and ethanol residues. The entire sterilization was done in a sterile laminar flow hood. To break the dormancy of seeds, the seeds underwent a vernalization process for three days at a temperature of four degrees Celsius in a refrigerator. 20 to 30 seeds were plated on each square petri plate containing half-strength Murashige-Skoog (MS) medium with p.H 5.7 and agar. The plates were placed vertically in a Percival growth chamber with continuous white light and relative humidity of 70 percent. The temperature was maintained at 22 degrees Celsius.

2.7. Primers used for genotyping:

	LP	RP
scl21_SALK_146085	AGATAACAACTTACGTGCCGG	TGAATCTACGTCAGCCATGAAC
pat1-2	TAAATCACACGCCGGTTTAAG	AAGCTCCCAACCGTTGAATAG
scl5_SALK_152973	CAGGTCTAGCACCAAGAGCAC	CGCTGAGTTCTGGTTTCTTTG
LBb1.3	ATTTTGCCGATTTCGGAAC	

Table 2: Genotyping primers

2.8. Imaging:

The leaf samples with fluorescent-tagged proteins were imaged using the Zeiss LSM 780 Multiphoton Microscope. 20 µg/ml propidium iodide (0.002%) (Sigma-Aldrich) dye was used to stain the leaf samples. The objective used for taking images was a 10× air objective. The Zeter zoom images of leaf samples were obtained using the Leica S8 APO stereo zoom microscope. The images were analyzed using the Fiji imaging software.

2.9. Assay:

12-day-old seedlings were chosen for the responses of DNRR. The pair of leaves from the first and second rosette were excised using angled Vannas scissors, and the detached leaves with petiole were placed on half-strength MS media without any

hormonal supplement. The leaf samples were placed in two different ways; in one, the adaxial side was in contact with the media, and in the other, the abaxial side.

Chapter 3:

RESULTS:

3.1. Genotyping results:

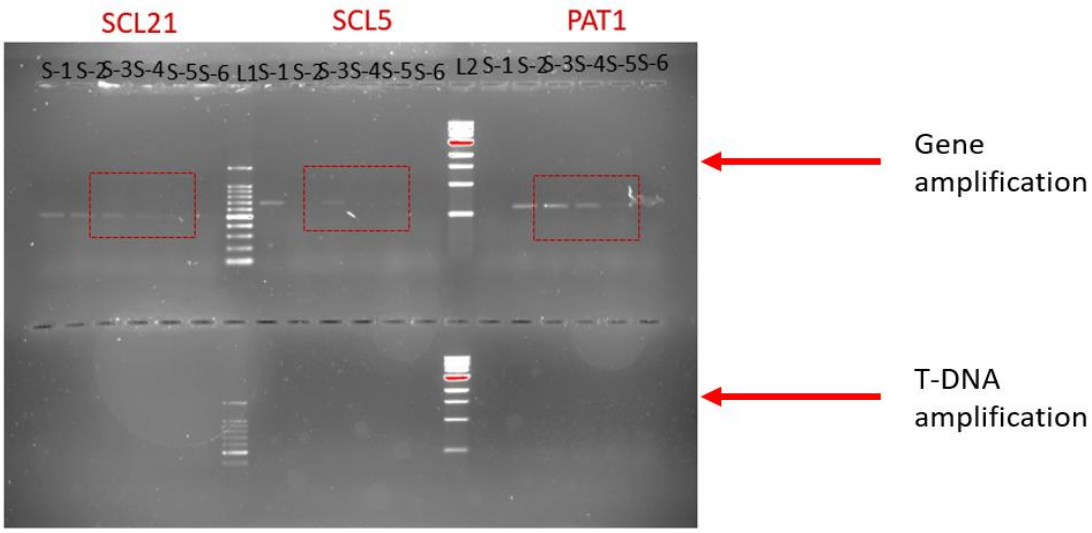


Figure 8: Gel image of col wt sample for genotyping.1 percent agarose gel. L1-100bp ladder, L2- 1 kb ladder

The gel electrophoresis results indicate that the control (col wT) only displayed bands for the SCL 5, SCL 21, and PAT1 gene amplification i.e. LP+RP and not for the T-DNA insertions i.e. for LP+RP (Figure). This implies that the control is functioning normally.

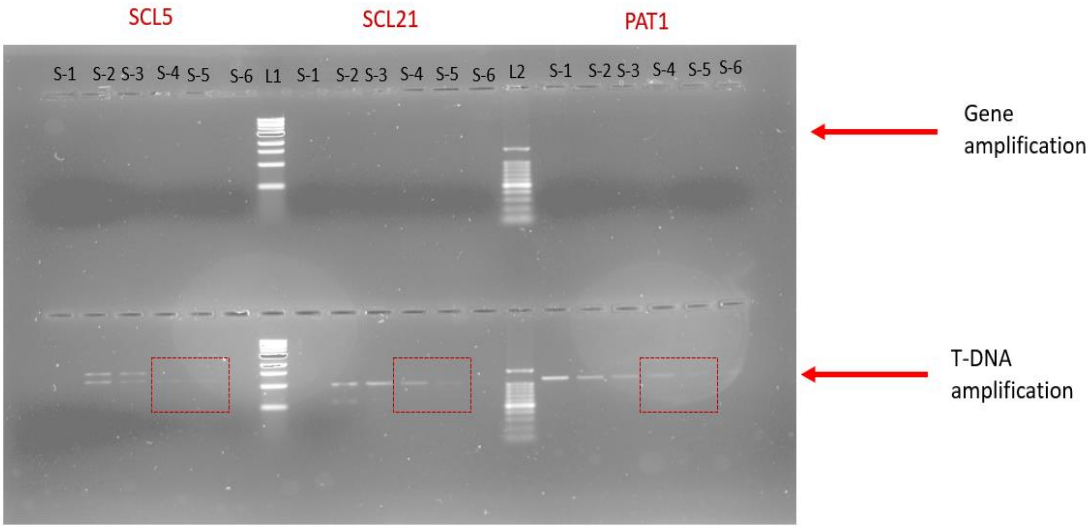
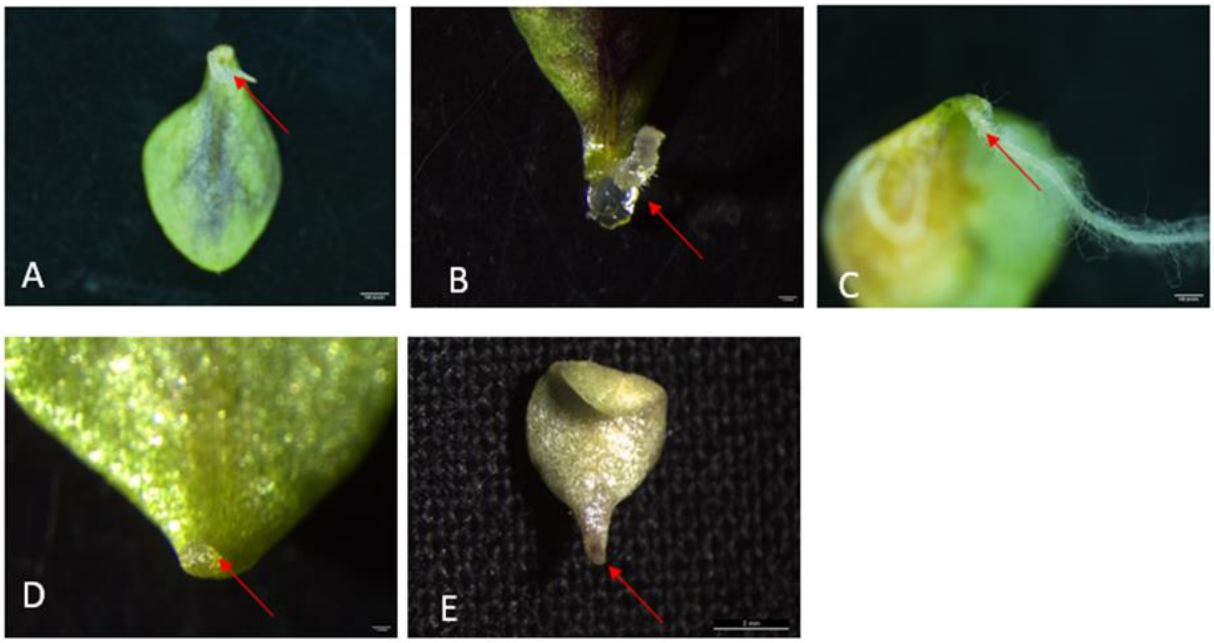


Figure 9: Gel image of *scl5scl21pat* triple mutant .1 percent agarose gel. L1- 1 kb ladder. L2- 100 bp ladder

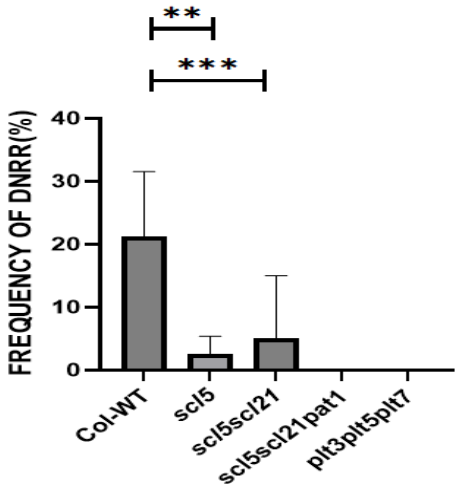
Figure 9 shows that there were bands in lane S-4 for all three T-DNA inserts of the mutant without any specific bands. Meanwhile, the non-template control S-6 showed no band. The *scl5,scl21, pat1* triple mutant gel image showed bands for only T-DNA insertions of respective genes i.e for LBP + RP. There was no band for the gene, i.e., for LP + RP. This indicated that the triple mutant is homozygous. The band length of gene amplification as per the Salk Institute Genome Analysis Laboratory (SIGnAL) database are *SCL5*-1179 bp, *SCL21*-1021 bp, *PAT 1*-1133 bp. The estimated length for T-DNA insertions as per SIGnAL database is *scl5*- 529 to 829bp, *scl21*-501 to 801 bp*, pat-1*- 481 to 781bp. The bands obtained for gene amplification and T-DNA insertions correspond to their respective band lengths as per the SIGnAL database.

3.2. Assay results:

a. Touch Assay



a)

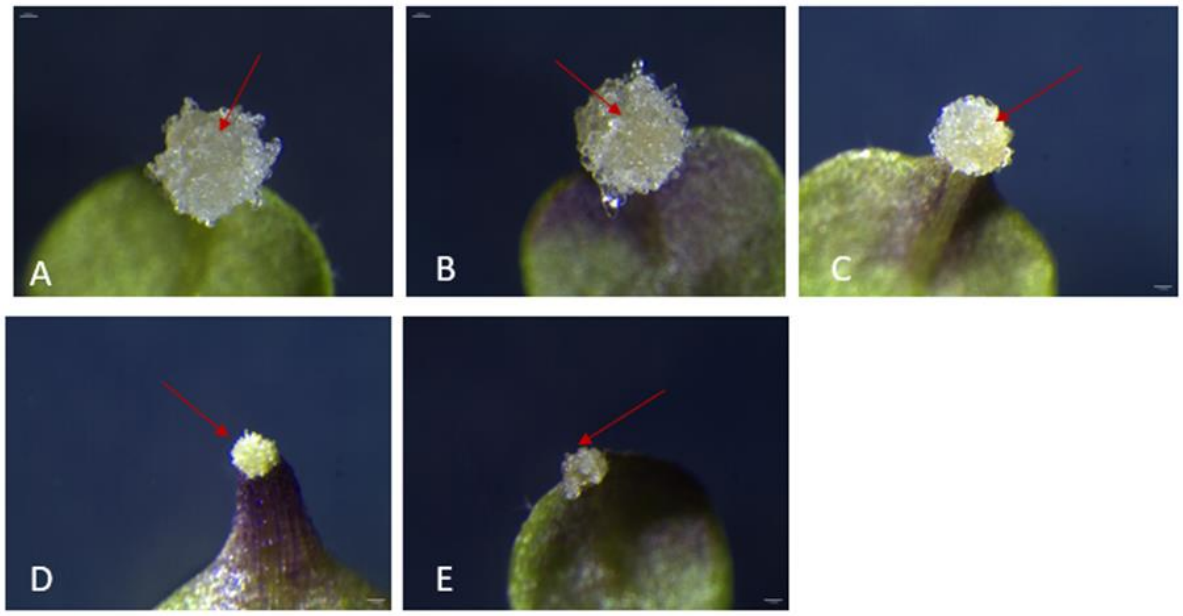


(b)

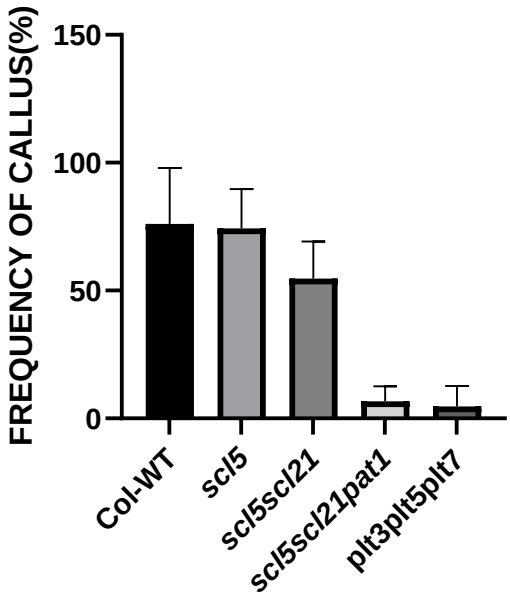
Figure 11: a). With contact leaf samples (A-E), A-*Col-wt (n=60), B*-scl5 (n=60), C-*scl5scl21 (n=60)*, D-*scl5scl21pat1 (n=64),* E-*plt3,plt5,plt7(n=57)*. b) DNRR frequency.

Scl5 (n=60) and scl*5,scl21*(n=60) showed reduced DNRR frequency, whereas the *scl5,scl21,pat1* (n= 64) tripled mutant showed no DNRR response. *scl5,scl21,pat1* response is similar to the *plt3,plt5,plt7* (n=57) - negative control. Mutations in *SCL5, SCL21,* and *PAT1* genes hinder the process of DNRR. This indicates for normal functioning of *SCL5, SCL21,* and *PAT1* genes is necessary for DNRR. Col Wt was used as a positive control.

b. Non-touch assay:



a)



b)

Figure 11: a) Without contact leaf samples (A-E), A-*Col-WT*(n=20), B-*scl5(n=19)*, C-*scl5scl21 (n=20)*, D-*scl5scl21pat1(n=27)*, E-*plt3,plt5,plt7 (n=16)*. b) callus vs wound healing frequency

The leaves with their cut end of petiole that were not in contact with substrate showed a low frequency of Wound healing response (WHR) compared to callus formation. WHR is an immediate localized response where the wound is sealed to prevent infections and restore structural integrity. On the other hand, a callus is an undifferentiated mass of cells. In *scl5* single mutant callus formation frequency was

comparable to col wt (positive control). *scl5*,*scl21* displayed reduced callus formation frequency, with respect to the col wt. *scl5,scl21,pat1* showed a significantly lower frequency of callus formation compared to col . This was similar to the *plt3plt5plt7*-negative control response.

3.3. Expression pattern analysis:

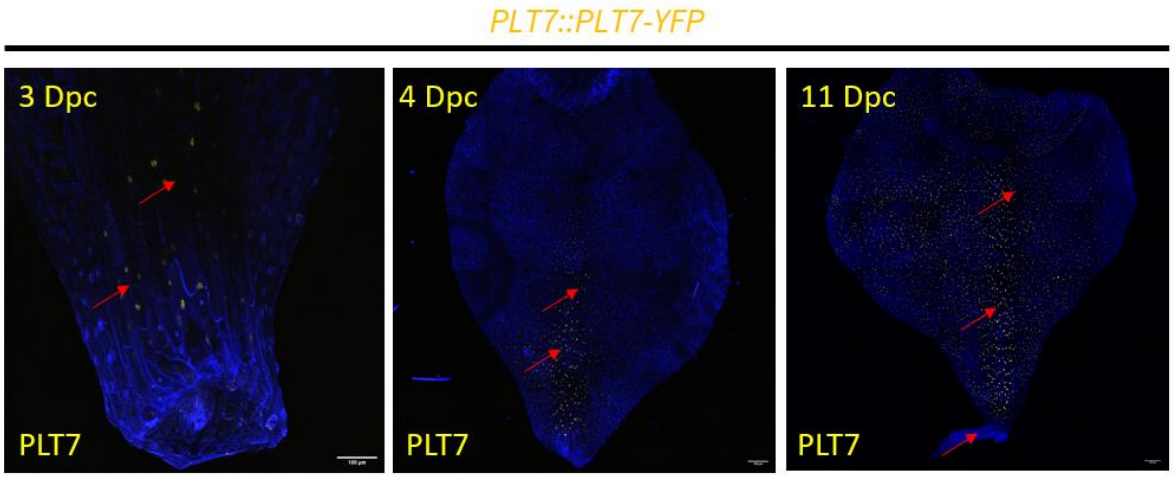


Figure 12: With contact *PLT7* expression (n=4): Imaging by: Akansha Ganguly

The expression of *PLT7* was first observed around 3 dpc (Day post cut). It was concentrated at the petiole above the cut end. The *PLT7* expression started to spread through the vascular bundle around the 4 dpc. It spread across the leaf at the later dpc.

It was observed that the expression of *PLT7-YFP* was upregulated systematically. Its expression pattern began in the petiole and expanded to the apex and lemma. In contrast to the minimal expression pattern of *PLT3* and *PLT5 (*Shanmukhan et al., 2021), *PLT7* expression was found to be spread across the leaf margin at early time points. This suggests that the DNRR process may operate in a *PLT7*-dependent manner.

3.4. Plasmid digestion:

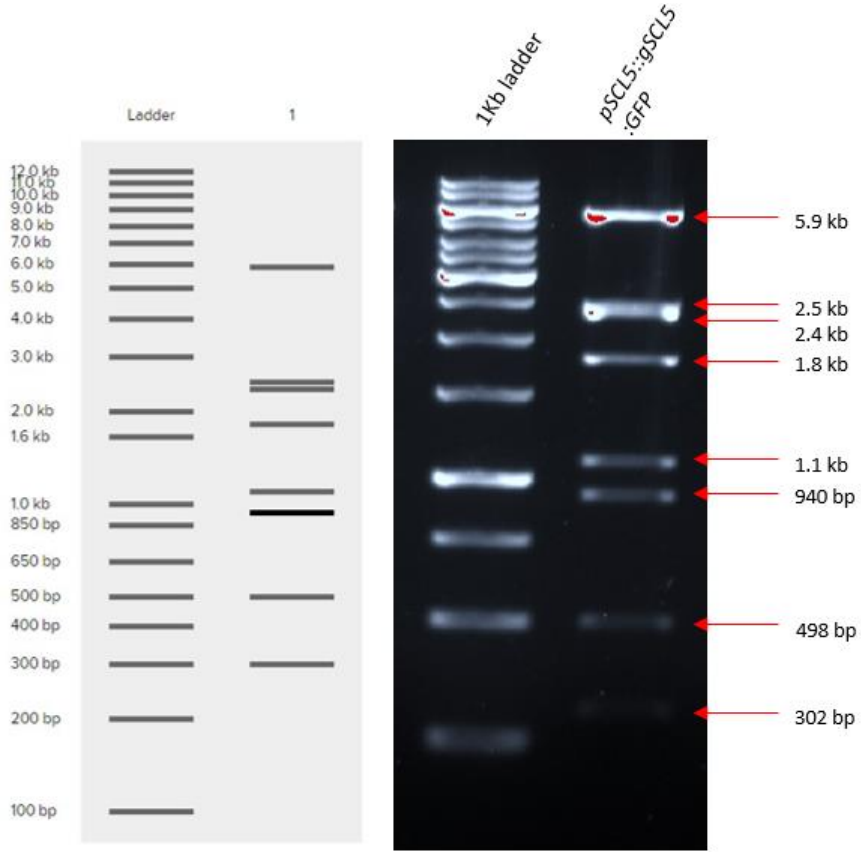


Figure 13: Digestion map of *pSCL5::gSCL5:GFP*

The translational reporter construct *pSCL5::gSCL5:GFP* was digested with restriction enzymes Apol1, Xoh1 and EcoR1.

Chapter 4:

DISCUSSION AND FUTURE PLANS:

Plants' ability to regenerate can be classified as one of the most unique characteristics in nature. Plants can show different regenerative responses depending on the cues provided. The formation of de novo organs from excised organs has been studied extensively, yet the underlying mechanisms are not completely understood to date (Wan et al., 2023).

The formation of de novo root from an excised leaf is known as de novo root regeneration (DNRR) (Chen et al., 2014). This process has been found to act in a touch-dependent manner. Depending on whether the leaf is placed in an adaxial or abaxial manner, the outcome of the regenerative response is entirely different. In case the cut end is in contact with a substrate, it leads to the formation of a de novo root, whereas if it's not in contact, then it leads to the formation of an undifferentiated mass of cells known as callus (Shanmukhan et al., 2021). These responses for wound healing are intriguing, but the mechanism is mostly unknown. Interestingly, overexpression of the *PLETHORA (PLT7)* family gene - *PLT7* can induce a root formation in an excised leaf even without the cut end being exposed to any substrate (Shanmukhan et al., 2021). Therefore, *PLT7*-driven DNRR might bypass mechano-dependent cell fate. To understand how *PLT7* functions in an excised leaf, it is essential to elucidate the possible interacting co-factors governing its function. This can help us understand how PLT7-driven DNRR is initiated and how it affects the cell fate during regeneration.

On comparing the mass spectroscopy data (Anju S, unpublished data) and the RNA sequencing data (Pan et al. 2019), *SCL 5*, a grass family protein that recently was found to be crucial in root regeneration (Bisht et al.,2023), can be a potential interacting partner of *PLT7*. To validate this, the DNRR responses of *scl* mutants were first studied. The *scl5* and *scl5scl21* mutants display a low frequency of DNRR responses compared to col wt in a contact-dependent context. But surprisingly, the *scl5sc21pat1* triple mutant failed to yield any DNRR response. These results indicate that long with *SCL5* and *SCL21* the normal functioning of *PAT1* is also necessary for the DNRR process.

Similarly, the *scl5sc21pat1* mutant displayed a low callus formation frequency compared to the WHR frequency. This suggests that the normal functioning of *SCL5, SCL21,* AND *PAT1* genes is necessary for the DNRR. The expression pattern of *PLT7-YFP* is observed to be distributed throughout the excised leaf during the initial time points, contrasting with the minimal expression of *PLT3* and *PLT5*, which are predominantly localized to the petiole during DNRR (Shanmukhan et al., 2021). This supports the hypothesis that *PLT7*-mediated regeneration may circumvent the contact-dependent DNRR mechanism. However, further validation with a larger sample size is needed to confirm the above results.

Future Studies:

For future studies

- We will be transforming the *pSCL5::gSCL5:GFP* construct into col wt plant and analyze the expression pattern of *SCL5-GFP*
- Additionally, we will be studying whether the expression patterns of *SCL5* and *PLT7* co-localize
- Complementary studies to understand expression patterns of *SCL5* and *PLT7* in mutants
- We will be crossing *scl5* mutant with *plt7* mutant to form *scl5plt7* double mutants. Later we will be doing genotypic and phenotypic characterization of *scl5plt7* double mutant

We can also use mass spectroscopy and Co-IP techniques to investigate further and explore other potentially interacting partners of *PLT-7*-driven DNRR. Overall, *SCl5* can potentially be an interacting pattern of *PLT7* during DNRR, but more in-depth studies are needed to confirm it.

REFERENCES:

Achard, Patrick, et al. "Integration of plant responses to environmentally activated phytohormonal signals." *Science* 311.5757 (2006): 91-94.

Alberts, Bruce, et al. "The plant cell wall." Molecular Biology of the Cell. 4th edition. Garland Science, 2002.

Azpiroz-Leehan, Ricardo, and Kenneth A. Feldmann. "T-DNA insertion mutagenesis in Arabidopsis: going back and forth." Trends in Genetics 13.4 (1997): 152-156.

Bisht, Anchal, et al. "PAT1-type GRAS-domain proteins control regeneration by activating DOF3. 4 to drive cell proliferation in Arabidopsis roots." The Plant Cell 35.5 (2023): 1513-1531.

Braybrook, Siobhan A., and John J. Harada. "LECs go crazy in embryo development." Trends in plant science 13.12 (2008): 624-630.

Chatfield, Steven P., et al. "Incipient stem cell niche conversion in tissue culture: using a systems approach to probe early events in WUSCHEL-dependent conversion of lateral root primordia into shoot meristems." The Plant Journal 73.5 (2013): 798-813.

Chen, Xiaodong, et al. "A simple method suitable to study de novo root organogenesis." Frontiers in Plant Science 5 (2014): 208.

Du, Yujuan, and Ben Scheres. "PLETHORA transcription factors orchestrate de novo organ patterning during Arabidopsis lateral root outgrowth." Proceedings of the National Academy of Sciences 114.44 (2017): 11709-11714.

Gurley, Kyle A., and Alejandro Sánchez Alvarado. "Stem cells in animal models of regeneration." International Journal of Biomedical and Health Sciences 8.4 (2021).

Hofmann, Nancy R. "A structure for plant-specific transcription factors: the GRAS domain revealed." (2016): 993-994.

Horstman, Anneke, et al. "AINTEGUMENTA-LIKE proteins: hubs in a plethora of networks." *Trends in plant science* 19.3 (2014): 146-157.

Ikeuchi, Momoko, et al. "Plant regeneration: cellular origins and molecular mechanisms." Development 143.9 (2016): 1442-1451.

Juliani, H. Rodolfo, et al. "Evaluation of micropropagation for the introduction into cultivation and conservation of Lippia junelliana, an endemic aromatic plant from Argentina." Industrial Crops and Products 34.2 (2011): 1353-1357.

Kareem, Abdul, et al. "PLETHORA genes control regeneration by a two-step mechanism." Current Biology 25.8 (2015): 1017-1030.

Laitinen, Roosa AE, and Zoran Nikoloski. "Genetic basis of plasticity in plants." Journal of Experimental Botany 70.3 (2019): 739-745.

Long, Yun, et al. "New insights into tissue culture plant-regeneration mechanisms." Frontiers in plant science 13 (2022): 926752.

Mathew, Mabel Maria, and Kalika Prasad. "Model systems for regeneration: Arabidopsis." Development 148.6 (2021): dev195347.

Nanda, Amrit K., and Charles W. Melnyk. "The role of plant hormones during grafting." Journal of plant research 131.1 (2018): 49-58.

Nessler, Craig L. "Somatic embryogenesis in the opium poppy, Papaver somniferum." PHysiologia plantarum 55.4 (1982): 453-458.

Omary, Moutasem, Rotem Matosevich, and Idan Efroni. "Systemic control of plant regeneration and wound repair." New Phytologist 237.2 (2023): 408-413.

Pan, Jing, et al. "Control of de novo root regeneration efficiency by developmental status of Arabidopsis leaf explants." Journal of Genetics and Genomics 46.3 (2019): 133-140.

Papavramidou, Niki. "Hepatic regeneration in Greek mythology." World Journal of Meta-Analysis 7.3 (2019): 77-79.

Peng, Jinrong, et al. "The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses." *Genes & development* 11.23 (1997): 3194-3205.

Poss, Kenneth D. "Advances in understanding tissue regenerative capacity and mechanisms in animals." Nature Reviews Genetics 11.10 (2010): 710-722.

Price, H. James, and Roberta H. Smith. "Somatic embryogenesis in suspension cultures of Gossypium klotzschianum Anderss." Planta 145 (1979): 305-307.

Price, H. James, and Roberta H. Smith. "Somatic embryogenesis in suspension cultures of Gossypium klotzschianum Anderss." Planta 145 (1979): 305-307.

Santuari, Luca, et al. "The PLETHORA gene regulatory network guides growth and cell differentiation in Arabidopsis roots." The Plant Cell 28.12 (2016): 2937-2951.

Shanmukhan, Anju Pallipurath, et al. "Regulation of touch-stimulated de novo root regeneration from Arabidopsis leaves." Plant Physiology 187.1 (2021): 52-58.

Somssich, Marc, et al. "CLAVATA-WUSCHEL signaling in the shoot meristem." Development 143.18 (2016): 3238-3248.

Sun, Run-Ze, et al. "A role of age-dependent DNA methylation reprogramming in regulating the regeneration capacity of Boea hygrometrica leaves." Functional & integrative genomics 20 (2020): 133-149.

Varapparambath, Vijina, et al. "Mechanical conflict caused by a cell-wall-loosening enzyme activates de novo shoot regeneration." Developmental Cell 57.17 (2022): 2063-2080.

Wan, Qihui, et al. "WOX11: The founder of plant organ regeneration." Cell Regeneration 12.1 (2023): 1.

Wang, Ting-Ting, et al. "Genome-wide analysis of the GRAS gene family and functional identification of GmGRAS37 in drought and salt tolerance." Frontiers in plant science 11 (2020): 604690.

Xu, Lin. "De novo root regeneration from leaf explants: wounding, auxin, and cell fate transition." *Current opinion in plant biology* 41 (2018): 39-45.