Ahmedabad
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Characterisation of *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK)* in Finger Millet *(Eleusine coracana L.)*

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Contents

Introduction

- Current challenge in plant tissue culture:
  - **Recalcitrancy**
- Solution- tissue culture strategies which focus on particular developmental stages:
  - Somatic embryogenesis (SE)
  - Meristem formation

- Morphogenesis
  - Organised, spatial development of embryos, tissues and organs
  - Tightly controlled process
  - Involves network of genes acting together
- Morphogenic genes
  - Genes that control plant growth and development (regulate totipotency and regeneration)
  - Often encode transcription factors, signaling molecules, and other proteins that influence cell division, differentiation, and morphogenesis
- By understanding and manipulating these genes, scientists can improve plant growth, development, and stress tolerance

Introduction

- **Millets**
  - Small-seeded C4 grasses
  - Highly nutritious
  - Capable of thriving in harsh environments
  - Grown in semi-arid regions
  - Crucial role in food security
- **Finger millet** (*Eleusine coracana L.*)
  - Known as "Ragi"
  - Climate-resilient crop
  - Calcium-rich

- Millets are under-researched, despite their advantages and are less responsive to tissue culture protocols

Finger millet

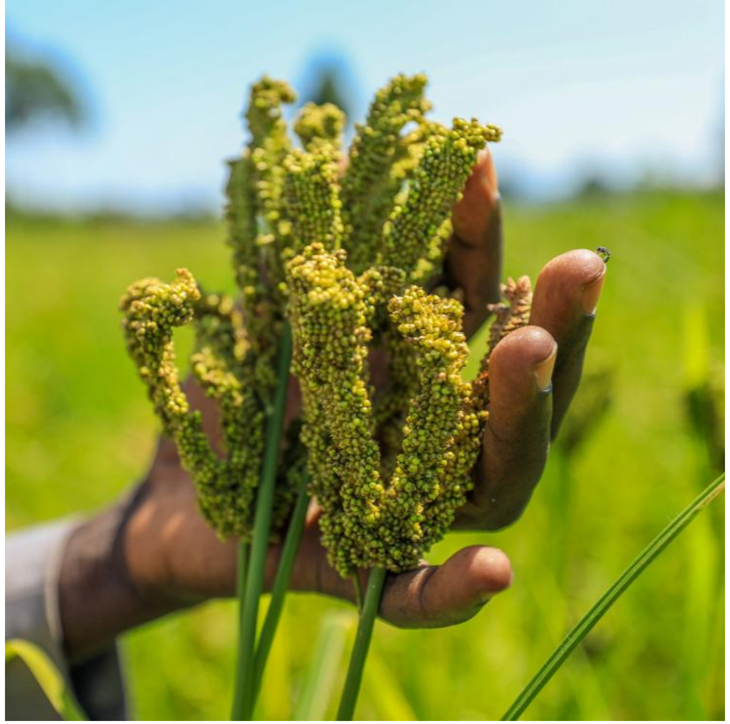


Photo credits: https://www.icrisat.org/crops/finger-millet/overview

Objectives

1. Identify key morphogenic genes affecting plant growth and development.
2. Understand the diversity and conservative nature of the SERK1 protein across plant species.
3. Study the expression pattern of *SERK1* in different tissue types:
    a) Using publicly available databases.
    b) Using finger millet samples grown in tissue culture.

Review of Literature

- Morphogenic genes identified:
  - *SERK1 (SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1)*
  - *AGL15 (AGAMOUS-LIKE 15)*
  - *WUS (WUSCHEL)*
  - *STM (SHOOT MERISTEMLESS)*
  - *BBM (BABYBOOM)*
  - *EMK (EMBRYOMAKER)*
  - *RKD4*
  - *LEC1 and LEC2 (LEAFY COTYLEDON 1 and 2)*
  - *FUSCA3 (FUS3)*
  - *CUC1 and CUC2 (CUP-SHAPED COTYLEDON)*
  - *ESR1 and ESR2 (ENHANCER OF SHOOT REGENERATION)*

Review of Literature

- About *SERK*s:
    - Key gene in plant morphogenesis and tissue culture responses
    - Code for LRR-RLKs which regulate:
        - Plant growth, root development, male fertility, stomatal development and immune responses
    - Role in somatic embryogenesis
        - Involved in callus formation and plant regeneration processes
    - Linked to stress responses
    - Molecular marker for somatic embryogenesis
        - Expression patterns during somatic and zygotic embryogenesis

- Enhances tissue culture efficiency when overexpressed
    - Enhanced embryogenic callus response and other morphological features
- SERK family has not yet been characterized in Finger Millet, an important crop grown in diverse environments

Materials and Methods

1. SERK sequence and phylogenetic analysis
2. Baseline expression analysis of *SERK1* using publicly available database
3. RNA isolation from fresh tissue samples
4. cDNA synthesis
5. Quantitative Real-time PCR

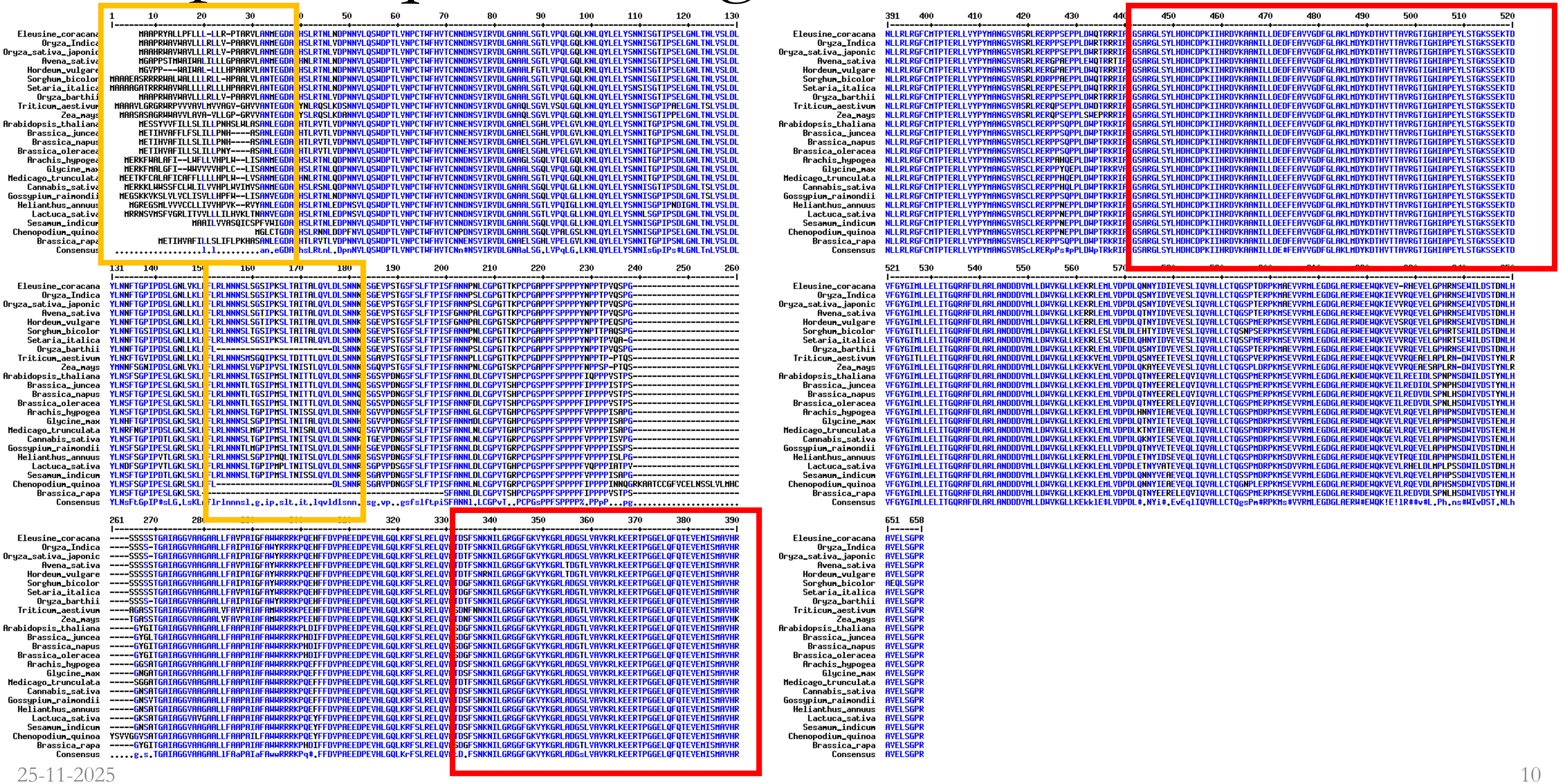
SERK Sequence and Phylogenetic Analysis

- Sequence accession:
  - *A. thaliana* SERK1 protein sequence (NP_177328.1)
  - BLASTp against 24 crop species (Plants Ensembl)
  - Selection criteria:
    - >80% ID and e-value < 0.00001
- Multiple Sequence Alignment:
  - Alignment using MUSCLE algorithm (MEGA 11)
  - Alignment visualisation (MultAlin)
- Phylogenetic tree:
  - Maximum likelihood (Bootstrap: 1000 and JTT model) (IQ-TREE)

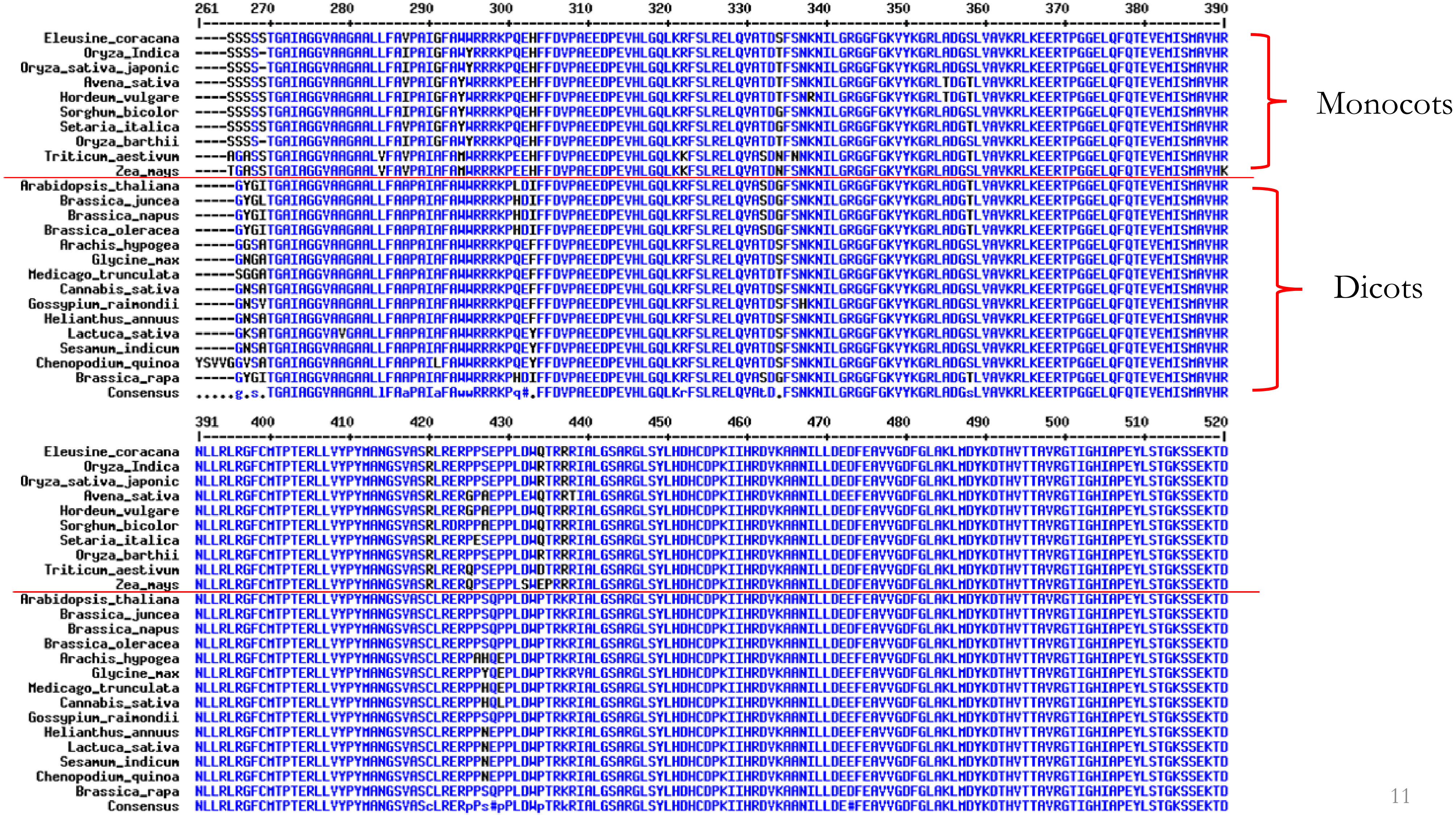
SERK1 protein sequence was accessed for the following species:

Eleusine coracana	*Brassica napus*
Avena sativa	*Brassica oleracea*
Hordeum vulgare	*Brassica rapa*
Oryza barthii	*Arachis hypogaea*
Oryza sativa indica	*Glycine max*
Oryza sativa japonica	*Medicago truncatula*
Setaria italica	*Gossypium raimondii*
Sorghum bicolor	*Helianthus annuus*
Triticum aestivum	*Lactuca sativa*
Zea mays	*Cannabis sativa*
Arabidopsis thaliana	*Chenopodium quinoa*
Brassica juncea	*Sesamum indicum*

Multiple Sequence Alignment



Multiple Sequence Alignment



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        Oryza_Indica  ----SSSS-TGAIAGGVAAGAALLFAIPAIGFAWYRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
Oryza_sativa_japonic  ----SSSS-TGAIAGGVAAGAALLFAIPAIGFAWYRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
        Avena_sativa  ----SSSSSTGAIAGGVAAGAALLFAVPAIGFAYWRRRKPEEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNKNILGRGGFGKVYKGRLTDGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
      Hordeum_vulgare  ----SSSSSTGAIAGGVAAGAALLFAIPAIGFAYWRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNRNILGRGGFGKVYKGRLTDGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
      Sorghum_bicolor  ----SSSSSTGAIAGGVAAGAALLFAIPAIGFAYWRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDGFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
      Setaria_italica  ----SSSSSTGAIAGGVAAGAALLFAVPAIGFAYWRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDGFSNKNILGRGGFGKVYKGRLADGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
       Oryza_barthii  ----SSSS-TGAIAGGVAAGAALLFAIPAIGFAWYRRRKPQEHFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
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      Brassica_napus  -----GYGITGAIAGGVAAGAALLFAAPAIAFAWWRRRKPHDIFFDVPAEEDPEVHLGQLKRFSLRELQVASDGFSNKNILGRGGFGKVYKGRLADGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
   Brassica_oleracea  -----GYGITGAIAGGVAAGAALLFAAPAIAFAWWRRRKPHDIFFDVPAEEDPEVHLGQLKRFSLRELQVASDGFSNKNILGRGGFGKVYKGRLADGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
     Arachis_hypogea  -----GGSATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
         Glycine_max  -----GNGATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
 Medicago_truncultata  -----SGGATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDTFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
     Cannabis_sativa  -----GNSATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
 Gossypium_raimondii  -----GNSVTGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSHKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
   Helianthus_annuus  -----GNSATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEFFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
      Lactuca_sativa  -----GKSATGAIAGGVAVGAALLFAAPAIAFAWWRRRKPQEYFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
      Sesamum_indicum  -----GNSATGAIAGGVAAGAALLFAAPAIAFAWWRRRKPQEYFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
  Chenopodium_quinoa  YSVVGGVSATGAIAGGVAAGAALLFAAPAILFAWWRRRKPQEYFFDVPAEEDPEVHLGQLKRFSLRELQVATDSFSNKNILGRGGFGKVYKGRLADGSLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
       Brassica_rapa  -----GYGITGAIAGGVAAGAALLFAAPAIAFAWWRRRKPHDIFFDVPAEEDPEVHLGQLKRFSLRELQVASDGFSNKNILGRGGFGKVYKGRLADGTLVAVKRLKEERTPGGELQFQTEVEMISMAVHR
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                      391     400       410       420       430       440       450       460       470       480       490       500       510       520
                      |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
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Oryza_sativa_japonic  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRERPPSEPPLDWRTRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
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      Hordeum_vulgare  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRERGPAEPPLDWQTRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
      Sorghum_bicolor  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRDRPPAEPPLDWQTRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
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       Oryza_barthii  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRERPPSEPPLDWRTRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
    Triticum_aestivum  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRERQPSEPPLDWDTRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
            Zea_mays  NLLRLRGFCMTPTERLLVYPYMANGSVASRLRERQPSEPPLSWEPRRRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
Arabidopsis_thaliana  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPSQPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
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 Medicago_truncultata  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPHQEPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
     Cannabis_sativa  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPHQLPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
 Gossypium_raimondii  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPSQPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
   Helianthus_annuus  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPNEPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
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      Sesamum_indicum  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPNEPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
  Chenopodium_quinoa  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPNEPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEEFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
       Brassica_rapa  NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPPSQPPLDWPTRKRIALGSARGLSYLHDHCDPKIIHRDVKAANILLDEDFEAVVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKTD
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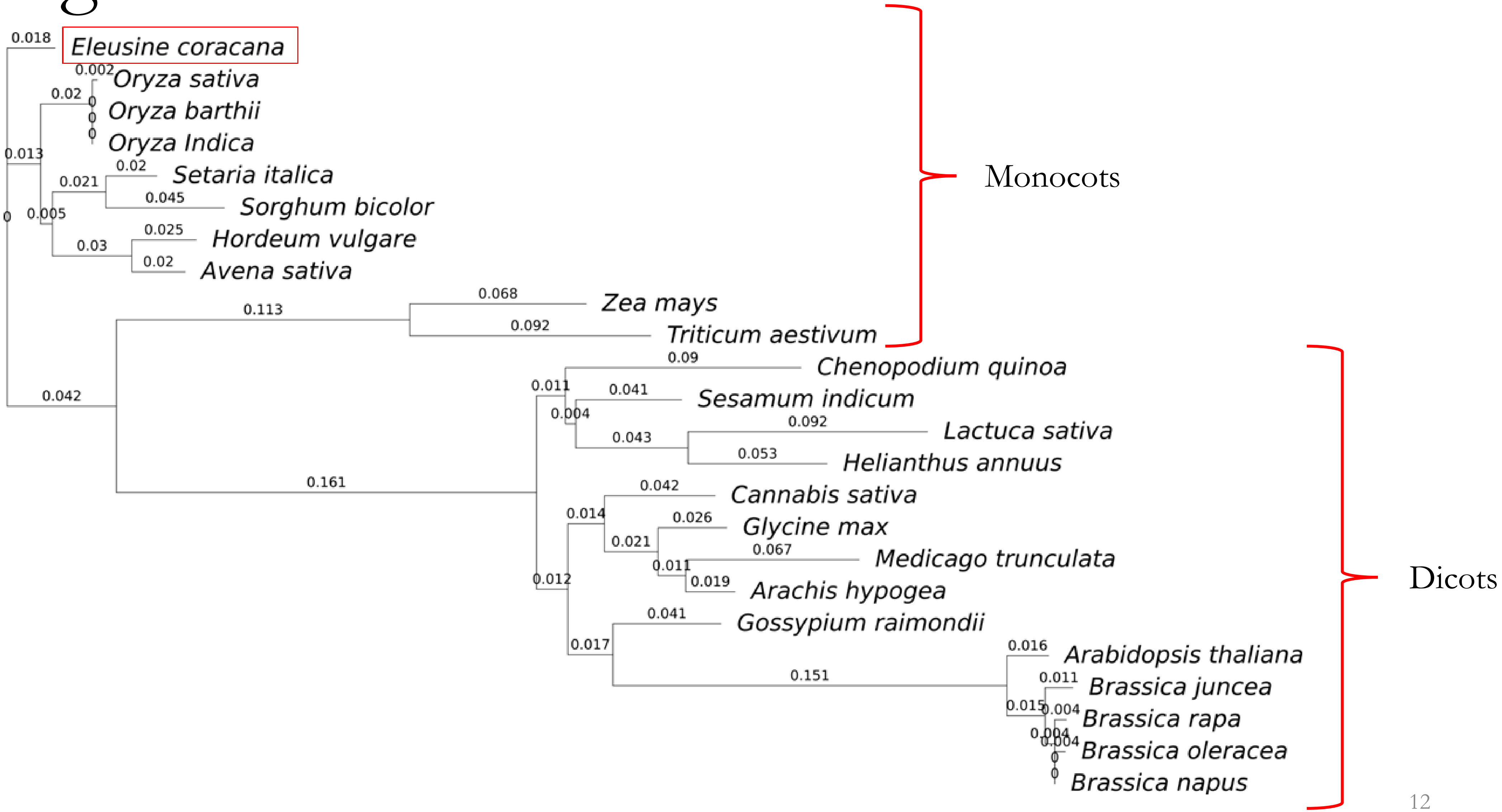
Phylogenetic Tree

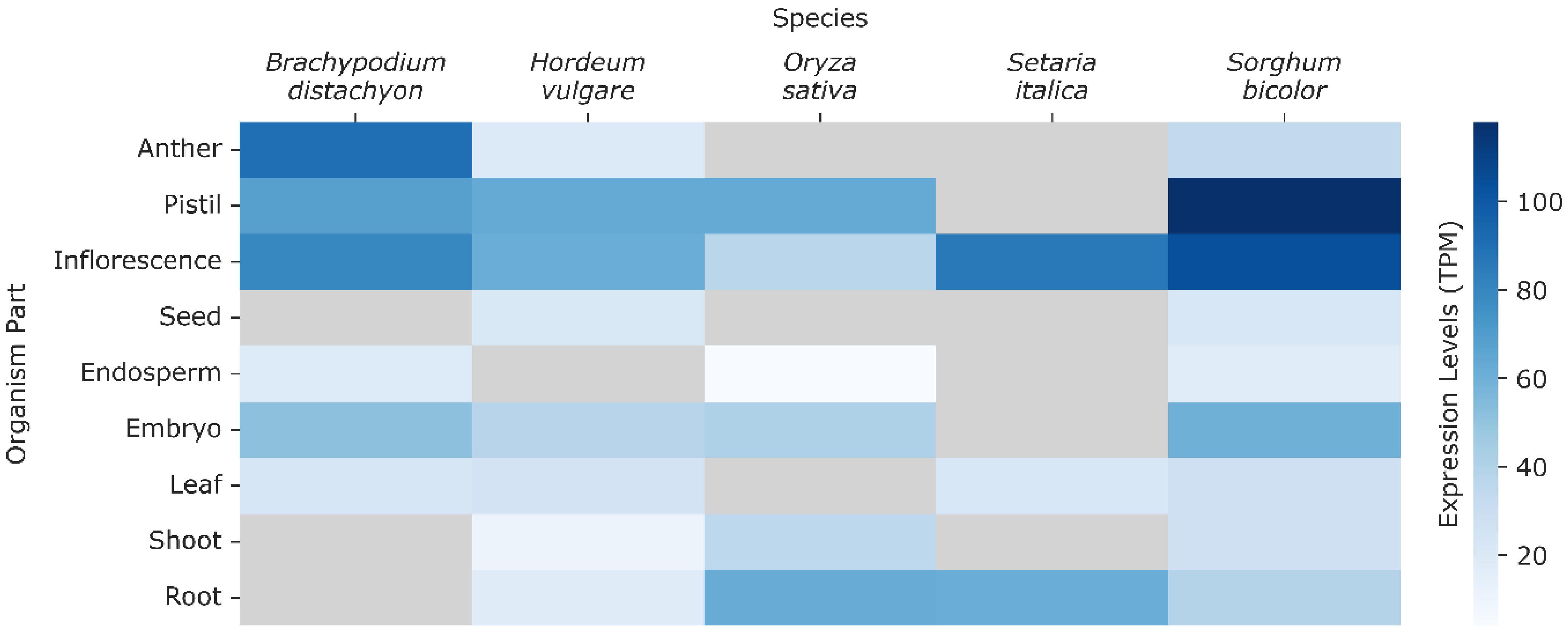


Baseline Expression Analysis of *SERK1* Using Publicly Available Database

- EMBL-EBI Expression Atlas Database
- Baseline expression data from RNA-Seq transcription profiling of different tissues
    - *Brachypodium distachyon, Hordeum vulgare, Oryza sativa, Setaria italica,* and *Sorghum bicolor*

- Expression patterns visualised in heatmap

Baseline Expression Analysis of *SERK1* Using Publicly Available Database



Grey boxes indicate the absence of data.

Plant Material

Sample list:

No.	Sample	Sample Name
1	GN 9 Callus	C1
2	SN5 Callus	C2
3	GNN7 Callus	C3
4	Leaf 1	L1
5	Leaf 2	L2
6	Flag Leaf	FL
7	Inflorescence 1	I1
8	Inflorescence 2	I2

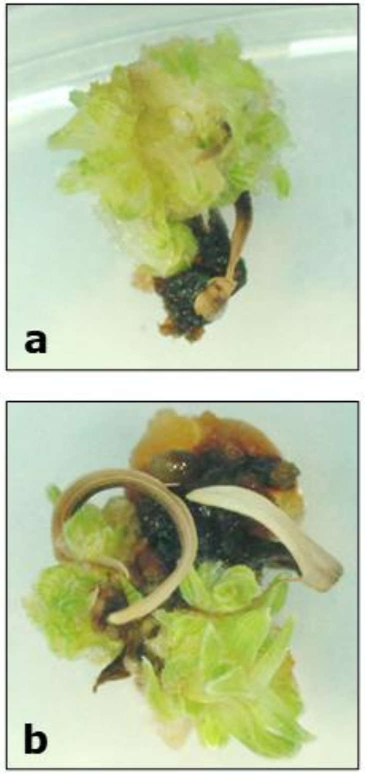


Photo credits: Kshitija Mishra & Liya Philip

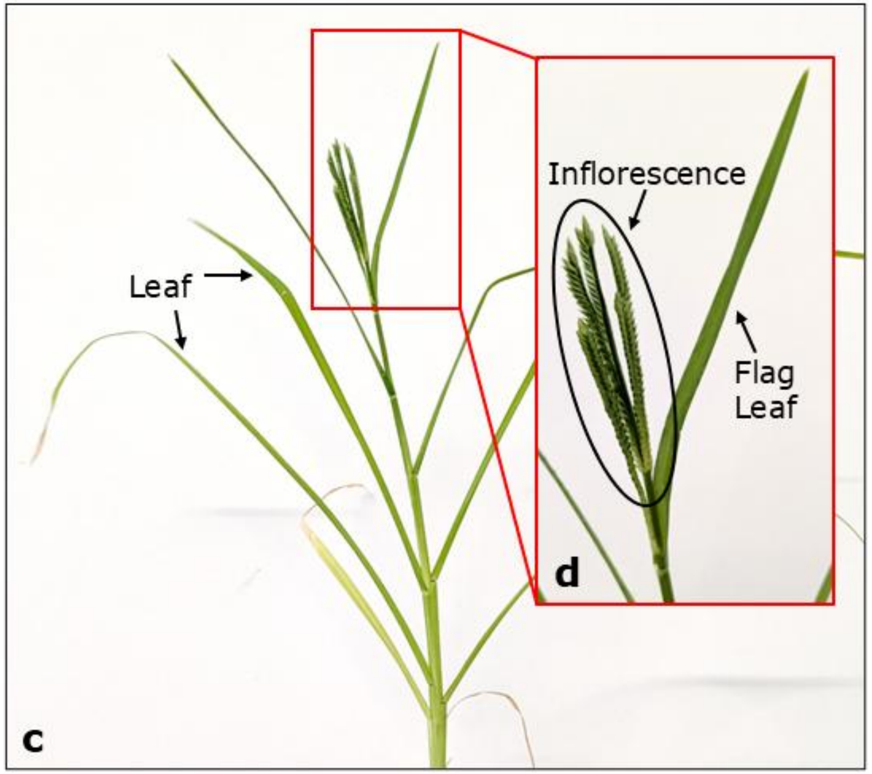


Photo credits: Siya Bhandari

RNA Isolation

- Homogenisation in liquid nitrogen
- TRIzol method

- Quantification:
    - Spectrophotometric methods (NanoDrop and Qubit)

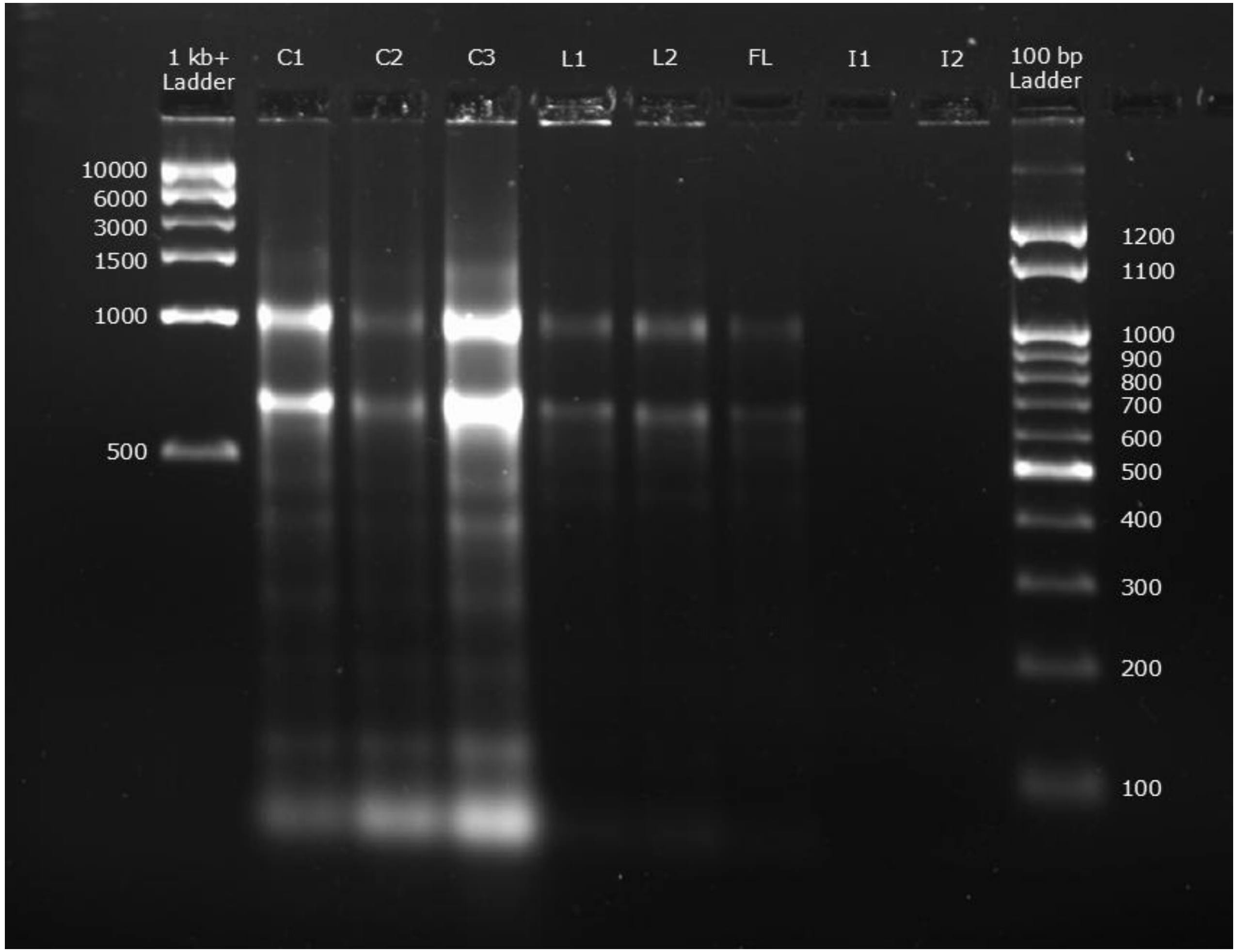
No.	Sample	ng/µl	A 260/280	A 260/230	Qubit Quant (ng/µl)
1	C1	1591.1	2.04	0.96	1096
2	C2	652.9	1.91	0.19	716
3	C3	813.1	2.02	0.48	1044
4	L1	100.6	1.81	0.52	86.7
5	L2	342.5	1.97	0.46	314
6	FL	103.9	1.80	0.38	116
7	I1	345.5	1.34	0.42	52.8
8	I2	418.7	0.97	0.16	46

- Quantitative analysis

RNA Isolation

- Quality check:
  - Agarose gel electrophoresis
  - Capillary gel electrophoresis (TapeStation)
- Qualitative analysis

No.	Sample	RIN
1	C1	7.6
2	C2	6.8
3	C3	7.5
4	L1	7.3
5	L2	8.0
6	FL	6.3
7	I1	-
8	I2	-



cDNA Synthesis & Quantitative Real-time PCR (qPCR)

- Bio-Rad iScript™ cDNA Synthesis Kit

5x iScript Reaction Mix	4 µl
iScript Reverse Transcriptase	1 µl
RNA sample (100 pg/µl)	15 µl
Total	**20 µl**

- qPCR using SYBR Green-based detection chemistry

2x iQ™ SYBR® Green Supermix	5 µl
Forward primer (5 µM)	0.5 µl
Reverse primer (5 µM)	0.5 µl
Nuclease-free water	3 µl
cDNA	1 µl
Total	**10 µl**

- Primers:

Primer Name	**Target Gene**	**Sequence (5'-3')**
qEcSERK-F1	*SERK1*	AGGAGTGGCAGAAGGTAGAA
qEcSERK-R1	*SERK1*	GGAGATTGTCAGTCGAGTCAAG
qEcActin-F	*β-actin*	CCAGTCCAAGAGAGGTATT
qEcActin-R	*β-actin*	CAACACGAAGCTCATTGTA

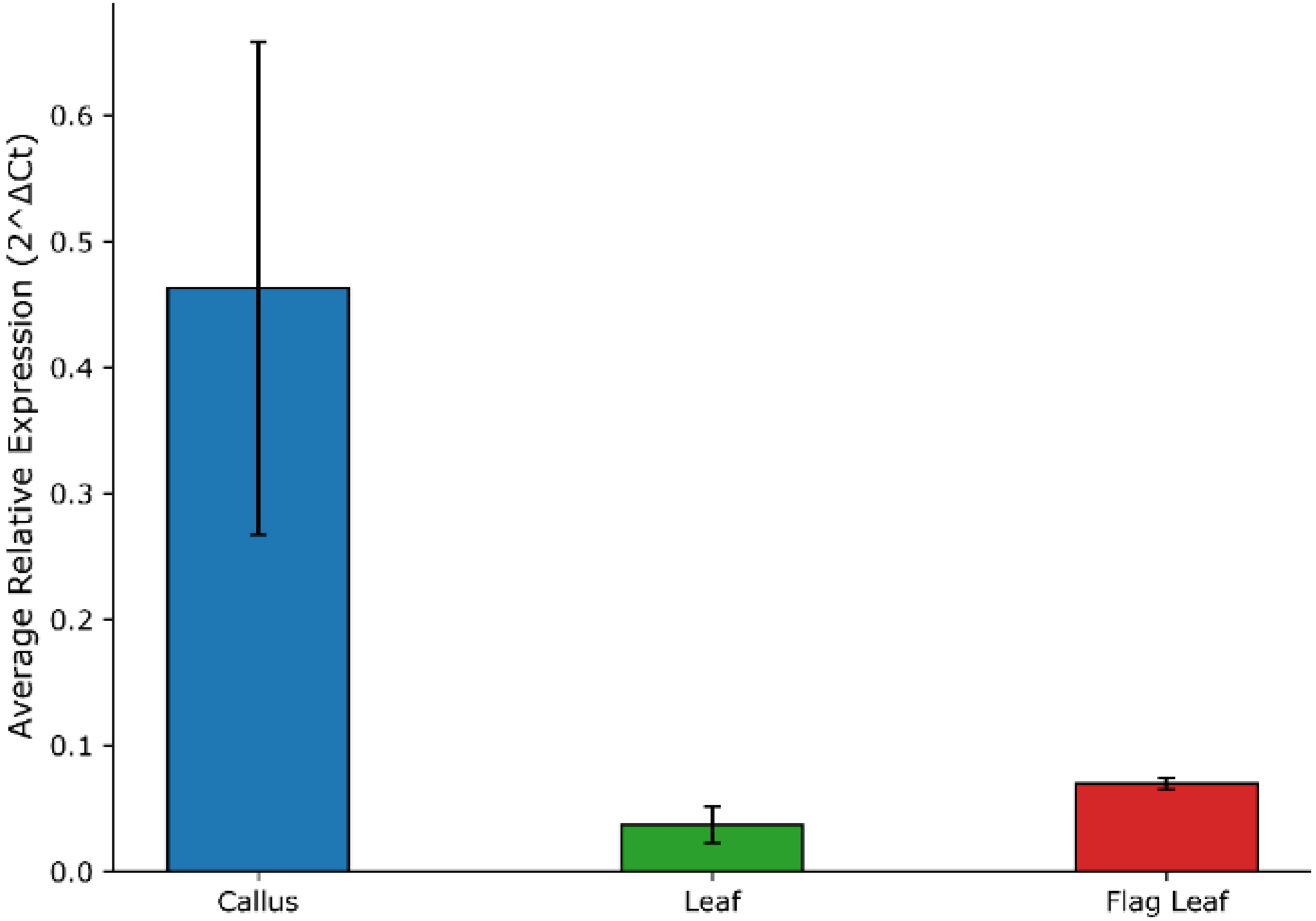
- Relative quantification using ΔCt method and fold change

qPCR Analysis

	Average Ct Value
Reference gene (*β-actin*)	23 ± 2.1
Callus	24 ± 0.9
(Vegetative) Leaf	27.5 ± 1.3
Flag Leaf	28 ± 0.08

$\Delta Ct = Ct_{\text{reference gene}} - Ct_{\text{target gene}}$

Avg. Relative Expression = $2^{\Delta Ct}$



Limitations

- Low number of biological replicates
- No statistical validation
- Exclusion of inflorescence samples due to RNA quality

Conclusion

- SERK1 protein is highly conserved
- Tissue-specific expression
- Linked to tissue regeneration and somatic embryogenesis
- Provides a molecular basis for improving tissue culture response

Future Perspectives

- Extend the study to more tissue types, especially reproductive
- Analyse variety-specific *SERK1* expression
- Investigate expression under abiotic stress
- Characterise *SERK* gene family and other morphogenic genes
- Apply gene editing/transformation tools

Acknowledgements

- Dr Bhuvan Pathak (Advisor)
- SBRI team: Dr Samarth Bhatt, Dr Vishal Nanavaty, Dr Nilay Shah, Mr Aditya Patel
- Navsari Agricultural University: Dr Swati Patel and Dr Sanjay Jha
- Lab mates, friends, and family
- Ahmedabad University & professors

Thank you.

Questions?