"Genes Identification involved in Flowering In *Stevia rebaudiana* by using Transcriptomic Data"

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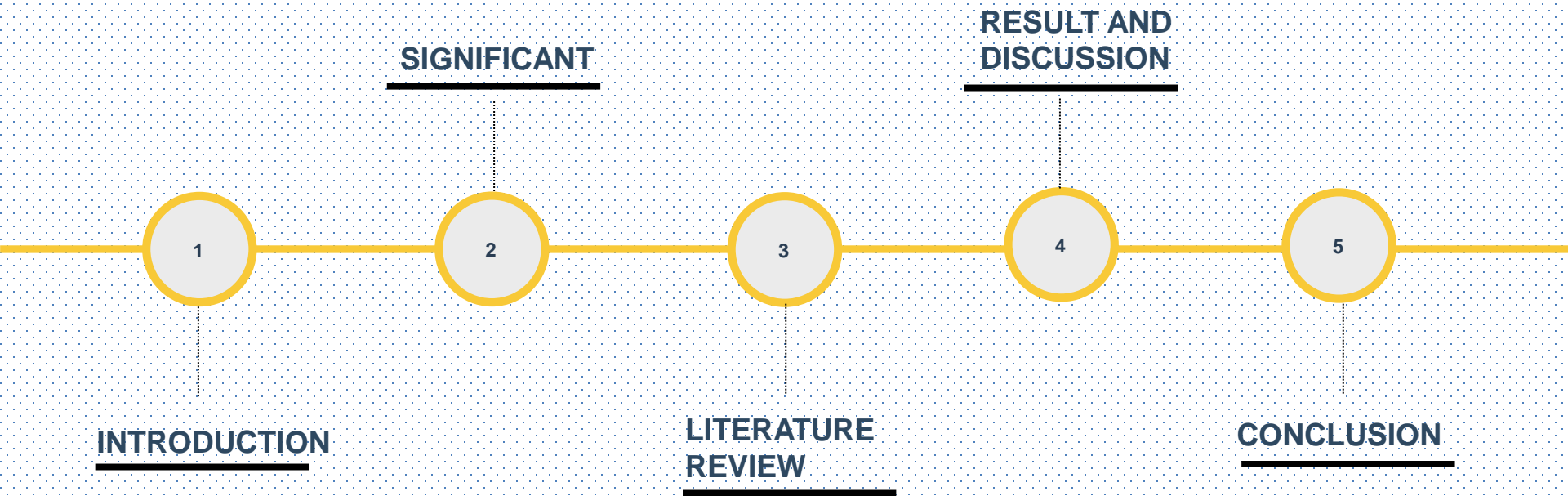Asst. Prof. Dr. Nurul Hidayah Samsulrizal IIUM Kuantan

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Garden of Knowledge and Virtue

INSTITUT BIOLOGI SISTEM
INBIOSIS
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SIGNIFICANT

RESULT AND DISCUSSION

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INTRODUCTION

LITERATURE REVIEW

CONCLUSION

RESEARCH OBJECTIVE

1. **To generates transcriptome library for transcriptomic analysis** of Stevia rebaudiana.

2. **To identify genes that involved in flowering process** by using bioinformatics tools.

PROBLEM STATEMENT

- **Formation of leaves dry weight will increase remarkably if the flowering of plant can be delayed** (Kim et. al., *2002*)**.**

- **However, there is still less evidence of data that can explain the role of flowering genes in S. rebaudiana.**

- **The development of Next Generation Sequencing (NGS) methodologies has offer useful strategies to overcome this problem**

HYPOTHESIS

Number of genes that involves in flowering process in *Stevia rebaudiana* will be identified which involves constructing, sequencing and analyses transcriptome library

SIGNIFICANT

- **Research shows that stevioside level are the highest at the time of flower bud formation and lower at time preceding and following flower bud formation** (Court *et. al.*, 2008)**.**

- **The outcome of this study will help in manipulation of flowering process especially in increasing the yield of steviol glycosides during harvest process**

Stevia rebaudiana

GROWTH ENVIRONMENT

- A mean annual temperature around 22°C, with minimum and a maximum temperature of 17°C and 26°C respectively correspond to humid climatic zone with a rainfall-deficient (Kinghorn *et. al.*, 2012).

CHEMICAL CONSTITUENT

1. Chemical constituent are steviolbioside, rebaudiosides, dulcosides and stevioside which considered sweetest compound in Stevia
2. 300 times sweeter than sugarcane sugar. (Morita *et al.*, 2009)
3. 0 calories sweetener containing mainly steviol glycoside (Mark, 2009)

IMPORTANCE

- strong demand for highly sweet, non-caloric and non-carcinogenic substances
- substitute sucrose for dietary daily intake that exhibit sucrose like taste

Bioinformatics tool/software

De novo assembly

- **Trinity** were used for de novo assembly of the Illumina reads. Trinity is a software of a novel method for the efficient de novo reconstruction and assembly of transcriptomes.

Homology Sequence Search Using Blast

- sequence similarity search program that can be used via a web interface or as a stand-alone tool to compare a user's query to a database of sequences

Domain Search Using InterPro

as an integrated documentation resource for protein families, domains and functional sites, to rationalise the complementary efforts of the individual protein signature database projects

Blast2GO software

- **B2G** joints in one application GO annotation based on similarity searches with statistical analysis and highlighted visualization on directed acyclic graphs

METHODOLOGIES
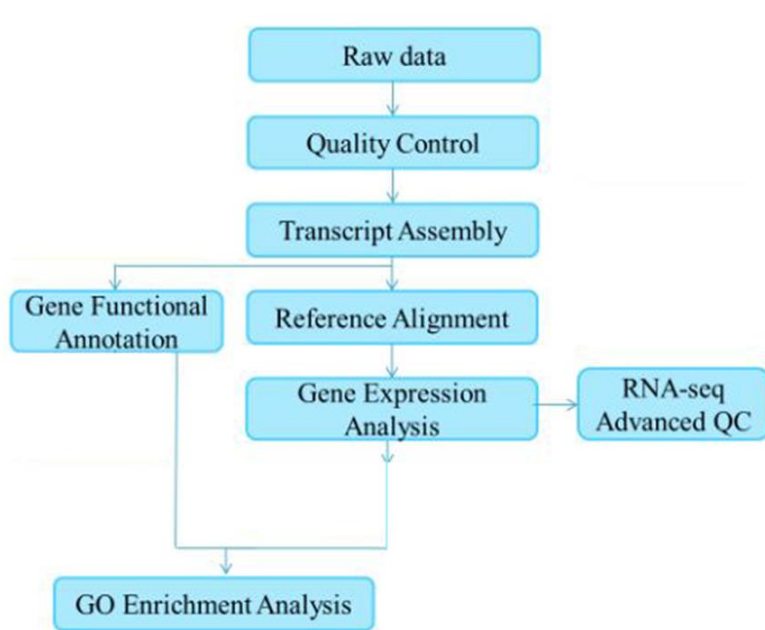
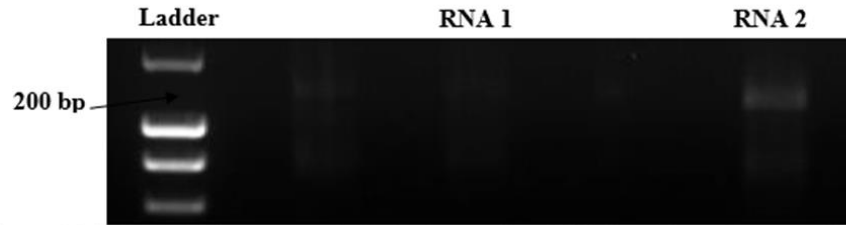| 1	Plant Preparation
2	RNA Extraction from Plant
3	Gel Electrophoresis of The Sample
4	Quantification by Using Nanodrop Spectrometer
5	Bioinformatics Method

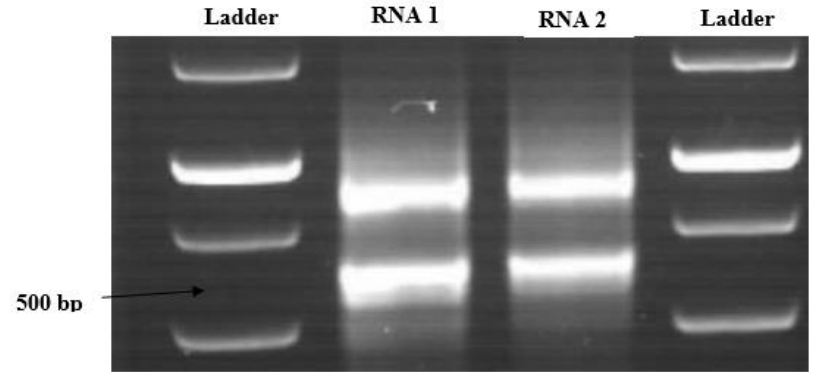## RNA extraction & Electrophoresis

Figure 4.1 EtBr-stained agarose gel displaying unclear RNA band from the controlled leaves using conventional method. Ladder: Hyperladder 100bp, RNA 1: extracted RNA of non-flowering *S. rebaudiana*. RNA 2: extracted RNA of flowering *S. rebaudiana*.

Figure 4.2 EtBr-stained agarose gel displaying unclear RNA band from the controlled leaves using Geneaid Kit. Ladder: Hyperladder 1kb, RNA 1: extracted RNA of non-flowering *S. rebaudiana*. RNA 2: extracted RNA of flowering *S. rebaudiana*

Data Quality Control
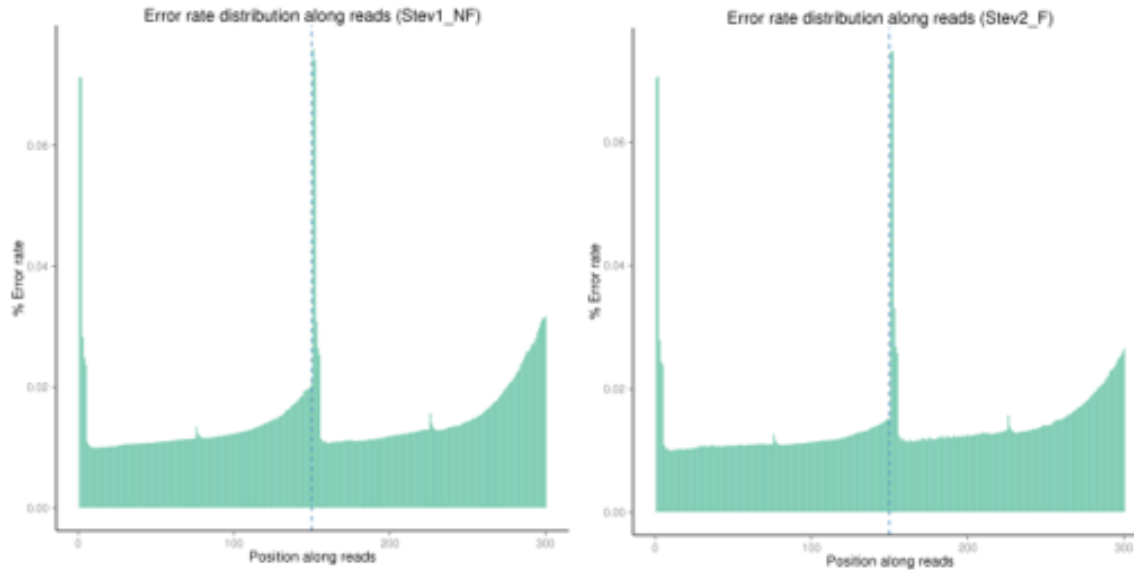
Figure 4.3 shows the graphs of error rate distribution along the reads of non-flowering and flowering *S. rebaudiana*
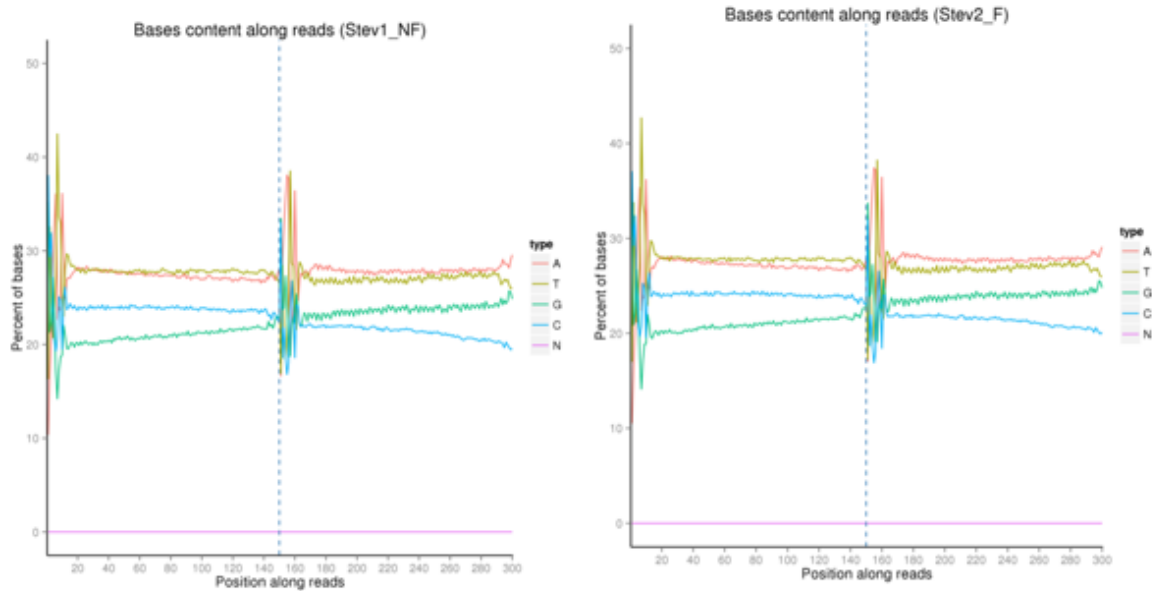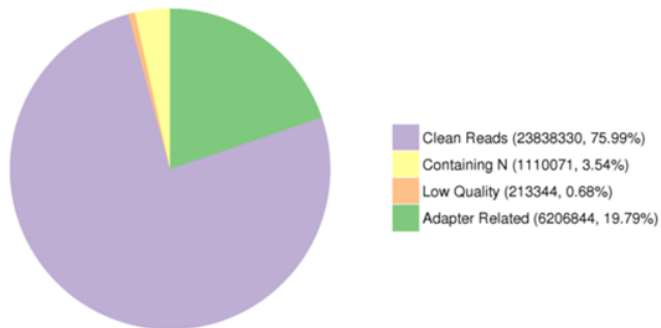
GC Content DIstribution

Figure 4.4 shows the graphs of GC distribution along the reads of non-flowering and flowering *S. rebaudiana*

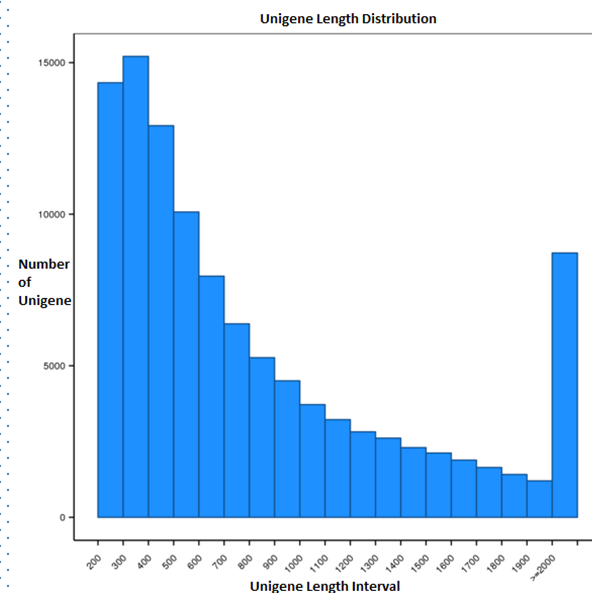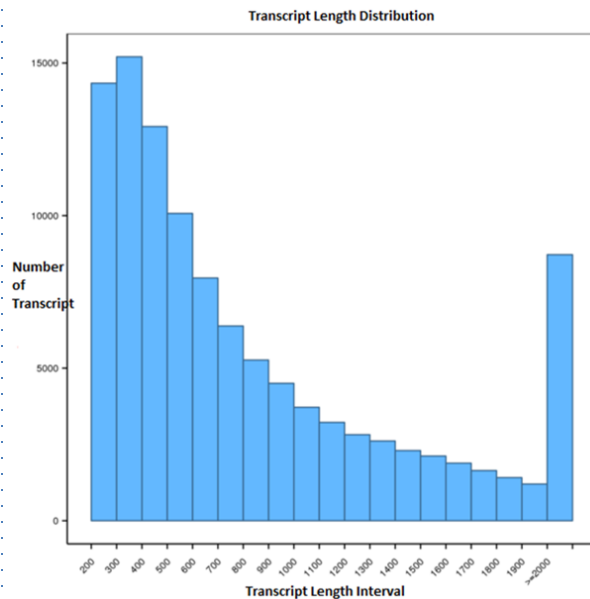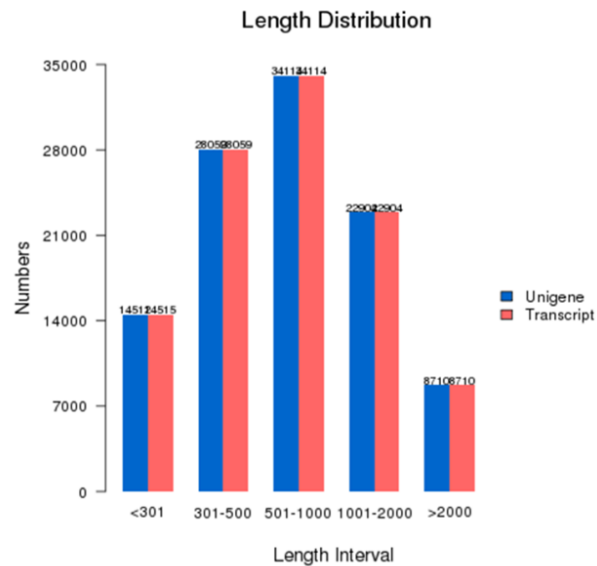## Data Filtering

Classification of Raw Reads (Stev1_NF)

- Clean Reads (23838330, 75.99%)
- Containing N (1110071, 3.54%)
- Low Quality (213344, 0.68%)
- Adapter Related (6206844, 19.79%)

Classification of Raw Reads (Stev2_F)

- Clean Reads (22138647, 82.84%)
- Containing N (455670, 1.70%)
- Low Quality (159694, 0.60%)
- Adapter Related (3972029, 14.86%)

Transcript Length Distribution

Functional gene annotation
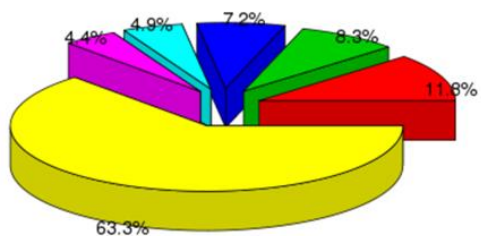
	Number of Unigenes	Percentage (%)
Annotated in NR	73959	68.29
Annotated in NT	38099	35.17
Annotated in PFAM	52335	48.32
Annotated in GO	52994	48.93
Annotated in KOG	26800	24.74
Annotated in all Databases	12144	11.21
Annotated in at least one Database	79708	73.59
Total Unigenes	108299	100
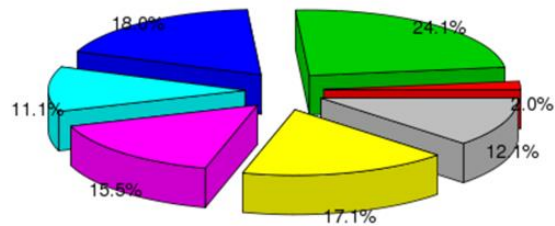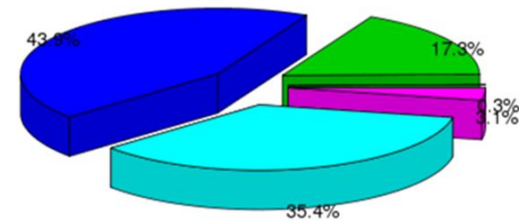
Functional gene annotation

Gene Expression Analysis

Sample name	Total reads	Total mapped
Flowering	44277294	35636530(80.48%)
Non-flowering	47676660	37635756(78.94%)
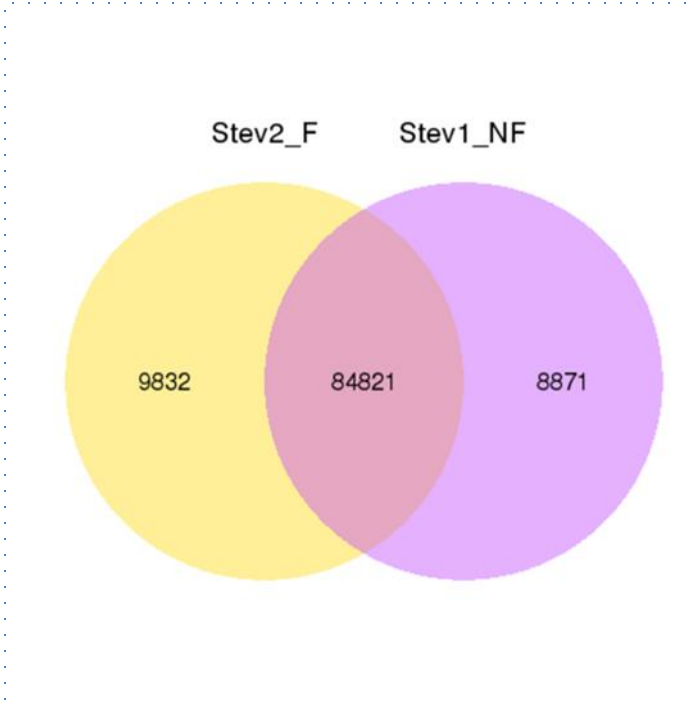
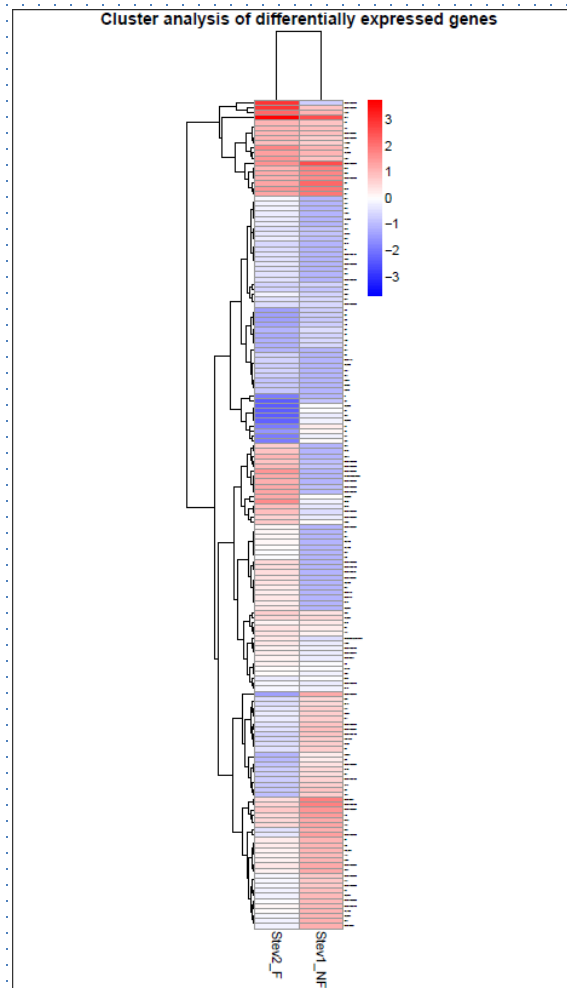## Gene Expression Analysis

Read count value obtained from the gene expression analysis is used as the input data to do differential expression analysis.
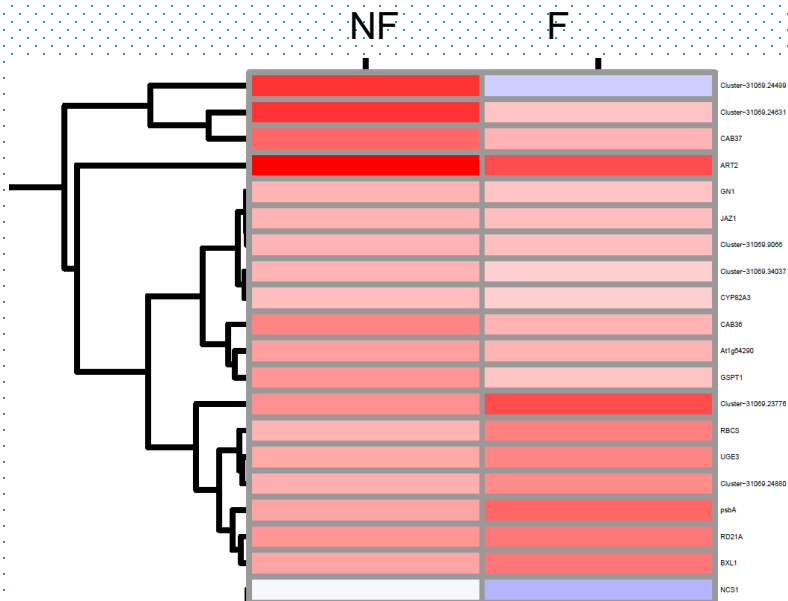
For samples without biological replicates, TMM is first used to normalize the read count value, and DEGseq is used to do the analysis

For experiments without biological replicates, the threshold is normally set as: [log2(Fold Change)] > 1 and q-value < 0.005.

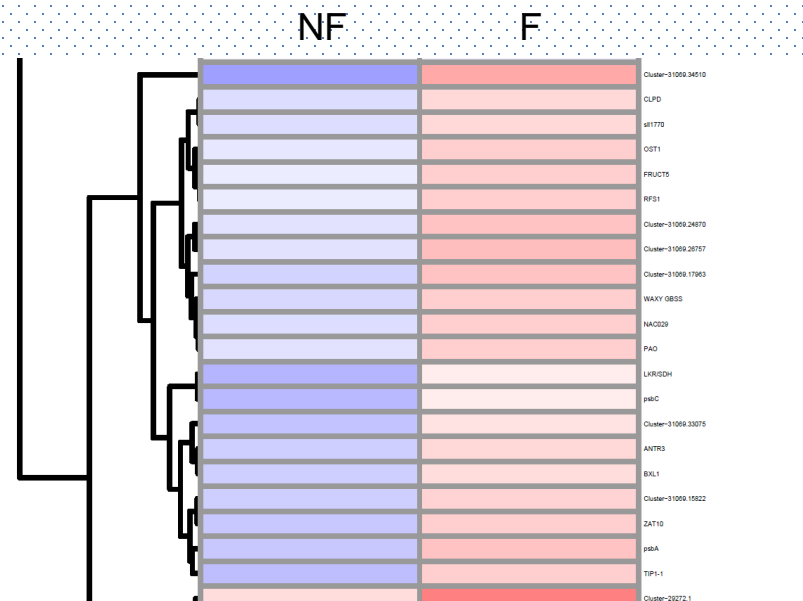Cluster analysis of differentially expressed genes

Gene Expression Analysis

NF   F

Both data of NF and F contain upregulated genes

NF   F

Only F data contain upregulated genes

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Upregulated flowering genes	Downregulated flowering genes
(At4g27290)	(UGE3)
(GASA6)	(SAG21)
(PAO)	(CTN)
(ABA2)	(ACC1)
(TIFY10A)	(LHY)
(UPF1)	(PAO)
(FRS6)	(ABA2)
(CAB37)	(ZAT10)
(CBL2)	(NAC029)
(SRK2E)	(SRK2E)
(TIF3E1)	

Genes in F data
(SAG21)
(LHY)
(ZAT10)
(NAC029)

RESULT & DISCUSSION

Genes	Function	Expression
1. SENESCENCE-ASSOCIATED GENE 21 (SAG21) genes	1. Mediates tolerance to oxidative stresses (e.g. hydrogen peroxide H_2O_2 2. Prevents premature aging (e.g. senescence and flowering)	Expressed in roots, stems leaves and flowers, but not in seeds (Mowla et. al, 2006)
2. *LATE ELONGATED HYPOCOTYL (LHY)* genes	1. Transcription factor involved in the circadian clock, binds to the promoter region of APRR1/TOC1 and TCP21/CHE to repress their transcription	Expressed in leaves, roots, stems, flowers and siliques (James et. al, 2008)
3. ZINC FINGER PROTEIN (ZAT10) genes	1. Transcriptional repressor involved in abiotic stress responses. Can repress the stress responsive genes DREB1A and LTI78	Expressed in roots, stems and leaves (Lippuner V., Cyert M.S., Gasser C.S. 2006)
4. NAC TRANSCRIPTION FACTOR 29 (*NAC029* gene)	1. Transcription activator that binds to, and transactivates the promoter of the abscisic aldehyde oxidase AAO3. 2. Promotes chlorophyll degradation in leaves	Expressed in senescing leaves, petals and sepals. (Guo Y., Gan S., 2006)

Protein Interaction Analysis
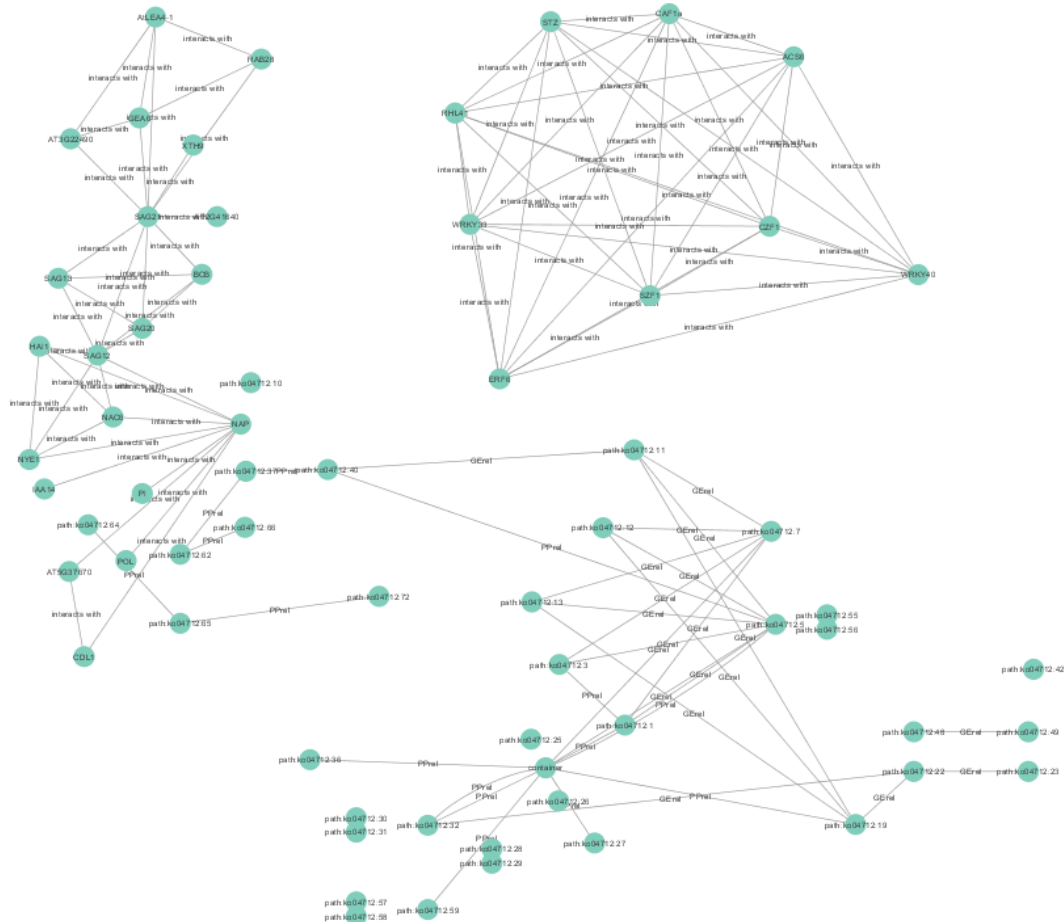
❝❞ Conclusion

LATE ELONGATED HYPOCOTYL (LHY) genes, **SENESCENCE-ASSOCIATED GENE 21 (SAG21)** genes, **ZINC FINGER PROTEIN (ZAT10)** genes, and **NAC TRANSCRIPTION FACTOR 29** genes was found highly expressed in flowering sample of *S. rebaudiana* compared to delayed flowering

,, References

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2. Court, W. E., & Hardman, R. (2006). *Medicinal and aromatic plants: Industrial profiles. Vol.15. bayanbox.ir*, 13. Retrieved from https://sci-hub.tw/http://bayanbox.ir/download/4311739981762865014/A.-DouglasKinghorn-Stevia-The-Genus-Stevia-Medicinal-and-Aromatic-Plants Industrial-Profiles-2001.pdf, p. **171**
3. Kinghorn, A.D. and Soejarto, D.D. (2002) Stevioside. In Alternative Sweeteners (2nd edn, Revised and Expanded), L.O'Brien Nabors and R.C.Gelardi (Eds), Marcel Dekker, Inc., New York, p. **157–171**
4. Mark Stibich. (2009). About Stevia Sweetener-Is it better than sugar? [Online]. Available: http://longevity.about.com/od/lifelongnutrition/a/stevia-extract.htm
5. Morita, T., Morita, K., and Koichiro, K. (2009). Variety of Stevia rebaudiana Bertoni with a high content of Rebaudioside – A plant. Publication No:US 2009/0214753 A1. United State Patent Application Publication

Acknowledgement

Thank you!