Today's Talk

Tips for Giving Talks

Background of DNA methylation and Plant phenotype

Hypothesis and Results

Summary/Conclusion

Future Experiments

Questions

Shuya Wang
MCDB191 Epigenetics
8th January 2024

What constitutes a good presentation

What constitutes a good presentation?
- **Introduction** (10 min):
  - Explain the **relevant background/techniques** needed to understand the paper.
  - **Use material from other papers and reviews** on the reading list or other papers.

- **Paper** (20-30 min):
  - Convey the **paper's main message** concisely while **critically** analyzing the validity of the data presented.
  - **Don't have to explain every figure** of the paper in great detail.
  - Try to **understand the materials and methods,** but you don't need to introduce every detail of the techniques used.
  - Try to end with a **statement of the paper's main findings and** the **next logical questions** to ask in this field.

What constitutes a good presentation

General Tips:

- **You are responsible for the information you present on your slides:** If you put a figure on your slide, your audience will expect you to be able to explain it and answer questions on reasoning, method, and implications, etc.
- **Its better to say more with less**
  - People will not read slides with paragraphs of text, and you will lose their attention.
- **High contrast is best for people to read your slides**
  - Black on White, for example
  - Avoid highlighter colors like this: NO
- **Be conscientious of time limits for presentations and stick to those limits**

LETTER

Loss of *Karma* transposon methylation underlies the mantled somaclonal variant of oil palm

Meilina Ong-Abdullah[1], Jared M. Ordway[2], Nan Jiang[2], Siew-Eng Ooi[1], Sau-Yee Kok[1], Norashikin Sarpan[1], Nuraziyan Azimi[1], Ahmad Tarmizi Hashim[1], Zamzuri Ishak[1], Samsul Kamal Rosli[1], Fadila Ahmad Malike[1], Nor Azwani Abu Bakar[1], Marhalil Marjuni[1], Norziha Abdullah[1], Zulkifli Yaakub[1], Mohd Din Amiruddin[1], Rajanaidu Nookiah[1], Rajinder Singh[1], Eng-Ti Leslie Low[1], Kuang-Lim Chan[1], Norazah Azizi[1], Steven W. Smith[2], Blaire Bacher[2], Muhammad A. Budiman[2], Andrew Van Brunt[2], Corey Wischmeyer[2], Melissa Beil[2], Michael Hogan[2]†, Nathan Lakey[2], Chin-Ching Lim[3], Xaviar Arulandoo[3], Choo-Kien Wong[4], Chin-Nee Choo[4], Wei-Chee Wong[4], Yen-Yen Kwan[5], Sharifah Shahrul Rabiah Syed Alwee[5], Ravigadevi Sambanthamurthi[1] & Robert A. Martienssen[6]

Simplified overview of the paper

Epigenetic abnormality

↓

Faulty gene expression

↓

Faulty gene product

↓

Altered Phenotype

↓

Major crop loss
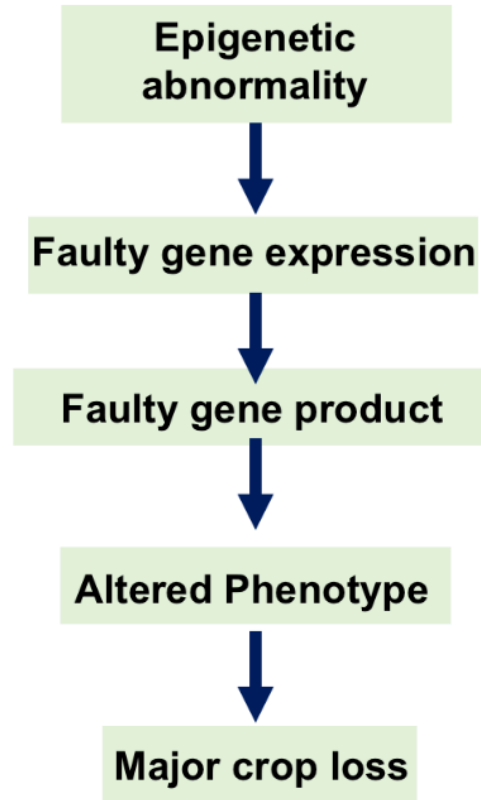
DNA cytosine methylation is an epigenetic modification
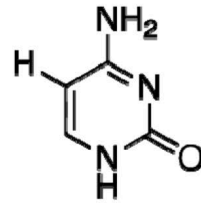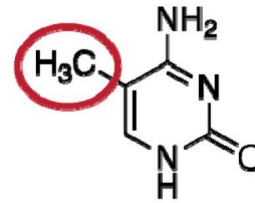
Cytosine   methylated Cytosine

5' ATGT**C** ^{m} **G**TAGTTC ^{m} TGATTGTATTC ^{m} ATTTTC 3'

- CG, CHG, CHH (H= A, T, C)

DNA cytosine methylation

Gene Silencing   **Transposon Silencing**   **Genome integrity**
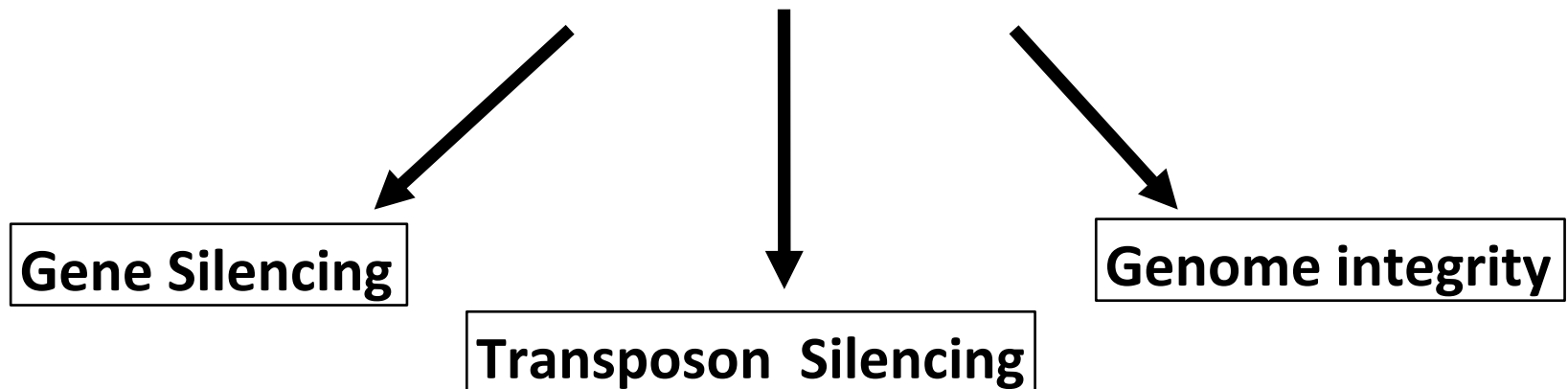
Crop in question: African oil Palm tree

- **Palm oil is the source of**
  - Edible vegetable oil
  - Biofuel
  - Used in other products – cosmetics, soap etc.

- **The mesocarp of the fruit is the source of Palm oil**

Mesocarp

Weckx et al. Front. In plant Sci. 2019
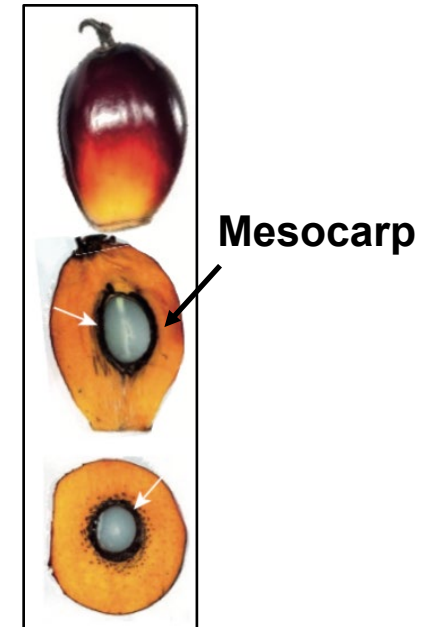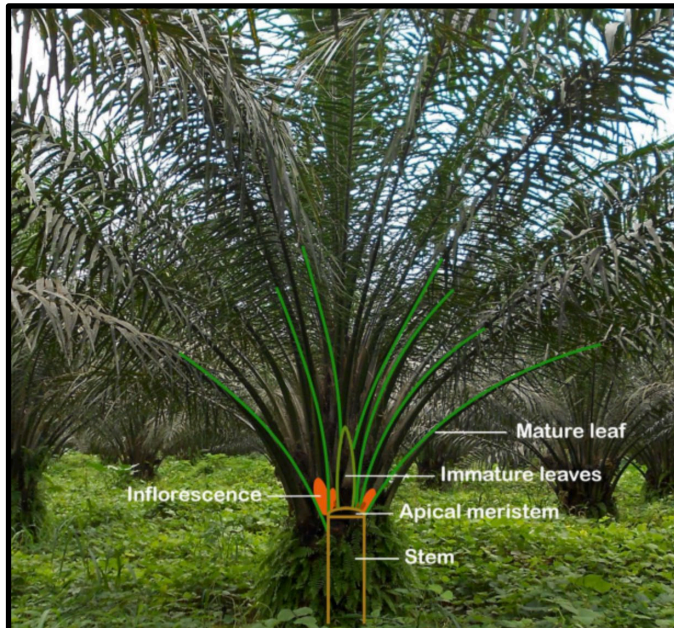
Micropropagation of high yielding varieties of oil Palm

Collect immature apex leaf tissue

Ortets

Mantled

~5 years

~2-3 years

~2-3 years
High frequency

Ramets

Normal fruit

Weckx et al. Front. In plant Sci. 2019
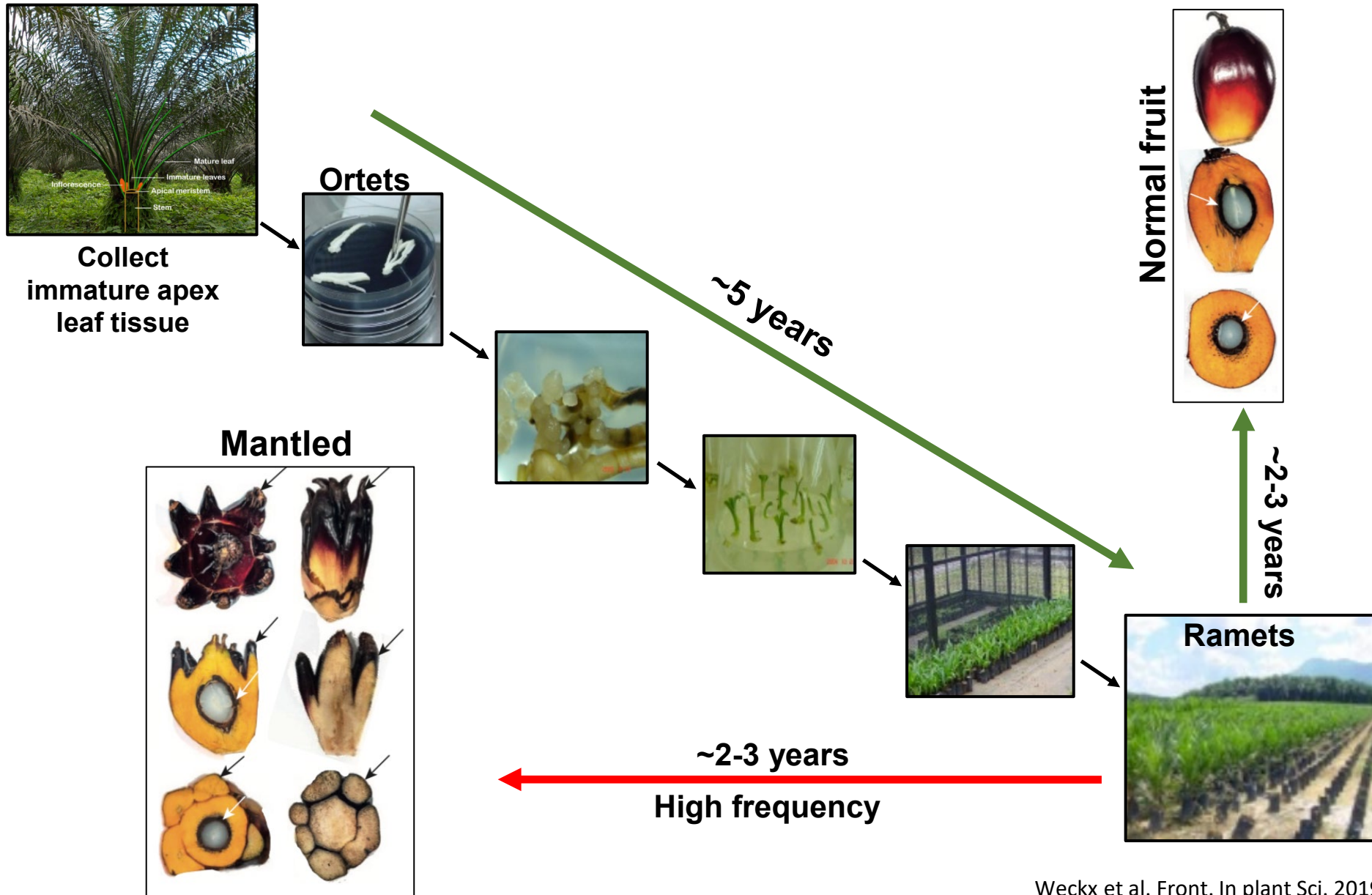
Abnormality in epigenetic modifications might lead to mantled phenotype

➤ **In plant tissue culture genetically identical clones but having variation in phenotype are called Somaclonal Variants**

Normal fruit

Mantled

Fertile Mantled

Parthenocarpic mantled
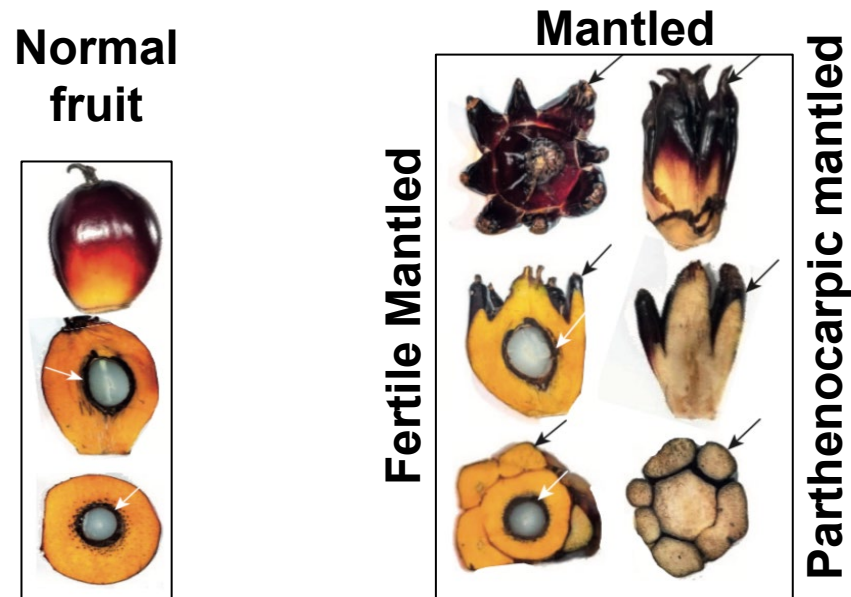
1. **Non-Mendelian inheritance of the mantled phenotype**
2. **Mantled trees exhibit global loss of DNA methylation**

What is the cause of the mantled phenotype?

Hypothesis :

Abnormality in DNA methylation at a specific locus leads to mantle phenotype

Weckx et al. Front. In plant Sci. 2019

Epigenome wide Association Study (EWAS)

	Normal Ramets	Mantled ramets
Epigenome	DNA methylation	DNA methylation

Compare
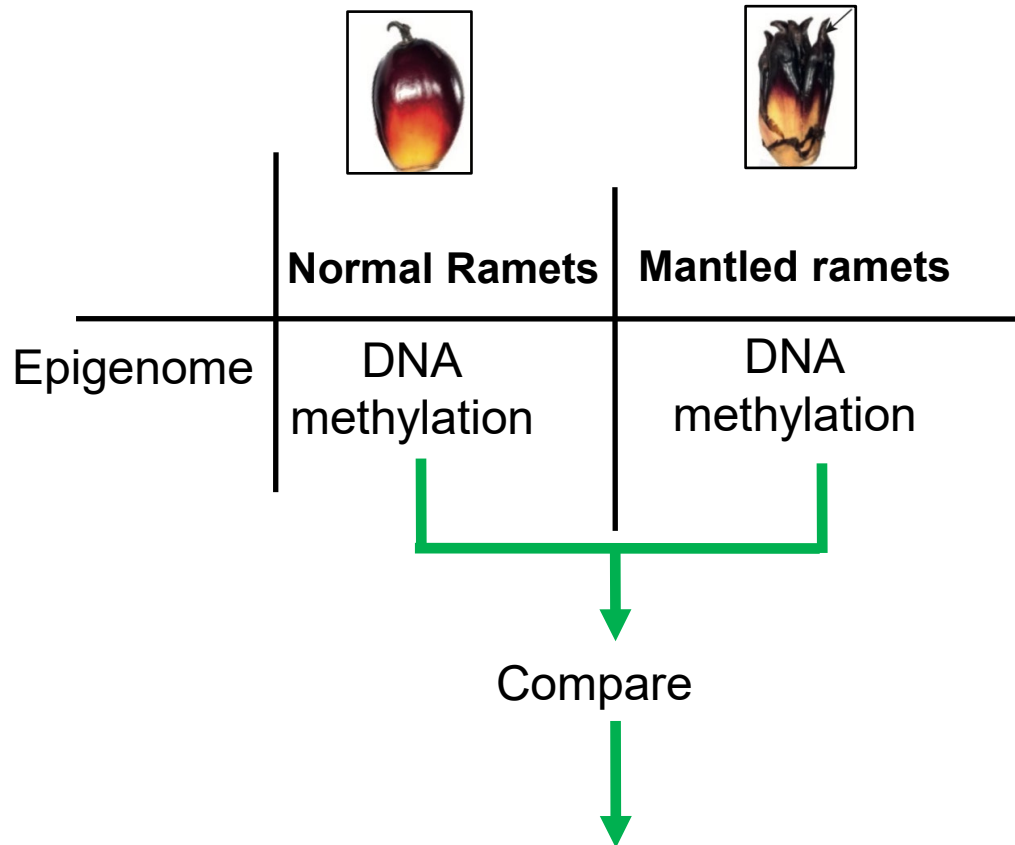
Identification of locus with epigenetic variation among normal and mantled lines

➢ **Genome wide DNA methylation maps : Methylation sensitive restriction enzymes followed by hybridizing it to genomic sequences**

Methylation sensitive restriction enzyme-based assay for DNA methylation analysis

Normal plant

Plants with phenotype

1. Methylation sensitive restriction enzyme (McrBC) : Digests DNA with methylated cytosines

X   Y   Z

X   Y   Z

X   Y   Z

X   Y   Z
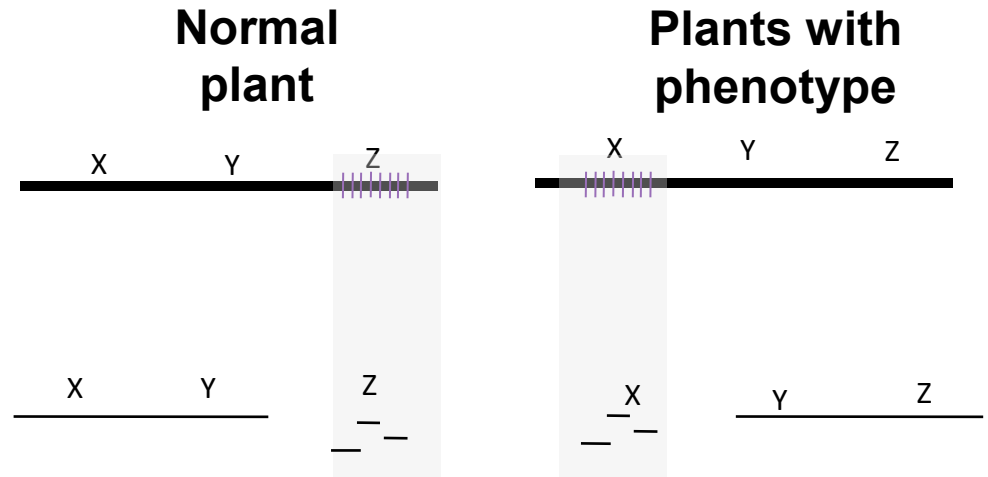
2. Determine the regions of genomic DNA that got digested

DNA Microarray

Several thousand hypomethylated regions found form EWAS studies

	Normal	Mantled
Total biological replicates	43 ramets	54 ramets
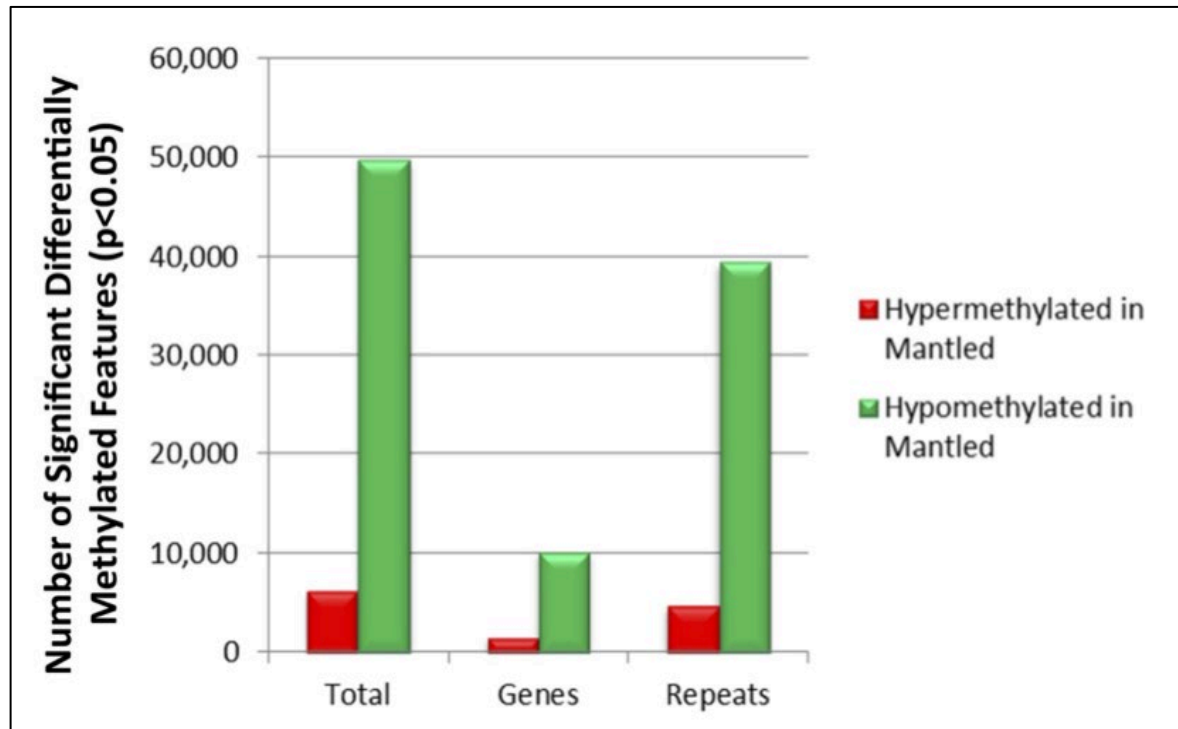
One differential methylated region (DMR) in all four mantled populations

Source Population	Normal (n=43)	Mantled (n=54)
Red	15	15
Brown	6	14
Blue	14	15
Green	8	10
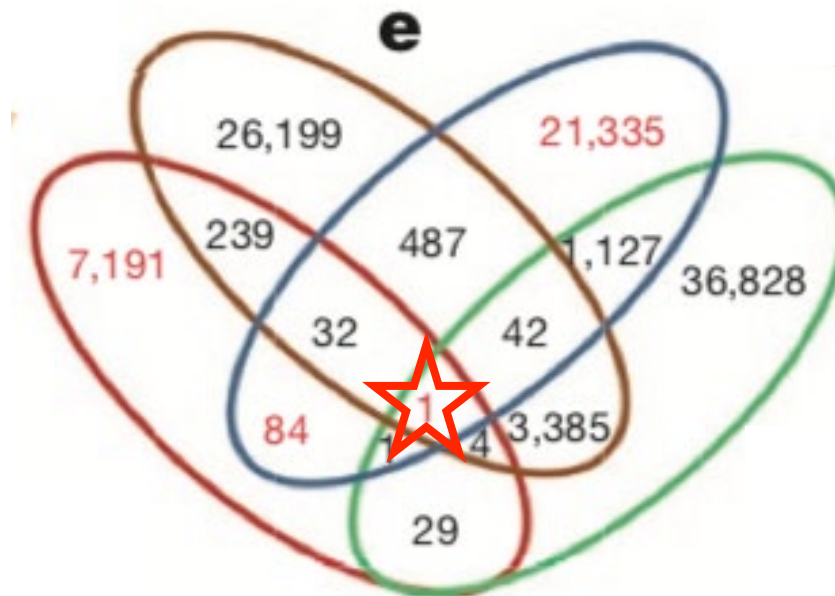
The DMR lies within the 35 kb intron 5 of *EgDEF1* gene
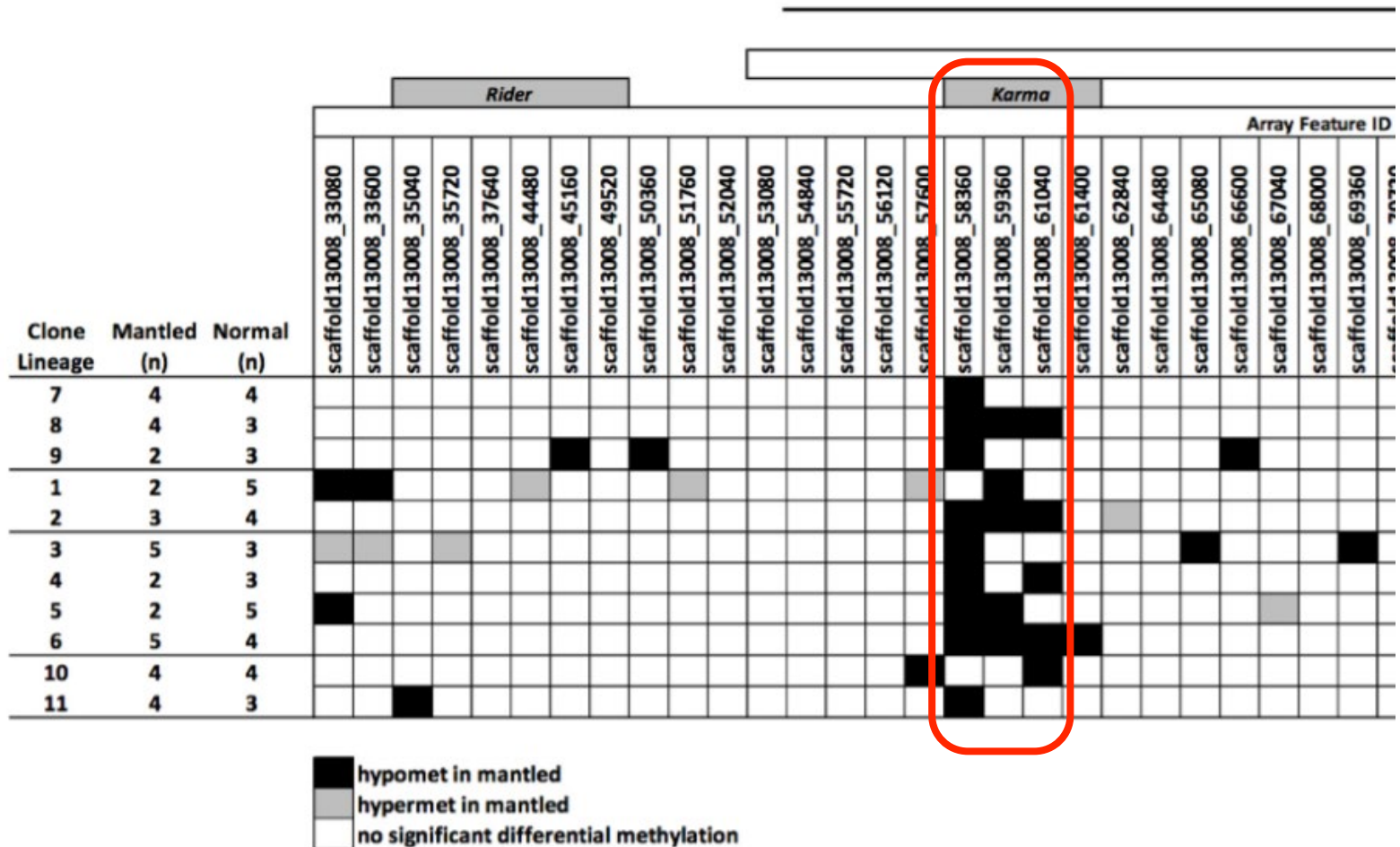
DNA methylation profile of the whole genome at the single nucleotide level

Whole genome bisulfite sequencing

Cytosine   methylated Cytosine

Bisulfite treatment

U   C
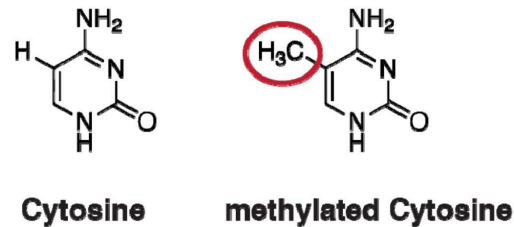
Sequencing the converted DNA

Aligning to a reference genome

Quantify the percentage of methylation for each cytosine of the genome

CHG methylation was dramatically reduced in the mantled clones

5' ATGTC^{m}GTAGTTC^{m}TGATTGTATTC^{m}ATTTTC 3'
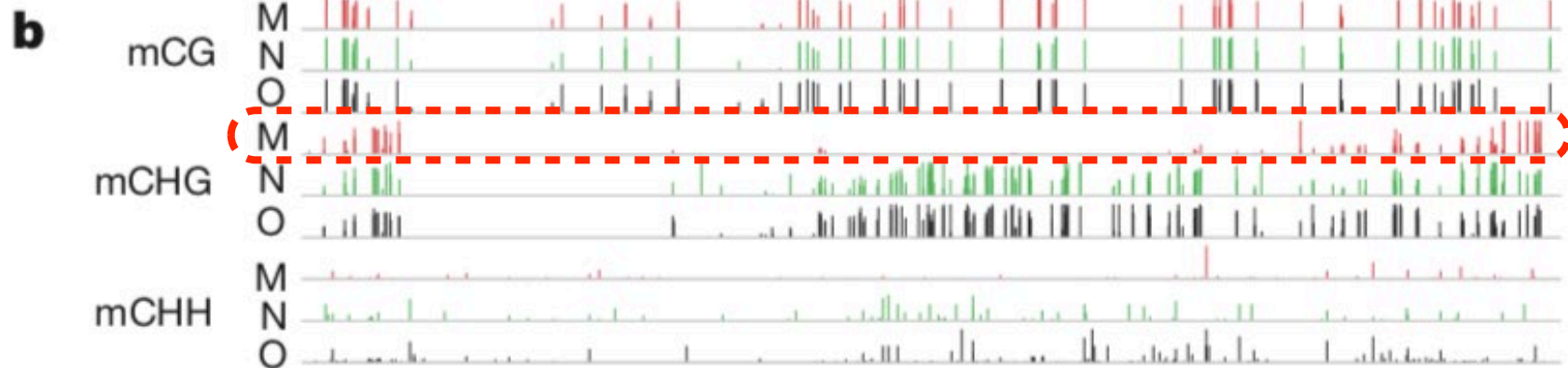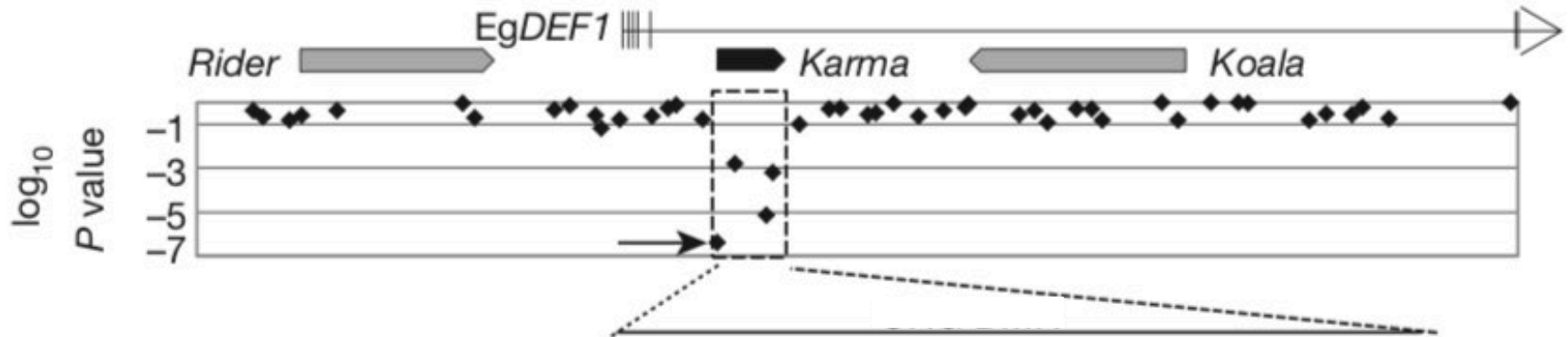
DNA hypomethylation at Karma: Strong predictor of mantled fruits

Methylation sensitive restriction enzyme assays : BbvI and RsaI
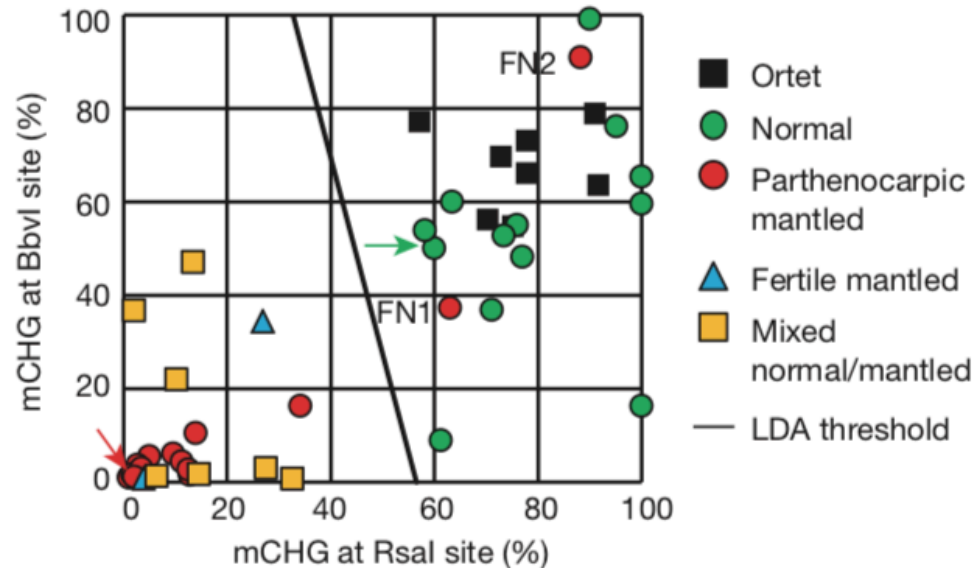
Linear discrimant analysis: **93% sensitive and 100% specific for mantle**

Mantled hypermethylated epiallele: Good karma
Mantled hypomethylated epiallele : Bad Karma

Splice acceptor site (methylated in all 4 four populations)

Karma sequence

Is there any relationship between DNA methylation at Karma and splicing of the gene *DEF1* (*MANTLE*)?

DNA methylation ?

A novel transcript (kDEF1) is identified that gets spliced at the proximal Karma acceptor site in the mantled ramets

Primer	Sequence
1	CTTTCCGACACCAAGACCA
2	GTTCGTCACAGAAAATACCATGT
3	CAAGTAGCGGATAGAGAGGCTTAC
4	TCTTCTGATCGCCTTGCTAGA
5	ACCGGATCAAAGACCGTAAAG
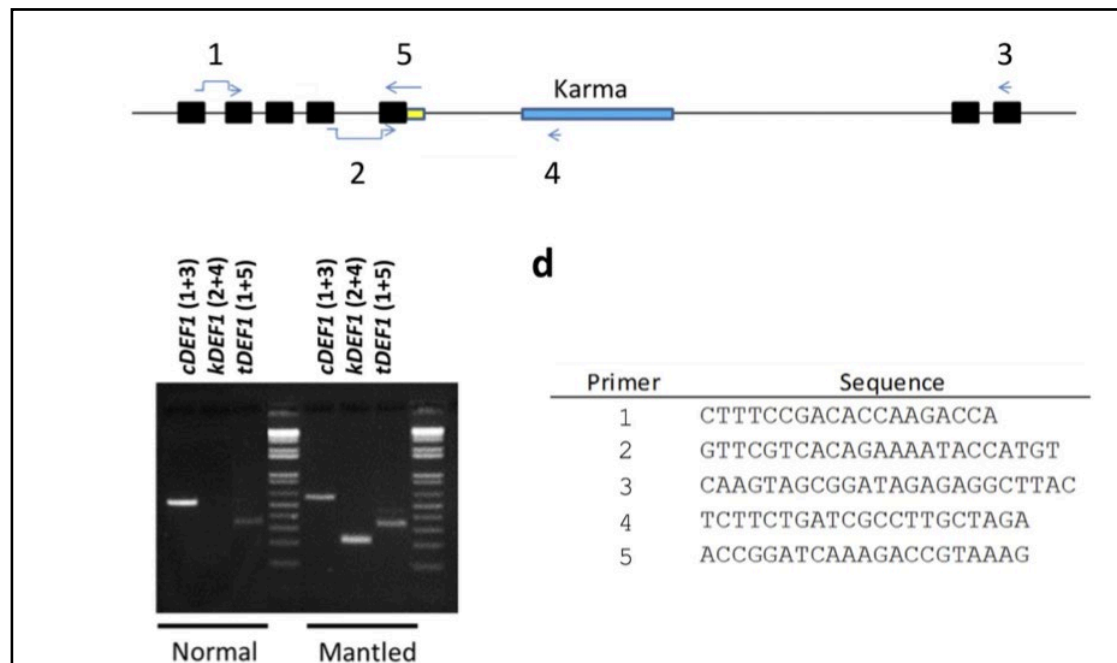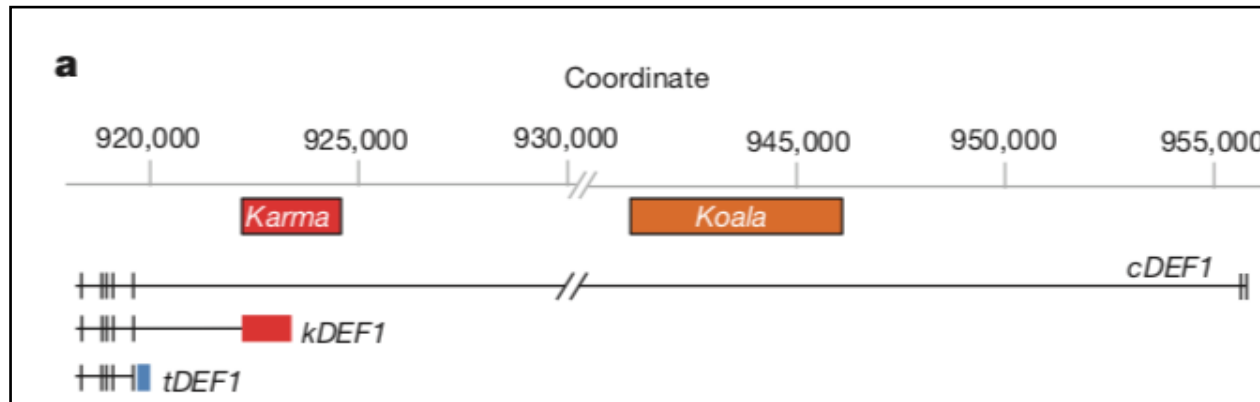
kDEF1 is only restricted to developmental stage 3 to 5 of mantled immature female inflorescence
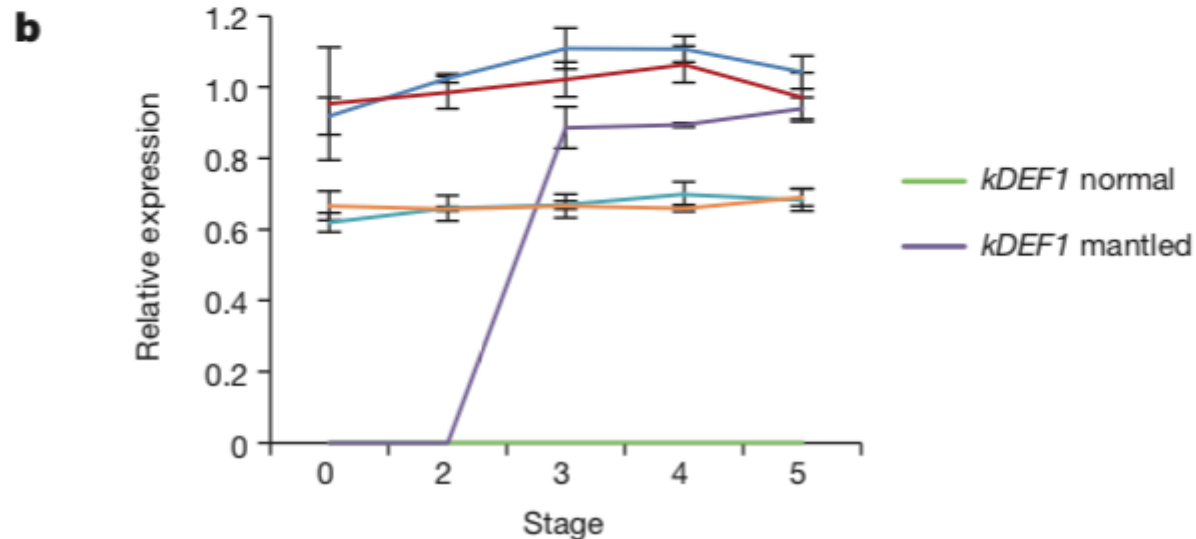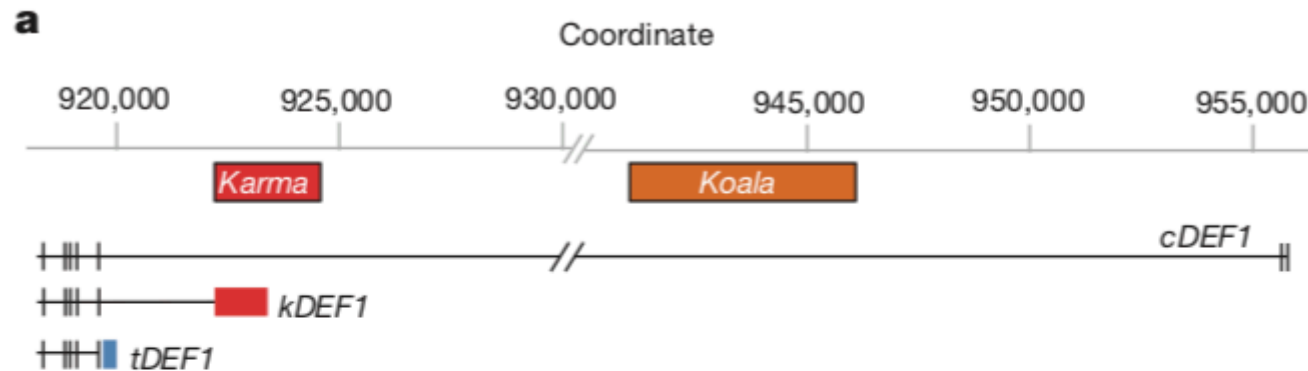
Why is *Karma* not methylated in the mantled phenotype?
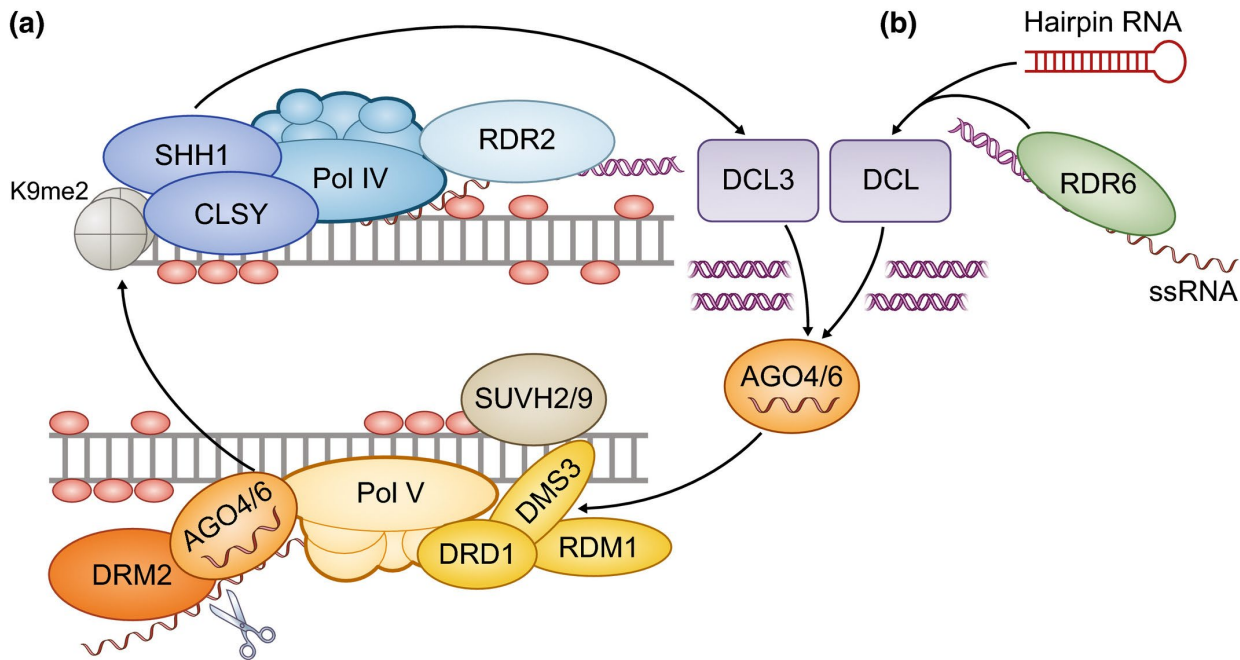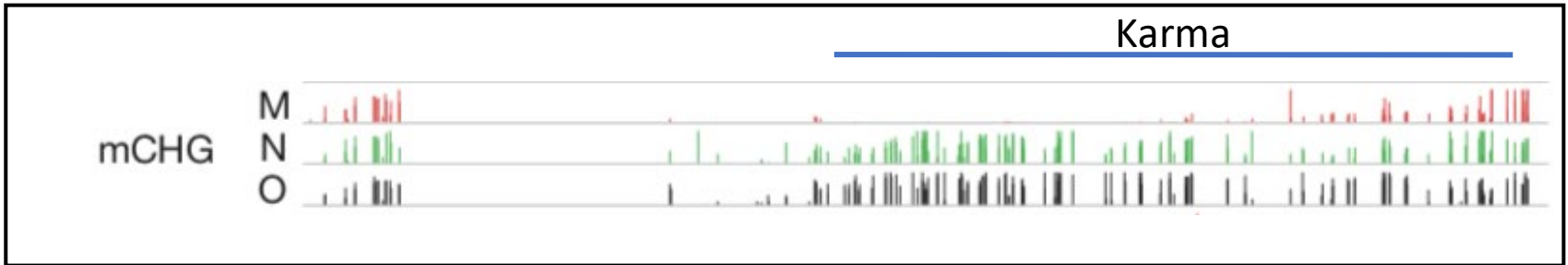
Reduction in small RNAs at *Karma* observed in tissue cultured or mantled samples
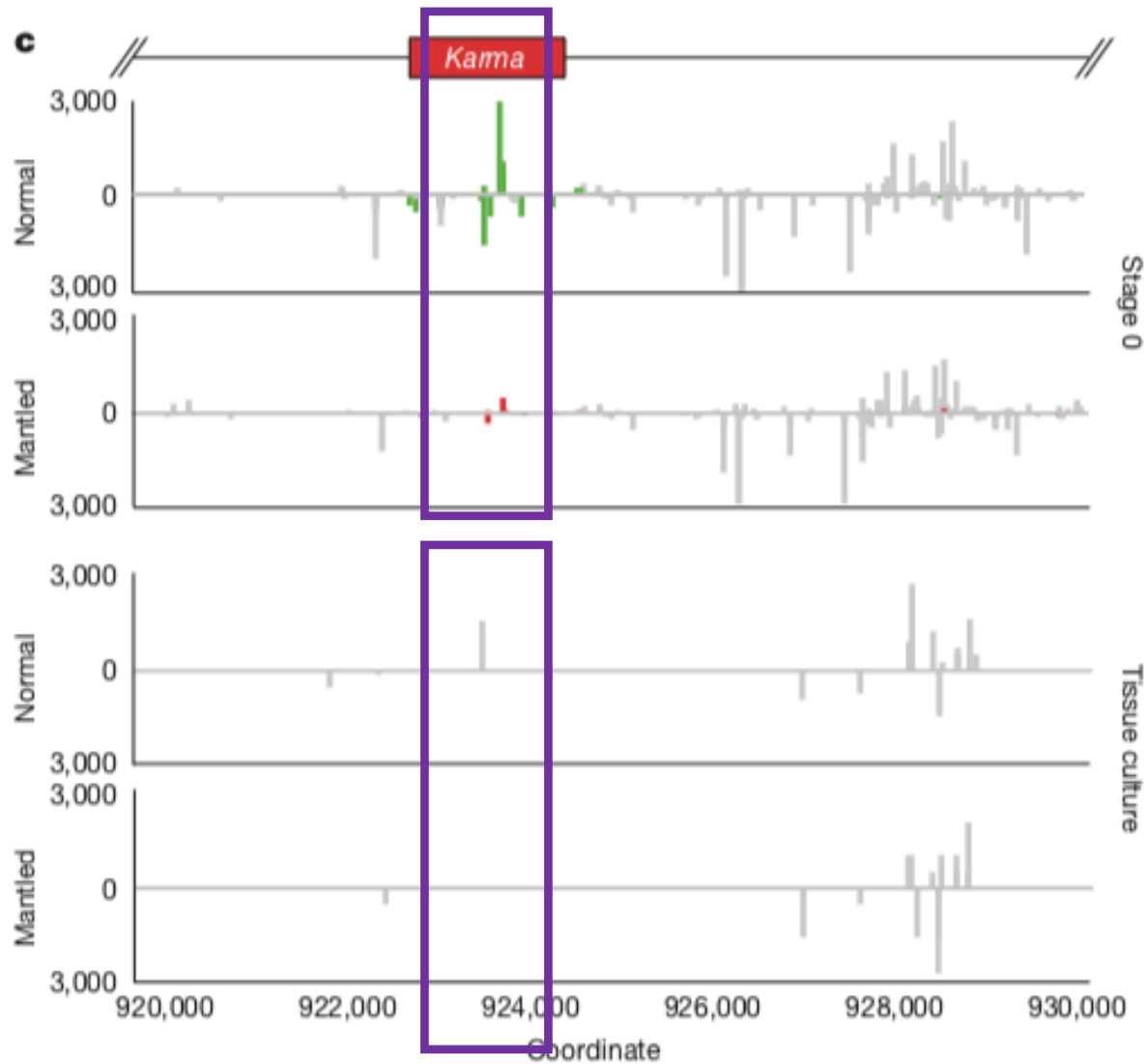
Loss of small RNAs at *Karma* observed in mantled and later stages of inflorescence development
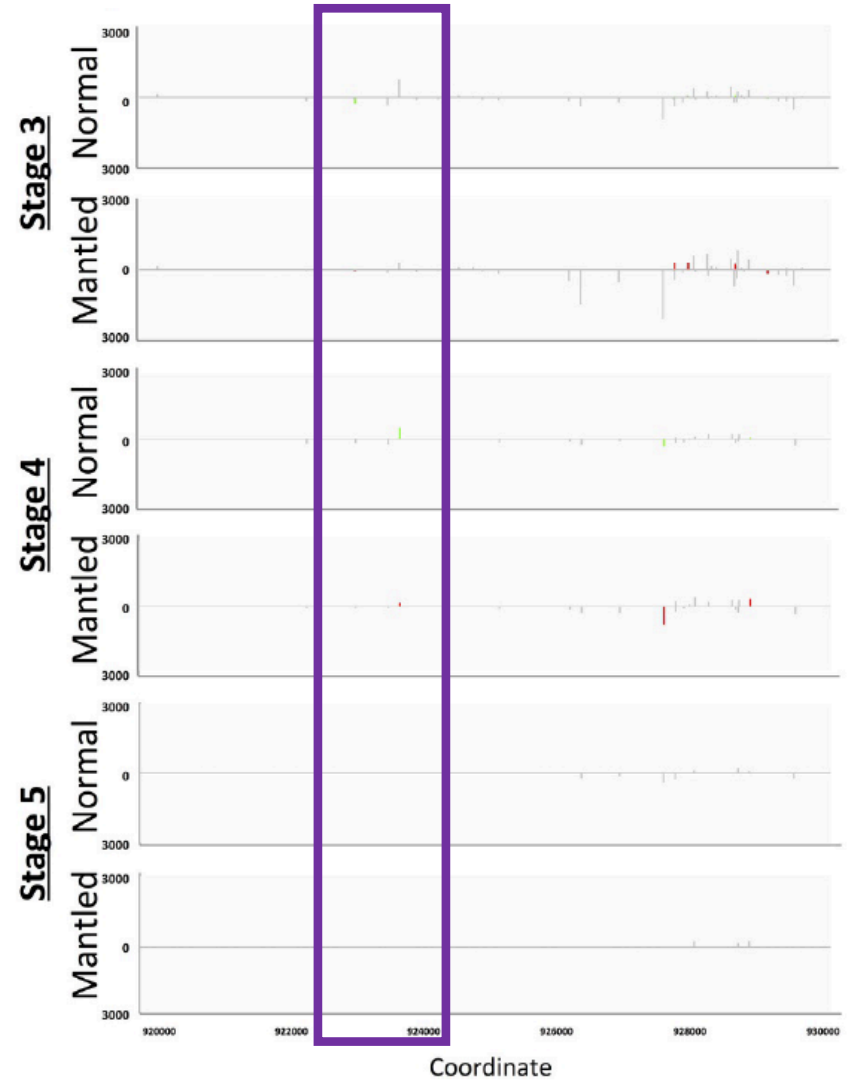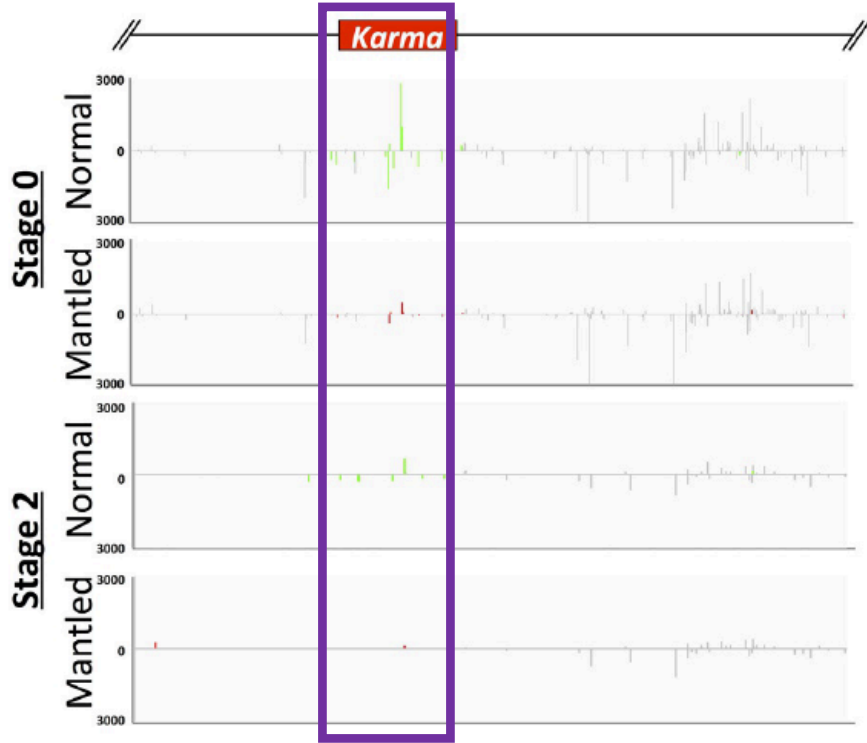
Summary

1. DNA hypomethylation at Karma is the underlying cause of mantled phenotype.

2. DNA hypomethylation at Karma effects alternative splicing of the *MANTLED* gene

3. The spliced product may have a role in the mantled phenotype

4. Small RNAs specific to Karma may also play a role in DNA hypomethylation at Karma

5. Absence or presence of DNA methylation at Karma is a strong predictor of the mantle phenotype: *Good Karma or Bad Karma*

Model for mantled phenotype

Normal fruit development

Meristem (Stage 0) → Small RNAs → DNA methylation at Karma → Properly processed *MANTLED* transcript → Correct gene products
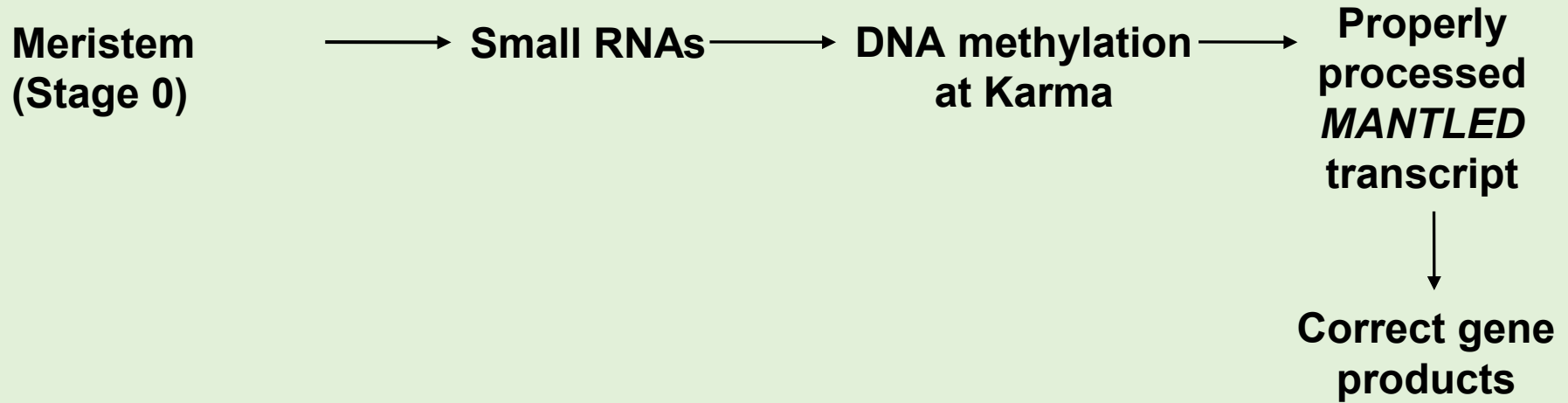
Mantled fruit development

Tissue Culture —X→ Small RNAs —X→ DNA methylation at Karma → Alternatively processed transcript → Truncated gene products
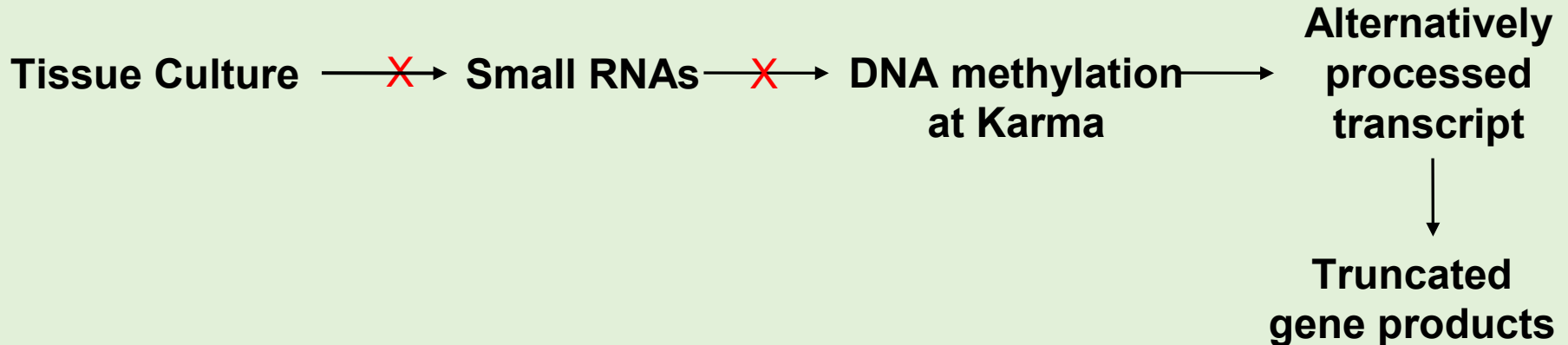
Future Experiments

1. Targeted epigenetics to cure the mantled phenotype using CRISPR based tools

2. Develop PCR based assay to identify mantled phenotype at early stages of tissue culture (Orion genomics)

3. DNA Methylation is mostly presently in the promoters and in the exons of a gene. Interesting case to study the role of methylation in the intron of a gene and its role in splicing

Questions?

Broad overview of the story of the paper

Epigenetic abnormality	Loss of DNA methylation
↓	↓
Faulty gene expression	Splicing defect
↓	↓
Faulty gene product	Truncated transcript
↓	↓
Altered Phenotype	Mantled phenotype
↓	↓
Major crop loss	Low oil production
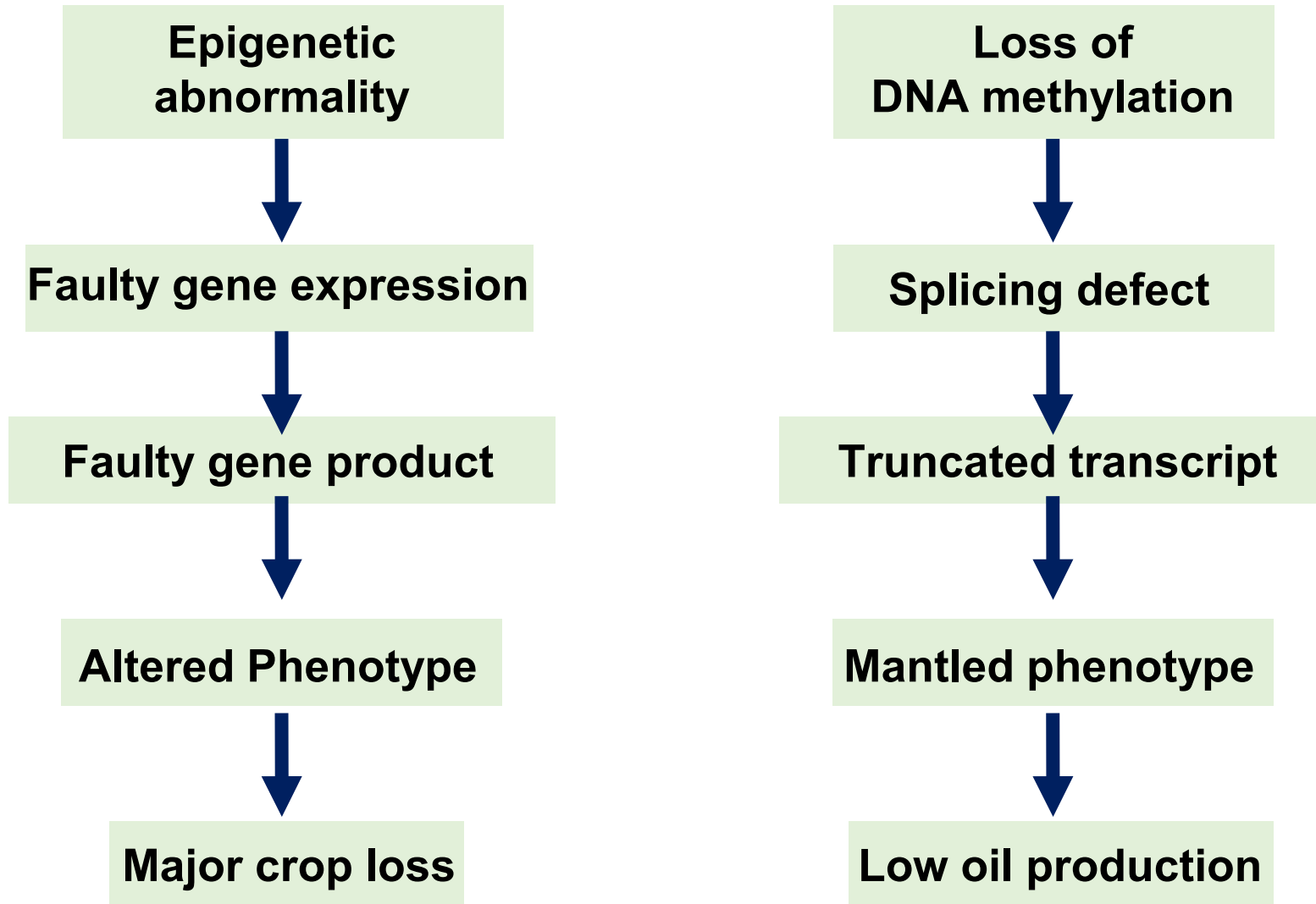
Alernative Splicing
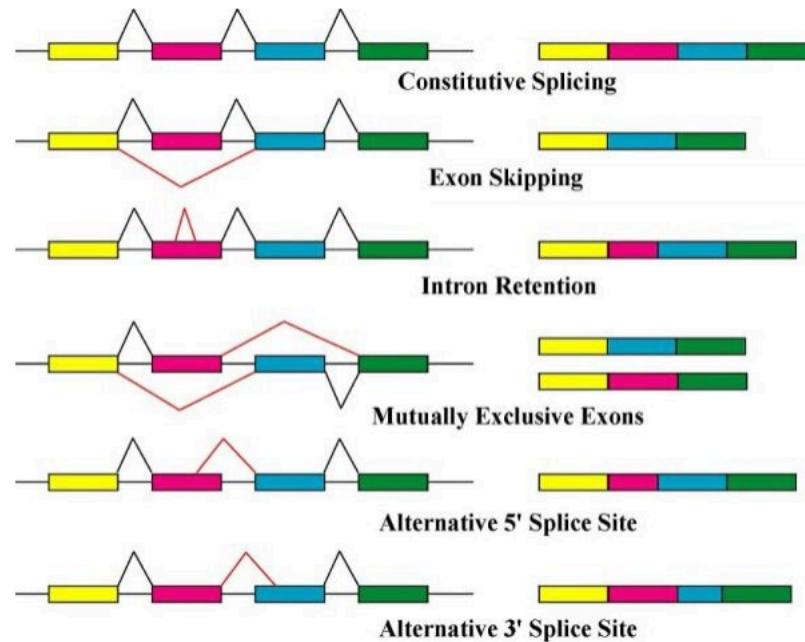
Methylation sensitive restriction enzyme-based assay followed by hybridization to DNA microarray

Normal plant

Plants with phenotype

X   Y   Z   X   Y   Z

1. Methylation sensitive restriction enzyme (McrBC) : Digests DNA with methylated cytosines

X   Y   Z   X   Y   Z

Red tag   **Green tag**
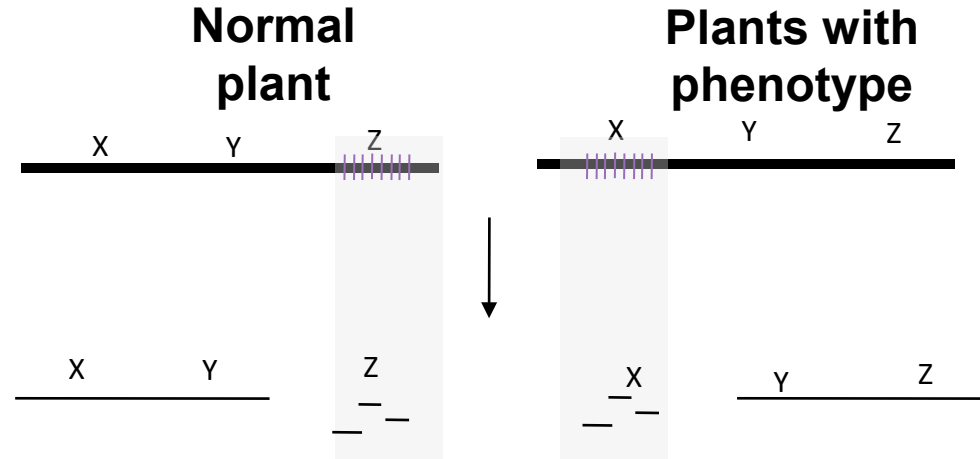
2. Label digested DNA using fluorescent tags
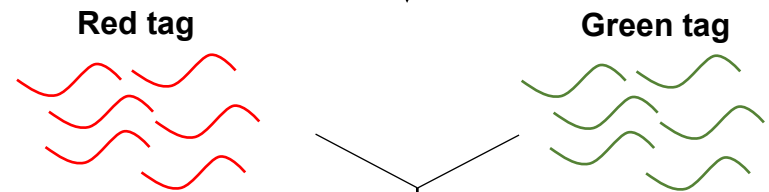
3. Mixing and hybridization

DNA microarray

(X) (Y) (Z)

Wells with X, Y, Z specific probes
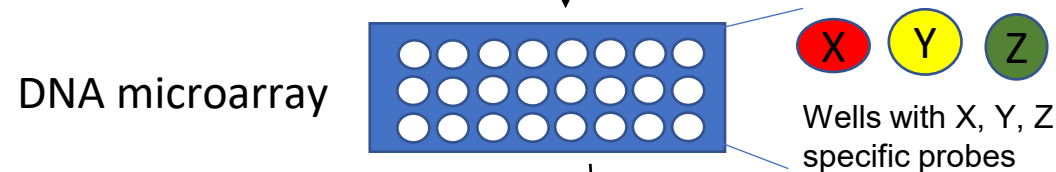
4. Assemble data to form DNA methylation maps and compare
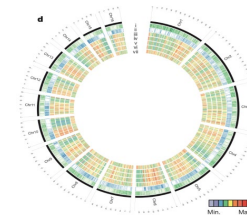
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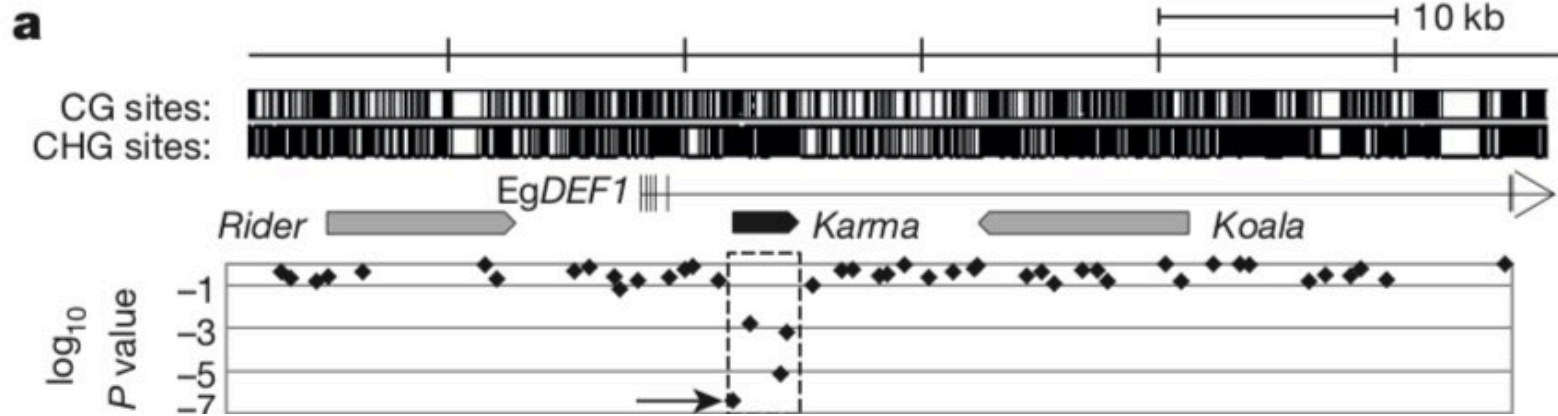
Unreported repetitive element:
Karma **line element (3.2kb)**

Splice acceptor site
(methylated in all 4 four
populations

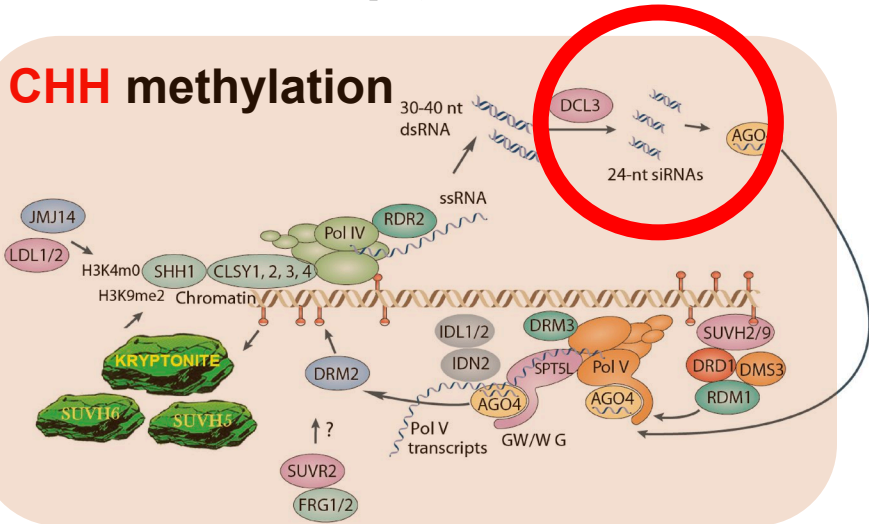# Four interlinked self-reinforcing methylation systems